The 12th International Conference on Protein Phosphatase

<joint symposium>

International Symposium on Innovative Research for Genome-Based Drug Discovery and Cancer Therapeutics

Program and Abstracts

October 27th (Thu) - 30th (Sun), 2016

NOVEMBER HALL (main hall),
Main Campus, KINDAI UNIVERSITY

Co-organized by Japanese Association for Protein Phosphatase Research &
MEXT-supported Program for Strategic Research Foundation at Private Universities
Sponserd by the Japanese Biochemical Society (JBS), Biofrontier Symposium

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12th International Conference on Protein Phosphatase Organizing committee

Reiko Sugiura, Chair
Masanori Hatakeyama
Hitosh Nakagama
Kazuyasu Sakaguchi
Toshio Watanabe, Chair
Takashi Matozaki
Masaharu Noda
Hiroshi Shima

International Symposium on Innovative Research for Genome-Based Drug Discovery and Cancer Therapeutics Organizing committee

Reiko Sugiura, Chair
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Masahiro Iwaki
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Atsufumi Kawabata
Mitsuhiro Kinoshita
Takashi Masuko
Hiroyuki Moriyama
Takashi Nakayama
Kazuto Nishio
Shigeo Suzuki
Hideo Takasashi

Welcome Message

Reiko Sugiura, M.D.

Chair and Professor, Departmentof Pharmaceutical Sciences, Kindai University

Chair, Organizing Committee, ICPP12

Head of Organizing Committee, MEXT-Supported Program

for the Strategic Research Foundation at Kindai University

Dear participants of the 12th International Congress on Protein Phosphatase

On behalf of the Organizing Committee, I'm honored to host the 12th International Congress on Protein Phosphatase (ICPP12) at Kindai University, Osaka, Japan, October 27-30, 2016.

The theme of this year's ICPP12 is "Protein phosphatase for Translational Research" and our goal is to ensure that protein phosphatase research will contribute to promoting human health and advancing medicine. This congress will be jointly organized by the ongoing MEXT-Supported Program for the Strategic Research Foundation at Private Universities at Kindai University. The aim of this program is to establish a research foundation in Kindai University, that will serve as a cross-disciplinary platform for strategic cancer research, including the elucidation of molecular basis and the regulatory mechanisms of cancer as well as the discovery of innovative therapeutic approaches for cancer treatment. We anticipate that the outcomes of this program will promote basic and clinical cancer research, which will contribute to cancer prevention as well as the development of new products for the pharmaceutical industries.

Because phosphorylation events executed by phosphatases and kinases are the most attractive mechanistic targets for cancer therapy, I believe that this congress will be instrumental in taking up the challenge of uniting basic research with clinical application research focused on defeating cancer. We believe the Kindai project will serve as a driver in developing, strengthening and disseminating evidence-based practices to the world.

Kindai University is one of the largest comprehensive Universities in Japan and for the last 3 years the number of applicants has exceeded one hundred thousand, ranking it number 1 in Japan. This fact, along with its world renowned research in farm raising tuna and research excellence in the life sciences makes Kindai University one of the most highly regarded universities in Japan.

As an innovative feature, this congress will provide a forum for young scientists to attend and present their research. Therefore, we have decided to give prominence to a wide range of workshops by speakers selected from graduate, undergraduate, young academic staff, and senior-high-school students. I believe this inclusive feature of ICPP12 will promote lively discussion and serve as a motivational force to help researchers and young scientists/students to maximize their potential.

I am very much looking forward to seeing you in October in 2016 at KINDAI University, Japan!!

Heartfelt Thanks to our 2016 ICPP Conference

Sponsors and Grant Funding!

Sponsors (alphabetical order)

・Abcam plc. (アブカム株式会社)
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Grant Funding (alphabetical order)

・Japanese Biochemical Society (JBS), Biofrontier Symposium
(JBS バイオフロンティアシンポジウム)
・MEXT-supported Program for the Strategic Research Foundation at Private Universities (私立大学戦略的研究基盤形成支援事業)
・Nakatsuji Foresight Foundation (一般財団法人中辻創智社)

General Information

Congress	The 12th International Conference on Protein Phosphatase & International Symposium on Innovative Research for Genome-Based Drug Discovery and Cancer Therapeutics
Date	October 27th (Thu) – 30th (Sun), 2016
Venue	NOVEMBER HALL (main hall), Main Campus, KINDAI UNIVERSITY Kowakae 3-4-1, Higashiosaka City, Osaka 577-8502, JAPAN TEL: +81-6-6721-2332
Oral Presentation	Please bring **a USB memory stick**, or **your own computer with a mini D-sub 15-pin connector**.
Short Oral Presentation	Please bring **a USB memory stick**. Alloted time time is 3 mins (No discussion time).
Poster Presentation	A poster board, 180 cm in height and 90 cm in width, is available for each presenter. Adhesive tapes are provided by secretariat. Please set up your poster before the 28th morning symposium session.
Group Photo	A group photo is scheduled from 15:30 following the “Symposium 6” on October 29th.
Reception	Reception will be held at KURE (Kindai University Restaurant) from 18:30 on October 29th.
Grant Funding	Japanese Biochemical Society (JBS) MEXT-supported Program for the Strategic Research Foundation at Private Universities Nakatsuji Foresight Foundation

Information for Participants

1. Registration

   Registration desk is located on the 1st floor in NOVEMBER HALL.

☆ Registration Desk Opening Hours:

Date	Hours
October 27th (Thu)	13:00 – 19:00
October 28th (Fri)	8:00 – 19:00
October 29th (Sat)	8:00 – 18:30
October 30th (Sun)	8:00 – **11:00**

2. Cloak

   Our cloak is located on the 2nd floor in NOVEMBER HALL. Valuables and PC cannot be accepted.

☆ Cloak Opening Hours:

Date	Hours
October 27th (Thu)	13:00 – 19:00
October 28th (Fri)	8:00 – 19:00
October 29th (Sat)	8:00 – 18:30
October 30th (Sun)	8:00 – **13:00**

3. Drink and Sweet Treat Service

   Drink and sweet treat service will be available for all participants in the 1 st floor in NOVEMBER HALL.

4. Prohibitions

   Photography, video recording, sound recording, and twittering the presented data are prohibited. Smoking is prohibited except the designated area.

5. Wireless Local Area Network

   NOVEMBER HALL is not equipped with Free Wi-Fi.

Session Chair and Presentation Guidelines

〈**Session Chair Guidelines**〉

1. Arrive Early

   If possible, we kindly ask chairs to arrive at the session room about 5 minutes prior to the start of the session.

2. Start on Time and Stick to the Schedule

   Please start the session on time.

3. Allotted Times

   For “Opening lecture” and “Special seminar” allocation of time for each presentation is left to the discretion of the chairs.

〈**Oral Presentation Guidelines**〉

☆ Language: English

☆ Allotted Time

Symposium: Presentation 15, 20, or 25 mins + Q&A 5 mins

Symposium: Presentation 12 mins + Q&A 3 mins

Young Investigators’ Session: Presentation 7 mins + Q&A 3 mins

Poster Short Talk: 3 mins (No discussion time)

☆ Process and Timing

・Symposium/ Young Investigators’ Session

1 ring: 2 minutes* to the end of presentation

2 rings: End of presentation - Start of discussion

3 rings: End of discussion - Time for next presentation

* Young Investigators’ Session: 1 minutes

・Poster Short Talks

1 ring: 2 minutes from the start of presentation

2 rings: 2.5 minutes from the start of presentation
(End of presentation)

3 rings: 3 minutes from the start of presentation
(Time for next presentation)

☆ Presentation Data

Please prepare your data in Microsoft PowerPoint (Windows PowerPoint 2010/2013/2016 or Mac PowerPoint 2011/2016).

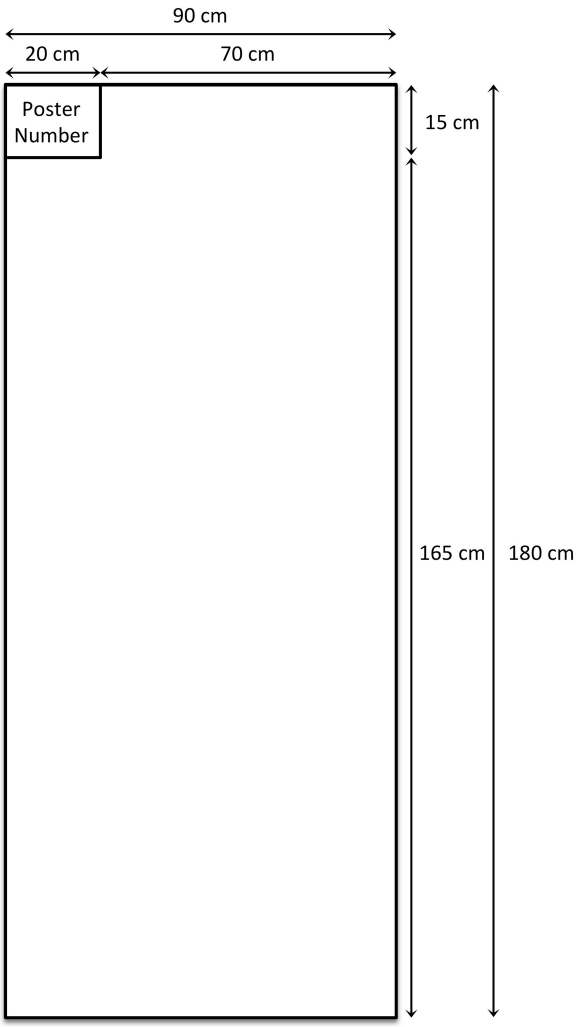
☆ If you use the Secretariat's PC

1. Please bring your data to the "PC Connection Desk" by the main stage.
   - If you will make a presentation in the morning
     Please bring your data **by the previous evening**.
   - If you will make a presentation in the afternoon
     Please bring your data **by the end of lunch time.**
2. Only USB flash drives are accepted.
3. Windows 10 or MacOS 10.12 (Sierra) is the only operating system available for the presentations.
4. Your presentation data file should be named as <Presentation number>_<Name> (eq. O-27_Sugiura).
5. If your presentation contains any movie files, you are also requested to bring your own computer.
6. Audio playback is not possible.
7. If your presentation data is linked to other files (i.e. still or moving images, graphs, etc.), those linked files should also be saved in the same folder, and the links checked beforehand.
8. The Secretariat is responsible for destroying all copies of any data after the session.

