Joint International Symposium on

TGF-β Family and Cancer

Signal Network in Tumor Microenvironment

2015 1/12Mon.13Tue

International Congress Center EPOCHAL TSUKUBA
(Tsukuba, Ibaraki)

Organizer

Kohei Miyazono
Department of Molecular Pathology,
Graduate School of Medicine, The University of Tokyo

Chairman

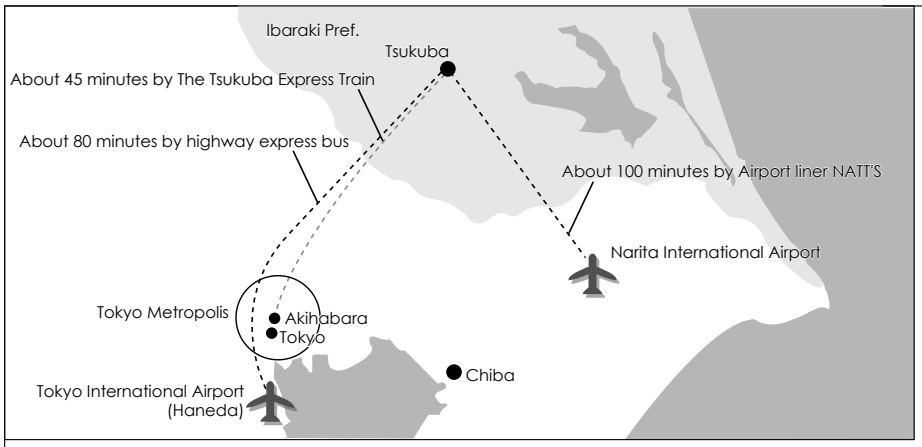
Mitsuyasu Kato
Department of Experimental Pathology, Faculty of Medicine, University of Tsukuba

Contents

General Information

Congress Name	Joint International Symposium on TGF-β and Cancer The 4th International Symposium by JSPS Core-to-Core Program “Cooperative International Framework in TGF-β Family Signaling” International Symposium by Grant-in-Aid for Scientific Research on Innovative Areas by MEXT “Integrative Research on Cancer Microenvironment Network”
Theme	TGF-β Family and cancer Signal Network in Tumor Microenvironment
Date	January 12（Mon）- 13（Tue）, 2015
Venue	International Congress Center "EPOCHAL TSUKUBA"（Tsukuba, Ibaraki） 2-20-3 Takezono,Tukuba, Ibaraki 305-0032, Japan TEL. +81-29-861-0001
Organizer	Kohei Miyazono Department of Molecular Pathology, Graduate School of Medicine, The University of Tokyo
Chairman	Mitsuyasu Kato Department of Experimental Pathology, Faculty of Medicine, University of Tsukuba
Language	English（no simultaneous interpretation provided）
Sponsor	Japan Society for the Promotion of Science, Core-to-Core Program Grant-in-Aid for Scientific Research on Innovative Areas by MEXT
Admission	Free
Banquet	Time: January 12（Mon） 18:20 - 20:30 Place: International Congress Center EPOCHAL TSUKUBA "Espoir"
Homepage	http://www.c2ctgfb2015-4thsymposium.net

Access Map



NOTE: **Seats for the bus *from Tsukuba to Narita* must be reserved in advance. Or call "Kanto-Tesudo, Tsukuba Office" (phone: +81-29-822-5345).**

Reservations are accepted up to a month in advance, and no later than 7 P.M. on the previous day.

Reservations are not necessary for buses heading to Tsukuba, or Haneda Airport.

From Haneda Airport

By taking a highway express bus

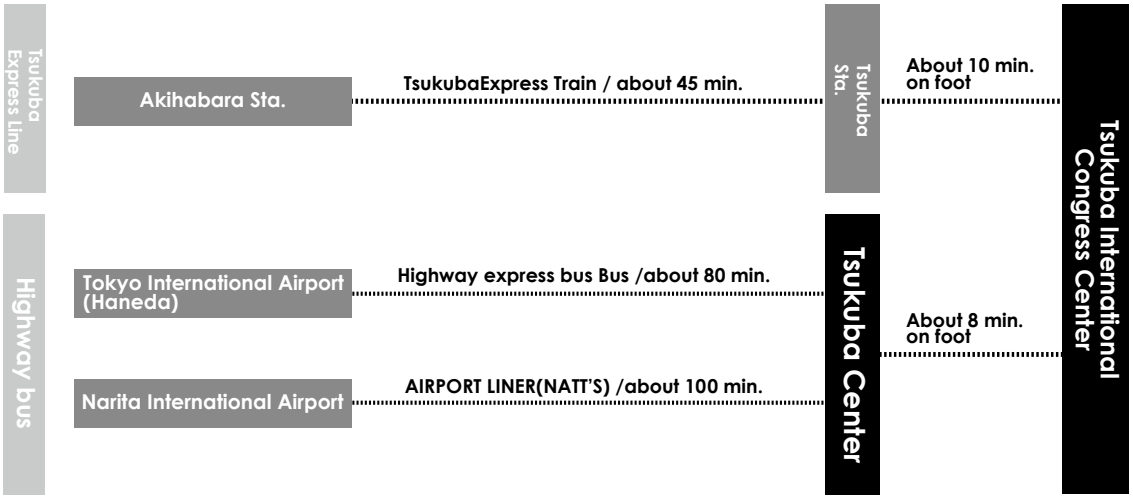
Board a highway express bus bound for Tsukuba Center (80 mins).
Bus route, timetable, and bus stops: http://hnd-bus.com/route/tsukubacenter.html
How to buy a ticket: http://hnd-bus.com/guide/ticket.html
NOTE: *1: You need to buy the ticket at the bus counter on the 1st floor before boarding.
*2: Do not recommend the use during rush hours.

By taking Monorail + JR + Tsukuba Express Train

Board Tokyo Monorail at Haneda Airport Terminal 1 to Hamamatsu-Cho Station (20 mins). Transfer to JR Yamanote Line and get off at Akihabara station (10 mins). And find out a sign "TX" for Tsukuba Express, located deep underground at the east side of the Akihabara station. It takes about 5 mintes from JR(Akihabara Sta.) to TX on foot.
Board TX to Tsukuba Station Terminal (45 mins).
10 mins walk from Tsukuba Station.

From Narita Airport

Board the Airport liner NATT'S at **the bus stop #8 in Terminal 1 (1st floor) or #10 in Terminal 2 (1st floor)** and get off at Tsukuba Center (100 mins).



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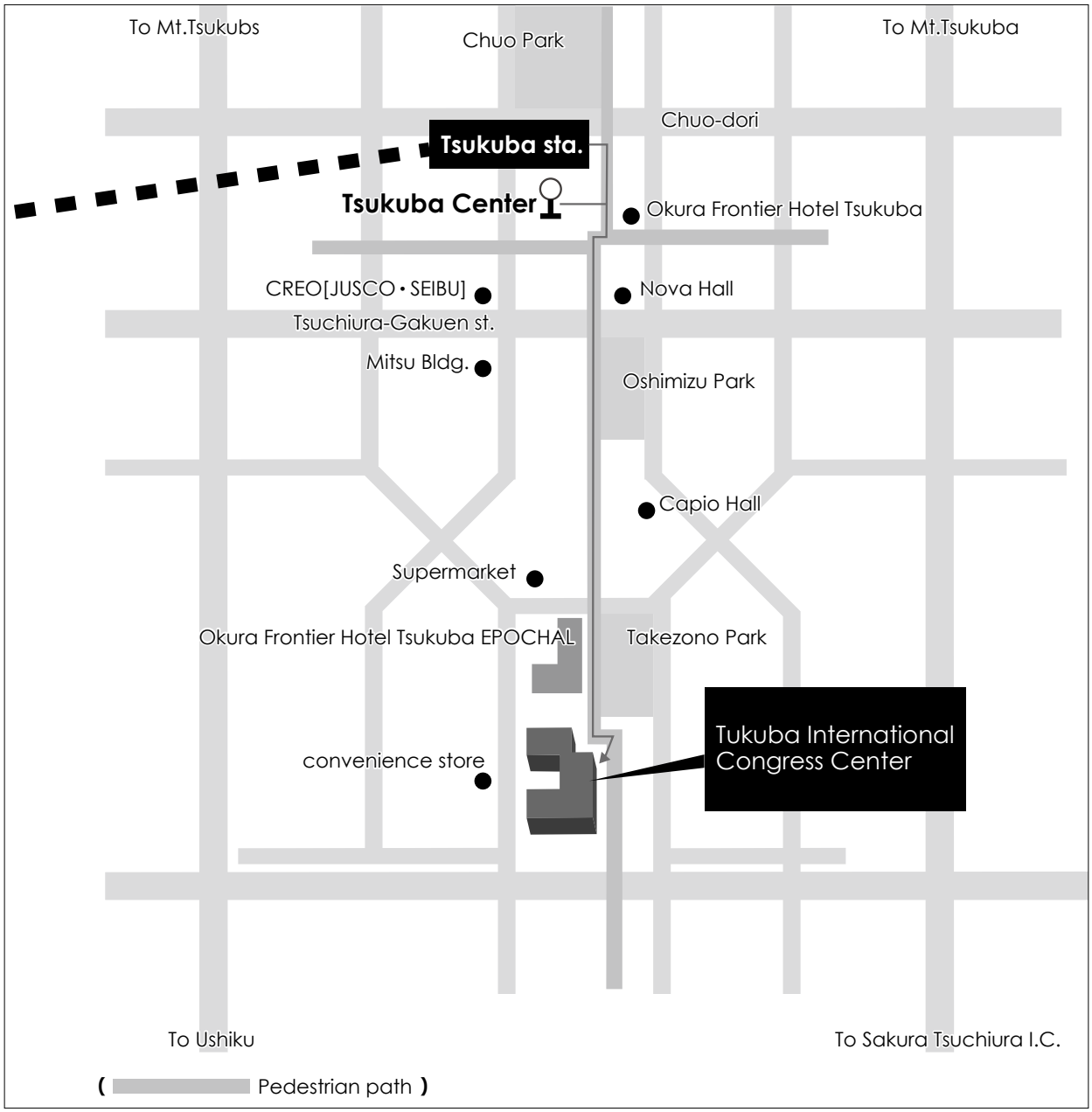


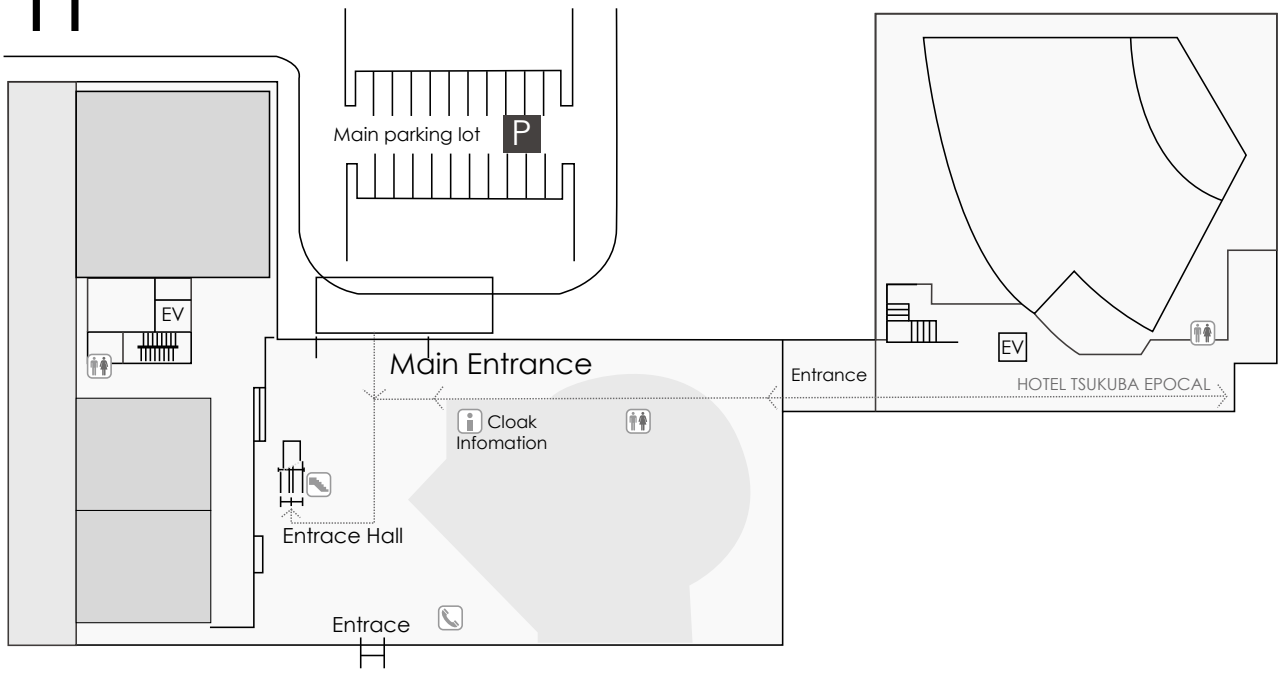
Walking Direction (From Tsukuba Center Bus Terminal / Tsukuba sta.)

For the Tsukuba International Congress Center
Please go up the escalator at Exit A3 to the pedestrian path and walk south approximately 800 m along the path, which is about 8 mins walk.

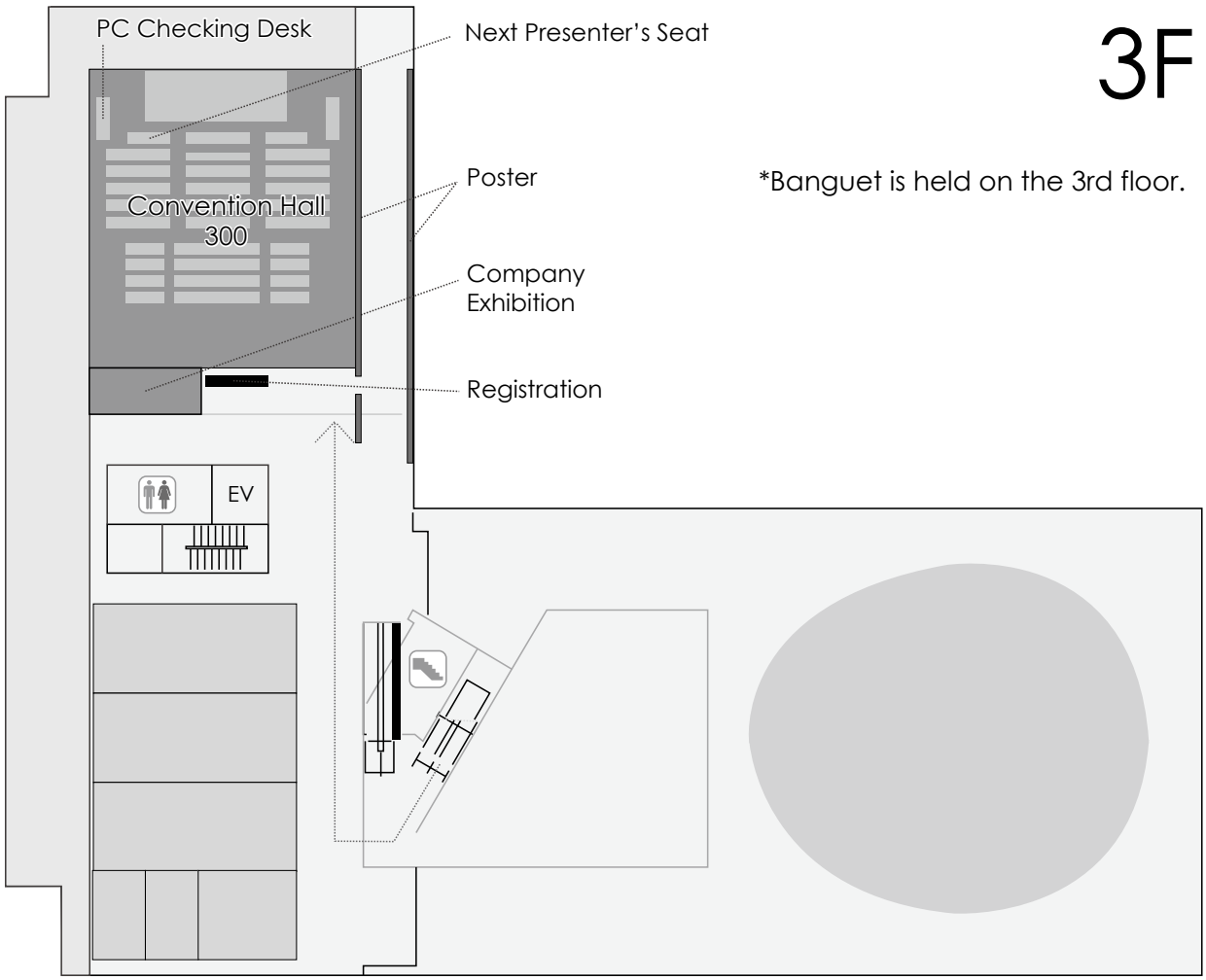
For HOTEL TSUKUBA EPOCHAL
Almost the same direction above. You will see the hotel on your right just before the Tsukuba International Congress Center.

Floor Map

1F



3F



*Banguet is held on the 3rd floor.

Instructions for Presenters and Chairpersons

Instruction for Chairpersons

Please be at the「Next Chairperson's Seat」10 minutes before your in-charged session starts.
Chairpersons are asked to remain within the time allocated for the session and each presentation.

Instruction for Oral Presenter

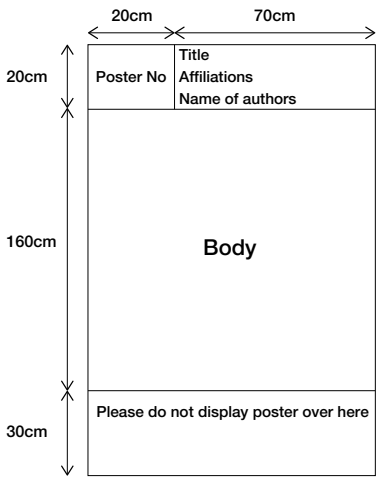
- Allocated time for Oral Presentation

Session 1・Short talks	15 minutes for presentation, 5 minutes for discussion
Session 2・3・4・5・6・7	25 minutes for presentation, 5 minutes for discussion
Special Lecture	30 minutes for presentation, 10 minutes for discussion

- Please visit the「PC Checking Desk」30 minutes before your session starts and be at the「Next Presenter's Seat」10 minutes before your presentation starts.
- Please bring your **own PC** and AC adaptor for your presentation.
- The only type of connecter available at the venue is「Mini D-sub 15pin 3」. Please provide your own connecter if your PC does not accept the Mini D-sub 15pin 3 type connecter.
- Please prepare a USB flash memory for data back-up.
- Presentation by using tablet terminator is not acceptable.
- For presenters who are not able to bring their own PC, PC will be prepared in the venue. Please provide your data in USB flash memory. The OS and Application for the data are Power Point 2007/2010/2013.
  Please save your data under your name.
- Please bring your own PC if your presentation includes Video (exclusive of Power Point animation function).
- Sound cannot be played.

Instruction for Poster Presenters

- Posters should be displayed on the assigned board during the poster set-up time.
- Poster presenters should be ready in front of your poster panel 5 minutes before the session starts.



Poster preparation

- All poster presenters will be provided with 90 cm wide and 210 cm high boards.
- Only English language is available.
- Presenters should prepare a header indicating or including the title, name of all authors and their affiliations.
- Presentation number of your poster, pins and ribbon will be provided at the poster session area.

Poster schedule

Poster Set-up	January 12 (Monday) 8:00~8:50
Poster Viewing1	January 12 (Monday) 10:40~11:40 (poster with odd number)
Poster Viewing2	January 12 (Monday) 15:10~16:10 (poster with even number)
Poster Award	January 12 (Monday) 18:20~20:30 (Banquet)
Poster Removal	January 13 (Tuesday) 15:40~16:00

Invited Speakers

Hiroyuki Aburatani (Japan)
Shigeru Chiba (Japan)
Marie-José Goumans (the Netherlands)
Eiji Hara (Japan)
Beate Heissig (Japan)
Carl-Henrik Heldin (Sweden)
Mitsuyasu Kato (Japan)
Yoshihiro Kawasaki, Tetsu Akiyama (Japan)
Seong-Jin Kim (Korea)
Gou-Young Koh (Korea)
Daizo Koinuma (Japan)
Mizuko Mamura (Japan)
Hiroyuki Mano (Japan)
Hozumi Motohashi (Japan)
Aristidis Moustakas (Sweden)
Masafumi Muratani (Japan)
Hiroaki Suga (Japan)
Hiroyuki Suzuki (Japan)
Nobuyuki Takakura (Japan)
Tetsuro Watabe (Japan)
Seiji Yano (Japan)

Time Table

Monday, January 12

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8:15 -
Registration（International Congress Center EPOCHAL TSUKUBA）
Poster mounting

Opening Remarks
8:50 - 8:55
Mitsuyasu Kato (University of Tsukuba, Japan)

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Session 1. Short talks（selected from poster abstracts） Chair: Shinae Kondoh (Tokyo Institute of Technology, Japan)
9:00 - 9:20
Yutaro Tsubakihara (Ehime University, Japan)
Arkadia induces ubiquitylation and degradation of Smad6
9:20 - 9:40
Kyung-Min Yang (CHA University, Korea)
Cytoplasmic DRAK1 overexpressed in HNSCC cells inhibits TGF-β1 tumor suppressor activity
9:40 - 10:00
Takahiro Kuchimaru (Tokyo Institute of Technology, Japan)
Non-invasive imaging of activation of HIF and TGF-β/Smad signaling in breast cancer progression
10:00 - 10:20
Ai Takemoto (Cancer Chemotherapy Center, JFCR, Japan)
Aggrus-induced platelet aggregation promotes tumor metastasis by enhancing EMT
10:20 - 10:40
Masamitsu Konno (Osaka University, Japan)
Embryonic MicroRNA-369 regulates pyruvate kinase splicing form by stabilizing translation of splicing

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10:40 - 11:40
Poster Viewing 1 / Coffee Break

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12

11:40 - 13:00
Lunch

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Session 2. TGF-β signaling Chair: Keiji Miyazawa（University of Yamanashi, Japan）
13:00 - 13:30
Carl-Henrik Heldin（Uppsala University, Sweden）
Mechanism of TGFβ-induced epithelial-mesenchymal transition
13:30 - 14:00
Aristidis Moustakas（Uppsala University, Sweden）
Regulation of BMP signaling during cell differentiation in human cancer

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14:00 - 14:30
Mitsuyasu Kato（University of Tsukuba, Japan）
Roles of MafK and Gpnmb in malignant progression of breast cancer

Special Lecture 1 Chair: Carl-Henrik Heldin（Uppsala University, Sweden）
14:30 - 15:10
Seong-Jin Kim（CHA University of Medicine and Science, Korea）
Effects of Smad3 linker and C-tail phosphorylation on tumorigenesis and metastasis

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15:10 - 16:10
Poster Viewing 2 / Coffee Break

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Session 3. Cancer and metabolism-1 Chair: Takeshi Imamura（Ehime University, Japan）
16:10 - 16:40
Eiji Hara（Japanese Foundation for Cancer Research, Japan））
Cellular senescence and cancer: a gut microbial connection
16:40-17:10
Hiroyuki Suzuki（University of Tsukuba, Japan）
Roles of THG-1/Tsc22D4 in squamous cell carcinoma development

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Special Lecture 2 Chair: Mitsuyasu Kato（University of Tsukuba, Japan）
17:10 - 17:50
Hiroaki Suga（The University of Tokyo, Japan）
Non-traditional drug discovery using artificial macrocycles for cancer therapeutics

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18:00 - 18:10 Group Photo

18:20 - 20:30
Banquet（International Congress Center EPOCHAL TSUKUBA）
Greetings from the President of University of Tsukuba : Kyosuke Nagata (University of Tsukuba, Japan)

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Poster Award

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Tuesday, January 13

8:00 - 8:30
JSPS Core-to-Core Business Meeting（place: room:304）

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8:30 -
Registration（International Congress Center EPOCHAL TSUKUBA）

