Dear Colleagues and Friends

It is my distinct pleasure to welcome everyone to the 4th Annual Tsukuba Global Science week. This year's event coincides with the University of Tsukuba's 40th + 101st anniversary and is a part of the celebration. Our history extends back 141 years when it was founded by the Meiji government as Japan's first institution of higher education and premier Normal School, or school for educating teachers. Forty years ago in October 1973, our university was relocated from its location in Tokyo to Tsukuba City where it was reborn as a comprehensive institution of higher education.

Since its establishment, the University of Tsukuba has aimed for interdisciplinary education and research and to be a university open to society and the world. In accordance with these principles, we are delighted to host this year's Tsukuba Global Science Week, which will be held from October 2nd-4th and includes many participants not only from a diverse range of academic fields, but also from many different countries. What started out as an event involving presentations from mainly researchers in the fields of medicine and medical sciences, this year's Global Science Week will have presentations and sessions related to chemistry, life sciences, human and systems biology, neuroscience, food security, public health and nursing, art and ecology, policy and planning, anthropology, education, and medicine and medical sciences. Furthermore, presenters and participants include academics, researchers and students from France, Germany, United Kingdom, Hungary, Laos, Taiwan, United States, Brazil, Australia, Vietnam, and of course Japan.

We are also proud that Tokyo has just been selected to host the 2020 Summer Olympics. In line with the Olympic spirit of inspiring youth to build a peaceful and better place, we trust that this event that brings such wide-ranging knowledge and research together will offer the opportunity to gain insights into and solutions for today's complex and global-reaching issues.

Lastly, I would like to thank all of you for your participation and sincerely hope that you will enjoy this invaluable research and academic program. I look forward to meeting with each of you during the week and in enhancing the partnerships between our institutions.

Caroline F. Benton

Caroline F. Benton
Vice President
University of Tsukuba

Dear Friends and Colleagues,

I extend a warm welcome to all delegates attending our Tsukuba Global Science Week. University of Tsukuba reached its 40th anniversary this year, and has been leading internationalization of higher education and interdisciplinary research in Japan. We hope to provide an opportunity for all of the participating institutions to strengthen our networks. In the age of expanding global problems such as population ageing, energy shortage and the destruction of the environment, our conference encourages your participation in divergent fields of sessions. We have relatively small numbers of attendees on divergent but related human topics. That is the greatest feature of this event and I believe to give attendees an excellent opportunity of expanding your horizons as well as international friendship, mutual respect and future collaboration to solve the problem for the global wellness.

Interdisciplinary research is one of the most important approaches for new discoveries and propels us to fearlessly confront challenges against the global problems. Conferences serve as a platform to exchange each others' research experience in the different fields.

The Graduate School of Comprehensive Human Sciences and The School of Integrative and Global Majors of the University of Tsukuba hope this interactive event will serve as an exchange forum to create the opportunity for all attendees to discuss on the topics outside of their specialties, encounter new field of work, understand their needs, become aware of the requirement of new developments, and share great ideas.

Mitsuyasu Kato
Associate Provost (Medical Programs)
Graduate School of Comprehensive Human Sciences
University of Tsukuba

Tsukuba Global Science Week 2013: PROGRAM

Wednesday, October 2

Venue	Main Convention Hall	Conference Room 303	Conference Room 304	Conference Room 406(405)
AM	8:30-8:40 **Opening Remarks** 8:40-8:55 **Globalization of Universities** 8:55-9:25 **Our Partner Universities** 9:25-9:40 **Photo Session**			
	9:40-13:25 **Integration of Chemistry and Life Science** P. 7	10:00-11:40 **Art and Ecology** P. 45		10:00-12:50 **Food Security and Human Health** P. 73
	13:25-14:25 **Lunch**	11:40-13:00 **Lunch**		12:50-14:30 **Lunch** -13:30-14:20 **Millipore Luncheon Semina-**
PM	14:25-15:10 **Keynote Lecture (Life Science Workshop)** P. 25 15:15-18:30 **Life Science Workshop Ⅰ & Ⅱ** P. 25	13:00-17:00 **Art and Ecology** P. 45	13:00-17:50 **Policy and Planning Sciences** P. 55	14:30-18:00 **Food Security and Human Health** P. 73
	18:30- **Reception** -Our Partner Universities-			

Thursday, October 3

Venue	Main Convention Hall	Conference Room 303	Conference Room 101	Conference Room 406(405)
AM	8:30-9:30 **Keynote Lecture (Public Health / Nursing)** P. 133			
	9:35-12:45 **Integration of Chemistry and Life Science** P. 7	10:00-12:00 **Technology, Anthropology, Umwelt** P. 109		
	12:45-13:45 **Lunch**	12:00-13:00 **Lunch**		
PM	13:45-14:30 **Dance performance** P. 219 14:40-18:25 **Student Presentations (Oral)** P. 165	13:00-18:00 **Technology, Anthropology, Umwelt** P. 109	13:00-17:40 **Developing a future-oriented global doctor/researcher** P. 119	13:00-18:20 **Public Health / Nursing** P. 133
	18:30- **Reception**			

Friday, October 4

Venue	Main Convention Hall	Conference Room 303		Conference Room 406(405)
AM	8:30-11:15 **Student Presentations (Oral)** P. 165			10:00-11:30 **Public Health / Nursing** P. 133
	11:15-13:15 **Lunch** **Student Presentations (Poster)** P. 165			11:30-12:30 **Lunch**
PM	13:15-13:30 **Profile of UCI** 13:30-14:30 **Keynote Lecture (Life Science Workshop)** P. 25 14:40-16:10 **Life Science Workshop Ⅲ** P. 25 16:20-18:20 **Life Science Workshop Ⅳ** P. 25 18:20-18:30 **Closing Remarks**	13:00-17:00 **The Present Conditions and Issues of the Teacher Training in Japan and Vietnam** P. 157		12:30-17:00 **Public Health / Nursing** P. 133
	18:30- **Reception**			

Tsukuba Global Science Week 2013: Time Schedule

Wedenesday, October 2

TIME	Main Convention Hall		
	Opening Remarks		
8:30-	Caroline Benton Mituyasu Kato	University of Tsukuba	
	Globalization of Japanese Universities		
8:40-	TBA	MEXT	
	Our Partner Universities		
8:55-	Kyosuke Nagata	University of Tsukuba	
	Jacob Levin	UCI	
	Vincent Dousset	Bordeaux Segalen University	
9:25-	**Photo Session**		
	Integration of Chemistry and Life Science		
Chair: Masaki Kita			
9:40-	Tadeusz Molinski	UCSD	
10:10-	Reiko Oda	Bordeaux University / CNRS	
10:40-	Paul Alewood	University of Queensland	
11:10-	**- Break -**		
Chair: Hideo Kigoshi			
11:25-	Hiroshi Nagase	University of Tsukuba	
11:55-	Jennifer Prescher	UCI	
Chair: Tito Akindele			
12:25-	Katsunori Tanaka	RIKEN	
12:55-	Yasuteru Urano	The University of Tokyo	
13:25-	**- Lunch -**		
	Keynote Lecture (Life Science Workshop)		
Chair: Naomichi Okamura			
14:25-	Vincent Dousset	Bordeaux Segalen University	
15:10-	**- Break -**		
	Life Science Workshop Ⅰ (Cell Signaling)		
Chairs: Shih-Tomg Ding and Hiroshi Hasegawa			
15:15-	Sayuri Miyamoto	University of Sao Paulo	
15:45-	Tsunaki Hongu	University of Tsukuba	
16:15-	Michael Kann	Bordeaux Segalen University	
16:45-	**- Break -**		
	Life Science Workshop Ⅱ (Chromatin)		
Chairs: Tsai-Kun Li and Koji Hisatake			
17:00-	Shu-Chun Teng	NTU	
17:30-	Jing-Jer Lin	NTU	
18:00-	Kyoko Yokomori	UCI	
18:30-	**- Reception -** ・Our Partner Universities		

TIME	Conference Room 303	
	Art and Ecology	
10:00-	Opening Remarks: Shinichi Tamagawa	
Chair: Toshiharu Omuka		
10:10-	Kunihiro Ando	University of Tsukuba
10:40-	Momoyo Kaijima	University of Tsukuba
11:10-	Terumi Hotokeyama	University of Tsukuba
11:40- -13:00	**- Lunch -**	
Chair: Toshiharu Omuka		
13:00-	James Nisbet	UCI
14:30-	**- Break -**	
Chair: Toshiharu Omuka		
14:40-	Andrew Wilson	University of Queensland
16:10-	**- Break -**	
16:20- -17:00	Discussion	

TIME	Conference Room 304	
	Policy and Planning Sciences	
Session 1 Chair: Akiko Yoshise		
13:00-	Hajime Seya	National Institute for Environmental Studies
13:30-	Morimitsu Kurino	WZB
14:00-	Yoshitsugu Yamamoto	University of Tsukuba
14:30-	Zaifu Yang	University of York
15:00-	**- Break -**	
Session 2 Chair: Morito Tsutsumi		
15:10-	Sho Kuroda	University of Tsukuba
15:25-	Takahiro Yoshida	University of Tsukuba
15:40-	Kazuki Tamesue	University of Tsukuba
15:55-	Daisuke Murakami	University of Tsukuba
16:10-	Akihiro Tanaka	University of Tsukuba
16:25-	**- Break -**	
Session 3 Chair: Shun Watanabe		
16:35-	Takaaki Fujikura	University of Tsukuba
16:50-	Manabu Iwata	University of Tsukuba
17:05-	Hiroto Yonenoh	University of Tsukuba
17:20-	Ayumi Igarashi	University of Tsukuba
17:35-	Yoichi Izunaga	University of Tsukuba

TIME	Conference Room 406(405)	
	Food Security and Human Health	
10:00-	Opening Remarks: Hiroshi Ezura	
Chair: Yooich Kainoh		
10:10-	Michel Hemould	Bordeaux Segalen University / INRA
10:40-	Hiroko Isoda	University of Tsukuba
11:10-	**- Break -**	
Chair: Kazuya Morikawa		
11:20-	DeMar Taylor	University of Tsukuba
11:50-	Nguyen Thi Quynh	ITB, Vietnam Academy of Science and Technology
12:20-	Duong Hoa Xo	Biotechnology Center of HCMC
12:50- -14:30	**- Lunch -** (13:30-14:20) **Millipore Luncheon Seminar**	
Chair: Chiaki Matsukura		
14:30-	Frederic Delmas	Bordeaux Segalen University / INRA
15:00-	Pierre Baldet	Bordeaux Segalen University / INRA
15:30-	Hiroshi Ezura	University of Tsukuba
16:00- -18:00	Poster Session *Conference Room 405	

Thursday, October 3

TIME	Main Convention Hall		TIME	Conference Room 303		TIME	Conference Room 101		TIME	Conference Room 406(405)	
Public health / Nursing			**Technology, Anthropology, Umwelt**			**Developing a future-oriented global doctor/researcher**			**Public Health / Nursing**		
Keynote Lecture Chair: Naomi Wakasugi											
8:30-	Francois Dabis	Bordeaux Segalen University									
9:30-	**- Break -**										
Integration of Chemistry and Life Science											
Chair: Takeo Usui											
9:35-	Elmar Schiebel	University of Heidelberg	1. Conference Introduction								
10:05-	Hirokazu Arimoto	Tohoku University	10:00-	Yasushi Uchiyamada	University of Tsukuba						
Chair: Yasuhiro Shinkai											
10:35-	Shiou-Chuan Tsai	UCI	2. Networks, Agents and Potentialities								
11:05-	**- Break -**		10:10-	Paul Hansen	University of Tsukuba						
Chair: Yasuhiro Shinkai				Sumiko Yamazaki	University of Tsukuba						
11:15-	Takeo Usui	University of Tsukuba		Discussant1: Gergely Mohacsi	Osaka University						
Chair: Mitsuru Okuwaki											
11:45-	Bela Gyurcsik	University of Szeged	12:00-	**- Lunch -**							
12:15-	Yoshito Kumagai	University of Tsukuba	3. Living with 3.11 / the Unexpected			Session Ⅰ Chair: Hiroyuki Nishiyama			Session I Chair: Masao Ichikawa		
12:45-	**- Lunch -**		13:00-	Shuhei Kimura	University of Tsukuba	13:00-	Yoshiyuki Sankai	University of Tsukuba	13:00-	Yukiko Wagatsuma	University of Tsukuba
Dance Performance				David Sprague	National Institute for Agro-Environmental Sciences	13:35-	Thomas Ahlering	UCI			
13:45-	John Crawford	UCI		Discussant2: Takashi Osugi	Hitotsubashi University	14:00-	-Discussion -		13:30-	Sysavanh Phommachanh	University of Health Science, Laos
14:30-	**- Break -**					14:20-	**- Break -**		14:00-	Do Van Dung	University of Medicine and Pharmacy at HCMC
Student Presentations (oral)				Discussant3: Xiaogang Sun	University of Tsukuba	Session Ⅱ Chair: Osamu Ohneda			14:30-	**- Break -**	
14:40-	Mai Thi Nhu Tran	University of Tsukuba				14:40-	Steve Cramer	UCI	Session II Chair: Yukiko Wagatsuma		
14:55-	Dinh Thi Huong Tra	University of Tsukuba	15:00-	**- Break -**		15:15-	**- Break -**		14:45-	Mayfong Mayxay	University of Health Science, Laos
15:10-	Farhana Ferdousi	University of Tsukuba	4. Our Lifeworld beyond Umwelt			Session Ⅲ Chair: Akira Matsumura			15:15-	Scott Benson	University of Utah
15:25-	Tai, Yu-Ling	NTU	15:20-	Yasushi Uchiyamada	University of Tsukuba	15:35-	Masataka Sakane	University of Tsukuba	15:45-	Nanako Tamiya	University of Tsukuba
15:40-	**- Break -**			Valerie Olson	UCI	15:50-	Jacob Levin	UCI	16:15-	**- Break -**	
15:55-	Yumi Abiko	University of Tsukuba		Discussant4: Toshihiro Kameda	University of Tsukuba	16:10-	**- Break -**		Session Ⅲ Chair: Nanako Tamiya		
16:10-	Shinichi Kadota	University of Tsukuba				Session Ⅳ Chair: Masayuki Masu			16:30-	Reiko Hayashi	National Institute of Population and Social Security Research
16:25-	Ariungerel Gerelchuluun	University of Tsukuba		Discussant5: Koji Hirose	University of Tsukuba	16:30-	Masato Sugano	University of Tsukuba	17:00- -18:20	G30 Student Seminar on Global Aging	
16:40-	Sisley Austin	Bordeaux Segalen University				16:40-	Chiho Tokunaga	University of Tsukuba			
16:55-	**- Break -**					16:50-	Makoto Tanaka	University of Tsukuba			
17:10-	Koki Hagiwara	University of Tsukuba	5. Open Discussion: Problems and Methodologies			17:10-	Thomas Cesario	UCI			
17:25-	Vo Nguyen Thanh Thao	University of Tsukuba	17:30- -18:00	All Participants							
17:40-	Rie Nakamoto-Matsubara	University of Tsukuba									
17:55-	Abhishek Kurup	UCI									
18:10-	Sara Brightwell	University of Edinburgh									
18:25-	**- Break -**										
18:30-	**- Reception -**										

Friday, October 4

Main Convention Hall

Student Presentations (oral & Poster)

8:30-	Tsuyoshi Setogawa	University of Tsukuba
8:45-	Kouta Niizuma	University of Tsukuba
9:00-	Yosuke Masuda	University of Tsukuba
9:15-	Tax Gábor	University of Szeged
9:30-	Katherine Long	University of Edinburgh
9:45-	**- Break -**	
10:00-	Trinh Nhu Thuy	University of Tsukuba
10:15-	Hiroshi Ohno	University of Tsukuba
10:30-	Yuki Miura	University of Tsukuba
10:45-	Sarah Vosen	University of Bonn
11:00-	Chang, Chia-Chun	NTU
11:15-	**- Lunch -**	
-13:15	**Student Poster Presentations**	

Profile of UCI (Educational Programs in Graduate Schools) Chair: Caroline Benton

13:15-	Arthur D. Lander	UCI

Life Science Workshop Ⅲ (Systems Biology)

Keynote Lecture Chair: Akira Shibuya

13:30-	Arthur D. Lander	UCI
14:30-	**- Break -**	

Chairs: Fuminori Tsuruta and Ryosuke Ohniwa

14:40-	Kazuhisa Ichikawa	The University of Tokyo
15:10-	Michael Lazarus	University of Tsukuba
15:40-	Akira Sasaki	The Graduate University for Advanced Studies
16:10-	**- Break -**	

Life Science Workshop Ⅳ (Re-programing and Neuroscience)

Chairs: Satoru Takahashi and Ken Nishimura

16:20-	Keisuke Kaji	University of Edinburgh
16:50-	Bernd Fleischmann	University of Bonn
17:20-	Charles ffrench-Constant	University of Edinburgh
17:50-	Peter Klivenyi	University of Szeged

Closing Remarks

18:20-	Yasunori Kanaho	University of Tsukuba
18:30-	**- Reception -** ・Mugenjuku ・Award Ceremony	

Conference Room 303

The Present Conditions and Issues of the Teacher Training in Japan and Vietnam

13:00-	The Opening	
13:15-	Greetings of the opening: Akitoshi Teuchi	

Chair: Akitoshi Teuchi

13:30-	Hoang Van Can	Ho Chi Minh City University of Education
14:10-	Le Thi Minh Ha	Ho Chi Minh City University of Education
14:50-	**- Break -**	
15:00-	Naohiro Higuchi	University of Tsukuba
15:40-	Takao Ando	University of Tsukuba
16:20-	Discussion	
-17:00		

Conference Room 406(405)

Public Health / Nursing

Student Presentations

10:00-	Felipe Sandoval	University of Tsukuba
10:15-	Kyoko Tokoro	University of Tsukuba
10:30-	Hiromi Sugawara	University of Tsukuba
10:45-	Milleanni Dominguez	University of Tsukuba
11:00-	Daisuke Fukuta	University of Tsukuba
11:15-	Grant Sunada	University of Utah
11:30- -12:30	**- Lunch -**	

Session Ⅳ Chair: Keiko Sugimoto

12:30-	Mi Ja Kim	University of Illinois at Chicago
13:00-	Misuzu Gregg	Kobe City College of Nursing
13:30- -15:00	**- Break -**	

Session Ⅴ Chair: Keiko Sugimoto

15:00-	Carol E. Ferrans	University of Illinois at Chicago
15:30-	Michiyo Mizuno	University of Tsukuba
16:00-	Kayuri Furuya	University of Tsukuba
16:30-	Rie Wakimizu	University of Tsukuba

Integration of Chemistry and Life Science: Leading Edge of Chemical Biology

- The 2nd Human Biology Symposium -

Integration of Chemistry and Life Science: Leading Edge of Chemical Biology - The 2nd Human Biology Symposium -

Wednesday, October 2

Venue: Main Convention Hall

Chair: Dr. Masaki Kita	
9:40-10:10	"Natural Products. Structure, Synthesis and Discovery at the Nanomole Scale" Dr. Tadeusz Molinski University of California, San Diego, USA
10:10-10:40	"Bio-inspired chiral nano structures based on molecular self-assemblies: towards functional hybrid materials" Dr. Reiko Oda Bordeaux University / CNRS, France
10:40-11:10	"Toxin Disulfide Bond Mimetics" Dr. Paul Alewood The University of Queensland, Australia
11:10-11:25	**Coffee Break**
Chair: Dr. Hideo Kigoshi	
11:25-11:55	”Synthesis of Novel Triplets with 1,3,5-Trioxazatriquinane Skeleton Using Nitrogen Clamp” Dr. Hiroshi Nagase University of Tsukuba, Japan
11:55-12:25	"Developing novel chemical reporters and biocompatible chemistries for multiplexed imaging" Dr. Jennifer A. Prescher University of California, Irvine, USA
Chair: Dr. Tito Akindele	
12:25-12:55	"Exploring the chemistry and biology of unsaturated imines" Dr. Katsunori Tanaka RIKEN, Japan
12:55-13:25	"Novel spirocyclization-based “activatable” fluorescence probes: From in vivo imaging of tiny tumors to super-resolution imaging" Dr. Yasuteru Urano The University of Tokyo, Japan

Thursday, October 3

Venue: Main Convention Hall

Chair: Dr. Takeo Usui	
9:35-10:05	"GTP regulates the microtubule nucleation activity of γ-tubulin" Dr. Elmar Schiebel University of Heidelberg, Germany
10:05-10:35	"Endogenous small-molecule regulator of antibacterial autophagy" Dr. Hirokazu Arimoto Tohoku University, Japan
Chair: Dr. Yasuhiro Shinkai	
10:35-11:05	"Architecture and dynamics of mega-synthases involved in primary and secondary metabolism" Dr. Shiou-Chuan Tsai University of California, Irvine, USA
11:05-11:15	**Coffee Break**
Chair: Dr. Yasuhiro Shinkai	
11:15-11:45	"Explore the function of microtubule dynamics by bioprobe" Dr. Takeo Usui University of Tsukuba, Japan
Chair: Dr. Mitsuru Okuwaki	
11:45-12:15	"Fine tuning of the allosteric activation in the nuclease domain of colicin E7" Dr. Béla Gyurcsik University of Szeged, Hungary
12:15-12:45	"A unique redox signal transduction pathway regulated by reactive sulfur species" Dr. Yoshito Kumagai University of Tsukuba, Japan

Natural Products. Structure, Synthesis and Discovery at the Nanomole Scale

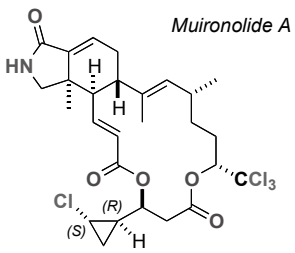
Tadeusz Molinski

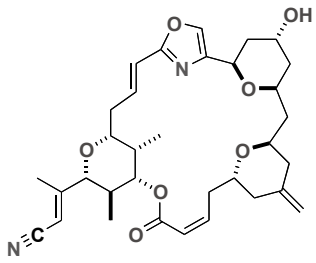
Department of Chemistry and Biochemistry, and Skaggs School of Pharmacy and Pharmaceutical Sciences University of California, San Diego, La Jolla, CA 92093-0358, USA.

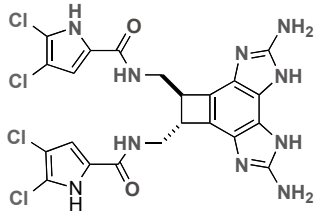
tmolinski@ucsd.edu

Marine invertebrates such as sponges and tunicates produce a wide variety of antitumor and antifungal compounds.[i]. Technological advancements in NMR, integrated with chiroptical methods and chemical synthesis, enable exploration of biodiversity and discovery of new marine natural products at extremely low abundances; sample amounts approaching ~1 nanomole.

So-called 'nanomole-scale' natural products discovery embraces integrated approaches for structure elucidation to achieve full characterization of new chemical entities from rare sources with total sample yields of only ~ 8–90 μg.[ii,iii,iv] The structure activity relationships of biologically active natural products and their analogs are expanded through the use of micro-scale techniques coupled with synthesis.







Procurement of rare natural products through synthesis enables investigation of their biological properties. Recent accomplishments in the synthesis of marine alkaloids natural – through conventional methodology, and 'non-natural' alkaloids by harnessing the innate bio-synthetic capacity of marine sponges alkaloids –will be described.

(i). Molinski, T.F. "Antifungal Compounds from Marine Organisms." *Curr. Med. Chem.: Anti-Infect. Agents* **2004**, *3*, 197-220.

(ii). Molinski, T. F. "Nanomole-Scale Natural Products Discovery" *Curr. Opin. Drug Discov. Devel.* **2009**, *12*, 197-206.

(iii). Molinski, T. F. "Microscale Methodology for Structure Elucidation of Natural Products." *Curr. Opin. Biotechnol.* **2010**, *21*, 819-826.

(iv). Molinski, T. F. "NMR of Natural Products at the 'Nanomole-Scale'. *Nat. Prod. Rep.* **2010**, *27*, 321-329

Bio-inspired chiral nano structures based on molecular self-assemblies: towards functional hybrid materials

Rumi Tamoto[1] Dmytro Dedovets[1,2], Emilie Pouget[1], Marie-Helène Delville[2], Marie-Christine Durrieu[1], Christian Bergaud[3], and Reiko Oda[1*],

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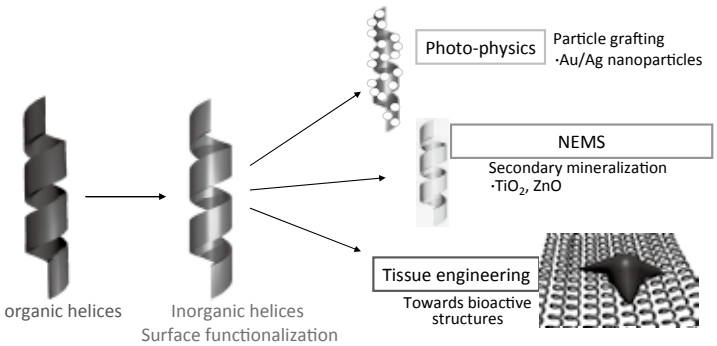
[2]*Institut de Chimie de la Matiere Condensee de Bordeaux - CNRS-UPR 9048, 87 Ave du Dr. Schweitzer, 33608 Pessac, France*

[3]*Laboratoire d'Analyse et d'Architecture des Systèmes - LAAS-CNRS, 7, avenue du Colonel Roche, 31077 Toulouse Cedex 4, France*

If chirality in one of the key factors in molecular recognition in chemical and biological systems, the chiral systems are also envisaged to play an important role in nanotechnologies. In the present work, we investigate how different parameters play roles on the formation of various supramolecular self-assemblies of amphiphilic molecules, focusing on the formation of chiral nanostructures. Starting from achiral surfactant, the transcription of the chirality of the complexing ion gives rise to the expression of chirality at the supramolecular level.[1] The morphologies of the nanohelices and nanoribbons formed are very sensitive to external and molecular factors. We focus here on the study of these morphologies, controlled by the conter-ion enantiomeric excess.



I will then discuss the possible inorganic transcription of these chiral structures: by use of sol-gel techniques, the nanohelices are covered with a thin layer of silica forming well-defined hybrid organic/inorganic nano-objects.[2] Indeed, while the organic self-assembly process gives access to extremely rich polymorphisms, their sensitivity to external perturbations can lead to the destruction of these structures. Inorganic transcription of these structures enhances significantly the possible applications while keeping the complexity of the morphologies. After showing the transcription mechanism, we will discuss the use of these structures for three applications currently studied in our group: the development of semiconductor helices for NanoElectroMachanical Systems (NEMS), the formation of systems taking advantage of their particular optical properties, as well as a very recent study concerning the use of these structures in the field of tissue engineering.[3]

[1]Kiagus-Armad, R., Brizard, A., Tang, C., Blatchly, R., Desbat, B., Oda, R Chem. Eur. J., 2011, 17, 9999-10009 b) Aime, C., Tamoto, R., Satoh, T., Grelard, A., Dufourc, E. J., Buffeteau, T., Ihara, H., and Oda, R. Langmuir 2009, 25(15), 8489–8496 c) R. Oda, M. Laguerre, I. Huc, F. Artzner J. AM. CHEM. SOC. (2008) *130* (44), 14705–14712 d) A. Brizard, C. Aimé, T. Labrot, I. Huc, D. Berthier, F Artzner, B. Desbat, R. Oda, J. AM. CHEM. SOC. (2007), 129, 3754-3762, e) R. Oda, I. Huc, M. Schmutz, S. J. Candau, F. C. Mackintosh, *Nature, 399 (1999) 566-569.*

[2]Delclos, T. Aimé, C. Pouget, E. Brizard, A. Huc, I. Delville, M.-H. and Oda R. (2008). Nanoletters, 8, (7): 1929-1935

[3]R. K. Das, O. F. Zouani, C. Labrugere, R. Oda, M.- C. Durrieu, Influence of Nanohelical Shape and Periodicity on Stem Cell Fate *ACS NANO* 2013, 7, 4, 3351–3361

Toxin Disulfide Bond Mimetics

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Many organisms including snakes, spiders, scorpions, cone snails, anemones and some mammalian species have evolved venom as either a defence mechanism or a weapon for prey capture. These venoms typically contain a complex cocktail of bioactive disulfide-bond rich small polypeptides which target a wide range of receptors including enzymes, ion channels, GPCRs and transporters. Of interest to drug designers is their high potency and selectivity combined with their resistance to many proteases.

These disulfide-bond rich toxins have small polypeptide chains typically between 10-80 amino acids that are highly constrained by two to five disulfide bridges and are structurally well-defined. Their high potency and *exquisite selectivity* for ion channels and receptors has led to several drug candidates undergoing preclinical and clinical trials.

In this presentation I will describe the replacement of disulfide bonds in highly bioactive conotoxins by thioether bonds and selenoether bonds. This has led to mimetics that have similar or improved potency to the native molecule plus exceptional stability when exposed to reducing environments and in plasma. Together, these results underpin the development of more stable and potent peptide mimetics suitable for new drug therapies, and highlight the application of this technology more broadly to disulfide-bonded peptides and proteins.

Synthesis of Novel Triplets with 1,3,5-Trioxazatriquinane Skeleton Using Nitrogen Clamp

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Many twin drugs were reported and symmetrical twin drugs can simultaneously fit to the symmetrical binding sites of the protein complex to afford increased activity. Non-symmetrical twin drugs may bind each relevant binding site to give dual action.

However, twin drugs can only play one role, by affording either an increase of activity or a dual action. If a rigid triplet drug (trimer drug) is available, the drug could be expected to exert both features of increased activity and dual action.

Recently, we have reported a synthetic method for rigid triplet drug with 1,3,5-trioxazatriquinane skeleton bearing three naltrexone units.

We have been interested in the structure and pharmacological effects of the unique triplet molecules and synthesized the various triplets. One of the homo triplets, **KNT-93** showed about 20 times potent analgesic effect than that of molphine in an mouse acetic acid writhing test. We also succeeded in synthesizing hetero triplets with morphinan skeletons, mono, di and tri cap-trimers. These triplets were applied to construction of compound libraries. In the binding asssays for opioid receptor as one of the candidate receptors, 9 hit compounds were obtained in 42 compound libraries and one of the hit compounds, SYK-146 showed almost same affinity and selectivity for opioid κ receptor as those of U-50488H which was used as the standard κ agonist made by Upjohn company. These triplets has the novel 1,3,5-trioazatriquinane skeleton having nitrogen atom in the center of the skeleton and can arrange aromatic rings to 6 directions. The structures of the top drugs in the world have the typical structures, have a nitrogen in the center of the molecules and two aromatic rings on the right and left sides. Our triplets have the similar structures to these top drugs. So, we expected that our triplets could apply them to any other receptors and enzymes assays.

Developing novel chemical reporters and biocompatible chemistries for multiplexed imaging

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The chemical reporter strategy has emerged as a popular method to equip glycans, lipids, and other biomolecules with visual probes in living systems. This strategy involves the incorporation of metabolites endowed with unique chemical handles (i.e., "chemical reporters") into target biomolecules. Following incorporation, these reporter groups can be specifically detected in a second step utilizing highly selective (i.e., bioorthogonal) reactions. The chemical reporter strategy, while powerful, has been largely limited to visualizing one biological feature at a time owing to a lack of unique reporters and reactions. Recently, we have identified a new chemical reporter—cyclopropene—that can be used concurrently with existing bioorthogonal probes. We synthesized a panel of cyclopropenes and discovered that both mono- and di-substituted scaffolds are stable in aqueous environments and in the presence of biological nucleophiles. Furthermore, these highly strained molecules are capable of reacting with tetrazines and other dienes under physiological conditions. In fact, the cyclopropene-tetrazine reaction rate is on par with some of the most popular ligation chemistries used for biological labeling in live animals. We also demonstrated that cyclopropenes can be metabolically introduced onto live cell surfaces and imaged with tetrazine probes. In the near future, we plan to utilize cyclopropenes in tandem with other chemical reporters for multi-component labeling studies (e.g., monitoring host-pathogen interactions and immune cell communication networks).

Exploring the chemistry and biology of unsaturated imines

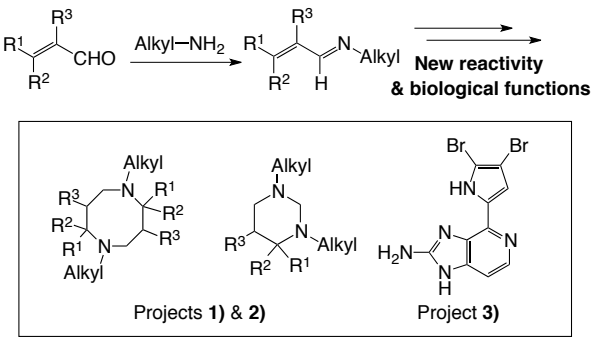
Katsunori Tanaka

Biofunctional Synthetic Chemistry Laboratory, RIKEN

We are synthetically exploring the overlooked reactivity of the *N*-alkyl unsaturated imines, which are readily derived from the various aldehydes and primary amines in biosystems. The new reactivity of imines could then be used to challenge to unveil the biosystems and to perform the multi-step synthesis of the biologically active molecules directly in live animals. Our recent examples are disclosed.

1. New reactivity of N-alkyl unsaturated imines: application to alkaloid synthesis

Recently we found that α,β-unsaturated imines, obtained from aldehydes and aminoalcohols or diamines, could readily participate in the [4+4] reaction yielding the 1,5-diazacyclooctanes. When the [4+4] products were further treated with formaldehyde, the six-membered pyrimidines were produced through [4+2] reaction between the "regenerated" imines from unsaturated aldehydes and formaldehyde. Imino [4+4]/[4+2] cascade reaction was then applied to asymmetric synthesis of variously substituted cyclic and linear alkaloids.



2. Reactivity of unsaturated imines from polyamines and their biological function

We have discovered that imino [4+4] or [4+2] reactions could regulate the biosystems. In fact, the [4+4] products derived from the polyamines and acrolein inhibited the cell growth and caused oxidative. Considering that the acrolein could be generated from the polyamines through the metabolic pathway, our results strongly suggest a new mechanism underlying acrolein-mediated oxidative stress and diseases.

3. Challenges to one-pot natural products synthesis in live animals

We explored the new reactivity of the unsaturated imines derived from the guanidine, on the basis of rather rare arginine post-translational modification by lipid metabolites. We succeeded in the first library synthesis of the 2-aminoimidazole derivatives, and the method was applied to the one-pot total synthesis of the alkaloid natural products, i.e., ageladines as the promising anti-angiogenic MMP inhibitors (Matrix metalloproteinase), through the multiple transformations starting from the simple starting materials. Such one-pot synthesis could allow us to synthesize the bioactive natural products on the target proteins in live animals.

Novel spirocyclization-based “activatable” fluorescence probes: From in vivo imaging of tiny tumors to super-resolution imaging

Yasuteru URANO

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Basic Research Program, Japan Science and Technology

Fluorescence imaging is one of the most powerful techniques currently available for continuous observation of dynamic intracellular processes in living cells. Suitable fluorescence probes are naturally of critical importance for fluorescence imaging, but only a very limited range of biomolecules can currently be visualized because of the lack of flexible design strategies for small molecule-based fluorescence probes. Recently, we found that hydroxymethyl rhodamine green (HMRG) was strongly fluorescent in aqueous solution at pH 7.4, while mono-amidated HMRG derivatives were colorless and non-fluorescent due to the preferred spirocyclized structure.

Based on above findings, we have developed various novel aminopeptidase-sensitive probes which were applicable for living cell system, including gGlu-HMRG, a novel HMRG-based “activatable” fluorescence probe for γ-glutamyltranspeptidase (GGT). We could establish a novel and highly activatable strategy for sensitive and fast-responding fluorescence imaging of tiny tumors in vivo by spraying gGlu-HMRG onto tissue surfaces that are suspected of harboring tumors, creating high signal contrast between the tumor and the background within 1 min.

We have also developed a novel class of fluorophores which spontaneously and repeatedly blink with proper average lifetime of fluorescent state without any additives and special conditions, by optimizing the equilibrium constants of intramolecular spirocyclization and the rate constants of ring-closure reaction. These are quite suitable for STORM super-resolution imaging technique, and indeed, super-resolution images of microtubules and RecA filaments on plasmid DNA could be obtained under normal oxygen conditions without any additives of thiols.

References

1. Kamiya M, et al. ***J. Am. Chem. Soc.***, 133, 12960-12963 (2011).
2. Urano Y, et al., ***Sci. Transl. Med.***, 3, 110ra119 (2011).
3. Sakabe M, et al. ***J. Am. Chem. Soc.***, 135, 409-414 (2013).
4. Kenmoku S, et al. ***J. Am. Chem. Soc.***, 129, 7313-7318 (2007).
5. Urano Y, et al., ***Nat. Med.***, 15, 104-109 (2009).
6. Urano Y, et al., ***J. Am. Chem. Soc.***, 127, 4888-4894 (2005).

GTP regulates the microtubule nucleation activity of γ-tubulin

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Microtubules (MTs) are hollow cylinders consisting of tubulin, a heterodimer of α- and β-tubulin that have essential functions in cell organization, motility and chromosome segregation in mitosis and meiosis. In addition, tubulin is the target of anti-cancer drugs such as taxol. *In vivo* MTs are composed of 13 tubulin protofilaments, strings of consecutive αβ-αβ tubulin subunits. MTs are dynamic, constantly grow and shrink by polymerization and depolymerization. In addition, MTs can assemble *de novo* from tubulin subunits. In cells, *de novo* formation of MTs is initiated by γ-tubulin, an additional member of the tubulin superfamily. γ-tubulin assembles together with additional proteins (GCP2-6) into ring-like complexes that probably function as a template for MT formation.

Both subunits of the αβ-tubulin heterodimer bind the nucleotide GTP. GTP binding to α-tubulin has a structural role, while β-tubulin binds to and hydrolyses GTP, to regulate MT dynamics. γ-tubulin also binds GTP; however, the importance of this association remains elusive. To address the role of GTP binding to yeast γ-tubulin, Tub4, we systematically mutagenized the GTP contact residues in Tub4. Tub4^{GTP}-mutant proteins that displayed greatly reduced GTP affinity still assembled into the small γ-tubulin complex. However, *tub4^{GTP}* mutants were no longer viable, had defects in the interaction between γ-tubulin and the αβ-tubulin heterodimer, decreased MT nucleation activity and generated serious defects in MT organization. *In vitro* and *in vivo* data show that only γ-tubulin loaded with GTP nucleates MTs. Our results suggest that GTP recruitment to γ-tubulin enhances its interaction with tubulin in a similar manner as GTP recruitment to β-tubulin.

Endogenous small-molecule regulator of antibacterial autophagy

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With the increasing number of drug-resistant bacteria continues to rise, the development of new antibacterial agents has become extremely important. Although the pharmaceutical industry is making efforts in this field, not much research is being conducted on novel bactericides. We have been studying novel compounds that can eliminate intracellular bacteria by stimulating the host innate immune response.

Elimination of intracellular bacteria requires mechanisms of action that are substantially different from those used against extracellular bacteria.[1] Moreover, recent studies have shown that some bacteria have mechanisms to resist our immune responses. For example, *Mycobacterium tuberculosis* can inhibit the fusion of phagosomes with lysosomes, thereby avoiding lysosomal digestion. Other strains such as *Listeria* and group A *Streptococcus* can escape from the phagosomes to the cytoplasm. Interestingly, starvation- or cytokine-induced autophagy has been shown to overcome the trafficking block imposed by *M. tuberculosis* in macrophages.[2] Because treatment with a lipopolysaccharide along with a cytokine is known to induce autophagy in cells, we hypothesized that small molecules that are formed in host cells under such inflammation conditions may also be involved in the clearance mechanism of intracellular bacteria. In my presentation, I will be talking about an autophagy-inducing small molecule that enhances the elimination of invading group A *Streptococcus* from infected macrophages.[3]

1) O. A. Mascaretti, *Bacteria versus antibacterial agents: an integrated approach*, ASM press (2003).
2) M. G. Gutierrez *et al., Autophagy Is a Defense Mechanism Inhibiting BCG and Mycobacterium tuberculosis Survival in Infected Macrophages, Cell* 119, 753-766 (2004).
3) C. Ito, T. Nozawa, T. Akaike, I. Nakagawa, H. Arimoto *et al.*, *submitted*.