☆ If you bring your own PC **("Poster Short Talk"s are not accepted)**

1. You are requested to leave your computer to our skilled technicians in the session room **at least 10 mins before your session starts**.
2. Please bring your AC adapter with you.
3. The Secretariat will prepare a Mini D-sub 15 pin PC cable connector. If your PC is not compatible with this cable connector, please bring an adaptor to connect your PC to the Mini D-sub 15 pin PC cable connector.
4. Please make sure to unlock your password on your computer.
5. Please also bring your presentation data on a media (USB flash drives are accepted) as a backup file.

〈**Poster Presentation Guidelines**〉



1. The panel size is shown in the figure on the right.
   Height: 180 cm
   Width: 90 cm
2. Please print out the title of your presentation as well as the presenter's name(s) and affiliation in English and affix this information at the top of the poster display space.
3. Poster must be prepared in **English**.
4. Poster numbers are already indicated on display panels.
5. Adhesive tapes for putting up posters are provided by secretariat. Please do **not** use tacks or glue.
6. Please attach the ribbon on your chest and stand in front of your poster panel during the poster presentation.
7. Posters remaining after the removal time will be removed by the secretariat.
8. The Organizer and Secretariat will accept no responsibility for any theft, loss or damage of posters.

☆ Poster Set up/Removal time

Set up time：Oct. 27th (Thu) 14:00～Oct. 28th (Fri) 9:00

Removal time：Oct. 30th (Sun) 12:20～13:20

Program

October 27 (Thu)

Opening Remarks **14:50～15:00**

Reiko Sugiura (Organizer, Kindai University, Japan)

Opening Remarks **15:00～15:10**

Hitoshi Shiozaki (President, Kindai University, Japan)

Opening Lecture **15:10～16:00**

Chair: Reiko Sugiura (Kindai University, Japan)

S-01 **Applying tyrosine phosphatase inhibitors in cancer therapeutics**
15:10～16:00 Michel Tremblay (McGill University, Canada)

Coffee Break **16:00～16:20**

Symposium1 – Phosphatase- **16:20～18:00**

Chair: Takashi Matozaki (Kobe University, Japan)
Nicholas Tonks (Cold Spring Harbor Laboratory, USA)

O-01 **Parafibromin, a substrate of SHP2, is a transcriptional platform**
16:20～16:45 **that integrates morphogen signaling pathways**
Masanori Hatakeyama (The University of Tokyo, Japan)

O-02 **Protein phosphatase PP1-NIPP1 limits the DNA-repair capacity**
16:45～17:15 Mathieu Bollen (University of Leuven, Belgium)

O-03 **Ppp6c deficiency predisposes mouse skin tissue**
17:15～17:35 **to carcinogenesis**
Hiroshi Shima (Miyagi Cancer Center Research Institute, Japan)

O-04 **PTPRZ activity is critical for maintaining oligodendrocyte**
17:35～18:00 **precursor cells and glioblastoma stem cells**
Masaharu Noda (National Institute for Basic Biology, Japan)

October 28 (Fri)

Symposium 2 – Immunity/Disease- **9:00～10:40**

Chair: Mathieu Bollen (University of Leuven, Belgium)
Shirish Shenolikar (Duke-NUS Medical School, Singapore)

O-05 **Control of Lymphocytes by Protein Phosphatase-6 and SAPS1**
9:00～9:30 David Brautigan (University of Virginia, USA)

O-06 **A novel mechanism involving SHP-1 for selective suppression of**
9:30～9:50 **B cells reactive to Sm/RNP, a lupus-related self-antigen**
Takeshi Tsubata (Tokyo Medical and Dental University, Japan)

O-07 **Human hemato-lymphoid system development in human**
9:50～10:05 **cytokine knock-in mice engrafted with adult donor-derived CD34+ cells**
Yasuyuki Saito (Kobe University, Japan)

O-08 **The roles of PTPROt in chronic lymphocytic leukemia**
10:05～10:25 Ari Elson (The Weizmann Institute of Science, Israel)

O-09 **PP6 and alpha4 regulate the apoptosis of immature B cells**
10:25～10:40 **induced by BCR crosslinking**
Seiji Inui (Kumamoto University, Japan)

Break **10:40～10:50**

Poster Short Talk (Odd number) Young Investigators **10:50～11:30**

Chair: Yasuyuki Saito (Kobe University, Japan)

PS-01(P-01) **A novel A-kinase anchoring protein, BIG3, coordinates estrogen**
10:50～10:53 **signalling in breast cancer cells**
Tetsuro Yoshimaru (Tokushima University, Japan)

PS-03(P-03) **Protein tyrosine phosphatase Shp2 deficiency in the**
10:53～10:56 **glomerular podocytes attenuates lipopolysaccharide-induced kidney injury**
Shinichiro Koike (University of California Davis, USA)

PS-05(P-05) **Therapeutic application of anti-SIRPα antibody in cancer**
10:56～10:59 **treatment**
Tadahiko Yanagita (Kobe University, Japan)

PS-07(P-07) **ERK-RSK mediated phosphorylation of FilGAP regulates cell**
10:59～11:02 **migration by promoting the conversion from lamellipodia to membrane blebbing downstream of epidermal growth factor signaling**
Koji Tsutsumi (Kitasato University, Japan)

PS-09(P-09) **Role of SIRPα in the homeostasis of fibroblastic reticular cells**
11:02～11:05 **by dendritic cells in the spleen**
Datu Respatika (Kobe University, Japan)

PS-11(P-11) **Mangiferin induced the apoptosis via suppression of NIK/NF-κB**
11:05～11:08 **pathway in human multiple myeloma cells**
Toshiki Kino (Kindai University, Japan)

PS-13(P-13) **Mangiferin, a novel nuclear factor kappa B inducing kinase**
11:08～11:11 **inhibitor, suppresses metastasis in a mouse metastatic melanoma model**
Tomoya Takeda (Kindai University, Japan)

PS-15(P-15) **Functional Analysis of the Puf family RNA-binding protein**
11:11～11:14 **Pumilio in stress responses and the inositol phospholipid signaling pathway**
Masahiro Inari (Kindai University, Japan)

PS-17(P-17) **Cross-species reaction of anti-human LAT1 with LAT1 of**
11:14～11:17 **crab-eating monkey**
Shiho Ueda (Kindai University, Japan)

PS-19(P-19) **Influence of radixin knockdown on drug efflux transporters of**
11:17～11:20 **cancer cells**
Yuta Inoue (Kindai University, Japan)

PS-21(P-21) **A CC3 variant of lymphotactin/XCL1 (XCL1- CC3) is an effective**
11:20～11:23 **CTL-inducing adjuvant for cancer immunotherapy**
Shinya Yamamoto (Kindai University, Japan)

PS-23(P-23) **The BCR crosslinking-induced phosphorylation of Bcl-xL and**
11:23～11:26 **apoptosis are controlled by alpha4 in immature B cell**
Kano Tanabe (Kumamoto Health Science University, Japan)

Poster presentation (Odd number) **11:30～12:30**

Lunch / Poster viewing **12:30～13:30**

Symposium 3 -Cellular Signaling and Stress Responses- **13:30～15:20**

Chair: Masaharu Noda (National Institute for Basic Biology, Japan)
Tzu-Ching Meng (Academia Sinica, Taiwan)

O-10 **Multiple Roles for eIF2alpha Phosphatases in the Unfolded**
13:30～14:00 **Stress Response**
Shirish Shenolikar (Duke University, USA)

O-11 **Characterization of a Gtr/Rag-independent and glutamine-**
14:00～14:20 **responsive TORC1 activation mechanism in yeast**
Tatsuya Maeda (The University of Tokyo, Japan)

O-12 **Mitochondrial stress sensing and cellular response**
14:20～14:40 Kohsuke Takeda (Nagasaki University, Japan)

O-13 **Neural specific RNA-binding proteins grasp the translation**
14:40～15:00 **regulatory networks**
Tohsinobu Fujiwara (Kindai University, Japan)

O-14 **Two bistable switches of cell division generated by**
15:00～15:20 **kinase/phosphatase antagonism**
Satoru Mochida (Kumamoto University, Japan)

Coffee Break **15:20～16:00**

Young Investigators' Session 1 -Cancer Therapeutics- **16:00～17:00**

Chair: Zhenghe Wang (Case Western Reserve University, USA)
Tatsuya Maeda (The University of Tokyo, Japan)

O-15 **Molecular mechanisms for the upregulation of Cav3.2 T-type**
16:00～16:10 **calcium channels involved in neuropathic pain**
Shiori Tomita (Kindai University, Japan)

O-16 **Targeting HMGB1 and its downstream molecules for treatment**
16:10～16:20 **of oxaliplatin-induced peripheral neuropathy**
Maho Tsubota (Kindai University, Japan)

O-17 **Sulfonic acid formation of the active-site cysteine directs**
16:20～16:30 **ubiquitin proteasome system-mediated degradation of myocardial protein tyrosine phosphatases**
Chun-Yi Yang (National Taiwan University, Taiwan)

O-18 **Overexpression of HIF-1alpha is involved with melphalan-**
16:30～16:40 **resistance in multiple myeloma cells**
Masanobu Tsubaki (Kindai University, Japan)