Session 3. Cancer and metabolism-2 Chair: Takeshi Imamura（Ehime University, Japan）
08:40 - 09:10
Hozumi Motohashi（Tohoku University, Japan）
Functional Nexus between Keap1-Nrf2 System and Cellular Metabolism

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Session 4. Hematology and Immunology Chair: Susumu Itoh (Showa Pharmaceutical University, Japan)
9:10 - 9:40
Shigeru Chiba（University of Tsukuba, Japan）
Clonal origin of microenvironmental cells in malignant lymphoma
9:40 - 10:10
Beate Heissig（The University of Tokyo, Japan）
The fibrinolytic system modulates the cytokine and cellular environment during cancer progression and chronic inflammation

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10:10 - 10:40
Mizuko Mamura（Tokyo Medical University, Japan, Kyungpook National University, Korea）
Dynamic Smad signaling networks in immune cell regulation

10:40 - 11:00
Coffee Break

Session 5. Genome-wide approach Chair: Hiroyuki Aburatani (The University of Tokyo, Japan)
11:00 - 11:30
Hiroyuki Aburatani（The University of Tokyo, Japan）
Coordinated chromatin regulation by developmental signals during cardiomyocyte differentiation

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11:30 -12:00
Daizo Koinuma（The University of Tokyo, Japan）
Identification of Smad regulatory factors through integrated analysis of ChIP-seq data
12:00 - 12:30
Masafumi Muratani（University of Tsukuba, Japan）
Ectopic induction of developmental gene regulatory network by cryptic promoters in gastric cancer

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12:30 - 13:30
Lunch

13
Session 6. Angiogenesis Chair: Yasufumi Sato（Tohoku University, Japan）
13:30 - 14:00
Marie-José Goumans（Leiden University, the Netherlands）
Impaired macrophage polarization in endoglin haplo-insufficiency leading to defective tissue repair
14:00 - 14:30

14
Tetsuro Watabe（Tokyo University of Pharmacy and Life Sciences, Japan）
Roles of signal networks during the formation and maintenance of tumor vasculature
14:30 - 15:00
Nobuyuki Takakura（Osaka University, Japan）
Maintenance of the stemness in cancer stem cells requires CD44 expressed by tumor stromal cells via tissue hypoxia

Special Lecture 3 Chair: Kohei Miyazono（The University of Tokyo, Japan）
15:00 - 15:40

15
Gou-Young Koh（KAIST, Korea）
Targeting tumor vasculatures

15:40 - 16:00
Coffee Break / Poster unmounting

Session 7. Cancer therapy Chair: Naoya Fujita（Japanese Foundation for Cancer Research, Japan）
16:00 – 16:30

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Seiji Yano（Kanazawa University, Japan）
Bone microenvironment confers Hsp90 inhibitor resistance in the metastatic small cell lung cancer
16:30 - 17:00
Hiroyuki Mano（The University of Tokyo, Japan）
Targeting essential growth drivers in human cancer
17:00 – 17:30

17
Yoshihiro Kawasaki, Tetsu Akiyama（The University of Tokyo, Japan）
MYU, a novel target lncRNA for Wnt/c-Myc signaling, mediates CDK6 induction to promote cell cycle progression

Closing Remarks
17:30 - 17:40
Kohei Miyazono（The University of Tokyo, Japan）

Program

Monday, January 12

08:15– **Registration** (International Congress Center EPOCHAL TSUKUBA)
Poster mounting

Opening Remarks

08:50 – 08:55 Mitsuyasu Kato (University of Tsukuba, Japan)

Session 1. Short talks (selected from poster abstracts)

Chair: Shinae Kondoh (Tokyo Institute of Technology, Japan)

09:00 – 09:20 **Yutaro Tsubakihara** (Ehime University, Japan)
(**poster no.26)** *Arkadia induces ubiquitylation and degradation of Smad6*

09:20 – 09:40 **Kyung-Min Yang** (CHA University, Korea)
(**poster no.01)** *Cytoplasmic DRAK1 overexpressed in HNSCC cells inhibits TGF-β1 tumor suppressor activity*

09:40 – 10:00 **Takahiro Kuchimaru** (Tokyo Institute of Technology, Japan)
(**poster no.03)** *Non-invasive imaging of activation of HIF and TGF-β/Smad signaling in breast cancer progression*

10:00 – 10:20 **Ai Takemoto** (Cancer Chemotherapy Center, JFCR, Japan)
(**poster no.37)** *Aggrus-induced platelet aggregation promotes tumor metastasis by enhancing EMT*

10:20 – 10:40 **Masamitsu Konno** (Osaka University, Japan)
(**poster no.60)** *Embryonic MicroRNA-369 regulates pyruvate kinase splicing form by stabilizing translation of splicing*

10:40 – 11:40 ***Poster Viewing 1 (odd number) / Coffee Break***

11:40 – 13:00 ***Lunch***

Session 2. TGF–β signaling

Chair: Keiji Miyazawa (University of Yamanashi, Japan)

13:00 – 13:30 **Carl–Henrik Heldin** (Uppsala University, Sweden)
Mechanism of TGFβ-induced epithelial-mesenchymal transition

13:30 – 14:00 **Aristidis Moustakas** (Uppsala University, Sweden)
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Program

Special Lecture 1

Chair: Carl–Henrik Heldin (Uppsala University, Sweden)

14:30 – 15:10 **Seong–Jin Kim** (CHA University of Medicine and Science, Korea)
Effects of Smad3 linker and C-tail phosphorylation on tumorigenesis and metastasis

15:10 – 16:10 ***Poster Viewing 2 (even number) / Coffee Break***

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Chair: Takeshi Imamura (Ehime University, Japan)

16:10 – 16:40 **Eiji Hara** (Japanese Foundation for Cancer Research, Japan)
Cellular senescence and cancer: a gut microbial connection

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Roles of THG-1/Tsc22D4 in squamous cell carcinoma development

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Non-traditional drug discovery using artificial macrocycles for cancer therapeutics

18:00 – 18:10 ***Group Photo***

18:20 – 20:30 ***Banquet*** (International Congress Center EPOCHAL TSUKUBA)

Greetings from the President of University of Tsukuba: Kyosuke Nagata

Poster Award

Program

Tuesday, January 13

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Session 3. Cancer and metabolism-2

Chair: Takeshi Imamura (Ehime University, Japan)

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10:40 – 11:00 ***Coffee Break***

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12:30 – 13:30 ***Lunch***

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Chair: Yasufumi Sato (Tohoku University, Japan)

13:30 – 14:00 **Marie–José Goumans** (Leiden University, the Netherlands)
Impaired macrophage polarization in endoglin haplo-insufficiency leading to defective tissue repair

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Special Lecture 3

Chair: Kohei Miyazono (The University of Tokyo, Japan)

15:00 – 15:40 **Gou–Young Koh** (KAIST, Korea)
Targeting tumor vasculatures

15:40 – 16:00 ***Coffee Break***
Poster unmounting

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Chair: Naoya Fujita (Japanese Foundation for Cancer Research, Japan)

16:00 – 16:30 **Seiji Yano** (Kanazawa University, Japan)
Bone microenvironment confers Hsp90 inhibitor resistance in the metastatic small cell lung cancer

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17:00 – 17:30 **Yoshihiro Kawasaki, Tetsu Akiyama** (The University of Tokyo, Japan)
MYU, a novel target lncRNA for Wnt/c-Myc signaling, mediates CDK6 induction to promote cell cycle progression

Closing Remarks

17:30 – 17:40 **Kohei Miyazono** (The University of Tokyo, Japan)

Oral Presentations

Mechanism of TGF*β*-induced epithelial-mesenchymal transition

Carl-Henrik Heldin

Ludwig Institute for Cancer Research,
Uppsala University

Transforming growth factor *β* (TGF*β*) affects growth, survival and differentiation of most cell types. Following ligand-induced hetero-tetramerization of type I and type II serine/threonine kinase receptors, Smad2 and 3 are phosphorylated whereafter they form complexes with Smad4, which are translocated to the nucleus where they regulate the transcription of certain genes. Smad signaling leads to growth arrest as well as epithelial-to-mesenchymal transition (EMT) of epithelial tumor cells (Heldin et al. 2012. FEBS Lett 586: 1959-1970). TGF*β* also activates non-Smad pathways, including MAP kinase pathways, and cleavage of the type I receptor releasing the intracellular receptor domain which then is translocated to the nucleus where it induces several genes coding for proteins involved in invasion of cancer cells, e.g. snail and MMP1 (Mu et al. 2011. Nature Commun. 2: 330; Gudey et al. 2014. Sci. Signal. 7: ra2). EMT is an important part of the tumor promoting effects of TGF*β*. We found important roles of the DNA architectural protein HMGA2 (Thuault et al. 2006. J. Cell Biol. 174: 175-183), the transcription factors Snail and Twist (Tan et al. 2012. J. Biol. Chem. 287: 7134-7145) and the hyaluronan synthase 2 (Porsch et al. 2013. Oncogene 32: 4355-4365) in Smad induced EMT. Moreover, HMGA2 epigenetically silences the gene for the epithelial marker E-Cadherin, as is evident by the hypermethylation of its promoter and gain of repressive (e.g. H3K9me3) and loss of activating (e.g. H3K4me3) histone marks (Tan et al., in revision). We are now attempting to find ways of selectively inhibiting TGF*β*-induced EMT to develop treatment regimens for patients with advanced cancers (Carthy et al., in progress).

Carl-Henrik Heldin, Ph.D.

Director, Uppsala Branch of the Ludwig Institute for Cancer Research and Professor in Molecular Cell Biology at Uppsala University.

1980 Ph.D. thesis at Uppsala University.
1981 Scientist position from the Swedish Cancer Society.
1986 Director, Uppsala Branch of the Ludwig Institute for Cancer Research.

Recent selected publications

1. Porsch H, Mehic M, Olofsson B, Heldin P and Heldin CH (2014). PDGF β-receptor, TGFβ type I receptor and CD44 modulate each others signaling and stability. ***J.Biol.Chem***. 289, 19747-19757.

2. Gudey SK, Sundar R, Mu Y, Wallenius A, Zang G, Bergh A, Heldin CH and Landström M (2014). TRAF6 stimulates the tumor-promoting effects of TGFβ type I receptor through polyubiquitination and activation of presenilin 1. ***Sci.Sign***. 7, ra2.

3. Mu Y, Sundar SK, Thakur N, Ekman M, Gudey SK, Yakymovych M, Hermansson A, Dimitriou H, Bengoechea-Alonso MT, Ericsson J, Heldin CH and Landström M (2011). TRAF6 ubiquitinates TGFβ type I receptor to promote its cleavage and nuclear translocation in cancer. ***Nature Commun***. 2, 330.

4. Lönn P, van der Heide L, Dahl M, Hellman U, Heldin CH and Moustakas A (2010). PARP-1 attenuates Smad-mediated transcription. ***Mol.Cell*** 40, 521-532.

5. Moustakas A and Heldin CH (2009). The regulation of TGFβ signal transduction. ***Development*** 136, 3699-3714.

Regulation of BMP signaling during cell differentiation in human cancer

Aristidis Moustakas

Department of Medical Biochemistry and Microbiology
Uppsala University and Ludwig Cancer Research

The aim of our studies is to decipher molecular mechanisms that explain how cancer cell differentiation is controlled and how differentiation processes link to tumor-initiating cell self-renewal. Signal transduction by bone morphogenetic proteins (BMP) proceeds via serine-threonine kinase receptors, intracellular Smad protein effectors and kinase cascades.

We demonstrate for the first time how the nuclear enzyme poly(ADP)ribosyl polymerase 1 (PARP1) cooperates with additional members of the PARP family and Smad proteins of the BMP family to regulate signaling output.

We have also analyzed the crosstalk between the tumor suppressor kinase LKB1 and BMP pathways that operates at the level of the BMP receptor function in cell models, Drosophila melanogaster wing development and human lung cancer.

Finally, we will present data about the role of BMP signaling in limiting the tumorigenic potential of brain tumors such as glioblastoma mutliforme.

Aristidis Moustakas, Ph.D.

Professor, Uppsala University and Ludwig Cancer Research

URL:http://www.imbim.uu.se/Research/Biochemistry+and+Molecular+Cell+Biology+/Moustakas_Aristidis/?languageId=1
and http://www.ludwigcancerresearch.org/location/uppsala-branch/aristidis-moustakas-lab

1985 B.Sc. Department of Biology, Aristotelian University of Thessaloniki, Greece
1991 Ph.D. Department of Genetics and Cell Biology, University of Minnesota, USA
1991 Postdoctoral fellow, Whitehead Institute for Biomedical Research, Cambridge, USA
1996 Adjunct Assistant Professor, Department of Medicine, University of Crete, Greece
1998 Assistant Member, Ludwig Institute for Cancer Research-Uppsala Branch, Sweden
2004 Associate Member,Ludwig Institute for Cancer Research-Uppsala Branch, Sweden
2010 Full Member, Ludwig Institute for Cancer Research-Uppsala Branch, Sweden
2010 Senior Investigator of the Swedish Cancer Society-Uppsala University, Sweden
2011 Lecturer, Department of Medical Biochemistry and Microbiology, Uppsala University, Sweden
2013 Professor, Department of Medical Biochemistry and Microbiology, Uppsala University, Sweden

Recent selected publications

1. Morén A, Raja E, Heldin CH, Moustakas A. (2011) The kinase LKB1 associates with Smad4 and negatively regulates TGF-β signalling. ***J Biol Chem.*** 286, 341-353.

2. Lönn P van der Heide LP, Dahl M, Hellman U, Heldin CH, Moustakas A. (2010) PARP-1 attenuates Smad-mediated transcription. ***Mol Cell.*** 40, 521-532.

3. Thuault S, Tan E-J, Peinado H, Cano A, Heldin CH, Moustakas A. (2008) HMGA2 and Smads coregulate SNAIL1 expression during induction of epithelial-to-mesenchymal transition. ***J Biol Chem.*** 283, 33437-33446.

4. Gal A, Sjöblom T, Fedorova L, Imreh S, Beug H, Moustakas A. (2008) Sustained TGFβ exposure suppresses Smad and non-Smad signalling in mammary epithelial cells, leading to EMT and inhibition of growth arrest and apoptosis. ***Oncogene.*** 27, 1218-1230.

5. Kowanetz M, Lönn P, Vanlandewijck M, Kowanetz K, Heldin CH, Moustakas A. (2008) TGFβ induces SIK to negatively regulate type I receptor kinase signaling. ***J Cell Biol.*** 182, 655-662.

Roles of MafK and Gpnmb in malignant progression of breast cancer

Mitsuyasu Kato

Department of Experimental Pathology, Faculty of Medicine
University of Tsukuba

Breast cancer is classified into 5 molecular subtypes: luminal A, luminal B, HER2-enriched, basal-like, and normal breast-like, by the status of estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2). Basal-like breast cancer is often referred to as triple-negative breast cancer (TNBC), because the cancer cells in this subtype typically lack expression of ER, PR, and HER2. TNBC metastasizes frequently to bone, lung, pleura, and liver, and has poor prognosis. Therefore, further molecular characterization of TNBC is eagerly awaited, to aid the development of novel molecular targeting therapies. Here, we show that transcription factor MafK and the target gene Glycoprotein nmb (Gpnmb), a cell surface type I transmembrane protein are highly expressed in TNBC cells and that knockdown of these genes significantly suppresses the tumorigenic potential of breast cancer cells. Expression of MafK or Gpnmb confers epithelial-mesenchymal transition (EMT), sphere formation and promotes de-differentiated tumor progression on non-tumorigenic breast epithelial NMuMG cells without changing proliferative activity of the cells under ordinary cell culture conditions. Gpnmb has hemi immunoreceptor tyrosine-based activation motif (hemITAM) and mutation on the tyrosine residue in this motif totally abrogates the induction of EMT, sphere formation and tumorigenic activity of Gpnmb. These findings highlight the essential roles that Gpnmb plays in the malignant progression of breast cancer cells.

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Recent selected publications

1. Nakano N, Maeyama K, Sakata N, Itoh F, Akatsu R, Nakata M, Katsu Y, Ikeno S, Togawa Y, Thanh Thao Vo Nguyen TT, Watanabe Y, Kato M and Itoh S. C18 ORF1: A Novel Negative Regulator of TGF-β Signaling. ***J Biol Chem***, 289, 12680-12692, 2014.

2. Vo Nguyen TT, Watanabe Y, Shiba A, Noguchi M, Itoh S and Kato M. TMEPAI/PMEPA1 enhances tumorigenic activities in lung cancer cells. ***Cancer Sci***, 105, 334-341, 2014.

3. Okita Y, Kamoshida A, Suzuki H, Itoh K, Motohashi H, Igarashi K, Yamamoto M, Ogami T, Koinuma D, and Kato M. Transforming Growth Factor-β induces transcription factors MafK and Bach1 to suppress expression of the heme oxygenase-1 gene. ***J Biol Chem***, 288, 20658-20667, 2013.

4. Nakano N, Itoh S, Watanabe Y, Maeyama K, Itoh F, and Kato M. Requirement of TCF7L2 for TGF-β-dependent transcriptional activation of the TMEPAI gene. ***J Biol Chem***, 285, 38023-38033, 2010.

5. Watanabe Y, Itoh S, Goto T, Ohnishi E, Inamitsu M, Itoh F, Satoh K, Wiercinska E, Yang W, Shi L, Tanaka A, Nakano N, Mommaas AM, Shibuya H, ten Dijke P and Kato M. TMEPAI, a transmembrane TGF-β-inducible protein, sequesters Smad proteins from active participation in TGF-β signaling. ***Mol Cell***, 37, 123-134, 2010.

Effects of Smad3 linker and C-tail phosphorylation on tumorigenesis and metastasis

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Smad3, a major intracellular mediator of transforming growth factor-β (TGF-β) signaling, functions as both a positive and negative regulator in carcinogenesis. In response to TGF-β the TGF-β receptor phosphorylates serine residues at the Smad3 C-tail. Cancer cells often contain high levels of the mitogen-activated protein kinase (MAPK) and the cyclin-dependent kinase (CDK) activities, which can lead to the Smad3 linker region becoming highly phosphorylated at the basal state. Recently, we have demonstrated that mutation of the Smad3 linker phosphorylation sites markedly inhibited tumor growth, but significantly increased lung metastasis of breast cancer cell lines. In contrast, mutation of the Smad3 C-tail phosphorylation sites promoted tumorigenesis, but markedly inhibited lung metastasis. *In vitro* study revealed that the mutation of the Smad3 linker phosphorylation sites greatly intensifies the TGF-β-induced epithelial-mesenchymal transition (EMT) with an increased invasive activity, growth arrest and apoptosis together with reduction in the size of putative cancer stem cell subpopulation. These responses were completely reversed by the mutation of the C-tail phosphorylation sites, suggesting that the linker phosphorylation negatively regulates the canonical TGF-β signaling. Microarray analysis revealed that mutation of the Smad3 linker phosphorylation sites markedly induced genes including CRYAB , DEFE103B, and CXCR7 that might be involved in metastasis. Our results deepens the current understanding on cancer progression, and provides a therapeutic rationale for development of putative small molecule inhibitors or other compounds targeting Smad3 phosphorylation sites at the C-tail or linker region.

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2007-2010	Director, Lee Gil Ya Cancer and Diabetes Research Institute, and Distinguished Professor of Medicine, Gachon University of Medicine and Science, Korea
2007-present	Adjunct Professor, Ireland Cancer Center, Case Western Reserve University School of Medicine, Cleveland, OH, USA
2010-present	Director, Cancer Research Institute, and Distinguished Professor of Medicine, CHA University of Medicine and Science, Korea
2012-present	Visiting Professor, Tsukuba University, Japan

Recent selected publications

1. Yang, K.-M. et al. (2012) DRAK2, a Novel TGF-β-Inducible Protein, Participates in a Negative Feedback Loop to Control TGF-β/Smads Signaling by Binding to Type I TGF-β Receptor. ***Cell Reports***, 2, 1286-1299

2. Hong, S. et al(2013) Smad7 induces IRF1-dependent transcriptional activation of caspase 8 to restore TRAIL-mediated apoptosis. ***J. Biol. Chem***. 288, 3560-3570

3. Yoon, K. et al. (2013) Comprehensive genome- and transcriptome-wide analyses of mutations associated with microsatellite instability in Korean gastric cancers. ***Genome Res***. 23, 1109-1117

4. Yang, K.-M. et al. (2013) Loss of TBK1 induces epithelial-mesenchymal transition in the breast cancer cells by ER-a downregulation. ***Cancer Res***. 73, 6679-6689

5. Jung, S. M. (2013) Smad6 recruits the A20 deubiquitinating enzyme to act as a negative regulator of the TGF-β1-mediated noncanonical TRAF6-TAK1-p38 MAPK/JNK pathway. ***Nat Commun***. 2. 460

6. Kim, T.-A. et al. (2014) Smad7-Skp2 complex orchestrates c-Myc stability, impacting on the cytostatic effect of TGF-β. ***J. Cell Sci.*** 127, 411-421

7. Bae, E. et al. (2014) Definition of Smad3 phosphorylation events that affect malignant and metastatic behaviors in breast cancer cells. ***Cancer Res***. in press.

Cellular senescence and cancer: a gut microbial connection

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Obesity is associated with an increased risk of several types of cancer, but the mechanisms connecting obesity and tumorigenesis remain poorly understood. Here, we show that dietary or genetic obesity induces alterations of gut microbiota, thereby increasing the levels of deoxycholic acid (DCA), a gut bacterial metabolite known to cause DNA damage in mice. The enterohepatic circulation of DCA provokes DNA damage and consequent cellular senescence in hepatic stellate cells (HSCs), which in turn, secretes various inflammatory and tumour promoting factors in the liver, thus facilitating hepatocellular carcinoma (HCC) development in mice after exposure to chemical carcinogen. Interestingly, signs of senescence-associated secretory phenotype (SASP) were also observed in the HSCs in the area of HCC arising in patients with non-alcoholic steatohepatitis (NASH), implying that a similar pathway may contribute to at least certain aspects of obesity-associated HCC development in humans as well. We believe that these findings provide valuable new insights into the development of obesity-associated cancer and open up-new possibilities for its control. In this symposium, I will provide an overview of our work and discuss the next steps, focusing on the potential clinical implications of these findings.

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2003 Professor, Institute for Genome Research, University of Tokushima, Japan
2008 Division Chief, Division of Cancer Biology, The Cancer Institute, Japanese Foundation for Cancer Research, Japan

Recent selected publications

1. Imai, Y., Takahashi, A., Hanyuu, A., Hori, S., Sato, S., Naka, K., Hirao, A., Ohtani, N. and *Hara, E. (2014) Crosstalk between the RB-pathway and AKT signalling forms a Quiescence-Senescence switch. ***Cell Reports*** 7, 194-207

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(2013) Obesity-induced gut microbial metabolite promotes liver cancer through senescence secretome. ***Nature*** 49, 97-101

3. Takahashi, A., Imai, Y., Yamakoshi, K., Kuninaka, S., Ohtani, N., Yoshimoto, S., Hori, S., Tachibana, M., Anderton, E., Takeuchi, T., Shinkai, Y., Peters, G., Saya, H. and *Hara E. (2012) DNA damage signaling triggers degradation of histone methyltransferases through APC/CCdh1 in senescent cells. ***Molecular Cell*** 45, 123-131

4. Yamakoshi, K., Takahashi, A., Hirota, F., Nakayama, R., Ishimaru, N., Kubo, Y., Mann, D.J., Ohmura, M., Hirao, A., Saya, H., Arase, S., Hayashi, Y., Nakao, K., Matsumoto, M., Ohtani, N. and *Hara, E. (2009) Real-time in vivo imaging of p16Ink4a reveals cross-talk with p53. ***Journal of Cell Biology*** 186, 393-407

5. Ohtani, N., Imamura, Y., Yamakoshi, K., Hirota, F., Nakayama, R., Kubo, Y., Ishimaru, N., Takahashi, A., Hirao, A., Shimizu, T., Mann, D.J., Saya, H., Hayashi, Y., Arase, S., Matsumoto, M., Nakao, K. and *Hara, E. (2007) Visualizing the dynamics of p21Waf1/Cip1 cyclin-dependent kinase inhibitor expression in living animals. ***Proc. Natl. Acad. Sci. USA*** 104, 15034-15039

Roles of THG-1/Tsc22D4 in squamous cell carcinoma development

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Carcinoma cells exhibit a high level of robustness against environmental stresses, metabolic disorders and therapeutic efforts. Here, we provide a novel mechanism connecting oncogenic signaling and robustness by THG-1, a Tsc-22 family protein. THG-1 localized in the basal layer of normal squamous epithelium and overexpressed in squamous cell carcinomas. THG-1 knockdown suppresses the cell proliferation, invasiveness and tumorigenicity. THG-1 is phosphorylated by the receptor tyrosine kinase-Ras-ERK pathway, which is required for oncogenic Ras-mediated tumorigenesis. Furthermore, THG-1 interacts with several factors that regulate the cytoprotection, metabolism and microenvironment. These findings highlight the pivotal role of THG-1 as a novel regulator of cellular robustness and tumorigenssis under the oncogenic signaling pathway.