Architecture and dynamics of mega-synthases involved in primary and secondary metabolism

Chi Nguyen[2†], Robert W. Haushalter[1†], D. John Lee[1], Phineus R. L. Markwick[1,3,4], Joel Bruegger[2], Grace Caldara-Festin[2], Kara Finzel[1], David R. Jackson[2], Fumihiro Ishikawa[1], Bing A. O'Dowd[1], J. Andrew McCammon[1,4], Stanley J. Opella[1], Shiou-Chuan Tsai[2*], and Michael D. Burkart[1*]

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The carrier protein (CP) transports the growing fatty acid chain between enzyme domains of fatty acid synthase (FAS), polyketide synthase (PKS) and non-ribosomal peptide synthetase (NRPS) in primary and secondary metabolism. Because these mega-synthases operate upon CP-bound substrates, CP must stabilize and transport the growing chain. The transient nature of CP-enzyme interactions imposes a major obstacle to gaining high-resolution structural information about biosynthesis directed by the mega-synthases, and a new strategy is required to properly study protein-protein interactions. In this work, we describe the application of mechanism-based probes that allow site-selective covalent crosslinking of CP to downstream enzymes. We report the 1.9 Å crystal structure of a crosslinked complex of an acyl carrier protein (ACP) with its enzyme partner, in which ACP exhibits two different conformations representing snapshots of ACP in action: the 4'-phosphopantetheine (PPant) group of ACP first binds an arginine-rich groove of its partner enzyme, followed by an ACP helical conformational change that locks the ACP and its partner enzyme in place. Residues at the interface of ACP and its partner are further identified and validated by solution-phase NMR techniques, including HSQC perturbation and RDC measurements. These not only support the interpretation of crystal structures but also provide a dynamic animation of ACP in action. Combined with molecular dynamic simulation, we show for the first time that the mega-synthase extrudes the sequestered substrate from the ACP binding pocket by repositioning helix III. Extensive sequence conservation of carrier proteins suggest that the mechanistic insights gleaned from our studies will prove general for fatty acid, polyketide and non-ribosomal biosyntheses. Here the foundation is laid for defining the dynamic action of carrier protein activity in primary and secondary metabolism, providing insight into pathways that can play major roles in the treatment of cancer, obesity and infectious disease.

Explore the function of microtubule dynamics by bioprobe

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Microtubules play important roles in mitosis, cell signaling and motility in eukaryotes. Therefore, tubulin inhibitors have been recognized as antitumor agents. Two major class compounds were used in the clinic, taxanes and *vinca* alkaloids, which bind different sites of β-tubulin and show opposite effects *in vitro*. However, these compounds sometimes cause severe side effects, including peripheral neuropathy, due to the extreme effects on microtubules. Because neuropathy is a common dose-limiting toxicity of these microtubule-targeting drugs, it is important to develop compounds without leading to the lesion. Recently, it has been proposed that drugs with subtle effects on microtubules that only modulate microtubule dynamics may entail fewer side effects and be clinically useful.

Glaziovianin A is an isoflavone isolated from the leaves of *Ateleia glazioviana* (Legminosae) as a cytotoxic compound against human leukemia cells. Glaziovianin A inhibited the cell cycle progression in M-phase with abnormal spindle structure. Recently, we found that glaziovianin A is a novel microtubule dynamics inhibitor which decreases both elongation and shortening rates of microtubule and drastically increase pause populations in cells. The inhibition of microtubule dynamics by glaziovianin A resulted in not only mitotic arrest but also perturbation of endosome transport which induced the prolonged EGFR phosphorylation and the EGF-mediated apoptosis. These results suggested that microtubule dynamics is important for not only bipolar spindle formation but endosome transport and maturation, and that the microtubule dynamics inhibitors, including glaziovianin A induce apoptosis by two pathways, mitotic arrest and accumulation of activated receptor kinases in early endosomes.

1. Yokosuka, A., *et al. Bioorg. Med. Chem. Lett.*, **17**, 3091-3094 (2007)
2. Ikedo, A., *et al. Bioorg. Med. Chem. Lett.,* **20**, 5402-5404 (2010)
3. Hayakawa, I., *et al. Bioorg. Med. Chem.*, **20**, 5745-5756 (2012)
4. Chinen, T. et al *ACS Chem. Biol.*, **8**, 884-889 (2013)

Fine tuning of the allosteric activation in the nuclease domain of colicin E7

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Colicin E7 metallonuclease is a bacterial toxin, the nuclease domain (NColE7) of which enters and kills the target cell. NColE7 belongs to the HNH family of nucleases. The HNH motif serving as the catalytic centre is situated at the C-terminus of NColE7. Our previous investigations clearly showed that the deletion of the KRNK section – containing three positively charged residues – from the N-terminus of NColE7, i.e. in ΔN4-NColE7 results in the lost of catalytic activity [1-3]. Such an allosteric effect may be applied in controlling the nuclease function – being an essential feature of artificial nucleases constructed from NColE7 and proteins that specifically recognize individual DNA sequences, such as zinc finger or TALE domains. Chimeric enzymes of this kind may become the future tools of gene targeting and therapy [4]. The study of the interactions between the N- and C-termini of NColE7 provides a necessary knowledge to understand the essential components of the control mechanism. Therefore, in our present study we have introduced a series of mutations in the N-terminal loop of NColE7 to check the role of the amino acids considered to be important from the point of view emphasized above. We will demonstrate how the exchange of selected amino acids at the N-terminus affect the structure, metal ion and DNA binding affinity, and thereby the catalytic activity of the enzyme.

References

[1] A. Czene, E. Németh, I.G. Zóka, N.I. Jakab-Simon, T. Körtvélyesi, K. Nagata, H.E.M. Christensen, B. Gyurcsik, J. Biol. Inorg. Chem. 2013, 18, 309.
[2] B. Gyurcsik, A. Czene, H. Barát-Jankovics, N.I. Simon-Jakab, K. Ślaska-Kiss, A. Kiss, Z. Kele, Protein Exp. Pur. 2013, 89, 210.
[3] A. Czene, E. Tóth, B. Gyurcsik, H. Otten, J.-C.N. Poulsen, Leila Lo Leggio, S. Larsen, H.E.M. Christensen, K. Nagata, Acta Cryst. Sect F. 2013, F69, 551.
[4] B. Gyurcsik, A. Czene, Future Med. Chem. 2011, 3, 1935.

A unique redox signal transduction pathway regulated by reactive sulfur species

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A variety of different signal transduction pathways are involved in cell survival, cell proliferation and apoptosis, and it is currently believed that the redox signaling pathways involving the modification of cysteine thiols in proteins (e.g., S-oxidation, S-nitrosylation, and S-glutathiolation) are associated with redox homeostasis, cellular protection against oxidative stress and inflammation. Although there are about 214,000 unique cysteine residues in the human genome, only 10–20% of protein thiols are thought to be readily oxidized, and we have hypothesized that these reactive cysteine thiols could also react with electrophiles to form protein adducts through C-S bonds, because electrophiles covalently bound to proteins through reactive thiols as referred to "sensor proteins". In this way, it is envisaged that electrophiles could modulate a redox signal transduction pathway consisting of an effector molecule (e.g., protein kinase, transcription factor) that is negatively regulated by a sensor protein.

Using 1,2-naphthoquinone (1,2-NQ) and methylmercury (MeHg) as model environmental electrophiles, we found that these electrophiles can activate/disrupt a number of redox transduction pathways, including the EGFR/ERK, Akt/CREB and Nrf2/ARE pathways, through the covalent modification of the sensor protein thiols. Although CBS and CSE are found to play crucial roles in catalyzing the formation of hydrogen sulfide (H_2S), we thought that this gaseous molecule would predominantly exist in its deprotonated form (HS^-) under physiological conditions, and any environmental electrophiles invaded into cells would therefore be effectively deactivated by this nucleophilic species. Consistent with this, we identified the 1,2-NQ-SH and 1,2-NQ-S-1,2-NQ adducts during the reaction of 1,2-NQ with NaSH and even Na_2S_4, suggesting that polysulfides as well as HS^- could be used as scavenger molecules for electrophiles. Consequently, the overexpression and knockdown of CBS in A431 cells enhanced and reduced activation of EGFR/ERK signaling mediated by 1,2-NQ. Our findings strongly suggest that reactive sulfur species are critical nucleophiles in the regulation of the redox signal transduction pathways mediated by environmental electrophiles.

References

1) Kumagai Y et al. The chemical biology of naphthoquinones and its environmental implications. ***Annu Rev Pharmacol Toxicol*** 52: 221-247, 2012.
2) Nishida M et al. Hydrogen sulfide anion regulates redox signaling via electrophile sulfhydration ***Nature Chem Biol*** 8: 714-724, 2012.

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Wednesday, October 2 — Venue: Main Convention Hall

Keynote Lecture
Chair: Dr. Naomichi Okamura

14:25-15:10	"Lessons on CNS immunology by in vivo imaging" Dr. Vincent Dousset Bordeaux Segalen University, France
15:10-15:15	**Coffee Break**

Session Ⅰ: Cell Signaling
Chairs: Dr. Shih-Torng Ding and Dr. Hiroshi Hasegawa

15:15-15:45	"An overview about the Brazilian network to study redox process in biomedicine: Redoxome" Dr. Sayuri Miyamoto University of São Paulo, Brazil
15:45-16:15	"Grp1-Arf6 axis regulates tumor angiogenesis and growth through HGF-induced endothelial β1 integrin recycling" Dr. Tsunaki Hongu University of Tsukuba, Japan
16:15-16:45	"Parvoviruses Cause Nuclear Envelope Breakdown by Activating Key Enzymes of Mitosis" Dr. Michael Kann Bordeaux Segalen University, France
16:45-17:00	**Coffee Break**

Session Ⅱ: Chromatin
Chairs: Dr. Tsai-Kun Li and Dr. Koji Hisatake

17:00-17:30	"Telomere Replication in Yeast and Cancer Cells" Dr. Shu-Chun Teng National Taiwan University, Taiwan
17:30-18:00	"G-quadruplex structure stabilizers inhibit tumorigenic properties of cancer cells through multiple cellular pathways" Dr. Jing-Jer Lin National Taiwan University, Taiwan
18:00-18:30	"SMC complexes: chromatin regulation and human disease" Dr. Kyoko Yokomori University of California, Irvine, USA

Friday, October 4 — Venue: Main Convention Hall

Session Ⅲ: Systems Biology

Keynote Lecture
Chair: Dr. Akira Shibuya

13:30-14:30	"Networks, Importance, and Control" Dr. Arthur D. Lander University of California, Irvine, USA
14:30-14:40	**Coffee Break**

Chairs: Dr. Fuminori Tsuruta and Dr. Ryosuke Ohniwa

14:40-15:10	"Predictive dynamic regulation of MT1-MMP at invadopodia by mathematical modeling and computer simulation" Dr. Kazuhisa Ichikawa The University of Tokyo, Japan
15:10-15:40	"Why coffee wakes us up? − The role of adenosine A2A receptors in sleep-wake regulation" Dr. Michael Lazarus University of Tsukuba, Japan
15:40-16:10	"Projecting evolutionary trajectory of influenza A virus: Multidimensional scaling and individual based antigenic drift model" Dr. Akira Sasaki The Graduate University for Advanced Studies, Japan
16:10-16:20	**Coffee Break**

Friday, October 4	**Venue: Main Convention Hall**
Session Ⅳ: Re-programing and Neuroscience Chairs: Dr. Satoru Takahashi and Dr. Ken Nishimura	
16:20-16:50	"Routes to iPS cells" Dr. Keisuke Kaji The University of Edinburgh, UK
16:50-17:20	"Stem cells and the Heart: Plasticity of the Heart during Development, Identification of Stem Cells *in Vivo*" Dr. Bernd K. Fleischmann University of Bonn, Germany
17:20-17:50	"Manipulation of tissue and inflammation biology to enhance CNS regeneration" Dr. Charles ffrench-Constant The University of Edinburgh, UK
17:50-18:20	"Role of PGC1α in neurodegeneration" Dr. Peter Klivenyi University of Szeged, Hungary

Lessons on CNS Immunology by in vivo imaging

Professor Vincent Dousset, MD, PhD

Director of Translational Research and Advanced Imaging Laboratory
http://trail.labex-univ-bordeaux.fr/
University of Bordeaux - France

Imaging modalities targeting biological markers of inflammation try to display and measure abnormalities while increasing sensitivity and\or specificity by new methodologies. The strength is to reveal measure these markers in living beings, in physiological condition and to be able to follow their evolution in time, spontaneously or following therapies. It might be applied to animals in basic, preclinical and translational research for diverse diseases of the SNC, which imply an inflammatory phenomenon. The weakness is to keep a quite low resolution in the range of millimeter.

The magnetic resonance imaging (MRI) is by far the most successful technique in the revealing of markers of inflammation. What are they ?

Oedema is probably the most accessible marker by the MRI because of intrinsic sensibility to water, the MRI being based on the relaxation of the atoms of hydrogen of water molecules.

Blood-brain barrier (BBB) pemeability might be reveal by the use Gadolinium-chelated contrast agents. In normal situation, gadolinium does not cross the BBB. In case of inflammation, the gadolinium crosses the BHE in a passive way between endothelial cells, following the water exchanges intensified by inflammation.

Cells presenting macrophagic activity during the successive phases of the inflammation are detectable by iron nanoparticles, a specific contrast agent that get in cytoplasmic vesicles of macrophages. The positron emission tomography (PET) could also reveal some markers of inflammation but is of little use in clinical practice. A radiobiological tracer called PK11195 through benzodiazepines receptors might reveal inflammation-activated microglial cells.

Brain metabolites might be studied by magnetic resonance Spectroscopy.

Astrogliosis proliferation, although not so easy to establish, can studied by technics such as diffusion imaging and magnetization transfer.

Brain atrophy is now widely investigated and measured in several diffuse and chronic CNS diseases as a result of neuro-inflammation or neurodegeneration.

We will review cases of multiple sclerosis, acute disseminated encephalomyelitis, optic neuromyelitis, immune recovery inflammatory syndrome, amyloid related imaging abnormalities, neurolupus and rhombencephalitis.

What is the future of CNS imaging in immune and other brain diseases ?

- Improving **diagnosis** through *in vivo* characterization of the cellular and molecular mechanisms of diseasein individual patients
- **Monitoring treatments** or target delivery of therapeutic agents and performing longitudinal evaluation of targeted therapies
- Substituting **interventional imaging** procedures for surgery
- Serving as **surrogate markers** of drug efficacy for clinical trials
- **Personalizing** patient care
- Exploring **populations** to better understand diseases
- Attracting medical **industries** to create wealth and employment

An overview about the Brazilian network to study redox process in biomedicine: Redoxome

Sayuri Miyamoto

Department of Biochemistry, Institute of Chemistry, University of São Paulo

Redoxome, is a Brazilian network of researchers that was launched in 2006 through a "Millenium Project" funded by a federal funding agency (CNPq). Currently, Redoxome is comprised by 25 laboratories and is part of the "National Institute for Science and Technology" (INCT, funded by federal agencies - CNPq and Capes – and by São Paulo state agency - FAPESP) (http://www2.iq.usp.br/redoxoma/). More recently, the Redoxome investigators from São Paulo were selected to constitute one of the "Research, Innovation and Dissemination Centers" (CEPID, funded by FAPESP). The headquarters of Redoxome group is located at the Institute of Chemistry, University of São Paulo, São Paulo, Brazil. Our network of researchers is working towards the integration of basic multidisciplinary research on redox process, the transfer of technology through collaboration with industry and government, and extension activities in scientific education. One of the major goals of Redoxome is to contribute toward the development of new biomarkers and antioxidant strategies with clinical applicability. To achieve these goals, the group is working within four specific research themes: 1) reactive oxygen species (ROS) generation and control in biological systems; 2) chemical reactivity of reactive oxygen species (ROS) in biological environments and consequent changes in structure and function of biomolecules; 3) mechanism and networks involved in redox signaling processes relevant to human disease. 4) diagnostic and therapeutic applications of redox processes. Through these interconnected studies Redoxome hopes to bridge meaningful conceptual gaps in the field and to allow technological and educational advances. In this presentation, I will show some recent and relevant publications of the group.

Grp1-Arf6 axis regulates tumor angiogenesis and growth through HGF-induced endothelial β1 integrin recycling

Tsunaki Hongu[1], Shigetomo Fukuhara[2], Masatsugu Ema[3], Satoru Takahashi[3], Susumu Itoh[4], Mitsuyasu Kato[4], Naoki Mochizuki[2] & Yasunori Kanaho[1]

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The mammalian small GTPase ADP-ribosylation factor (Arf) family consists of six related gene products, Arf1-6, which are divided into three classes based on the sequence homology. ClassI includes Arf1, Arf2 and Arf3, classII Arf4 and Arf5, and classIII Arf6. The sole member of class III, Arf6, localizes to the plasma membrane and endosomal compartments, and plays important roles in a wide variety of cellular events, including exocytosis, endocytosis, actin cytoskeleton reorganization and phosphoinositide metabolism. Although these functions for Arf6 have been extensively characterized at the cellular and molecular levels, the physiological functions of Arf6 at the whole organ level remains to be clarified. We show here that Arf6 regulates tumor neoangiogenesis induced by hepatocyte growth factor (HGF), but not other angiogenic factors, utilizing endothelial cell-specific *Arf6*$^{-/-}$ mice. In *Arf6*$^{-/-}$ endothelial cells, HGF-stimulated β1 integrin recycling was abolished, resulting in inhibition of spreading, migration and focal adhesion formation. This function of Arf6 is regulated by its guanine nucleotide exchange factor Grp1, and the pharmacological inhibition of Grp1-Arf6 axis efficiently suppressed tumor vascularization. Taken together, our findings reveal uncovered mechanism of angiogenesis regulated by HGF, and an importance of Arf6 in tumor vascularization.

Parvoviruses Cause Nuclear Envelope Breakdown by Activating Key Enzymes of Mitosis

Manvi Porwal[1,2], Sarah Cohen[4], Kenza Snoussi [2#]; Ruth Popa-Wagner[4], Fenja Anderson[1,5], Nathalie Dugot-Senant[6], Harald Wodrich[2], Christiane Dinsart[7], Jürgen A. Kleinschmidt[4], Nelly Panté[3] and Michael Kann[1,2*]

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Disassembly of the nuclear lamina is essential in mitosis and apoptosis requiring multiple coordinated enzymatic activities in nucleus and cytoplasm. Activation and coordination of the different activities is poorly understood and moreover complicated as some factors translocate between cytoplasm and nucleus in preparatory phases. Here we used the ability of parvoviruses to induce nuclear membrane breakdown to understand the triggers of key mitotic enzymes. Nuclear envelope disintegration was shown upon infection, microinjection but also upon their application to permeabilized cells. The latter technique also showed that nuclear envelope disintegration was independent upon soluble cytoplasmic factors. Using time-lapse microscopy we observed that nuclear disassembly exhibited mitosis-like kinetics and occurred suddenly implying a catastrophic event irrespective of cell- or type of parvovirus used. Analyzing the order of the processes allowed us to propose a model starting with direct binding of parvoviruses to distinct proteins of the nuclear pore causing structural rearrangement of the parvoviruses. The resulting exposure of domains comprising amphipathic helices was required for nuclear envelope disintegration, which comprised disruption of inner and outer nuclear membrane as shown by electron microscopy. Consistent with Ca^{++} efflux from the lumen between inner and outer nuclear membrane we found that Ca^{++} was essential for nuclear disassembly by activating PKC. PKC activation then triggered activation of cdk-2, which became further activated by caspase-3. Collectively our study shows a unique interaction of a virus with the nuclear envelope, provides evidence that a nuclear pool of executing enzymes is sufficient for nuclear disassembly in quiescent cells, and demonstrates that nuclear disassembly can be uncoupled from initial phases of mitosis.

Telomere Replication in Yeast and Cancer Cells

Zih-Jie Shen, Meng-Hsun Hsieh, Shu-Chun Teng

Department of Microbiology, College of Medicine, National Taiwan University

Telomeres are dynamic DNA-protein complexes that protect the ends of linear chromosomes. Most telomeric DNA is synthesized by the enzyme telomerase. While most somatic cells do not express telomerase and therefore have limited life span, cancer cells can bypass the crisis either through telomerase reactivation or through an alternative recombination pathway for telomere lengthening (ALT).

Telomerase is mainly activated by Cdk1/Tel1/Mec1 on telomeric binding protein Cdc13 from late S to G2 phase of the cell cycle. Cdk1 phosphorylates residues 308 and 336 of Cdc13. Phenotypic analysis in vivo revealed that the mutations in the Cdc13 S/TP motifs phosphorylated by Cdk1/Mec1/Tel1 caused cell cycle delay and telomere shortening and these phenotypes could be partially restored by the replacement with a negative charge residue. Furthermore, the Cdk1-mediated phosphorylation was required to promote the regular turnover of Cdc13. Hypernegatively charged domain of Cdc13 contributed by Cdk1, Tel1 and Mec1 may provide an optimal interface to recruit the potential positively charged domain near the amino acid 444 lysine residue of Est1 in the telomerase complex.

Moreover, our previous studies have identified more than 15 factors required for the ALT pathway in yeast. Among them we found that Sgs1 is specifically sumoylated under the stress of DNA double strand breaks. The major SUMO attachment site in Sgs1 is lysine 621, which lies at the Top2 and Rpa1 binding domain. Sumoylation of K621 was found to be uniquely required for Sgs1's role in telomere-telomere recombination. Additionally, both other topoisomerases are required for telomere recombination. Our results demonstrate that the RecQ helicase-topoisomerase complexes coordinate to resolve the replicative tension during the movement of recombinational fork in ALT cells. Blockage of the telomerase and ALT pathways should therefore inhibit the cell cycle of all types of cancers.

G-quadruplex structure stabilizers inhibit tumorigenic properties of cancer cells through multiple cellular pathways

Jing-Jer Lin

Institute of Biochemistry and Molecular Biology, National Taiwan University College of Medicine, Taipei, 100, Taiwan

Carbazole derivatives that stabilized G-quadruplex DNA structure formed by human telomeric sequence have been designed and synthesized. Among them, 3,6-bis(1-methyl-4-vinylpyridinium) carbazole diiodide (BMVC) and 3,6-bis(4-methyl-2-vinylpyrazinium) carbazole diiodine (BMVC4) bound and stabilized G-quadruplex structure and had potent inhibitory effects against telomerase activity. The cellular effects of both BMVC and BMVC4 were characterized in cancer cells. These two compounds repressed tumor progression through both telomere-dependent and telomere-independent pathways. In addition to their induction of telomere shortening, these compounds also induced senescence by activation of DNA damage response pathway that was independent of their telomerase inhibitory activity. Interestingly, the expressions of *Wnt-1* were also repressed by BMVC and BMVC4 through formation of a G-quadruplex structure at the *Wnt-1* promoter. As a consequence, the expression levels of β-catenin, a mediator of the Wnt-mediated signaling pathway, and downstream target genes MMP7 and survivin were also down-regulated upon BMVC or BMVC4 treatments. Moreover, the migration and invasion activities of cancer cells were inhibited by BMVC and BMVC4. Together our results indicated the G-quarduplex stabilizers BMVC and BMVC4 have the potential to be further developed as a chemotherapeutic agent against both telomerase-positive and telomerase-negative ALT cancer cells.

SMC complexes: chromatin regulation and human disease

Kyoko Yokomori, Ph.D.

Department of Biological Chemistry, School of Medicine, University of California, Irvine

The DNA genome stored in the form of chromatin or chromosomes undergoes dynamic structural changes during cell cycling and cellular differentiation that are critical for both proper maintenance and output of genetic information. Our laboratory investigates the mechanisms underlying these changes and how they affect both mitotic and interphase genomic functions in mammalian cells. In particular, we focus on the Structural Maintenance of Chromosomes (SMC) family of proteins, essential proteins that physically associate with chromatin and modulate higher-order chromatin structures. Six SMC proteins (SMC1-6) are conserved in eukaryotes, and form three SMC heterodimers (SMC1-SMC3, SMC2-SMC4, and SMC5-SMC6). These heterodimers serve as cores for three separate types of multiprotein complexes: cohesins, condensins, and the SMC5-SMC6 complex, respectively. In mammalian somatic cells, there are two cohesins (cohesin-SA1 and cohesin-SA2) and two condensins (condensin I and condensin II), with each pair performing both redundant and distinct functions. These complexes are involved in different aspects of mitotic chromosome organization as well as DNA repair to protect genomic integrity. Cohesins and condensins further participate in developmental gene regulation, as their impairment is associated with human developmental disorders. We have previously studied the roles of SMC complexes in mitosis, and now our research focuses on their interphase functions in DNA repair and gene regulation. We utilize laser microirradiation and endonuclease systems to study the responses of SMC complexes to different types of DNA damage in vivo. We also study gene regulatory functions of SMC complexes linked to the human developmental disorders facioscapulohumeral muscular dystrophy (FSHD) and Cornelia de Lange Syndrome (CdLS) using biochemical and high-throughput genomic approaches in patient cells and in a mouse model. Through these studies, we hope to elucidate fundamental elements of chromatin and nuclear structural regulation critical for human health and disease.

Networks, Importance, and Control

Arthur D. Lander, M.D., Ph.D.

Center for Complex Biological Systems, and Departments of Developmental & Cell Biology and Biomedical Engineering, University of California, Irvine, CA, USA

The start of the 21st Century marks a singular shift in the way that biological scientists interact with data. In the past, most experiments were designed to test individual hypotheses by gathering just the data necessary. Improved technology now makes it possible to gather massive amounts of data first, and only later confront the issue of which hypotheses to test. Although the ability to gather terabytes of data cheaply has been broadly welcomed by biologists, it brings with it a new challenge: how to decide what observations are important. This is a serious problem because biological data are inherently noisy, and the more hypotheses one attempts to test at once, the more true signals become statistically indistinguishable from noise. Getting around this problem requires using prior knowledge to prioritize one's data and hypotheses, but what prior knowledge should be used? I will explain how answering this question—arguably the greatest challenge yet taken on by the young field of Systems Biology—is harder than it looks. The difficulty arises, in large part, because biological systems are selected to perform well in the face of perturbations of many kinds. This general robustness directly undermines the ability of observable patterns of causal influence to reveal biological organization. This is especially true when such organization exists for the purpose of control, which is very often just what we may expect evolution to select for. I will argue that, in the end, Systems Biologists will only be able to tame "big data" when they can use what they have learned about fundamental biological design principles at the small-scale to anticipate the architectures of biological systems at the large-scale.

Predictive dynamic regulation of MT1-MMP at invadopodia by mathematical modeling and computer simulation

Kazuhisa Ichikawa

Mathematical Oncology, The Institute of Medical Science, The University of Tokyo

It is reported that metastasis causes 90% of human cancer deaths. Thus, the blockade of metastasis is an important therapeutic target. Although the phenomena and the mechanisms of metastasis are complex, its initial step is the degradation of extracellular matrix (ECM) and opening of small holes, through which cancer cells can move to a distant location. MT1-MMP is a potent ECM-degrading membrane type metalloproteinase, which is concentrated at the invasive machinery, invadopodia. The activity of MT1-MMP is inhibited by an intrinsic inhibitor TIMP-2. However, TIMP-2 binds the proform of MMP-2, proMMP-2, forming a ternary complex of MMP-2:TIMP-2:proMMP-2, and proMMP-2 in the complex is converted to an active form, MMP-2, through shedding by TIMP-2-free MT1-MMP. Activated MMP-2 undergoes ECM degradation. Thus TIMP-2 has a dual role in the ECM degradation, inhibition and promotion of ECM degradation.

We ran computer simulations of ECM degradation by MT1-MMP at invadopodia, and found that a repetitive insertion of MT1-MMP with an interval of several tens of seconds was required for the ECM degradation. This interval was unexpectedly short. Then we performed FRAP experiments on the turnover of MT1-MMP focusing on a single invadopodium. The analyses of the fluorescent signal revealed that the recovery is composed of fast and slow processes with the time constants of 26.0 and 259 sec, respectively. Simulations of the reconstructed model according to our FRAP experiments showed ECM degradation consistent with the observation as in our preliminary simulations. We next sought a reason why such quick replenishment of MT1-MMP was required for the ECM degradation, and found that there was a sharp transient activity of MT1-MMP with the half width narrower than 4.5 sec. If we computationally eliminated the sharp transient, ECM degradation was greatly reduced showing the critical role of the sharp transient in the ECM degradation. These computer simulations and experimental results show the highly dynamic nature of MT1-MMP in the invadopodia. We will propose a possible therapeutic treatment to prevent the invasion based on our results.

Why coffee wakes us up? − The role of adenosine A_{2A} receptors in sleep-wake regulation

Michael Lazarus

WPI-International Institute for Integrative Sleep Medicine (WPI-IIIS), University of Tsukuba

Sleep is one of the most mysterious of the physiological functions of the brain. Sleep or sleep-like states seem to exist in all complex organisms that have a central nervous system. The sleeping habits of humans, however, are unique in the sense that we often defy sleep and stay awake for occupational or recreational reasons, although we are tired during that time. The motivation to stay awake and active in our modern society is ever growing and often accompanied by the use of psychoactive substances, most prominently caffeine, but also various prescription drugs and substances of abuse. The key area governing these behaviors is the basal ganglia, which are subcortical nuclei involved in motor function, habit formation, and reward/addictive behaviors, all of which depend on wakefulness.

One of the mechanisms of sleep induction involves the activation of A_{2A} receptors by adenosine. A_{2A} receptors are highly expressed on striatopallidal neurons in the indirect pathway of the basal ganglia, in which dopamine D_2 receptors are co-expressed with A_{2A} receptors. The extent to which A_{2A} receptors in the basal ganglia contribute to the regulation of sleep and wakefulness is not known. We investigated the role of A_{2A} receptors in the basal ganglia for sleep-wake regulation by using powerful tools for site-specific gene manipulations, including conditional A_{2A} receptor knockout mice based on the Cre/lox technology; focal A_{2A} receptor knockdown in rats through the local infection with adeno-associated virus carrying short-hairpin RNA of A_{2A} receptors; and modulation of neuronal activity through in-vivo stimulation with genetically engineered receptor-channel systems, e.g. designer receptors exclusively activated by a designer drug (DREADD) or optogenetics.

Our studies have revealed that the arousal effect of caffeine critically depends on A_{2A} receptors on neurons in the shell of the nucleus accumbens and that transient activation of nucleus accumbens neurons promotes sleep. These observations strongly suggest that A_{2A} receptors in the nucleus accumbens are key structural elements for the control of sleep and wakefulness. These findings further suggest the intriguing possibility that the ventral striatum may be a key site through which sleep and wakefulness are regulated by behavioral processes and, by extension, that motivational state may be an important fundamental regulator of sleep and wake (Trends Neurosci, doi: 10.1016/j.tins.2012.07.001; Curr Opin Neurobiol, doi: 10.1016/j.conb.2013.02.001).

Projecting evolutionary trajectory of influenza A virus: Multidimensional scaling and individual based antigenic drift model

Akira Sasaki

Department of Evolutionary Studies of Biosystems, The Graduate University for Advanced Studies

Antigenicity of influenza A viruses is known to evolve rapidly by changing variable amino acid sites of hemagglutinin, which enable them to escape host immune response and to spread every year in the host population. Predicting the evolutionary direction of the viral antigenicity is very difficult particularly because of high dimensionality of its phenotypic space (antigen space) due to combinational diversity of variable amino acid sites. Recently a technique called multidimensional scaling (MDS) is focusing attention in the evolutionary studies of influenza, which translate the genetic and immunological distances between viral strains into distance in a low dimensional model space. Application of MDS to over 30 years evolution of A Hong Kong influenza viruses (H3N2 subtype) has revealed a linear trend in their evolutionary trajectory. In this paper, I study possible factors that are responsible for an extracted linear trend in MDS map in the phylodynamical trajectory of influenza virus evolution, by using extensive simulations of individual-based model (IBM) for the coupled dynamics of viral antigenic drift and specific immune defense of hosts. Analyses revealed that if phylogenetical tree shape of IBM simulation is "cactus like" as is observed in influenza A Hong Kong virus phylogeny, a linear trend is realized if MDS is applied to the Hamming distance relationship between strains. We also discuss how big turns between linear trends, observed both in real data and simulation data, are related to the phylogenetical changes of virus population.

Routes to iPS cells

Keisuke Kaji

MRC Centre for Regenerative Medicine, University of Edinburgh, UK

Generation of induced pluripotent stem cells (iPSCs) is a novel technology with the great potential to revolutionize medicine. While iPSCs have already been widely used for drug screening and disease modeling, little is known about the mechanism of this cellular reprogramming. The low efficiency and the heterogeneity of this de-differentiation process have hampered further molecular analysis. To overcome this problem, we adopted a strategy called 'secondary reprogramming'. Briefly, using mouse embryonic fibroblasts (MEFs), we first generated an iPSC line with random integration of a piggyBac transposon carrying a doxycycline (dox)-inducible polycistronic reprogramming factors (c-Myc, Klf4, Oct4, Sox2 linked with 2A-peptides = MKOS). This iPSC line was used to generate MEFs (secondary MEFs) which can reprogram upon addition of dox. This strategy enabled us to achieve more homogeneous reprogramming factor induction. In combination with novel cell surface markers, CD44 and ICAM1, and a pluripotency marker Nanog-GFP reporter, we demonstrate that reprogramming progresses in a step-wise manner and the behavior of each intermediate subpopulation is predictable. RNA-sequencing analysis of these populations demonstrates two waves of pluripotency gene upregulation, and unexpectedly, transient upregulation of several epidermis-related genes, demonstrating that reprogramming is not simply the reversal of the normal developmental processes. This novel high-resolution analysis enables the construction of a detailed reprogramming route map, and the improved understanding of the reprogramming process will lead to new reprogramming strategies.

Stem cells and the Heart: Plasticity of the Heart during Development, Identification of Stem Cells *in Vivo*

Martin Breitbach[1], Kenichi Kimura[1,2], Alexandra Raulf[1], Christopher J. Fuegemann[1], Michael Hesse[1], Osamu Ohneda[2], Bernd K. Fleischmann[1]

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Cardiovascular diseases are one of the leading causes of morbidity and mortality, therefore novel therapeutic approaches are required to restore myocardial contractility and prevent heart failure upon myocardial infarction. We are exploring different strategies to enhance myocardial plasticity. Our studies demonstrate that the neonatal heart has in contrast to the adult a surprising degree of myocardial plasticity. This is due to the proliferative activity of cardiomyocytes and the presence of resident progenitor populations with myogenic potential. Currently we are analyzing the differences in stem-/progenitor cells and cardiomyocyte cell cycle activity. Of high interest for the clinical use are mesenchymal stem cells (MSCs), because of their multipotency and straightforward isolation procedures. However, their localization and function *in vivo* remains elusive, because of a lack of specific MSC markers. We have generated a new transgenic mouse model, where the expression of EGFP is under control of the CD73 promoter (BAC) to identify MSCs *in vivo*. $EGFP^+$ cells are found in different tissues of embryonic- and adult CD73-BAC-EGFP mice, such as in developing bones at the sites of endochondral ossification. In long bones sinusoidal reticular cells are labeled, that form elongated caverns throughout the BM; these express typical MSC markers like Sca-1 and PDGFRα and are presumably hematopoietic niche cells. This is underscored by co-localization of $c\text{-}kit^+$ hematopoietic progenitor cells with these $EGFP^+$ structures. Furthermore, primary cultures from epiphysis, BM, white fat, and other organs give rise to adherently growing $EGFP^+$ cells. These differentiate into mesenchymal lineages, underlining enrichment of MSCs in the $EGFP^+$ cell fraction and residence of MSCs in various tissues. Our long-term goal is the identification of the MSC niches to better understand the biology of MSCs and their physiological role.

Manipulation of tissue and inflammation biology to enhance CNS regeneration

Charles ffrench-Constant

MRC Centre for Regenerative Medicine, University of Edinburgh

Strategies to enhance regeneration in the CNS can be developed from studies of three different areas of biology - stem cell biology, tissue biology and inflammation. Whilst the first has received much attention, the latter two have been the subject of fewer studies. Using axon regeneration and remyelination, both required for effective tissue restoration following white matter lesions, as examples I will describe experiments showing how manipulation of extracellular matrix receptors on regenerating growth cones can be used to turn an inhibitory extracellular matrix environment into one that is permissive for growth, and also how the study of the macrophage/microglial response to demyelination leads to the identification of novel factors that promote remyelination and so provide targets for novel treatments in CNS repair.

Role of PGC1α in neurodegeneration

Peter Klivenyi

Department of Neurology, University of Szeged, Szeged, Hungary

Peroxisome proliferator-activated receptor-γ coactivator-1α (PGC-1α) is a transcriptional coactivator that is a key master regulator of mitochondrial biogenesis. Recent evidence highlighted that PGC1α deficiency may contribute to the mitochondrial dysfunction in neurodegenerative diseases.

To study the role of PGC1a first we systematically evaluated the neuropathological alterations of mice lacking the expression of the full-length PGC-1α protein (FL-PGC-1α) but expressing an N-truncated fragment. The immunohistochemical pattern of neurodegeneration-related proteins did not differ between FL-PGC-1α knockout and wild-type animals, and there was a complete lack of protein deposits or ubiquitin-positive inclusions. The analysis of neuropathological alterations revealed widespread vacuolation predominating in the cerebral white matter, caudate-putamen, thalamus and brainstem, and reactive astrogliosis in the brainstem and cerebellar nuclei. This morphological phenotype was thus reminiscent of human mitochondrial encephalopathies, especially the Kearns-Sayre syndrome.

In behaviour tests several alterations can be detected in these mice, such as reduced spontaneous locomotor activity, reduced anxiety, while they showed no memory deficit.

To test the interaction of environmental toxins and PGC1α expression we administered a mitochondrial complex II inhibitor, 3-nitropropionic acid to these mice and found a significant elevation of the PGC1α expression in the striatum but not in the cortex and the cerebellum.

All these data indicate massive involvement of PGC1α system in different form of neurodegeneration, which may produce a new therapeutic target for further drug development.

MEMO

Art and Ecology

Art and Ecology

Wednesday, October 2		Venue: Conference Room 303
Opening Remarks		
10:00-10:10	Dr. Shinichi Tamagawa	University of Tsukuba, Japan
Chair: Dr. Toshiharu Omuka		
10:10-10:40	"Reconstruction of Earthquake Disaster by forestry resources The construction of a temporary house by using the construction method of wooden Panel-Houses and Reconstruction houses for The Great east Japan earthquake 2011" Dr. Kunihiro Ando	University of Tsukuba, Japan
10:40-11:10	"Cultivation Art in Hospital" Dr. Momoyo Kaijima	University of Tsukuba, Japan
11:10-11:40	"Painting on mud and lime mortar walls" Dr. Terumi Hotokeyama	University of Tsukuba, Japan
11:40-13:00	**- Lunch -**	
13:00-14:30	"Planetary Visions: Land Art, Ecology, and the Whole Earth" Dr. James Nisbet	University of California, Irvine, USA
14:30-14:40	**Coffee Break**	
14:40-16:10	"Queensland House: local idiom" Andrew Wilson	University of Queensland, Australia
16:10-16:20	**Coffee Break**	
16:20-17:00	Discussion	

Reconstruction of Earthquake Disaster by forestry resources The construction of a temporary house by using the construction method of wooden Panel-Houses and Reconstruction houses for The Great east Japan earthquake 2011

Kunihiro Ando

Emeritus Professor of Tsukuba University

The wooden Panel (Itakura)-house as a temporary house was intended to break away from the dependence of nuclear energy and petroleum, using much of wooden resources and to increase employment of local carpenters and craftsman. The structure and facades were constructed by japanese cedar and natural fibers as insulating material. The steep roof made a buffer space under the roof, which intended to serve as a community space and to regulate the thermal environment. The loft space serves as storage for the limited floor space.

The using term of temporary houses are maximum 4 years. Those temporary houses are planed to reuse as a permanent residence toward the rehabilitation from the earthquake. Therefore the reconstructable structure and recyclable material for facade were chosen. The wooden Panel Houses were constructed supplementar with neils and bolts without lamination.