O-19 **FGFR gene alterations in lung squamous cell carcinoma are**
16:40～16:50 **potential targets for the multikinase inhibitor nintedanib**
Kazuko Sakai (Kindai University, Japan)

O-20 **Antiepileptic drug use is associated with a decreased risk of**
16:50～17:00 **cancer: Data mining of spontaneous reporting and claims**
Mai Fujimoto (Kindai University, Japan)

Coffee Break **17:00～17:20**

Symposium 4 -Phosphatase- **17:20～18:20**

Chair: Satoru Mochida (Kumamoto University, Japan)
David Brautigan (University of Birginia, USA)

O-21 **Roles of protein phosphatases in cell polarity control**
17:20～17:50 Takashi Toda (Hiroshima University, Japan)

O-22 **Identifying the human calcineurin signaling network**
17:50～18:20 Martha Cyert (Stanford University, USA)

October 29 (Sat)

Symposium 5 -Molecular Basis of Cancer- **9:00～10:40**

Chair: Fawaz Haj (University of Califolnia Davis, USA)
Takeshi Tsubata (Tokyo Medical and Dental University, Japan)

O-23 **PTPRT is a tumor suppressor that regulates intestinal stem cell proliferation**
9:00～9:25 Zhenghe Wang (Case Western Reserve University, USA)

O-24 **Epigenetic Regulation by Notch Signaling in Glioma**
9:25 ～9:50 Yutaka Kondo (Nagoya City University, Japan)

O-25 **Modest attenuation of DNA damage repair delays therapy-related cancer in mouse model**
9:50～10:10 Hitoshi Okada (Kindai University, Japan)

O-26 **A Surprising Role for PTP1B in Breast Cancer**
10:10～10:40 Benjamin Neel (Perlmutter Cancer Center, USA)

Break **10:40～10:50**

Poster Short Talk (Even number) Young Investigators **10:50～11:30**

Chair: Takeshi Ijuin (Kobe University, Japan)

PS-02 (P-02) **Phyllotaxis Patterns**
10:50～10:53 Risa Yamada (Nara Woman's University Secondary School, Japan)

PS-04 (P-04) **Inhibition of NF-kappaB by mangiferin increased the sensitivity of human multiple myeloma cells to anticancer drugs**
10:53～10:56 Yoshika Tomonari (Kindai University, Japan)

PS-06 (P-06) **Mutation and Inhibition of Hsp90 affect stress granule assembly and MAPK signaling ~Implications of anti-cancer mechanisms of Geldanamycin~**
10:56～10:59 Takumi Ikehata (Kindai University, Japan)

PS-08 (P-08) 10:59～11:02
Skb5, an SH3 domain adaptor protein, plays a regulatory role in the PKC/MAPK signaling pathway by controlling the intracellular localization of the MAPKKK Mkh1
Chisato Ikeda (Kindai University, Japan)

PS-10 (P-10) 11:02～11:05
Global gene expression profiling reveals unexpected spectrum of effects of a novel immune modulator FTY720 ~Possible involvement of iron homeostasis as an antitumor property of FTY720~
Kanako Hagihara (Kindai University, Japan)

PS-12 (P-12) 11:05～11:08
Anti-cancer drug discovery using fission yeast genetics identified a novel analog of 1'-Acetoxychavicol Acetate (ACA) with a potent anti-tumor activity against human melanoma cells
Kazuki Matsuura (Kindai University, Japan)

PS-14 (P-14) 11:08～11:11
SET/I2PP2A Is a Prognostic Marker and a Potential Therapeutic Target for Gastric Cancer
Shuhei Enjoji (Yamaguchi University, Japan)

PS-16 (P-16) 11:11～11:14
Modification of PP2A Methylation Status Assay and Implication for Protein Phosphatase Methylesterase-1 (PME-1) as a Therapeutic Target for a Subset of Melanoma
Ryotaro Yabe (Yamaguchi University, Japan)

PS-18 (P-18) 11:14～11:17
Identification of specific inhibitors for oncogenic protein phosphatase PPM1D from G-quadruplex DNA aptamer library
Atsushi Kaneko (Niigata University, Japan)

PS-20 (P-20) 11:17～11:20
Inhibition of p53-inducible Ser/Thr phosphatase PPM1D induces differentiation of human testicular embryonal carcinoma cell line
Rui Kamada (Hokkaido University, Japan)

PS-22 (P-22) 11:20～11:23
Effect of inhibition of p53-inducible Ser/Thr phosphatase PPM1D on neutrophil differentiation
Fuki Kudoh (Hokkaido University, Japan)

PS-24 (P-24) **Identification of aldolase A as a novel diagnosis biomarker for**
11:23～11:26 **colorectal cancer based on proteomic analysis using formalin-fixed paraffin-embedded tissue**
Kanta Sato (Kindai University, Japan)

Poster presentation (Even number) **11:30～12:30**

Lunch / Poster viewing **12:30～13:30**

Symposium 6 - Therapeutic Strategies for Cancer- **13:30～15:30**

Chair: Michel Tremblay (McGil University, Canada)
Benjamin Neel (Perlmutter Cancer Center, NY, USA)

O-27 **Applying Kinase-Phosphatase Interplay in Ca^{2+} signaling for**
13:30～13:55 **Cancer Therapeutics: A pas de deux**
Reiko Sugiura (kindai University, Japan)

O-28 **Structual basis for PTPN3-p38gamma complex involved in colon**
13:55～14:20 **cancer progression**
Tzu-Ching Meng (Academia Sinica, Taiwan)

O-29 **Illuminating Cellular Phosphorylation Signaling by Kinase-**
14:20～14:45 **Centric Phosphoproteomics**
Yasushi Ishihama (Kyoto University, Japan)

O-30 **Identification of binding molecules for Ser/Thr phosphatases**
14:45～15:00 **using structurally rigid libraries**
Yoshiro Chuman (Niigata University, Japan)

O-31 **Drugging the undruggable:exploiting PTP1B as a therapeutic**
15:00～15:30 **target**
Nicholas K. Tonks (Cold Spring Harbor Laboratory, Cold Spring Harbor, USA)

Coffee Break / Group Photo **15:30～16:00**

Young Investigators' Session 2 -Cancer Signaling - **16:00～17:00**

Chair: Takashi Toda (Hiroshima University, Japan)
Tzu-Ching Meng (Academia Sinica, Taiwan)

O-32 **Skb5, an SH3 adaptor protein, regulates PKC/MAPK signaling via spatial regulation of MAPKKK**
16:00～16:10 Yuki Kanda (Kindai University, Japan)

O-33 **CCR4 is involved in Th17 cell expansion and Th17-mediated induction of antitumor CTLs in mice bearing intradermal B16 melanoma**
16:10～16:20 Kazuhiko Matsuo (Kindai University, Japan)

O-34 **Anticancer-drug screening utilizing fission yeast genetics identified Acremomannolipin A, a Calcium signalling modulator with anti-tumor activity**
16:20～16:30 Ryosuke Satoh (Kindai University, Japan)

O-35 **The coupling mechanism between translation and mRNA degradation mediated by RNA-binding protein HuD**
16:30～16:40 Akira Fukao (Kindai University, Japan)

O-36 **Potential role of glycosylation in regulating biological function of PTPRA**
16:40～16:50 Deepa Murali (Academia Sinica, Taiwan)

O-37 **Role of Src family kinases in regulation of intestinal epithelial homeostasis**
16:50～17:00 Shinya Imada (Kobe University Graduate School of Medicine, Japan)

Coffee Break **17:00～17:20**

Special Seminar **17:20～18:10**

Chair: Masanori Hatakeyama (The University of Tokyo, Japan)

S-02 **Cancer heterogeneity and plasticity based on cancer stem cell biology**
17:20～18:10 Hideyuki Saya (Keio University, Japan)

Saturday Night Party at KURE **18:30～20:30**

October 30 (Sun)

Symposium 7 –Metabolism/Diseases- **9:00～10:45**

Chair: Hiroshi Shima (Miyagi Cancer Center Research Institute, Japan)
Ari Elson (The Weizmann Institute of Science, Israel)

O-38 9:00～9:20
Protein tyrosine phosphatase 1B deficiency in podocytes protects against hyperglycemia-induced renal injury
Fawaz G. Haj (University of California Davis, USA)

O-39 9:20～9:40
Lacking of dopamine D2L receptor causes vulnerability against chronic stress in mice
Kohji Fukunaga (Tohoku University, Japan)

O-40 9:40～10:00
Regulation of adipocyte differentiation and lipid droplet formation by PPM1D phosphatase
Kazuyasu Sakaguchi (Hokkaido University, Japan)

O-41 10:00～10:15
The R3 receptor-like protein tyrosine phosphatase subfamily negatively regulates insulin signaling by dephosphorylating the insulin receptor at specific sites
Takafumi Shintani (National Institute for Basic Biology, Japan)

O-42 10:15～10:30
Skeletal muscle enriched inositol polyphosphate phosphatase (SKIP) links ER stress to insulin resistance
Takeshi Ijuin (Kobe University, Japan)

O-43 10:30～10:45
Loss of NDRG2/PP2A complex induces global abnormalities in protein phosphorylation in cancer development and progression
Tomonaga Ichikawa (University of Miyazaki, Japan)

Coffee Break **10:45～11:00**

Young Investigators' Session 3 -Phosphorylation and Dephosphorylation-

11:00～11:40

Chair: Kohsuke Takeda (Nagasaki University, Japan)
Kazuyasu Sakaguchi (Hokkaido University, Japan)

O-44 11:00～11:10
The protein phosphatase 6 catalytic subunit (Ppp6c) is indispensable for proper postimplantation embryogenesis
Honami Ogoh (Nara Women's University, Japan)

O-45 11:10～11:20
In vivo regulation of GSK3β activity, as revealed by quantitative measurements of its phosphoisotypes
Ambika Krishnankutty (Tokyo Metropolitan University, Japan)