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Recent selected publications

1. Okita Y, Kamoshida A, Suzuki H, Itoh K, Motohashi M, Igarashi K, Yamamoto M, Ogami T, Koinuma D and Kato M Transforming Growth Factor-β Induces Transcription Factors MafK and Bach1 to Suppress Expression of the Heme Oxygenase-1 Gene. ***J.Biol.Chem***. 288:20658-20667,2013

2. Sonkoly E, Wei T, Loriè EP, Suzuki H, Kato M, Törmä H, Ståhle M and Pivarcsi A Protein kinase C-dependent upregulation of miR-203 induces the differentiation of human keratinocytes. ***J Invest Dermatol***. 130: 124-134, 2010

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Non-traditional drug discovery using artificial macrocycles for cancer therapeutics

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The genetic code is the law of translation, where genetic information encoded in RNA is translated to amino acid sequence. The code consists of tri-nucleotides, so-called codons, assigning to particular amino acids. In cells or in ordinary cell-free translation systems originating from prokaryotes, the usage of amino acids is generally restricted to 20 proteinogenic (standard) kinds, and thus the expressed peptides are composed of only such monomers. To overcome this limitation, we recently devised a new means to reprogram the genetic code, which allows us to express non-standard peptides containing multiple non-proteinogenic amino acids in vitro. This lecture will describe the most recent development in the genetic code reprogramming technology that enables us to express natural product-like non-standard peptides. The technology involves (1) efficient macrocyclization of peptides, (2) incorporation of non-standard amino acids, such as N-methyl amino acids, and (3) reliable synthesis of libraries with the complexity of more than a trillion members. When the technology is coupled with an in vitro display system, referred to as RaPID (Random non-standard Peptide Integrated Discovery) system, the non-standard macrocyclic peptide libraries with a variety ring sizes and building blocks can be screened (selected) against various drug targets inexpensively, less laboriously, and very rapidly. In this lecture, I shall describe recent successes in selecting highly active macrocycles against various drug targets related to cancer therapeutics.

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Bachelor of Engineering (1986) Department of Applied Chemistry, Okayama University, Okayama
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Master of Engineering (1989) Department of Applied Chemistry, Okayama University, Okayama (with Dr. Sigeru Torii)
Ph.D. (1994), Department of Chemistry, Massachusetts Institute of Technology, Cambridge, MA (with Dr. Satoru Masamune)

Professional Appointments

Post-doctoral Fellow (1994-1997), Harvard Medical School/Massachusetts General Hospital, Boston, MA (with Dr. Jack W. Szostak)
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Honors and Awards

2012 President Award of Science Council of Japan for New Inventions in Government-Industry-Academic Relation, Cabinet Office
2012 Boehringer Ingelheim Lectureship at Montreal University
2013 Award of The Chemical Society of Japan for Creative Work
2013 Lilly Distinguished Lecturer at Colorado State University
2014 Akabori Memorial Award 2014, Japanese Peptide Society

Recent selected publications

1. Y. Goto, Y. Ito, Y. Kato, S. Tsunoda, H. Suga* "One-pot synthesis of azoline-containing peptides in a cell-free translation system integrated with a posttranslational cyclodehydratase" ***Chemistry & Biology*** 21, 766–774 (2014). DOI: http://dx.doi.org/10.1016/j.chembiol.2014.04.008
2. N. Terasaka, G. Hayashi, T. Katoh, H. Suga* "An orthogonal ribosome-tRNAs pair via the engineering of peptidyl transferase center" ***Nature Chemical Biology*** 10, 555-557 (2014).
3. C.J. Hipolito, N.K. Bashiruddin, H. Suga* "Protein cocrystallization molecules originating from in vitro selected macrocyclic peptides" ***Current Opinion in Structural Biology*** 26, 24-31 (2014). http://dx.doi.org/10.1016/j.sbi.2014.03.001
4. A. Kodan, T. Yamaguchi, T. Nakatsu, K. Sakiyama, C.J. Hipolito, A. Fujioka, R. Hirokane, K. Ikeguchi, B. Watanabe, J. Hiratake, Y. Kimura, H. Suga, K. Ueda, H. Kato. "Structural basis for gating mechanisms of a eukaryotic P-glycoprotein homolog" ***Pro. Nat. Acad. Sci***. 111, 4049-4054 (2014).
5. T. Passioura, T. Katoh, Y. Goto, H. Suga* "Selection-based discovery of druglike macrocyclic peptides" ***Annual Review in Biochemistry***, Feb. 12 (2014).
6. Y. Tanaka, C.J. Hipolito, A.D. Maturana, K. Ito, T. Kuroda, T. Higuchi, T. Katoh, H.E. Kato, M. Hattori, K. Kumazaki, T. Tsukazaki, R. Ishitani, H. Suga*, O. Nureki "Structural basis for the drug extrusion mechanism by a MATE multidrug transporter" ***Nature*** 496, 247-51 (2013).

Functional Nexus between Keap1-Nrf2 System and Cellular Metabolism

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Nrf2 is a potent transcriptional activator coordinately regulating cytoprotective genes. While Nrf2 activation is beneficial to our health, Nrf2 is responsible for the malignant progression of cancers. We found that Nrf2 not only enhances survival of cancers by activating cytoprotective genes but also redirects glucose and glutamine into anabolic pathways by activating metabolic genes, which are advantageous for cancer proliferation. We also found that active PI3K-Akt signaling enables Nrf2 to induce the metabolic genes and modulate metabolism. Thus Nrf2 behaves as a facultative accelerator of cell proliferation through boosting metabolic reprogramming under the sustained activation of proliferative signals. Interestingly, recent cancer genome analyses identified cancer-prone mutations in genes involved in glucose metabolism. Among them, isocitrate dehydrogenase 1 (*IDH1*) mutations in anaplastic glioma patients correlate with better prognosis. To clarify the functional interaction between *IDH1* mutations and NRF2 pathway, we examined expressions of *NRF2* and its target genes in surgically resected anaplastic glioma and found that they were reduced in *IDH1*-mutant glioma. We introduced a mutant IDH1 to T98 glioma-derived cells and examined its effect on the NRF2 activity. Expressions of NRF2 and its target genes were lower in cells with the mutant IDH1 versus those with wild-type IDH1. Recruitment of NRF2 to target gene loci was reduced in the former cells. These results indicate that NRF2 activity is limited in the presence of the *IDH1* mutation and implicate that the better prognosis of anaplastic glioma patients with *IDH1* mutations is attributable to the suppression of NRF2 pathway. Thus, alteration in cellular metabolism, in turn, affects the Nrf2 activity, implying a novel nexus connecting cellular metabolism and stress response.

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Recent selected publications

1. Kanamori M, Higa T, Sonoda Y, Murakami S, Dodo M, Kitamura H, Taguchi K, Shibata T, Watanabe M, Suzuki H, Shibahara I, Saito R, Yamashita Y, Kumabe T, Yamamoto M, Motohashi H*, and Tominaga T. Activation of the NRF2 pathway and its impact on the prognosis of anaplastic glioma patients. ***Neuro-Oncology*** in press (*corresponding author)

2. Shirasaki K, Taguchi K, Unno M, Motohashi H*, and Yamamoto M. (2014) Nrf2 promotes compensatory liver hypertrophy after portal vein branch ligation in mice. ***Hepatology*** 59, 2371-2382 (*corresponding author)

3. Mitsuishi Y, Taguchi K, Kawatani Y, Shibata T, Nukiwa T, Aburatani H, Yamamoto M, and Motohashi H*. (2012) Nrf2 redirects glucose and glutamine into anabolic pathways in metabolic reprogramming. ***Cancer Cell*** 22, 66-79, 2012. (*corresponding author)

4. Motohashi H*, Fujita R, Takayama M, Inoue A, Katsuoka F, Bresnick EH and Yamamoto M. (2011) Molecular determinants for small Maf protein control of platelet production. ***Mol Cell Biol*** 31, 151-162. Erratum in: ***Mol Cell Biol*** 32, 2041, 2012 (*corresponding author)

5. Motohashi H*, Kimura M, Fujita R, Inoue A, Pan X, Takayama M, Katsuoka F, Aburatani H, Bresnick EH and Yamamoto M. (2010) NF-E2 domination over Nrf2 promotes ROS accumulation and megakaryocytic maturation. ***Blood*** 115, 677-686 (*corresponding author)

Clonal origin of microenvironmental cells in malignant lymphoma

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Angioimmunoblastic T-cell lymphoma (AITL) is a distinct subtype of T-cell lymphoma, the tumor cells of which are considered to arise from follicular helper T (TFH) cells. Histologic hallmarks of this cancer include prominent high endothelial venules, meshwork formation by follicular dendritic cells, and proliferation of reactive B cells. These rich microenvironmental structures as well as functioning nature of the tumor cells suggest a potential communication between the tumor cells and non-tumor components in the tumor tissue.

We recently identified novel recurrent mutations in *RHOA* in 70% of the AITL cohort. The *RHOA* mutations were strikingly concentrated at Gly17, with vast majority being replacement with Val (G17V). RHOA is a founder molecule in the RHO small GTPase family; nevertheless, the G17V mutant RHOA did not bind GTP, and further, inhibited the GTP binding by wild-type RHOA. We also confirmed dominant-negative nature of the G17V mutant RHOA, potentially changing the classical concept on the role of RHO family GTPase proteins in cancer.

The Darwinian model tells us a selection of the specific *RHOA* mutation-carrying clone in the particular environmental context within AITL. Interestingly, our findings implicated that both tumor cells and environmental cells of AITL arise in the same clonal origin. We identified mutations in *TET2*, encoding an enzyme converting methylcytosine to hydroxymethylcytosine in DNA, in almost all cases carrying the G17V *RHOA* mutation. We unexpectedly found that approximately half the paired normal bone marrow (BM) samples, free from tumor cell infiltration, had the *TET2* mutations identical to those found in the counterpart tumor sample. In most cases, the allele frequencies were similar in tumor and normal BM samples, implying that the paired tumor and normal BM samples contained the same *TET2* mutation-carrying clonal cells at similar frequencies. Therefore, the origin of AITL tumor cells is likely to be BM progenitor cells capable of giving rise to TFH cells and all blood lineage cells. As predicted, the same *TET2* mutations were identified in both Laser-microdissected tumor cell- and reactive B cell-enriched populations, indicating that the progenies of *TET2* mutation-carrying clonal cells participate in the microenvironmental structure of AITL. In contrast, the G17V *RHOA* mutations were restricted to the tumor samples, and particularly, to the tumor cell-enriched population within the tumor tissue.

It is an interesting issue whether and how epigenetic dysregulation by the *TET2* mutations in both tumor cells and surrounding reactive cells affects the tumor - microenvironmental communication. Also, an open question is raised whether and how such communication assists the clonal growth of TFH cells carrying G17V mutant RHOA.

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Recent selected publications

1. Kato T, Sakata-Yanagimoto M, Nishikii H, Miyake Y, Yokoyama Y, Asabe Y, Kamada Y, Ueno M, Obara N, Suzukawa K, Hasegawa Y, Kitabayashi I, Uchida K, Hirao A, Yagita H, Kageyama R, Chiba S. Hes1 suppresses acute myeloid leukemia development through FLT3 repression. ***Leukemia***, in press

2. Sakata-Yanagimoto M, Enami T, Yoshida K, Shiraishi Y, Ishii R, Miyake Y, Muto H, Tsuyama N, Sato-Otsubo A, Okuno Y, Sakata S, Kamada Y, Nakamoto-Matsubara R, Tran NB, Izutsu K, Sato Y, Ohta Y, Furuta J, Shimizu S, Komeno T, Sato Y, Ito T, Noguchi M, Noguchi E, Sanada M, Chiba K, Tanaka H, Suzukawa K, Nanmoku T, Hasegawa Y, Nureki O, Miyano S, Nakamura N, Takeuchi K, Ogawa S, Chiba S. Somatic RHOA mutation in angioimmunoblastic T cell lymphoma. ***Nat Genet*** 46:171-5, 2014

3. Yoshida K, Sanada M, Shiraishi Y, Nowak D, Nagata Y, Yamamoto R, Sato Y, Sato-Otsubo A, Kon A, Nagasaki M, Chalkidis G, Suzuki Y, Shiosaka M, Kawahata R, Yamaguchi T, Otsu M, Obara N, Sakata-Yanagimoto M, Ishiyama K, Mori H, Nolte F, Hofmann WK, Miyawaki S, Sugano S, Haferlach C, Koeffler HP, Shih LY, Haferlach T, Chiba S, Nakauchi H, Miyano S, Ogawa S. Frequent pathway mutations of splicing machinery in myelodysplasia. ***Nature*** 478:64-9, 2011

4. Sakata-Yanagimoto M, Sakai T, Miyake Y, Saito TI, Maruyama H, Morishita Y, Nakagami-Yamaguchi E, Kumano K, Yagita H, Fukayama M, Ogawa S, Kurokawa M, Yasutomo K, Chiba S. Notch2 signaling is required for proper mast cell distribution and mucosal immunity in the intestine. ***Blood*** 117:128-34, 2011

5. Nakahara F, Sakata-Yanagimoto M, Komeno Y, Kato N, Uchida T, Haraguchi K, Kumano K, Harada Y, Harada H, Kitaura J, Ogawa S, Kurokawa M, Kitamura T, Chiba S. Hes1 immortalizes committed progenitors and plays a role in blast crisis transition in chronic myelogenous leukemia. ***Blood*** 115:2872-81, 2010

The fibrinolytic system modulates the cytokine and cellular environment during cancer progression and chronic inflammation

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The serine protease plasmin generated from its zymogen plasminogen and activated by plasminogen activators is best known for its function as a key enzyme of the fibrinolytic cascade. But plasmin can also change the activation status of growth factors (e.g. latent TGF-β activation in tumor cells), of growth factor receptors (e.g. TGFR), and of matrix metalloproteinases (MMPs). Plasminogen activator inhibitor-1 (PAI-1) gene transcription is regulated by Smad3 and 4 binding to its promoter region. PAI-1 is the principal inhibitor of tissue type plasminogen activator and urokinase.

We show that plasmin regulates myelomonocytic cell recruitment into cancer or inflammatory tissues by enhancing the activity of the chemokines monocyte chemotactic protein 1 and CXCL5. Infiltrating myelomonocytic cells can release inflammatory cytokines like TNF-α. It was reported by others that Smad7 expression is increased in response to TNF-α. We found that plasmin controls the progression of TNF-α-associated diseases like chronic colitis, and graft-versus host disease after bone marrow transplantation through MMP-dependent and -independent pathways. We propose that plasmin alters the cellular and cytokine environment, and thereby control growth factor-associated cancer progression and chronic inflammatory diseases.

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2002 Senior Research Associate, Department of Hematology-Oncology, Weill Medical College of Cornell University, US
2003 Assistant Professor, Department of Transfusion Medicine, Juntendo University School of Medicine, Japan
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2004 Project Research Associate, Stem Cell Regulation, Center for Experimental Medicine, The Institute of Medical Science, The University of Tokyo, Japan
2007 Project Assistant Professor, Stem Cell Regulation, Center for Experimental Medicine, The Institute of Medical Science, The University of Tokyo, Japan
2008 Project Assistant Professor, Frontier Research Initiative, The Institute of Medical Science, The University of Tokyo, Japan
2010 Project Associate Professor, Frontier Research Initiative, The Institute of Medical Science, The University of Tokyo, Japan
2012 Associate Professor, Department of Stem Cell Dynamics, Center for Stem Cell Biology and Regenerative Medicine, The Institute of Medical Science, The University of Tokyo

Recent selected publications

1. Sato A, Nishida C, Sato-Kusubata K, Ishihara M, Tashiro Y, Gritli I, Shimazu H, Munakata S, Yagita H, Okumura K, Tsuda Y, Okada Y, Tojo A, Nakauchi, H, Takahashi S, Hattori K, Heissig B. Inhibition of plasmin attenuates murine acute graft-versus-host disease mortality by suppressing the matrix metalloproteinase-9-dependent inflammatory cytokine storm and effector cell trafficking. Leukemia, 2014, ***Leukemia***. 2014 May 5. doi: 10.1038/leu.2014.151. [Epub ahead of print].
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3. Nishida C, Kusubata K, Tashiro Y, Gritli I, Sato A, Ohki-Koizumi M, Morita Y, Nagano M, Sakamoto T, Koshikawa N, Kuchimaru T, Kizaka-Kondoh S, Seiki M, Nakauchi H, Hattori K, Heissig B. MT1-MMP plays a critical role in hematopoiesis by regulating HIF-mediated chemokine-/cytokine gene transcription within niche cells. ***Blood***, 119, 5405-16, 2012.
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Dynamic Smad signaling networks in immune cell regulation

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Transforming growth factor-β (TGF-β) has been characterized as the most potent immunosuppressive cytokine in inflammatory lesions including tumor microenvironment. Various abnormalities in TGF-β signaling pathway via Smads have been reported to be crucial for carcinogenesis. However, roles of Smad-mediated TGF-β signaling in immune cell subsets remain largely undetermined. Here, we discuss our recent findings on the diverse roles of Smads in the regulation of I> dendritic cells (DC), II> cytotoxic T lymphocytes (CTL), and III> IL-17-producing CD4+ T helper cells (Th17). I> We found that DC progenitors such as mouse bone marrow CD115+ lineage marker- cells and human monocytes lost the expression of Smad3 along the differentiation into DCs and Smad2 was the specific TGF-β receptor-regulated Smad to suppress the maturation and functions of DCs. Accordingly, DC-specific Smad2 deletion significantly enhanced anti-tumor immunity. II> TGF-β signaling via the common Smad: Smad4 in combination with Smad3, but not Smad2, suppressed the Eomes gene, the essential transcription factor for CTL, and thereby suppressed the differentiation and functions of CTLs. TGF-β antagonism enhanced anti-tumor CTL activity by inducing ubiquitin-mediated degradation of Smad4 in CD8+ T cell-specific manner. III> Smad2 and Smad3 oppositely regulated Th17 differentiation, independently of Smad4. We found that linker-phosphorylated Smad2 acted as a transcription coactivator of STAT3, whereas C-terminally unphosphorylated Smad3 acted as a transcription corepressor of STAT3. These findings suggest that Smads orchestrate immune regulation in highly cell-type-specific dynamic signaling networks.

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2000-2005	Visiting Fellow (2000-2003), Research Fellow (2003-2005), Laboratory of Cell Regulation and Carcinogenesis, Center for Cancer Research, National Cancer Institute, National Institutes of Health, USA
2005-2007	Assistant Professor, Clinical Immunology, Major of Advanced Biomedical Applications, Graduate School of Comprehensive Human Science (Department of Rheumatology and Allergology), University of Tsukuba, Japan
2007-2012	Lee Gil Ya Cancer and Diabetes Institute, Gachon Medical and Science University, Korea
2011~present	Adjunct Professor, Department of Molecular Pathology, Tokyo Medical University, Japan
2012-2014	Department of Microbiology, CHA University/Internal Medicine, Bundang CHA Hospital, South Korea
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Recent selected publications

1. Yoon JH, Jung SM, Park SH, Kato M, Yamashita T, Lee IK, Sudo K, Nakae S, Han JS, Kim OH, Oh BC, Sumida T, Kuroda M, Ju JH, Jung KC, Park SH, Kim DK, Mamura M. Activin receptor-like kinase5 inhibition suppresses mouse melanoma by ubiquitin degradation of Smad4, thereby derepressing eomesodermin in cytotoxic T lymphocytes. ***EMBO Mol Med***. 11:1720-1739, 2013

Coordinated chromatin regulation by developmental signals during cardiomyocyte differentiation

Hiroyuki Aburatani

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Cell identity is regulated by defined combinations of transcription factors, which bind to cis-regulatory elements and generate cell-specific transcriptional networks. These networks are encoded and memorized in chromatin as the epigenome. When cells differentiate into certain lineages, their identity dynamically changes along with their transcriptional networks and epigenomes, although the underlying mechanisms remain to be understood. Here, we applied epigenome and single cell transcriptome analysis during cardiomyocyte differentiation of mouse embryonic stem cells and P19CL6 cells, an embryonic carcinoma cell line that can robustly differentiate into cardiomyocytes. We demonstrate that Wnt/β-catenin signaling switches the transcriptional networks and re-organizes the epigenomes to specify mesodermal and cardiac lineages from the pluripotent state. ChIP-seq analysis of β-catenin and other transcription factors and their protein interaction analysis show that β-catenin augments its signal by activating Wnt3A transcription and induces mesodermal transcription factors, such as Brachyury. These factors cooperatively bind to mesoderm-specific regulatory elements and recruit chromatin modifiers, such as chromatin remodeling factors and histone acetyltransferases, to establish and maintain the differentiated state. FAIRE-seq analysis revealed stage-specific increase in chromatin accessibility at these sites. Collectively, cell identity and its specificity is determined by coordination of chromatin regulators through development.

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Recent selected publications

1. Totoki Y, et al. Trans-ethnic mutational landscape of hepatocellular carcinoma genomes. ***Nature genetics*** accepted

2. Isogaya K, et al. A Smad3 and TTF-1/NKX2-1 complex regulates Smad4-independent gene expression. ***Cell Res***. 24:994-1008. 2014

3. Kakiuchi M, et al. Recurrent gain-of-function mutations of RHOA in diffuse-type gastric carcinoma. ***Nature genet***. 46:583-7. 2014

4. Inoue T, et al. Cross-enhancement of ANGPTL4 transcription by HIF1 alpha and PPAR beta/delta is the result of the conformational proximity of two response elements. ***Genome Biol.*** 15:R63. 2014

5. Johnson BE, et al. Mutational Analysis Reveals the Origin and Therapy-Driven Evolution of Recurrent Glioma. ***Science***. 343:189-93. 2014

6. Goda S, et al. Control of Histone H3 Lysine 9 (H3K9) Methylation State via Cooperative Two-step Demethylation by Jumonji Domain Containing 1A (JMJD1A) Homodimer. ***J Biol Chem***. 288:36948-56. 2013

7. Sato T, et al. PRC2 overexpression and PRC2-target gene repression relating to poorer prognosis in small cell lung cancer. ***Sci Rep***. 3:1911. 2013

8. Kaneda A, et al. Epstein-barr virus infection as an epigenetic driver of tumorigenesis. ***Cancer Res***. 72:3445-50. 2012

9. Wang L, et al. Whole-exome sequencing of human pancreatic cancers and characterization of genomic instability caused by MLH1 haploinsufficiency and complete deficiency. ***Genome Research*** 22:208-19. 2012

Research interest

Cancer genomics and epigenomics

Identification of Smad regulatory factors through integrated analysis of ChIP-seq data

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Co-localization of cell-type-specific transcription factors and Smad family protein in the genome is important for context-dependent outputs of transforming growth factor-β (TGF-β) signals. We are investigating what kinds of tissue- and cancer-specific factors regulate TGF-β-Smad signaling at genome wide level. Usefulness of ChIP-seq-based studies for proper identification of regulatory mechanisms as well as target genes of Smad signaling will be discussed by showing the examples of our studies in lung adenocarcinoma cells and diffuse-type gastric cancer cells. First, our quantitative analysis of ChIP-sequencing data of Smad3, Smad4 and thyroid transcription factor-1 (TTF-1) in lung adenocarcinoma cells revealed that TTF-1 dissociates Smad3-Smad4 complex in the nucleus and inhibits Smad3 from binding to chromatin in a subset of its target regions. TTF-1 also co-localizes with Smad3 and regulates distinct target regions in the absence of TGF-β. Our integrated approach also lead to the successful identification of several direct target genes of Smad3 and TTF-1 whose expressions are well correlated with prognosis of the patients and have essential roles in mTOR signaling and cellular metabolism. Second, comparative analysis of the in vitro and in vivo Smad binding regions in diffuse-type gastric cancer cells was performed to search for potential regulatory pathway(s) of TGF-β signaling in xenografted tumors. Our approaches will provide an efficient way of identifying specific regulators of TGF-β signaling in a given context.