Considering the experience of construct as temporal house, we proposed the wooden panel house in Miyagi prefecture and built one model house “Sanriku Santaro”. It intends to use forest resource of artificial Japanese cedar actively which has not been used for a long time, and to activated forest industry for along for a long term and sustainably. The worry is about the short term concentration of craftsman for rebuilding after the earthquake. As a support for such kind of situation, we try to organize new systems which support the timber production like cutting, sawing and construction by negotiate with the authorities of the residence. It intends to decrese the cost of construction and rebuilt the connection of residence and regional society.

Cultivation Art in Hospital

Momoyo Kaijima

Associate Professor, Faculty of Art and Design, University of Tsukuba

Faculty of Art and Design started the art in University of Tsukuba Hospital in 2003 as a theme of design class. In 2005 new curriculum of the Art and Design Produce which gives the site for art and design studio for the students, was started and the students group «Asparagus» for Hospital art and design was founded. At first they renovated the part of corridor as art space «Seed of Humanity» and did art workshop with the hospital staff and patient for the better QOL in Hospital. In 2007 the field was expanded to Tsukuba Medical Center Foundation. In 2011 the project of art in new Kayaki building, University of Tsukuba Hospital are started. And for it the art working group of professor in Faculty of Art and Design started to discuss of the wider issue of the hospital art in practical experiment for the art works and researches. The importance of art in hospital are there points; art with time, collaboration with patient and medical staff, cultivating art.

In this fall we carry out human resources development curriculum for art in hospital. We hope that we can contribute to art in hospital in the future.

Painting on mud and lime mortar walls

Terumi Hotokeyama

Associate Professor, Faculty of Art and Design, University of Tsukuba

Mud and lime mortar walls are natural building supporting materials traditionally used in houses. These are the produce of human wisdom to form the living space synergistically with nature.

This presentation focuses two of our activities: oil painting directly on mud walls and fresco painting using Japanese lime mortar. These paintings were produced on walls of existing old houses. The greatest challenge associated with these is how to stably fix pigments in mud and lime mortar walls.

1. Oil painting on the mud wall

Before painting murals in oil directly on the mud wall, the surface shall be treated by sizing. In the course of our production, the mud wall was sized in reference to techniques and materials used in Buddhist murals in Bamiyan, Afghanistan.

2. Fresco painting using Japanese lime mortar

The mural was produced by buon fresco technique using Japanese lime mortar. In buon fresco painting, pigment is mixed with water and laid on wet, fresh lime mortar. It is the only technique to fix pigment particles in the wall without medium addition by the chemical process called calcite resulted from calcium hydroxide contained in lime mortar reacted with carbon dioxide in atmosphere.

Future challenges

Oil painting on the mud wall achieved success to stably fix oil paints on the wall with successful sizing treatment.

On the other hand buon fresco technique using Japanese lime mortar made a certain level of success in fixing pigments. However, observation revealed that pigment fixation needs some more improvement to attain calcite as seen in murals produced by the original buon fresco technique.

Japanese lime mortar has different composition from Italian one (malta meaning lime mortar), either of which requires the specific painting technique. Toward achievement of buon fresco technique using Japanese lime mortar, various auxiliary works should be added in the painting process.

Planetary Visions: Land Art, Ecology, and the Whole Earth

James Nisbet

Assistant Professor
Department of Art History
University of California, Irvine

This paper will revise the long-established relationship between minimalist sculpture and land art by addressing the role of ecology and systems theory in the transition between these two artistic movements. Such issues resonate with different forms of envisioning the earth from outer space during this period, as in the closing scene of Stanley Kubrick's *2001: A Space Odyssey* and the cover of Steward Brand's iconic *Whole Earth Catalog*. Careful consideration of these "planetary visions" shows that the earliest works of land art all address a shared thematic of how singular objects operate within holistic ecosystems. This insight extends to such canonical earthworks as Heizer's *Double Negative* (1969–1970) and Robert Smithson's *Spiral Jetty* (1970), both of which recast the sculptural scale of minimalism as a way to mediate the body of the individual spectator with the magnitude of the earth itself. Throughout the late 1960s, such grand, utopian visions persist in imagining the planet as both a singular physical object and a systematic network. It is from this cultural imaginary that land art emerged as a means of negotiating these tensions through the convergence of landscape, technology, and sculpture.

The Queensland House: local idiom

Andrew Wilson[1]

[1]*School of Architecture, University of Queensland, Australia*

As a type, the Queensland House is a relatively recent phenomenon that appeared in the first half of the nineteenth century, a local idiom that is found across Queensland and Northern New South Wales in Australia. It reflects the timber industry's success over the brick industry, politically in the State and the abundance of hardwood and softwood timber that was relentlessly exploited by the British colonists along the coast of Queensland, particularly in South-east Queensland and Northern New South Wales. Although there are variations of the type, it is interesting that it can be found in country areas, particularly along the coast and in towns and cities. In the middle of the twentieth century, Brisbane, the capital of Queensland was a field of detached timber Queensland houses.

The Queenslander is arguably the strongest vernacular tradition in Australia notwithstanding aboriginal shelters that influenced the first structures built in the new British colony. There are house and cottage variations. Timber sawmillers like James Campbell and Sons developed prefabricated house systems that could be bought from their Redicut House Catalogue, and transported by rail across the State as a stacked kit of parts.

Typical characteristics include use of logs or stumps as foundations, that provided storage space under the house. Other characteristics include the raised platform, verandas, corrugated iron roof, and potential for additions over time and changes of use within the house, including enclosing verandas to become bedrooms, kitchens or extensions to living spaces. Building on stumps allowed for the possibility of moving the house to another location. The Queenslander can be a simple form or accretion of parts. In some cases toilets, bathrooms and kitchens were initially located in separate pavilions outside and incorporated into the house at a later date.

MEMO

MEMO

Policy and Planning Sciences

Policy and Planning Sciences

Wednesday, October 2

Venue: Conference Room 304

Session 1
Chair: Dr. Akiko Yoshise

13:00-13:30	"A new method to measure effects of flood hazard on land prices" Dr. Hajime Seya National Institute for Environmental Studies, Japan
13:30-14:00	"An experimental study on the incentives of the probabilistic serial mechanism" Dr. Morimitsu Kurino Berlin Social Research Center, Germany
14:00-14:30	"Characterization of Aggregation Functions in Voting Mechanism" Dr. Yoshitsugu Yamamoto University of Tsukuba, Japan
14:30-15:00	"An Efficient and Incentive Compatible Dynamic Auction for Multiple Complements" Dr. Zaifu Yang University of York, UK
15:00-15:10	**Coffee Break**

Session 2: Student Presentation
Chair: Dr. Morito Tsutsumi

15:10-15:25	"Delineating spatial submarket based on spatial heterogeneity in price structure" Sho Kuroda University of Tsukuba, Japan
15:25-15:40	"Eigenvector-based spatial filtering approach to a multinomial discrete choice model" Takahiro Yoshida University of Tsukuba, Japan
15:40-15:55	"Incorporating spatial non-stationarity into a spatial interaction model" Kazuki Tamesue University of Tsukuba, Japan
15:55-16:10	"A Multi-scale Land Price Analysis Using an Eigenvector-based Approach" Daisuke Murakami University of Tsukuba, Japan
16:10-16:25	"An LP-based algorithm to test copositivity" Akihiro Tanaka University of Tsukuba, Japan
16:25-16:35	**Coffee Break**

Session 3: Student Presentation
Chair: Dr. Shun Watanabe

16:35-16:50	"Analysis on ambiguity aversion and gender of traders who lean toward an intermediated trade in search market" Takaaki Fujikura University of Tsukuba, Japan
16:50-17:05	"Effects of the heterogeneity of link weights on the evolution of cooperation" Manabu Iwata University of Tsukuba, Japan
17:05-17:20	"Selection of Opponents in the Prisoner's Dilemma in Dynamic Networks --- An Experimental Approach---" Hiroto Yonenoh University of Tsukuba, Japan
17:20-17:35	"Computational complexity of a solution for directed graph cooperative games" Ayumi Igarashi University of Tsukuba, Japan
17:35-17:50	"A cutting plane algorithm for modularity maximizatio with heuristics for separation problem" Yoichi Izunaga University of Tsukuba, Japan

A new method to measure effects of flood hazard on land prices

Hajime Seya[1]

[1]*Center for Global Environmental Research, National Institute for Environment Studies*

The negative effect of flood hazard on land prices is preferable from the view point of reducing disaster risk. One of the conventional ways to verify such effect is using hedonic approach, where land prices are regressed on explanatory variables, and marginal implicit price (MIP) of flood hazard is calculated. However, conventional hedonic analysis with basic multiple regression model suffers from two problems. The first problem is omitted variables bias—the effect other than flood hazard, which correlate with the flood hazard variable, are only partially controlled (excluded from the model), resulting in the biased coefficient estimate. The second problem is in that we can only obtain the averaged MIP, and differences in MIPs by location points are usually ignored. Here, we show that these two problems can be improved using the techniques of spatial statistics.

The conventional way to control omitted variable effects is introducing zone specific dummy variables to one' s model. However, in this approach, the variation of omitted variables inside the zones cannot be considered. Hence instead, we use the another way—spatial statistical models to model the spatial dependent structure of the omitted variables. The results show that the positive effect of flood hazard variable on land prices in industrial zones of the Tokyo metropolitan area is inverted to negative, if we use the spatial model. With regard to the second problem, we use the new eigenvector based spatial filtering technique, which gives the coefficient estimate for each observed point. The results show that although the averaged effect of flood hazard on land prices is negative in residential zones, there may exist local positive effects in the flood prone zones in Tokyo. In such zones, flood hazard may not be naturally capitalized into land prices, resulting in large damage when river flood once occurred.

An experimental study on the incentives of the probabilistic serial mechanism

David Hugh-Jones[1], Morimitsu Kurino[2], Christoph Vanberg[3]

[1]*Department of Government, University of Essex*
[2]*Research Unit of Market Behavior, WZB Berlin Social Research Center*
[3]*Department of Economics, University of Heidelberg*

We report an experiment on the Probabilistic Serial (PS) mechanism for allocating indivisible goods. The PS mechanism, a recently discovered alternative to the widely used Random Serial Dictatorship mechanism, has attractive fairness and efficiency properties if people report their preferences truthfully. However, the mechanism is not strategy-proof, so participants may not truthfully report their preferences. We investigate misreporting in a set of simple applications of the PS mechanism. We confront subjects with situations in which theory suggests that there is an incentive or no incentive to misreport. We find little misreporting in situations where misreporting is a Nash equilibrium. However, we also find a significant degree of misreporting in situations where there is actually no benefit to doing so. These findings suggest that the PS mechanism may have problems in terms of truthful elicitation.

Characterization of Aggregation Functions in Voting Mechanism

Yoshitsugu Yamamoto

Faculty of Engineering, Information and Systems, University of Tsukuba

Balinski and Laraki proposed the majority judgment as a new voting mechanism in their book *Majority Judgment : Measuring, Ranking, and Electing.* The key ideas are the common language that is an ordered set consisting of a finite number of assessment words and the aggregation function that transforms the vector of words reported by the jury to a final grade. They assumed anonymity, weak monotonicity, strong monotonicity, unanimity and strategy-proofness on the aggregation function and proved that the unique aggregation function is the order function. In this talk we will raise two issues about their aggregation function, and show that they are resolved by relaxing the strong monotonicity assumption. Furthermore we will show that the anonymous, weakly monotonic and strategy-proof aggregation function is completely determined by the set of final grades when the jury splits deeply.

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An Efficient and Incentive Compatible Dynamic Auction for Multiple Complements

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This article proposes an efficient and incentive compatible dynamic auction for selling several complementary goods to finitely many bidders. The goods are traded in discrete quantities. The seller has a reserve price for every bundle of goods and determines which bundles to sell based on current prices. The auctioneer announces a current price for every bundle of goods and a supply set of goods, every bidder subsequently responds with a set of goods demanded at these prices, and then the auctioneer adjusts prices. We prove that even when bidders can exercise their market power strategically, this dynamic auction always induces them to bid truthfully as price-takers, resulting in an efficient allocation, its supporting Walrasian equilibrium price for every bundle of goods, and a generalized Vickrey-Clarke-Groves payment for every bidder.

Delineating spatial submarket based on spatial heterogeneity in price structure

Sho Kuroda[1], Morito Tsutsumi[2]

[1]*Graduate School of Systems and Information Engineering, University of Tsukuba*
[2]*Faculty of Engineering, Information and Systems, University of Tsukuba*

Hedonic pricing method, based on capitalization hypothesis, has been widely used for purpose of property appraisal or valuation of non-market goods and many related work has pointed out the effectiveness of considering submarket or segmentation in terms of proper estimation of hedonic price function. This study proposes a method that endogenously delineates property spatial/ geographical market segmentation or submarket. This segmentation algorithm is formulated as an optimization problem whose objective functions is goodness-of-fit to a price function that takes segmentation structures as decision variables, in order to deal with the spatial heterogeneity of price structures. Using this scheme, we achieve improvements in accuracy and parameter estimation adequateness for the real estate price function and realize a segmentation that satisfies geographical contiguity or spatial proximity. We applied this approach to the observed dataset and confirmed its potency in coping with spatial heterogeneity and such problems as instability in segmentation.

Eigenvector-based spatial filtering approach to a multinomial discrete choice model

Takahiro Yoshida[1] and Morito Tsutsumi[2]

[1]*Graduate School of Systems and Information Engineering, University of Tsukuba*
[2]*Faculty of Engineering, Information and systems, University of Tsukuba*

It is vital to deal appropriately with spatial dependence, that is, when using regression models dealing with spatial data. Recent studies on spatial statistics and spatial econometrics have suggested many methods to consider spatial dependence in quantitative data. However, these methods, termed spatial discrete choice models, typically require a computationally burdensome, iterative calculation for parameter estimation. In addition, spatial "multinomial" discrete choice models are still being finalized, and these methods are not well established.

Hence, the present study takes another approach, namely eigenvector-based spatial filtering (ESF), in which spatial dependence can be considered only by introducing eigenvectors of a modified spatial weight matrix as explanatory variables. This approach facilitates easy practical implementation because it does not require a special parameter estimation technique and can be used with standard statistical software packages after selecting eigenvectors. The modified spatial weight matrix is directly related to the Moran's *I* statistics, which is known as a spatial dependence measure, and therefore, has significant merits in interpretation. Further, linear combinations of the selected eigenvectors and their estimated coefficients can be interpreted as potential explanatory variables.

Hence, in the present study, we apply ESF to a multinomial discrete choice model and compare its predictive accuracies, in terms of the Akaike Information Criterion and the hit ratio, and its computation time to that of the conventional model. The obtained results suggest that compared to the conventional model, those measures are substantially improved with ESF.

Incorporating spatial non-stationarity into a spatial interaction model

Kazuki Tamesue[1] and Morito Tsutsumi[2]

[1]*Graduate School of Systems and Information Engineering,University of Tsukuba*
[2]*Faculty of Engineering, Information and Systems, University of Tsukuba*

Geographically weighted regression (GWR) is used in a variety of fields to capture spatial variation or spatial non-stationarity of a regression analysis by allowing parameters to vary across space; however, application to spatial interaction model is much more complex. The complexity comes from the fact of measuring distances between flows, whereas each flows contains two regions. Because an origin-destination flow is a pairwise of origin and destination region, the weighting of not only origin regions but also destination regions is preferable in order to consider geographical neighbors of flows in the GWR framework. In this study, we propose GWR for an unconstrained gravity model, in which a weighting kernel is a mixture of origin-based and destination-based based kernel. Weighting specification for the model is somewhat equivalent to that of geographically and temporally weighted regression, where a spatio-temporal kernel is constructed with a combination of spatial and temporal weight matrix; therefore the estimation procedure would be carried out in a standard manner of GWR. The estimation results using an interprefecture migration flow data of Japan is examined to see the effectiveness of our proposed method.

A Multi-scale Land Price Analysis Using an Eigenvector-based Approach

Daisuke Murakami[1]

[1]*Graduate School of Systems and Information Engineering, University of Tsukuba*

Urban structure is determined by many geographical phenomena including urban sprawl, conurbation, and gentrification of the urban core. Each of these phenomena can be prominent in certain spatial scales, and accordingly, the method that considers spatial scale would be helpful for urban geographical analysis.

This study analyzes spatial components in land prices, which seem to reflect urbanization well, by applying an eigenvector-based approach that captures multi-scale spatial structures. The target area is Tokyo and the period covered is 1995–2006. The analysis clarified the following geographical properties of land prices: (1) while global spatial components are substantial, medium- or local-scale components are also prominent in certain scales; (2) global components strengthen and local components weaken gradually; (3) some global high-rise areas in western Tokyo have been absorbed gradually into the global high-rise area that includes central Tokyo; (4) fine-scale components are remarkable in the area around Shinjuku; and (5) a toroidal low-rise area with its center in central Tokyo is confirmed in a medium-scale component. These results would be helpful in discussions on urban structures. Further, these results suggest the effectiveness of the eigenvector-based method for urban analysis.

An LP-based algorithm to test copositivity

Akihiro Tanaka, Akiko Yoshise

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A symmetric matrix is called copositive if it generates a quadratic form taking no negative values over the positive orthant, and the linear optimization problem over the set of copositive matrices is called the copositive programming problem.

Recently, many studies have been done on the copositive programming problem which (cf, Dur (2010)).

Among others, several blanch and bound type algorithms have been provided to test copositivity since it is known that the problem for deciding whether a given matrix is copositive is co-NP-complete (cf. Murty and Kabadi (1987)).

In this talk, we propose a new blanch and bound type algorithm for this testing problem.

Our algorithm is based on solving linear optimization problems over the nonnegative orthant, repeatedly.

Numerical experiments suggest that our algorithm is promising for determining upper bounds of the maximum clique problem.

Analysis on ambiguity aversion and gender of traders who lean toward an intermediated trade in search market

Takaaki Fujikura[1], Kazuhito Ogawa[2], Eizo Akiyama[3]

[1]*Graduate School of Systems and Information Engineering, University of Tsukuba*
[2]*Faculty of Sociology, Kansai University*
[3]*Faculty of Engineering, Information and Systems, University of Tsukuba*

Intermediaries emerge often times in a search market wherein traders (sellers and buyers) search for their trading partners, make a pair and negotiate in the price. Intermediaries have the right of pricing, buy goods at a bid price from a seller and sell them to a buyer at an ask price. Intermediaries make such bid and ask prices known to the public extensively. Since it is as such, while paying attention to the intermediaries' posted price, traders will be faced with the situation as to whether they should make deal with the intermediaries or choose to search for a trading partner and make a direct transaction with such partner, if any, without dealing with any of those intermediaries.

The purpose of the present study is to explore what kind of traders tend to choose intermediated trade in the aforementioned situation. In this research, we conducted a series of experiments wherein we assumed the situation in which traders can come to know benefits of direct transaction only through their experiences. Concurrently, we found that the traders with higher ambiguity aversion like an intermediated trade and that the female favors the intermediated trade more than the male.

Effects of the heterogeneity of link weights on the evolution of cooperation

Manabu Iwata[1], Yan Zexin[2], Hiroto Yonenoh[1], Eizo Akiyama[3]

[1]*Graduate School of Systems and Information Engineering, University of Tsukuba*
[2]*NTT DATA Corporation*
[3]*Faculty of Engineering, Information and Systems, University of Tsukuba*

Understanding the evolution of cooperation in natural and social systems has been the focus of a great deal of attention in various fields. One of the key rules for the evolution of cooperation is the network reciprocity, the mechanism that promotes cooperation when individuals of a population interact only with individuals in a subset of the population. There have been many researches which investigate the effect of the network reciprocity on the evolution of cooperation.

However, few of these studies have mainly considered the heterogeneity of the frequency of interaction between individuals, while in most real cases individuals have heterogeneous contact frequency.

We focus on the "link weight" between individuals (which represents their contact frequency), and explore how the "heterogeneity of link weights" affects the evolution of cooperation.

Simulation results showed that cooperation can be more promoted in the network with a fair level of heterogeneous link weights, than in the network with homogeneous ones. Also we find that the level of cooperation varies with the allocation pattern of link weights. We analyze these phenomena by the mechanism similar to so-called "multilevel selection": regarding the individuals connected by strong links as subgroups of a population, and the weak links between individuals as the connections of subgroups.

Selection of Opponents in the Prisoner's Dilemma in Dynamic Networks --- An Experimental Approach---

Hiroto Yonenoh[1], Eizo Akiyama[2]

[1]*Graduate School of Systems and Information Engineering, University of Tsukuba*
[2]*Faculty of Engineering, Information and Systems, University of Tsukuba*

Cooperation is ubiquitous among humans and many other species. However, standard evolutionary game theory predicts that all individuals eventually defect (betray) in Prisoner's Dilemma situation. As a mechanism to solve this issue, reciprocity in dynamic networks, where individuals choose whom to interact with, has attracted attention in recent years.

To investigate how a human subject selects her neighbors (opponents) to play the Prisoner's Dilemma within a social network, we conducted a human-subject experiment. The results are as follows: (1) A subject is more likely to dismiss the links to her neighbors most frequently when the subject chooses C and when the neighbor chooses D; (2) a subject who has more neighbors is less likely to dismiss links than a subject who has fewer neighbors; and (3) a subject is more likely to create links to (= select) opponents who have more neighbors than to opponents who have fewer neighbors.

Computational complexity of a solution for directed graph cooperative games

Ayumi Igarashi[1] and Yoshitsugu Yamamoto[2]

[1]*Graduate School of Systems and Information Engineering, University of Tsukuba*
[2]*Faculty of Engineering, Information and Systems, University of Tsukuba*

The central question of cooperative game theory is how to allocate the total benefit gained from cooperative behavior of players, and many allocation concepts have been proposed to date. Computational complexity is one of the most important criterion to judge whether a given solution concept is appropriate. An allocation concept is not applicable in real settings if it requires computation time proportional to exponential of the problem size.

We study cooperative games with directed graph structure from the viewpoint of computational complexity. Khmelnitskaya et al. introduced a cooperative game on a directed graph that furnishes the game with a communication structure. They proposed a single-valued solution concept, and named it the average covering tree value. To construct the average covering tree value, they introduced a so-called covering tree of a directed graph. The average covering tree value is defined as the average of marginal contribution vectors, each of which corresponds to a covering tree.

Concerning the computational complexity issue, we show that calculating the average covering tree value is a #P –complete problem. The proof uses a reduction from counting the number of all linear extensions of an arbitrary partial order, which has been shown by Brightwell et al. to be #P -complete. This result implies that an efficient algorithm to calculate the average covering tree value is unlikely to exist.

A cutting plane algorithm for modularity maximization with heuristics for separation problem

Yoichi Izunaga[1], Yoshitsugu Yamamoto[2]

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[2]*Faculty of Engineering Information and Systems, University of Tsukuba*

As social network services grow, clustering on graphs has been attracting more attention, and since Newman and Girvan proposed the modularity as a graph clustering measure, modularity maximization problem became one of the central subjects of research. The NP-hardness of the modularity maximization problem was shown by Brandes et al. We formulate the modularity maximization problem as a set partitioning problem. Since this formulation has to take into account all nonempty subsets of the node set, it has variables, where n is the number of nodes. We can hardly secure the computational resource to hold the problem when is large. We propose an algorithm for the problem based on the linear programming relaxation, and solve the dual of the linear programming relaxation by using a cutting plane method. The separation problem of determining a cutting plane to add ends up as a non-convex quadratic programming in binary variables, which is hard to solve exactly. Hence we propose to use a heuristic algorithm to solve the separation problem. To mediate the slow convergence that cutting plane methods usually suffer, we also propose a method for simultaneously adding multiple cutting planes which may complement well each other.

MEMO

Food Security and Human Health

Food Security and Human Health

Wednesday, October 2

Venue: Conference Room 406 (405)

Opening Remarks

10:00-10:10 Dr. Hiroshi Ezura — University of Tsukuba, Japan

Chair: Dr. Yooich Kainoh

10:10-10:40 "The Mini Zinc Finger protein MIF2 regulates COP9-signalosome activity. Can we use it for controlling cell proliferation?"
Dr. Michel Hernould — Bordeaux Segalen University / INRA, France

10:40-11:10 "*Rosmarinus officinalis* polyphenols produce anti-depressant like effect through monoaminergic and cholinergic functions modulation"
Dr. Hiroko Isoda — University of Tsukuba, Japan

11:10-11:20 **Coffee Break**

Chair: Dr. Kazuya Morikawa

11:20-11:50 "PCR-RFLP patterns of COI gene established for accurate and rapid identification of invasive tephritid fruit flies infesting sweet oranges in Nigeria"
Dr. DeMar Taylor — University of Tsukuba, Japan

11:50-12:20 "Maximizing plant biomass and secondary metabolite production efficiency in closed environment for food security and human health"
Dr. Nguyen Thi Quynh — Institute of Tropical Biology, Vietnam Academy of Science and Technology Hochiminh City, Vietnam

12:20-12:50 "Collecting, preserving and developping medicinal herbs for research and pharmaceutical production in Vietnam"
Dr. Duong Hoa Xo — Biotechnology Center of Ho Chi Minh City

12:50-14:30 **Lunch** 【13:30-14:20】 Millipore Luncheon Seminar

Chair: Dr. Chiaki Matsukura

14:30-15:00 "Controls of Embryo De-greening Through Mendel's I locus"
Dr. Frederic Delmas — Bordeaux Segalen University / INRA, France

15:00-15:30 "Deciphering the Metabolism of Ascorbate in Tomato: What Strategies for the Future?"
Dr. Pierre Baldet — Bordeaux Segalen University / INRA, France

15:30-16:00 "Miraculin tomato, a possible diet of human health"
Dr. Hiroshi Ezura — University of Tsukuba, Japan

Poster Session Venue: Conference Room 405

16:00-18:00

"Associative learning of color in host finding of Ascogaster reticulata Watanabe (Hymenoptera: Braconidae)"
Risa Kawamata — University of Tsukuba, Japan

"Development of a method to control *Varroa* mites with semiochemicals in honey bee larval diet"
Haruna Kazama — University of Tsukuba, Japan

"Effects of Host Plants of Herbivorous Insect Mythimna separata on its Larval Parasitoid Cotesia kariyai"
Kazumu Kuramitsu — University of Tsukuba, Japan

"Functional Analysis of Host Plant Volatiles in the Regulation of Ovipositional Behavior in the Yellow Peach Moth Conogethes punctiferalis"
Zhixin Luo — University of Tsukuba, Japan

"PCR-RFLP patterns of COI gene established for accurate and rapid identification of invasive tephritid fruit flies infesting sweet oranges in Nigeria."
Ikechukwu Eugene Onah — University of Tsukuba, Japan / University of Nigeria

Wednesday, October 2

Venue: Conference Room 406 (405)

Poster Session Venue: Conference Room 405

16:00-18:00	"Behavioral responses of the egg-larval parasitoid Ascogaster reticulata to tea leaves treated with the host's reproductive organs" Narisara Piyasaengthong University of Tsukuba, Japan
	"Biosynthetic Pathway for C15-dienal Sex Pheromone" Takuya Uehara University of Tsukuba, Japan / JSPS
	"Sex pheromone of Rehimena surusalis composed of Type I and Type II components" Ryokuhei Yamazaki University of Tsukuba, Japan
	"Effect of *Argania spinosa* press-cake on melanogenesis in B16 murine melanoma cells" Thouria Bourhim University of Tsukuba, Japan
	"Antidepressant-like effect of rosmarinic acid through MKP-1 down-regulation" Shinji Kondo University of Tsukuba, Japan
	"Cymbopogon shoenanthus ethanol extract has a melanogenesis-regulatory effect on human epidermal melanocytes" Sakura Eri B. Maezono University of Tsukuba, Japan
	"Molecular mechanism analysis of insulin resistance improvement effect of Cyanidin-3-glucoside in 3T3-L1 adipocyte" Toshiya Matsukawa University of Tsukuba, Japan
	"Inhibitory effect of olive oil-in-water emulsions from Montpellier in Southern France on β-hexosaminidase release and characterization of their physicochemical properties" Hideko Motojima University of Tsukuba, Japan
	"Downregulation of the *MITF* gene expression in human and murine melanoma cell lines: mechanism for the antimelanoma effect of daphnane diterpene hirsein B" Myra O. Villareal University of Tsukuba, Japan
	"Ameliorative effect of calcium channel blocker on the pathological conditions of mice with pregnancy-associated hypertension" Haojun Xu University of Tsukuba, Japan
	"Toward production of parthenocarpic GM tomato" Kentaro Ezura University of Tsukuba, Japan
	"The expression pattern of ADP-glucose pyrophosphorylase small subunit gene (AgpS1) in tomato (Solanum lycopersicum L.) plant" Yukihisa Goto University of Tsukuba, Japan
	"Phytohormone and its regulatory gene expression of tomato changed by domestication" S.Hao University of Tsukuba, Japan
	"Functional analysis of phosphoenolpyruvate carboxykinase (PEPCK) in tomato plant" Yongxing Huang University of Tsukuba, Japan
	"Toward establishment of quantitative method of tomato osmotin, a functional material for human health" Sayumi Komuro University of Tsukuba, Japan
	"Suppression of ADP-glucose Pyrophosphorylase Small Subunit Gene (***AgpS1***) Affects Sugar Accumulation in Tomato Fruit" Miki Sato University of Tsukuba, Japan
	"*SlGAD2* and *SlGAD3* are the key genes regulating GABA accumulation in tomato fruits" Mariko Takayama University of Tsukuba, Japan

The Mini Zinc Finger protein MIF2 regulates COP9-signalosome activity. Can we use it for controlling cell proliferation?

Julie Leblond-Castaing[a], Adrien Sicard[a], Frederic Delmas[a], Jérome Verdier[a], Frédéric Gévaudant[a], Christian Chevalier[b] and Michel Hernould[a]

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A multifunctional protein complex, called the COP9 signalosome (CSN), plays a conserved key role in the integration of endogenous and exogenous signals to regulate several developmental processes in Eukaryotes such as cell division, stress perception and response, etc. The 450 kDa CSN complex is composed of 8 subunits that are paralogous to each of the components of the 26S proteasome lid. Based on the homology between CSN and the 26S proteasome, it has been proposed that CSN is involved in the regulation of ubiquitin-dependent proteolytic processes. CSN interacts with SCF (Skp1–cullin1–F-box protein) -type E3 ubiquitin ligases (CRLs) that recognize protein substrates for subsequent degradation and recruit E2 ubiquitin-conjugating enzymes. The signaling pathways involving CSN have been extensively explored. However, the proper regulation of the COP9-signalosome remains to be elucidated. We demonstrate that the Mini Zinc Finger protein IMA/MIF2, which is involved in the regulation of multihormonal pathways during plant development, is able to interact with the subunit 5 (CSN5) of the CSN complex. CSN5 harbors the unique endoprotease activity of the CSN complex that is necessary for the regulation of the CRL activity. The characterization of the CSN5/MIF2 interaction demonstrates an adapter/cargo role for the MIF proteins which inhibit CSN activity through the nuclear externalization of the CSN5. Despite the key role of MIF2 in plant development and its impact in biomass production, we are also evaluating the potential role of the MIF2 protein to control the COP9-signalosome activity in animal cells for a medical use.

Rosmarinus officinalis polyphenols produce anti-depressant like effect through monoaminergic and cholinergic functions modulation

Hiroko ISODA

[1]*Graduate School of Life and Environmental Sciences, University of Tsukuba.*
[2]*Alliance for Research on North Africa (ARENA), University of Tsukuba.*

The Mediterranean diet is considered as one of the healthiest diets in the world. Such health benefits are attributed in part to the large usage of herbs and aromatic plants in the daily life cuisine. Beside their nutritional, flavoring and protective properties against microbial and oxidative degradation of foods, these herbs are believed to have several medicinal and pharmacological benefits. Among these culinary herbs, *Rosmarinus officinalis* (*R. officinalis*), a common spice used worldwide for culinary and medicinal purposes was demonstrated by several reports to exert numerous health benefits due to its various phytochemical constituents. *Rosmarinus officinalis* (*R. officinalis*), a culinary aromatic and medicinal plant, is very rich in polyphenols and flavonoids with high antioxidant properties. This plant was reported to exert multiple benefits for neuronal system and alleviate mood disorder. In our previous study, we demonstrated that *R.officinalis* and its active compounds, luteolin (Lut), carnosic acid (CA), and rosmarinic acid (RA), exhibited neurotrophic effects and improved cholinergic functions in PC12 cells in correlation with mitogen-activated protein kinase (MAPK), ERK1/2 signaling pathway. The current study was conducted to evaluate and understand the anti-depressant effect of *R. officinalis* using tail suspension test (TST) in ICR mice and PC12 cells as *in vitro* neuronal model. Proteomics analysis of PC12 cells treated with *R.officinalis* polyphenols (ROP) Lut, CA, and RA revealed a significant upregulation of tyrosine hydorxylase (TH) and pyruvate carboxylase (PC) two major genes involved in dopaminergic, serotenergic and GABAergic pathway regulations. Moreover, ROP were demonstrated to protect neuronal cells against corticosterone-induced toxicity. These results were concordant with decreasing immobility time in TST and regulation of several neurotransmitters (dopamine, norepinephrine, serotonin and acetylcholine) and gene expression in mice brain like TH, PC and MAPK phosphatase (MKP-1). To the best of our knowledge this is the first evidence to contribute to the understanding of molecular mechanism behind the anti-depressant effect of *R.officinalis* and its major active compounds.

PCR-RFLP patterns of COI gene established for accurate and rapid identification of invasive tephritid fruit flies infesting sweet oranges in Nigeria.

Ikechukwu Eugene Onah[1,2] and DeMar Taylor[3]

[1]*Graduate School of Life and Environmental Sciences, University of Tsukuba*
[2]*Department of Zoology and Environmental Biology, University of Nigeria*
[3]*Faculty of Life and Environmental Sciences, University of Tsukuba*

Horticulture plays a major role in the economy of many nations as a source of income, ensure food security, and create jobs. Citrus is the most widely grown fruit crop in Nigeria and tephritid fruit flies have been identified as the major constraint to citrus production. Many species of tephritid fruit flies that appear morphologically similar differ in their biological behavior and thus have quite different potential impacts on food production and implications for biosecurity and market access. Moreover, the immature life stages lack morphological diagnostic features and are the most likely life stages to be intercepted in food produce. These limitations have led taxonomists and quarantine officials to seek viable alternative ways of fruit fly identification including the use of molecular markers such as PCR-RFLP of COI gene. To this end, we extracted genomic DNA from citrus fruit flies in Nigeria, amplified the COI gene, identified and constructed for the first time RFLP patterns for accurate and rapid diagnosis of the different species. The identification was confirmed by sequencing COI gene and comparing with sequences from GenBank.

The invasive tephritid *Bactrocera invadens*, *Ceratitis anonae*, and a cryptic *Ceratitis* sp were identified as key sweet orange fruit flies in Nigeria.

Maximizing plant biomass and secondary metabolite production efficiency in closed environment for food security and human health

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Agricultural and food systems are positioned to continue playing an important role in solving today's problems in food security, human health, energy supply and community development. The United Nations estimates that there will be nine billion people to feed by 2050 while the earth is confronting with arable land and freshwater resource limitation. In order to meet the needs of burgeoning global population, novel agricultural practices as well as proper national policies are vital for food security purposes. Additionally, global consumers also want to have safe food products and good for their health. Urban agriculture, a viable and sustainable strategy, can help growers to produce high quality food plants but still maintain beauty, diversity, and safe environment long into the future. Urban agriculture, worldwide in large cities like Tokyo, includes open gardens, rooftop greenhouses, or fully enclosed growth rooms within buildings using artificial light sources. Until 2012, this indoor system, named "plant factory with artificial light" or PFAL, has been operated in more than 150 locations in Japan for year-round production of leaf vegetables, herbs, root crops, medicinal plants, or miniature ornamental plants of about 30 cm high or less. In PFAL system, with no soil debris and no insect, the labor cost for hygienic processing of fresh food is drastically reduced as PFAL products contain no pesticide residue. With stable nutrient composition, leaf vegetables produced in PFAL can be used as foods for babies, elderly and hospitalized people. Herbs and medicinal plants produced in PFAL will also be used to produce food and drink additives, traditional medicine, or cosmetics, etc.

For future approach of PFAL in Vietnam, we are now studying conditions for maximizing plant biomass and secondary metabolite accumulation of medicinal plants in closed (*in vitro*) environment. Results from these studies will attribute to PFAL application in Vietnam.

Collecting, preserving and developping medicinal herbs for research and pharmaceutical production in Vietnam

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Being a tropical country, Vietnam is abundant in resource of medicinal herbs. However, this resource is being rapidly decreased, many herbal species are facing the extinction. Therefore, the preservation and development of pharmaceutical herbs for research and production is highly important and necessary. In this report, the medicinal herbs from different regions in Vietnam have been collected, planted and valuated in greenhouse conditions. Some value species of medicinal herbs were propagated *in-vitro* using tissue culture technique. The technique of gene transfer mediated by *Agrobacterium rhizogenes* was initially applied on Ngoc Linh ginseng (*Panax vietnamensis* Ha et Grushv.), an endemic medicinal herb of Vietnam, in order to increase the yield of root biomass. We have collected 45 species of medicinal herbs and they have grown well in the greenhouse condition. In addition, 5 species among these herbs have been successfully cultured in *in-vitro* condition including red knotweed (*Polygonum multiflorum* Thunb.), Black turmeric (*Curcuma zedoaria* Rosc.), Ceylon Leadwort (*Plumbago zeylanica* L.) and Tuber stephaniae glabrae (*Stephania* spp.). Importantly, we have successfully established saponin-producing cell lines derived from roots of transgenic Ngoc Linh ginseng.

Keywords: *Panax vietnamensis*, *Polygonum multiflorum*, *Curcuma zedoaria*, *Plumbago zeylanica*, *Stephania*, *in-vitro* cultured medicinal herbs

Controls of Embryo De-greening Through Mendel's I locus

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Chlorophyll is the most abundant pigment on earth providing plants with the photosynthetic ability to sustain growth and productivity. Although retention of the green chlorophyll pigment is considered a desirable crop trait to reduce stress induced by disease and drought, presence of chlorophyll in mature seeds can be an undesirable trait that can affect seed maturation, seed oil quality and meal quality. Occurrence of mature green seeds in oil crops such as canola and soybean due to unfavorable weather conditions during seed maturity is known to cause severe losses in revenue. Various attempts to alleviate this green seed problem by targeting the known chlorophyll biosynthetic and degradation genes have proven unsuccessful. One recently identified candidate that controls the chlorophyll degradation machinery is the stay-green gene, *SGR1*, which was mapped to Mendel's *I* locus. Defect in SGR1 leads to leaf stay-green phenotypes in Arabidopsis and rice, although the role of SGR1 in seed de-greening and the signaling machinery that converges on SGR1 have remained elusive. To decipher the gene regulatory network that controls de-greening in Arabidopsis, we have used a mutant approach to demonstrate that embryo de-greening is achieved by the SGR family and that this whole process is regulated by a transcription factor known to be involved in seed development. This discovery has uncovered for the first time, a mechanistic role during seed de-greening and thus targeting of this pathway could provide a solution to the green seed problem in various oil seed crop species.

Deciphering the Metabolism of Ascorbate in Tomato: What Strategies for the Future?

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Ascorbic acid (AsA) is a major antioxidant in plants. While its metabolism is now well characterized, the regulation is still poorly understood. Fruits are the major source of vitamin C for Human. In the aim to decipher the AsA metabolism and its regulation in fruits, tomato was chosen as a model of fleshy fruits using several strategies. Among the key steps of the major AsA biosynthesis pathway described in Arabidopsis, known as the Wheeler & Smirnoff pathway, are the GDP-D-mannose epimerase (GME) and the GDP-L-galactose phosphorylase (GGP), both represented by two genes in tomato. We performed the characterization of these enzymes through reverse genetic approaches in tomato plants. (1) An RNAi strategy was selected for the *GME* genes. In addition to a reduction of AsA content, transgenic RNAi tomato lines exhibited growth phenotypes resulting from cell division and expansion defects, exacerbated fragility and loss of fruit firmness related to modifications of the cell wall structure and composition. These findings point out the intimate linkage of ascorbate and non-cellulosic cell wall biogenesis in plants. (2) A TILLING strategy was chosen for the *GME* and *GGP* genes using EMS tomato mutant collection created in the MicroTom genotype. Among mutants identified, two KO mutants were for the *GGP* genes but none for *GME*. In the homozygous GGP mutants, reduced AsA content as well as bleaching phenotypes induced after a short exposure to high light were observed. Such a response to high light was expected regarding to ascorbate function in plants. (3) To get insights the complex relationships between AsA biosynthesis/recycling and other metabolic pathways in the fruit a fruit system biology approach named VTC Fruit project was undertaken. Tomato plants obtained by RNAi-targeting several AsA-related genes were characterized at the transcriptomic, proteomic, and metabolomics levels. Pair-wise Pearson correlation analyses allowed identifying 15 candidate genes possibly involved in the regulation of the ascorbate pathway. (4) Recently, we started a forward genetic approach using the EMS collections to screen for plants producing fruits with high and low AsA level among 1000 families of tomato mutants.