O-46 11:20～11:30
Protein kinase N (PKN) family-dependent regulation of hepatic cytochrome P450 2C and metabolic profile analysis in PKN mutant mice through targeted metabolomics by LC-MS/MS
Atsushi Kawase (Kindai University, Japan)

O-47 11:30～11:40
Down-regulation of ErbB2/ErbB3 heterodimer via ERK-mediated phosphorylation of ErbB2 Thr-677 in the juxtamembrane domain
Yuki Kawasaki (University of Toyama, Japan)

Break **11:40～11:50**

Award Lecture **11:50～12:10**

Chair: Toshio Watanebe (Nara Women's University, Japan)

O-48 11:50～12:10
Shp2 in Forebrain Neurons Regulates Synaptic Plasticity Locomotion, and Memory Formation in Mice
Shinya Kusakari (Tokyo Medical University, Japan)

Closing Remarks **12:10～12:20**

Toshio Watanabe (Nara Women's University, Japan)
Reiko Sugiura (Kindai University, Japan)

<Poster> October 28 (Fri)

Poster (Odd number) **11:30～13:30**

P-01 (PS-01) **A novel A-kinase anchoring protein, BIG3, coordinates estrogen signalling in breast cancer cells**
Tetsuro Yoshimaru (Tokushima University, Japan)

P-03 (PS-03) **Protein tyrosine phosphatase Shp2 deficiency in the glomerular podocytes attenuates lipopolysaccharide-induced kidney injury**
Shinichiro Koike (University of California Davis, USA)

P-05 (PS-05) **Therapeutic application of anti-SIRPα antibody in cancer treatment**
Tadahiko Yanagita (Kobe University Graduate School of Medicine, Japan)

P-07 (PS-07) **ERK-RSK mediated phosphorylation of FilGAP regulates cell migration by promoting the conversion from lamellipodia to membrane blebbing downstream of epidermal growth factor signaling**
Koji Tsutsumi (Kitasato University, Japan)

P-09 (PS-09) **Role of SIRPα in the homeostasis of fibroblastic reticular cells by dendritic cells in the spleen**
Datu Respatika (Kobe University, Japan)

P-11 (PS-11) **Mangiferin induced the apoptosis via suppression of NIK/NF-κB pathway in human multiple myeloma cells**
Toshiki Kino (Kindai University, Japan)

P-13 (PS-13) **Mangiferin, a novel nuclear factor kappa B-inducing kinase inhibitor, suppresses metastasis in a mouse metastatic melanoma model**
Tomoya Takeda (Kindai University, Japan)

P-15 (PS-15) **Functional Analysis of the Puf family RNA-binding protein Pumilio in stress responses and the inositol phospholipid signaling pathway**
Masahiro Inari (Kindai University, Japan)

P-17 (PS-17) **Cross-species reaction of anti-human LAT1 with LAT1 of crab-eating monkey**
Shiho Ueda (Kindai University, Japan)

P-19 (PS-19) **Influence of radixin knockdown on drug efflux transporters of cancer cells**
Yuta Inoue (Kindai University, Japan)

P-21 (PS-21) **A CC3 variant of lymphotactin/XCL1 (XCL1- CC3) is an effective CTL-inducing adjuvant for cancer immunotherapy**
Shinya Yamamoto (Kindai University, Japan)

P-23 (PS-23) **The BCR crosslinking-induced phosphorylation of Bcl-xL and apoptosis are controlled by alpha4 in immature B cell**
Kano Tanabe (Kumamoto Health Science University, Japan)

P-25 **Cav3.2 T-type calcium channels as therapeutic targets for the oxaliplatin-induced peripheral neuropathy**
Takaya Miyazaki (Kindai University, Japan)

P-27 **Interleukin-6-induced neuroendocrine-like differentiation of human prostate cancer cells: cell signaling and upregulation of Cav3.2 T-type calcium channels**
Kazuki Fukami (Kindai University, Japan)

P-29 **A role of macrophage-derived HMGB1 in paclitaxel-induced peripheral neuropathy in mice**
Risa Domoto (Kindai University, Japan)

P-31 **Evaluation of the measurement method of intracellular calcium ion concentration in fission yeast**
Fumihiko Ogata (Kindai University, Japan)

P-33 **Extra-mitochondrial function of cleaved PGAM5**
Ayane Yamaguchi (Nagasaki University, Japan)

P-35 **Ca^{2+}/calmodulin-dependent protein kinase phosphatase (CaMKP/PPM1F) interacts with neurofilament L and inhibits its filament association**
Hana Ozaki (Hiroshima University, Japan)

P-37 **Evidence that Warburg effect functions as anti-cancer barrier**
Nobuhiro Tanuma (Miyagi Cancer Center Research Institute, Japan)

P-39 **Interaction of SHP2 with ALK regulates oncogenicity of neuroblastoma cells**
Ryuichi Sakai (Kitasato University School of Medicine, Japan)

P-41 **Down-regulation of ErbB2/ErbB3 heterodimer via ERK-mediated phosphorylation of ErbB2 Thr-677 in the juxtamembrane domain**
Yuki Kawasaki (University of Toyama, Japan)

P-43 **Analyses of the molecular function of PABP interacting protein 1 (PAIP-1) in translational regulation**
Tomohiko Aoyama (Nagoya City University, Japan)

P-45 **Elucidation of elementary processes in which RNA-binding protein HuD stimulates the cap- poly(A) dependent translation**
Hiroshi Ohtsuka (Nagoya City University, Japan)

P-47 **High affinity RNA for capping enzyme of *Saccharomyces cerevisiae***
Yuka Yamada (Kindai University, Japan)

P-49 **Butyrate response factor 1 induces translation repression independently of ARE-mediated mRNA decay**
Miwa Takechi (Kyoto University,Japan)

P-51 **Analyses of cell type specific translation from IRES mRNA derived from two different poliovirus strains**
Akitoshi Sadahiro (Kyoto University, Japan)

P-53 **Effect of interaction between RNA binding protein HuD and SMN protein on protein synthesis**
Daisuke Ikeda (Nagoya City University, Japan)

P-55 **Inhibitory Effects of Oligostilbenoids from the Bark of Shorea roxburghii on Malignant Melanoma Cell Growth: Implications for Novel Topical Anticancer Candidates**
Takashi Morita (Kindai University, Japan)

P-57 **Mysterious eukaryotic translation initiation factor eIF4H**
Takumi Tomohiro (Nagoya City, Japan)

P-59 **Novel target molecules for treatment of cancer of unknown primary**
Yoshihiko Fujita (Kindai University, Japan)

P-61 **Evaluation of binding structures predicted by SDO-VS method**
Yusuke Namba (Kindai University, Japan)

P-63 **Potential role of glycosylation in regulating biological function of PTPRA**
Deepa Murali (Academia Sinica, Taiwan)

<Poster> October 29 (Sat)

Poster Session(Even number) **11:30～12:30**

P-02 (PS-02) **Phyllotaxis Patterns**
Risa Yamada (Nara Woman's University Secondary School, Japan)

P-04 (PS-04) **Inhibition of NF-kappaB by mangiferin increased the sensitivity of human multiple myeloma cells to anticancer drugs**
Yoshika Tomonari (Kindai University, Japan)

P-06 (PS-06) **Mutation and Inhibition of Hsp90 affect stress granule assembly and MAPK signaling ~Implications of anti-cancer mechanisms of Geldanamycin~**
Takumi Ikehata (Kindai University, Japan)

P-08 (PS-08) **Skb5, an SH3 domain adaptor protein, plays a regulatory role in the PKC/MAPK signaling pathway by controlling the intracellular localization of the MAPKKK Mkh1**
Chisato Ikeda (Kindai University, Japan)

P-10 (PS-10) **Global gene expression profiling reveals unexpected spectrum of effects of a novel immune modulator FTY720 ~Possible involvement of iron homeostasis as an antitumor property of FTY720~**
Kanako Hagihara (Kindai University, Japan)

P-12 (PS-12) **Anti-cancer drug discovery using fission yeast genetics identified a novel analog of 1'-Acetoxychavicol Acetate (ACA) with a potent anti-tumor activity against human melanoma cells**
Kazuki Matsuura (Kindai University, Japan)

P-14 (PS-14) **SET/I2PP2A Is a Prognostic Marker and a Potential Therapeutic Target for Gastric Cancer**
Shuhei Enjoji (Yamaguchi University, Japan)

P-16 (PS-16) **Modification of PP2A Methylation Status Assay and Implication for Protein Phosphatase Methylesterase-1 (PME-1) as a Therapeutic Target for a Subset of Melanoma**
Ryotaro Yabe (Yamaguchi University, Japan)

P-18 (PS-18) **Identification of specific inhibitors for oncogenic protein phosphatase PPM1D from G-quadruplex DNA aptamer library**
Atsushi Kaneko (Niigata University, Japan)

P-20 (PS-20) **Inhibition of p53-inducible Ser/Thr phosphatase PPM1D induces differentiation of human testicular embryonal carcinoma cell line**
Rui Kamada (Hokkaido University, Japan)

P-22 (PS-22) **Effect of inhibition of p53-inducible Ser/Thr phosphatase PPM1D on neutrophil differentiation**
Fuki Kudoh (Hokkaido University, Japan)

P-24 (PS-24) **Identification of aldolase A as a novel diagnosis biomarker for colorectal cancer based on proteomic analysis using formalin-fixed paraffin-embedded tissue**
Kanta Sato (Kindai University, Japan)

P-26 **Regulation of Beclin 1 Phosphorylation and Autophagy by PP2A and DAPK3**
Nobuyuki Fujiwara (Yamaguchi University, Japan)

P-28 **Analysis of a direct cell-cell communication signal that regulates glial activation in the brain**
Tomomi Nozu (Gunma University, Japan)

P-30 **Small-molecule inhibition of PTPRZ reduces tumor growth in a rat model of glioblastoma**
Akihiro Fujikawa (National Institute for Basic Biology, Japan)