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2012 Project Associate Professor, Department of Molecular Pathology, Graduate School of Medicine, The University of Tokyo
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Recent selected publications

1. Isogaya, K., Koinuma, D., Tsutsumi, S., Saito, R. A., Miyazawa, K., Aburatani, H., and Miyazono, K. (2014) A Smad3 and TTF-1/NKX2-1 complex regulates Smad4-independent gene expression. ***Cell Res***. 24, 994-1008.

2. Arase, M., Horiguchi, K., Ehata, S., Morikawa, M., Tsutsumi, S., Aburatani, H., Miyazono, K., and Koinuma, D. (2014) Transforming growth factor-β-induced lncRNA-Smad7 inhibits apoptosis of mouse breast cancer JygMC(A) cells. ***Cancer Sci***. 105, 974-982

3. Morikawa, M., Koinuma, D., Tsutsumi, S., Vasilaki, E., Kanki, Y., Heldin, C. H., Aburatani, H., and Miyazono, K. (2011) ChIP-seq reveals cell type-specific binding patterns of BMP-specific Smads and a novel binding motif. ***Nucleic Acids Res*** 39, 8712-8727.

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Ectopic induction of developmental gene regulatory network by cryptic promoters in gastric cancer

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Recent exome-wide cancer genome sequencing projects led identification of cancer-associated mutations and gene copy number changes in different types of human cancer. However, it is still unclear how non-coding regulatory regions such as enhancers and unannotated functional genomic regions are regulated in native cancer tissue. In this talk, I will present insights learned from integrative genome and epigenome analysis of primary gastric cancer tissues.

To profile somatic alternations of regulatory regions and underlying genetic variations, long-read ChIPseq for 5 histone modifications, marking gene promoters, enhancers, transcribed regions and repressed regions, were performed on 5 pairs of cancer and matched normal tissues. One of the prominent features found in cancer epigenome was widespread usage of non-canonical gene promoter regions. These 'cryptic' promoters were often associated with expressed exons which were linked to nearby genes, creating altered form of 1st exon for transcripts. Interestingly, cancer-associated cryptic promoters were enriched near developmental genes. These results indicated that cryptic promoters drive ectopic activation of developmental pathways which are normally inactive in normal adult stomach tissue. It also may provide mechanistic explanation for the link between embryonic gene regulatory network and cancer.

Epigenetic and genetic events were explored to obtain clues for cryptic promoter activation. Analysis of transcription factor binding sites showed that cancer-associated promoters were enriched at targets of Suz12 and Ezh2, polycomb complex 2 components which has been implicated in human cancer. Genetic polymorphisms and somatic mutations found in ChIPseq reads were filtered for allele-specific regulation and overlap with cancer-associated regulatory regions to identify candidate functional genetic variants. I will further discuss use of allele frequency information to address the issues on tissue heterogeneity and analysis of tumor microenvironment.

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2005 Research Fellow, Cancer Research UK London Research Institute, UK

2008 Research Associate, Genome Institute of Singapore, Singapore

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Recent selected publications

1. Muratani, M., Deng, N., Ooi, W.F., Lin, S.J., Xing, M., Xu, C., Qamra, A., Tay, S.T., Malik, S., Wu, J., Lee, M.H., Zhang, S., Tan, L.L., Chua, H., Wong, W.K., Ong, H.S., Ooi, L.L., Chow, P.K., Chan, W.H., Soo, K.C., Goh, L.K., Rozen, S., Teh, B.T., Yu, Q., Ng, H.H., Tan, P. (2014) Nanoscale chromatin profiling of gastric adenocarcinoma reveals cancer-associated cryptic promoters and somatically acquired regulatory elements. ***Nature Communications***. 5:4361.

2. Kumar, V., Muratani, M., Rayan, N.A., Kraus, P., Lufkin, T., Ng, H.H., Prabhakar, S. (2013) Insights from uniform, optimal signal processing of next-gen tag profiles. ***Nature Biotechnology***. 31(7), 615-22.

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Impaired macrophage polarization in endoglin haplo-insufficiency leading to defective tissue repair.

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Introduction : Endoglin is a co-receptor of Transforming Growth Factorβ (TGFβ) and is crucial for the formation of new blood vessels. Endoglin-haploinsuffiency is the leading cause of the severe vascular disease Hereditary hemorrhagic telangiectasia 1 (HHT-1). The aim of this study is to investigate whether endoglin haplo-insufficiency impairs macrophage polarization towards a regenerative phenotype to induce angiogenesis and tissue repair in ischemic disease.

Materials and Methods : HHT-1 was studied in Eng+/- mice and in HHT-1 patients with endoglin mutation. In Eng+/- and Eng+/+ mice, angiogenesis and tissue repair were studied by the induction of hindlimb ischemia, myocardial infarction or in matrigel plugs. Macrophage phenotypes were screened after a differentiation assay in-vitro after stimulation with GM-CSF or in tissue sections with immuno-histochemistry.

Results : Angiogenic capacity was impaired in Eng+/- mice, which was associated by the infiltration of macrophages. Ischemic muscle or heart sections of Eng+/- mice showed prolonged inflammation 7 days postischemia. We can explain this by an extended polarization of bone marrow-derived monocytes (BMM) from Eng+/- mice towards pro-inflammatory macrophage (CD11b+/LY6Chi/CD206-) at the expense of regenerative macrophages. In addition, stimulation of macrophages with TGFβ resulted in the polarization of the more regenerative phenotype only in WT BMM-cultures. This TGFβ response was blunted in macrophages from Eng+/- mice, but we were able to rescue it by the inhibition of the Bone Morphogenetic Protein (BMP)-pathway (by LDN-193189). Subsequently, LDN also improved blood flow recovery and myocardial function in Eng+/- mice after hind limb ischemia and MI, respectively. Interestingly, blood monocytes from HHT-1 patients show increased number of CD14+/CD16+ monocytes, which is associated with pro-inflammatory/non-regenerative phenotype.

Discussion and Conclusions : Endoglin haplo-insufficiency results in defective tissue repair due to impaired polarization of regenerative macrophages, directed by TGFβ. The defective responses were rescued by the inhibition of another TGFβ familiy member, BMP, and thereby counterbalance its pathway. These results have major implications for the treatment of HHT-1 patients, as we show the same impaired macrophage polarization.

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1994 PhD student at the Netherlands Institute for developmental biology in Utrecht.
1999 Postdoctoral researcher at the Ludwig Institute for Cancer Research in Uppsala, Sweden.
2000 Postdoctoral researcher at the Netherlands Cancer Institute, Amsterdam.
2003 Assistant professor at the department of Cardiology, UMCU, Utrecht.
2008 Associate professor at the department of Molecular cell Biology, LUMC.
2012 Full professor at the department of Molecular cell Biology, LUMC

Recent selected publications

1. Grewal N, Gittenberger-de Groot AC, DeRuiter MC, Klautz RJ, Poelmann RE, Duim S, Lindeman JH, Koenraadt WM, Jongbloed MR, Mohamed SA, Sievers HH, Bogers AJ, Goumans MJ. Bicuspid aortic valve: phosphorylation of c-Kit and downstream targets are prognostic for future aortopathy. ***Eur J Cardiothorac Surg***. 2014 Nov;46(5):831-9.

2. Tijsen AJ, van der Made I, van den Hoogenhof MM, Wijnen WJ, van Deel ED, de Groot NE, Alekseev S, Fluiter K, Schroen B, Goumans MJ, van der Velden J, Duncker DJ, Pinto YM, Creemers EE. The microRNA-15 family inhibits the TGFβ-pathway in the heart. ***Cardiovasc Res***. 2014 Oct 1;104(1):61-71.

3. Mosqueira D, Pagliari S, Uto K, Ebara M, Romanazzo S, Escobedo-Lucea C, Nakanishi J, Taniguchi A, Franzese O, Di Nardo P, Goumans MJ, Traversa E, Pinto-do-Ó P, Aoyagi T, Forte G. Hippo pathway effectors control cardiac progenitor cell fate by acting as dynamic sensors of substrate mechanics and nanostructure. ***ACS Nano***. 2014 Mar 25;8(3):2033-47.

4. Karkampouna S, Kruithof BP, Kloen P, Obdeijn MC, van der Laan AM, Tanke HJ, Kemaladewi DU, Hoogaars WM, 't Hoen PA, Aartsma-Rus A, Clark IM, Ten Dijke P, Goumans MJ, Kruithof-de Julio M. Novel Ex Vivo Culture Method for the Study of Dupuytren's Disease: Effects of TGFβ Type 1 Receptor Modulation by Antisense Oligonucleotides. ***Mol Ther Nucleic Acids***. 2014 Jan 21;3:e142.

5. Kruithof BP, Kruithof-De-Julio M, Poelmann RE, Gittenberger-De-Groot AC, Gaussin V, Goumans MJ. Remodeling of the myocardium in early trabeculation and cardiac valve formation; a role for TGFβ2. ***Int J Dev Biol***. 2013;57(11-12):853-63.

Roles of signal networks during the formation and maintenance of tumor vasculature

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Tumor microenvironment is composed of multiple components including cancer cells, blood vessels, fibroblasts and so on. Recent reports have shown that a part of cancer-associated fibroblasts is originated from endothelial cells via endothelial-mesenchymal transition (EndMT). While multiple cytokines including transforming growth factor (TGF)-β have been implicated in EndMT, the molecular mechanisms underlying it remain to be fully elucidated. Here, we examined the effects of TGF-β signals on the EndMT of various types of endothelial cells. By addition of TGF-β2, MS-1 endothelial cells underwent EndMT characterized by increased expression of various mesenchymal markers such as smooth muscle α-actin (SMA). We found that the activation of Rho signals was found to be essential for the mesenchymal transition of MS-1 cells. We also found that TGF-β2 induces the expression of MRTF-A. Furthermore, we found that this TGF-β/Rho/MRTF-A axis is inhibited in tumor microenvironment by novel mechanisms in order to maintain the characteristics of tumor endothelial cells. These findings suggest that TGF-β-induced EndMT plays important roles in determining the characteristics of tumor microenvironment.

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2009 Associate Professor, Department of Molecular Pathology, Graduate School of Medicine, The University of Tokyo, Japan
2013 Professor, Laboratory of Oncology, School of Life Sciences, Tokyo University of Pharmacy and Life Sciences, Japan

Recent selected publications

1. Miyazaki H, Yoshimatsu Y, Akatsu Y, Mishima K, Fukayama M, Watabe T, Miyazono K. (2014) Expression of platelet-derived growth factor receptor β is maintained by Prox1 in lymphatic endothelial cells and is required for tumor lymphangiogenesis. ***Cancer Science***. 2014 105:1116-1123.

2. Yoshimatsu Y, Lee YG, Akatsu Y, Taguchi L, Suzuki HI, Cunha SI, Maruyama K, Suzuki Y, Yamazaki T, Katsura A, Oh SP, Zimmers TA, Lee SJ, Pietras K, Koh GY, Miyazono K, Watabe T. (2013) Bone morphogenetic protein-9 inhibits lymphatic vessel formation via activin receptor-like kinase 1 during development and cancer progression. ***Proc Natl Acad Sci U S A***. 110:18940-18945.

3. Mihira H, Suzuki HI, Akatsu Y, Yoshimatsu Y, Igarashi T, Miyazono K, Watabe T. (2012) TGF-β-induced mesenchymal transition of MS-1 endothelial cells requires Smad-dependent cooperative activation of Rho signals and MRTF-A. ***Journal of Biochemistry***. 143:199-206.

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5. Kokudo T, Suzuki Y, Yoshimatsu Y, Yamazaki T, Watabe T, Miyazono K. (2008) Snail is required for TGFβ-induced endothelial-mesenchymal transition of embryonic stem cell-derived endothelial cells. ***Journal of Cell Science***. 121:3317-3324.

Maintenance of the stemness in cancer stem cells requires CD44 expressed by tumor stromal cells via tissue hypoxia

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In the normal organ, stem cells localize in the specific foci so called niche and niche cells support stemness of stem cells by providing molecular cues to them. In the cancer microenvironment, it has been elucidated that cancer stem cells (CSCs) self-renew and differentiate into cancer cells to generate tumors. It has been suggested that CSCs also localize in the CSC niche and maintain immature status as stem cells affected by niche molecules. Therefore, unmasking CSC niche is critical to understand how differentiation and proliferation of CSCs are regulated by molecular cues from niche cells. We reported that CSCs are identified by their high promoter activity of PSF1 gene, a member of DNA replication factors, GINS. Using reporter gene composed with PSF1 promoter and EGFP, we found that CSCs frequently localize in the perivascular area. In the tumor, most of blood vessels are immature phenotype in which endothelial cells (ECs) are not covered with mural cells such as pericytes. However, alpha smooth muscle actin (αSMA) positive mural cell like myofibroblastic cells (Here, we call these cells as CAFs) can be observed near vasculature. We found that CSCs show drug resistance when they localize near CAFs in the perivascular area. We characterize these CAFs and found that CAFs express CD44 abundantly when they are in the hypoxic condition. We analyzed the function of CD44 on CAFs by generating co-culture system of CSCs with CAFs and elucidated that CD44 on CAFs is involved in the maintenance of stemness in CSCs. Moreover, drug resistance of CSCs was cancelled in tumors which are generated by the inoculation of cancer cells into CD44 mutant mice. In the session, we will discuss whether improvement of hypoxia into normoxia by the normalization of tumor vasculature affects malignancy of tumor associating with characteristic change of CAFs.

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1997 Graduated from Kyoto Univ. Graduate School of Medicine and obtained Ph.D.
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1999 Assistant Professor, the same as above
2000 Associate Professor, the same as above
2001 Professor, Cancer Research Institute, Kanazawa University
2006 Professor, Research Institute for Microbial Disease, Osaka University

Recent selected publications

1. Kinugasa Y, Matsui T, Takakura N. CD44 expressed on CAFs is a functional molecule supporting the stemness and drug resistance of malignant cancer cells in the tumor microenvironment. ***Stem Cells*** 32; 145-156, 2014

2. Muramatsu F, Kidoya H, Naito H, Sakimoto S, Takakura N. microRNA-125b inhibits tube formation of blood vessels through translational suppression of VE-cadherin. ***Oncogene*** 32:414-421, 2013

3. Kidoya H, Kunii N, Naito H, Muramatsu F, Okamoto Y, Nakayama T, Takakura N. The apelin/APJ system induces maturation of the tumor vasculature and improves the efficiency of immune therapy. ***Oncogene*** 31: 3254-3264, 2012

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Targeting tumor vasculatures

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Tumor vessels are malformed, leaky, highly branched and somewhat disintegrated from multiple vascular remodeling. These features are mainly caused by abnormally high VEGF-A levels in the tumor microenvironment, and blockade of VEGF-A has been shown to suppress the pathological characteristics of tumor vessels. However, the inhibition of vascularization by blockade of VEGF-A/VEGFR2 signaling frequently promotes rebound hypoxia, micro-invasion, drug-resistance and metastasis, all of which arise as additional challenges to cancer treatment using current anti-VEGF therapy. Since angiopoietin-2 (Ang2) level is high in the tumor microenvironment and plays a supportive role in VEGF-A-induced pathological vascular remodeling, single blockade of Ang2 or simultaneous blockade of VEGF-A and Ang2 are currently being developed and tested in experimental and clinical settings. The benefits of double anti-angiogenic protein (DAAP), which simultaneously blocks VEGF-A and Ang2, over single blockade in suppressing tumor angiogenesis, metastasis and vascular leakage will be addressed. Moreover, a promising antibody that induces Tie2 activation for effective tumor vessel normalization will be introduced. Nevertheless, such anti-angiogenic therapies against tumor progression still seem not so effective, selective and limited in clinics. To overcome these limitations, we have uncovered the role of RhoJ, a new endothelial-enriched Rho GTPase, during tumor progression. RhoJ blockade offers a double assault on tumor vessels by both suppressing tumor angiogenesis and disrupting the preformed tumor vessels, through the activation of the RhoA-ROCK (Rho kinase) signaling pathway in tumor endothelial cells, consequently leading to functional failure of tumor vasculatures. These results identify RhoJ blockade as a selective and effective therapeutic strategy for targeting tumor vasculature with minimal side effects.

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1995-2001	Assistant Professor/Associate Professor, Department of Physiology, Chonbuk National University Medical School
1997-2003	Director, National Creative Research Initiatives for Endothelial Cells, Korean Minister of Science and Technology
2001-2003	Associate Professor, Department of Life Sciences, Pohang University of Science and Technology (POSTECH)
2003-2010	Professor
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Recent selected publications

1. Park D-Y, Lee J, Park I, Choi D, Lee S, Song S, Hwang Y, Hong KY, Nakaoka Y, Makinen T, Kim P, Alitalo K, Hong Y-K, Koh GY* (2014) Prox1 as a biosensor reflecting the integrity of Schlemm's canal. ***J. Clinical Investigation*** 124:3960-3974

2. Kim C, Yang H, Fukushima Y, Saw PE, Lee J, Park I, Kataoka H, Heo WD, Kim I, Jon S, Adams R, Nishikawa S, Uemura A, Koh GY* (2014) Vascular RhoJ is an effective and selective target for tumor angiogenesis and vascular disruption. ***Cancer Cell*** 25:102-117.

3. Kim H, Kataru RP, Koh GY* (2014) Inflammation-associated lymphangiogenesis: A double-edged sword? ***J. Clinical Investigation*** 124:936-942, 2014 (Review).

4. Lee J, Kim KE, Kim H, Kim KH, Jang JY, Chang W, Suda T, Mochizuki N, Nakaoka Y, Komuro I, Yoo O-J, Koh GY* (2013) Angiopoietin-1 guides directional angiogenesis through integrin signaling pathway. ***Science Translational Medicine*** 5:203ra127

5. Kataru RP, Kim H, Jang C, Choi DK, Koh BI, Kim M, Gollamudi S, Kim YK, Lee SH, Koh GY* (2011) T lymphocytes negatively regulate lymph node lymphatic vessels through interferon-gamma. ***Immunity*** 34:96-107.

6. Kim M, Koh YJ, Kim KE, Koh BI, Nam DH, Alitalo K, Kim I, Koh GY* (2010) Regulation of chemo-resistant melanoma cell metastasis by a lymphatic metastatic niche: a key role of CXCR4 signaling. ***Cancer Research*** 70:10411-10421.

7. Koh YJ, Kim HZ, Hwang SI, Lee JE, Oh N, Jung K, Kim M, Kim KE, Kim H, Lim NK, Jeon CJ, Lee GM, Jeon BH, Nam DH, Sung HK, Nagy A, Yoo OJ, Koh GY* (2010) Double anti-angiogenic protein, DAAP, targeting VEGF-A and angiopoietins in tumor angiogenesis, metastasis, and vascular leakage. ***Cancer Cell*** 18:171-184.

Bone microenvironment confers Hsp90 inhibitor resistance in the metastatic small cell lung cancer.

Seiji Yano[1], Kouji Fukuda[1], Eiji Hara[2], Shinji Takeuchi[1]

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Small-cell lung cancer (SCLC) accounts for nearly 15% of lung cancer cases and presents aggressive clinical behavior characterized by rapid growth and metastatic spread to multiple organs. About 70% of patients with SCLC are diagnosed as extensive disease with distant metastases. Hsp90 is a 90 kDa molecular chaperon whose association is required for the stability and function of numerous "client proteins". Here, we determined therapeutic potential of Hsp90 inhibitors against SCLC. Hsp90 inhibitors inhibited viability of human SCLC cell lines, regardless their chemo-sensitivity, via decreased protein expression of AKT and CRAF. In the in vivo imaging multiple-organ metastasis model with the human SCLC cell line SBC-5, treatment with hsp90 inhibitor 17-DMAG remarkably inhibited the liver metastasis, but not bone metastasis. Several bone derived growth factors, including TGF-β, protected SBC-5 cells from 17-DMAG and preserved expression of AKT and CRAF. Combined use of bisphosphonate with Hsp90 inhibitor controlled the liver metastasis and the bone metastasis as well. These findings suggest that therapeutic effects of Hsp90 inhibitors may be different among organs and should be carefully evaluated in clinical trials in SCLC.

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1995 PhD, Third Department of Internal Medicine, University of Tokushima
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1997 Visiting Assistant Professor, Department of Cancer Biology (Professor IJ Fidler), MD Anderson Cancer Center, University of Texas, Houston TX.
2000 Lecturer, Department of Internal Medicine and Molecular Therapeutics, University of Tokushima
2007 Professor, Division of Medical Oncology, Cancer Research Institute, Kanazawa University Head, Kanazawa University Hospital Cancer Center

Recent selected publications

1. Nakagawa T, Takeuchi S, Yamada T, Ebi H, Sano T, Nanjo S, Ishikawa D, Sato M, Hasegawa Y, Sekido Y, Yano S. EGFR-TKI resistance due to BIM polymorphism can be circumvented by in combination with HDAC inhibition. ***Cancer Res***, 2013 73:2428-34.
2. Yamada T, Takeuchi S, Fujita N, Nakamura A, Wang W, Li Q, Oda M, Mitsudomi T, Yatabe Y, Sekido Y, Yoshida J, Higashiyama M, Noguchi M, Uehara H, Nishioka Y, Sone S, Yano S. Scaffold Aki1, a novel therapeutic target for lung cancer with epidermal growth factor receptor mutations. ***Oncogene***, 2013;32:4427-35.
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Targeting essential growth drivers in human cancer

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While there may be several "driver" mutations in a given cancer genome, targeting essential growth drivers that tumor cells cannot live without would be one of the ideal ways to treat this intractable disorder. To identify such essential drivers, we developed a highly sensitive functional screening system with retroviral cDNA expression libraries. Application of this technology to a non-small cell lung cancer (NSCLC) specimen identified the *EML4-ALK* fusion oncogene. Wild-type *ALK* encodes a receptor-type protein-tyrosine kinase, but a small inversion within the short arm of chromosome 2 leads to the production of a constitutively active, and highly oncogenic fusion kinase.

In response to such observation, a large number of *ALK* inhibitors are currently under development or clinical trials, and a marked efficacy of the first inhibitor (crizotinib) was already reported with a response rate of ~60%. Only four years after our initial discovery of *EML4-ALK*, crizotinib was approved, as of August 26, 2011, as a therapeutic drug against lung cancer by U.S. FDA, which was the record-breaking speed in the history of cancer drug development.

Furthermore, from a patient with NSCLC who was once effectively treated with crizotinib but underwent rapid relapse after 6 months' treatment, we discovered secondary mutations within EML4-ALK that confer resistance to ALK inhibitors. One of the acquired mutations was an L1196M substitution that is placed at the identical position to Thr-790 in EGFR and Thr-315 in ABL; the "gatekeeper" position in the ATP-binding pocket. Our discovery of the gatekeeper mutation swiftly led to the development of the second generation of ALK inhibitors. One of such inhibitors, alectinib, demonstrated a magical response rate of 93.5%, and has been already approved in Japan.

In this presentation I will further present our recent finding of other essential growth drivers, RAC1/2 mutations, identified through a coupled approach of functional screening with massive cDNA resequencing.