Miraculin tomato, a possible diet of human health

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The utility of plants as biofactories has progressed in recent years. Some recombinant plant-derived pharmaceutical products have already reached the marketplace. However, with the exception of drugs and vaccines, a strong effort has not yet been made to bring recombinant products to market, as cost-effectiveness is critically important for commercialization. Sweet-tasting proteins and taste-modifying proteins have a great deal of potential in industry as substitutes for sugars and as artificial sweeteners. The taste-modifying protein, miraculin, functions to change the perception of a sour taste to a sweet one. Miraculin is a glycoprotein found in red berries known as miracle fruit (*Richadella dulcifica*; synonym *Synsepalum dulcificum*), produced by a tropical shrub native to West Africa. Miraculin itself is not sweet, but it has a taste modifying activity and is capable of converting sour taste to sweet taste. After chewing the red berry miracle fruit, lemons taste as sweet as oranges. The name 'miracle fruit' was derived from this unique and attractive property, and the isolated active substance was named miraculin (Kurihara and Beidler 1968). This taste-modifying function can potentially be used not only as a low-calorie sweetener but also as a new seasoning that could be the basis of a new dietary lifestyle. However, miraculin is far from inexpensive, and its potential as a marketable product has not yet been fully developed. For the last several years, biotechnological production of this taste-modifying protein has progressed extensively. We are succeeded in highly accumulating miraculin in transgenic tomato fruits, which accumulation level is reached to more than 10% of total soluble protein. We also developed a plant factory for effective production of the transgenic tomato. In this presentation, the characteristics of miraculin and recent advances in its production using transgenic plants are summarized, focusing on such topics as the suitability of plant species as expression hosts, the cultivation method for transgenic plants, the method of purifying miraculin and future advances required to achieve industrial use.

Associative learning of color in host finding of *Ascogaster reticulata* Watanabe (Hymenoptera: Braconidae)

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Insect parasitoids are used as natural enemies in biological control of insect pests that cause various damages to plants or crops. Parasitoids are said to use several cues (e.g. visual, chemical, tactile stimuli) for host searching. Many studies on the searching behavior of parasitoids have focused on chemical cues, but several studies have shown that parasitoids also use visual cues. *Ascogaster reticulata* is an egg-larval parasitoid of *Adoxophyes honmai* (Lepidoptera: Tortricidae), known as a pest of tea plants. We conducted several experiments on color learning in *A. reticulata*.

In this study, we tested whether females of *A. reticulata* can associate color cues with the presence of the host. The female wasp was trained to lay eggs on the host egg mass placed on a piece of paper (1x2 cm) of a certain color. After training, parasitoids were presented with two pieces of paper including the color used for training and new colors in a choice test without host egg masses. Results showed that *A. reticulata* can distinguish different color combinations and significantly chose blue (vs. green) and black (vs. green). This study also suggests that the learning ability of *A. reticulate* may differ with color combinations. Based on these results, *A. reticulata* appears to use color as a cue in some situations to increase host searching efficiency in nature.

Development of a method to control *Varroa* mites with semiochemicals in honey bee larval diet

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Varroa destructor (Acari: Varroidae) is well known as an ectoparasitic mite of the honey bee *Apis mellifera* L. and appears to be a crucial agent for the colony collapse disorder (CCD) of honey bees in the world. Some acaricides are used to control mites. However, because of resistance development and chemical contamination of honey products, alternative control strategies using natural products including semiochemicals have been underscored for integrated pest management. Mites prefer drone broods to queen broods and immature larvae, suggesting some chemical agents (repellents) that originate in non-hosts may contribute to host selection by mites. In this study, we focused on the repellent activity of lipids in honeybee jelly, glandular secretions of nurse bees that are fed to each caste of larvae in cells.

Behavioral responses of mites were investigated in worker bee larvae that were treated topically with jelly from different caste larval cells, lipid extracts and the main components of the extracts. Jelly, the extracts or authentic lipids at different concentrations were applied to mature worker larvae as a substrate, and then mites were placed individually on the substrates. Bee larvae treated with solvent only were also tested as controls. Mite behavioral responses were recorded for 5 min after treatments. Mite responses were assessed by the following four criteria, 1) duration of walking on substrates, 2) duration of arrestment on substrates, 3) duration until moving off substrates and 4) the number of mites that moved off the substrates.

Varroa mites were significantly repelled by royal jelly and its ether extracts in bioassays, while drone jelly showed no repellency against mites even at higher dosages. These results suggest that repellency resulted from chemical components contained exclusively or at higher concentrations in royal jelly. This was also supported by chemical analysis of the lipid components in the jelly, and bioassays with authentic compounds of the jelly lipids. All authentic chemical tested in this study never disturbed oviposition by queens and nursing by worker bees even when treated at 300 mg/1500 cells. Whether these repellents are effective for controlling mite behavior in hives will be confirmed by field tests.

Effects of Host Plants of Herbivorous Insect *Mythimna separata* on its Larval Parasitoid *Cotesia kariyai*

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Previous studies show that host plant species of herbivore affect the success rate of parasitism by its parasitoids. However, this phenomenon is known only in limited species, such as tiger moth, parsnip webworm. In this study, we demonstrated the effects of host plant species of rice armyworm *Mythimna separata* (Lepidoptera: Noctuidae) on the successful parasitism rate by the larval endoparasitoid *Cotesia kariyai* (Hymenoptera: Braconidae). The rice armyworm *Mythimna separata* is a pest of some poaceous plants. In this study, we used five plant species, i.e., corn, rice, barley, kidney beans and Japanese radish as food for armyworm. At the first, we gave five plant species for rice armyworm individually from 5th instar until pupation to examine effects of host plant species on the survival rate of rice armyworm. Then we recorded survival rate of each cases. Next, we gave five plant species for parasitized rice armyworm individually from 5th instar until pupation of host larvae to examine the effects of host plant species on the success rate of parasitism by *C. kariyai*. Then we recorded survival rate of both armyworm and *C. kariyai*. As the result, survival rate of rice armyworm was affected by its food plants (corn> rice >barley > kidney beans > J. radish). On the other hand, survival rate of parasitized rice armyworm with J. radish was higher than any other plant species. These results suggest that even if J. radish is not an optimal food for unparasitized, it protect the larvae from the risk of killing by parasitoids.

Functional Analysis of Host Plant Volatiles in the Regulation of Ovipositional Behavior in the Yellow Peach Moth *Conogethes punctiferalis*

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Host plant volatiles induce oviposition in the yellow peach moth Conogethes punctiferalis (Honda and Matsumoto 1984), but detailed functions of the volatiles in a series of oviposition behavioral responses are still unclear. The complete process leading to oviposition was explored in a wind tunnel and divided into five behavioral components; take off flight from release point, halfway flight, hovering close to source, landing and egg-laying. Subsequently, the effects of host plant odors on these behavioral components were analyzed by providing continuous or transient odor stimuli during each behavioral component. Plant odors accelerated the take off flight and increased orientation to the stimulus source in hovering and landing. These odors also stimulated females to lay more eggs resulting in a significantly longer time for egg-laying with no decrease in the time necessary to lay each egg. The absence of plant odors delayed take off flight and interrupted subsequent behavioral responses. These results indicate host plant stimuli induce orientation of flight and stimulate egg-laying, and may also independently regulate each component of the behavioral responses through the central nervous system for oviposition in the yellow peach moth.

PCR-RFLP patterns of COI gene established for accurate and rapid identification of invasive tephritid fruit flies infesting sweet oranges in Nigeria.

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Horticulture plays a major role in the economy of many nations as a source of income, ensure food security, and create jobs. Citrus is the most widely grown fruit crop in Nigeria and tephritid fruit flies have been identified as the major constraint to citrus production. Many species of tephritid fruit flies that appear morphologically similar differ in their biological behavior and thus have quite different potential impacts on food production and implications for biosecurity and market access. Moreover, the immature life stages lack morphological diagnostic features and are the most likely life stages to be intercepted in food produce. These limitations have led taxonomists and quarantine officials to seek viable alternative ways of fruit fly identification including the use of molecular markers such as PCR-RFLP of COI gene. To this end, we extracted genomic DNA from citrus fruit flies in Nigeria, amplified the COI gene, identified and constructed for the first time RFLP patterns for accurate and rapid diagnosis of the different species. The identification was confirmed by sequencing COI gene and comparing with sequences from GenBank.

The invasive tephritid *Bactrocera invadens*, *Ceratitis anonae*, and a cryptic *Ceratitis* sp were identified as key sweet orange fruit flies in Nigeria.

Behavioral responses of the egg-larval parasitoid *Ascogaster reticulata* to tea leaves treated with the host's reproductive organs

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Biological control, as one of the approaches to integrated pest management (IPM), has been proved to be an effective pest control measure to limit the growth of pest population. Parasitoids, commonly known as natural enemies of various pest insects, need to locate their hosts by using not only kairomones from their hosts, but also synomones released by herbivore-damaged plants. Induction of odors from plants after herbivore damage in tritrophic interactions may vary, depending on the plant and the herbivore species. Host searching behaviors of *Ascogaster reticulata*, a solitary egg-larval parasitoid of the smaller tea tortrix *Adoxophyes honmai,* can be stimulated by *A. honmai* egg mass deposition on the under surface of the tea leaves. This process does not result in any visually recognizable damage. Naïve female parasitoids also responded by an intensive zigzag searching to the tea leaf treated with reproductive organs of mated female host. Elicitors in the female reproductive organs of *A. honmai* could induce tea leaves to produce the cues that arrested parasitoids between 24 and 48 hr after the treatment on the leaf. The concentration of homogenized reproductive organ is important for the induction of tea leaves because the time spent by parasitoid searching on tea leaves treated with higher concentrations was significantly longer than the lower ones.

Biosynthetic Pathway for C15-dienal Sex Pheromone

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Many species of moths release species-specific sex pheromones to attract conspecific male moths. The pheromones with functional groups are most commonly made from even-carbon chain fatty acids such as C_{16} or C_{18} acids. However, we have found a novel sex pheromone component 9,11-pentadecadienal, which cannot be explained by known pathways. We here investigated the sex pheromone precursors of *Dolbina tancrei* and propose a possible pathway.

Common pheromone component in hawk moth such as 10,12-hexadecadienals are synthesized from C_{16} fatty acids through several modification steps. We postulated two pathways for the changes of *D. tancrei*; (1) C_{16} precursor conversion into C_{15} fatty acid, or (2) modification of the steps in biosynthesis of 10,12-hexadecadienal. To determine the pathways we analyzed fatty acids in the pheromone gland extracts. Glycerolipids and free fatty acids from the extracts were converted to the corresponding methyl esters and analyzed by GC-MS and DMDS or MTAD derivatization.

Although C_{15} fatty acids existed in the extracts, monoenyl C_{15} intermediates that can be pheromone precursors were not detected in the extracts. Moreover, the existence of 11-hexadecenoic and 10, 12-hexadecadienoic acids supports second postulated pathway. These results suggest that 9,11-pentadecadienal is biosynthesized from palmitic acid via 11-hexadecenoic and 10,12-hecadecadienoic acids rather than from C_{15} fatty acids.

Sex pheromone of *Rehimena surusalis* composed of Type I and Type II components

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As *Rehimena surusalis* is a pest insect of Malvaceae plants such as *Hibiscus syriacus*, identification of their sex pheromones is useful for developing pheromone lures for pest management. Based on laboratory assay results, Sumiuchi (2005) reported that pheromone components of *R. surusalis* are *E*10, *Z*12-16:Ald, *E*10, *Z*12-16:OH and *E*10, *Z*12-16:Ac, but these components never showed any attractive activity in field trials. In this study, we revised pheromone components and found a new formulation which showed remarkable activity in field assasys.

We identified three compounds as candidates of sex pheromone components of *R. surusalis*. From pheromone extracts of virgin females by using gas chromatography (GC) coupled with an electroantennographic detector (EAD) and a GC-mass spectrometry (GC-MS), *E*10, *Z*12-16:Ald, and *E*10, *Z*12-16:Ac were confirmed and furthermore, a new compound, Z3,Z6,Z9-23:HC was found as an active candidate of sex pheromone component. The natural ratio of these components in the extracts was 1:5:14, and male moths were, also, specifically attracted to a ternary blend of *E*10,*Z*12-16:Ald, *E*10,*Z*12-16:Ac, *Z*3,*Z*6,*Z*9-23:HC at a this ratio in field tests, whereas single and binary blend of either compound had a weak attractancy.

Analogous blends of polyunsaturated, long-chain hydrocarbons (Type II) with much shorter chain aldehydes or alcohols (Type I) recently have been discovered in some species in Crambidae and Pyralidae. These hybrid blend of components from two distinct chemical structure may be widespread model in Crambidae and Pyralidae.

Effect of *Argania spinosa* press-cake on melanogenesis in B16 murine melanoma cells

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Melanin plays an important role in preventing ultraviolet-light induced skin damage. However, excessive biosynthesis of melanin causes aesthetic problem and various related pigment disorders. *Argania spinosa* is an endemic Moroccan plant commonly used in folk medicine and several biological effects of this plant have been reported. Many studies were interested on argan oil, while few ones have been dealing with the by-product of this plant. In this work, we evaluate the effect of argan press-cake, the main by-product of argan oil extraction, on melanogenesis in B16 murine melanoma cells. Cells treated with argan press-cake ethanol extract showed a significant inhibition of melanin biosynthesis in a time-dependent manner without cytotoxicity. To determine whether the inhibitory activity of argan press-cake was related to the expression levels of melanogenesis related proteins, including tyrosinase, tyrosinase related protein 1 and the dopachrome tautomerase, we used western blot assay. Our results showed that argan press-cake extract down-regulated the expressions of these proteins below the control level in cells. These results indicate that argan press-cake may be effective in topical application for treating hyperpigmentation disorders.

Antidepressant-like effect of rosmarinic acid through MKP-1 down-regulation

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Depression is caused by corticosterone (CORT) hypersecretion. For treatment, antidepressants have been generally-prescribed to increase neurotransmitters in synaptic cleft. However, the antidepressants also have anticholine effect as side effect. Meanwhile, several polyphenols are known to increase neurotransmitters and diminish the side effect. A previous study have shown that the polyphenols rosmarinic acid (RA)promotes ERK1/2 phosphorylation which is relevant to BDNF transcription, and tyrosine hydroxylase (TH) and pyruvate carboxylase (PC) expressions relevant to producing neurotransmitter and enhancing energy metabolism, respectively, in PC12 cells. In addition, it was recently reportedthatMKP-1 works as a negative regulator of ERK1/2 phosphorylation and causes depressive behavior. In the present study, we investigated the antidepressant-like effect of RA through MKP-1 down-regulation and its mechanism was analyzed *in vitro* and *in vivo*. As a result, RA increased the viability of PC12 cell seven after treatment with CORT. *In vivo*, oral administration of RA to mice decreased the immobility time of mice in tail suspension test (TST) which is related to depressive behavior. After TST, serum CORT levels in RA-administrated mice decreased and the dopamine and noradrenaline in the brain increased, but not adrenaline. Furthermore, the mRNA expression of MKP-1 was down-regulated, and while BDNF, TH and PC were up-regulated. In conclusion, RA suppresses the depressive behavior related to the following effects. RA does not only increase the neurotransmitters concentration but also increase the cell viability by decreasing CORT, up-regulating the BDNF through the MKP-1 down-regulation, and promote neuronal cell proliferation by enhancing energy metabolism.

Cymbopogon shoenanthus ethanol extract has a melanogenesis-regulatory effect on human epidermal melanocytes

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The melanin in the skin renders the perfect protection against UV-induced photo damage due to its antioxidant and radical scavenging properties in addition to its optical and chemical filtering properties. A normal amount of melanin in the skin is important as abnormally high or low levels of melanin could be a sign of pigmentary diseases. In this study, we evaluated the effect of *Cymbopogon shoenanthus* (CS) on melanin biosynthesis. Human epidermal melanocytes (HEM) were treated with 1/1000 v/v of CS ethanol extract and subjected to melanogenesis assay to determine its effect on melanin biosynthesis. Proteins and RNAs extracted from CS-treated HEM were subjected to western blotting and real-time PCR to quantify the expressions of the enzymes that mediate melanin biosynthesis (tyrosinase, tyrosinase-related protein 1, and dopachrome tautomerase) as well as the expressions of MAPKs ERK1/2 and p38 which are known to positively regulate melanogenesis. Results showed that CS had significant stimulatory effect on melanin biosynthesis in HEM. The melanogenesis-regulatory effect was due to the effect of CS on melanogenic enzymes' expressions and MAPKs activation in HEM. CS ethanol extract can therefore be used as a possible treatment for hypopigmentation but further studies will be performed to determine the effect of using combinations of bioactive compounds in the extract.

Molecular mechanism analysis of insulin resistance improvement effect of Cyanidin-3-glucoside in 3T3-L1 adipocyte

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Insulin resistance (IR) is a physiological condition in which cells fail to respond to the normal actions of insulin resulting to a decrease in glucose uptake in adipocyte and skeletal muscle. IR is the main cause of Type 2 diabetes mellitus (T2DM). The improvement of IR is therefore necessary for the improvement and prevention of T2DM. Recently, some polyphenols derived from plants have improvement effect on insulin resistance. Cyanidin-3-glucoside (Cy3G) is a typical anthocyanin contain of blueberry, red wine, black soybean and so on. Black soybean extract (contains Cy3G as its main compound) has IR improvement effect on diabetes model mouse. In this research, we focused on the analysis of IR improvement effect of Cy3G *in vitro*. 3T3-L1 preadipocyte cell line is frequently used to study differentiation and function of adipocyte. Cy3G treated 3T3-L1 preadipocytes differentiated into small adipocytes. In addition, the adipocytokine production level indicated an increased adiponectin and decreased tumor necrosis factor α (TNF-α) and reactive oxygen species (ROS) production. Moreover, Cy3G treatment increased glucose transporter4 (GLUT4) mRNA level and glucose uptake. Furthermore, phosphorylation of Insulin Receptor and Akt were increased. These results indicate that Cy3G decreases intracellular production of TNF-α and ROS by inducing preadipocytes to differentiate into small adipocytes and increase glucose uptake through the activation of the insulin signaling pathway.

Inhibitory effect of olive oil-in-water emulsions from Montpellier in Southern France on β-hexosaminidase release and characterization of their physicochemical properties

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Olive oil polyphenols are natural antioxidants and anti-inflammatory compounds present in extra virgin olive oil. In this study, the inhibitory effect of *Picholine* olive oil from Montpellier in Southern France on the chemical mediator, β-hexosaminidase, release in type I allergy, using rat basophilic leukemia (RBL-2H3) cells, was investigated. Phenolic compounds present in *Picholine* and *Lucques* olive oil were measured using HPLC. Oil-in-water (O/W) emulsions were prepared separately from two different types of olive oil, and their physical properties, such as droplet size, size distribution, and surface tension were indicated. Inhibitory effect of β-hexosaminidase release from RBL-2H3 cells of *Picholine* and *Lucques* olive O/W emulsion was assayed, and the effect of *Picholine* olive O/W emulsion on genes expressions in RBL-2H3 cells was investigated using DNA microarray. Our findings indicated that *Picholine* olive oil had higher flavonoids content, especially apigenin content, compared with *Lucques* olive oil. The prepared emulsion of *Picholine* olive oil resulted in a considerable small size distribution with average droplet size of 170 nm compared to Lucques olive oil O/W emulsion. The *Picholine* olive oil O/W emulsions showed higher inhibitory effect on the β-hexosaminidase release than that of *Lucques* olive oil. Additionally, *Picholine* olive O/W emulsion decreased the expressions of genes related to type I allergy such as interleukins and transcription factors in RBL-2H3 cells.

Downregulation of the *MITF* gene expression in human and murine melanoma cell lines: mechanism for the antimelanoma effect of daphnane diterpene hirsein B

Myra O. Villareal[1], Junkyu Han [1,2], and Hiroko Isoda[2]

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Melanoma is one of the main causes of death in patients with skin cancer. As the incidence of melanoma worldwide continue to increase, treatment beyond the usual radiation therapy and chemotherapy are being considered. Microphthalmia-associated transcription factor (MITF) is important in melanocyte development, function, and survival. However, it is also an amplified oncogene and the reduction of MITF activity has been suggested to sensitize melanoma cells to chemotherapeutic agents. Hirsein B (HB) can downregulate the expression of *Mitf* in B16 cells and in this study, its effect on MITF expression and in aggressive melanoma cells is determined. *MITF* mRNA was quantified using real-time PCR and ERK1/2 activation in HB-treated human melanoma cells SK-MEL-5 and SK-MEL-28. Furthermore, the effect of HB on MITF activation was determined by western blotting. Cell cycle assay was performed to determine any effect on the cycling of the cells. Results show that HB significantly decreased *MITF* mRNA levels in SK-MEL-5 cells but not in SK-MEL-28 cells. However, although HB failed to downregulate the MITF gene expression in SK-MEL-28, it increased activated ERK2 levels indicating an MITF protein degradation. Murine and melanoma cells exhibit differences in functions such as cell proliferation, cell cycle, among others. Results of cell cycle analysis showed that HB has no significant effect on the cell cycle in both human and murine cells lines. The results of this study highlights the potential of HB as a potential therapeutic agent that can target MITF thereby sensitizing melanoma cells to chemotherapeutic agents against melanoma.

Ameliorative effect of calcium channel blockeron the pathological conditions of mice with pregnancy-associated hypertension

Haojun Xu[1, 2], Junji Ishida[2], Tomohiro Ishimaru[2], Altansarnai Baasanjav[2], Misuzu Hashimoto[2], Kazuyuki Noguchi[2, 3], and Akiyoshi Fukamizu[1, 2]

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Pregnancy-induced hypertension (PIH) is characterized by elevated blood pressure in late pregnancy with significant amount of protein in the urine. PIHis estimated to affect 5% of all pregnancies in Japan. Recently, it is thought that angiotensin II, a potent vasoconstrictorproduced by the renin-angiotensin system (RAS), plays an important role in progression ofPIH whilethe inhibition of RAS during pregnancy is contraindicationbecause of the risk of adverse effects on fetal development.

Previously, we have generated pregnancy-associated hypertensive (PAH)mice, which exhibit high blood pressure during late pregnancy byoverproduction of angiotensin II in maternal circulation. PAH mice also show maternal organ damages such as, proteinuria,cardiac hypertrophy, morphological changes in placenta,and fetal intrauterine growth retardation (IUGR), which is similar to the symptom in PIH patients.In this study, to evaluate the effect of a L-type specific calcium channel blocker on maternal organ abnormalities and fetal growth,we administrated amlodipineto PAH mice from 13 days of gestation (E13), the day before the blood pressure start to elevate,to E19, the day before parturition. Amlodipine significantly lowered the blood pressure and urinary protein level of PAH mice in late pregnancy with improvement of cardiac failure,placental abnormalities and fetal IUGR. These results suggesteda variety of ameliorative effect of calcium channel blocker on the progressive pathology of pregnancy-associated hypertension in mice.

Toward production of parthenocarpic GM tomato

Kentaro Ezura[1], Ji-Seong Kim[2], Tohru Ariizumi[2], Hiroshi Ezura[2]

[1]*Graduate school of Life and Environmental Sciences, University of Tsukuba*
[2]*Faculty of Life and Environmental Sciences, University of Tsukuba*

Tomato is widely produced in the world, and its production is the largest among all vegetables, although it is easily suffering from harsh environmental conditions.Parthenocarpy is the fruit formation in the absence of pollination and/or fertilization and a desirable trait for fruit-bearing crops. Several genetic-engineered parthenocarpic lines are produced by manipulating levels of phytohormones. However, these induced parthenocarpic tomato still have undesirable pleiotropic traits. Controllable parthenocarpic fruit development without undesirable side effects would be of great value. This research aims to create genetically modified parthenocarpic tomatoes by expressing parthenocarpic genes under a control of pistil or early fruit specific promoter to induce efficient parthenocarpy but without side effects.

In the present study, to isolate pistil-specific genes in tomato, we performed RNA-Seq based transcriptome analysis using 25 samples,identifying genes specifically expressed within the pistils. In this analysis, 1714 genes were initially identified as pistil-specific genes.80 were chosen for the RT-PCR analysis, revealing that 14 genes were highly specifically expressed only in the pistil. 4 genes were further selected as candidate and the chimeric constructs inducing parthenocarpy are under construction using promoters of these four pistil specific genes. Our research will be benefit for food production under harsh environmental conditions,while contribute for human health.

The expression pattern of ADP-glucose pyrophosphorylase small subunit gene (*AgpS1*) in tomato (*Solanum lycopersicum* L.) plant

Yukihisa Goto, Satoko Nonaka, Hiroshi Ezura, Chiaki Matsukura

Graduate School of Life and Environment Sciences, University of Tsukuba

ADP-glucose pyrophosphorylase (AGPase) is a key regulatory enzyme in starch biosynthesis in plant. In this research, 2,885bp of the predicted promoter sequence for the *AgpS1* gene encoding the AGPase small subunit was isolated from tomato. Sequence analyses revealed a number of known *cis-elements* related to responses to salt and dehydration stress and sugar repression. The spatial expression pattern and tissue/organ specificity of *AgpS1* were analyzed in during development using promoter-GUS transgenic tomato plants. Based on GUS staining, the obtained sequence directed broad expression in both sink and source tissues/organs. In source leaf and early developing fruit, GUS staining was observed in all tissues, except for epidermal tissue. In contrast, GUS staining tended to be confined to vascular tissues in seedling, stem, fruit stalk and ripening fruit. In particular, a patchy staining pattern was observed in the phloem of the stem and fruit stalk, suggesting that *AgpS1* is expressed in the phloem companion cells in those organs. These results also suggest that AGPase mainly functions in the vascular tissue of those organs. In this study, to explore a factor regulating *AgpS1* gene in immature-green fruit, we also analyzed the effect of various fruit components such proline, GABA, polyamine and ions. Quantitative RT-PCR analyses revealed that the *AgpS1* transcription was strongly induced by proline treatment. The gene also slightly responded to GABA but not to polyamines. Those results indicated proline has an important role on regulation of *AgpS1* expression under the salt stress condition.

Phytohormone and its regulatory gene expression of tomato changed by domestication

S.Hao[1], T.Ariizumi[1], H.Hirakawa[2], K.Shirasawa[2], M.Kojoma[3], H.Sakakibara[3] and H.Ezura[1]

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Tomato is an economically important vegetable crop that contains many cultivars result from history of domestication and is now cultivated worldwide. Tomato cultivated varieties (*Solanum lycopersicum*)are different from its wild relatives in many reproductive and vegetative characters such as color and shape of fruits, although the mechanism for the domestication discriminating the cultivated varieties and the wild relatives has been poorly understood. In order to shed light on this mechanism, we compared morphology, levels of phytohormones and expression of genes associated with hormone metabolism and signaling between them. This research used 3 cultivated tomatoes, Micro-Tom, Ailsa Craig and M82, and 3 wild relatives, *Solanum pimpinellifolium*, *S. peruvianum* and *S. pennellii*, all of these are known to ancestors of cultivated tomatoes. Three cultivate varieties and *S. pimpinellifolium* bear red ripe fruits whereas the others bear green ripe fruits. Since fruit size of *S. lycopersicum* is in general larger than wild relatives, we expect hormone levels differ between them. Phytohormone analysis indicated that salicylic acid (SA) and jasmonic acid (JA) are higher in green fruits of the cultivate varieties and wild relatives than those in ripe red fruits, suggesting that defense mechanism might be active in wild relatives. In contrast, cytokinin (CK) content was higher in ripe red fruits of cultivated species than those in green fruits. There was no clear correlation between the fruit size and gibberellin (GA) and auxin (AUX), both of which are known to be important for cell expansion. Our results suggested that the fruit size is not under direct control of absolute levels of GA and AUX.

Functional analysis of phosphoenolpyruvate carboxykinase (PEPCK) in tomato plant

Yongxing Huang, Satoko Nonaka, Hiroshi Ezura, Chiaki Matsukura

Graduate School of Life and Environmental Sciences, University of Tsukuba

Phosphoenolpyruvate carboxykinase (PEPCK) is a key rate-limiting enzyme regulating metabolism pathway of gluconeogenesis for sugar synthesis. However, its physiological role still remained to be elucidated. To clarify the physiological function of PEPCK and the contribution of gluconeogenesis on sugar accumulation in tomato, RNAi transgenic lines were generated with suppressed *PEPCK* expression driven by CaMV 35S constitutive promoter and ripening fruit-specific E8 promoter (designed as *35Spro::PEPCK*^{RNAi} and *E8pro::PEPCK*^{RNAi} , respectively). Detail phenotypic characterization of the RNAi plants revealed the constitutive suppression of *PEPCK* caused growth suppression especially in germination stage. In contrast to wild type, significant suppression of seedling length was observed in the *35Spro::PEPCK*^{RNAi} lines for 10 days after sowing (DAS) seedlings. This growth suppression was maintained by 30DAS with the suppression by 23-30% in the RNAi lines compared to the wild-type plants. Those results indicated the gluconeogenesis pathway was a main sugar resource for seedling growth in tomato. Soluble sugar contents in red-ripe fruit tended to decrease whereas malate content tended to increase in the RNAi lines than wild-type. In the fruit at 42 days after flowering (DAF) of the *35Spro::PEPCK*^{RNAi} T_2 lines, the sugar contents remarkably decreased by 65% and 54% in fructose and glucose, and 72% reduction in sucrose. Total sugar accumulation was 59-46% lower in the RNAi lines than that of the wild type fruit. In the *E8pro::PEPCK*^{RNAi} T_2 lines, malate content in the fruit at 42 DAF was 12-33% higher in the RNAi lines than wild-type fruit. Those results indicates gluconeogenesis from organic acids to sugars occurs in ripening tomato fruit and it is inhibited by the suppression of *PEPCK* expression, suggesting that PEPCK plays a regulatory role in sugar-acid ratio in tomato fruit.

Toward establishment of quantitative method of tomato osmotin, a functional material for human health

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Osmotin belongs to the PR5 family proteins with a molecular mass of 26-kDa. Osmotin, is an adiponection-like protein, and is considered to be a functional material for promoting human health. In previous studies, osmotin has shown to be induced in response to both biotic and abiotic stresses in plants. Although there are several reports on osmotin genes in plants, the quantitative method of osmotin protein is still not established. Tomato is an excellent food staff and must contain osmotin protein. However, the quantitative method of tomato osmotin is not established. In this research, we firstly isolated candidate genes coding osmotin in tomato by bioinformatics approach, and analyzed the functions using transgenic tomato plants. Two out of 19 candidate genes were highly expressed during tomato fruit development. Transgenic tomato plants expressing the gene showed increased tolerance to salt, indicating the gene encodes osmotin protein in tomato. Subsequently we have generated antibody against the gene product, and confirmed the specificity to product of osmotin gene. Now we are trying to establish quantitative method of tomato osmotin using the antibody, and are going to present a current progress of this study.

Suppression of ADP-glucose Pyrophosphorylase Small Subunit Gene (*AgpS1*) Affects Sugar Accumulation in Tomato Fruit

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[1]*Graduate School of Life and Environmental Sciences, University of Tsukuba*
[2]*Institut national de la recherche agronomique (INRA), Bordeaux, France*

Sweetness is an important facor for the good taste of tomato fruits. Our early works reported i) starch accumulation in early developing fruit is important for the sugar content in red-ripe fruit, and ii) ADP-glucose pyrophosphorylase (AGPase), a key enzyme for starch biosynthesis in plant, is involved in the starch accmulation in tomato fruit. However, the physiological function of AGPase and its encoding genes have not been well investigated in tomato to date. With this aim, in the present study, we generated RNAi transgenic tomato lines with suppressed expression of the *AgpS1* gene, which encodes the AGPase small subunit, and investigated metabolic alterations in developing fruits focusing on sugar, sugar phosphate, and organic acids. Metabolic characterization revealed total sugar content and glucose-1-phosphate in the starch-deficient transgenic fruits decreased by 30% and 20% compared to the wild-type fruit in red-ripe stage. Additionally, fruit malate content increased by OO% and XX% in the RNAi lines compared to the wild-type fruit at immature-green and ripening stages, when the respiratory activity increases. Those results indicated i) contribution of starch accumulated in early developing fruit is about 30% in red-ripe fruit, ii) glucan phosphorylase is involved in starch degradation process in the fruit during ripening stages. The increase of malate content in the transgenic fruit suggested malate plays a complementary role in respiratory-active organs instead of starch.

SlGAD2 and *SlGAD3* are the key genes regulating GABA accumulation in tomato fruits

Mariko Takayama[1], Satoshi Koike[1], Miyako Kusano[2], Ryo Nakabayashi[2], Atsushi Fukushima[2], Kazuki Saito[2, 3], Chiaki Matsukura[1], Hiroshi Ezura[1]

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γ-aminobutyric acid (GABA) is a ubiquitous non-protein amino acid that is well known to be an inhibitory neurotransmitter in vertebrates. In human body, it is effective on lowing blood pressure and relieving stress. Therefore, GABA has received attention as a health-promoting functional compound, and several GABA-enriched food such as chocolate have been commercialized so far. In plant, tomato accumulates relatively high level of GABA during fruit development. However, the molecular mechanism of GABA accumulation and its physiological role in tomato fruits is still unclear. In some species, glutamate decarboxylase (GAD) is reported to be the key enzyme for GABA biosynthesis. In our previous studies, we identified three *GAD* genes (*SlGAD1*, *SlGAD2*, and *SlGAD3*) from tomato fruits and found out that the expression pattern of *SlGAD2* and *SlGAD3* were in good agreement with the change of GABA content during fruit development. In this study, to reveal whether these *SlGAD* genes involve in GABA biosynthesis actually, we have generated transgenic plants with RNAi constructs targeting to reduce the expression of each *SlGAD* gene. In the result, the GABA content in fruits of *RNAi-GAD2* and *RNAi-GAD3* lines were significantly lower than that of WT while no prominent change was observed in that of *RNAi-GAD1*. These results indicate that *SlGAD2* and *SlGAD3* expressions are important for GABA biosynthesis in tomato fruits.

MOMO

MOMO

Technology, Anthropology, Umwelt

Technology, Anthropology, Umwelt

Thursday, October 3 — **Venue: Conference Room 303**

1. Conference Introduction		
10:00-10:10	Dr. Yasushi Uchiyamada	University of Tsukuba, Japan
2. Networks, Agents and Potentialities		
10:10-12:00	"Fuzzy Bounds: Ethnography at the Limits of Social Metaphor" Dr. Paul Hansen	University of Tsukuba, Japan
	"Fermentation Makes Foods and Relationships: Laotian Sociality in a Village in Cambodia" Dr. Sumiko Yamazaki	University of Tsukuba, Japan
	Discussant 1: Dr. Gergely Mohacsi	Osaka University, Japan
12:00-13:00	**- Lunch -**	
3. Living with 3.11 / the Unexpected		
13:00-15:00	"A Changing Landscape after the Tsunami: Vision, Risk, and Infrastructure" Dr. Shuhei Kimura	University of Tsukuba, Japan
	"Mapping probability, reality, and risk for policy goals and public information" Dr. David S. Sprague	National Institute for Agro-Environmental Sciences, Japan
	Discussant 2: Dr. Takashi Osugi	Hitotsubashi University, Japan
	Discussant 3: Dr. Xiaogang Sun	University of Tsukuba, Japan
15:00-15:20	**Coffee Break**	
4. Our Lifeworld beyond Umwelt		
15:20-17:30	"Rethinking Umwelt and Individuation in the wake of 3/11" Dr. Yasushi Uchiyamada	University of Tsukuba, Japan
	"Can the oyster point? The indexical lives of animals in ecosystem science and politics" Dr. Valerie Olson	University of California, Irvine, USA
	Discussant 4: Dr. Toshihiro Kameda	University of Tsukuba, Japan
	Discussant 5: Dr. Koji Hirose	University of Tsukuba, Japan
5. Open Discussion: Problems and Methodologies		
17:30-18:00	All Participants	

Fuzzy Bounds: Ethnography at the Limits of Social Metaphor

Paul Hansen

University of Tsukuba, Assistant Professor of Anthropology

My research focuses on animal-human-technology linkages; industrial dairy farms in Hokkaido, canine companions in Osaka, with comparative research beginning in Jamaica in the winter of 2014. Working on topics where the object of study routinely transgresses what are assumed to be the normative physical and affective boundaries of non-human and human, the concepts of network, agent, and potentiality have proven to be both essential and problematic. This is especially the case when one conducts research that is largely rooted in anthropological ethnography; a mode of inquiry dependent not only upon the *observation* of lives, but on long-term *participation* in and with those lives. In short, and like lab or medical work and in contrast with the lion's share of other social science methodologies, observing ought to be accompanied by *doing* for anthropological ethnographers.

Anthropologists exist in *umwelten* that are often contradictory. On the one hand I am an embodied field researcher exploring how animal-human-technology is experienced by self and others, on the other hand I am an educator and writer expected to communicate these findings, often to other social scientists or neophyte researchers, in largely shared if occasionally opaque *lingua franca*. In this space of betwixt and betweenness there are limits as to how deeply one can commit to shared modes of discourse. Building off of what Bruno Latour has coined the "default social" in social science practices, the critical work of anthropologists Tim Ingold and Paul Rabinow, and utilizing my own fieldwork experiences, this paper argues for the greater explanatory potential of conceptualizing networks as meshwork and the need to refer to agents or "actants" not by abstract categories but accounting for specific and historically situated and developed capacities and potentials.

Fermentation Makes Foods and Relationships: Laotian Sociality in a Village in Cambodia

Sumiko Yamazaki

Faculty of Humanities and Social Sciences, University of Tsukuba

In this paper I utilize ethnographic cases to describe how Laotian fermented foods and social relationships are made. I also evaluate the similar process and structure by which these delicious fermented foods and good relationships are created and sustained.

First I outline the process whereby plural agents communicate in making a particular fermented food. Laotian people, a minority group in Cambodia, are good at preparing a fermented food called *padeek*. *Padeek* is basically made from freshwater fishes, salt, and rice bran. The ingredients are put in a crock for fermenting. Laotian people are proud of the good taste of their *padeek*'s and describe it as "right delicious" and "raw-smelling but good smelling". Good tasting and good smelling *padeek* is a compound of plural agents such as a crock, salt, rice bran, fermentation bacteria, maggots and time. The communication of the agents gradually leads to fermentation. The people can follow the tracks of satisfactory fermentation by noticing some indexes of the agents' transformation such as appearance of maggots, a rise in the water level and of course the smell. This ready-made fermented food is eaten by the people and if some amount of it is leftover at the end of the year then it will be added with new ingredients next year. Thus, *padeek* is continuously changed, yet it leaves open the past path of communication.

How to make good human relationships in this society resembles how to make delicious fermented foods. The agents for fermentation are relevant to both. Theoretically speaking, the creative activities of plural agents through time make delicious fermented foods and good human relationships. This process does not occur if we premise making on the autonomy of individual components; in other words, fermented foods cannot be made from the combination of the ingredients alone. Just as good relationships cannot be made only from combination of a person and person, Delicious or good compounds are made through agents communicating with other agents over time.

A Changing Landscape after the Tsunami: Vision, Risk, and Infrastructure

Shuhei Kimura

University of Tsukuba

The "unexpected" tsunami on March 11, 2011 overwhelmed Japan. In the aftermath of the disaster, many people said that Human cannot match Nature. Although saying this may be rooted in emotive overtones, it is indisputable that the tsunami urged us to reconsider the arrangement of humans and nonhumans which was seen to make the event "unexpected." The focus of this paper will be on the landscape of a coastal town where the "reconstruction" of this infrastructural arrangement is in progress at multiple levels.