P-32 **A simple method for preparing nonphosphorylated protein kinases using E. coli strain BL21 (DE3, pλPP) which constitutively expresses λPPase**
Kazutoshi Akizuki (Kagawa University, Japan)

P-34 **Role of Chondroitin Sulfate (CS) Modification in the Regulation of Proteintyrosine Phosphatase Receptor Type Z (PTPRZ) Activity: PLEIOTROPHIN-PTPRZ-A SIGNALING IS INVOLVED IN OLIGODENDROCYTE DIFFERENTIATION**

Kazuya Kuboyama (National Institute for Basic Biology, Japan)

P-36 **Disease-associated EED Ile363Met mutation increases susceptibility to hematologic malignancies**

Takeshi Ueda (Kindai University, Japan)

P-38 **Elucidation of adipogenesis by the H3K27 histone demethylase Utx**

Kazushige Ota (Kindai University, Japan)

P-40 **IL-18 amplifies macrophage M2 polarization, leading to enhancement of angiogenesis via up-regulation of osteopontin**

Takuro Kobori (Kindai University, Japan)

P-42 **The role of histone demethylase KDM4b in breast cancer stem cell**

Akiyoshi Komuro (Kindai University, Japan)

P-44 **A Genome-wide Screen Reveals Genes Involved in Calcium Signaling and Glycosylation for Tolerance to SKB (Sugiura Kagobutsu B), a Novel Glycolipid with Potent Anti-tumor Activity**

Ayako Kita (Kindai University, Japan)

P-46 **Lung epithelial cell apoptosis induced by increased ectodomain shedding of cell adhesion molecule 1 in the lungs of emphysema and idiopathic interstitial pneumonia**

Azusa Yoneshige (Kindai University, Japan)

P-48 **Expression of amino-acid transporters, adhesion molecules and oncogene products in human cancers revealed by novel mAb**

Takuya Imaida (Kindai University, Japan)

P-50 **The diagnosis of an innovation cancer by the antibody secretion hybridoma transplantation and establishment of the treatment system**
Kazuma Terashima (Kindai University, Japan)

P-52 **Aiming at establishment of discrimination method of a male and female of pistachio**
Erina Komeda (Nara Women's University Secondary School, Japan)

P-54 **Hepatoprotective triterpene saponin constituents from roots of Bupleurum falcatum**
Takuya Konno (Kindai University, Japan)

P-56 **Limonoids from Brazilian folk medicine, Andiroba, with fat metabolizing activity in hepatocytes**
Kiyofumi Ninomiya (Kindai University, Japan)

P-58 **Diterpenoids from the Aerial Part of Isodon trichocapus with Melanogenesis Inhibitory Activity**
Yoshiaki Manse (Kaminomoto Co., Ltd., Japan)

P-60 **In situ photopolymerization of polyacryrlamide gel for specific entrapment and analysis of a phosphate compounds using microchip electrophoresis**
Sachio Yamamoto (Kindai University, Japan)

P-62 **The role of cyclophilin A as a novel therapeutic target for colorectal cancer**
Tetsushi Yamamoto (Kindai University, Japan)

P-64 **Sulfonic acid formation of the active-site cysteine directs ubiquitin proteasome system-mediated degradation of myocardial protein tyrosine phosphatases**
Chun-Yi Yang (National Taiwan University, Taiwan)

Time Table

	<Day1> Oct. 27th (Thu)	<Day 2> Oct. 28th (Fri)	<Day 3> Oct. 29th (Sat)	<Day 4> Oct. 30th (Sun)
9:00		Symposium 2 -Immunity/Disease- 9:00～10:40	Symposium 5 -Molecular Basis of Cancer- 9:00～10:40	Symposium 7 -Metabolism/Diseases- 9:00～10:45
10:00				
		Break 10:40～10:50	Break 10:40～10:50	Coffee Break 10:45～11:00
11:00		Poster Short Talk (Odd number) 10:50～11:30	Poster Short Talk (Even number) 10:50～11:30	Young Investigators' Session 3 -Phosphorylation and Dephosphorylation- 11:00～11:40
		Poster Presentation (Odd number) 11:30～12:30	Poster Presentation (Even number) 11:30～12:30	Break 11:40～11:50
12:00				Award Lecture Closing Remarks 11:50～12:20
		Lunch (and Poster Viewing) 12:30～13:30	Lunch (and Poster Viewing) 12:30～13:30	
13:00	Reception Starts 13:00～	Symposium 3 -Cellular Signaling and Stress Responses- 13:30～15:20	Symposium 6 -Therapeutic Strategies for Cancer- 13:30～15:30	
14:00				
15:00	Opening Remarks 14:50～15:10 Opening Lecture 15:10～16:00	Coffee Break 15:20～16:00	Coffee Break & Group Photo 15:30～16:00	
16:00	Cofee Break 16:00～16:20	Young Investigators' Session 1 -Cancer Therapeutics- 16:00～17:00	Young Investigators' Session 2 -Cancer Signaling- 16:00～17:00	
	Symposium 1 -Phosphatase- 16:20～18:00			
17:00		Cofee Break 17:00～17:20	Cofee Break 17:00～17:20	
		Symposium 4 -Phosphatase- 17:20～18:20	Special Seminar 17:20～18:10	
18:00				
19:00			Saturday Night Party at KURE 18:30～20:30	
20:00				

Opening Lecture

S-01

Applying protein tyrosine phosphatase inhibitors in cancer therapeutics

Author(s)

Michel L. Tremblay

Author's affiliation(s)

Goodman Cancer Research Centre and Dept. of Biochemistry, McGill University, Montreal, Canada

Abstract

The protein tyrosine phosphatase (PTP) gene family encodes for 107 genes in the human genome. This remarkable diversity is reflected in the function of these enzymes in important regulatory axes ranging from growth factor and cytokine signaling, to cell-cell interaction, cytoskeletal regulation and cell specification. Not surprisingly, the past 30 years of PTP studies have led to the identification of broad numbers of disease contexts where members of this family were found to be either mutated or inappropriately expressed, including cancer, metabolic, neurological and immune diseases.

Their diverse functions have also provided exciting opportunities to employ modulators of these enzymes to treat several diseases. Unfortunately, they remain largely impervious to targeting in clinical applications due to similarity of the catalytic domain, presence of charged residues, poor inhibitor uptake and other issues.

In this presentation, I will review several approaches that we have undertaken to target specific PTP enzymes in disease. Among those that I will discuss are our efforts to comprehend the roles and additive effects of two small intracellular PTPs, PTP1B and TC-PTP, and the therapeutic relevance of targeting these in prostate and

pancreatic cancers, particularly through our new immunotherapy platform.

A second major interest of the laboratory has been to examine the oncogenic mechanisms of the trio of PTP4A enzymes, with a focus on our recent finding of their modulatory activity on the CNNM magnesium sensors. This represents a novel paradigm in cellular metabolism that places the PTP4A/CNNM protein complexes at the center of oncometabolism, infectious disease, and normal mammalian physiology.

Our overarching conclusion on PTP function is that protein tyrosine phosphatases act as finely tuned sensors of signaling output. Their stoichiometry is key to maintaining homeostasis. Therefore, their study and targeting must reflect this dosage effect since such functions are often poorly exposed in gene knock-out disease models, yet they are likely to open novel approaches in translational applications.

Special Seminar

S-02

Cancer heterogeneity and plasticity based on cancer stem cell biology

Author(s)

Hideyuki Saya

Author's affiliation(s)

Division of Gene Regulation, Institute for Advanced Medical Research, Keio University School of Medicine, Tokyo, Japan

Abstract

For several types of cancer, it is considered that a select subpopulation with stem-cell like properties gives rise to all other cells in the tumor mass. Cancer stem cells (CSCs) are therefore, by definition, a major contributor to tumor heterogeneity. However, CSCs themselves can display both intra- and intertumoral diversity and undergo phenotypic reprogramming in response to environmental cues.

The present study explores the metabolic characteristics of brain tumor stem cells, with a specific focus on their adaptation to nutrient and oxygen availability. We present our findings from an induced cancer stem cell model of glioma, based on orthotopic implantation of murine *Ink4a/Arf* -/- neural stem cells overexpressing HRasV12 into the brains of syngeneic mice. Our results show that brain tumors can contain stem cells with different metabolic characteristics and that this diversity can help survival in conditions of nutrient limitation. Moreover, in a subgroup of tumor stem cells, hypoxia can induce a reversible change of the main metabolic pathway.

In addition, we have recently found that differentiation property is a critical factor for tumorigenic activity of cancer stem cells. Based on our findings, we attempted to establish the transdifferentiation approach for treatment of cancer stem cells by using mouse osteosarcoma stem cell model.

Symposium / Young Investigators' Session

O-01

Parafibromin, a substrate of SHP2, is a transcriptional platform that integrates morphogen signaling pathways

Author(s)

Masanori Hatakeyama, Ippei Kikuchi, Atsushi Takahashi

Author's affiliation(s)

The University of Tokyo, Japan

Abstract

The Wnt, Hedgehog (Hh), and Notch morphogen pathways play essential roles in the development, homeostasis, and pathogenesis of multicellular organisms. During signal transmission in tissues and organs, these pathways often functionally interplay one another for cell fate specification of individual cells. Nevertheless, molecular mechanisms that intracellularly coordinate these signal inputs remain poorly understood. Here, we found that parafibromin, a component of the RNA polymerase II-associated factor (PAF) complex, serves as a nuclear platform/scaffold that intracellularly coordinates signal outputs of the Wnt, Hh, and Notch signaling pathways. We revealed that parafibromin competitively interacts with the Wnt-signal effector β-catenin and the Hh-signal effector Gli1, thereby regulating transactivation of Wnt- and Hh-target genes in a mutually exclusive manner. On the other hand, parafibromin can simultaneously bind to the Notch-signal effector Notch intracellular domain (NICD) and β-catenin, enabling cooperative activation of Notch- and Wnt-target genes in the cell. Furthermore, the transcriptional platform function is strictly regulated by tyrosine phosphorylation status of parafibromin, which is reciprocally regulated by SHP2 tyrosine phosphatase and PTK6 tyrosine kinase. Thus, parafibromin integrates and converts signals conveyed by these morphogen pathways into appropriate transcriptional outputs in a tyrosine phosphorylation/dephosphorylation-regulated manner.