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2013 Professor, Department of Cellular Signaling, Graduate School of Medicine, The University of Tokyo

Recent selected publications

1. Yoshimi, A., Toya, T., Kawazu, M., Ueno, T., Tsukamoto, A., Iizuka, H., Nakagawa, M., Nannya, Y., Arai, S., Harada, H., Usuki, K., Hayashi, Y., Ito, E., Kirito, K., Nakajima, H., Ichikawa, M., Mano, H. and Kurokawa, M. (2014) Recurrent CDC25C mutations drive malignant transformation in FPD/AML. ***Nat. Commun***. 5, 4770

2. Kawazu, M., Ueno, T., Kontani, K., Ogita, Y., Ando, M., Fukumura, K., Yamato, A., Soda, M., Takeuchi, K., Miki, Y., Yamaguchi, H., Yasuda, T., Naoe, T., Yamashita, Y., Katada, T., Choi, Y.L. and Mano, H. (2013) Transforming mutations of RAC guanosine triphosphatases in human cancers. ***Proc. Natl. Acad. Sci. U S A*** 110, 3029-3034

3. Takeuchi, K., Soda, M., Togashi, Y., Suzuki, R., Sakata, S., Hatano, S., Asaka, R., Hamanaka, W., Ninomiya, H., Uehara, H., Choi, Y.L., Satoh, Y., Okumura, S., Nakagawa, K., Mano, H. and Ishikawa, Y. (2012) RET, ROS1 and ALK fusions in lung cancer. ***Nat. Med***. 18, 378-381

4. Choi, Y.L., Soda, M., Yamashita, Y., Ueno, T., Takashima, J., Nakajima, T., Yatabe, Y., Takeuchi, K., Hamada, T., Haruta, H., Ishikawa, Y., Kimura, H., Mitsudomi, T., Tanio, Y. and Mano, H. (2010) EML4-ALK mutations in lung cancer that confer resistance to ALK inhibitors. ***N. Engl. J. Med***. 363, 1734-1739

5. Soda, M., Choi, Y.L., Enomoto, M., Takada, S., Yamashita, Y., Ishikawa, S., Fujiwara, S., Watanabe, H., Kurashina, K., Hatanaka, H., Bando, M., Ohno, S., Ishikawa, Y., Aburatani, H., Niki, T., Sohara, Y., Sugiyama, Y. and Mano, H. (2007) Identification of the transforming EML4-ALK fusion gene in non-small-cell lung cancer. ***Nature*** 448, 561-566

MYU, a novel target lncRNA for Wnt/c-Myc signaling, mediates CDK6 induction to promote cell cycle progression

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Aberrant activation of Wnt/β-catenin signaling is a major driving force in colon cancer. Wnt/β-catenin signaling induces c-Myc expression, leading to cell proliferation and tumorigenesis. However, the mechanisms underlying c-Myc-induced oncogenesis remain to be established. Here we identify a novel direct target of c-Myc, MYU (c-Myc-upregulated long non-coding RNA) and show that MYU is upregulated in most colon cancers and required for the tumorigenicity of colorectal tumor cells. We further demonstrate that MYU associates with hnRNP K to stabilize CDK6 expression, and thereby promotes the G1-S transition. These results suggest that the MYU-hnRNP K-CDK6 pathway functions downstream of Wnt/c-Myc signaling and plays a critical role in the proliferation and tumorigenicity of colorectal tumor cells.

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Recent selected publications

1. Tsuji S*, Kawasaki Y*, Furukawa S, Taniue K, Hayashi T, Okuno M, Hiyoshi M, Kitayama J, Akiyama T. (* co-first author) (2014) The miR-363-GATA6-Lgr5 pathway is critical for colorectal tumorigenesis. ***Nat. Commun***. 5:3150.

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3. Kawasaki Y, Tsuji S, Muroya K, Furukawa S, Shibata Y, Okuno M, Ohwada S, Akiyama T. (2009) The adenomatous polyposis coli-associated exchange factors Asef and Asef2 are required for adenoma formation in Apc(Min/+)mice. ***EMBO Reports***. 10:1355-1362.

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5. Kawasaki Y, Sagara M, Shibata Y, Shirouzu M, Yokoyama S, Akiyama T. (2007) Identification and Characterization of Asef2, a guanine-nucleotide exchange factor specific for Rac1 and Cdc42. ***Oncogene***. 26:7620-7627.

6. Kawasaki Y, Sato R, Akiyama T. (2003) Mutated APC and Asef are involved in the migration of colorectal tumour cells. ***Nat. Cell Biol***. 5:211-215.

7. Kawasaki Y, Senda T, Ishidate T, Koyama R, Morishita T, Iwayama Y, Higuchi O, Akiyama T. (2000) Asef, a link between the tumor suppressor APC and G-protein signaling. ***Science***. 289:1194-1197.

Poster Presentations

Poster Session

TGF-β family and cancer

01 Kyung-Min Yang (CHA University, Seoul, Korea)
Cytoplasmic DRAK1 overexpressed in HNSCC cells inhibits TGF-β1 tumor suppressor activity

02 Kazuhito Naka (Kanazawa University)
Oral TGF-β signaling inhibitor eradicates CML stem cells

03 Takahiro Kuchimaru (Tokyo Institute of Technology)
Non-invasive imaging of activation of HIF and TGF-β/Smad signaling in breast cancer progression

04 Koji Miyabayashi (The University of Tokyo)
A role of BMP signaling in pancreatic cancer

05 Fumihiko Murai (The University of Tokyo)
Functional analysis of transforming growth factor (TGF)-β signal in small cell lung cancer

06 Yuichiro Yokoyama (The University of Tokyo)
Autocrine BMP-4 protects colorectal cancer cells from apoptosis through down-regulation of Bim

07 Shingo Maeda (Kagoshima University)
BMP-induced PEG10 regulates level of metalloproteinases and invasion of chondrosarcoma cells

08 Erna Raja (The University of Tokyo)
BMP receptor ALK2-PTCH1-DLX2 axis inhibits glioblastoma

09 Tsubasa Sakurai (The University of Tokyo)
RBM47 inhibits Nrf2 activity to suppress tumour growth in lung adenocarcinoma

10 Jun Nishida (The University of Tokyo)
Functional analysis of TGF-β and renal cancer-initiating cells

11 Ryo Tanabe (The University of Tokyo)
Identification of genes involved in BMP-Induced differentiation of glioma-initiating cells

12 Luna Taguchi (The University of Tokyo)
Roles of TGF-β signal in renal cancer cells

13 Kazunobu Isogaya (The University of Tokyo)
Regulatory mechanism of Smad3 target genes expression by FoxA1/2 in lung adenocarcinoma cell lines

14 Kazuki Yoneyama (Tokyo University of Pharmacy and Life Science)
Tumor necrosis factor-a enhances TGF-β-induced endothelial-mesenchymal transition

15 Yukihide Watanabe (University of Tsukuba)
TMEPAI cross regulation with multiple signaling pathways during tumorigenesis

16 Chiaki Furuta (Tokyo University of Pharmacy and Life Science)
Hypoxic conditions potentiates TGF-β signaling in Lewis lung carcinoma cells

TGF-β family signaling

17 (Anna Maria) Anita Morén (Uppsala branch TS group)
Complex formation and ubiquitination of Smad2 and HECT-WW domain E3-ligases

18 Nobuo Sakata (Showa Pharmaceutical University)
Transcriptional repression mechanisms of TGF-β signaling and ATBF1 on the *AFP* promoter

Poster Session

19 Mitsuyoshi Motizuki (University of Yamanashi)
Determination of functional domains in Smad3 by using synthetic peptide blockers

20 Zhaojun Xu (University of Yamanashi)
Transcriptional activation via the Smad-binding elements

21 Masao Saitoh (University of Yamanashi)
Roles of Stat3 in Snail induction by the synergism between Ras and TGF-β

22 Keiichi Hirono (University of Toyama)
The role of BMP10 during differentiating cardiomyocyte from iPSCs

23 Akihiro Katsura (The University of Tokyo)
The role of TGF-β signaling in extracellular miRNA secretion and cell-to-cell communication

24 Ji-Won Lee (Ehime University)
Super-resolution analysis of cell cycle phase-dependent phosphorylation of Smads

25 Tadayoshi Hayata (University of Tsukuba)
Dullard/Ctdnep1 is a Smad1-interacting protein to suppress BMP signaling

26 Yutaro Tsubakihara (Ehime university)
Arkadia induces ubiquitylation and degradation of Smad6

27 Shin yamamoto (Ehime University)
Analysis of the role of Smurf 1/2 in osteoblast differentiation

28 Mayu Arase (The University of Tokyo)
FAIRE-seq analysis of TGF-β-induced EMT in mouse mammary gland epithelial cells

Cancer biology(angiogenesis /lymphangiogenesis)

29 Tomohisa Sakaue (Ehime university, PROS)
BTB proteins as an adaptor for Cul3-based ubiquitin ligases multiply regulate angiogenesis

30 Yasuhiro Yoshimatsu (Tokyo University of Pharmacy and Life Sciences)
Prox1 controls platelet-derived growth factor signals during tumor lymphangiogenesis

31 Ling Zheng (University of Tsukuba)
Molecular function of THG-1/Tsc22D4 in tumor angiogenesis

32 Fumiko Itoh (Tokyo University of Pharmacy and Life Sciences)
TGF-β regulates lymphangiogenesis

33 Erika Abe (Tokyo University of Pharmacy and Life Science)
TC-1 is a novel Smad binding protein in lymphangiogenesis

Cancer biology (podoplanin/aggrus CLEC)

34 Caname Iwata (The University of Tokyo)
Podoplanin/aggrus maintains vascular integrity in the brain

35 Shogo Tamura (University of Yamanashi)
A novel function of CLEC-2 for megakaryopoiesis: CLEC-2/PDPN niche promotes megakaryocyte expansion

36 Ai Takemoto (Japanese Foundation for Cancer Research)
Aggrus-induced platelet aggregation promotes tumor metastasis by enhancing EMT

37 Toshiaki Shirai (University of Yamanashi)
CLEC-2 facilitates hematogenous tumor metastasis, but not tumor growth or lymphogenous metastasis

Poster Session

38 Nagaharu Tsukiji (University of Yamanashi)
Unexpected role of platelets in lung development depending on a platelet activation receptor, CLEC-2

Cancer biology (EMT)

39 Tomokazu Kimura (University of Tsukuba)
GPNMB impacts on invasive properties of bladder cancer

40 Sinh Duy Nguyen (University of Yamanashi)
Role of ets1 and ZEB1/SIP1 in EMT of basal-like subtype breast cancer

41 Zhihong Chen (University of Yamanashi)
Biochemical activities of truncated ESRP1 mutants

42 Yukari Okita (University of Tsukuba)
Gpnmb generates epithelial-mesenchymal transition and tumorigenesis in breast cancer cells

Cancer biology (tumor microenvironment)

43 Hiroshi Seno (Kyoto University Graduate School of Medicine)
The role of Nardilysin in intestinal tumorigenesis

44 Kei Takahashi (The University of Tokyo)
Analysis of pancreatic microenvironment with an orthotopic model and in vivo bioluminecence imaging

45 Douaa Dhahri (University of Tokyo, The Institute of Medical Science)
Mesenchymal Stem Cells expansion by the fibrinolytic system: implications in cancer microenvironment

46 Naohiro Nishida (Osaka University Graduate School of Medicine)
Hypoxia induced long non-coding RNAs are involved in cancer progression

47 Eisaku Kondo (Niigata University Graduate School of Medical and Dental Sciences)
MSC in panceratic tumor microenviroment and development of MSC-targeting CPP

48 Satoshi Muraoka (Hiroshima University)
Biological activity of senescence associated exoxomes in tumor microenvironment

Cancer biology (therapy)

49 Yu Okubo (Tokyo University of Pharmacy and Life Scienences)
Exploration of cancer-targeting antibodies using an HSV-based screening system

50 Hiroaki Uchida (Tokyo University of Pharmacy and Life Sciences)
Augmentation of oncolytic potential of a fully retargeted HSV by introduction of syncytial mutations

51 Ryota Takahashi (The University of Tokyo)
Soluble VCAM-1 predicts efficacy of gemcitabine treatment in pancreatic cancer

52 Makoto Hayashi (Kobe University)
ALDH1 contributes to acquisition of resistance to paclitaxel in gastric cancer cells

53 Takuma Suzuki (Tokyo University of Pharmacy and Life Sciences)
Development of highly specific cancer-retargeted HSV by modification of multiple glycoproteins

54 Takeshi Fukuhara (Tokyo University of Pharmacy and Life Sciences)
IL13Ra2, therapeutic target of melanoma, identified by unique antibody screening technology

Poster Session

55 Mao Komai (The University of Tokyo)
Identification of Interleukin 13 Receptor alpha 2 (IL13Ra2) as a novel marker of human melanoma

56 Hayato Okamoto (Tokyo University of Pharmacy and Life Science)
Interleukin 13 receptor alpha 2 expression increases the growth of human melanoma xenograft in mice

57 Jun Koseki (Osaka University)
Computational analysis predicts imbalanced IDH1/2 expression associate with 2-HG-inactivating β-oxygenation enzyme

58 Yuta Matsuura (Tokyo University of Pharmacy and Life Science)
Development of Anti-EpCAM ScFv toxin for cancer therapeutics

Cancer biology (miscellaneous)

59 Akira Suzuki (Kyushu Univ)
Function of MOB1A/1B in murine liver

60 Masamitsu Konno (Osaka University)
Embryonic MicroRNA-369 regulates pyruvate kinase splicing form by stabilizing translation of splicing

61 Akiyoshi Komuro (The University of Tokyo)
Identification of a novel fusion gene, HMGA2-EGFR in glioblastoma

62 Koichi Kawamoto (Osaka University Graduate School of Medicine)
Murine insulinoma cell-conditioned medium with BETA2/NeuroD1 transduction efficiently induces the differentiation into pancreatic β cells

63 Phu Thien Truong (University of Tsukuba)
Age-dependent decrease of DNA hydroxymethylation in human T cells

64 Tran Bich Nguyen (University of Tsukuba)
Identification of cell-type-specific mutations in angioimmunoblastic T-cell lymphoma

65 Rie Nakamoto-Matsubara (University of Tsukuba)
Detection of the G17V RHOA mutation in angioimmunoblastic T-cell lymphoma using quantitative allele-specific polymerase chain reaction

TGF-β family and cancer

01 Cytoplasmic DRAK1 overexpressed in HNSCC cells inhibits TGF-β1 tumor suppressor activity

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Head and neck squamous cell carcinoma (HNSCC) is an extremely aggressive cancer with a poor prognosis and low patient survival. Because chemotherapy for advanced HNSCC is often ineffective, discovering new therapeutic targets that are important for HNSCC development and progression and elucidating their molecular mechanisms are required. In the present study, we describe the role of DRAK1 (death-associated protein kinase-related apoptosis-inducing kinase 1) as a novel negative regulator of the transforming growth factor-β (TGF-β) tumor suppressor signaling pathway for the first time in human HNSCC cells. DRAK1 was significantly overexpressed in primary human HNSCCs and in HNSCC cell lines. Through gain- and loss-of-function experiments, we demonstrated that the DRAK1 expression level regulated TGF-β1-induced transcriptional activity and expression of the tumor suppressor gene p21Waf1/Cip1. DRAK1 depletion enhanced TGF-β1-induced growth inhibition in vitro and suppressed tumorigenicity in xenograft models in vivo. Mechanistically, DRAK1 was predominantly localized in the cytoplasm and bound to Smad3, thereby interrupting Smad3/Smad4 complex formation, which is the core process for the induction of tumor suppressor genes by TGF-β1. Thus, our findings suggest that cytoplasmic DRAK1 increases tumorigenic potential through inhibition of TGF-β1-mediated tumor suppressor activity in HNSCC cells and may be a potential therapeutic target for HNSCCs.

02 Oral TGF-β signaling inhibitor eradicates CML stem cells

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Recent strategies for treating chronic myelogenous leukemia (CML) patients have focused on investigating new combinations of tyrosine kinase inhibitors (TKIs) as well as identifying novel translational research agents that can eradicate CML stem cells. However, little is known about the therapeutic benefits and risks such CML stem cells targeting therapies might bring to CML patients. In this study, we investigated the therapeutic potential of an oral TGF-β signaling inhibitor to suppress CML stem cells in vivo. Compared to TKI treatment alone, administration of TKI plus TGF-β signaling inhibitor to CML-affected mice significantly delayed disease relapse and prolonged survival. Although treatment with TGF-β signaling inhibitor alone appeared to reduce CML stem cell numbers by driving their commitment to mature CML cell differentiation, the TGF-β signaling inhibitor combined with a TKI dramatically decreased CML stem cell frequency with no evidence of mature CML cell proliferation. Collectively, these results indicate that the TGF-β signaling inhibitor may be a promising candidate for a new therapeutic that can greatly benefit CML patients by working in combination with TKIs to eradicate CML stem cells.

03 Non-invasive imaging of activation of HIF and TGF-β/Smad signaling in breast cancer progression

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Understanding the role of the cellular microenvironment in tumor progression offers attractive therapeutic strategies because the microenvironmental factors are often shared among various tumor types. In vivo optical imaging with genetic coded reporters enables non-invasive, real-time, longitudinal studies of molecular signal activity in living animals. We here demonstrate a new multi-reporter system (MRS) to visualize activity of two important factors characterizing tumor microenvironment, namely hypoxia-inducible factor (HIF) and TGF-β/Smad signaling. We established a MDA-MB-231 human breast cancer cell line, which stably express a Renilla luciferase reporter driven by a HIF-dependent promoter, a firefly luciferase reporter driven by a TGF-β-dependent promoter and a mRuby2 reporter, which constitutively expresses a red fluorescent protein, to monitor tumor burden. Using this cell line, we monitored the activity of HIF and TGF-β/Smad signaling during tumor progression in mouse cancer models. The results may help us better understand the malignant processes involved in HIF and TGF-β/Smad signaling.

04 A role of BMP signaling in pancreatic cancer

Koji Miyabayashi[1], Hideaki Ijichi[1], Ryota Takahashi[1], Dai Mohri[1], Keisuke Yamamoto[1], Yoshinari Asaoka[1], Keisuke Tateishi[1], Yousuke Nakai[1], Hiroyuki Isayama[1], Harold L Moses[2], Kazuhiko Koike[1]
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TGF-β signaling has a crucial role in pancreatic tumorigenesis and progression, and almost all of pancreatic cancers carry at least one genetic alteration of TGF-β related genes, such as SMAD4, TGFBR2, SMAD3, and BMPR2. However, the role of BMP signaling in pancreatic cancer remains unclear. We have already established pancreas-specific Tgbr2 knockout mice in the context of Kras activation, which clinically and histopathologically recapitulate human PDAC. With regard to PDAC, Smad4 mutation or deletion is more commonly observed, however the Smad4 knockout mice with activating Kras mutation was reported to show cystic type tumor of pancreas. Therefore, our Kras+Tgfbr2KO might be the closest approximation of the human PDAC in terms of histology. We examined the effect of BMP signaling on the tumorigenesis of PDAC using this mouse model.
The immunohistochemistry of murine pancreas tissues demonstrated that Smad1/5/8 was phosphorylated in ADM and PanIN lesions and more strongly phosphorylated in PDAC lesion. In ADM assay using C57BL/6, Bmp4 and Bmp7 increased the acinar to ductal transdifferentiation and Tgf beta decreased the transdifferentiation. In ADM assay with pancreas explants from Ptf1acre/+;Tgfbr2flox/flox and Tgfbr2flox/flox, deletion of Tgfbr2 increased the acinar to ductal transdifferentiation and DMH1, BMPR inhibitor decreased the ductal formation. These results suggest that BMP signaling plays an important role on the initiation of pancreatic cancer.

05 Functional analysis of transforming growth factor (TGF)-β signal in small cell lung cancer

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TGF-β regulates growth and differentiation of many types of cells. Although TGF-β acts as a tumor suppressor in many types of cancer, the roles of TGF-β in the progression of small cell lung cancer (SCLC) have not been fully elucidated. In the present study, several analyses revealed lower expression of TGF-β type II receptor (TβRII) in most of SCLC cells or SCLC tissues than in normal lung cells or normal lung tissues. *In vitro* cell growth and *in vivo* tumor growth were suppressed by TGF-β-mediated apoptosis when wild type TβRII was overexpressed in SCLC cells. Moreover, TGF-β-target genes were identified by chromatin immunoprecipitation sequencing (ChIP-seq) analysis and molecular biological analysis. TGF-β directly regulated expression of several novel genes via a Smad-dependent manner, which might be important for TGF-β-mediated apoptosis of SCLC cells. These results suggested that TGF-β negatively contributes to tumor progression of SCLC through the induction of apoptosis of SCLC cells.

06 Autocrine BMP-4 protects colorectal cancer cells from apoptosis through down-regulation of Bim

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Bone morphogenetic proteins (BMPs), members of the TGF-β family, are multi-functional cytokines, which are known to be involved in many types of cancer. In this study, the roles of BMPs during colorectal cancer progression were investigated.
First, we examined which BMP isoforms were produced by colorectal cancer cells. RT-PCR analyses and ELISA assays revealed that several colorectal cancer cells produced BMP-4 in an autocrine manner. Xenograft experiments demonstrated that knockdown of BMP-4 in colorectal cancer cells attenuated the phosphorylation of Smad1/5, which in turn inhibited in vivo tumor formation. Knockdown of BMP-4 or treatment with BMP inhibitors induced apoptosis of colorectal cancer cells under serum free condition. Moreover, expression of apoptosis-related BH3-only protein, Bim in these cells was enhanced by inhibition of endogenous BMP signaling.
These findings suggest that colorectal cancer cells produce BMP-4 in an autocrine manner and this autocrine signaling is important for survival of them.

07 BMP-induced PEG10 regulates level of metalloproteinases and invasion of chondrosarcoma cells

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Chondrosarcoma is the second most common malignancy of bone with a poor response to chemotherapy or radiation therapy, characterized by developing invasion and metastasis. Paternally expressed gene 10 (*PEG10*) is a retrotransposon-derived gene exclusively conserved in mammals, which is overexpressed in various human cancers. It interacts with receptors of TGF-β family to suppress the signaling. Here, we found expression of *PEG10* to be increased in human chondrosarcoma cell-lines SW1353 and Hs 819.T, compared to normal human chondrocytes C28/I2. The expression was suppressed or enhanced by treating with TGF-β1 or BMP-6, respectively. Knockdown of *PEG10* mildly decreased expression of chondrogenic marker *COL2A1*, whereas it showed no effect on the cell growth or motility. Importantly, in chondrosarcoma cells, BMP-6 induced expression of metalloproteinase *(MMP)-1* and *MMP-13*, which are important collagenases in cell invasion, while induction of *PEG10* siRNA further enhanced both of the expression. As a result, *PEG10* silencing promoted invasion of chondrosarcoma cells *in vitro*. Our data suggest that, although BMP signaling promotes expression of MMPs, it simultaneously induces PEG10 to suppress expression of MMPs and cell invasion of chondrosarcoma cells as a negative feedback manner.