The devastation publically exposed the formation and the insufficiency of the existing disaster preparedness system; an assemblage of constructions, the communication system, discipline, and others. Thus, along with the rehabilitation of local community life, Japan's government promoted a comprehensive reassessment of disaster risk and sought to restructure of this system. New hazard maps representing "maximum hazard" potential were published as a result of the review. In parallel with the revision, the so-called "multi-layered protection system against tsunamis" was launched in the devastated areas. In this framework, higher seawalls will be constructed, new architectural zoning will be implemented, and information about disaster risks will be distributed.

While a long-term vision is changing the landscape on a large scale, another slow, back-and-forth alteration is under way. As a bitter lesson from past tsunamis, when local fishermen made enough money they relocated their houses to higher ground. After the devastation of March 11, they placed a set of wooden stakes to mark the border line of the area inundated by the sea, thinking that when the stakes decay, their children would rebuild them, and by rebuilding them, they would remember the disaster. In this way, their life-sized memories and visions are accumulated and embedded in the landscape.

In the course of "reconstruction," multiple artifacts like seawalls, houses, and monuments are added to the landscape. The conjunctions of the artifacts serve as infrastructures of the visions of the landscape with different scales and temporalities. My aim is to explore ways to mediate 'vision' through examining the infrastructures.

Mapping probability, reality, and risk for policy goals and public information

David S. Sprague

Ecosystem Informatics Division, National Institute for Agro-Environmental Sciences, Tsukuba

Maps are essential for assessing risk because hazards vary across space. In the broadest sense, almost any map can be interpreted as a type of hazard map. Weather maps show rain, topographic maps show cliffs, and even tourist guide maps in effect show uninteresting places to avoid. Deliberately designed hazard maps show clear dangers, such as flood, landslide, crime, traffic accidents, and pollution, among many undesirable possibilities. After the Great East Japan Earthquake of March 2011, both experts and the public have become acutely aware of maps depicting earthquakes, tsunamis, and radiation. Many of these maps are intended to show some type of determinative reality, that may be the existing present-day reality, the past reality of historical events, or the unfolding reality of ongoing events. Predictive maps envision a future reality, but given the uncertainties of the future, often depict probabilities, such as the estimated chance of an event extending over a region at a future time, or the chance of an individual experiencing an event at a particular location over a set time period. However, the efficacy of hazard maps is itself difficult to predict. Maps are made from a bewildering variety of data, based on differing methodologies by experts of many scientific fields, sometimes for multiple government agencies each attempting to fulfill a distinct policy goal. Furthermore, upon seeing a map, the audience freely interprets it to fit their own needs, fears, and lives, in ways both intended and unintended by map makers. Thus, map makers must nurture a fine-tuned sense for the ever-changing needs of the public, as well as those of each policy goal, in addition to analytic geographic expertise. Map makers should remember that hazard maps are not merely informational but force viewers to face difficult choices that affect the very foundations of their lives.

Rethinking Umwelt and Individuation in the wake of 3/11

Yasushi Uchiyamada

Faculty of Humanities and Social Sciences, University of Tsukuba

I was made to rethink the relevance (and irrelevance) of Jakob von Uexküll's Umwelt in the wake of 3/11. Various events with different tempo-topographical order/scale/extension (e.g., earthquake, tsunami, subsidence, unrolling Fukushima Daiich nuclear disaster, human tribulations, revisions and re-revisions of nuclear policy), triggered off by the gigantic seismic tremor measuring 9 on the Richter scale, point to deficiencies in the ways in which we make sense of, and simultaneously act upon, and hence constitute, human-environment relations, or more precisely, human habitat reinforced with foolproof protective measures composed of latest technologies, folkways, artificial objects, productive activities, geological forces, climatic factors, and various other agents (including humans and nonhumans), some of which are visible and tangible, while the greater part of it remains invisible and intangible.

Umwelt is relevant. Every animal, be it a tick, a jackdaw, a girl, takes only a limited qualities of objects in its environment as the carriers of a perception mark and effect mark. Each animal, including place-bound humans, lives in an impoverished Umwelt, or a bubble, as a system of signs and actions. An animal is at home in its Umwelt, which is a world of a Kantian subject. In Kesenuma, I met a high school teacher who saved the lives of students with his timely reading of perception marks and taking quick actions (i.e. running to the hills) in the tsunami-prone environment. As a native of a fishing village, he distrusted the "unrealistic" safety instructions, and intuitively followed the familiar perceptive/effective signs in his environment. But Umwelt does not urge a Kantian subject to leave its bubble. The world becomes unconnected with surroundings when perception marks start sending non- corresponding information. The Fukushima Daiichi nuclear disaster is a case in point.

On August 8th, 2013, we were belatedly informed that 300 tons of radioactive water had been leaking daily to the Pacific Ocean for more than 2 years (*Le Monde édition abonnés* broke the news one day earlier). Our environment is no longer the same, nor is organism. Time's arrow! Umwelt presupposes *a priori* existence of organisms and their environments. It is a harmonious world of interactions of existing individuals. It does not have a space for ontogenesis. Let me introduce Gilbert Simondon's concepts of individuation and metastability: individuals continuously emerge out of individuation, which is relation itself. The seemingly functional circle of Umwelt is grounded on precarious metastability that is the state of individuating individuals. The knowledge about individuation too individuates in the process of individuation. This is a rough outline of what I would discuss on October 3rd.

Can the oyster point?
The indexical lives of animals in ecosystem science and politics

Valerie Olson

University of California, Irvine

How do new forms of human/animal relations illuminate where and why "ecosystem" wins out over "environment" and "ecology" as a large-scale scientific and social organizing concept? This paper examines emerging activities in the post-BP oil disaster Gulf of Mexico to provide one perspective on that question. It traces how the aligned activities of scientists, policy-makers, community members, and non-human entities constitute a contemporary Gulf ecosystem that incorporates formerly separate natural, economic, and political systems. New ways to model, quantify, and politicize relations between oysters and humans provide an example of these emerging processes and their effects in re-defining what it means to be a living part of the Gulf of Mexico ecosystem. As flows of post-disaster research and restoration funds create new relations among far flung Gulf of Mexico social worlds, the role of Gulf mollusks in those activities make visible powerful and scalable formalizations of ecosystemic intersubjectivities and ontological intimacies. The Gulf oyster has gone from having two loosely linked identities as a scientific subject and economic object to a new life as a fully integrated "ecosystem being." For people monitoring them from Florida, US to Campeche, Mexico, oyster life signs and activities indicate ecosystem health for human and non-human beings, its water filtration organs index ecological and economic value systems, and its shells and reefs are shaping ecological engineering projects and politics. The new indexical lives of Gulf oysters are connected to multiple visions of "ecosystem," in particular those of Jacob Uexküll, Alfred Tansley, and Howard Odum in his Gulf-based work. As a result, this case also shows how the ecosystem concept being mobilized as a social organizing concept may not be scientifically precise or orthodox. Instead, it evidences a politically useful discursive flexibility by incorporating mechanistic, organismic, deterministic, and stochastic models of spatial wholeness that allow, among other things, Gulf water and oil/gas systems to be understood as functionally and "naturally" interconnected.

MEMO

Developing a future-oriented global doctor/researcher

Developing a future-oriented global doctor/researcher

Thursday, October 3 — **Venue: Conference Room 101**

Session Ⅰ: Robotic technology in medicine Chair: Dr. Hiroyuki Nishiyama	
13:00-13:35	Dr. Yoshiyuki Sankai University of Tsukuba, Japan
13:35-14:00	"Surgical “Robotics” The University of California, Irvine Experience 2002-2013 " Dr. Thomas E. Ahlering University of California, Irvine, USA
14:00-14:20	- Discussion -
14:20-14:40	**Coffee Break**
Session Ⅱ: Topics in regeneration medicine Chair: Dr. Osamu Ohneda	
14:40-15:15	"Brain Repair" Dr. Steven Cramer University of California, Irvine, USA
15:15-15:35	**Coffee Break**
Session Ⅲ: Clinical/basic Research in University of Tsukuba / University of California, Irvine Chair: Dr. Akira Matsumura	
15:35-15:50	"Integration of Translation, Innovation, and Education" Dr. Masataka Sakane University of Tsukuba, Japan
15:50-16:10	"Expanding the Funding and Collaboration Space of the Future Researcher" Dr. Jacob Levin University of California, Irvine, USA
16:10-16:30	**Coffee Break**
Session Ⅳ: What to do for international communication of medical students Chair: Dr. Masayuki Masu	
16:30-16:40	”That’s one small step for mankind, one giant leap for a man” Dr. Masato Sugano University of Tsukuba, Japan
16:40-16:50	"My Experience at the University of Irvine" Dr. Chiho Tokunaga University of Tsukuba, Japan
16:50-17:10	"English Education for Undergraduate Medical Students" Dr. Makoto Tanaka University of Tsukuba, Japan
17:10-17:40	"CLINICAL RESEARCH IN THE MEDICAL SCHOOL CURRICULUM" Dr. Thomas Cesario University California, Irvine, USA

Yoshiyuki Sankai

University of Tsukuba,
Professor, Director of Center for Cybernics Research
CEO, Cyberdyne. Inc.

Cybernics is a new domain of interdisciplinary academic field of human-assistive technology to enhance, strengthen, and support human's cognitive and physical functions, which challenges to integrate and harmonize humans and robots (RT: robotics technology) with the basis of information technology (IT) in a functional, organic, and social manner. We aim to develop the frontier science Cybernics, which is centered on cybernetics, mechatronics, and informatics, and it challenges to integrate neuroscience, robotics, systems engineering, information technology, "kansei" engineering, ergonomics, physiology, social science, law, ethics, management. The goal of the Program represents a Grand Challenge that makes breakthroughs in the innovative creation and fusion of forefront researches based on information science. A pioneering achievement of Cybernics is Robot Suit HAL (Hybrid Assistive Limbs) developed by Yoshiyuki Sankai. HAL is the world's first cyborg type robot that improves, support, and enhances the physical motion of human bodies by detecting the weak bioelectrical signal through the body from the brain which generates the nerve signal to control the musculoskeletal system. In this talk, I will deliver the outline Cybernics approach based on our experienced and introduce the work performed in Cybernics. And, I may present what we have done related in this fields.

Surgical "Robotics"
The University of California, Irvine Experience
2002-2013

Dr. Thomas E. Ahlering[1] and Dr. Ralph V. Clayman[2]

[1]Professor and Vice Chair, Department of Urology, University of California, Irvine
[2]Professor of Urology and Dean, School of Medicine University of California, Irvine

Dr. Ralph V. Clayman is world renown for developing and introducing laparoscopy to Urology. In 1991, Dr. Clayman performed the first human laparoscopic nephrectomy. After 5 years of work in the lab working on a porcine model, he developed the tools and skills to take the immense leap of removing a gall bladder to removing a kidney laparoscopically. As a visionary Dr. Clayman introduced remarkable advancements including laparoscopic partial nephrectomy, donor nephrectomy, total nephrouretectomy and radical prostatectomy. However, he recognized the limitations of laparoscopic **radical prostatectomy** due to difficult dissection and extensive suturing both of which limit the application of laparoscopy.

In 2002, Drs. Clayman and Ahlering were introduced to the da Vinci robot recognizing the potential of this remarkable new device that with laparoscopic instruments a surgeon could operate with many important advantages over both open and laparoscopic techniques. This new robot offered 3D visualization with up to 12x magnification, intuitive surgical manipulation and increased degrees of freedom with dissection, cutting and suturing. On June 18th, 2002 they performed the first robot assisted laparoscopic radical prostatectomy (RARP) at university in the western United States.

Since then UC Irvine under the surgical guidance of Dr. Ahlering has introduced many key technique changes and improvements that have reduced complications and improved patient outcomes. These improvements include specific techniques to dramatically reduce the technical challenges of the urethro-vesical anastomosis, techniques to reduce positive surgical margins at the apex, a better understanding of thermal and traction injury to the neurovascular bundle (NVB) and introduction of the concept of hypothermia to reduce inflammation based injury to the urinary sphincters and NVB.

Brain Repair

Steven C. Cramer, MD

University of California, Irvine USA
October 3, 2013

An emerging area of clinical neurotherapeutics pertains to restorative therapies, or brain repair. Much of the research to date has focused on conditions marked by neural injury such as stroke, where increasing evidence suggests that these therapies have the potential to favorably modify long-term outcomes. In contrast to neuroprotective therapies, which aim to modify the extent of acute injury, restorative therapies begin when injury is fixed, and aim to promote neural repair. Many classes of restorative therapy are under study, some in human clinical trials, and some still mainly investigated at the preclinical stage. Examples include growth factors, small molecules, stem cells, brain stimulation, and robotic devices. Effects of restorative therapies are optimized when these are prescribed in accordance with key principles of neural repair, such as patient stratification, temporal change in biological target, the value of a neural systems approach, and the need for experience-dependent experience. This rich area of translational neuroscience investigation provides an outstanding opportunity for the training of tomorrow's physician scientists.

Integration of Translation, Innovation, and Education

Masataka Sakane[1], Koichi Hashimoto[1,2]

[1]Critical-path Research and Education Integrated Leading Center, University of Tsukuba
[2]Course of clinical research and regional innovation donated by the Japan Agriculture Ibaraki Public Welfare Federation

To Establish a new system of research and education based on close collaboration with medical schools and the University of Tsukuba Hospital, Critical-path Research and Education Integrated Leading Center, so called the CREIL center was organized in 2006.

Our missions are following,

1. Platform for effective CPR.
2. Offering a wide range of educational courses in medical graduate schools for CPR.
3. Emphasizing clinical research and the use of novel biomaterials, and new diagnostic and therapeutic devices/materials.
4. Installation of a medical technology training
5. Reinforcement of an executive system to facilitate the realization of objectives by continuous innovation, such as the establishment of new institutes using donated funds.

In Lecture, we introduce the present status and future direction of the CREIL center from the point view of Ttanslation, Innovation, and Education.

Expanding the Funding and Collaboration Space of the Future Researcher

Jacob E. Levin, PhD

Office of Research, University of California, Irvine

Researchers worldwide are facing mounting challenges in their efforts to support their work, and many are ill-prepared to confront those challenges. In the United States, funding streams are flat or shrinking, and becoming ever more competitive. Agencies are placing additional administrative, compliance and reporting requirements on grantees and prospective applicants, and their priorities are rapidly evolving, increasingly towards large-scale, multi-investigator, interdisciplinary efforts. For the researcher of the future to remain competitive for research funding, they must master a range of skills not part of traditional scientific education, and must be able to build partnerships, and become fluent in scientific disciplines outside of their training and experience. Identifying these required skills and incorporating them into researcher training, or creating an institutional support structure that provides them, is crucial to the prosperity of any modern research university.

The field of research development - individuals at Universities and research institutions who facilitate research program strategy, team building and in particular efforts to attract extramural funding - has been expanding vigorously in recent years, as evidenced by the growth of the National Organization of Research Development Professionals (NORDP – www.nordp.org), which only 3 years after its founding has over 500 members at nearly 300 institutions in 9 countries. Research development staff assist and train faculty and researchers in the grant-writing process, and help them navigate the shifting interests and requirements of funding agencies. They build partnerships: on campus, between institutions, and internationally, and help open new avenues of research support and collaboration. The perspective and experience of a research development office can inform and guide the growth of the successful future researcher, arming them with the tools they need to thrive in the world's frontier research milieu.

“なんでもないようなこと”
That’s one small step for mankind, one giant leap for a man

Sugano Masato M.D.

University of Tsukuba
Department of Pathology

“It was already a year ago, I joined 100days of clinical clerkship in University of California, Irvine, and somehow returned to Tsukuba”

This is how my report(or essay) of this clinical clerkship in UCI Begins and it was published on the University of Tsukuba Alumni Communication in the millennium year. Of course, over all was about what I have learned in UCI, but the medicine/medical-education that I saw and experienced was not the subject, and nor the subject of this review. One reason that I am not to mention this matter, i.e., the difference in medicine/medical-education of USA and Japan maybe that 10-years is a time long enough to change the scene, but the biggest reason that I don`t(or more correctly, “cannot”) argue this matter is because even after 14year-career as a physician, including the years I spent as a basic medical researcher, I am not quite sure what actually “Japanese” medicine/medical education means. So this time again, I want to talk about just what I felt in UCI during the rotation in 1999 and see what this experience can do to a man.

My Experience at the University of Irvine.

Chiho Tokunaga

Department of cardiovascular surgery, University of Tsukuba

I had joined an elective program to do the clinical clearkship at the university of Irvine medical center when I was at 6th grade of medical school at 1996.

I decided to join this program because I would like to clarify my curiosity about the medical practices in USA and wanted to see the difference between Japan and USA.

Also I expected to see more advanced medical skills and knowledge. I was very happy when I selected to participate in this program.

I spend 4 weeks at OBGYN department, 2weeks each at pediatric surgery, surgical ICU and CCU. Almost all the doctors were so nice to me. Since I was interested in surgery, pediatric surgery was the most impressive rotation. There are a female attending, and she allowed me to scrub in many cases and sometime let me suture the skin. She encouraged me a lot that I have a good hand as a surgeon. Those words helped me to decide choosing career as a surgeon later.

Surgical intensive care rotation was also very interesting. I tried to make a presentation about my patients at morning round, write admission note and everyday charts. At SICU, I practiced with medical students at UCI and they helped me a lot to have done such a heavy task for me. It was fun working with medical student in same generation. I was impressed so much that they work so hard to learn new things to become a better doctor.

Practicing at UCI medical center was a great learning opportunity for me. I greatly enjoyed working with a wonderful doctors and students.

Altogether, I had a thoroughly enjoyable experience at Irvine. I am very happy to hear that we will have an exchange program with UCI again. I hope that lot of medical students have an opportunity to learn in abroad and get a global prospect in their future.

English Education for Undergraduate Medical Students

Makoto Tanaka

Professor and Chairman, Department of Anesthesiology
Vice Dean, School of Medicine
University of Tsukuba

Text: The University of Tsukuba was reborn in 1973 as a comprehensive university based on new educational concepts in the heart of Tsukuba Science City. Since then it came to be a center of cutting-edge research and education, human development, collaboration among industry, government and academia, and needless to say, an international center of intellectual activity. It has promoted constant reforms in order to create a flexible education that would meet the needs of the next generation. Our university also intends to contribute to the international society by demonstrating leadership as an innovative and unique education. English education in the school of medicine is no exception.

In this talk, I would like to introduce some of the unique features of our English education for undergraduate medical students. In addition, achievement of our international student exchange program/overseas clinical clerkship program is presented.

CLINICAL RESEARCH IN THE MEDICAL SCHOOL CURRICULUM

Thomas C Cesario MD

University California, Irvine

The medical student of today is being taught only a tiny fraction of what he or she will be required to know and use during the course of their medical career. Even though this was true for the doctors trained in years past, the power of the computer and the pace of modern medical research have made my last statement a reality . How then are we to prepare these students not only to acquire this new knowledge but also to determine which of this information is valuable and which is useful to them. The current medical school course is already crowded with necessary information and it is difficult to add additional course work. Hence it is unlikely committees charged with approving courses would approve another course Further, while students often get a passing familiarity with some of the tools necessary to evaluate new information from research they are not required to continually use this during their clinical years, thus these tools are often forgotten. Because of the need, which I believe will only increase in the future, to understand research articles, I think it is necessary for students in each of their clinical courses, including those in both medical and surgical specialties, to be asked to read and interpret articles in current literature and to present their evaluation of this work. That should include strengths and weaknesses of these reports, potential applications and possible future studies. It is my hope that students required to do this will develop a habit of routinely evaluating , in their own minds, the new information which they will encounter. Hopefully by doing so, they will avoid the pit falls of ordering unnecessary tests, procedures or treatments that may be both costly and harmful to their patients.

MEMO

MEMO

Public Health / Nursing
"Global Challenges in Public Health & Nursing"

Public Health / Nursing
"Global Challenges in Public Health & Nursing"

Thursday, October 3	**Venue: Main Convention Hall / Conference Room 406 (405)**	
Keynote Lecture Chair: Dr. Naomi Wakasugi	**Main Convention Hall**	
8:30-9:30	"Can we reach the end of AIDS?" Dr. Francois Dabis	Université Bordeaux Segalen, France
-13:00	**- Lunch -**	
Session Ⅰ Chair: Dr. Masao Ichikawa	**Conference Room 406 (405)**	
13:00-13:30	"The effect of meteorological factors on fetal growth" Dr. Yukiko Wagatsuma	University of Tsukuba, Japan
13:30-14:00	"Oral Health among Pregnant Women in the Attapeu Province.,Lao PDR" Dr. Sysavanh Phommachanh	University of Health Sciences, Lao PDR
14:00-14:30	"The effect of weather on cardio vascular disease hospital admissions among elderly people in Thai Nguyen Province, Vietnam" Dr. Van Dung Do	The University of Medicine and Pharmacy at Ho Chi Minh city, Vietnam
14:30-14:45	**Coffee Break**	
Session Ⅱ: Chair: Dr. Yukiko Wagatsuma	**Conference Room 406 (405)**	
14:45-15:15	"Dengue in Vientiane and Luang Prabang: Two Community Surveys on Knowledge, Attitude and Practice on Dengue Prevention" Dr. Mayfong Mayxay	University of Health Sciences, Lao PDR
15:15-15:45	”Building a Successful Comprehensive Global Health Program – A Different Way to Make a Difference” Dr. Scott Benson	University of Utah, USA
15:45-16:15	"Population aging in Asia and sharing the experience of Japan -through the example of JICA project in Thai" Dr. Nanako Tamiya	University of Tsukuba, Japan
16:15-16:30	**Coffee Break**	
Session Ⅲ: Chair: Dr. Nanako Tamiya	**Conference Room 406 (405)**	
16:30-17:00	Dr. Reiko Hayashi	National Institute of Population and Social Security Research
17:00-18:20	G30 Student Seminar on Global Aging	

Friday, October 4	**Conference Room 406 (405)**	
Student Presentations		
10:00-10:15	"A Study on the Relation of Sociodemographics and Social Support with Depression among Informal Carers in the Republic of Chile, from a Nationwide Survey" Felipe Sandoval	University of Tsukuba, Japan
10:15-10:30	"Cross-Sectional Study of Subjective Symptoms in Women within One Year after childbirth" Kyoko Tokoro	University of Tsukuba, Japan

Friday, October 4	Conference Room 406 (405)
Student Presentations	
10:30-10:45	”How Hospitalization in the Forensic Psychiatric Ward Affect Attitude toward Medicine and Self-Efficacy” Hiromi Sugawara University of Tsukuba, Japan
10:45-11:00	”Sexual and Reproductive Issues in the Brazilian Community in Japan: An Analysis of Adolescents in the Brazilian Schools” Milleanni Dominguez University of Tsukuba, Japan
11:00-11:15	”Validity and Reliability of the Japanese Version of the Alcohol and Alcohol Problems Perception Questionnaire” Daisuke Fukuta University of Tsukuba, Japan
11:15-11:30	"Community engaged global health through academic partnerships among six countries" Grant Sunada University of Utah, USA
11:30-12:30	**- Lunch -**
Session Ⅳ Chair: Dr. Keiko Sugimoto	
12:30-13:00	"Strategies for Quality Nursing Doctoral Education" Dr. Mi Ja Kim University of Illinois at Chicago, USA
13:00-13:30	"An evaluation study of doctoral nursing programs in Japan" Dr. Misuzu F. Gregg Kobe City College of Nursing, Japan
13:30-15:00	**Coffee Break**
Session Ⅴ Chair: Dr. Keiko Sugimoto	
15:00-15:30	"Creating the Ferrans and Powers Quality of Life Index" Dr. Carol E. Ferrans University of Illinois at Chicago, USA
15:30-16:00	"The origins of oncology nursing studies and the application of their findings to the intrinsic quality of Japanese cancer patients" Dr. Michiyo Mizuno University of Tsukuba, Japan
16:00-16:30	"The support system to children receiving a comprehensive anticancer therapy utilizing a proton beam tratment" Dr. Kayuri Furuya University of Tsukuba, Japan
16:30-17:00	"Perceptions and needs of childhood cancer patients, mothers, and staffs for their long-term treatment and school re-entry in Japan" Dr. Rie Wakimizu University of Tsukuba, Japan

Can we reach the end of AIDS?

Francois Dabis

Bordeaux School of Public Health (ISPED) and INSERM U897,
Université Bordeaux Segalen, France

HIV has been identified 30 years ago. The pandemic has reached all parts of the world since then, although with different modalities and epidemiologic characteristics, leading to the "know your epidemic" concept.

The science of prevention has gone through many advances in the past 20 years, with clear successes constituting today a high-quality panel of biomedical interventions. This includes HIV counseling and testing (C&T), ARV-based PMTCT, medical male circumcision (MMC), ART for treatment and prevention of serodiscordant couples as well as vaginal microbicides and pre-exposure prophylaxis.

The use of ARV combinations for treatment of adults and children has become a universal standard of care in the past 10 years with a progressive enlargement of its indications, exemplified by the WHO 2013 recommendations.

The national and international responses have not generally followed the science at the right speed. The resources allocated have constantly remained below the forecasted needs. The uptake of treatment and PMTCT services and to a lesser extent of MMC is now high in the most affected populations throughout the world. It is only recently that both HIV incidence and AIDS mortality have started to decline globally according to UN statistics.

Two approaches have helped shaping the global response. First, the formulation of unified public health guidelines for treatment and prevention, using more and more the principles and findings of systematic review and meta-analyses that are regularly updated. Second, mathematical modeling has been heavily developed. These tools can assist policy makers in projecting the response to the many interventions validated or in development. Treatment as prevention (TasP) is still an intervention in debate with ongoing modeling and field research at population level.

Ending the epidemic requires consensus on the targets to be achieved. The currently available interventions offer clear opportunities to achieve sizeable changes in the face of the HIV/AIDS pandemic within 20 years. Ongoing research will provide soon the missing links that could speed up and ease this global response to reach an AIDS-free generation.

The effect of meteorological factors on fetal growth

Yukiko Wagatsuma[1], Miki Kagami[1], Enbo Ma[1]

[1]*Department of Clinical Trial and Clinical Epidemiology, University of Tsukuba*

Climate change is one of the defining challenges of the century and increasingly recognized as a public health policy. Previous studies reported the relationship between the increased risk of infections and climate related disasters. Many countries have a high burden of climate-sensitive diseases, but public health capability to respond is not always optimal. Major diseases that are sensitive to climate change often become serious among vulnerable population. Household food security and maternal malnutrition are known to be linked with child mortality and growth. Low birth weight (LBW, <2500g) is a major determinant of mortality, morbidity and disability in neonates, infancy and childhood and has long term impact on health outcomes in adult life. The prevalence of LBW is estimated to be 16% worldwide with a range of 3-40% and occurs mostly in developing countries. The incidence of LBW in Bangladesh, predominantly the result of intrauterine growth restriction, is one of the highest in the world. Seasonal fluctuation of birth weight has been observed. Few studies reported the relationship between fetal growth and ambient temperature during pregnancy. However, there is no consensus regarding which meteorological element has an effect on fetal growth, thus the size at birth. Furthermore, it has not been clear which timing of such insults during the pregnancy, if exist, is critical for intrauterine fetal growth restriction. This study aimed to examine whether meteorological elements have an influence upon intrauterine fetal growth restriction in Bangladesh.

This study found a significant effect of temperature on fetal size. The regression analysis showed if temperature increased at the 1st and the 2nd trimesters, birth length was shorter. For birth weight, this study found as temperature increased at 26-30 weeks of gestation, birth weight decreased. Further studies are required to confirm the relationship.

Oral Health among Pregnant Women in the Attapeu Province.,Lao PDR

Sysavanh Phommachanh[1], Borisouth Phommakhoth[2], Vansy Vilayvone[1], Mayfong Mayxay[1]

[1]Faculty of Postgraduate Studies, University of Health Sciences
[2]Department of Provincial Health Office, Attapeu Provincial Hospital

Oral disease is a major problem affecting people's health in Lao PDR. Pregnant women are more at risk of being oral disease than other group. Very few studies have been conducted on women's oral health, especially women in the rural area of Laos. The aim of this study is to explore the prevalence and associated factors with oral disease among pregnant women. An analytical cross-sectional study was designed-using questionnaire form with oral health inspection among pregnant women in the community from July to September 2012.

Average age of the study population was 24 years old (range: 16 to 42 years). Most of participants were housewives/famers (78.8%), low education (62.4%), and 52.0% of them were Lao-lum. 28.2 % of pregnant women had at least one oral disease. Occupation, ethnic, and religion were significantly associated with oral disease during pregnancy (OR=2.1 95% CI= 1.4-3.2, P-Value= 0.000; OR= 2.2, 95% CI= 1.5-3.2, P-Value= 0.000; OR=2.1, 95% CI= 1.4-3.1, P-Value= 0.000). Pregnant women who had high income was more likely to protect themselves from oral disease compared to those had less income (OR= 0.6, 95% CI= 0.2-0.4, P-value < 0.001; OR= 0.6, 95% CI= 0.4-0.8, P-value=0.001. Antenatal care visit was a protective factor against oral disease during pregnancy (OR=0.7, 95% CI=0.5-0.9P-Value= 0.005); either past or current smokers was 2 times more likely to have oral disease when compared to those who have never smoked (OR=2.1, 95% CI= 1.4-3.1, P-value <0.001).

Oral health problem is common among pregnant women in Attatpeu Province, which were more likely to occur with pregnant women in the second trimester than the first and the third trimesters. Smoking behavior in women should be paid more attention. Promoting ANC visit at least one time could prevent the oral disease.

Keywod: Oral diease, risk factors, pregnancy

The effect of weather on cardio-vascular disease hospital admissions among elderly people in Thai Nguyen Province, Vietnam

Ngan Giang Pham[1], Bao Giang Kim[2], Van Dung Do[3]

[1]*Department of Science and Training, Ministry of Health*
[2]*Institute of Preventive Medicine and Public Health, Ha-noi Medical University*
[3]*Department of Medical statistics, The University of Medicine and Pharmacy at Ho Chi Minh city*

Background

Climate change has been discussed in many scientific papers for its potential severe effects on human life. For example, unusual weather variability as expected worsened by climate change, have severe direct consequences on people's health such as increasing the mortality and frequency of diseases. Among those, cardiovascular diseases are highly prevalent among elderly.

Objectives

This study aimed to find out the relationship between weather variables such as number of hours of bright sunshine, humidity, temperature, evaporation, rainfall and CVD hospital admissions among the elderly population in Thai Nguyen province, a northern province of Vietnam.

Methods

Retrospective data of CVD cases were obtained from data base of 4 hospitals in Thai Nguyen province for a period of 5 years from 2008 to 2012. Number of CVD hospital admissions in each day were counted and merged with daily weather data of this period. The generalized additive model was used to estimate the effect and lag structure of weather variables on the number of CVD hospital admissions, adjusted to time trends with regression splines, day of the week and public holiday. To account for possible delayed effects, we examined the impact of weather up to 7 days before each admission.

Results

Temperature, evaporation, humidity (on the same day) are associated with increasing number of CVD. The number of hour of bright sunshine (on the same day and the two days before admission) with decreasing the number of CVD cases. The daily variability of temperature, evaporation, humidity and sunshine link with 9%, 3%, 3% and 3% of number of CVD cases, respectively.

Conclusion

Weather factors, especially high temperature, contribute significantly to the number of CVD hospital admissions among elderly.

Dengue in Vientiane and Luang Prabang: Two Community Surveys on Knowledge, Attitude and Practice on Dengue Prevention

Mayfong Mayxay[1], Phonesavanh Philasouk[1], Wanyuan Cui[2], Sounthone Thammavong[3], Khamphong Khensakhou[5], Viengnakhone Vongxay[1], Latdaphone Inthasoum[1], Vanphanom Sychareun[1] and Gregory Armstrong[2]

[1]Faculty of Postgraduate Studies, University of Health Sciences, Vientiane, Lao PDR
[2]Nossal Institute for Global Health, The University of Melbourne, Melbourne, Australia
[3]Pak-Ngum District Health Office, Vientiane Capital, Lao PDR

Background: Dengue remains an important cause of morbidity in Laos. Good knowledge, attitudes and practices (KAP) among the public regarding dengue prevention are required for the success of disease control. Little is known about dengue KAP among the Lao general population.

Objective: To assess the KAP of Lao villagers on dengue prevention in two different geographical areas of Laos (Vientiane–the central and Luang Prabang-the northern parts of Laos).

Methods: Two studies were conducted in two different geographical areas of Laos. In Vientiane, the study was conducted in a peri-urban Pak-Ngum district and in Luang Prabang this was carried out in the municipality of Luang Prabang town. Two-stage cluster sampling method was used to select a sample of participants to represent the general community in each study site. Participants were surveyed using an interviewer-administered questionnaire.

Results: In Vientiane, although 97% of the participants heard of dengue, there was a lack of depth of knowledge on dengue: 33% of them did not know that malaria and dengue were different diseases, 32% incorrectly believed that *Aedes* mosquito transmits malaria, 36% could not correctly report that *Aedes* mosquitoes bite most frequently at sunrise and sunset; and <10% of them recognized that indoor water containers could be *Aedes* mosquito breeding sites. Self reported prevention methods were quite high yet observation of the participants' yards showed use of prevention methods to be only moderate. There was an association between good knowledge and better practices, but good knowledge was associated with worse attitudes. In Luang Prabang, the proportion of the villagers with correct behavior on dengue prevention (by interview) was 58% but this was only 44% from the actual observation of their yards. Knowledge on dengue prevention was inversely correlated with behavior on dengue prevention suggesting that better knowledge does not necessarily lead to correct practice.

Conclusions and Recommendations: There is a lack of depth of knowledge regarding dengue in Laos and observation methods revealed that more needs to be done by community members themselves to prevent the spread of *Aedes* mosquitoes. Observation should only be used to assess practice on dengue prevention rather than reporting by respondents.

Building a Successful Comprehensive Global Health Program – A Different Way to Make a Difference

L. Scott Benson

Division of Public Health, Department of Family and Preventive Medicine
And
Division of Infectious Diseases, Department of Internal Medicine
University of Utah

The program for Comprehensive Global Health (CGH) at the University of Utah is a cross-disciplinary, multi-partner collaboration led by the Division of Public Health within the University of Utah School of Medicine, through the Office of Comprehensive Global Health. Working with University of Utah affiliated partners including the Colleges of Nursing, Education, Pharmacy, and Engineering as well as the Honors College and Graduate School, the CGH harnesses the expertise of doctors, faculty, and students to address the triad of global human development challenges – health, education, and economic sustainability.

Together, the CGH works to improve health around the world and create sustainable change through educational exchanges, developing capacity building models and advancing the science of global health practice in collaboration with local partners. By using scholarship as a foundation, the CGH engages in mutually beneficial partnerships that focus on collaboration and sustainable outcomes that transcend local culture, political philosophies and geographic boundaries.

The CGH program has implemented a unique process that has been created over the last decade that is based upon long-term partnerships with in-country government representatives, university and hospital faculty, local community leaders and residents. In collaboration with our local partners, we assess and prioritize local needs, evaluate potential solutions, prioritize projects and create sustainable programs that we extend into local communities in cooperation with partners. Each program is evaluated for success, relevance and worthiness for replication in other parts of the country and in other countries around the world where similar need exists.

The CGH is earning international attention for its ability to empower host country educators, medical practitioners and community leaders to build capacity and create measurable, sustainable change, while honoring the culture, policies and practices of both the host and visiting countries. The CGH is currently operating in Ghana, Peru, China, India and Armenia, as well as locally in the State of Utah.

Population aging in Asia and sharing the experience of Japan -through the example of JICA project in Thai

Nanako Tamiya[1], Yumiko Miyashita[1]

[1]*Department of Health Services Research, Faculty of Medicine, University of Tsukuba*

Rapid aging in the developing countries as well as Japan is a growing issue in Asia. Ten countries has reached the so-called aging society with 7% or more of the elderly (aged 65 or older) population rate by 2010: Japan(23%), Hong Kong(13%), Korea(11%), Taiwan(11%), North Korea(10%), Singapore(9%), Thailand(9%), China(8%), Sri Lanka(8%) and Macao(7%).

In Thailand, for example, the number of the elderly has increased 6-fold from 1 million in 1950 to 5.9 million in 2010. By 2035, the elderly population is expected to be 15.3 million and reached the same level of the current Japan.

The main provider of the long-term care for the elderly in Thai or most of the Asian coutries is a family member, and volunteers in the community support them. However, sometimes there is a gap between necessary care and the actual care by such lay people. Furthermore, due to the current social change, such as declining birth rate and urbanization, the capacity of family carers is expected to decline and the way of caregiving is to be reconsidered.

Under such circumstances, Japan International Cooperation Agency (JICA) and Ministry of Public Health (MOPH) and Ministry of Social Development and Human Security (MSDHS) launched the Project on Long-term Care Service Development for the Frail Elderly and Other Vulnerable People (LTOP) in January 2013.

LTOP aims to propose a sustainable long-term care system for the frail elderly people by making use of the integrated community-based services with sharing the knowledge and experience of Japan, the world fastest aging country. Model services including care management system, home help services, day services and short stay services are to be developed in 6 pilot project sites and will be disseminated to the entire nation and other ASEAN countries.

A Study on the Relation of Sociodemographics and Social Support with Depression among Informal Carers in the Republic of Chile, from a Nationwide Survey

Felipe SANDOVAL & Nanako TAMIYA

Health Services Research, Faculty of Medicine, University of Tsukuba

Objective: We aim to clarify the relation of social support and depression among informal carers of community-dwelling elderly people in the Republic of Chile.

Methods: This is an inferential study, using logistic regression on secondary data from 445 community-dwelling caregivers of elderly from all over Chile. The Duke-UNC Functional Support Scale Questionnaire was used to measure perceived social support as exposure and the Center for Epidemiologic Studies Depression Scale (CES-D) for depression of caregivers as outcome. Gender, age, education, urbanization, health insurance status of the carers and their relation with the recipient were used as covariates, along the dependency of the recipient.

Results: 77.5% of the carers perceived a normal level of social support; 47.4% of the caregivers present depression. Logistic regression shows significant beneficial factors that decrease likelihood of being depressed are: a normal level of social support (Odds Ratio, OR: 0.363, 95% Confidence Interval, CI:0.220~0.600) compared to low support; age of the carer (OR:0.980, 95% CI:0.962~0.999); years of education (OR:0.924, 95% CI:0.870~0.980); national health insurance (OR:0.207, 95% CI:0.090~0.477), and other health insurance (OR:0.179, 95% CI:0.038~0.836) compared to those uninsured. Significant harmful factors that increase the likelihood of being depressed are: female sex of the caregiver (OR:2.148, 95% CI:1.175~3.928), hours of care (OR:1.026, 95% CI:1.004~1.049), and being the partner of the recipient, as opposed to other carers (OR:2.679, 95% CI:1.307~5.491).

Conclusion: Higher levels of perceived social support were associated with lower prevalence of depression in community-dwelling caregivers. Interventions to enhance perceived social support might be helpful for improving mental health among informal caregivers, in addition to support female carers, uninsured, and who care for longer hours.

Cross-Sectional Study of Subjective Symptoms in Women within One Year after childbirth

Kyoko TOKORO & Yoko EMORI

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Background Puerperium is associated with great changes in a woman's health. Even when a woman has had a normal childbirth, there may be physical and mental symptoms. Many studies have investigated women's health in puerperium; however, there is little research focused on their physical and mental symptoms. Postpartum women are healthy in general, but they can have a variety of symptoms.

Previous studies have reported 4 categories.

(1) Symptoms due to the impact of delivery: vaginal bleeding, anemia, edema, feeling the descent of the uterus, hemorrhoids, rectal prolapse, pain during urination, urinary incontinence, pain in the vulva, pain in the pubic bone

(2) Symptoms of daily life, including childcare, fatigue, lack of sleep, headache, general malaise, neck stiffness, eye strain, back pain

(3) Symptoms due to breastfeeding: breast pain, nipple trouble

(4) Symptoms due to sexual intercourse: pain with sexual intercourse

In Japan, women usually go to the hospital one month after childbirth to consult about their physical condition. The checklist in the hospital is general, for example, weight loss, blood pressure, urinary findings, and breast examination. There is no detailed examination in the hospital. Thereafter, most women do not have a consultation about themselves in the hospital or healthcare office.