O-02 Invited

Protein phosphatase PP1-NIPP1 limits the DNA-repair capacity

Author(s)

Mathieu Bollen

Author's affiliation(s)

Laboratory of Biosignaling & Therapeutics, University of Leuven, Leuven, Belgium

Abstract

PP1 is a member of the PPP superfamily of protein Ser/Thr phosphatases. It is a ubiquitously expressed enzyme that catalyzes over half of all protein dephosphorylation events in eukaryotic cells. In mammals PP1 interacts with over 200 PP1-interacting proteins (PIPs) that determine when and where the phosphatase acts. One of these PIPs is NIPP1, for nuclear inhibitor of PP1. To study the *in vivo* function of NIPP1 we have generated NIPP1 knockout models. A total NIPP1 knockout in mice is early embryonic lethal. However, the conditional knockout of NIPP1 in liver epithelial cells or skin keratinocytes has no major spontaneous phenotype, except for a moderate expansion of the stem-cell compartment. Strikingly, such NIPP1-deprived cells display a strongly enhanced DNA-repair capacity and a nearly complete resistance to mutagen-induced carcinogenesis. Conversely, the expression of a PP1-NIPP1 fusion in HeLa cells causes replication stress, as illustrated by the appearance of slow and stalled replication forks, and the accumulation of double-strand DNA breaks. Importantly, replication stress was not observed after the expression of PP1-NIPP1 fusions with mutated substrate-binding or PP1-anchoring domains of NIPP1. This strongly suggests that replication stress induced by PP1-NIPP1 stems from the dephosphorylation of FHA ligands by associated PP1. We are currently identifying the relevant substrates. Thus, our data indicate that PP1-NIPP1 limits the DNA-repair capacity.

O-03

Ppp6c deficiency predisposes mouse skin tissue to carcinogenesis

Author(s)

Hiroshi Shima[1,2], Yuki Momoi[1], Honami Ogoh[3], Koreyuki Kurosawa[1,2], Yui Inoue[1], Nobuhiro Tanuma[1,2], Toshio Watanabe[3]

Author's affiliation(s)

[1]Miyagi Cancer Center Research Institute, Japan

[2]Tohoku University School of Medicine, Japan

[3]Nara Women's University, Japan

Abstract

We generated skin keratinocyte-specific Ppp6c conditional knockout mice and performed 2-stage skin carcinogenesis analysis. Ppp6c deficiency induced papilloma formation with DMBA only, and development of those papillomas was significantly accelerated compared to that seen following DMBA/TPA treatment of wild-type mice. NFκB activation either by TNFα or IL-1β was enhanced in Ppp6c-deficient keratinocytes. Overall, we conclude that that Ppp6c deficiency predisposes mice to skin carcinogenesis initiated by DMBA. This is the first report showing that such deficiency promotes tumor formation in mice. Then we asked whether Ppp6c loss acts as a tumor promoter in UVB-induced squamous cell carcinogenesis. Following UVB irradiation, mice with Ppp6c-deficient keratinocytes showed a higher incidence of skin squamous cell carcinoma than did control mice. Time course experiments showed that following UVB irradiation, Ppp6c-deficient keratinocytes upregulated expression of p53, PUMA, BAX, and cleaved caspase-3 proteins. UVB-induced tumors in Ppp6c-deficient keratinocytes exhibited a high frequency of both p53- and γH2AX-positive cells, suggestive of DNA damage. Our analysis suggests that PP6 deficiency underlies molecular events that drive outgrowth of initiated keratinocytes harboring UVB-induced mutated p53. Understanding PP6 function in preventing carcinogen and UV-induced tumorigenesis could suggest strategies to prevent and treat these conditions.

O-04

PTPRZ activity is critical for maintaining oligodendrocyte precursor cells and glioblastoma stem cells

Author(s)

Masaharu Noda[1,2]

Author's affiliation(s)

[1]**National Institute for Basic Biology, Japan**

[2]**The Graduate University for Advanced Studies (SOKENDAI), Japan**

Abstract

Protein tyrosine phosphatase receptor type Z (PTPRZ; human orthologue, PTPRZ1), dephosphorylates p190 RhoGAP, thereby acting as a counterpart of FYN in differentiation of oligodendrocyte precursor cells (OPCs) to oligodendrocytes. PTPRZ isoforms expressed in the CNS are modified with chondroitin sulfate (CS) chains. We found that the expression of pleiotrophin (PTN), an inhibitory ligand of PTPRZ, peaked at P10, corresponding to the onset of myelination during mouse brain development. Advanced or delayed myelination was observed in Ptprz-deficient or Ptn-deficient mice, respectively.

We established NG2 proteoglycan-positive OPC-like cell line, OL1. Upon PTN application, PTPRZ receptor isoforms (PTPRZ-A/B) turned to punctate localization in the plasma membrane, causing inactivation of PTPRZ and oligodendrocyte differentiation. The same effect was observed by the removal of CS chains with chondroitinase ABC, indicating that the CS moiety prevents PTPRZ from spontaneously clustering, and that the positively-charged PTN induces PTPRZ clustering, potentially by neutralizing electrostatic repulsion between negatively-charged CS chains.

A recent single-cell sequencing study of primary human glioblastomas suggested a close association between high expression of PTPRZ1 and cancer stemness. PTPRZ knockdown decreased sphere-forming ability of glioblastoma cells, along with a decrease in the expression of a stem cell marker, SOX2. We recently found a cell-permeable small molecule, NAZ2329, that specifically inhibits both PTPRZ and PTPRG. This compound expectedly induced differentiation of OL1 cells and delayed tumor growth in a xenograft mouse model of C6 glioblastoma cells.

O-05 Invited

Control of Lymphocytes by Protein Phosphatase-6 and SAPS1

Author(s)

David L. Brautigan

Author's affiliation(s)

Center for Cell Signaling, Department of Microbiology, Immunology & Cancer Biology. University of Virginia, Charlottesville, VA, USA

Abstract

Protein phosphatase-6 (PP6) is one member of the PPP protein Ser/Thr phosphatase family, conserved as an essential gene among eukaryotes starting with Sit4 in yeast. PP6 is distinct from its closest PPP relatives PP2A and PP4, because of specific association with conserved regulatory subunits called SAPS (*Sit4-Associated Proteins*). SAPS1 and PP6 participate in signaling that links TNFα to NF-κB and enhance the stability of Iκ-Bε. We discovered by proteomics that SAPS1 associates with one of three ANKRD (Ankyrin-repeat domain) subunits to form trimeric PP6 holoenzymes. SAPS1 also co-immunoprecipitates with DNA-PK (DNA-dependent protein kinase) and is required for DNA-PK activation in response to irradiation. We have produced SAPS1 deficient mice that display accelerated lethality in response to whole body irradiation.

We examined lymphocyte development in these *SAPS1*$^{-/-}$ mice. There were no differences in T or B cells in the primary lymphoid organs or in the mature B or T cells in the spleen or blood of SAPS1 deficient mice compared to control mice, however there was an increase in eosinophils. Eosinophilia is often associated with type 2 immune responses and we discovered a huge increase in serum IgE in SAPS1 deficient mice, as well as an increase in CD4 T cells producing IL-4. Our hypothesis is that SAPS1/PP6 constrains CD4 T-helper 2 (Th2) cells from inappropriate Th2 differentiation. More recent results examined SAPS1/PP6-dependent changes in intracellular signaling of CD4+ T cells from SAPS1 deficient mice, based on changes in gene expression and proteomics.

O-06

A novel mechanism involving SHP-1 for selective suppression of B cells reactive to Sm/RNP, a lupus-related self-antigen

Author(s)

Chizuru Akatsu[1], Kenro Shinagawa[2], Nobutaka Numoto[2], Zhihong Liu[1], Mohammad Aslam[1], Nobutoshi Ito[2], Takeshi Tsubata[1]

Author's affiliation(s)

[1]Department of Immunology and [2]Department of Structural Biology, Medical Research Institute, Tokyo Medical and Dental University, Japan

Abstract

B cell specific SHP-1 knock-out causes lupus-like disease. CD72, a B cell inhibitory receptor, contains a C-type lectin-like domain (CTLD) in the extracellular part, and an SHP-1-recruiting ITIM in the cytoplasmic region. CD72 polymorphism is associated with lupus in both human and mice, and CD72-deficinet mice spontaneously develop lupus-like disease. Thus, SHP-1 appears to suppress development of lupus by mediating signal suppression induced by CD72. However, little is known about how CD72 regulates development of lupus. Here we showed that CD72 CTLD specifically recognizes Sm/RNP, a nuclear self-antigen corresponding to anti-Sm/RNP antibody crucial in lupus. The lupus-susceptible allelic form CD72c bound to Sm/RNP with lower affinity than lupus-resistant CD72a probably due to presence of negatively charged patch at the putative ligand binding region in CD72c revealed by crystallography and homology modeling. We further showed that CD72 negatively regulates BCR signaling and B cell activation induced by Sm/RNP but not other antigens. Finally, immunization of Sm/RNP induced production of anti-Sm/RNP antibody in CD72-difficient but not sufficient mice. These results clearly demonstrate that CD72 inhibits B cell response to Sm/RNP thereby suppressing production of pathogenic autoantibody to Sm/RNP. Thus, CD72 prevents development of lupus by bridging SHP-1 with the lupus self-antigen Sm/RNP.