08 BMP receptor ALK2-PTCH1-DLX2 axis inhibits glioblastoma

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Glioblastoma is a common and malignant form of brain cancer with a poor prognosis. It is a heterogenous tumour consisting of both highly proliferating cells and glioma initiating stem cells (GSCs). BMP4 induces GSC differentiation into astrocytes and reduces gliomagenesis (1), however, the mechanism is largely unknown. Our study aims to find novel gene candidates downstream of BMP4 that could be modulated for GSC elimination and therapy.
Cell surface receptors are more readily targeted molecules. To begin with, our study examines the functional relevance of BMP receptors expressed in glioma, ALK2, ALK3 and ALK6. Surprisingly, expression of constitutively active ALK2 inhibits cell proliferation and induces apoptosis in our glioma cell models, TGS01 and TGS04. Apoptosis is marked by elevated levels of cleaved Parp1, cleaved caspase 3 and annexin V binding to the cell membrane, as measured by western blotting and flow cytometry. Moreover, ALK2-induced apoptosis is accompanied by induction of phosphorylated p38 MAPK and a lower phosphorylation of Rb.
Our laboratory further conducted microarray analysis from which we identified PTCH1 (Sonic hedgehog receptor) and DLX2 (a pro-neurogenesis transcription factor) as genes up-regulated by both BMP4 and ALK2 receptor. Gain of function of PTCH1 and DLX2 by lentiviral transduction lead to significantly reduced glioma cell proliferation and enhanced expression of neuronal-related genes. In addition, our results suggest that PTCH1 positively regulates DLX2 mRNA level, indicating that DLX2 may function downstream of PTCH1.
In summary, while BMP type I receptors ALK2, ALK3 and ALK6 are similar in structure and function, we observed that ALK2 plays a unique role to induce apoptosis in glioblastoma and the mechanism may involve PTCH1 and DLX2.
Reference:
[1] Piccirillo SG, Reynolds BA, Zanetti N, Lamorte G, Binda E, Broggi G, Brem H, Olivi A, Dimeco F, Vescovi AL. (2006) Nature 444(7120): 761-5.

09 RBM47 inhibits Nrf2 activity to suppress tumour growth in lung adenocarcinoma

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Recent findings on the roles of RNA binding proteins have uncovered a new layer of post-transcriptional regulation of RNA during cancer progression. We here aimed to identify a new RNA binding protein regulated by transforming growth factor (TGF)-β and reveal its roles in cancer.
A transcriptome-wide screen of RNA-binding proteins in mammary gland epithelial cells stimulated by TGF-β resulted in the identification of RNA binding motif protein 47 (RBM47) as the most strongly suppressed gene by TGF-β. Expression of RBM47 correlated significantly with good prognosis in patients with lung adenocarcinoma. Integrated RNA-seq and RNA-immunoprecipitation (RIP)-seq of lung adenocarcinoma cells revealed that RBM47 preferentially suppressed the expression of cell metabolism-related genes, many of which were the direct targets of Nrf2. RBM47 bound to Keap1 and Cullin3 mRNAs, and knockdown of RBM47 inhibited their protein expression, which led to enhanced binding of Nrf2 to target genomic regions. Both mitochondrial respiration rates and the side population in lung cancer cells increased in the absence of RBM47. These results, together with the enhanced tumour formation and metastasis of xenografted mice as a result of RBM47 loss, suggested tumour suppressive roles for RBM47 through the inhibition of Nrf2 activity.

10 Functional analysis of TGF-β and renal cancer-initiating cells

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The concept of cancer-initiating cells (CICs) has been raised in recent studies. CICs are thought to be a small population of undifferentiated cancer cells that have stem cell-like abilities and have been considered as a suitable target of cancer therapy. Although CICs in many types of cancers have been analyzed, there are few reports of CICs in clear cell renal cell carcinomas (ccRCCs).
In this study, we demonstrated that TGF-β3 suppressed the tumor forming ability of ccRCCs in soft agar. Expression levels of major stem cell markers in several ccRCC cells were determined by quantitative RT-PCR analysis and flow cytometry. Though several markers, such as CD44 and some ABC transporters, were expressed in cell type-dependent manner, CD24 and ALDH1A1 were commonly expressed in ccRCC cells we examined. In addition, TGF-β3 decreased the expression of these stem cell markers in ccRCC cells.
These findings suggested that TGF-β3 negatively regulates the tumor forming ability of ccRCC cells through the suppression of CIC activity.

11 Identification of genes involved in BMP-induced differentiation of glioma-initiating cells

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Glioblastoma multiforme (GBM) is one of the most malignant forms of glioma. We previously investigated the effects of BMP-4 on our glioma-initiating cell (GIC) model *in vitro* and *in vivo*. The results suggest that BMP signaling deprives of *in vivo* tumorigenic activity through loss of stemness properties of GICs (Komuro A, et al., unpublished).
However, the mechanisms underlying the BMP-induced differentiation of GICs are not fully understood. In this study, DNA microarray analysis and RNA-Seq analysis identified novel target genes of BMP signaling in GICs. Using meta-analysis for the prognostic value of the genes, the candidate genes were identified whose expression levels were correlated with prognosis in GBM. Paired related homeobox 1 (PRRX1), one of the candidates, has been reported as a regulator of stemness in adult neural stem/progenitor cells (Shimozaki K, et al., J.Neurosci, 2013).
To evaluate the influence of *PRRX1*, overexpression and knockdown of *PRRX1* were performed. These experiments revealed that *PRRX1* acted downstream of BMP-4 to lose the sphere formation ability of GICs and reduced expression levels of *PROM1*. Our findings indicate that *PRRX1* has an essential role in losing stemness properties of GICs and is associated with differentiation and tumorigenicity of GICs.

12 Roles of TGF-β signal in renal cancer cells

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Deletions of TGF-β signal components were not detected in clear cell renal cell carcinoma (ccRCC) cells we examined. Although TGF-β did not alter the proliferative ability of ccRCC cells, morphological changes of those cells were observed by the treatment of TGF-β. Phalloidin staining revealed that stress fiber formation was increased in TGF-β stimulated ccRCC cells. Real-time PCR analysis also revealed that TGF-β up-regulated the expression of mesenchymal markers, including N-cadherin, Vimentin and Fibronectin. These findings suggest that TGF-β stimulates the process of epithelial-mesenchymal transition (EMT) in ccRCC cells. We are currently investigating the effect of TGF-β signal inhibition on tumor forming ability in vivo by use of dominant negative receptor-expressing ccRCC cells.

13 Regulatory mechanism of Smad3 target genes expression by FoxA1/2 in lung adenocarcinoma cell lines

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FoxA1/2 are Forkhead box proteins with winged helix domain which can bind to DNA in a similar manner to histones. They are called "pioneer factors", since they can open chromatin and recruit other transcriptional factors. They play a very important role during tumorigenesis as well as development. Especially, FoxA2 is frequently down-regulated in lung adenocarcinoma, and it prevents Epthelial Mesenchymal Transition(EMT) by suppressing Slug expression in lung adenocarcinoma cell lines.
Here, we show that FoxA1/2 partially affect Smad3 binding induced by TGF-β stimulation in lung adenocarcinoma cell lines, using ChIP-sequencing. TGF-β signaling also changes FoxA1/2 binding profiles.
On the other hand, knock-down of FoxA2 disrupts Smad3 binding to the promoters of these target genes. RNA-sequence data show that knock-down of FoxA1 or 2 up-regulates part of TGF-β target genes without TGF-β stimulation, and alters response to TGF-β signaling. These findings suggest that FoxA1/2 affect chromatin comformation around target genes and regulate their expressions.

14 Tumor necrosis factor-α enhances TGF-β-induced endothelial-mesenchymal transition

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In the tumor microenvironment, infiltrating inflammatory cells secrete various cytokines including transforming growth factor (TGF)-β and tumor necrosis factor (TNF)-α. TGF-β have been implicated in the progression and metastases of epithelium-derived tumor cells by inducing epithelial mesenchymal transition (EMT). Similarly, TGF-β drives transformation of endothelial cells into cancer-associated fibroblasts that assist malignant tumorigenesis through endothelial mesenchymal transition (EndMT) in the tumor microenvironment. While we previously reported that TNF-α enhances TGF-β-induced EMT, the roles of TNF-α in EndMT are largely unknown. In this study, we examined the effect of TNF-α on TGF-β-induced EndMT using murine pancreatic endothelial cells, MS-1. When MS-1 cells were treated with TGF-β alone, expression of smooth muscle α-actin, a mesenchymal marker was induced. Treatment of TGF-β in combination with TNF-α significantly enhanced its expression. Decreased expression of vascular endothelial growth factor receptor (VEGFR) 2, an endothelial marker, by TGF-β was further enhanced by co-treatment with TNF-α. These results suggest that TNF-α is a potent inducer of TGF-β-induced EndMT.

15 TMEPAI cross regulation with multiple signaling pathways during tumorigenesis

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TMEPAI/PMEPA1 (transmembrane prostate androgen induced-RNA/ prostate transmembrane protein, androgen induced 1) is pro-tumorigenic and is highly expressed in most cancer cells examined, such as in lung, breast, colon, and renal cell carcinoma. Our previous reports showed that TMEPAI inhibits TGF-β signaling by sequestering the R-Smad from TGF-β type I receptor kinase, and that TMEPAI knockdown in lung cancer cells significantly reduces in vivo tumor growth and in vitro sphere formation. Constitutive TMEPAI expression in cancer cells requires autocrine TGF-β signaling and cooperative Wnt signaling. Here we demonstrate that EGF/Ras/MAPK signaling with TGF-β signaling cooperatively controls TMEPAI expression. Moreover, the transcription factor ELK-1 which is activated by the EGF/Ras/MAPK pathway binds to the first intron of the TMEPAI gene and enhances TMEPAI expression in cooperation with Smad3. Additionally, we show that TMEPAI has a novel function in the regulation of Akt activation and Wnt signaling pathways. Therefore, TMEPAI is involved in tumorigenesis through a complexity of actions.

16 Hypoxic conditions potentiates TGF-β signaling in Lewis lung carcinoma cells

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Transforming growth factor-β (TGF-β) is a potent growth inhibitor in normal epithelial cells, however a number of malignant tumors produce excessive amounts of TGF-β, which affects tumor microenvironment and further progresses tumorigenicity at the later stages of tumor. Although the tumor microenvironment becomes often hypoxic, which is linked to increased metastatic potential, it has not been well documented how hypoxia affects TGF-β signaling in tumor-associated microenvironment.
In this study, we investigated the effect of hypoxia on TGF-β signaling pathways in Lewis lung carcinoma (LLC) cells under hypoxic condition (1% O_2). When LLC cells were cultured under hypoxic without interruption for days, their shapes become spindle shapes. In addition their Smad2 phosphorylation upon TGF-β stimulation was stronger than that of cells cultured under normoxia. Concomitantly, TGF-β-induced $(CAGA)_{12}$-luc reporter activity was augumented in hypoxia, whereas TGF-β did not influence hypoxia-responsive reporter activity. Furthermore, expression of several TGF-β target genes was increased in hypoxic conditions. Our results demonstrate that hypoxia might potentiate TGF-β signaling to make tumors ultimately malignant.

TGF-β family signaling

17 Complex formation and ubiquitination of Smad2 and HECT-WW domain E3-ligases

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Signal transduction by transforming growth factor β (TGFβ) and Smad proteins controls basic biological processes including cell differentiation and proliferation. The inhibitory Smad7 negatively regulates the pathway by associating with the ubiquitin ligase Smurf2 and by targeting the TGFβ type I receptor for degradation. Smurf2 also negatively regulates Smad2 protein stability. The related ubiquitin ligase NEDD4-2 has also been implicated in regulation of TGFβ signaling. In this study we aimed at analyzing the relative contribution of the two related ubiquitin ligases, NEDD4-2 and Smurf2, as negative regulators of TGFβ signaling. Unexpectedly, we found that NEDD4-2 could ubiquitinate but failed to downregulate the type I receptor, whereas Smurf2 did downregulate the receptor. We also identified NEDD4-2 in complex with Smad2, Smad7 and Smurf2. These data propose that control of TGFβ signaling by NEDD4-2 and Smurf2 is coordinately spread between the membrane receptors and the nuclear Smads, providing a platform that continuously monitor the flow of this signaling pathway.

18 Transcriptional repression mechanisms of TGF-β signaling and ATBF1 on the *AFP* promoter

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The oncofetal glycoprotein, α-fetoprotein (AFP) is produced a large amount in hepatoma, but hardly detectable in normal tissue. AFP mRNA expression is down regulated in response to TGF-β signal transduction in HuH-7 cells. To further understand the molecular mechanisms of the transcriptional repression, we analyzed the effect of TGF-β signaling and AT motif binding factor 1 (ATBF1) on the transcriptional activities associated with human AFP gene in HepG2 cells. TGF-β signaling and ATBF1 have a synergistic effect on the transcriptional repression and the effect is due to the presence of ATBF1 binding elements, AT-motifs. One is localized in the enhancer region, another in the promoter region, respectively. Both AT-motifs are required to the transcriptional repression. TGF-β signaling transducer, Smad proteins (Smad2 and Smad3) bind to ATBF1 at the N-terminal and C-terminal region, respectively. Because N-terminal and C-terminal region of ATBF1 bind to Smad proteins, but lack DNA binding domain, both proteins inhibit the synergistic effect between TGF-β signaling and ATBF1 in a dose dependent manner. These results suggest that TGF-β signaling repress the transcription of AFP in co-operation with transcription factor ATBF1 through direct binding with R-Smads.

19 Determination of functional domains in Smad3 by using synthetic peptide blockers

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Transforming growth factor-β (TGF-β) plays crucial roles during embryonic development as well as in adult tissue homeostasis through eliciting various cellular responses in target cells. TGF-β signaling is principally mediated through the effector molecules Smad2 and Smad3 (R-Smads) that regulate expression of target genes in cooperation with other DNA-binding transcription factors (Smad cofactors). We previously found that Olig1 is a Smad cofactor which is involved in TGF-β-induced cell motility. We also found that Olig1 interacts with the L3 loop of Smad3 and a synthetic peptide, Thr371-His399, corresponding to the region selectively inhibits TGF-β-induced cell motility.
To determine functional domains in Smad3, peptide fragments covering various regions of MH-1 and MH-2 domains in Smad3 were synthesized, introduced into NMuMG cells and the effects of peptide on cellular responses induced by TGF-β were determined. Peptides Asn357-Arg368, Thr371-His399 and Gln405-Cys421 covering C-terminal region of Smad3 efficiently inhibited TGF-β-induced cell motility. Currently we investigate the effects of the peptides on other cellular responses.

20 Transcriptional activation via the Smad-binding elements

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Transforming growth factor (TGF)-β is a pleiotropic cytokine that plays crucial roles during embryonic development as well as in adult tissue homeostasis through induction of cellular responses in a wide variety of target cells. TGF-β stimulation induces activation of type I receptor to phosphorylate cytoplasmic Smad2 and Smad3. Smad2 and 3 then form a trimeric complex with Smad4, translocate into the nucleus and regulate target gene expression through association with specific DNA element. Several Smad-binding DNA sequence motifs such as CAGA and SBE have been reported. However, minimal requirements for Smad-dependent transcriptional activation have not been well elucidated.
In this study, we used the cyclic amplification of selected target (CASTing) method to determine binding sequences with endogenous Smad proteins expressed in mammalian cells with signaling from TGF-β receptor typeI (ALK5). We found that endogenous Smad3 binds to sequences that include either SBE or the CAGA motif. We further examined requirements for transcriptional activation through Smad-binding sequences by changing the number of repeats or the combination of SBE and CAGA. We will discuss minimal requirements for Smad-dependent transcriptional activation.

21 Roles of Stat3 in Snail induction by the synergism between Ras and TGF-β

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The epithelial-mesenchymal transition (EMT) is a crucial morphological event that occurs during the progression of epithelial tumors. EMT can be induced through induction of Snail, a key regulator of EMT, by transforming growth factor (TGF)-β. We have previously found that TGF-β remarkably induces Snail expression only in cooperation with Ras signals. However, the underlying mechanism of this synergism has not been determined yet. Here, we demonstrate that signal transducer and activator of transcription (Stat) 3 acts as one of mediator for the synergism between TGF-β signaling and Ras signals. Overexpression of Stat3 enhanced Snail induction by the synergism, whereas Stat3 siRNAs inhibited it. In Stat3-derivative mutants, Stat3 YF mutant that changes tyrosine 705 to phenylalanine did not affect Snail induction by the synergism. Several Stat3 mutants lacking transcriptional activity possessed the abilities similar to those of Stat3 YF mutant. In addition, Stat3 binding elements in Snail promoter regions were not required for Snail induction promoted by Stat3. Interestingly, protein inhibitor of activated Stat3 (PIAS3) inhibited Snail promoter activity enhanced by Stat3. Interaction of PIAS3 with Stat3 was reduced by TGF-β, whereas TGF-β promoted interaction of PIAS3 with Smad3, a crucial molecule of TGF-β signaling. These results suggest that Stat3 dissociated from PIAS3 enhances Snail induction by TGF-β in cooperation with Ras.

22 The role of BMP10 during differentiating cardiomyocyte from iPSCs

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Background: BMP10 is cardiac specific ligand expressed in the late stages of cardiac development responsible for cardiomyocyte proliferation, differentiation and compaction. BMP10 knockout mice shows the thin ventricle result from the arrest of proliferation of cardiomyocytes during embryonic stage, which resembles left ventricular noncompation (LVNC). Left ventricular noncompaction (LVNC) is a myocardial disease characterized by a pattern of prominent trabecular meshwork and deep intertrabecular recesses. We hypothesize that BMP10 plays an important role via regulating Smad dependent and independent pathways that involve cell cycle regulatory proteins and several major cardiac transcription factors that typically orchestrate normal cardiogenesis during differentiation.
Results: We investigated the role of BMP10 in cardiomyocytes differentiated from iPSCs. During cardiac development, BMP10 is expressed transiently in the ventricular trabecular myocardium from E9.0-13.5, a critical time span when cardiac development shifts from patterning to growth and chamber maturation. Our in vitro study showed that BMP10 promotes the proliferation of iPSCs derived cardiomyocytes (CMs) at day 9-12. BMP10 impairs the apoptosis of iPSCs derived cardiomyocytes. Exogenous BMP10 mildly affects the cardiac gene expression in iPSCs derived cardiomyocytes at day 8-10. Endogenouse BMP10 regulates the proliferation and differentiation of iPSCs derived cardiomyocytes around day 8.
Conclusion: Endogenous BMP10 may play a important role during early cardiac differentiation and proliferation.

23 The role of TGF-β signaling in extracellular miRNA secretion and cell-to-cell communication

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Transforming growth factor (TGF)-β is pivotal to cell-to-cell communication and plays important roles in diverse diseases such as cancer. Recent evidences highlight that extracellular microRNAs (miRNAs) embedded in extracellular vesicles are involved in intercellular crosstalk and cancer pathogenesis. However, little is known about how secretion of these RNAs is regulated by environmental stimuli. In this study, we report unique crosstalk between TGF-β signaling and regulation of extracellular miRNAs.
We first identified miR-221/-222 as the most suppressed miRNAs upon TGF-β stimulation in MS-1 mouse endothelial cells by global miRNA profiling analysis. Rapid suppression of intracellular miR-221/-222 levels was not attributable to active destabilization of these miRNAs, and was further associated with specific upregulation of extracellular miR-221/-222, thereby suggesting active secretion. Furthermore, this upregulation of extracellular miR-221/-222 was mediated by direct interaction with extracellular Ago2 but not by exosomes. Functional analyses demonstrated that these extracellular miRNAs with Ago2 is transferable to recipient cancer cells and supports in vivo growth of pancreatic tumor, and identified several tumor suppressive targets of miR-221/-222. Collectively, our findings illuminate dynamic contribution of TGF-β signaling to extracellular miRNA-mediated intercellular communication.

24 Super-resolution analysis of cell cycle phase-dependent phosphorylation of Smads

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BMPs and their intracellular signaling are well established to be involved in skeletogenesis, through regulating proliferation and differentiation of osteoblasts and chondrocytes. By immunofluorescence-based super-resolution microscopy, we observed that mitosis-specific signal of phosphorylated Smad1/5/8 in osteoblastic and chondrogenic cells. Our further analyses indicated that this phenomenon was independent from BMP2. Since, the lineage specific transcriptional factors such as Runx2 and MyoD showed their intense signals in mitotic phase. These findings suggest that phosphorylated Smad1/5/8 may be involved in mitotic retention of skeletal cells phenotype.

25 Dullard/Ctdnep1 is a Smad1-interacting protein to suppress BMP signaling

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BMP signaling plays crucial roles in cellular differentiation and various antagonists negatively regulates BMP signaling to maintain the appropriate signaling levels. We previously identified Dullard encoding phosphatase as a suppressor of BMP signaling through dephosphorylating activated BMP type I receptor and promoting degradation of BMP type II receptor. Regarding human pathology, CTDNEP1 has been proposed as a candidate gene for one of the medulloblastoma tumor suppressors. To further gain insight into the mechanism how Dullard suppresses BMP signaling, we investigated the epistatic relationship between Dullard and the components of BMP signaling using MC3T3-E1 osteoblastic cells. Overexpression of Dullard suppressed BMP-responsive reporter activity induced by constitutively active ALK3 and constitutively active Smad1 (caSmad1) independently of its phosphatase activity. Overexpression of Dullard suppressed nuclear localization of phospho-Smad1, while knockdown of Dullard enhanced that. Overexpression of Dullard impaired nuclear localization of caSmad1, while Dullard and caSmad1 staining colocalized in the cytoplasm. Immunoprecipitation experiment showed that both wild-type and phosphatase-inactive mutant of Dullard interacted with Smad1 in Xenopus embryo. These results provide with the evidence that Dullard can function as Smad1-interacting protein to suppress BMP signaling downstream of receptors through, at least in part, modulating localization of Smad1.

26 Arkadia induces ubiquitylation and degradation of Smad6

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Bone morphogenetic protein (BMP) and transforming growth factor-β (TGF-β) signaling pathways are tightly regulated by the ubiquitin-proteasome system. Arkadia is a RING type E3 ubiquitin ligase which induces the degradation of Smad7, c-Ski and SnoN, resulting in enhancement of both the signaling pathways.
The differentiation of osteoblasts are regulated by BMP and TGF-β signaling pathways in different manners. BMP signaling enhances the differentiation, while TGF-β signaling inhibits the differentiation of ostoeblasts. Arkadia is thought to be controlling both BMP and TGF-β signaling pathways in osteoblasts, but it has not been elucidated how the effects on both pathways results in the osteoblastic differentiation.
To clarify the role of Arkadia in bone metabolism, we prepared lentiviruses which express short-hairpin RNAs against Arkadia. Differentiation of osteoblasts were inhibited by the knock-down of Arkadia in absence of TGF-β signaling. This result suggest that Arkadia enhances BMP signaling in the differentiation of osteoblasts, because Arkadia target protein Smad7, c-Ski and SnoN are repressors for both TGF-β and BMP signaling. Some studies have been reported in which Arkadia binds to Smad6. In this study, we showed that Arkadia induces ubiquitylation and degradation of Smad6.

27 Analysis of the role of Smurf 1/2 in osteoblast differentiation

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The osteoblast differentiation is regulated by transforming growth factor-β(TGF-β) and bone morphogenetic proteins (BMPs). The positive regulation of BMPs in the osteoblast differentiation is recognized in vivo and in vitro, but the function of TGF-β in the osteoblast differentiation is not fully understood. It is known that TGF-βand BMP signal are regulated by E3 ubiquitin ligases such as Smurf1 and Smurf2. The role of Smurf proteins is considered to be both promotion and inhibition during osteoblast differentiation. It has been reported that in the analysis of Smurf1 knockout mice, their bone formation showed a mild increase, but knockout of Smurf1 did not affect the Smad pathway. On the other hand, in the experiments of Smurf2 knockout mice, no obvious abnormalities of phenotype in bone formation. Our hypothesis is that Smurf1 and Smurf2 work complementary in the bone formation including osteoblast differentiation. To reveal the role of Smurf 1/2 in osteoblast differentiation, it is necessary to investigate the effect to osteoblast differentiation when Smurf1 and Smurf2 are suppress. We prepared a shRNA lentiviral vectors for Smurf1 and Smurf2, and Smurf2 knockout mice, and we will examine the loss of function for Smurf1/2 in vivo and in vitro.