Aim: To research subjective symptoms in women within one year after childbirth.

To estimate the factors that relate to the symptoms.

Methods

Design: Cross sectional study

Setting: This study was conducted in 7 Maternity facilities in Hitachinaka city, Hitachi city, and Shimotsuma city in Ibaraki prefecture. It includes hospitals, healthcare centers, and childcare facilities. Data were collected from women within one year of childbirth by means of a questionnaire in which there were 58 items of "subjective symptoms ", and related problems. The questionnaire was returned by mailing method or placement method. Data were analyzed by chi-squared test.

Result: 1198 postpartum women within one year after childbirth were recruited. 513 questionnaires were returned, a return rate of 42.8%. Those on which the child's age was not written or more than half was blank were excluded, leaving 502 questionnaires.

60% or more responded "sleepy", "I want to lie down" "stiff shoulders" and "headache". The symptoms continued from just after childbirth to one year. More than 40% responded "frustrated, worry, anxiety, sleepy, tired eyes". Results were compared before and after 4 months. The frequency of the symptoms was divided into 3 types by the periods after childbirth. "Sleepy, yawn, headache, stiff shoulder, back pain" continued throughout one year. "Perineal pain, anal pain when defecate, vaginal pain when have intercourse, wrist pain when holding the baby" were symptoms within 4 months after childbirth. "Feel disoriented, frustrated, distracted, cannot try anything difficult, increase in mistakes" associated with mental concentration were common from 4 months after childbirth.

Conclusion

Women up to one year after childbirth are unlikely to seek medical care or be in trouble. Nevertheless there can be many symptoms, and they are different in the different periods after childbirth. It is important that health care providers know women's symptoms in the periods after childbirth, and when consulting with them to ask about individual physical and mental symptoms to recognize their condition in detail.

How Hospitalization in the Forensic Psychiatric Ward Affect Attitude toward Medicine and Self-Efficacy

Hiromi SUGAWARA & Chizuru MORI

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Background: Act on Medical Care and Treatment for Persons who Have Caused Serious Cases Under the Condition of Insanity stated from 2005 in Japan. The purpose of this Act is to promote their reflection and rehabilitation in society. To achieve this goal, Attitude toward medicine is most important factor, and Self-efficacy about living in Community is a key factor to continue psychiatric medicine.

Aim: The objective of this study was to examine how affect Attitude toward medicine and Self-efficacy in the Forensic psychiatric medicine.

Method: Seventy-one participants who discharged from Forensic psychiatric ward from Oct 2007 to Jun 2013 were recruited this survey. Thirty-nine participants (82.1%male and a mean age of 42.7years) completed Drug Attitude Inventory 30 item version (DAI-30) and Self-Efficacy for Community Life scale (SECL) at admission and discharge day. Also, we investigated their demographic information

Result: Cronbach's alpha coefficient were 0.81, 0.85for DAI-30 and SECL, respectively. The mean score of DAI-30 were 3.64(SD:12.3), 17.3(SD:11.4) for at admission and discharge day. DAI-30 were statistically significant different between 2 periods ($p<0.001$). The mean of SECL subcategories at admission were 73.1 (SD:12.6), 82.3 (SD:16.2), 74.8 (SD:15.2), 69.9 (SD:25.3), 58.5 (SD:27.9) for Daily life, Behavior about medical treatment, Coping behavior about symptoms, Social life, and Personal relations. The mean of SECL subcategories at discharge day were 80.7 (SD:11.9), 87.4 (SD:11.2), 84.7 (SD:11.3), 82.0 (SD:16.0), 74.1 (SD:18.1) for Daily life, Behavior about medical treatment, Coping behavior about symptoms, Social life and Personal relations. Except Behavior about medical treatment, all scores of SECL subcategories were significant different from baseline ($p<0.005$).

Discussion: Patient's Attitude toward medicine was promoted during hospitalization. But their Self-Efficacy for Behavior about medical treatment was not significant different between at admission and discharge day. The mean of score of Self-Efficacy for Behavior about medical treatment at admission was high compared other subcategories. It suggests they already had high Self-Efficacy. They could keep Self-Efficacy for Behavior about medical treatment high and more concerned antipsychotics during in the Forensic psychiatric medicine.

Sexual and Reproductive Issues in the Brazilian Community in Japan : An Analysis of Adolescents in the Brazilian Schools

Milleanni DOMINGUEZ & Yoko EMORI

Maternity Nursing and Midwifery, Faculty of Medicine, University of Tsukuba

Objective: to understand the kinds of information about sex and sexuality that the Japanese Brazilian adolescents studying at two Brazilian schools in Japan, and to determine whether they are in risk for unsafe sexual behaviors.

Methodology: Information was collected using focus group interviews.

Results: Parents were a reliable source of information about sex, but only few participants talked to them about the matter. Also, the school seemed to bring superficially the theme sex, which makes the students full of doubts. Considering the Japanese environment, the high price and difficulty of finding contraceptives and the barrier provoked by the incomprehension of the Japanese language were mentioned as hardships that adolescents had to lead.

Conclusion: Due to the lack of comprehension of the language and socio-cultural factors from both Japanese and Brazilian population, there is no interaction among the two populations, and Brazilians tend to live in a small Brazil inside Japan. This way, Brazilians have no access to health services and programs aimed for the Japanese. The challenge in seek for efficient ways to transmit information on sex and sexuality still remains as a polemic subject for educators and health professionals.

Validity and Reliability of the Japanese Version of the Alcohol and Alcohol Problems Perception Questionnaire

Daisuke FUKUTA, Yoshimi OHMORI, Tomokazu SUGAYA, & Chizuru MORI

Psychiatric and Mental Health Nursing, Faculty of Medicine, University of Tsukuba

Background: Nurses are expected to have enough knowledge and responsibility and to play an appropriate role toward people with alcohol problems in treatment. In Europe, the attitudes scale of the health profession working with people with alcohol problems has been used widely. It has been reported nurses have a negative emotion to people with alcohol problems in Japan, it is necessary to evaluate the attitudes of nurses by using the scale.

Aim: The purpose of this study is to investigate the validity and reliability of the Japanese version of the Alcohol and Alcohol Problems Perception Questionnaire (AAPPQ-J).

Method: **Participants** Nurses and assistant nurses (n=886) in the nine psychiatric hospitals.

Design The questionnaires were mailed to the hospitals. The same questionnaires were sent to one hospital (n=60) for performing test-retest reliability two weeks after the first mailing.

The Alcohol and Alcohol Problems Perception Questionnaire (AAPPQ)

The AAPPQ was developed to measure the attitudes of health professionals working with drinkers (Cartwright A, 1980). The AAPPQ consists of the 30 items. Items are designed to measure six subscales: role adequacy, role legitimacy, role support, motivation, task specific self-esteem and work satisfaction. The AAPPQ was translated into Japanese and back-translated after obtaining the permission of the author.

Analysis A factor-analysis (the principal factor method and promax rotation) was carried out to assess the validity of the AAPPQ-J. Correlation coefficient was computed for the test-retest reliability.

Ethical considerations This study was approved by University of Tsukuba Faculty of Madicine, Ethics Committee.

Key Findings: Response rate was 86.0% (n=762), effective answer was 581. The six items with low correlation coefficients were discarded, and the AAPPQ-J was reduced to 24-item. Five factors were extracted from a factor-analysis: role adequacy, role support, work interest and concern, corresponding difficulty and role legitimacy. The alpha coefficient of the entire 24-item was 0.90, subscales ranged from 0.52 to 0.97.

Implications: The AAPPQ-J was shown to be a valid and reliable scale which can be used to measure attitudes of psychiatric nurses. In addition, the language and cultural factors by the translation affected the interclass correlation coefficient of the six items, and motivation, task specific self-esteem and work satisfaction were gathered into two subscales of ‘work interest and concern’ and ‘corresponding difficulty’ respectively.

References Cartwright A. The attitudes of helping agents toward the alcoholic client; the influence of experience, support, training and self esteem. *British Journal of Addiction* 1980; 75: 413–31.

Community engaged global health through academic partnerships among six countries

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Division of Public Health, Department of Family and Preventive Medicine, College of Medicine, University of Utah

Text: Many global health efforts have focused on delivering short-term medical care to individuals and lacked community-level buy in. For over ten years, the University of Utah has built mutually beneficial, community-engaged partnerships with other academic organizations and communities in Armenia, China, Ghana, India, and Peru. These projects are modeled after the partnership between the University of Utah and the Barekuma Collaborative Community Development Project in rural Ghana. Together, these partners provide positive educational experiences for all participants; understand and solve health related issues at the community level; and promote self-reliance, independence, and local confidence in local infrastructure. Partners work toward shared goals during four phases: 1) establishing partnerships between medical schools, 2) partnering between public health and community experts, 3) launching community development programs, and 4) evaluating and sustaining relationships and efforts. Results are analyzed and presented in academic journals and conferences and among community leaders and members. Research methods, interventions, and outcomes of each project are unique to needs and cultural context of each community and country.

Strategies for Quality Nursing Doctoral Education

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Professor and Dean Emerita
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Background: Rapid growth of nursing doctoral programs has caused many nurse leaders to be concerned about the quality of nursing doctoral education (QNDE). In the world, there were 333 nursing doctoral programs in 2012 compared with 286 in 2005.

Purpose: This cross-cultural study aimed to compare the perception of faculty and students/graduates on the quality of nursing doctoral education, identify factors of the four domains of the quality of nursing doctoral education that influence the quality of education, and analyze the relationship of quality of education to scholarly performance of nursing schools.

Methods: Seven countries participated in the cross sectional on-line questionnaire survey except for Japan that used paper questionnaire after translation. All schools used the same study procedure and the QNDE questionnaire. In the USA study, Importance Performance Analysis (IPA) was performed to identify domains that are important to the QNDE. The performance was measured based on the survey response results, but the importance was estimated using path analysis test. While the importance measures signify central role each domain played in the quality of nursing doctoral education, performance measures indicated achievements/outcomes of each school in four domains of the QNDE.

Results: A total of 101 deans/schools, 414 faculties, 1,149 students/graduates responded from seven countries. In the USA study, program and faculty were identified as the most important domains of QNDE. The most important item in the program domain was supportive environment for students' learning, while the item in the faculty domain was that faculty members mentored and assisted students to understand the value of programs of research and scholarship. The percentage of faculty members with research grant was significant for all domains of QNDE ($p<.05$), and time to graduation was also significant in explaining overall qualities ($p<0.01$).

Conclusion: Global strategies are needed to strengthen the program and resources for nursing doctoral education. Colleagues from these seven countries and others need to collaborate and develop recommendations for their respective government and funding agencies to increase the number of qualified nursing faculty, and strengthen financial resource and infrastructure for high quality doctoral education in nursing.

An evaluation study of doctoral nursing programs in Japan

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[4]Oita University of Nursing and Health Sciences

To assess the quality of doctoral nursing programs in Japan, the Japan Association of Nursing Programs in Universities (JANPU) conducted a survey of the programs using the Japanese version of the "Survey of the Quality of Nursing Doctoral Education" developed by Kim et al. in 2006. The questionnaire was translated into Japanese and a back translation method was used to verify.

Forty-six doctoral nursing programs existed in 2008 and 28 programs (60.9%) agreed to participate in this study. The study participants were 17 out of 28 directors (60.7%), 85 faculty members out of 276 (30.8%), 127 doctoral students out of 304 (41.8%), and 24 graduates out of 116 (20.7%).

The questionnaire had 3 sections: program (17 items), faculty (12 items), and resources (9 items). Regarding the evaluation of program, 79.1% of the graduates agreed that there were sufficient numbers of faculty members to facilitate learning, while only 36.1% of faculty members and 49.6% of students agreed. Regarding the evaluation of the faculty, more than 40% of students answered that the faculty members did not spend enough time for their own dissertation research. Significant differences among the evaluators were shown in the greatest number of items that related to the quality of resources. The students and the graduates identified that resources were adequate than the faculty.

As to the results of overall evaluation, evaluation by the graduates was more positive than the students and faculty for curriculum, quality of faculty teaching, and overall quality of the doctoral program. There were significant differences among groups of evaluators about the quality of faculty teaching. Students and graduates highly rated faculty teaching while the faculty themselves assessed it as adequate.

The overall quality of doctoral nursing programs in Japan was perceived as good by all of the groups of evaluators; however, more faculty development and a better education system are needed for continued improvement in the future.

Creating the Ferrans and Powers Quality of Life Index

Carol Estwing Ferrans, PhD, RN, FAAN

Tsukuba Global Science Week 2013

PURPOSE: Describe the development trajectory of the Ferrans and Powers Quality of Life Index (QLI).

BACKGROUND: First published in 1985, the Ferrans and Powers Quality of Life Index is one of the earliest and best-known instruments for quality of life internationally. It has been translated into 21 languages and used in more than 30 countries. In addition to the Generic Version for the general population, 14 illness versions have been developed. More than 200 research studies have been published to date using the QLI. In addition, the QLI has been used in clinical practice to assess treatment effects and guide health care. In cancer care, baseline QLI scores have been demonstrated to be predictive of survival, independent of cancer stage, in breast, colon, prostate, pancreas, and lung cancer. It also is one of the most popular instruments for cardiac rehabilitation and pulmonary rehabilitation programs in the United States.

METHODS: The primary goal was to provide an understanding of the impact of illness from the patient's viewpoint, in order to capture the "patient's voice" as accurately and credibly as possible. The QLI is composed of two paired sections, one measures satisfaction with various aspects of life, and the other measures the importance of those same aspects of life to the patient. Importance ratings are used to weight the paired satisfaction responses, so that scores reflect satisfaction with the aspects of life that are most valued by the patient. The incorporation of the patient's values made this instrument unique among quality of life instruments and measures of health status. The steps in development and testing will be described, using both quantitative and qualitative methods. The work of our team as well as others will be presented, to provide a full characterization.

RESULTS: *Responsiveness to change (sensitivity)* has been demonstrated in 27 published intervention studies. In these studies, QLI scores changed significantly over time, as shown by comparisons before and after an experimental intervention or therapeutic treatment. *Reliability*: In 48 studies, internal consistency reliability was supported by Cronbach's alphas ranging from .73 to .99 for the QLI. Stability reliability also has been demonstrated over 2 to 4 week time periods. *Validity*: Support for content validity was provided by acceptably high scores using the Content Validity Index. Convergent validity was supported by strong correlations between QLI scores and measures of life satisfaction (ranging from .61 to .93). Factor analysis with 349 Americans revealed four dimensions underlying the QLI: health and functioning, social and economic, psychological/spiritual, and family. The factor analytic solution explained 91% of the total variance. Factor analysis of the four primary factors revealed one higher order factor, which represented quality of life. Subsequent factor analysis with a group of 284 Norwegian women supported the original four factors. In addition, evidence of construct validity was provided by demonstrating that cancer patients who had less pain, less depression, or who were coping better with stress had significantly better QLI scores than patients who did not.

CONCLUSION: The QLI is an instrument that measures quality of life from the patient's perspective, with its unique scoring algorithm for combining satisfaction and importance. Robust evidence for reliability and validity across numerous illness populations, cultural groups, and languages over 25 years provides confidence in its psychometric properties and encourages its use in research and clinical practice.

The origins of oncology nursing studies and the application of their findings to the intrinsic quality of Japanese cancer patients

Michiyo Mizuno

Faculty of Medicine, University of Tsukuba

Although cancer patients experience numerous ordeals, people have a natural resilience that allows them to overcome many difficulties. My qualitative studies on the meaning of health for cancer survivors and the function of hope for hematology/oncology patients revealed that when both cancer patients and survivors try to adapt to their cancer experience, they should be aware of their illness-related problems, understand the substance of the problems, and acquire the skills required to solve the problems as they cope with them. My research team developed a program to support the performance of cancer patients' own adaptation tasks with approaches targeting their cognitive behaviors.

On the other hand, in a questionnaire survey conducted on bereaved family members of cancer patients, I found that cancer patients and their family members do not share detailed information about a patient's disease with each other, but many bereaved family members are not convinced of the justifiability of having done so. Japanese people are poorly skilled at asking others about their mind and are not able to speak articulately about their own mind. Meanwhile they believe they can understand others' minds without directly asking them.

We thought that health-related quality of life (HR-QOL) could be used not only as an indicator of the effect of a support program, but also as a means for nurses and physicians to share patients' appraisal of and level of satisfaction with their current health state with the patients themselves. QOL is both multidimensional and subjective. Therefore, if a measure of HR-QOL that adequately reflects the patients' perspective is selected, it can provide nurses and physicians with information that can be used to start carefully talking with patients about their actual health and illness. These dialogues may strengthen Japanese patients' resilience. Therefore, we are planning a study of these concepts.

The support system to children receiving a comprehensive anticancer therapy utilizing a proton beam tratment

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[2]University of Tsukuba Hospital
[3]Tsukuba Medical Center Hospital

The progress of the treatment result of the childhood cancer is remarkable and improves a lifesaving rate in 70%. The number of childhood cancer experienced people (survivors) is in this way said to act as one of 400-1000 people of the young adult in our country. For the QOL (Quality of Life) improvement of the children with cancer, an action to minimize late-effects in the close is required. In late years, as one method, the use of the proton beam treatment has been introduced. In University of Tsukuba, we started the clinical study for not only adults but also children in the 1980s.

In the adult domain, they can receive proton beam treatment in ten hospitals of this country. On the other hand, for the childhood cancer, they can receive proton beam treatment only in 3 facilities. The reasons are as follows: they need to give intensive chemotherapy and concurrent proton beam therapy, infants need sedation during proton beam treatment. Because there are few facilities, patients and families have to move in the far distance and have to stay for 2-3 months to receive the treatment.

Although each child and his/her family have to adapt to their new environment after changing the hospital. They have not enough time because of a time-intensified, high density treatment plan. We have set a support system up for them to achieve their comprehensive, high density anticancer therapy including proton beam treatment as follows: preparation by the cooperation with the proton beam center staff and the children's ward staff, the reclamation through the proton beam irradiation enforcement period trains more. We report the concrete content.

Perceptions and needs of childhood cancer patients, mothers, and staffs for their long-term treatment and school re-entry in Japan.

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[5]Division of Pediatric Hematology and Oncology, Ibaraki Children's Hospital

In the present study, better ways to support children to go back to school after a long-term hospitalization due to pediatric cancer were discussed.

Semi-structured interviews were conducted with pediatric patients themselves, their mothers, hospital school teachers, and primary nurses to obtain a true picture of their perceptions and needs concerning the children's return to school. The recorded interviews were transcribed into verbatim records and content analyses were performed by the method developed by Krippendorff (1989).

As a result, 10 and 15 categories of thoughts were extracted from children and mothers, respectively, with 7 of these categories common to children and mothers, and 10 and 8 categories of thoughts were extracted from the teachers and nurses, respectively, with 4 of these common to teachers and nurses.

The study showed that the children and mothers, who take being watched over and receiving coordination by teachers and nurses from the beginning of hospitalization, undergo some important changes of perceptions until discharge from hospital before they eventually go back to school. Teachers and medical staff should be aware of this process when they make efforts in giving support in a broad sense to pediatric cancer patients to return to school, including helping them with learning.

At the same time, it is also important that teachers and medical staff turn to and solve issues that have now been discovered in the study: these include distress and dilemma regarding return to school faced by each professional and problems related to the support system in the ward and hospital.

MEMO

The Present Conditions and Issues of the Teacher Training in Japan and Vietnam

The Present Conditions and Issues of the Teacher Training in Japan and Vietnam

Friday, October 4	Venue: Conference Room 303
1. The opening	
13:00-13:15	Operation check of the apparatus for presentation
Greetings of the opening	
13:15-13:30	Dr. . Akitoshi Teuchi University of Tsukuba, Japan
Chair: Dr. Akitoshi Teuchi	
13:30-14:10	"CURRENT SITUATIONS AND ISSUES OF THE TRAINING OF PRIMARY TEACHERS IN HO CHI MINH CITY UNIVERSITY OF EDUCATION" Dr. Hoang Van Can Ho Chi Minh City University of Education, Vietnam
14:10-14:50	"THE PRESENT CONDITIONS AND ISSUES OF THE TEACHER TRAINING OF SCHOOL FOR THE DISABLED IN VIETNAM" Dr. Le Thi Minh Ha Ho Chi Minh City University of Education, Vietnam
14:50-15:00	**Coffee Break**
15:00-15:40	"Primary Teacher Training in Japan and University of Tsukuba" Dr. Naohiro Higuchi University of Tsukuba, Japan
15:40-16:20	"The Present Conditions and Issues of the Teacher Training of School for the Disabled in Japan " Dr. Takao Ando University of Tsukuba, Japan
16:20-17:00	-Discussion-

Current Situations and Issues of the Training of Primary Teachers in Ho Chi Minh City University of Education

Hoang Van Can[1]

Vice - Rector of Ho Chi Minh City University of Education

1. Current situation

- Ho Chi Minh City University of education was established on October 27, 1976, The Department of Primary Education was established in May 1995. The Department of Primary Education started its Bachelor program in 1995 and post-graduate program in September 2012.
- Total number of staff: 18 (16 lecturers, 2 office administrators), including 1 Associate Professor, 3 PhDs, 5 Senior Lecturers, 11 Masters and 4 Bachelors. The Department has 2 academic sections, namely General Science Team and Teaching Methodology Team. Number of students who are currently enrolled: Full time undergraduate students: 600, Part-time undergraduate students: 5200 and Post-graduate students: 30
- The Department of Primary Education has training and research partners in Belgium and Australia (The Free University of Brussels - Belgium (study of primary students with dyslexia) and Trinh Foundation Australia (Research on Speech Therapy for Children with difficulties in pronouncing).

2. Current matters and long-term issues need resolving:

- Standardization issues and the qualifications of the teaching staff, especially their foreign language competence in compliance with the Common European framework of reference for languages.
- The problem of regular pedagogical training for students on a monthly basis, semester and full program to meet the requirements and graduation criteria.
- Partnerships in training with the departments of Primary Education of other domestic and international pedagogical universities.
- Developing training programs (second degree program) for those wishing to learn about primary education.
- Tightening its close link with other Primary Education programs at localities and provincial departments of education and training.
- Issues in education for integration (for primary education teacher students) at the demand of education in Vietnam.

3. Prospects for teaching and scientific research

- Improving the quality of curriculum framework, detailed programs across disciplines and interdisciplinary training of primary school teachers (with a focus on mainstream undergraduate and postgraduate programs) .
- Building project on starting a doctoral program on Primary Education Teacher Training Program in 2015.
- Developing a project for training Primary Education teachers using English language (2014).

Conclusion: All graduate students have a stable job after graduation. The Department of Primary Education is one of the leading academic units of Ho Chi Minh City University of Education, gaining high reputation in Ho Chi Minh City regarding education and training of Primary Education teachers.

HCMC, August 29, 2013
Assoc. Prof. Hoang Van Can

The Present Conditions and Issues of the Teacher Training of School for the Disabled in Vietnam

Le Thi Minh Ha[1] Dean; Vo Thi My Dung[1] Head of Department of Specialized Subjects [1]of Faculty of Special Education, Ho Chi Minh City University of Education, Vietnam.

1. Situation of special education teacher training in VN

According to the survey of children with disabilities (CWD) in *8 socio-economic areas of Vietnam in 2005* conducted by the Ministry of Education and Training, the number of children with disabilities are estimated in 2015 as follows: the number of children with disabilities from 0 to 16 years is 969.489, of which CWD aged 0-5 is 67.767; CWD aged 6-11 is 332.825; CWD aged 12-16 is 568.896. According to an incomplete statistics report, at present, Vietnam has approximately 1.2 million CWD.

2. The present conditions of the teacher training of school for the disabled in Vietnam

The teacher is a core force determines the success of the Special Education. To meet the learning needs of children with disabilities aforementioned, 400.898 teachers need training / retraining of Special Education. Accordingly, the Special Education teachers are allocated according to the following levels: Pre-15.248, 158.092 Primary, Secondary 227.558. Time of 2002-2003, in 6 cases of Vietnam Teachers are respectively established science to teacher education Special Education. From 2006 to 2013 there were 8 GV Special Education courses from the Special Education in the country with about 2654 teachers in schools. Thus, compared to the learning needs of children with disabilities, the number of Special Education teachers are not trained to meet the learning needs of children with disabilities in Vietnam. This is a remarkable challenge for Special Education in Vietnam.

3. Issues of teachers of special education training

The training capacity of the universities of education is limited and teaching staff recruitment for special education faculty is difficult. Vietnam has a law, an educational policy for people with disability and a national program about inclusive education. However, in reality, the law and policy is not well implemented. For instance, inclusive schools do not recruit teachers of special education as special needs teachers (because there is not salary code for this group).

Primary Teacher Training in Japan and University of Tsukuba

Naohiro Higuchi

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University of Tsukuba

Japanese teacher training is generally carried out in the university. The curriculum is prescribed by the law. It consists of the teaching subjects and the specialized subjects. The teaching practice is carried out for four weeks. The students who get the necessary credits can take the teacher license. It has no national examination to take the license but has the teaching staff examination by the local educational board or an every private school.

The task of primary teacher training is the balance of a theory and practice. The academic professors don't teach in the primary school, so they tend to teach the theory mainly. The teaching practice period is so short that students have difficulty to teach and manage the classroom when they become a novice teacher. Many universities establish the graduated school but examinees are few. And there are many teachers in their twenties, while few in their thirties and forties. The balance is not so good that it is impossible for the novice teacher to study in the primary school.

The root of the University of Tsukuba is Normal School established in 1872. The school name has changed to Tokyo University of Education, it committed to train the high school teachers. But College of Education in University of Tsukuba has started the primary teacher training course since 2012. The number admitted annually is 35 including 15 students as the primary teacher training course. There is a traditional primary school attached as the laboratory school since 1873. Students are given lectures from the laboratory school teachers. But the primary teacher training course schedule is so tight that students have to have the classes even in the holidays. And the laboratory school is in Tokyo, so students have to go to Tokyo.

The Present Conditions and Issues of the Teacher Training of School for the Disabled in Japan

Takao Ando[1]

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The establishment of special education system and special education school

Japan' educational policy interests after world war II were on the establishment of special education system and on establishing schools for the disabled prescribed by School Education Law Article 1 in 1947. In accordance with the law that was financial grounds to establish a school for the disabled in 1956, one or more schools for the intellectual disabled, the physically disabled and the health impaired were established in each prefecture. Because schools for the visual impaired and the hearing impaired had been already established after the 1870s, the number of the special education school for the disabled increased drastically in the 1960s. The remarkable increase in number of the schools for the disabled raised the demand for the teacher training.

The present conditions and issues of teacher training

Principle of teacher training: The teacher training in Japan will be carried out based on the principle of the opening system under the postwar education reform at a university.

Present conditions: The Ministry of Education drew up a plan to maintain four-years special school teacher training courses at the national universities. It took twenty years from 1953 through 1973 to maintain the teacher training course of the special education school in all faculties of education of national universities.

Under the revised School Education Law, a system was reformed from special education to special needs education in 2007. The school for special needs education can accepts students with several kinds of disabilities and works as a center of education for children with disabilities in each local community. Now there are about 1,000 schools for special needs education. The demand for teacher training of special needs education school increases still more especially. Teachers of the school for special needs education are trained at not only the national university but also the private university accredited by the Ministry of Education.

Issues: The license for schools for special needs education is (1) to require the basic knowledge and understanding of all disabilities, including LD etc., (2) to ensure the teacher's specialty on 1 or more particular disabilities. It becomes the important problem how we realize advancement of the specialty to support a variety of educational needs.

MEMO

Student Presentations
-The 8th Tsukuba Medical Science Research Meeting-

Student Presentations
- The 8th Tsukuba Medical Science Research Meeting -

Thursday, October 3
Venue: Main Convention Hall

Student Oral Presentations

14:40-14:55	"MafB deficiency impairs the apoptotic cell clearance" Mai Thi Nhu Tran	University of Tsukuba, Japan	**O-1**
14:55-15:10	"Induction of the single nucleotide mutation by CRISPR/Cas9 in mice" Dinh Thi Huong Tra	University of Tsukuba, Japan	**O-2**
15:10-15:25	"A Household Survey on Larval Habitats of Aedes Mosquitoes in Dhaka, Bangladesh" Farhana Ferdousi	University of Tsukuba, Japan	**O-3**
15:25-15:40	"Activation of focal adhesion kinase by direct interaction with β4 integrin in an EGFR-Src dependent pathway in tumor progression" Tai, Yu-Ling	National Taiwan University, Taiwan	**O-4**
15:40-15:55	**Coffee Break**		
15:55-16:10	"Removal of 2-tert-butyl-1,4-benzoquinone from Keap1-TBQ adduct by glutathione-mediated S-transarylation" Yumi Abiko	University of Tsukuba, Japan	**O-5**
16:10-16:25	"TAF-I regulates the transcription of interferon-stimulated genes through it's histone chaperone activity" Shinichi Kadota	University of Tsukuba, Japan	**O-6**
16:25-16:40	"Clinical Proton beam induced DNA damage and its repair mechanism" Ariungerel Gerelchuluun	University of Tsukuba, Japan	**O-7**
16:40-16:55	"A Common Host-Pathogen Interaction as Broad-Spectrum Antiviral Drug Target" Sisley Austin	Bordeau Segalen University, France	**O-8**
16:55-17:10	**Coffee Break**		
17:10-17:25	"Skeletal Muscle Regeneration Research for Diaphragmatic Hernia Treatment" Koki Hagiwara	University of Tsukuba, Japan	**O-9**
17:25-17:40	"Autocrine Transforming Growth Factor-β Induces TMEPAI and Promotes Tumor Formation in Lung Cancer Cells" Vo Nguyen Thanh Thao	University of Tsukuba, Japan	**O-10**
17:40-17:55	"A Novel Diagnostic Method For AITL By Detecting RHOA G17V Hotspot Mutation Using Allele-Specific Real-Time PCR" Rie Nakamoto-Matsubara	University of Tsukuba, Japan	**O-11**
17:55-18:10	"ErbB2 Signaling and ECM Stiffness Cooperate to Drive Breast Tumor Progression in 3D" Abhishek Kurup	University of California, Irvine, USA	**O-12**
18:10-18:25	"Identifying positive regulators of reprogramming using RNA interference" Sara Brightwell	The University of Edinburgh, UK	**O-13**

Friday, October 4
Venue: Main Convention Hall

Student Oral Presentations

8:30-8:45	"Reward value coding in the monkey orbitofrontal cortex" Tsuyoshi Setogawa	University of Tsukuba, Japan	**O-14**
8:45-9:00	"Identification and characterization of a novel CD300 molecule, CD300H" Kouta Niizuma	University of Tsukuba, Japan	**O-15**
9:00-9:15	"Initial clinical experience of movable, ceiling-mounted, intraoperative MRI" Yosuke Masuda	University of Tsukuba, Japan	**O-16**

Friday, October 4

Venue: Main Convention Hall

Student Oral Presentations

9:15-9:30	"Studying the effect of various Propionibacterium acnes strains on the cellular functions of human keratinocytes"		
	Gábor Tax	University of Szeged, Hungary	**O-17**
9:30-9:45	"Non-cell autonomous effects on integrin signaling in the chick embryonic CNS"		
	Katherine Long	The University of Edinburgh, UK	**O-18**
9:45-10:00	**Coffee Break**		
10:00-10:15	"Characterization of human adipose tissue-derived mesenchymal stem cells for diabetic complications"		
	Trinh Nhu Thuy	University of Tsukuba, Japan	**O-19**
10:15-10:30	"Crucial role of Elovl6 in brain development and function"		
	Hiroshi Ohno	University of Tsukuba, Japan	**O-20**
10:30-10:45	"ACAP3 functions as an Arf6-specific GAP to control neurite outgrowth in hippocampal neuron"		
	Yuki Miura	University of Tsukuba, Japan	**O-21**
10:45-11:00	"Improvement of vascular function by magnetic nanoparticle-assisted gene and endothelial cell transfer in vessels"		
	Sarah Vosen	University of Bonn, Germany	**O-22**
11:00-11:15	"Both CTPS1 and CTPS2 Can Form Cytoophidium in Mammalian Cells"		
	Chang, Chia-Chun	National Taiwan University, Taiwan	**O-23**
11:15-13:15	**- Lunch -**		

Student Poster Presentations

11:15-13:15	"Pbp1 is involved in the Ccr4 and Khd1-mediated regulation of cell wall synthesis through the association with ribosome"		
	Yuichi Kimura	University of Tsukuba, Japan	**P-1**
	"Enhanced Proliferation of Adipose-derived Stem Cells by Defined Medium Supplemented with Growth Factors"		
	Chang, Y-T	National Taiwan University, Taiwan	**P-2**
	"Functions of RNA polymerase I transcription factor UBF in nucleolar formation"		
	Shuhei Ueshima	University of Tsukuba, Japan	**P-3**
	"Identify the Topoisomerase II Isozyme-specific Targeting Agents and Investigate their Biological Responses"		
	Hsu, Huang-Ling	National Taiwan University, Taiwan	**P-4**
	"DNA-binding Activity of CTCF, an Insulator Protein Regulated by Phosphorylation in Mitosis"		
	Takeshi Sekiya	University of Tsukuba, Japan	**P-5**
	"Calcium Influx-induced Proteolytic Cleavage of Topoisomerase 2β"		
	Chen, Yi-Chun	National Taiwan University, Taiwan	**P-6**
	"EZH2, a component of polycomb complex, regulates nuclear export of the influenza virus M1 and the viral genome"		
	Masamitsu N. Asaka	University of Tsukuba, Japan	**P-7**
	"RecF Act as Regulators in Transcription-associated R-loop Formation and their Biological Implications"		
	Leu, Wan-Ning	National Taiwan University, Taiwan	**P-8**
	"In cellulo analysis of parvovirus-mediated changes of nuclear envelope integrity by real time microscopy"		
	Kenza Snoussi	University of Tsukuba, Japan	**P-9**
	"Activation of Proteolytic Systems Contributes to Cadmium Toxicity"		
	Yen, Chia-Hau	National Taiwan University, Taiwan	**P-10**
	"Involvement of Fcα/μ receptor (CD351) in autoantibody production"		
	Yuichi Yoshizawa	University of Tsukuba, Japan	**P-11**

Friday, October 4

Venue: Main Convention Hall

Student Poster Presentations

11:15-13:15	"A Negative Feedback of the HIF-1α Pathway via Interferon-stimulated Gene 15 and ISGylation" Zi Ying Valerie Tay	National Taiwan University, Taiwan	**P-12**
	"Deficient expression of HIF-2α in spleen results in compensatory angiogenesis by upregulation of HIF-1α" Ikki Tsuboi	University of Tsukuba, Japan	**P-13**
	"Activin Signaling is Required in the Wound Healing of Aeolosoma viride" Hou, Fen-Han	National Taiwan University, Taiwan	**P-14**
	"MafB is required for maintenance of differentiated state of microglia in vitro" Ryusuke Koshida	University of Tsukuba, Japan	**P-15**
	"A Portable Microfluidic Device for Rapid Diagnosis of Cancer Metastatic Potential" Chen, Li-Yu	National Taiwan University, Taiwan	**P-16**
	"Fatty acid synthase inhibitor cerulenin suppresses human liver cancer cell lines in vitro" Naoki Sano	University of Tsukuba, Japan	**P-17**
	"Interaction between SIK2 and PP2A Regulates the Activity of CaMKI" Lee, Chia-Wei	National Taiwan University, Taiwan	**P-18**
	"Role of MafB in macrophage dendritic cell progenitor (MDP) differentiation" Zeinab Kosibaty	University of Tsukuba, Japan	**P-19**
	"Extratumoral Acidification as a Promoter for the Development of Stemness Properties in Cancer" Chiang, Chi-Feng	National Taiwan University, Taiwan	**P-20**
	"Direct Conversion of Mouse Liver Cells to Insulin-producing Cells by Defined Factors" Yutaro Itoh	University of Tsukuba, Japan	**P-21**
	"Lysophosphatidic Acid Inhibits TPA Induced Megakaryopoiesis in K562 Cells Through the Activation of LPA Receptor 2" Ho, Ya-Hsuan	National Taiwan University, Taiwan	**P-22**
	"Detoxification of methylmercury by hydrogen sulfide producing enzyme in vitro and in vivo" Eiko Yoshida	University of Tsukuba, Japan	**P-23**
	"Soluble DNAX accessory molecule-1 is a novel predictive biomarker for acute graft-versus-host disease" Minoru Kanaya	University of Tsukuba, Japan	**P-24**
	To Be Announced	National Taiwan University, Taiwan	**P-25**
	To Be Announced	National Taiwan University, Taiwan	**P-26**
	To Be Announced	National Taiwan University, Taiwan	**P-27**

O-1

MafB deficiency impairs the apoptotic cell clearance

Mai Thi Nhu Tran[1,2], Michito Hamada[1,2], Risako Shiraishi[1] and Satoru Takahashi[1,2]

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Macrophage is one of the main phagocytes recognize and engulf apoptotic cells to maintain peripheral immune tolerance. The failure of apoptotic cell clearance by macrophages induces autoimmunity. Although most of apoptotic cell recognition factors have been identified, the mechanism of transcriptional regulation of these factors remains unknown. MafB is a transcription factor that affects proliferation and differentiation of macrophage. Here we show that MafB involves in apoptotic cells clearance. The depletion of MafB in macrophage decreases the expression of apoptotic cell recognition genes, especially *C1qa*, *C1qb*, *C1qc*, leads to the impairment of phagocytosis both *in vitro* and *in vivo*. While wild-type serum is able to recover phagocytosis in *Mafb*$^{-/-}$ macrophages, C1q-depleted serum could not rescue this phenotype. In addition, promoter analysis of *C1q* genes showed that MafB directly regulates expression of *C1qa*, *C1qb* and *C1qc*. In *Mafb*$^{-/-}$ mice, production of autoantibody is increased. Our results indicate that MafB has a pivotal role in phagocytosis of apoptotic cells via C1q regulation, and they give therapeutic strategies for autoimmune diseases.

O-2

Induction of the single nucleotide mutation by CRISPR/Cas9 in mice

Dinh Thi Huong Tra

Laboratory Animal Resource Center, Human Biology Ph.D. Program

The genome editing with engineered nucleases (GEEN) is a new technology for producing gene modified cells, plants, and animals. It has been reported that 3 kinds of engineered nucleases (ZFN, TALENs and CRISPR/Cas) are useful for producing knockout (KO) mouse. Because it is easier to design and construct CRISPR/Cast than ZFN and TALEN, CRIPR/Cas will be mainly used for gene modification in various animals in future.

Before GEEN development, KO mice are exclusively produced by gene targeting method. In the method, genetic mutations are induced by spontaneous homologous recombination in mouse embryonic stem cells (mESc). KO mice are generated via germline chimera production with specific gene-mutated mESc. While this method is popular and stable, it is costly and time consuming. In contrast, GEEN induce gene mutation by directly injecting DNA or mRNA of site-specific engineered nucleases into the one-cell embryo without any selection gene (e.g. Neomycin registrant gene). Moreover, if co-injection of CRISPR/Cas and single strand oligo DNA donor, it is possible to induce small-scale gene mutation which you want.

The single nucleotide mutations (SNMs) are associated with a variety of human diseases. However, since creation of SNM is more difficult than that of standard null mutation, SNMs-induced mice are hardly used. To establish the method for generating mice with SNMs, we try to generate "Albino" C57BL/6 mice by CRISPR/Cas induced SNM in *Tyr* gene. The *Tyr* gene codes for enzyme tyrosinase that is necessary for melanine production. A couple of SNMs (291: G to T, 369: G to C) in *Tyr* is caused of Albino phenotype.

To induce 291 SNMs (G to T) in *Tyr*, we first designed and constructed CRISPR/Cas expression vector. Activity of CRISPR/Cas for *Tyr* was confirmed by EGxxFP system in HEK293T cells. We then injected CRISPR/Cas expression DNA vector and single strand oligo DNA donor into 154 one-cell embryos from C57BL/6 mice. As result, 35 mice were obtained and 2 mice showed Albino phenotype. This result suggests that disease model animals carrying SNMs could be generated with comparative ease by CRISPR/Cas mediated genome editing.