O-07

Human hemato-lymphoid system development in human cytokine knock-in mice engrafted with adult donor-derived CD34+ cells

Author(s)

Yasuyuki Saito[1,2], Takashi Matozaki[1], Richard A. Flavell[3], Markus G. Manz[2]

Author's affiliation(s)

[1]Kobe University Graduate School of Medicine, Japan

[2]University Hospital and University of Zurich, Switzerland

[3]Yale University, USA

Abstract

Transplantation of human CD34+ hematopoietic stem and progenitor cells into immunodeficient mice constitutes a human hemato-lymphoid system. This model is called as a humanized mouse model and holds great promise for studying human health and diseases in vivo. In the model, interaction of human CD47 with signal regulatory protein α (SIRPα) on mouse macrophages is thought to be critical for elimination of human cells through activation of protein tyrosine phosphatase SHP-1. We previously demonstrated that expression of human SIRPA transgene in immunodeficient mice substantially improved human cell engraftment. However, the development of human innate immune cells, including myeloid cells and NK cells, particularly from adult donor-derived CD34+ cells, has not been supported in the models. Here we generated the novel Rag2-/-Il2rg-/- (RG) immune-deficient strain, named MISTRG or MSTRG, in which human homologues of M-CSF, TPO, with or without IL-3/GM-CSF were inserted in addition to the expression of human SIRPA transgene. We transplanted adult donor-derived CD34+ cells into sublethally irradiated MISTRG and MITRG mice. These mice supported higher engraftment of human CD45+ hematopoietic cells in both lymphoid and non-lymphoid organs compared with control NOD/Scid Il2rg-/- or RG SIRPA transgenic mice 10-16 weeks after transplantation. Moreover, these mice displayed the development of human myeloid cells and NK cells in various organs in vivo. Furthermore, the development of mature lymphoid cells was also supported in the model. Thus, this novel humanized mouse model might enable us to study (patho-) physiology of the human hematopoietic and immune systems that are derived from individual patients in vivo.

O-08

The roles of PTPROt in chronic lymphocytic leukemia

Author(s)

Ari Elson[1], Jean Wakim[1], Esther Arman[1], Shirly Becker-Herman[2], Matthias P. Kramer[2], Idit Shachar[2]

Author's affiliation(s)

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Abstract

The hematopoietic tyrosine phosphatase PTPROt is a putative tumor suppressor in B cell chronic lymphocytic leukemia (CLL), where its expression is reduced. In order to examine the role of PTPROt in CLL we abrogated expression of PTPROt in mice and followed progression of CLL in them. Unexpectedly, complete loss of PTPROt delayed disease detection and progression and lengthened survival, indicating that PTPROt fulfills a novel tumor-promoting role in CLL. PTPROt-deficient tumor cells exhibited reduced B-cell receptor (BCR) signaling and increased apoptosis and autophagy. Inhibition of BCR/Src family kinases (SFK) in CLL cells induced apoptosis in a dose-dependent manner, indicating these events are linked causally. Complete loss of PTPROt thus reduces SFK activity, leading to reduced BCR signaling and reduced tumor cell survival, in agreement with the weakened CLL phenotype of PTPROt-deficient mice. These findings uncover non-redundant, cell-autonomous roles for PTPROt in support of BCR signaling and survival of CLL cells. In contrast, loss of only one Ptpro allele induced the opposite phenotype - earlier detection and progression of CLL and reduced mouse survival, consistent with the putative tumor suppressing role of PTPROt. Tumor cells from mice lacking one Ptprot allele exhibited normal BCR signaling and cell death, suggesting that their more aggressive disease is associated with its earlier initiation or dissemination. PTPROt thus functions in CLL as an obligate haploinsufficient tumor suppressor, a class of gene products whose expression levels determine their functions as tumor promoters or tumor suppressors. Partial loss of PTPROt generates the strongest disease phenotype, suggesting that its intermediate expression levels in CLL in humans are selected for.

O-09

PP6 and alpha4 regulate the apoptosis of immature B cells induced by BCR crosslinking

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Abstract

BCR crosslinking induces apoptosis in immature B cells. The mechanism of apoptosis-induction was investigated with an immature B cell line WEHI-231. An apoptosis inhibiting molecule Bcl-xL was phosphorylated at Ser62 and decreased by ubiquitination upon BCR crosslinking. This reduction of Bcl-xL was enhanced by a phosphatase inhibitor oadaic acid. JNK phosphorylated the Ser62 of Bcl-xL and PP6 inhibited this phosphorylation. JNK phosphorylated Bcl-xL when co-transfected to 293T cells. A phosphatase regulator, alpha4 associates with PP2Ac and PP6c and regulates the function of phosphatases. Alpha4 associated with both JNK and PP6 and enhanced the phosphorylation of Bcl-xL induced by JNK in 293T cells. Dominant negative form of alpha4 was prepared and transfected into WEHI231 cells. WEHI-231 cells expressing dominant negative form of alpha4 showed stronger resistance to apoptosis induced by BCR crosslinking. These results suggest that PP6 and alpha4 regulate the apoptosis of immature B cells induced by BCR crosslinking.

O-10 Invited

Multiple Roles for eIF2alpha Phosphatases in the Unfolded Stress Response

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Abstract

Transient repression of mRNA translation is a universal response of all eukaryotic cells to perturbations in their metabolic or growth environment. This "integrated stress response" temporarily slows general protein synthesis and allows cells to redirect their efforts towards translating mRNAs encoding stress response proteins that are required to overcome the "stress". Failure to execute this complex translational and transcriptional response triggers cell death and likely contributes to a wide variety of chronic human diseases. The repression of global mRNA translation is mediated via the phosphorylation of a single serine-51 on the eukaryotic initiation factor, eIF2α. Subsequently, the transcriptional and translational upregulation of GADD34 (Growth Arrest- and DNA Damage-induced transcript 34), a regulator of protein phosphatase-1 (PP1), assembles an eIF2α phosphatase that restores general protein synthesis. Cells also express another eIF2α phosphatase, containing the regulatory subunit, CReP (constitutive repressor of eIF2α phosphorylation). This presentation will review our current understanding of the distinct roles of GADD34- and CReP-containing eIF2α phosphatases in the control of transcritome and translatome in unstressed cells and in stressed cells experiencing the unfolded protein response. The data highlight some of the challenges and opportunities in therapeutic targeting of eIF2α phosphatases with small molecules to treat diabetes, cancer and neurodegenerative disorders.

O-11

Characterization of a Gtr/Rag-independent and glutamine-responsive TORC1 activation mechanism in yeast

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Abstract

Evolutionary-conserved TOR complex 1 (TORC1) responds to nutrients, especially amino acids, to activate cell growth. In yeast Saccharomyces cerevisiae, various nitrogen sources activate TORC1 with different efficiencies although the mechanism remains elusive. It was reported that leucine activates TORC1 via the heterodimer of the small GTPases Gtr1 and Gtr2, the orthologs of the mammalian Rag GTPases. More recently, an alternative Gtr-independent mechanism that may respond to glutamine was reported to activate TORC1. The molecular detail of the alternative mechanism is unknown although PI(3)P-binding FYVE domain-containing Pib2 is suggested to participate. In studying the nutrient-responsive TORC1 activation mechanism, the lack of an in vitro assay hinders associating particular nutrient compounds with the TORC1 activation status, as nutrients are continuously metabolized and the metabolites are interconverted in vivo. We developed a TORC1 kinase assay that reproduces, to our knowledge for the first time, the nutrient-responsive TORC1 activation in vitro. Among the amino acids tested, only glutamine, and perhaps cysteine, showed ability to activate TORC1 in this assay. The glutamine-responsiveness in the assay system was independent of the Gtr-mediated mechanism, and dependent on the Vps34 PI3 kinase and PI(3)P-binding FYVE domain-containing Pib2. These results suggest that our in vitro TORC1 kinase assay recapitulates the Gtr-independent and glutamine-responsive TORC1 activation mechanism that involves Pib2 recruited onto the vacuolar membrane via PI(3)P produced by Vps34.

O-12

Mitochondrial stress sensing and cellular response

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Abstract

To respond properly to cellular stress, it is crucial that all organelles within a cell cooperatively function. Among them, mitochondria have been regarded as central organelles in cellular stress response because they, not only act as ATP-generating powerhouses to support cell survival, but also are involved in apoptosis execution. More recently, importance of mitochondria as central regulators of metabolism, such as carbohydrate, lipid and amino acid metabolism, has been re-recognized. Thus, mitochondria appear to play important roles in cellular stress response through the "fine-tuning" of various cellular functions beyond their roles in the alternative decision between death and survival. We have been focusing on phosphoglycerate mutase family member 5 (PGAM5), which we believe senses mitochondrial dysfunction and mediates appropriate signals to other organelles and molecules to regulate cellular response to mitochondrial stress. PGAM5 is a new type of protein phosphatase that exists in the inner mitochondrial membrane through its N-terminal transmembrane (TM) domain and is cleaved within the TM domain in response to the loss of mitochondrial membrane potential. We recently found the conditions under which cleaved PGAM5 was released from mitochondria and showed that the released PGAM5 distributed in the cytosol and nucleus. Interestingly, cytosolic PGAM5 regulated stabilization of microtubules, and nuclear PGAM5 interacted with and regulated SRm160/SRRM1, a nuclear protein that regulates processing and transport of mRNA. These results suggest the role of PGAM5 as a signaling intermediate between mitochondrial stress and cellular response to it.

O-13

Neural specific RNA-binding proteins grasp the translation regulatory networks

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Abstract

Vital roles of RNA-binding proteins (RBPs) have emerged for posttranscriptional gene regulation in neurons. The neuronal Hu protein family represents such RBPs and targets several mRNAs containing adenine/uridine-rich elements. Nevertheless, little is known about the mode of involvement of Hu proteins in neuronal differentiation. HuD is one member of the neuronal Hu family of proteins and promotes neuronal differentiation.