28 FAIRE-seq analysis of TGF-β-induced EMT in mouse mammary gland epithelial cells

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The epithelial-mesenchymal transition (EMT) occurs in response to Transforming growth factor (TGF)-β stimulation and facilitates tumor migration and invasion. In this study, we performed global mapping of the open chromatin regions in mouse mammary gland epithelial EpH4 and EpRas cells using formaldehyde-assisted isolation of regulatory element (FAIRE)-sequencing (seq) to analyze the mechanisms of transcriptional regulation during TGF-β-induced EMT. Calculated FAIRE regions of some of mesenchymal markers and epithelial markers were regulated by TGF-β and Ras signaling. We found an Ets binding motif was enriched in the FAIRE regions of EpRas cells treated with TGF-β. We then focused on Ets family proteins ETV4 and ETV5 (ETV4/5) predominantly expressed in EpRas cells and found that double knockdown of ETV4/5 decreased the number of living cells treated by TGF-β. In line with this observation, GSEA analysis of RNA-seq data revealed that double knock down of ETV4/5 resulted in up-regulation of cytostasis-related genes. Moreover, down-regulation of E-cadherin mRNA by TGF-β was inhibited in the absence of ETV4/5. Taken together, these findings suggest a mechanism of TGF-β-induced EMT as well as cytostasis that involves regulation of open chromatin regions.

Cancer biology

►angiogenesis / lymphangiogenesis

29 BTB proteins as an adaptor for Cul3-based ubiquitin ligases multiply regulate angiogenesis

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Angiogenesis is essential in several physiological and pathological processes including wound healing and tumor growth. Vascular endothelial growth factor (VEGF) is a prime stimulator of endothelial cell (EC) activation. Recently, we found CUL3-based E3 ubiquitin ligase complex in the downstream of VEGF signaling (Ohnuki et al, Blood 2012). CUL3 associates with BAZF, known as one of the BTB domain-containing proteins, which targets RBP-Jκ/CBF-1, the transcriptional factor of Notch signaling as a negative regulator of angiogenesis. As a result, the CUL3-BAZF complex positively controls angiogenesis by mediating between VEGF and Notch signaling in the ECs.
CUL3 functions as a scaffold protein that forms the E3 ligase complex with ROC1/BTBP. Although 183 BTBPs have been reported in human, only CUL3-BAZF complexes have been known in the EC so far. Indeed, CUL3 knockdown led more severe angiogenic inhibition rather than BAZF both in vitro and in vivo. These results suggest that CUL3 may multiply regulate angiogenesis. Therefore, to identify the novel CUL3-BTBP complex as an angiogenic regulator, the knockdown experiments were carried out using BTBP-siRNA libraries.

30 Prox1 controls platelet-derived growth factor signals during tumor lymphangiogenesis

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The lymphatic system plays important roles not only in the physiological processes, such as maintenance of tissue fluid homeostasis, but also in pathological processes including the lymph node metastasis of tumor cells. Therefore, understanding the molecular mechanisms underlying lymphatic vessel formation is crucial. Previous studies have shown that signals mediated by platelet-derived growth factor receptor β (PDGFRβ) have been implicated in lymphangiogenesis, the mechanisms explaining how PDGFRβ expression is maintained in LECs remain to be fully elucidated. In the present study, we show that PDGFRβ expression in LECs is maintained by Prox1 transcription factor. Knockdown of Prox1 expression in human dermal LECs decreased the expression of PDGFRβ, leading to the lowered migration of human dermal LECs towards PDGF-BB. Furthermore, we found that PDGF signals play important roles in inflammatory lymphangiogenesis in a chronic aseptic peritonitis model. Intraperitoneal administration of imatinib, a potent inhibitor of PDGFRβ, and transduction of PDGFRβ/Fc chimeric protein by an adenoviral system both reduced inflammatory lymphangiogenesis induced by thioglycollate in mice. We also found that the expression of PDGFRβ/Fc reduced tumor lymphangiogenesis in a BxPC3 human pancreatic cancer xenograft model. These findings suggest that PDGFRβ is one of the key mediators of lymphatic vessel formation acting downstream of Prox1.

31 Molecular function of THG-1/Tsc22D4 in tumor angiogenesis

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THG-1/Tsc22D4 is one of the Tsc22 family members. We found that THG-1 is expressed in squamous cell carcinoma cells and is phosphorylated by EGFR-Ras-ERK signaling pathway. The phosphorylation of THG-1 promotes cell proliferation, invasion and tumorigenicity. Furthermore our previous data showed that THG-1 knockdown reduces not only tumor sizes but also blood vessel formation in oncogenic Ras-mediated tumor. These results suggest that THG-1 is required for oncogenic Ras-mediated tumorigenesis and involves in tumor angiogenesis which is essential for tumor growth and survival. To investigate the molecular function of THG-1, we analyzed the interacting proteins of THG-1 and found that THG-1 binds to prolyl hydroxylase enzymes (PHDs) which regulate the stability of hypoxia inducible factor-1α (HIF-1α). HIF-1α is a transcription factor that induces vascular endothelial cell growth factor (VEGF). Our data showed that phosphorylated THG-1 interacts with PHD2 to inhibit the PHD-mediated degradation of HIF-1α and promotes HIF-1α stability in the presence of EGF signaling. These results indicated that THG-1 promotes angiogenesis by binding to PHD2 and regulating HIF-1α stability even in the normoxic condition.

32 TGF-β regulates lymphangiogenesis

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TGF-β signaling regulates angiogenesis and the maintenance of blood vessel integrity. However, it is not clear how TGF-β signaling affects lymphatic vessel formation. To address this issue, TGF-β type II receptor (TβRII) conditional knockout mice; TβRIIF/F, were crossed with Prox1-CreERT2 mice in which Cre recombinase is induced in lymphatic vascular endothelial cells (LECs) by tamoxifen (Tx). When Tx was administrated to pregnant mice, TβRIICKO embryos revealed edema in their back at E13.5. We stained blood and lymphatic vessels in TβRIICKO embryos with anti-PECAM-1 and anti-LYVE-1 antibodies, respectively. Interestingly, blood vessel seemed to be well-established, whereas lymphatic vessels could hardly be detected. To examine how TGF-β regulates lymphangiogenesis in adulthood, B16F10 melanoma cells were transplanted into footpad of TβRIICKO mice, followed by injection of Tx. TβRIICKO mice showed more LYVE-1 positive cells in the tumor area than the control, however reduced lung metastasis. These results indicated that TGF-β signaling is implicated in not only maintenance of lymphatic vessel integrities in embryos but also inhibiting lymphangiogenesis in adults.

33 TC-1 is a novel Smad binding protein in lymphangiogenesis

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The lymphatic system plays a pivotal role in maintaining tissue homeostasis by removal of needless fluid, whereas excessive lymphatic vessel formation is implicated in many diseases including inflammation and tumor metastasis. However, the molecular mechanisms that regulate lymphatic vessel formation remain unclear.
In order to elucidate molecular mechanisms by which VEGF-C regulates lymphatic vessel development, we tried to find key proteins which regulates VEGF-C induced lymphangiogenesis. Using total RNAs from HUVECs (human umbilical vein endothelial cells) and HDLECs (human dermal lymphatic endothelial cells) which were stimulated with VEGF-C for 24h, we performed microarray analysis to identify a lymphangiogenesis inhibiting molecule(s). Among them, we give attention on a gene named thyroid cancer protein-z (TC-1). Since TGF-β also regulates lymphangiogenesis as well as angiogenesis, we analyzed the role of TC-1 in TGF-β signaling and found that TC-1 interact with Smad3 and inhibit TGF-β signaling. Our results suggested that TC-1 might involve in lymphangiogenesis by inhibiting TGF-β signaling.

►podoplanin/aggrus CLEC

34 Podoplanin/aggrus maintains vascular integrity in the brain

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The mucin-type glycoprotein podoplanin (PDPN, also known as aggrus) is expressed in various tissue types, however its function in vivo is unclear. Recently, Herzog et al. reported that PDPN maintains vascular integrity in lymph nodes by interacting with platelet CLEC2 (Nature 2013). From this viewpoint, we realized that PDPN is universally expressed at the sites of blood-tissue barriers (BTBs). This fact strongly suggests that the role of PDPN on maintaining vascular integrity is not limited to lymph nodes, but may be generalized throughout the body. In this study, we tried to prove this concept, focusing on blood-brain barrier, one of the tightest BTBs in the body.
First we checked the expression of PDPN in murine brain by IHC and identified PDPN-positive astrocytes wrapping around the blood vessels. PDPN on astrocytes was also detected by FACS analysis. Next we treated mice with anti-PDPN neutralizing mAb in model of transient brain ischemia to examine the function of PDPN in brain. We observed that blood leakage, detected by fibrinogen staining, was dramatically enhanced by the mAb treatment. In addition, we found that PDPN expression in astrocytes was negatively regulated by BMP signaling.
These results suggest that astrocyte PDPN maintains vascular integrity in the brain.

35 A novel function of CLEC-2 for megakaryopoiesis: CLEC-2/PDPN niche promotes megakaryocyte expansion

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C-type lectin-like receptor 2 (CLEC-2) is a novel platelet activation receptor. Recently, podoplanin (PDPN, also called as aggrus) was identified as an endogenous CLEC-2 ligand. The response of CLEC-2 and PDPN plays crucial roles for the vascular/lymphatic vessel separation during embryonic development, the integrity of high endothelial venue (HEV) in lymph nodes, and tumor metastasis. CLEC-2 knockout (KO) and megakaryocyte/platelet specific conditional KO (cKO) mice show thrombocytopenic and mild anemic phenotypes. However, the pathogenesis of CLEC-2 KO to these hematological phenotypes has not been elucidated.
In this study, we first investigated the role of CLEC-2 in megakaryopoiesis. BM megakaryocytes (MKs) were significantly decreased in CLEC-2 cKO mice. Colony formation assay showed that recombinant PDPN stimulation accelerated expansions of MK progenitors. Next, we explored CLEC-2 ligand-expressing cells in bone marrow (BM), and found PDPN+ BM stromal cells. Subset of MK lineages located in the vicinity of these PDPN+ cells in vivo. In co-culture with BM stromal cells, expansions of WT MK progenitors were promoted compared with CLEC-2 cKO. These results suggest that MK lineages are maintained and their expansions were accelerated at CLEC-2/PDPN microenvironments in BM.

36 Aggrus-induced platelet aggregation promotes tumor metastasis by enhancing EMT

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Tumor cells and microenvironment interact mutually and contribute to tumor progression. In hematogenous metastasis, tumor cells interact with platelets, which induces platelet activation and forms tumor clumps. The aggregation leads to protection from immunological assaults and blood sheer stress, and to embolization in microvasculature in distant organs. And platelets contain factors that support the tumor cell malignancy. TGF-β is known to be a platelet-containing factor that can induce EMT. On EMT, cells lose the epithelial phenotype, instead gaining an invasive and migratory mesenchymal phenotype, which is supposed to be critical for metastasis. As Aggrus can induce platelet aggregation, Aggrus would promote tumor metastasis by stimulating TGF-β release. So, we examined Aggrus-contribution to EMT. Firstly, we found epithelial tumor cells showed EMT by treatment with released fraction from Aggrus-expressing cell activated platelets. Secondly, the EMT was suppressed by knockdown or anti-Aggrus antibodies as well as inhibition of TGF-β signaling. Consistently in vivo metastasis was suppressed by the inhibition of Aggrus and TGF-β. These findings indicate a mechanism by which platelets contribute metastasis and suggest Aggrus and TGF-β as the targets for anti-metastatic drugs.

37 CLEC-2 facilitates hematogenous tumor metastasis, but not tumor growth or lymphogenous metastasis

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Aims: Podoplanin expressed on the surface of tumor cells facilitates hematogenous metastasis by activating platelets. We have shown that C-type lectin like recptor 2 (CLEC-2) is a podoplanin receptor in platelets. We aim to investigate a role of CLEC-2/podoplanin interaction in hematogenous and lymphogeous metastasis and tumor growth.
Results: Lung metastasis of a podoplanin-positive melanoma cell line, B16F10 injected into mice via the tail vein was significantly inhibited in CLEC-2-deficient chimeric mice. Co-culture of B16F10 with wild type platelets, but not that with CLEC-2-deficient platelets, significantly increase proliferation of B16F10. However, neither tumor growth nor lymphatic metastasis was inhibited in CLEC-2-depleted mice with B16F10 inoculated into the right flank. The number of functional tumor vessels and survival rates was significantly increased in CLEC-2-depleted mice.
Conclusion: CLEC-2 facilitates hematogenous tumor metastasis by binding to podoplanin, but not lymphogenous metastasis. Tumor growth is facilitated by CLEC-2 in vitro, but not in vivo, probably because tumor vessels are not occluded by thrombus in the absence of CLEC-2. We speculated that survival rates are increased because thrombotic tendency is inhibited in the absence of CLEC-2.

38 Unexpected role of platelets in lung development depending on a platelet activation receptor, CLEC-2

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Platelets are small anucleate blood cells which play a crucial role in physiological hemostasis and pathological thrombosis. We have previously identified a platelet activation receptor, C-type lectin-like receptor 2 (CLEC-2) as a receptor and its ligand podoplanin. Based on our previous data to show that podoplanin is also expressed on type I alveolar epithelial cells, mesothelium and lymphatic endothelial cells, and that CLEC-2 null mice die shortly after birth, we hypothesized that CLEC-2 also plays a role in lung development. CLEC-2 expression was detected only in platelets and megakaryocytes during lung development and CLEC-2 null mice showed malformation in alveolar septum. In addition, alveolar myofibroblasts did not migrate into alveolar septum in CLEC-2 null mice.
Platelets contain abundant cytokines, such as TGF-β in its alpha granules. We found that the mixture of cytokines from platelets can induce myofibroblast differentiation from lung mesothelial cells. Our results strongly suggest that platelet activation via association between CLEC-2 on platelets and lung podoplanin is required for normal lung development.

►EMT

39 GPNMB impacts on invasive properties of bladder cancer

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Glycoprotein non-metastatic melanoma protein B (GPNMB) is a type 1 transmembrane glycoprotein. It plays roles on epithelial-mesenchymal transition, anchorage-independent growth, and tumorigenicity in cancer cells. However, there are little evidences how GPNMB play role on cancer invasion. We evaluated how GPNMB impact on invasion by using bladder cancer.
We evaluated protein expression of GPNMB in nine cell lines using western blotting and examined their invasion properties. To investigate contribution to the aggressive phenotype, we knocked down GPNMB by short hairpin RNA or over expressed it and checked the effects on invasion. To find out the important domain for invasion, we made several point mutants of GPNMB and evaluated their effects on invasion as well.
Every bladder cancer cell line expressed GPNMB protein in each manner. There was not any correlation between GPNMB expression levels and invasiveness. However, the short hairpin RNA targeting GPNMB significantly suppressed cell growth and invasive ability. Conversely, overexpressing wild type GPNMB greatly promoted invasiveness. When we made hemITAM mutant of GPNMB, this mutant could not promote the invasion compared to wild type GPNMB.
GPNMB has a role in invasive properties of bladder cancer. The hemITAM is one of the important domains for bladder cancer invasion.

40 Role of Ets1 and ZEB1/SIP1 in EMT of basal-like subtype breast cancer

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Breast cancer cells of the basal-like subtype has been defined as a phenotype that is associated with epithelial mesenchymal transition (EMT), in which E-cadherin is repressed mostly by activities of transcription factors such as the zinc-finger factors family proteins δ-crystallin/E2box factor (δEF1)/zinc-finger E-box-binding homeobox (ZEB) 1 and Smad-interacting protein (SIP)1/ZEB2. Our previous study has shown that Ets1, a molecule downstream of ERK, activates ZEB1 promoter in mouse mammary epithelial cells (NMuMG cells). Here, we demonstrate that Ets1 also activates ZEB1 promoter in human breast cancer cells. Ets1 siRNAs can down-regulate expression of both ZEB1 and SIP1 , leading to partial recovery of epithelial phenotypes of basal-like breast cancer cells. These findings suggest that Ets1 can induce EMT in basal-like breast cancer cells through regulation of ZEB1 and SIP1.

41 Biochemical activities of truncated ESRP1 mutants

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It has been proven that ESRP1 is an mRNA splicing factor that regulates the formation of epithelial cell-specific isoforms and plays a crucial role in regulating epithelial-mesenchymal transition (EMT), however the mechanism has not been fully understood. To explore the role of ESRP1 in the regulation of EMT, here we constructed a series of ESRP1 truncate mutant vectors, and they are respectively named pcDEF3-E1-WT, pcDEF3-E1-RRM123, pcDEF3-E1-ΔC and pcDEF3-E1-ΔN. The recombinant vectors were transfected into SAS cells or HeLa cells, and expression of each protein was detected. These vectors provide necessary experimental materials for further investigation of regulation of ESRP1 during EMT. Biochemical activities of these mutants will be examined and discussed.

42 Gpnmb generates epithelial-mesenchymal transition and tumorigenesis in breast cancer cells

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Gpnmb (glycoprotein non-metastatic melanoma protein b) is a type I membrane protein which is highly expressed in many types of cancer including glioma, melanoma, breast cancer and so on. Previously we showed that transcription factor MafK is one of TGF-β target genes, which is responsible to induce epithelial-mesenchymal transition (EMT) and tumorigenicity to normal mouse mammary epithelium-derived NMuMG cells. To reveal the mechanism how MafK regulates EMT and tumor growth, we performed DNA microarray analysis, and identified Gpnmb as one of critical target genes of MafK to induce EMT and tumor formation. When Gpnmb was overexpressed in NMuMG cells, E-cadherin downregulated, cell migration and invasion activated, and typical EMT features were observed. Although the large number of transcription factors which regulate EMT has been reported, it is a little known that transmembrane protein induces EMT except for growth factor receptors. Moreover, enhanced expression of Gpnmb promotes sphere formation *in vitro* and tumor growth *in vitro*. Gpnmb-expressing xenografts show dedifferentiated morphology, high proliferating rates, and loss of E-cadherin. Furthermore, suppression of Gpnmb using siRNAs attenuates sphere-forming capacity in human breast cancer cells. These results indicate that Gpnmb is one of EMT inducers critical for tumorigenicity in breast cancer cells.

►tumor microenvironment

43 The role of Nardilysin in intestinal tumorigenesis

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Nardilysin (N-arginine dibasic convertase, NRD1), a metalloendopeptidase enhancer of protein ectodomain shedding, enhances the release of growth factors and cytokines via the regulation of ADAM (a disintegrin and metalloproteinase domain) 17 activity. Tumor necrosis factor (TNF)-α is activated through the shedding of membrane-bound pro-TNF-α in an ADAM17/NRD1-dependent manner.
To investigate the *in vivo* role of NRD1 in intestinal tumorigenesis, we first administered azoxymethane/dextran sulfate sodium (AOM/DSS) to wild type (WT) and *Nrd1* knockout (KO) mice. *Nrd1* KO mice scarcely developed colonic tumors. By the simple administration of DSS, *Nrd1* KO mice did not show apparent colitis. Although TNF-α was produced in DSS-treated colonic stroma, shedding and release of TNF-α was significantly reduced in Nrd1 KO mice. Consistently, ADAM17 activity was suppressed in *Nrd1* KO mice. Downstream targets of TNF-α, such as NF-ϰB, were also downregulated in Nrd1 KO mouse colonic mucosa. In contrast, *Villin-Nrd1* transgenic mice developed larger number of colonic tumors compared to WT mice concomitantly with severer colitis by AOM/DSS treatment. Furthermore, when Apc^{Min} mutation was inserted into *Nrd1* KO and *Villin-Nrd1* transgenic mice, the size and number of intestinal tumors were altered by the gene dosage of *Nrd1*.
These experiments suggested that NRD1 plays a pivotal role in intestinal tumor development possibly through the regulation of inflammatory responses in tumor microenvironment.

44 Analysis of pancreatic microenvironment with an orthotopic model and in vivo bioluminecence imaging

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It has been considered that tumor microenvironment plays an important role for cancer progression. To investigate the interactions between pancreatic cancer cells and pancreatic microenvironment during cancer progression in vivo, an orthotopic injection model was used in the present study. Several types of human pancreatic cancer cells expressing GFP and luciferase were inoculated orthotopically into the pancreas of nude mice. By use of in vivo bioluminescence imaging system, tumor progression was visualized. GFP-expressing cancer cells were isolated from the primary or metastatic tumors, and established as “high tumorigenic cancer cells”. Interestingly, the morphology of these high tumorigenic cancer cells was different from that of parental cancer cells. In addition, high tumorigenic cancer cells showed more aggressive characters, such as high proliferative ability and increased invasiveness. These results suggest that cancer cells are activated through the interactions with tumor microenvironment, and acquire more malignant phenotype. We are currently further investigating the gene expression profiles including TGF-β signaling and trying to identify some important factors for pancreatic cancer progression.

45 Mesenchymal stem cells expansion by the fibrinolytic system: implications in cancer microenvironment

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There is a growing body of evidence pointing to similarities between stem cells niche in the bone marrow and the cancer microenvironment since they both harbor the same cell types e.g. endothelial cells, immune cells, stromal cells and mensenchymal stem cells (MSCs). We have shown recently that the tissue-type plasminogen activator factor (tPA) can expand MSCs in the bone marrow through a cytokine crosstalk with endothelial cells. Knowing that MSCs are abundant in the cancer microenvironment where they support tumor growth, angiogenesis, EMT and metastasis, we hypothesize that tumor-derived tPA will enhance this MSCs supportive effect. The screening of different cancer cell lines showed that melanoma cells secrete the highest level of tPA. Since tPA generates plasmin which activates TGF-beta and MSCs secrete high levels of TGF-beta, our study is focused on studying this signaling pathway.

46 Hypoxia induced long non-coding RNAs are involved in cancer progression

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We demonstrate a functional link between oxygen deprivation and the modulation of long noncoding transcripts from ultra-conserved regions of whole genome, termed transcribed-ultraconserved regions. Interestingly, several hypoxia-upregulated these transcripts are also overexpressed in clinical samples from colon cancer patients. We show that these transcrips are predominantly nuclear and that the hypoxia-inducible factor (HIF) is at least partly responsible for the induction of several members of this group. One specific lncRNA is part of a retained intron of the host protein-coding gene, O-linked N-acetylglucosamine transferase (OGT) gene, which is overexpressed in epithelial cancer. This transcript supports cell proliferation specifically under hypoxic conditions and may be critical for optimal O-GlcNAcylation of proteins when oxygen tension is limiting. Our data gives a first glimpse of a novel functional hypoxic network comprising protein-coding transcripts and noncoding RNAs from the ultra-conserved region.

47 MSC in panceratic tumor microenviroment and development of MSC-targeting CPP

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Pancreatic cancer (invasive ductal carcinoma) is currently one of the most intractable malignancies, which tumor parenchyma is surrounded by dense fibrous tissues. In this area, we detected intermingled fibroblastoid cells consistent with the immunophenotype of bonemarrow-derived mesenchymal stem cells (MSC). We examined the cellular interaction between MSC and pancreatic cancer line BxPC3 by profiling the soluble proteins such as chemokines and receptor-ligands contained in cocultured supernatant. The result showed the several specific soluble factors seem to be upregulated through the cellular interaction between MSC and BxPC3 cells, which mainly facilitates growth promotion of cancer cells and/or tumor microenviromental components. Thus Based on these findings, we are now developing the novel cell-penetrating peptide (CPP) which is efficiently incorporated to the human MSC using the mRNA display technology. The MSC-penetrating peptide can be utilized as a non-invasive biotool for establishing novel DDS to target MSC in scirrhus cancer tissues.

48 Biological activity of senescence associated exoxomes in tumor microenvironment

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Exosomes are extracellular vesicles (EVs) released from various cells into the extracellular space. They are small particle membrane proteins with a diameter 50-100 nm and contain microRNA, and protein that could be transferred to target cells. Our objective is to elucidate biological function and role of senescence-associated exosome (SA-exosome) in tumor microenvironment. Our previous study demonstrated the SA-exosome suppressed invasion and proliferation of cancer cell. In this study, to clarify the biological mechanism of them, we examined protein and microRNA profiling of exosome released from young and senescence fibroblast TIG-3 cell. Exosomes were isolated from culture medium of senescence and young TIG-3 and analyzed by a quantitative proteomics approach using iTRAQ labeling and next generation sequencing. Some proteins and microRNA were differentially expressed in senescence fibroblast cell as compared with young fibroblast cell. These results indicate their proteins and microRNA were an important function to the suppression of cancer cell proliferation and invasion. This study elucidates not only biological mechanism of SA-exosome in tumor microenvironment but will aid development of therapeutic drug.