O-3

A Household Survey on Larval Habitats of *Aedes* Mosquitoes in Dhaka, Bangladesh

Farhana Ferdousi

Graduate School of Comprehensive Human Sciences, University of Tsukuba

Dengue fever (DF), one of the most important emerging arboviral diseases worldwide, is transmitted through the bite of container breeding mosquitoes *Aedes aegypti* and *Aedes albopictus*. In Bangladesh, DF has become a serious public health concern after the first large-scale outbreak in 2000. Since then, DF cases have been reported every year in all major cities of Bangladesh.

To identify important breeding habitats of *Aedes* mosquitoes, a household entomological survey was conducted in Dhaka from August through October 2000, the peak epidemic period of DF/DHF. Approximately 100 households (range 100-119) were randomly selected from each of the 90 administrative wards in Dhaka City Corporation. All water holding containers were inspected for *Aedes* larvae in all 3 locations of each household, ie, indoor, outdoor, and rooftop.

Of 9,222 households inspected, 1,306 households (14.16%) were found positive for *Aedes* larvae. Of 38,777 wet containers were examined, 2,216 wet containers (5.71%) were found infested with *Aedes* larvae. The overall house index (HI), breteau intex (BI) and container index (CI) were 14.16, 24.64 and 5.86 respectively. Positive wet containers were significantly higher in number in outdoor than in indoor and in rooftop. Among the positive containers, the most commons were earthen jars (19.89%), flower pots (16.24%), tires (14.88%), drums (9.82%), tanks (9.11%), and cans and bottles (8.23%). A total of 3,027,867 *Aedes* larvae were collected, among which 1,923,648 (63.5%) were *Aedes aegypti*. Number of *Aedes aegypti* was higher than the number of *Aedes albopictus* in all 3 locations (92.7:1, 1.4:1, and 9.9:1 in indoor, outdoor, and rooftop locations respectively). Independent type of household, having water storage system in the household, and having fully/partly shaded outdoor premise were significantly associated with household infestation of *Aedes* larvae.

The study results would reinforce the dengue vector control strategy to have focus on the containers that are consistent producers of *Aedes* larvae and houses that consistently have *Aedes* larvae in containers.

O-4

Activation of focal adhesion kinase by direct interaction with β4 integrin in an EGFR-Src dependent pathway in tumor progression

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Although β4 integrin and focal adhesion kinase (FAK) are individually known to be associated with tumor progression, only recently they have been suggested to function in the signaling pathway. Here, we demonstrate *in vivo* and *in vitro* physical and functional interactions between β4 integrin and FAK which involves in regulating tumorigenesis. An amino-terminal linker of FAK is essential for binding with cytodomain of β4 integrin in malignant cells rather than in less malignant cells, implicating the role of this interaction in malignancies. Furthermore, EGF signaling and Src activity are indispensable for β4 integrin-FAK complex formation and followed by the functional β4 integrin activate FAK in an adhesion-dependent manner. Finally, disruption of this complex reduces tumorigenesis, in concurrence with the decreases in phospho-AKT and phospho-p38. This study indicates a novel β4 integrin-FAK complex-mediated tumorigenesis in an EGFR-Src dependent pathway. The significance of this research is highlighted that co-overexpression of both β4 integrin and FAK in patients' cancerous tissues with colon cancer. Our findings may provide a new anti-cancer opinion by targeting the specific oncogenic pathway.

O-5

Removal of 2-*tert*-1,4-butylbenzoquinone from Keap1-TBQ adduct by glutathione-mediated *S*-transarylation

Yumi Abiko[1, 2] and Yoshito Kumagai[1]

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Butylated hydroxyanisole (BHA) is a phenolic antioxidant and classified class-2B carcinogen. It is readily undergoes *O*-dealkylation to produce 2-*tert*-butyl-1,4-hydroquinone (TBHQ), which readily auto-oxidizes to the electrophlic metabolite 2-*tert*-butyl-1,4-benzoquinone (TBQ). TBQ causes activation of Nrf2 together with *S*-arylation of its negative regulator Keap1 through Cys23, Cys151, Cys228, and Cys368 (Abiko Y *et al*., Toxicol Appl Pharmacol 255: 32-39, 2011). In a previous study, we found that glyceraldehyde-3-phosphate dehydrogenase (GAPDH) covalently modified with 1,2-naphthoquinone (1,2-NQ) undergoes *S*-transarylation by glutathione (GSH), resulting in a recovery of GAPDH activity through removal of the 1,2-NQ from modified GAPDH and a concomitant formation of a 1,2-NQH_2-SG that readily oxidizes to 1,2-NQ-SG. (Miura T *et al*., Chem Res Toxicol 24: 1836-1844, 2011). In the present study, we explored the possibility of GSH-dependent *S*-transarylation of Keap1-TBQ adduct. Pretreatment with L-buthionine-(*S*,*R*)-sulfoximine and *N*-acetylcysteine, which are inhibitor of GSH synthesis and enhancer of GSH level, prior to TBQ exposure of HepG2 cells suggested that the Keap1-TBQ adduct appears to undergo GSH-mediated *S*-transarylation because the resulting alterations in the intracellular GSH concentration affected Nrf2 activation and up-regulation of downstream protein such as Heme oxygenase-1 caused by TBQ. In support of this hypothesis, a cell-free study demonstrated that incubation of Keap1-TBQ adduct with GSH results in the removal of TBQ from Keap1 thiol groups, including Cys151 of Keap1 known to play a important role in Nrf2 activation, with the production of GSH adducts of TB(H)Q such as TBHQ-SG, TBHQ-diSG, and TBQ-diSG. These results suggest that GSH plays a role in reversible covalent modification of TBQ derived from BHA to Keap1 through the formation of a C-S bond (Abiko Y and Kumagai Y., Chem Res Toxicol 26(7): 1080-1087, 2013).

O-6

TAF-I regulates the transcription of interferon-stimulated genes through it's histone chaperone activity

Shinichi Kadota[1], and Kyosuke Nagata[2]

[1]*Department of Infection Biology (Molecular Virology), Graduate School of Comprehensive Human Sciences and Faculty of Medicine,*
[2]*University of Tsukuba*

Interferon (IFN) has the critical role in establishing cellular antiviral state by inducing transcription of IFN-stimulated genes (ISGs), which encode proteins involved in the antiviral activity, through the IFN signaling pathway called JAK-STAT pathway. STAT1, STAT2, and IRF9, as transcription factors involved in ISG transcription, form the ISGF3 complex followed by IFN stimulation, bind to IFN-stimulated response element (ISRE) sequences on the promoter of ISGs, facilitate formation of transcription initiation complexes, and thereby promote transcription of ISGs. It is believed that transcription is regulated by combinated effects of genetic and epigenetic mechanisms including nucleosome-positioning sequence, histone chaperones, ATP-dependent chromatin-remodeling factors, post-translational modifications, and histone variants. Reacent studies suggests that ISG transcription are also regulated by some of epigenetic mechanisms.

Template activating factor (TAF)-I was first identified as a stimulator of adenovirus DNA reprication *in vitro*, and subsequent studies revealed that TAF-I acts as histone chaperone for histone H3-H4 and linker histone H1. At least six histone H1 variants exist in somatic mammalian cells and are involved in the formation of higher-order chromatin structure. Histone H1 seems to be involved in the regulation of gene expression, but it is not clarified what kind of genes are regulated by the mechanisms involving histone H1. In this study, we focused on the function of TAF-I in ISGs transcription. TAF-I knockdown by using short-hairpin RNA specific for TAF-I induces the enhancement of ISG transcription and reduction of the amounts of histone H1 on ISG promoters. These results suggest that TAF-I regulates ISG transcription through it's histone H1 chaperone activity. The exact function of TAF-I in ISG transcription is under investigation.

O-7

Clinical Proton beam induced DNA damage and its repair mechanism

Ariungerel Gerelchuluun

Radiation Life Sciences Research Group, Biomedical Sciences Research field

The usage of protons in the treatment of various cancers has accelerated due to their excellent dose localization to tumors while preserving the surrounding healthy normal tissues. Although, its biological characteristics, in particular, DNA damage and repair mechanisms are not fully understood. It is known that the majority of DNA double-strand breaks (DSBs) induced by ionizing radiation are repaired either by non-homologous end-joining (NHEJ) or by homologous recombination (HR) pathways. However, how NHEJ and HR pathways contribute to the repair of DSB induced by clinical proton beams have not been clarified yet.

In this study, we aimed to identify the roles of NHEJ and HR pathways in the repair of the DNA lesions induced by proton, using Chinese hamster cells those are defective of either NHEJ or HR pathways. After irradiating these cells with γ-rays or different depth of clinical proton beams (plateau part and Spread out Bragg peak (SOBP)) with 200 MeV, we evaluated clonogenic survival, cell cycle distribution and genome stability maintenance using chromosomal aberration detection.

As a result, first we found that the extent of cell survival was clearly dependent on the presence or absence of NHEJ and HR pathway factors as well as the radiation quality. Interestingly, we noticed that the radiosensitivity of HR deficient cells does not affected by different depth of proton beam. In contrast, radiosensitivity of NHEJ pathway deficient cells were increased after in the middle of SOBP of proton beam, compared to other depth of proton beam. Second, in contrast to radiosensitivity, the extent of chromosomal aberrations was not significantly different between NHEJ and HR defective cells, as a function of LET, suggesting that not all chromosome aberrations lead to cell death. Taken together, utilization of a specific DSB repair pathway in cells is clearly dependent on the nature of the DNA lesions induced by various radiation sources.

Taken together, our results suggest that NHEJ might play a dominant role in repairing DNA lesions induced by proton beams.

O-8

A Common Host-Pathogen Interaction as Broad-Spectrum Antiviral Drug Target

Sisley Austin[1], Said Taouji[2], Fabienne Rayne[1], Eric Chevet[2], Harald Wodrich[1]

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[2]*Department of Research for the Study of Liver, Team Avenir, Inserm U889, Université of Bordeaux, France*

In contrast to braod-spectrum antibiotics, most antiviral drugs target very specific functions of specific viruses (e.g., protease inhibitors for the HIV-1 protease). Ideally to develop a broad acting antiviral treatment one has to identify a common drug target preserved in several unrelated virus families.

Here we identify late domains of the PPxY-type as new host-pathogen interaction and convert it into target for drug-screening. PPxY motifs interact with proteins containing WW domains and are present in several viral proteins where they serve to recruit cellular pathways. PPxY-WW domain interactions are involved in early (entry, sorting and gene expression) and the late phase (budding) of some virus families (e.g., Adeno-, Retro-, Rhabdo- and Filoviruses).

Adenoviruses are a major health threat for immunocompromised patients and we recently showed that the capsid protein VI which has a PPXY domain interacts with the cellular ubiquitin ligase Nedd4 via its WW domain to mediate efficient entry and gene expression.

Based on this observation we choose this interaction to develop new and safer drugs that specifically target the PPxY-WW interfaces required during Adenovirus replication and potentially other viruses.

We used Alphascreen technology for small compound screening. This assay uses the diffusion of singlet state oxygen from donor (photosensitizer) to acceptor (chemiluminescer) microbeads. Beads are coated with proteins of interest: the viral capsid protein VI and the cellular protein Nedd4. A signal is produced when the beads are brought by the PPxY-WW interaction occurring between the binding partners. A large range of chemical components was tested to find potential inhibitors with broad-spectrum antiviral drug potential.

O-9

Skeletal Muscle Regeneration Research for Diaphragmatic Hernia Treatment

Koki Hagiwara

Department of Pediatric Surgery

Diaphragmatic hernia, especially Bochdalek hernia, is a congenital malformation of the diaphragm occurring in 1/2200~1/12500 newborns per year. Due to the hole, digestive organs invade into the chest cavity and interrupt the left lung maturation. To treat this disease, patching the hole by an artificial material, such as Gore-Tex®, is commonly done. However, the patch will not expand with child growth resulting in a distortion of the diaphragm shape along with an immature left lung. Therefore, using skeletal muscle precursor cells, called skeletal myoblast, were focused on since they can grow with the children and the skeletal myoblast fate is limited into skeletal muscle majorly. First, gelatin hydrogel microspheres, which can gradually release basic fibroblast growth factor (bFGF) for 4 weeks, were injected into the rats' hind limb muscle tissue with the EGFP myoblasts. The purpose of using bFGF was to increase the transplanted cells' survival rate by enhancing angiogenesis. This resulted in improved EGFP cell survival rate, myogenesis, and angiogenesis; however, the remaining cell number seemed low still. Transplantation of myotubes might stay in target area rather than cells which tend to migrate. On a culture dish, myotube formation happened in random directions in comparison with the aligned skeletal muscle tissues in vitro. It was known that culturing in a rectangle groove pattern could align myotubes, but its optimal width varied by research. Therefore, 1000μm × 5, 10, 25, 50, 200, and 400μm grooves were made on a culture plate by azidophenol poly vinyl alcohol ridges. The result mainly demonstrated three types myotubes based on the groove width. The 5 and 10μm width grooves made net like structure myotubes. The 25 and 50μm could make a few straight myotubes. Then, the 200 and 400μm width groove grew a few very thick myotubes along with many very thin myotubes. Currently, an aligned triangular prism sheet for aligned myotube culture is being made of alginic acid. The aim of this study is making an aligned myotube sheet for transplantation. The alginic acid sheet is constructed by alternatively aligned triangular prisms which are coated by type I collagen for cell attachment. In near future, after the seeded myoblasts differentiate into myotubes, the sheet will be dissolved by sodium bicarbonate. Then, the aligned myotubes can be collected for transplantation.

O-10

Autocrine Transforming Growth Factor-β Induces TMEPAI and Promotes Tumor Formation in Lung Cancer Cells

Thanh Thao Vo Nguyen, Yukihide Watanabe, Susumu Itoh, and Mitsuyasu Kato

Graduate School of Comprehensive Human Sciences, Laboratory of Experimental Pathology

TMEPAI is a transmembrane prostate androgen-induced RNA that was originally identified as a prostatic RNA, the synthesis of which is induced by testosterone or its derivatives. We have recently identified TMEPAI as a direct target gene of TGF-β/Smad signaling that participates in negative feedback control of the duration and intensity of TGF-β/Smad signaling. TMEPAI is constitutively and highly expressed in many types of cancers and is associated with poor prognosis. Here, we report that TMEPAI is highly expressed in the lung adenocarcinoma cell lines Calu3, NCI-H23, and RERF-LC-KJ. Expression of TMEPAI in these cancer cells was significantly suppressed by a TGF-β receptor kinase antagonist, SB208, and by TGF-β neutralizing antibodies. These results suggest that constitutive expression of TMEPAI in these cancer cells depends on autocrine TGF-β stimulation. Knockdown of TMEPAI in Calu3 cells enhanced levels of Smad2 phosphorylation and significantly suppressed cell proliferation in the presence of TGF-β-stimulation, indicating that highly expressed TMEPAI suppresses levels of Smad phosphorylation in these cancer cells and reduces the growth inhibitory effects of TGF-β/Smad signaling. Furthermore, knockdown of TMEPAI in Calu3 and NCI-H23 cells suppressed sphere formation *in vitro* and tumor formation in subcutaneous tissues and metastatic lung tumor formation after tail vein injection in NOD-SCID mice *in vivo.* Together, these experiments indicate that autocrine TGF-β stimulates TMEPAI expression to promote tumorigenic activities in lung cancer cells.

O-11

A Novel Diagnostic Method For AITL By Detecting RHOA G17V Hotspot Mutation Using Allele-Specific Real-time PCR

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[Backgrounds] Angioimmunoblastic T-cell lymphoma (AITL) is a distinct subtype of T-cell lymphoma, characterized by generalized lymphadenopathy and frequent autoimmune-like manifestations. The diagnosis of AITL is sometimes challenging, because the tumor cell content is generally low and relatively large reactive lymphocytes are confused as tumor cells. We identified recurrent mutations in *RHOA* at c.G50T, predicting to generate p.G17V in 70% of AITL and PTCL-NOS harboring AITL features, which implies possibility of developing a new diagnostic method for AITL (M S-Y and SC, unpublished). [Purpose] To establish a novel cost-effective method to diagnose AITL, we performed allele-specific real time PCR to detect *RHOA* G17V mutation. [Methods] Genomic DNA was extracted from 119 AITL and PTCL-NOS samples, which include 47 periodate-lysine-paraformaldehyde (PLP)-fixed, 12 formalin-fixed-paraffin-embedded (FFPE) and 60 frozen tissues. Forty-one out of 60 genomic DNA samples, purified from frozen tissue, were amplified by RepliG kit (QIAGEN). Allele-specific primers for *RHOA* G17V mutant and wild-type sequences were designed by Wangkumhang's algorithm. The [mut] and [WT] values were individually measured by real time PCR using each primer set, and the [mut] / ([mut]+ [WT]) values were calculated. Mutant allele frequencies were determined by amplicon-based deep sequencing using MiSeq. [Results] The [mut] values were distributed from 1.5×10-7 to 7.6×10-2, and the [WT] values were from 7.9×10-5 to 1.3×10-2. The [mut]/ ([mut]+ [WT]) values were distributed from 1.9×10-4 to 8.5×10-1. We set a cut-off value to determine existence of mutation as 2.0% for MiSeq, and 1.3×10-2 for the [mut]/ ([mut]+ [WT]) value. Then we compared these two methods to detect *RHOA* G17V mutation. Forty-three cases were positive for the *RHOA* mutation in this study cohort by MiSeq, including 32 AITL and 11 PTCL-NOS cases. The [mut]/ ([mut]+ [WT]) values of DNA from FFPE samples tended to be lower than those from other samples, and 4 out of 12 FFPE samples determined as mutation-positive by MiSeq were not detected by our allele-specific realtime PCR. We therefore excluded the FFPE samples and analyzed the data of 107 DNA samples, purified from PLP-fixed and frozen tissues. Rank correlation coefficient was 0.753. Sensitivity and specificity were 97.4% and 97.1%, respectively. Positive and negative concordance rates were 94.9% and 98.6%, respectively. [Conclusions] We established a method to detect the *RHOA* G17V hotspot mutation for AITL. It is expected to be highly accurate and cost effective.

O-12

ErbB2 Signaling and ECM Stiffness Cooperate to Drive Breast Tumor Progression in 3D

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Evidence suggests that increased Extracellular Matrix (ECM) stiffness, acting via mechanotransduction, cooperates with oncogenes to sensitize mammary epithelial cells (MECs) towards malignancy and increase metastatic potential. The ErbB2 oncogene is of particular interest because it is over-expressed in 20-30% of invasive cancers and 85% of Ductal Carcinoma In Situ. Furthermore, ErbB2 downstream targets suggest cooperation with mechanoreceptor pathways.

In natural ECMs, stiffness is commonly regulated via changes in concentration or cross-linking, changes that have many additional, indirect effects. We therefore cultured MCF10A and MCF10A.ErbB2 cells in 3D Matrigel/Collagen gels in which stiffness was tuned independent of concentration. Optical tweezers active microrheology (AMR) was used to map ECM stiffness distribution and correlate it with adhesion signaling. MEC growth-arrested acini were exposed to increases in either stiffness or ErbB2 signaling or both. Acini morphology and molecular distributions were characterized by non-linear microscopy.

We observed a significant amplification of invasive behavior when ErbB2 signaling and ECM stiffness were increased together. Contrary to findings with 3D-*overlay*, 3D-*embedded* cultures also showed a significant increase in invasion with ErbB2 signaling alone. These results highlight the importance of the tumor microenvironment in increasing MEC sensitivity though the cooperation of mechanotransduction and oncogene pathways.

O-13

Identifying positive regulators of reprogramming using RNA interference

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Since Yamanaka and Takahashi first described the isolation of induced pluripotent stem cells (iPSCs) in 2006, researchers have invested a vast amount of time and resources into trying to understand the process of reprogramming. However, the exact mechanisms underlying the induction of somatic cells to pluripotency is still incompletely understood. With this in mind, we have undertaken a screening approach to identify shRNA that enhance the reprogramming process. We used a retrovirus based system to knock down candidate genes during reprogramming of mouse embryonic fibroblasts (MEF) containing doxycycline-inducible reprogramming factors and a Nanog-GFP reporter which is activated when cells become iPSCs. The initial round of screening with 140 shRNA vectors successfully identified several candidates that enhance reprogramming. One of these shRNA vectors exhibited both faster reprogramming kinetics as determined by activation of the Nanog-GFP reporter 2 to 3 days earlier and increased reprogramming efficiency giving rise to >5 fold more GFP+ colonies when compared with a control. Cell surface marker analysis with flow cytometry demonstrated that changes in CD44 and ICAM1 expression, which occur preceding Nanog-GFP expression, were also accelerated. Moving forward, we have carried out a microarray analysis to further investigate the functional importance of this knock down and its role in establishing the pluripotency transcriptional network during reprogramming.

O-14

Reward value coding in the monkey orbitofrontal cortex

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In our daily life, we often choose one of alternatives by considering their values and efforts to obtain them. To understand the neuronal mechanism of such decision-making process, we developed a decision-making schedule task and recorded single neuronal activity from monkey orbitofrontal cortex (OFC) which has been reported to be one of the important brain areas for reward-guided behaviors.

The monkey was initially trained to perform a reward schedule task. In this task, the monkey had to complete the schedule composed of 1, 2 or 4 trials of visual discriminations to earn 1, 2 or 4 drops of liquid reward. After the monkey learned this task, the decision-making schedule task was introduced. The decision-making schedule task was consisted of the decision-making part and the reward schedule part. In the decision-making part, two kinds of choice target were presented Brightness and length of the choice targets were proportional to amount of liquid reward (1, 2 or 4 drops) and required number of the visual-discrimination trials (1, 2 or 4 trials) to be performed, respectively. After choice targets were presented sequentially, these two targets were reappeared side by side simultaneously and the monkey was required to choose one of the two choice targets by touching the corresponding bar (left or right) in the chair. Following a choice of one target, the chosen reward schedule task was started.

A large proportion of recorded OFC neurons (134/137; 97.8%) responded to task events in the decision-making part and/or the reward schedule part of the task. Some neurons (20/134: 14.9%) showed characteristic activities during the sequential presentation of the choice targets in the decision-making part. 10/20 neuronal firings were correlated with the differential value of the two choice targets. The remaining 10 neurons showed larger (n=6)/smaller (n=4) responses when the two target values were close. The result suggests that OFC neurons have an important role in reward value information processing during the decision-making.

O-15

Identification and characterization of a novel CD300 molecule, CD300H

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Human CD300, a multigene family consisting of seven genes on a segment of human chromosome 17, are immunoreceptors preferentially expressed on myeloid lineage cells and mediate either activating or inhibitory signals, suggesting that they play important roles in immune regulation.

By analyzing NCBI database, we identified a previously un-annotated gene located in the CD300 gene cluster, and isolated a gene from a $CD14^+$ peripheral blood monocytes-derived cDNA by RT-PCR. This gene encodes an immunoglobulin-like receptor containing a short cytoplasmic domain and a positively charged lysine residue in its transmembrane region. This molecule encoded by this gene has provisionally been designated CD300H.

A monoclonal antibody against CD300H (TX93) was generated and used for the study of expression and function of CD300H. Expression analysis of CD300H on human PBMC by flow cytometry showed that CD300H was expressed on myeloid dendritic cells ($HLA\text{-}DR^+CD11c^+$) and $CD16^+$ monocytes. To identify the adaptor protein associated with CD300H, a human monocytic cell line, THP-1, expressing Flag-tagged CD300H were generated, and used for co-immunoprecipitation experiments. Immunoprecipitation of CD300H with anti-Flag mAb demonstrated that CD300H was associated with DAP12, but not with FcRγ chain in THP-1 cells, suggesting that CD300H may transduce activating signals via DAP12 in human dendritic cells and monocytes. Thus, CD300H may play an important role in regulation of inflammatory responses.

O-16

Initial clinical experience of movable, ceiling-mounted, intraoperative MRI

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Object. Intraoperative MRI (iMRI) is known to contribute to improve extent of removal and prevention of operative complication in a brain tumor surgery. Movable, ceiling-mounted iMRI has a great advantage because it is unnecessary to move patient, which provides safety for patient and less stress for all medical staff. In addition, a dual-room concept, which facilitate both surgical and diagnostic use, enable to share the cost. We have introduced movable, ceiling-mounted, high-field iMRI in dual room concept and present the initial clinical experience.

Methods. From January to August, 2013, thirty-one patients with brain tumor underwent surgical removal using the iMRI system. The pathologies of these patients were 6 low grade gliomas, 12 high grade gliomas, 2 brain metastases, 1 AT/RT, 2 pituitary adenomas, 1 craniopharyngioma, 1 cavernoma, 1 chondrosarcoma, 1 epidermoidcyst, and 2 menigiomas. In addition, biopsy was done for 2 patients with malignant lymphoma. Our system integrated VISIUS Surgical Theatre (IMRIS inc.) based on 1.5-tesla magnet with using seismic system and ceiling-mounted navigation system (Brain Lab inc.).

Result. In all patients, high-quality images including intraoperative tractography were rapidly and reproducibly acquired at various stages of the surgical procedures. Interruption time until starting MRI and restart of operation were 27±8 minutes and 83±15 minutes, respectively. Extent of removal increased in 19 of 29 cases using iMRI. There was no iMRI related complication.

Discussion. Movable, ceiling-mounted iMRI provides us high quality images that contribute to improve removal rate. Since, on surgical stage, iMRI is out side operation room, our surgical staff can concentrate for the procedure. On taking iMRI stage, transfer is very smooth even if initial experience. This system is user-friendly for all medical staff and provides us safety.

O-17

Studying the effect of various *Propionibacterium acnes* strains on the cellular functions of human keratinocytes

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Acne is a common multifactorial inflammatory skin disease of the pilosebaceous unit, in which the *Propionibacterium acnes (P. acnes)* bacterium is an important pathogenic factor.

To investigate the effect of the bacterium on the cellular properties of cultured immortalized keratinocytes (HPV-KER), we treated them with three *P. acnes* strains (*889, 6609, ATCC11828*) and analyzed their proliferation and viability in real-time, using the xCELLigence system.

We found that the non-pathogenic *P. acnes 6609* strain had no measurable effect in any applied doses. In contrast, the *P. acnes 889* strain in high doses induced increased cell proliferation. Later the pathogenic *P. acnes 889* and *ATCC11828* strains in high doses resulted the death of HPV-KER cells. This may be the effect of a pore-forming exotoxin (CAMP factor), expressed by these latter two strains.

We also analyzed the strain and dose specific signaling differences induced in HPV-KER cells, and found that the activation of NF-κB transcriptional factor dose dependently increases, shown by Western blotting. Parallel to that, the mRNA expression of the TNFα and IL-1α pro-inflammatory cytokines also increases at 6 hours post-treatment.

These results suggest that various *P. acnes* strains have strain and dose dependent effect on the cellular functions of human keratinocytes, and these may determine the severity of skin symptoms.

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O-18

Non-cell autonomous effects on integrin signaling in the chick embryonic CNS

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Integrins are known to regulate key aspects of stem cell behaviour. Previously we have shown integrin-α6 and β1, which heterodimerise into a laminin receptor, are highly expressed within the neural stem cell (NSC) niche of the embryonic ventricular zone (VZ). Loss of integrin-β1 (itgβ1) function within the VZ of the embryonic mouse results in NSC detachment and apoptosis. We found that disrupting itgβ1 in the VZ using blocking antibodies resulted in a change in the angle of NSC division and loss of apical process attachment within the VZ, decreasing asymmetric divisions. We also observed that RNAi-mediated knockdown of itgβ1 in the chick mesencephalon increased apoptosis and NSC detachment (unpublished), consistent with findings in the mouse. We would therefore predict that perturbing itgβ1 signalling will affect neurogenesis. To test this we are using *in ovo* electroporation of the chick embryonic midbrain to allow targeted expression of human itgβ1 constructs in the NSCs lining the ventricle of the developing mesencephalon (E2-E4/HH10-22). We observed that over-expression of a constitutively-active human itgβ1 resulted in a significant increase in neurogenesis. However, this increase in neurogenesis occurs as a non-cell autonomous effect; none of the additional neurons expressed GFP, the marker of transfection. Using microarray analysis of FACS sorted GFP+ and GFP- cells, allowing separation of itgβ1-expressing cells and their neighbours, we have identified up-regulation of ECM molecules shown to enhance neurogenesis. Together these results suggest a model whereby integrin signaling increases neurogenic signals in the GFP- cells whilst promoting self-renewal of the GFP + cells.

O-19

Characterization of human adipose tissue-derived mesenchymal stem cells for diabetic complications

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Diabetes mellitus type 2 is a metabolic disorder that is characterized by high blood sugar in context of insulin resistance. Patients with hyperglycemia often cause abnormalities in blood flow, microvascular cell loss, and lack of trophic factors. Consequently, it becomes ischemia and hypoxia leading to diabetic complications. Stem cell therapy is a new therapeutic approach of regenerative medicine to solve the problem of diabetic complications. Therefore, it is important to investigate whether stem cells from diabetic donors are similar as non-diabetic donors.

In this study, we demonstrated that adipose tissue-derived mesenchymal stem cells from diabetic donors (dAT-MSCs) had good ability to differentiate into osteoblasts, adipocytes, and chondrocytes. Compared to non-diabetic donor-derived AT-MSCs (nAT-MSCs), dAT-MSCs showed the abnormal adhesive phenomenon under prolonged hypoxic stress. We found that dAT-MSCs with ALDH high activity (Alde-High dAT-MSCs) showed the abnormal adhesive phenomenon, but not in dAT-MSCs with ALDH low activity (Alde-Low dAT-MSCs). The two transcription factors, early growth response factor-1 (EGR-1) and hypoxia inducible factor-2α (HIF-2α), were significantly increased in Alde-High dAT-MSCs under hypoxic condition compared to Alde-High nAT-MSCs. Additionally, EGR-1 and HIF-2α upregulated the expression of growth factors bFGF, VEGF, and TGFβ, as well as adhesive molecules CYR-61, VCAM-1, Ang-1, integrin αv in Alde-High dAT-MSCs than those of Alde-High nAT-MSCs under hypoxic condition.

Furthermore, Alde-High dAT-MSCs were impaired their wound healing ability in ischemic flap mouse model proved by necrosis area, Hematoxylin-Eosin staining, Lectin-TRITC, and immunohistochemistry staining. Notably, some target genes of EGR-1 in Alde-High dAT-MSCs were down-regulated in EGR-1 knockdown (shEGR-1) cells and adding PD98059, ERK1/2 inhibitor. Alde-High dAT-MSCs with shEGR-1 rescued wound healing ability in ischemic flap model.

Our results suggest that Alde-High dAT-MSCs may be impaired their functions under hypoxic conditions that may lead to microvascular damage and delayed wound healing *in vivo*. The loss of EGR-1 function in Alde-High dAT-MSCs restored cell function and wound healing ability. This work provides *in vitro* and *in vivo* evidence for a new therapeutic target of preventing vascular disease and ischemic tissue in high-risk diabetic population.

O-20

Crucial role of Elovl6 in brain development and function

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Background and Objective: ELOVL family member 6, elongation of very long chain fatty acids (Elovl6) is a microsomal enzyme that specifically catalyzes the elongation of C12-16 saturated and monounsaturated fatty acids (FAs). We have shown previously that Elovl6 is a major target for sterol regulatory element binding proteins in the liver and that it plays a critical role in the development of obesity-induced insulin resistance by modifying FA composition. Elovl6 is highly expressed also in brain. In this study, we investigated the roles of Elovl6 in brain development, function, and psychological diseases in mice.

Methods: We generated Elovl6 deficient mice and brain-specific Elovl6 deficient mice. We observed brain morphology by H&E stain, Klüver-Barrera stain, Golgi stain, and immunostaining for Neuron (Tuj1) and astrocyte (GFAP). We analyzed neurogenesis by 5-Bromo-2'-deoxyuridine-labeled (BrdU) incorporation. Behavioral aspects of the Elovl6 mutant mice were analyzed by Open field test, Morris water maze, Elevated plus maze, and Rotarod. We examined the role of Elovl6 in the proliferation and differentiation process in hippocampal primary culture cells.

Results: Elovl6 deficiency in brain caused decreased the contents of stearate (C18:0) and increased contents of palmitate (C16:0) compared to control. The weight of the brain, especially hippocampus, was significantly heavier in Elovl6 deficient mice than in wild- type mice. Mutant hippocampus exhibited decreased neural precursors, decreased spine density, and increased astrocyte. Memory impairment and enhanced anxiety were observed in Elovl6 deficient mice. Moreover, Elovl6 deficiency increased proliferation and differentiation of astrocyte and neuron in primary hippocampal culture cells.

Conclusion: Our results suggest that the abnormal regulation of fatty acid composition by Elovl6 can underlie impaired brain development, macrocephaly, and behavioral abnormalities such as anxiety and depression.

O-21

ACAP3 functions as an Arf6-specific GAP to control neurite outgrowth in hippocampal neuron

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The mammalian small GTPase ADP-ribosylation factor (Arf) family consists of six related gene products, Arf1-6, which are divided into three classes based on their sequence homology. Class I includes Arf1, Arf2 and Arf3, class II Arf4 and Arf5, and class III Arf6. The sole member of class III, Arf6, plays important roles in a wide variety of cellular events, including exocytosis, endocytosis, actin cytoskeleton reorganization, and neurite outgrowth in neuronal cells. Like other small G proteins, the GTP/GDP cycle of Arf6 is precisely regulated by its guanine exchange factor (GEF), which accelerates GTP binding to Arf6, and GTPase activating protein (GAP), which stimulates hydrolysis of bound GTP to GDP. Although previous studies have indicated that Arf6 is involved in neurite outgrowth in developmental neuron, it remains to be elucidated how GTP/GDP cycle of Arf6 is controlled in this process.

Here we show that one of Arf GAPs, ACAP3 functions as an Arf6-specific GAP and regulates Arf6-mediated neurite outgrowth in mouse hippocampus neuron. GTP-Arf pulldown assay revealed that ACAP3 possesses GAP activity for Arf6, but not for other classes of Arf family, Arf1 and Arf5. Moreover, ACAP3, unlike other ACAP family proteins, ACAP1 and 2, is highly expressed in the mouse brain. ACAP3 co-localized with Arf6 at the neurite tips of the primary cultured hippocampal neuron, and shRNA-mediated depletion of ACAP3 markedly suppressed the neurite outgrowth. Expression of shRNA-resistant ACAP3-WT, but not the GAP-inactive mutant R446Q, rescued the impairment of neurite outgrowth, suggesting that ACAP3 regulates neurite outgrowth in a GAP activity-dependent manner. Finally, we found that depletion of Arf6 also inhibited the neurite outgrowth, demonstrating that Arf6 itself is necessary for the neurite outgrowth.

These results indicate that ACAP3 functions as an Arf6 GAP in the hippocampal neuron to regulate neurite outgrowth by promoting hydrolysis of GTP bound to Arf6.

O-22

Improvement of vascular function by magnetic nanoparticle-assisted gene and endothelial cell transfer in vessels

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Cardiovascular diseases are amongst the leading causes of death worldwide. They preferentially develop after vascular injury due to atherosclerosis and are characterized by endothelial dysfunction.

In order to restore vascular function in injured vessels, we developed strategies for the site-directed transduction of native endothelium and for the site-specific endothelial cell replacement by the combined use of lentiviral vectors (LVs), magnetic nanoparticles (MNPs) and magnetic fields. In an ex vivo flow-loop system we could achieve a radially symmetric transduction of the endothelial layer of mouse aortas. When using therapeutically relevant genes such as endothelial nitric oxide synthase (eNOS) or vascular endothelial growth factor (VEGF), we could demonstrate their overexpression in vessels using qRT-PCR, Western Blot and ELISA analysis. Similarly, the endothelium of denuded arteries could be successfully replaced by MNP-loaded endothelial cells, which had been transduced beforehand in vitro and were found to overexpress eNOS. Enhanced nitric oxide (NO) production could be corroborated via the use of the NO fluorescence indicator DAF-FM and a cGMP ELISA. We could also demonstrate NO-dependent modulation of vascular tone in eNOS re-expressing vessels compared to eNOS-/-controls using isometric force measurements. Similarly, elevated VEGF levels in the endothelial layer of mouse aortas induced enhanced angiogenesis in an aortic ring assay.

Thus, we show promising strategies to restore physiological function in vessels for the treatment of endothelial dysfunction.

O-23

Both CTPS1 and CTPS2 Can Form Cytoophidium in Mammalian Cells

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Cytosine triphosphate synthase (CTPS) catalyzes the rate-limiting step in which an UTP is converted into a CTP with the consumption of an ATP and a glutamine during CTP *de novo* synthesis. Previously, independent groups reported a filamentous structure, which was composed by CTPS, in bacterium, yeast, fruit fly and mammalian cells. This structure was termed "cytoophidium" according to its special shape. In yeast, the appearance of cytoophidium altered along with the change in environmental nutrients and nucleotide concentrations, suggesting polymerization might be a way to regulate CTPS activity. Human genome contains two CTPS isoforms encoded by CTPS1 and CTPS2. These two isoforms are more than 74% identical. Knowledge about the interactions of CTPS1/CTPS2 and cytoophidium still remains unclear. Therefore, the aim of this study is to determine the localization of CTPS1 and CTPS2 proteins in mammalian cells. Firstly, CTPS1-GFP and CTPS2-GFP fusion proteins were generated to represent the localizations of CTPS1 and CTPS2. Ectopic expression of CTPS1-GFP and CTPS2-GFP did not affect the endogenous expression level of CTPS1 in human 293T cells, but promoted the formation of enlarged cytoophidium. Secondly, the glutamine transaminase domain from CTPS1-GFP and CTPS2-GFP constructs were deleted. Interestingly, truncated CTPS1-GFP (SD1-GFP) was diffused in cytoplasm, whereas large amounts of truncated CTPS2-GFP (SD2-GFP) accumulated in mitochondria. In addition, cells that expressed either SD1-GFP or SD2-GFP failed to form cytoophidium while endogenous CTPS1 expression level was not changed, argued that glutamine transaminase domain is crucial for the formation of cytoophidium in mammalian cells. Taken together, these results demonstrated that CTPS1 and CTPS2 show distinct distributions, but both of them are able to form cytoophidium, which provided basic information to study intracellular compartmentation and roles of the cytoophidium in cell metabolism.

P-1

Pbp1 is involved in the Ccr4 and Khd1-mediated regulation of cell wall synthesis through the association with ribosome

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The Saccharomyces cerevisiae Pbp1 [poly(A)-binding protein (Pab1)-binding protein] is believed to be involved in RNA metabolism and regulation of translation, since Pbp1 regulates a length of poly(A) tail and is involved in stress granule (SG) formation. However, a physiological function of Pbp1 remains unclear, since the *pbp1Δ* mutation has no obvious effect on cell growth. In this study, we showed that *PBP1* genetically interacts with *CCR4* and *KHD1*, which encode a cytoplasmic deadenylase and an RNA-binding protein, respectively. Ccr4 and Khd1 modulate a signal from Rho1 in the cell wall integrity pathway by regulating the expression of RhoGEF and RhoGAP, and the double deletion of *CCR4* and *KHD1* confers a severe growth defect displaying cell lysis. We found that the *pbp1Δ* mutation suppressed the growth defect caused by the *ccr4Δ khd1Δ* mutation. The *pbp1Δ* mutation also suppressed the growth defect caused by double deletion of *POP2*, encoding another cytoplasmic deadenylase, and *KHD1*. We then screened novel Pbp1-interacting factors and found that Pbp1 interacts with ribosomal proteins Rpl12a and Rpl12b. Similarly to the pbp1 mutation, the *rpl12aΔ* and *rpl12bΔ* mutations also suppressed the growth defect caused by the ccr4 khd1 mutation. Our results suggest that Pbp1 is involved in the Ccr4- and Khd1- mediated regulation of cell growth through the association with Rpl12a and Rpl12b.

P-2

Enhanced Proliferation of Adipose-derived Stem Cells by Defined Medium Supplemented with Growth Factors

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Insulin deficiency resulting from pancreatic β-cell destruction leads to type 1 diabetes which accounts for 5–10% of all cases of diabetes. Due to the developmental plasticity of mesenchymal stem cells (MSC), MSC-based therapeutic intervention has become a promising strategy to replace injured tissues such as islets. However, a major obstacle of applying MSC is required large amount of MSC for transplantation and significant cell loss after transplantation. To tackle this issue, we use adipose-derived stem cells (ADSC) owing to its ease of isolation, abundant source and multipotency and seek to further enhance its proliferative ability and differentiation into β-cells. Isolated ADSCs from porcine back fat region of subcutaneous adipose tissue (pADSC) were cultured in different basal medium supplemented with or without growth factors and determine the proliferation and pluripotency of pADSC by MTT and gene expression pattern, respectively. Our results demonstrated that pADSCs had higher proliferative rate and pluripotency when cultured in α-MEM compared to DMEM/F12. Supplementation with fibroblast growth factor 4 (FGF4), fibroblast growth factor 2 (FGF2) and insulin-like growth factor 1 (IGF-1) increased the pADSC proliferation. To conclude, in this study, we determined the optimal culture condition for pADSC in α-MEM supplemented with either FGF4, FGF2 or IGF-1. These pre-conditioned ADSCs provide a potential strategy to augment MSC proliferation and differentiation for transplantation and shed new light on treating type 1 diabetes or other autoimmune disease.

P-3

Functions of RNA polymerase I transcription factor UBF in nucleolar formation

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The nucleolus is a nuclear domain observable under light microscopy. The primary function of the nucleolus is ribosome biogenesis that includes ribosomal RNA precursor (pre-rRNA) transcription, modification, and processing. The nucleolar structure is disrupted upon entry into mitosis and formed in early G1 phase. Before nucleolar assembly, pre-rRNA and factors involved in pre-rRNA modifications and processing form RNA-protein complexes (RNP) and are accumulated in small granules outside of the nucleolus. These RNPs are then recruited to a chromosome region termed nucleolar organizer region (NOR) containing the rRNA gene repeats to form mature nucleolar structure. However, the molecular mechanism by which the RNPs are assembled to the NOR remains unknown. Our research goal is to elucidate the molecular mechanism of nucleolar formation. We first examined the association of RNPs with NOR by chromatin immunoprecipitation (ChIP) assay. The RNPs were found to be associated with NOR in asynchronous cells, and this association was disrupted in mitosis. The association between RNPs and NOR were recovered during early G1 phase, and this process was independent of pre-rRNA transcription. These results indicate that pre-rRNA transcription is dispensable for the interaction between RNPs and NOR. In order to identify a factor(s) mediating the association between RNPs and NOR, we focused on a transcription factor Upstream Binding Factor (UBF), because UBF was previously reported to be involved in maintenance of NOR integrity. ChIP assays with HeLa cells treated with UBF siRNA revealed the association of RNA polymerase I and nucleolar RNPs with NOR depending on UBF. To test whether UBF is necessary and sufficient to mediate the association between RNPs and NOR, Lac repressor-fused UBF1 was tethered on ectopically integrated Lac operator sequences, and the localization of nucleolar RNPs were examined. Unexpectedly, RNPs were not recruited to the ectopic UBF locus. These results indicate that UBF is necessary but not sufficient for the association between RNPs and NOR. We are trying to find a factor(s) collaborating with UBF to mediate RNPs-NOR interaction.

P-4

Identify the Topoisomerase II Isozyme-specific Targeting Agents and Investigate their Biological Responses

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Topoisomerase II (TOP2) plays crucial roles in cells and TOP2-targeting drugs are effective anticancer drugs but with side-effects. There are 2 types of TOP2-targeting agents: (i) Poisons stabilize TOP2 cleavable complex (TOP2cc) and induce DNA break; (ii) Inhibitors only interfere with catalytic activity and antagonize the poisoning action. In human cells, the alpha (α) and beta (β) isozymes share similar enzymatic action but play differential functions. Etoposide (VP-16), an active TOP2 poison, induces 2nd malignancies. Notably, TOP2b is mainly responsible for the VP-16-induced DNA sequence rearrangement and carcinogenesis as well as the doxorubicin-induced cardiotoxicity. We screened anthracenedione derivatives and identified potential TOP2 isozyme-specific poisons and/or inhibitors. First, MTT cytotoxic assay was used to detect cell viability of HL60 and TOP2-deficient MX2 cells after exposure to drugs. The inhibitory ability of TOP2 activity was assessed by an In Vitro relaxation assay. We then selected compound CL-14 that preferentially inhibits TOP2β relaxation activity for further study. Using comet assay we found that CL-14 induced minimal DNA break, but antagonized VP-16-induced DNA cleavage. TOP2β-mediated DNA cleavage induced by VP-16 In Vitro was also antagonized by CL-14. Together, we suggested that CL-14 is a potential TOP2β-specific inhibitor, which is clinically helpful in preventing TOP2-targeting side-effects. In agreement with above notion, our data also revealed that CL-14 still antagonized VP-16-induced DNA break in TOP2α-knockdown cells. A structure-based study has also been initiated to unravel the molecular mechanism(s) underlying this isozyme-specific action.

P-5

DNA-binding Activity of CTCF, an Insulator Protein Regulated by Phosphorylation in Mitosis

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In the cell nucleus, the genomic DNA is packed into chromatin structure with a variety of proteins. Chromatin components are not uniformly distributed on the DNA, but dynamically changed depending on nuclear events such as transcription, replication and cell cycle progression. In mitosis, chromatin was highly compressed. The condensation of chromatin reduces the accessibility of transcription factors and RNA polymerases to DNA. Mitosis is also characterized by protein phosphorylation. Many transcription-related proteins are excluded from condensed chromosomes through phosphorylation.

CTCF (CCCTC-binding factor) is a conserved and ubiquitously expressed nuclear protein. It was originally identified as a repressor of the chicken *c-myc* gene. The analysis of experimentally identified CTCF binding sites, suggests various functions of CTCF such as transcriptional activator, insulator, and mediator of interchromosomal interaction. Recent genome-wide analyses across multiple cell types or spices suggest that CTCF may play fundamental and conserved roles for gene expression.

Here we show by the biochemical fractionation of mitotic cells that CTCF is highly phosphorylated in mitosis and partially released from condensed chromosomes. We found that serine and threonine residues in the linker regions of the CTCF zinc finger domain were phosphorylated in mitosis. CTCF proteins purified from mitotic extracts was found to have reduced DNA binding activity *in vitro* and phosphatase treatment resumed the activity. Exogenously expressed mitotic phospho-mimetic mutants of CTCF showed reduced chromatin binding property in the interphase. From these results, we conclude that the mitotic phosphorylation of DNA-binding domains of CTCF reduces the affinity to the DNA.

P-6

Calcium Influx-induced Proteolytic Cleavage of Topoisomerase 2β

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In cells, Ca^{2+} homeostasis affects many kinases and proteases as well as subsequent cellular responses upon different stimuli. Here, we used a Ca^{2+} ionophore, ionomycin, to induce Ca^{2+} influx and mimic the microenvironment of elevated intracellular [Ca^{2+}]i. We had reported hTOP1 is a novel nuclear substrate of calpains with the possible involvement of calapin 2. Interestingly, hTOP2β but not hTOP2α was rapidly cleaved upon cellular exposure to ionomycin treatment. The cellular location of hTOP2β was also affected after ionomycin treatment. This proteolytic cleavage of hTOP2β requires the activation of calpains in the presence of Ca^{2+} -influx suggesting hTOP2β is a novel substrate to calpains. In addition, either overexpression of natural calpain inhibitor calpastatin or calpain 2-targeting siRNA could dramatically abolish the Ca^{2+}-influx induced cleavage of hTOP2β. Corresponding to the above observations, cells with calpain 2 knockdown had more protein level of hTOP2β than si-vector control cells implying that calpains might regulate the stability of hTOP2β protein. In addition, this calpain 2-mediated cleavage of hTOP2β might have a potential function to improve cell resistance to VP-16 (TOP2 poison) treatment. Consistently, we found calpain 2 knockdown cells are more sensitive to VP-16 treatment. Together, our results suggest that Ca^{2+} regulates hTOP2βstability and could serve as a sensitivity determent for TOP2 drugs.

P-7

EZH2, a component of polycomb complex, regulates nuclear export of the influenza virus M1 and the viral genome

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The influenza A virus genome consists of eight single-stranded negative-sense RNAs (vRNAs). vRNA forms vRNP complexes, composed of three viral RNA polymerase subunits and nucleoprotein. vRNP is replicated through cRNA into a large number of progeny vRNA in the nucleus. The progeny vRNA forms vRNP and interacts with M1. This complex is exported from the nucleus to the cytoplasm through the CRM1-dependent pathway mediated by viral protein NS2. It has been shown that vRNP and M1 interact with cellular chromatin structure and nuclear matrix, which are involved in regulation of transcription, replication and nuclear export of vRNA. It is also reported that some chromatin- related factors regulate the influenza virus growth. However, it is not clear where vRNP and vRNP-M1 complex are exactly localized in the chromatin and whether other chromatin-related factors participate viral life cycle.

In this study, we found that EZH2, a component of polycomb complex with the histone lysine methyltransferase (KMT) activity, binds to M1. From the results of RNA immunoprecipitation (RIP) assay, EZH2 also binds to the vRNP complex. To investigate the effect of EZH2 on the viral life cycle, we examined the viral growth in the HeLa cells in the presence or absence of siRNA for EZH2. The production of progeny virions was reduced by approximately 50% in EZH2-knockdown cells compared with control cells. Next, we investigated what step EZH2 affects in the viral life cycle. The transcription and replication levels of vRNA were unchanged in EZH2-knockdown cells. However, we found that knockdown of EZH2 results in the accumulation of M1 and the virus genome in the nucleus. The mechanism how EZH2 regulates the nuclear export of M1 and vRNP is under investigation.

P-8

RecF Act as Regulators in Transcription-associated R-loop Formation and their Biological Implications

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The RNA-DNA hybrid with a single-stranded DNA (ssDNA) region and two junction of single-stranded and double-stranded is called R-loop which might occur during transcription elongation. Our previous results revealed that transcription-associated R-loop as a target substrate for activation-induced cytidine deaminase (AID) for recombination. In addition, R-loop could also serve for a replication primer, but excess amount of R-loop in cells result in genome instability. Here, we employed bacterial model system to identify cellular factors that regulate R-loop formation and explore R-loop-associated functions by the AID-stimulated mutagenesis (ASM) assay. Our results showed that the ASM level was much higher in the RecF-activated strain JC7623 (*recBC sbcBC*). Moreover, activated RecF is also responsible for other biological process including plasmid-mediated lethality, run-away plasmid replication and cellular filamentation. All of above phenotypes are suppressed by additional expression of functional RNase H or TopA, two suppressors of R-loop formation suggesting that RecF may play a positive role in the R-loop formation. Further, our results showed that additional *recA* mutation in JC7623 cannot rescue the increased ASM fold and cellular filamentation, suggesting that RecF itself plays the major role independent of RecA in recombination and cellular filamentation. We then expressed and purified RecF proteins by using *in vitro* R-loop-forming assay. We showed that RecF proteins increased RNA transcripts and R-loop formation *in vitro*. Together, we suggested that RecF plays a positive role in R-loop formation and through the regulation of R-loop formation, RecF also participate in many cellular function including genome instability and survival.

P-9

In cellulo analysis of parvovirus-mediated changes of nuclear envelope integrity by real time microscopy

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Parvovirus H-1 (PV H-1), a member of parvoviridae family, is characterized by its oncolytic and immunomodulatory effects. Currently, it is used in clinical assays, phase I / II, for the treatment of glioblastoma. PV H-1 is a DNA virus that needs to reach the nucleus to replicate its genome and to induce cell lysis. However, the nuclear import mechanism of the viral capsid remains controversial. It was reported that parvovirus minute virus of mice when injected in *Xenopus* oocytes induces holes in nuclear envelope (NE). This observation brings the hypothesis that PV could use, for their nuclear transport, a mechanism based on NE breakdown (NEBD) that usually occurs physiologically during mitosis and apoptosis. Because of the low efficiency of PV release from the endosome into the cytoplasm upon infection, in the present study we bypassed natural entry by microinjection of PV H-1 in order to analyze nuclear phenomena *in cellulo*. To evaluate NE degradation and permeability, we co-injected fluorescent markers of different sizes (Dextran 10 and 40, IgG; Stokes diameter 2.3, 7.3 and 11 nm, respectively). We followed the distribution of the markers by real-time microscopy; technically limited to frames of 10 sec. In good agreement with the natural permeability of nuclear pores for small molecules we observed a rapid nuclear entry of Dextran 10 irrespectively to the presence of PV but an exclusion of Dextran 40 and IgG in 90% of the cells. In contrast, cytoplasmic PV coinjection resulted in a loss of NE integrity as demonstrated by a nuclear entry of Dextran 40 and IgG. Dextrans 40 and IgG entry occurred in parallel, indicating that immediately after the break-down is triggered, holes rapidly exceed the size of both fluorescent markers. The NE disintegration occurred at 5-7 minutes after microinjction, which is similar to the time of NE disintegration upon mitosis. We did not found any effect upon nuclear PV injection, supporting the notion that the nuclear phenomena are linked to the virus entry instead of viral egress.

P-10

Activation of Proteolytic Systems Contributes to Cadmium Toxicity

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The health risks posted by cadmium (Cd^{2+})-related toxicity have been studied extensively and this macromolecule-thiolating metal exhibits both cytotoxic and carcinogenic activities. At the cellular level, both oxidative stress and higher proteolytic activities (e.g., degradation of Na^+/K^+-ATPase and HIF-1α) have been observed after exposure to Cd^{2+}. However, the intracellular damage(s) and action mechanism(s) for Cd^{2+} toxicity remain to be investigated. The effects of acute Cd^{2+} exposure in the activation of cellular proteolytic activities and the generation of reactive oxygen species (ROS) were determined in cells. Pharmacological inhibition approach was specifically employed to analyze the potential roles of proteases and ROS and their interplay in the Cd^{2+}-induced proteolytic systems and cytotoxicity. Acute Cd^{2+} treatment effectively induced a time- and concentration-dependent degradation of topoisomerase I (TOP1) and TOP2α, but not GAPDH proteins. Using

these two DNA topoisomerases as the surrogate proteolytic markers coupled with the

pharmacological inhibition, we have demonstrated the involvements of 26S proteasome, metallo-, serine and cysteine proteases as well as reactive oxygen species (ROS) in the reduction of the topoisomerase levels during acute Cd^{2+} stress. Importantly, our results demonstrated that pharmacological inhibition of metallo-, serine or cysteine proteases protected cells from the Cd^{2+}-caused cell-killing suggesting the novel role(s) of activated proteolytic systems in mediating Cd^{2+} toxicity. Similarly, co-treatment of ROS scavenger N-acetylcysteine also reduced cellular Cd^{2+} toxicity.Conclusion: Our results thus suggested acute Cd^{2+} exposure can effectively activate several proteolytic systems which are, at least in part, responsible for the Cd^{2+}-caused intracellular damage and cytotoxicity. Our finding of the novel role(s) of metallo-, serine and cysteine proteases in the Cd^{2+}-mediated cell-killing might also rationalize a potential intervention of their pharmacological inhibitors in reducing the Cd^{2+}-related toxicity.

P-11

Involvement of Fcα/μ receptor (CD351) in autoantibody production

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Receptors for Fc portion of immunoglobulin play important roles in various immune responses. Although Fc receptors for IgG (FcγRI, FcγRIIb, FcγRIII, and FcγRIV) and IgE (FcεRI and FcεRII) have been extensively investigated, molecular and functional characteristics of an Fc receptor for IgM have been incompletely understood. We previously identified IgM Fc receptor, Fcα/μ receptor (Fcα/μR, CD351), expressed on B cells and follicular dendritic cells (FDCs) (Shibuya, et al., Nat Immunol, 2000). Since Fcα/μR-deficient mice showed increased germinal center formation and antibody productions against T-independent (TI) antigen (Honda, et al., PNAS, 2009), Fcα/μR negatively regulates humoral immune responses against TI antigens.

Evidences demonstrated that mice deficient in serum IgM showed increased anti-double strand DNA (dsDNA) IgG production. Moreover, serum IgM deficiency in autoimmune-prone MRL/MpJ-*Fas*$^{lpr/lpr}$ mice showed significantly increased IgG autoantibody productions against dsDNA and histone, and shortened life span with severe glomerulonephritis. Thus, IgM prevents autoantibody production and ameliorates immunological disorders. However, the involvement of Fc receptor for IgM in autoantibody production is largely unknown.

Because dsDNA belongs to a TI antigen and induce B cells to rapidly produce IgM, we speculated that, in response to TI type self-antigens, self-reactive IgM interacts with Fcα/μR and negatively regulates antibody production against TI antigens. To test this hypothesis, we generated mice lacking *Fcα/μR* gene with lymphoproliferation (lpr) mutation of *Fas* gene (lpr/*Fcα/μR*-KO mice) by intercrossing Fcα/μR-deficient mice with MRL/MpJ-*Fas*$^{lpr/lpr}$ mice, and analyzed the involvement of Fcα/μR in autoantibody production. Unexpectedly, we found that lpr/*Fcα/μR*-KO mice showed significantly decreased IgG autoantibody production against dsDNA, histone and cardiolipin, compare to those of control lpr/*Fcα/μR*-WT mice. Moreover, lpr/*Fcα/μR*-KO mice showed improved survival around ages of seven to ten months old, compare to that of lpr/*Fcα/μR*-WT mice. These results indicated that Fcα/μR accelerates, rather than decelerates, autoantibody production.

P-12

A Negative Feedback of the HIF-1α Pathway via Interferon-stimulated Gene 15 and ISGylation

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The interferon-stimulated gene 15 (ISG15)- and ubiquitin-conjugation pathways play roles in hypoxic and inflammatory responses. To identify interaction(s) between these two tumor microenvironments, we investigated the effect of ISG15 on the activity of the master hypoxic transcription factor HIF-1α. Interferon and desferoxamine treatments were used to induce the expression of ISGs and HIF-1α, respectively. Interactions between HIF-1α and the ISG15 and ISGylation system were studied using knockdown, immunoblotting, co-immunoprecipitation and pull-down analyses. Effects of the ISG15 and ISGylation system on the HIF-1α-directed processes were examined using reporter, RT-PCR and tumorigenic growth assays. We found that the level of the free form of HIF-1α is differentially regulated by interferon treatment and that the free ISG15 level is lower under hypoxia. Mechanism-directed studies demonstrated that HIF-1α not only interacts physically with ISG15 but is also ISGylated in multiple domains. ISG15 expression disrupts the functional dimerization of HIF-1α and HIF-1β. Subsequently, expression of the ISG15 and/or ISGylation system attenuates HIF-1α-mediated gene expression and tumorigenic growth. In summary, our results revealed crosstalk between inflammatory and hypoxic pathways through the ISGylation of HIF-1α. Based on these results, we propose a novel negative feedback loop for the HIF-1α-mediated pathway involving the regulation of HIF-1α via interferon-induced ISGylation.

P-13

Deficient expression of HIF-2α in spleen results in compensatory angiogenesis by upregulation of HIF-1α

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Hypoxic inducible factor (HIF) is involved in essential developmental and physiological processes via transactivation of target genes. HIF-1α is ubiquitously expressed in several types of cells, whereas HIF-2α is expressed in some limited cells, such as endothelial cells (EC). Knockout studies in mice demonstrate that HIF-1α and HIF-2α play redundant and/or nonredundant roles and inactivation of each one show distinct phenotype.

In order to know, how HIF-2α acts in hematopoiesis, we generated HIF-2α knockdown mice (*kd/kd*). Previously, we reported that the number of erythroid cells but not other lineages of hematopoietic cells were impaired in *kd/kd* compared to wild type mice (*WT).* We also found that the expression of VCAM-1, which is a target gene of HIF-2α, in *kd/kd* spleen EC caused defect of stromal cell-supported erythroblast development.

In this study, we generated HIF-2α knockdown/knockout mice (*kd/null*) in order to investigate how decreased expression of HIF-2α affects to erythropoiesis *in vivo*. Firstly, we examined erythroid cell number and found that erythroid cell number in *kd/null* was higher than that of *kd/kd*. But no significant difference of HIF-2α regulated erythropoietin concentration was observed in *kd/kd* and *kd/null*.

We next investigated the expression of VCAM-1 in spleen EC. In spite of the decreased expression of VCAM-1 in *kd/null* spleen EC, the expression of VCAM-1 in *kd/null* whole spleen was higher than that of *kd/kd*. And, we observed much number of spleen EC in *kd/null* compared to that in *kd/kd*, indicating that proliferation of spleen EC was induced in *kd/null* in spite of the decreased expression of HIF-2α.

In order to clarify the mechanism, we isolated primary EC from spleen and analyzed for the expression of angiogenic factors. Of note, we found HIF-1α and HIF-1α regulated angiogenic factors in *kd/null* spleen EC were expressed high compared to that of *WT* and *kd/kd* under hypoxic condition. This HIF-1α activation might be occurred as compensatory effect of attenuated HIF-2α in *kd/null* spleen, and resulted in increased number of spleen EC, leading increased number of erythroid cells in peripheral blood.

P-14

Activin Signaling is Required in the Wound Healing of *Aeolosoma viride*

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Activin, a TGFβ superfamily protein, is known to regulate cell proliferation, inflammation, apoptosis and wound healing, but these studies are limited to vertebrates. Previous researches demonstrate that activin is necessary for wound healing in mice and fin regeneration in zebrafish. Transgenic mice overexpressing activin healed faster, while follistatin (an activin antagonist) overexpressing transgenic mice healed less efficiently. In this research, we investigate the roles of activin in the wound healing of an invertebrate, *Aeolosoma viride*. *A.viride* is a small fresh water annelid, closely related to earthworms, that fully regenerates within 5 days after head amputation. The results showed activin is expressed in the epithelium of *A.viride* at all times, perhaps to maintain normal functions of the epithelium. Furthermore, *A. viride* treated with a chemical inhibitor of activin, SB 505124, obviously inhibited wound healing and impaired regeneration. Therefore, activin signaling is necessary for anterior regeneration and wound healing in *A. viride*, but its regulatory mechanisms requires further study.

P-15

MafB is required for maintenance of differentiated state of microglia *in vitro*

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Microglia are the tissue macrophages which are distributed in central nervous system (CNS) and act as immune cells. Recent studies revealed that microglia arise in yolk sac and migrate into CNS during early embryonic stage. However, molecular mechanisms underlying their differentiation are largely unknown.

MafB is a bZip transcription factor which belongs to large Maf family. MafB is highly expressed in monocyte/macrophage lineage. It has been reported that MafB negatively regulates the differentiation of osteoclasts and dendritic cells. On the other hand, the function of MafB in microglia is yet to be analyzed. We already generated MafB-deficient mice. However, they died soon after birth due to central respiratory failure, which limits our analytical approaches.

Here, we analyzed the phenotype of microglia in MafB-deficient embryonic brain. At embryonic day 18.5, No apparent differences are observed between wild-type and MafB-deficient microglia in respect to cell number, histological distribution and expression of differentiation marker such as CD11b and F4/80. Next, we performed mixed glial culture from embryonic brains. Most of wild-type microglia maintained the expression of F4/80 *in vitro*, whereas MafB-deficient microglia had a significantly increased $CD45^{+}/F4/80^{-}$ population. In addition, MafB-deficient microglia grew more rapidly than wild-type microglia. After isolation from mixed glial culture, most of wild-type microglia were adherent whereas many of MafB-deficient microglia were non-adherent. These data suggests that MafB-deficient microglia de-differentiate *in vitro*.

In conclusion, MafB is not essential for the differentiation of microglia during embryonic stage, but is required for the maintenance of their differentiated state *in vitro*.

P-16

A Portable Microfluidic Device for Rapid Diagnosis of Cancer Metastatic Potential

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If metastasis of lung cancer can be found and treated earlier, sufferers may have higher chances to prevail over it. Nevertheless, routine examinations such as chest radiography, computed tomography and biopsy cannot characterize the metastatic potential of lung cancer cells, losing critical diagnoses for defining optimal therapeutic strategies. In this study, we designed a portable microfluidic device for the rapid diagnosis of cancer metastatic potential. Featuring a micro-temperature control system and a bicarbonate buffered environment, our device discriminates surface detachment rate as an index of migratory ability of cells cultured on pH responsive chitosan. We labeled a series of three metastatic lung cancer subpopulations by fluorescent protein expression, and verified that our device is capable of separating cells by their metastatic ability. Since only a minimum amount of cells is needed, patients' specimen from biopsies, e.g. fine needle aspiration can be processed on site to offer immediate information for doctors. We expect that our design would provide valuable information in pre-operative evaluations as to help define therapeutic plans for lung cancer, as well as for other types of metastatic tumors.

P-17

Fatty acid synthase inhibitor cerulenin suppresses human liver cancer cell lines *in vitro*

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Department of surgery

Aims:The prognosis of primary liver cancer is poor.Sorafenib is the only chemotherapeutic agent to treat advanced liver cancer, but its indication is restricted with its high cost and side effects.A new chemotherapeutic agent is required.Fatty acid synthase(FAS) is highly expressed in many kinds of human tumors, including liver cancers.Previous studies have shown that cancer cell growth can be suppressed with a FAS inhibition cerulenin.We have investigated the potential use of cerulenin for chemotherapy in liver cancers *in vitro*.Method:Three human liver cancer cell lines, HepG2, Huh7 and Hep3B were treated with cerulenin for various concentration. Cell viability was assessed by WST-8 assay.Cell proliferation was assessed by BrdU assay. Apoptosis was evaluated by TUNEL staining.FAS and cell cycle proteins were evaluated by Western blot analysis.We injected HepG2 cells to SCID mice for establishing orthotopic liver cancer model.Results:Cerulenin significantly inhibited cell viability and proliferation dose dependently.Cerulenin also induced apoptosis of tumor cells and cell cycle arrest.We successfully developed an orthotopic tumor models in SCID mice.Conclusions: Cerulenin reduced growth of liver cancers *in vitro*.We established an orthotopic mouse xenograft model. Based on these studies, inhibiting FAS would be an effective strategy to prevent growth of liver cancers *in vivo*.

P-18

Interaction between SIK2 and PP2A Regulates the Activity of CaMKI

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Salt-inducible kinase 2 (SIK2) is an AMPK family member known to form stable complex with PP2A. However, the function consequences of this kinase-phosphatase interaction are largely unknown. Here we report that SIK2 through this association has a positive effect on the stability and activity of PP2A, as revealed by knockdown and overexpression experiments. In this capacity, SIK2 attenuates the association of the PP2A repressor, the PME-1 demethylase, thus preserving the methylation status of PP2A. Furthermore, *in vitro* phosphatase assay demonstrated that the SIK2-PP2A holoenzyme complex dephosphorylates and inactivates CaMKI, which acts as an activating kinase of PME-1. The functionally antagonistic SIK2-PP2A and CaMK1-PME-1 networks thus constitute a negative feedback loop that modulates the phosphatase activity of PP2A. Knockdown of SIK2 also led to activation of CaMKI and the consequent phosphorylation of HDAC5/Ser259, rendering sequestration of HDAC5 in the cytoplasm and activation MEF2C-mediated gene expression. These results thus implicate functional SIK2-PP2A complex in the regulation of MEF2C-dependent transcription. Furthermore, this study suggests that the tightly linked regulatory loop comprised of the SIK2-PP2A and CaMKI-PME-1 networks may function in fine-tuning cell proliferation and stress response.

P-19

Role of MafB in macrophage dendritic cell progenitor (MDP) differentiation

Zeinab Kosibaty

Anatomy and Embryology

MafB is a member of large Maf family of transcription factor that share basic region/leucine zipper DNA binding motifs and N-terminal activation domains. This protein is expressed in a wide variety of tissues. In hematopoietic system, MafB is selectively expressed in monocytes and macrophages and promotes macrophage differentiation cells. MafB negatively regulates receptor activator of nuclear factor kappaB (RANKL)-mediated osteoclast differentiation. It was reported that the Multicentric Carpotarsal Osteolysis is caused by mutations the amino-terminal transcriptional activation domain of MAFB, according to these results, it suggests that MafB play a critical role in osteoclastogenesis and myeloid progenitor fate.

Macrophage dendritic progenitor (MDP) is the first precursor population downstream of the common myeloid progenitor (CMP) that retains DC potential, it differentiates to dendritic cells, monocytes, macrophages and osteoclast, those cells are crucial for immune, inflammatory response and remodeling bone. However the origin and relationships between these cell types are still unclear, as well as the mechanism how MDP differentiations to give rise dendritic cells, osteoclast and monocytes.

To address the function of MafB in differentiation from progenitor cell to macrophage, we performed colony assay using fetal liver cells from *Mafb*$^{-/-}$ and *Mafb*$^{+/+}$ mice. The number of colonies which treatment with macrophage colony stimulating factor (M-CSF), were significantly increased in e14.5 fetal liver cells of *Mafb*$^{-/-}$ compared with *Mafb*$^{+/+}$. This data indicates that undefined cell population that could response to M-CSF was increased in *Mafb*$^{-/-}$. As MDP is known to differentiate into macrophage by stimulation with M-CSF, we hypothesized that it is MafB might regulate fate of MDP.

To examine the expression of MafB on hematopoietic progenitor specific *in vivo*, first we monitored GFP expression of GFP knock in *Mafb* deficient mice from bone marrow (BM) cells by FACS. The result showed that GFP fluorescence was observed in MDP population (Lin^{-} sca1^{-} c-kit^{low} CD115^{+} CD16/32^{+} CD34^{+}). Moreover, we performed that colony assay using lineage negative progenitor population. The data indicates that, the colony from lin^{-}c-kit^{-}sca-1^{-} cells, which was potentially developed from MDP were increased in *Mafb*$^{-/-}$ mice in presence of M-CSF, that suggested MafB may play an important role in MDP differentiation.

These results suggest that MafB may important role in MDP differentiation, considering that MafB regulates MDP differentiation into a specific precursor and this precursor gives rise to macrophage or osteoclast or both cells.

P-20

Extratumoral Acidification as a Promoter for the Development of Stemness Properties in Cancer

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Tumor microenvironment is an important factor for the development of cancer stemness properties towards malignant progression; albeit the widely appreciated association of extratumoral acidification with malignant tumor growth, however, only a handful of studies is dedicated to understand the relationship between microenvironment acidification and cancer stemness. Our laboratory has previously identified that acidification can modulate the TRIM28 protein via phosphorylation on the S473 residue, with concomitant change in partner association and protein distribution. Since TRIM28 is a chromatin modulator that participates in the maintenance of stemness properties of embryonic stem cells, we thus infer that acidification may invoke cancer cell stemness properties through hijacking the stemness related function of TRIM28. To test this hypothesis, we set to use xenograft models to analyze whether acidification challenge promotes the *in vivo* tumorigenicity of non-small cell lung cancer cells. To assess the role of TRIM28 modulation, we will also establish S473 phosphomimicking/non-phosphorylatable mutants replacement cell lines by the TALEN technology, and delineate the change in genomic occupancy as well as association network homeostasis of TRIM28 through chromatin immunoprecipitation assays. We believe our study will provide important insight into the development of stemness properties in cancer.

P-21

Direct Conversion of Mouse Liver Cells to Insulin-producing Cells by Defined Factors.

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Diabetes mellitus is characterized by an absolute and relative insulin insufficiency, resulting in the disturbance of glucose homeostasis accompanied with high blood sugar levels. A number of drugs are prescribed to improve glycemic control and treat diabetes, including administration of insulin itself. In addition, based on the clinical success of islet cell transplantation, generation of insulin-producing β-cells from different types of cells including iPS cells and its complementation are expected to achieve complete remission of the disease. We have been attempted to directly convert hepatocytes to β-cells using mouse models *in vivo*. In this study, we constructed single polycistronic adenovirus that can transduce three β-cell related transcription factors, Pdx1, Neurod, and Mafa, and examined whether the polycistronic vector using 2A peptides can be applied to *in vivo* conversion. To test this, the recombinant virus was transferred into wild-type mouse via tail vein injection, and three days after infection, liver tissue was collected and subjected to Western blotting analysis. Anti Pdx1, Neurod, and Mafa antibodies reacted on the appropriate band size respectively, indicating that gene transfer and self-cleavage of the 2A peptides were successfully carried out as expected. Next, to examine the overexpression of these β-cell related transcription factors can activate insulin gene transcription in mouse liver, the recombinant adenovirus was infected into MIP-GFP mouse, in which green fluorescent protein are expressed under the control of mouse *insulin I* gene, and GFP expression in liver was analyzed at day 3 and 7 after infection by flow cytometry. GFP expression was mainly detected in hepatocyte population at both time points, and interestingly only at day 7, we found that GFP-positive cells were divided into two distinct populations based on GFP expression levels. In conjunction with a recent finding that high GFP-expressing cells of MIP-GFP mice islets are functionally advanced than the other populations, high GFP-expressing cells in gene-transferred liver might be functionally maturated close to pancreatic β-cells. This hypothesis was also supported by the gene expression profiles that bright GFP cells highly express several genes related to glucose responsiveness, insulin transcription, processing and secretion compared to low GFP fraction. Taken together, these results suggest that Pdx1, Neurod, and Mafa gene combination can transiently convert liver cells to functionally mature β-cells which can secrete insulin respond to extracellular glucose levels.

P-22

Lysophosphatidic Acid Inhibits TPA Induced Megakaryopoiesis in K562 Cells Through the Activation of LPA Receptor 2

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Erythrocytes and megakaryocytes (MK) come from a common progenitor that undergoes lineage specification depending on the stimulus. Recent studies in our lab have demonstrated that lysophosphatidic acid (LPA) enhances erythropoiesis, but whether LPA is involved in megakaryopoiesis remains unclear. K562 leukemia cell line, under phorbol 12-myristate 13-acetate (TPA) treatment, displays MK characteristics, such as endomitosis, expression of CD41a and CD61, and enhancement of cell adhesion. Our data showed that expression of LPA receptor 2 (LPA_2) was relatively higher than LPA_1 and LPA_3 in K562 cells. After TPA induction, LPA_2 expression was increased at 24 hours but decreased at 48 hours. Moreover, we found that LPA_2 knockdown increased the quantity of $CD41a^+CD61^+$ cells and also enhanced cell adhesion. On the contrary, activation of LPA_2 signaling by mono-dodecylphosphate (MDP) decreased CD41a and CD61 expression at mRNA and protein levels, and endomitosis was also suppressed. These results suggested that LPA negatively regulates megakaryopoiesis through activating LPA_2 in K562 cells.

P-23

Detoxification of methylmercury by hydrogen sulfide producing enzyme *in vitro* and *in vivo*

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Methylmercury (MeHg) is an environmental electrophile that can be covalently bound to protein sulfhydryls to form protein–MeHg complexes. These covalent modifications, referred as "*S*-mercuration", are associated with disruption of enzyme function and neurotoxicity. Some of MeHg unbound and/or proteins modified by MeHg undergo interaction with deprotonated glutathione (GSH) to yield its GSH adducts that are rapidly excreted into extracellular space through MRP transporters. On the other hand, hydrogen sulfide (H_2S) is a gaseous, weakly acidic molecule mainly produced by cystathionine β-synthase (CBS) and cystathionine γ-lyase (CSE) in cells. Accumulated evidence has indicated that H_2S is protective in neural damage and heart failure, but mechanistic details remain to be elucidated. Most interestingly, H_2S is extensively dissociated into a nucleophile HS^- because of its p*K*a value = 6.76 while p*K*a value of GSH is 9.12, implying that most of GSH exists as its protonated form at physiological conditions. However, role of H_2S in chemical modification of cellular proteins and cytotoxicity during exposure to MeHg is poorly understood. In this study, we examined the contribution of H_2S derived from CBS and/or CSE to cellular protection against MeHg. Pretreatment with NaHS or overexpression of CBS reduced MeHg cytotoxicity, whereas transfection with CBS small interfering RNA enhanced MeHg toxicity in human neuroblastoma SH-SY5Y cells. These results suggest that H_2S produced by CBS in the cells represses MeHg-mediated cellular toxicity. Bismethylmercury sulfide ($(MeHg)_2S$), formed during the reaction of MeHg with H_2S, was identified as a metabolite of MeHg in SH-SY5Y cells exposed to MeHg and in the livers of rats treated with MeHg. MeHg covalently modified numerous proteins in SH-SY5Y cells in a concentration-dependent fashion, whereas $(MeHg)_2S$ did not. This metabolite had a weak cytotoxicity compared to MeHg in SHSY-5Y cells. Consistent with this, intraperitoneal administration of $(MeHg)_2S$ to mice had little effect on the death rate although the exposure to MeHg caused a time-dependent mortality.

P-24

Soluble DNAX accessory molecule-1 is a novel predictive biomarker for acute graft-versus-host disease

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Acute graft-versus-host-disease (aGVHD) is the most major complication of allogeneic hematopoietic stem cell transplantation (allo-HSCT). Diagnosis of aGVHD depends on clinical symptoms and pathological findings. There are some problems in the aGVHD diagnosis and treatment. First, prediction of aGVHD development is difficult before allo-HSCT. Second, differential diagnosis to distinguish between other allo-HSCT complications and aGVHD is not easy. At a present time, exploration and validation of biomarker for GVHD become active to compensate thrse problems of aGVHD diagnosis and therapy.

DNAM-1 is an adhesion molecule expressed on T cells and NK cells. We previously demonstrated that DNAM-1 was involved in development of aGVHD in mouse model (Proc Natl Acad Sci USA. 2010). Recently, we found the soluble form of DNAM-1 (soluble DNAM-1) in human serum. We also detected soluble DNAM-1 in culture supernatant when T cells were stimulated with anti-CD3 together with anti-DNAM-1 mAb. The western blotting assay showed that the molecular weight of soluble DNAM-1 was 45kDa, which was matched with molecular weight of extracellular lesion of human DNAM-1. Production of soluble DNAM-1 was inhibited in the presence of matrix-metalloprotease inhibitor. These data suggested that soluble DNAM-1 was produced form activated T cells by shedding manner. In clinical cases, we found the value of serum soluble DNAM-1 was relatively high in aGVHD patients. In statistical analysis (71patients), cumulative incidence of aGVHD in the group of the patients with high soluble DNAM-1 was significantly higher compared with that with low soluble DNAM-1 ($P<0.01$). Moreover, we discovered the value of serum soluble DNAM-1 before and after allo-HSCT could be a prediction marker for development of aGVHD.

MEMO

MEMO

Dance Performance

Dance Performance

Thursday, October 3	Venue: Main Convention Hall
"Responsive Environments for Movement"	
13:45-14:30	Choreography: Lisa Naugle Video: John Crawford Music: Kei Akagi and the Tokyo Trio Dancers: Landyn Endo and Ryan Thomas University of California, Irvine, USA

Responsive Environments for Movement

Abstract:

Active Space is a digital media arts research project investigating embodied interaction through movement and other forms of dynamic expression. Activities include the development of real-world performative environments that continuously sense, measure and respond to movement, allowing participants to engage and "play the space" as an instrument. The results of this research are applicable to the performing arts, education, telepresence, rehabilitation medicine, and other areas.

Active Space environments use a collection of custom real-time media objects developed by intermedia artist and software designer John Crawford in association with choreographer Lisa Naugle. The Active Space media objects include systems for multi-channel live video and audio processing, generative animation, musical composition, mediabase storage/retrieval and high bandwidth networking. Associated motion tracking objects perform real-time sensing and analysis of location, speed, duration and various other characteristics of movement. The results of this analysis are processed to generate video and audio in response to movement.

Motion tracking refers to a variety of approaches for real-time sensing and analysis of location, speed, duration and various other characteristics of movement. Motion capture is the technique of sampling movement in 3D space and creating graphical representations of the movement. Typical applications of motion capture tend to produce realistic animation. By integrating advanced motion tracking techniques, the aesthetic focus of our Active Space work goes beyond realism to explore notions of non-linear association, embodiment and reflexivity. The interplay between improvisational and compositional elements is of particular interest.

Short Bio:

John Crawford is an intermedia artist, interactive performance director, technology developer and projection designer. Intersecting software with digital media and theatrical performance, he uses computers and video to create interactive environments, painterly animations and motion graphics for dance, theatre and music. He originated the Active Space concept in 1994 to describe his interactive performance systems that produce visuals and music in response to movement.

As a professor of Dance and Media Arts at University of California, Irvine, he is a frequent participant in transdisciplinary research projects and multi-site telepresence events connecting performing arts and digital media practices with computer science and engineering. He founded the eMedia Studio, a distributed arts collaboratory in the California Institute for Telecommunications and Information Technology (Calit2) at UC Irvine, where he leads the digital culture initiative. His work has been performed and exhibited across North America and in Asia, Europe and South America.