►therapy

49 Exploration of cancer-targeting antibodies using an HSV-based screening system

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We have recently established a fully retargeted herpes simplex virus (HSV) platform that incorporates single-chain antibodies (scFv) into glycoprotein D (gD) to mediate virus entry through cancer antigens. For further development of this platform, it is helpful to have an efficient system to screen for antibodies that may mediate HSV entry when incorporated as scFvs into gD. To this end, we have created an HSV-based screening probe by genetic fusion of a gD mutant that is ablated for binding to the natural HSV entry receptors, herpesvirus entry mediator (HVEM) and nectin-1, and the antibody-binding C domain of Streptococcal protein G. The re-engineered gD was found to be expressed on the surface of transfected cells and capable of binding antibodies. We observed that recombinant virus expressing the re-engineered gD failed to enter HVEM- or nectin-1-transduced CHO-K1 cells. However, when conjugated with a specific antibody, the virus entered CHO-K1 cells expressing the cognate antigen, suggesting that entry of the re-engineered virus was dependent on interaction between the cellular antigen and recombinant gD-bound antibody. We then used this system to identify antibodies that could mediate virus entry by recognition of unknown receptors. We prepared a hybridoma library from the spleen of a mouse immunized with ACHN cells, a human renal adenocarcinoma cell line, mixed the re-engineered virus with the conditioned media from individual hybridoma clones, and added ACHN cells to the mixtures. The results showed that several of the media samples enabled virus entry while the majority had essentially no effect, strongly suggesting that the modified virus can be utilized as a screening probe for antibodies capable of mediating HSV entry through bridging the virus to non-canonical receptors. We have started to derive scFvs from the cognate hybridoma clones for fusion with detargeted gD. This novel antibody-screening system may lead to a new generation of fully cancer-retargeted oncolytic HSV vectors.

50 Augmentation of oncolytic potential of a fully retargeted HSV by introduction of syncytial mutations

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We previously reported that entry of herpes simplex virus (HSV) can be efficiently retargeted to cells expressing tumor-associated antigens, such as epidermal growth factor receptor (EGFR) and carcinoembryonic antigen (CEA), by re-engineering of two viral envelope glycoproteins, gD and gB. We tested the EGFR-retargeted virus for cell-to-cell spread on a panel of human pancreatic tumor lines. While the virus efficiently entered each of the lines, subsequent spread was not detected in all cases in spite of abundant cell surface expression of EGFR. To address this concern, we examined the possibility of enhancing retargeted virus spread through the introduction of one or more well-characterized syncytial mutations into specific viral glycoprotein genes. Syncytial mutations alter the typical plaque morphology resulting from cytopathic effect (CPE) to syncytia, i.e., multi-nucleated giant cells, and have been identified in the viral glycoprotein gB and gK genes; these mutations confer hyperactivity in cell-cell fusion. We introduced selected syncytial mutations into the retargeted virus genome and found that each mutation enabled robust viral spread by formation of giant syncytia even on those cells that did not show plaque formation by the parent EGFR-retargeted virus. Interestingly, the relative degrees of spread enhancement by the gB and gK mutations varied among the cell lines, suggesting cell-specific differences in the roles of these two glycoproteins in cell fusion. This observation raises the possibility that combining the gB and gK mutations will augment virus cell-to-cell spread on a broader range of tumor cell types. Our strategy is novel in that it exploits multiple mutant glycoproteins to achieve not only robust entry, but also efficient spread of a receptor-retargeted oncolytic HSV.

51 Soluble VCAM-1 predicts efficacy of gemcitabine treatment in pancreatic cancer

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Resistance to chemotherapy is often problematic in treating advanced pancreatic cancer. Measurable biomarkers to predict the efficacy of chemotherapy can be helpful in treating patients. We aimed to define a novel biomarker indicating efficacy of chemotherapy and to elucidate the mechanism of the resistance. We examined the levels of soluble factors in the plasma of genetically engineered mouse model of pancreatic cancer before and after treating with gemcitabine, and found that the soluble form of vascular cell adhesion molecule-1 (VCAM-1) is strikingly elevated after gemcitabine treatment. VCAM-1 expression in pancreatic cancer tissue was elevated compared to normal pancreas, and gemcitabine treatment led to increased VCAM-1 expression. We examined VCAM-1 levels in human blood plasma samples obtained from patients undergoing chemotherapy by gemcitabine, and found that patients with increased soluble VCAM-1 in blood plasma after initial gemcitabine treatment had shorter progression-free survival. Thus, VCAM-1 can be a good biomarker to predict efficacy of gemcitabine treatment, and may have a function in the context of resistance to chemotherapy.

52 ALDH1 contributes to acquisition of resistance to paclitaxel in gastric cancer cells

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Cancer initiating cells (CICs) are a population of cells that acquire stem cell-like properties, such as self-renewal, and reported to cause drug-resistance and radio-resistance, resulting in relapse of cancer. We previously reported that in gastric cancer, cells that highly express aldehyde dehydrogenase 1 (ALDH1) exhibits high tumorigenic activity. However, how ALDH1 regulates CIC properties is not fully understood. In this study, we report a role ofALDH1 in resistance to an anticancer drug paclitaxel (PTX) using human gastric cancer cell lines, including OCUM-2MLN and HSC-39 cells that highly express ALDH1 and have its high activity. We found that CIC markers, CD24, CD44 and CD133 were enriched in OCUM-2MLN with high ALDH activity. Microtubule depolymerization inhibitor Paclitaxel led to an increase of the cell population that have high ALDH activity, and up-regulated CD24 and CD133. ALDH inhibitor DEAB prevented PTX-induced expressions of CIC marker. Finally, DEAB enhanced sensitivity of OCUM-2MLN to PTX, leading to increase of cell death. These findings suggest that ALDH1, especially ALDH1A2 and ALDH1A3, regulates expression of CIC marker genes and inhibition of this pathway may be a novel therapeutic target for drug-resistant cancer cells.

53 Development of highly specific cancer-retargeted HSV by modification of multiple glycoproteins

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Oncolytic virotherapy has become very promising for cancer therapy. Herpes simplex virus (HSV) has been used as an oncolytic virus because of its broad host range and high oncolytic potential. Recently, we established an entry-retargeted HSV by insertion of a single-chain antibody (scFv) against epidermal growth factor receptor (EGFR) into the envelope glycoprotein D (gD), one of the essential glycoproteins for virus entry, and found that the retargeted HSV administered intravenously in a tumor-bearing mouse model accumulated in the tumors at 100-1,000 fold higher levels than in normal tissues. Envelope glycoprotein C (gC) plays an important role in virus adsorption to the cell surface prior to gD-mediated activation of the virus penetration mechanism through binding to heparan sulfate which is expressed on many types of cells. We hypothesized that replacement of the heparan sulfate-binding site of gC with another cancer-targeting scFv could further augment the preferential homing of the gD-modified EGFR-retargeted HSV to tumors after systemic administration. We have thus designed various scFv-incorporating gC constructs and observed specific binding of several of these to their respective cognate antigens. These results suggest that simultaneous modification of gC and gD employing distinct cancer-targeting scFvs will allow the construction of dually retargeted HSV vectors that can be expected to infect cancer cells with substantially increased specificity.

54 IL13Ra2, therapeutic target of melanoma, identified by unique antibody screening technology

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Current approach of cancer therapy, such as chemotherapy, radiotherapy and surgery, has been treated for many years, but it is still hard to cure completely. Especially in chemotherapy, one of long-lasting problem is the side effect of administrated drug, following the interference of regimen.
To overcome this issue, we developed unique targeting technology coupled with monoclonal antibody (MoAb) screening system that enable us to utilize both selectivity and specificity. To establish selective and specific antibody that target cancer biomarker, classical hybridoma library was upgraded with our unique probe that is recombinant toxin protein fused with Fc-binding protein (DT3C), to generate immunotoxin library. This powerful technology enabled us to screen potent MoAb in various cancer cell types.
Here we present; (A) principle of our targeting technology, and (B) discovery of anti-IL13Ra2 MoAb (clone NS66) that has potent activity as DT3C-based immunotoxin, (C) characterization of IL13Ra2 as novel melanoma biomarker as well as therapeutic target.

55 Identification of Interleukin 13 Receptor alpha 2 (IL13Rα2) as a novel marker of human melanoma

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Melanoma is the most devastating form of skin cancer and represents a leading cause of cancer death, particularly in young adults. Therefore, there is an urgent need to develop molecular biomarkers that identify high-risk melanoma patients. We performed monoclonal antibody screening using A375 human melanoma cells as antigens, and identified interleukin 13 receptor alpha 2 (IL13Rα2) as a novel marker for melanoma. To examine the expression of IL13Rα2 in melanoma, immunohistochemistry analysis with tissue microarray was used to evaluate expression of IL13Rα2 in 187 patients with melanoma. High membrane staining of IL13Rα2 was detected in 21 of 187 (11.23%) melanoma tissues. When searched in the PrognoScan database, it was revealed that IL13Rα2 expression was significantly associated with worse overall survival rates. These findings indicate that IL13Rα2 expression is correlated with clinic-pathological features of melanoma patients, and it may serve as a poor prognostic marker.

56 Interleukin 13 receptor alpha 2 expression increases the growth of human melanoma xenograft in mice

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Malignant melanoma is one of the untreatable cancers in which conventional therapeutic strategies including chemotherapy is hardly effective. Thus, identification of novel therapeutic targets that are involved in its progression is significant to develop effective therapeutic methods. Through the monoclonal antibody screening using A375 human melanoma cells as antigens, we, for the first time, revealed that a part of melanoma cells express interleukin 13 receptor alpha 2 (IL13Rα2), which is highly expressed in various types of solid tumors including glioma and pancreatic cancer, While IL13Rα2 has been implicated in the migration and metastasis of pancreatic cancer, its roles in the malignant melanoma has not yet been elucidated. In the present study, we aimed to examine the effects of IL13Rα2 on the tumor formation of melanoma cells using mouse xenograft model of human SK-MEL-28 melanoma cells that do not express IL13Rα2. When control SK-MEL-28 melanoma cells or SK-MEL-28 cells expressing IL13Rα2 (SK-IL13Rα2) were subcutaneously xenografted to immunodeficient mice, the size of tumors derived from SK-IL13Rα2 was significantly larger than that of SK-MEL-28 cells. Furthermore, we found that the tumors derived from SK-IL13Rα2 exhibited more angiogenic characteristics as compared with those from control cells. These results suggest that expression of IL13Rα2 enhances tumorigenicity by inducing angiogensis in malignant melanoma.

57 Computational analysis predicts imbalanced IDH1/2 expression associate with 2-HG-inactivating β-oxygenation enzyme

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We developed a novel methodology to computationally analyze gene expression in colorectal cancer (CRC), and identified novel sets of genes that are associated with patient survival. The study of OxPhos–related genes revealed that an imbalance between the expression of IDH1/2, defined as overexpression of one isoform in relation to the other, was associated with worse prognosis in CRC patients. This effect was further accentuated by reduced expression of the β-oxygenation enzyme, 3-D-hydroxyacyl-CoA dehydratase 4, which has been reported to contribute to metabolism of intracellular D2HG. The present computational analysis revealed a novel and potential mechanism of CRC development, through over-production of D2HG when there is an imbalance between IDH1/2 expression, resulting in decreased clearance of D2HG when the β-oxidization pathway is diminished. Additional validation analysis with other gene expression dataset has resulted in that IDH1/2 imbalanced expression had a shorter DFS compared with balanced expression. Altogether, these findings provide a strong rationale for studying this mechanism further in order to discover novel therapeutic targets for the treatment of CRC.

58 Development of Anti-EpCAM ScFv toxin for cancer therapeutics

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It has been crucial to generate better cancer therapeutics rather than small molecule bearing inevitable side effect. Recently, antibody therapy became clinically available with the advantage for its high specificity. Further, antibody-drug conjugate (ADC) has been extensively under development in combination of the benefits of both small molecule and monoclonal antibody (MoAb). Due to the limited number of clinically available therapeutics, there are increasing demands to isolate ADC orthogonal MoAbs that are capable and suitable for drug delivery function. To aim this, we utilized the immunotoxin probe (DT3C) carrying Fc-binding domain fused with diphtheria toxin to screen ADC orthogonal MoAbs. Among extensive screening, we could establish multiple hybridomas producing anti-EpCAM MoAbs, demonstrating DT3C-dependent killing activity against cancer cells. Since EpCAM was characterized as critical biomarker of cancer stem cell, we further genetically engineered to construct single-chain Fv (ScFv) integrated with toxin (or EGFP). Here we report and discuss the functionality and effectiveness of anti-EpCAM ScFv-toxins against cancer cells.

►miscellaneous

59 Function of MOB1A/1B in murine liver

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The Hippo pathway is an evolutionarily conserved kinase cascade involved in cell growth and differentiation. This pathway is very unique as it may sense the tissue architecture such as polarity or mechanical tensions from the cellular surrounding environment to control the size and the shape of an organ. Two kinases (MSTs and LATSs) and two adaptor proteins (Sav and MOBs) are the core components of this pathway. The MOB1A/1B are the binding partners and strong co-activators of LATS kinases family. We have recently reported MOB1B (or MOB1A) can complement MOB1A (MOB1B) loss of function, and loss of both MOB1A/1B in mice shows embryonic lethality (JCI 2012).We therefore generated liver-specific MOB1A/1B mutant mice (LMOB1A/1B KO mice) by crossing MOB1A/1B mutant mice to AlbCre Tg mice to find the function of MOB1A/1B in the liver. LMOB1A/1B KO mice show oval cells/immature cholangiocellular hyperplasia with inflammatory cells and fibrosis. Most of the mutant mice are lethal before weaning period. Residual mutant mice can live long, but all die within 60 weeks by cholangiocellular and hepatocellular carcinomas. Thus, MOB1A/1B are import ant for the normal liver homeostasis.

60 Embryonic MicroRNA-369 regulates pyruvate kinase splicing form by stabilizing translation of splicing

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The proper control of metabolic flow dictates the fate of differentiation of stem cells. Although many miRs exert the inhibitory effect predominantly in translation and partially in transcript stability of coding mRNA in a cellular context dependent manner, miR369, located in pluripotency-rerated aberrant silencing genomic regions on mouse chromosome 12qF could demonstrate a unique effect in the stabilization of the translation of target proteins in a cellular stress condition, the functional role of miR369 has been elusive. Whole-cell proteomic study revealed that miR369 stabilized the alternative splicing factor proteins such as hnRNPA2B1 for pyruvate kinase (PK) M2, a key enzyme for glycolysis. Knockout of miR369 resulted in disruption of the lineage-dependent differentiation of ESCs by excessive oxidative phosphorylation (OxPhos), which was rescued partially by exogenous expression of miR369 and Pkm2. Collectively, the miR369 Pkm2 axis may play a critical role in the preservation of an optimal OxPhos level during differentiation. Thus our data demonstrated that imprinted, metabolism-controlling miR369 play a critical role in the fine-tuning for stem cells.

61 Identification of a novel fusion gene, HMGA2-EGFR in glioblastoma

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Glioblastoma multiforme (GBM) is one of the most malignant forms of cancer. However, there is no effective targeted therapy with substantial survival benefit so far. Although TCGA reported a list of fusion genes in glioblastoma, their roles remain largely unknown. In this study, we obtained RNA-seq data from TGS-01 human glioma cells and identified a novel high mobility group A 2 (HMGA2) gene mutant, which fuses to the C-terminal portion of epidermal growth factor receptor (EGFR), including transmembrane domain and kinase domain (HMGA2-EGFR fusion gene). Interestingly, this fusion gene gives the transforming potential and high tumor-forming capacity in vitro and in vivo. Mechanistically, HMGA2-EGFR is auto-phosphorylated and up-regulates phosphorylation of STAT3 in the same way as EGFRvIII, a frequently occurring EGFR mutation in primary glioblastoma. Overexpressed HMGA2-EGFR enhances orthotopic tumor formation of the U87 human glioma cell line. Furthermore, EGFR kinase inhibitor Elrotinib blocks sphere formation of TGS-01 in vitro and inhibits tumor formation in vivo. These results may highlight this novel fusion gene as a new target for therapeutic intervention of glioma.

62 Murine insulinoma cell-conditioned medium with BETA2/NeuroD1 transduction efficiently induces the differentiation into pancreatic β cells

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Background: Adipose tissue-derived mesenchymal stem cells (ADSCs) are multipotent and can differentiate into pancreatic β-like cells in in vitro multi-step protocol. Therefore, ADSCs present a potential cell source for the treatment of diabetes mellitus. However, current protocols are insufficient to induce fully matured insulin-producing β cells. In this study, we assessed the effectiveness of overexpression of BETA2, a member of the basic helix–loop–helix transcription factor family, with murine insulinoma cell line-derived conditioned medium (MIN6-CM) to improve the differentiation capacity of ADSCs into insulin-producing cells. Method: Murine ADSCs were transduced with several transcriptional factors and stable transfectants were established. Supernatants of MIN6 cells were pooled, filtered, and employed as MIN6-CM. Differentiated cells were transplanted under the kidney capsule of recipient streptozotocin-induced diabetic mice. Next, blood glucose levels were monitored. Results: CM alone was sufficient to induce insulin mRNA expression in vitro. However, other TFs were not detected. ADSCs cultured with MIN6-CM induced insulin expressions in vitro, but other β cell-related genes were detected. However, BETA2 transduction in MIN6-CM resulted in robust expression of multiple β cell phenotypic markers. Moreover, insulin content analysis revealed insulin protein expression in vitro. Furthermore, in vivo transplant studies revealed the effectiveness of the simultaneous use of BETA2 transduction with the CM. Conclusion: These results suggest that the balance of cytokines and growth factors in addition to gene manipulation would benefit the efficient differentiation of ADSCs into pancreatic β cells. Our technology could provide a path to β cell differentiation and novel cell replacement-based therapies for diabetes.

63 Age-dependent decrease of DNA hydroxymethylation in human T cells

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Hydroxymethylcytosine (hmC) is a natural nucleobase, which is converted from methylcytosine (mC) by tet methylcytosine dioxygenase (TET) family (TET1-3) enzymes. Decrease of genomic hmC is postulated to confer a risk for myeloid-lineage as well as T-cell neoplasms, based on the fact that loss-of-function mutations of *TET2* gene were frequently identified in these diseases. Relationship between hmC and aging remains to be elucidated. Here we demonstrated that hmC content was decreased with age in human peripheral blood T cells of 53 volunteers. We further identified that the mRNA expression levels of *TET1* and *TET3* were decreased with age while those of *TET2* were not influenced by age. The genomic hmC content was correlated with the mRNA expression levels of *TET3* but not those of *TET1* and *TET2*. Our study suggests the presence of new epigenetic regulatory mechanisms in aging T cells.

64 Identification of cell-type-specific mutations in angioimmunoblastic T-cell lymphoma

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[Backgrounds] Angioimmunoblastic T-cell lymphoma (AITL) is a distinct subtype of peripheral T-cell lymphoma (PTCL). Regarding genetic lesions of AITL, Frequent mutations in *TET2*, *IDH2*, *DNMT3A* and *RHOA* have been identified. In some PTCL cases, *TET2* and *DNMT3A* mutations were identified in cell populations beyond the CD4+ T-lymphocytes, in which the tumor cells are contained, suggesting that *TET2* and *DNMT3A* mutations occurred earlier than the commitment to CD4+ T lymphocytes.
[Objective] We performed this study to identify the cell-type-specific mutations and further explore mutational profiles in AITL and AITL-related cancer.
[Methods] The dataset of targeted sequencing was analyzed for 76 genes in 79 PTCL samples. Mutational origin was analyzed by cell sorting and laser microdissection.
[Results] Targeted sequencing identified 168 mutations in 33 genes. Recurrent mutations, in addition to the already known frequent mutations in *RHOA*/*TET2*/*IDH2*/*DNMT3A* were found in *ODZ1* [4/79 (5%)], *Notch1, NAV2*, and *MTERFD3* [3/79 (4%) for each], *MLL2, TET3, FAT2*, and *LAMA2* [2/79 (3%) for each]. Cell sorting and laser microdissection, followed by amplicon sequencing, revealed that *TET2*/*DNMT3A* mutations were identified in both tumor cell-enriched and –depleted populations while *RHOA* and *IDH2* mutations were confined to tumor cell-enriched populations.
[Conclusion] Differentiation stages that mutational events arise are likely to be multiple in AITL and AITL-related lymphoma.

65 Detection of the G17V RHOA mutation in angioimmunoblastic T-cell lymphoma using quantitative allele-specific polymerase chain reaction

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Angioimmunoblastic T-cell lymphoma (AITL) and peripheral T-cell lymphoma, not otherwise specified (PTCL-NOS) are subtypes of T-cell lymphoma. Due to low tumor cell content and substantial reactive cell infiltration, these lymphomas are sometimes mistaken for other types of lymphomas or even non-neoplastic diseases. In addition, a significant proportion of PTCL-NOS cases reportedly exhibit features of AITL (AITL-like PTCL-NOS). Thus disagreement is common in distinguishing between AITL and PTCL-NOS. Using whole-exome and subsequent targeted sequencing, we recently identified G17V *RHOA* mutations in 60-70% of AITL and AITL-like PTCL-NOS cases but not in other hematologic cancers, including other T-cell malignancies. Here, we establish a sensitive detection method for the G17V *RHOA* mutation using a quantitative allele-specific polymerase chain reaction (qAS-PCR) assay. Mutated allele frequencies deduced from this approach were highly correlated with those determined by deep sequencing. This method could serve as a novel diagnostic tool for 60-70% of AITL and AITL-like PTCL-NOS.

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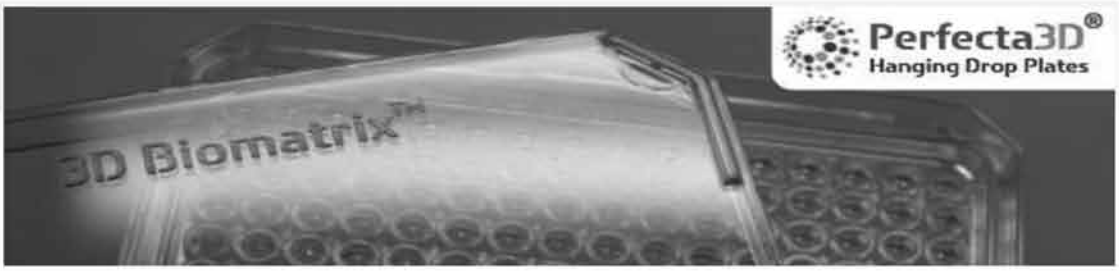
3次元培養用ハンギングドロッププレート

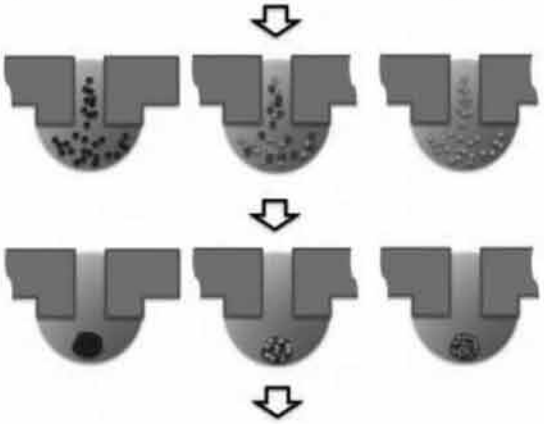


Perfecta 3D Hanging Drop Plateは細胞培養工学、幹細胞研究、癌研究、創薬と試験、HTS化学療法と放射線療法の研究等に非常に有用です。
プレートは96フォーマット、384フォーマットの2種類。 マニュアルでの操作の他自動分注装置でのドロップ作成が出来ます。上部からの操作で、培地交換・スフェロイドの回収も簡単です。

96-Well Plate

384Well Plate

Cancer Cell Lines. Primary Tumor Cells. Normal Primary Cells
Embryonic Stem (ES) Cells. Induced Pluripotent Stem (iPS) Cells. Progenitor Cells



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