We have previously shown that HuD enhances cap-dependent translation in a eIF4A- and poly(A)-dependent manner1. We have also shown that the stimulatory effect of HuD on neurite outgrowth in PC12 cells depends on the interaction of HuD with eIF4A- and poly(A)[1]. Recently, we show that miRISC promotes the release of both eIF4AI and eIF4AII from the target mRNA before dissociation of eIF4E and eIF4G and demonstrate that this is independent of deadenylation. Strikingly, both, the miRISC-induced release of eIF4A I and II from target mRNA and the miRISC-induced inhibition of cap-dependent translation can be counteracted by the RNA binding protein HuD via a direct interaction of HuD with eIF4A[2]. We further explored the underlying molecular interactions and found that RNA-bound HuD directly and specifically interacts with phosphorylated Akt1, and this interaction is required for HuD-induced neurite outgrowth in PC12 cells[3]. Here we show that phosphorylated Akt1 is recruited in a HuD- dependent manner into the cap-binding complex. We also observe that this leads to increased levels of phosphorylated eIF4B within the cap-binding complex suggesting that eIF4B phosphorylation is key for the control of translation via HuD. To address this hypothesis, we are currently performing in vitro translation assays using HeLa cell extracts that are depleted of eIF4B. The status of these experiments will be discussed.

[1]Fujiwara et al., Mol Cell 36, 1007-1017 (2009)

[2]Fujiwara et al., Mol Cell 56, 79-89 (2014)

[3]Fujiwara et al., Nucleic Acids Res. 40, 1944-1953 (2011)

O-14

Two bistable switches of cell division generated by kinase/phosphatase antagonism

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Abstract

The abrupt and irreversible transition from interphase to M phase is essential to separate DNA replication from chromosome segregation. This transition requires the switch-like phosphorylation of hundreds of proteins by cyclin-dependent kinase 1 (Cdk1):cyclin B (CycB) complex. Previous studies have ascribed these switch-like phosphorylations to the auto-activation of Cdk1:CycB through the removal of inhibitory phosphorylations on Cdk1-Tyr15. Here, we surprisingly find that Cdk1 auto-activation is dispensable for irreversible, switch-like mitotic entry due to a second mechanism, whereby Cdk1:CycB inhibits its counteracting phosphatase PP2A:B55. We show that the PP2A:B55-inhibiting Greatwall(Gwl)-Endosulfine(ENSA) pathway is both necessary and sufficient for full, switch-like phosphorylations of mitotic substrates. Using purified components of the Gwl-ENSA pathway in a reconstituted system, we find a sharp Cdk1 threshold for phosphorylation of a luminescent mitotic substrate. The Cdk1 threshold to induce mitotic phosphorylation is distinctly higher than the Cdk1 threshold required to maintain these phosphorylations - evidence for bistability. A combination of mathematical modeling and synthetic biological approaches show that the bistable behavior of the Gwl-ENSA pathway emerges from its mutual antagonism with PP2A:B55. Our results demonstrate that two complementary, functionally-redundant bistable mechanisms provide a robust solution for irreversible and switch-like mitotic entry.

O-15

Molecular mechanisms for the upregulation of Cav3.2 T-type calcium channels involved in neuropathic pain

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Abstract

Chemotherapy-induced peripheral neuropathy is one of the most common neurologic complications experienced by cancer patients receiving anti-cancer drugs. We have reported that Cav3.2 contributes to neuropathic pain caused by repetitive administration of paclitaxcel, an anti-cancer drug, and L5 spinal nerve cutting (L5SNC) in rats. In the present study, we analyzed the molecular mechanisms by which Cav3.2 participates in the neuropathic pain after L5SNC in rats. The mechanical nociceptive threshold significantly decreased on days 6-9 (early phase) and reached a bottom level on days 14-15 (persistent phase) after L5SNC. In both early and persistent phases, protein levels of Cav3.2 and early growth response 1 (Egr-1), known to transcriptionally promote Cav3.2 expression, were upregulated in the dorsal root ganglion (DRG) at an L4 level. On the other hand, ubiquitin specific protease 5 (USP5), known to suppress ubiquitination and proteasomal degradation of Cav3.2, was upregulated in the persistent, but not early, phases. Knockdown of Egr-1, but not USP5, in DRG suppressed the hyperalgesia and Cav3.2 upregulation in the early phase. On the other hand, knockdown of either Egr-1 or USP5 suppressed the hyperalgesia and Cav3.2 upregulation in the persistent phase. These results suggest that L5SNC-indued neuropathy involves the Egr-1-dependent transcriptional upregulation and/or USP5-mediated protection from proteasomal degradation of Cav3.2 in the sensory neurons at an L4 level. Thus, Cav3.2 plays a critical role in the neuropathic pain, and is considered a promising therapeutic target for neuropathic pain, possibly including chemotherapy-induced peripheral neuropathy.

O-16

Targeting HMGB1 and its downstream molecules for treatment of oxaliplatin-induced peripheral neuropathy

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Abstract

High mobility group box 1 (HMGB1), a nuclear protein, is passively released from necrotic cells or actively secreted by macrophages, and promotes inflammation or pain signaling by targeting multiple molecules including RAGE, TLR4 and CXCR4. We have demonstrated the involvement of macrophage-derived HMGB1 in the neuropathic pain induced by paclitaxel and vincristine, anti-cancer drugs. Here we asked if HMGB1 and its downstream molecules participate in the oxaliplatin-induced neuropathy. Oxaliplatin treatment decreased the mechanical nociceptive threshold, as assessed by von Frey test, an effect prevented by pretreatment with an anti-HMGB1 neutralizing antibody or recombinant human soluble thrombomodulin capable of inactivating HMGB1. Antagonists of RAGE, TLR4 or CXCR4 also suppressed the oxaliplatin-induced allodynia. On the other hand, liposomal clodronate that depletes macrophages, minocycline, an inhibitor of macrophage/microglia, or ethyl pyruvate, known to inhibit the release of HMGB1 from macrophages, did not affect the oxaliplatin-induced allodynia. Immunostaining showed no increase in the number of macrophages in the sciatic nerve after oxaliplatin treatment. In macrophage-like RAW264.7 cells, paclitaxel or vincristine, but not oxaliplatin, caused the release of HMGB. These data strongly suggest that HMGB1 derived from cells other than macrophages contributes to the oxaliplatin-induced neuropathic pain through the activation of RAGE, TLR4 or CXCR4. Apart from some distinct mechanisms with different anti-cancer drugs, we propose that HMGB1 and its downstream molecules could be therapeutic targets for the treatment of chemotherapy-induced peripheral neuropathy.

O-17

Sulfonic acid formation of the active-site cysteine directs ubiquitin proteasome system-mediated degradation of myocardial protein tyrosine phosphatases

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Abstract

Protein oxidation is a natural consequence of aerobic metabolism in cells. However, oxidative modification of amino acids may lead to loss of protein function. Such unwanted protein should be removed through intrinsic machinery, presumably mediated by degradation pathways which act to maintain the quality control of cellular proteins. One such removable protein might be induced by oxidation on cysteine (Cys) residues with a low pKa characteristic. However, to date it remains unclear whether irreversible Cys oxidation, if happens under pathophysiological conditions, can drive degradation of the targeted protein. In the present study, we have demonstrated that protein tyrosine phosphatases (PTPs) including PTP1B are constitutively oxidized to the sulfonic acid state (Cys-SO3H) in normal cardiomyocytes. We examined whether such highly oxidized PTPs are undergoing ubiquitin proteasome system (UPS)-mediated degradation. Interestingly, in the presence of proteasome inhibitors, we showed that proteolysis of PTP1B was inhibited. Irreversible oxidation of the active-site Cys215 was essential for degradation of PTP1B through the UPS pathway in cardiomyocytes. We further demonstrated the underlying mechanism of stepwise Cys215 oxidation followed by ubiquitination of PTP1B using an in vitro assay. Our findings thus provide new insights into a process through which Cys oxidation directs myocardial protein turnover via UPS-mediated degradation. Identification of a specific E3 ligase that targets sulfonic acid-modified PTP1B for ubiquitination is now under study.

O-18

Overexpression of HIF-1alpha is involved with melphalan-resistance in multiple myeloma cells

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Abstract

<Purpose> The acquisition of anti-cancer drug resistance is a major limitation of chemotherapy for multiple myeloma (MM) and it is thus important to identify the mechanisms by which MM cells develop such drug resistance. Our previous study showed that melphalan-resistance involves the overexpression of MDR1 and Survivin, and decreasing Bim expression in melphalan-resistant RPMI8226/L-PAM cells. However, the underlying mechanism of melphalan-resistance remains unclear. In this study, we investigated the mechanism of melphalan-resistance in RPMI8226/L-PAM cells.

<Methods> Cell viability was assessed by the trypan blue dye method. Signal molecules were determined by western blots.

<Results> We found that RPMI8226/L-PAM cells exhibit increased levels of HIF-1alpha expression between RPMI8226/L-PAM cells and their melphalan-susceptible counterparts. In addition, the inhibition of HIF-1alpha by inhibitors reversed the drug-resistance of RPMI8226/L-PAM cells via the suppressed the MDR1 and Survivin expression, and enhanced expression of Bim.

<Discussion> These results indicate that enhanced the expression of MDR1 and Survivin, and decreasing Bim expression via the HIF-1alpha plays a critical role in melphalan resistance in RPMI8226/L-PAM cells. Our findings suggest that HIF-1alpha inhibitors are potentially useful as anti-MDR agents for the treatment of melphalan-resistant MM.

O-19

FGFR gene alterations in lung squamous cell carcinoma are potential targets for the multikinase inhibitor nintedanib

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Abstract

Fibroblast growth factor receptor (FGFR) gene alterations are relatively frequent in lung squamous cell carcinoma (LSCC) and are a potential targets for therapy with FGFR inhibitors. However, little is known regarding the clinicopathologic features associated with FGFR alterations. The angiokinase inhibitor nintedanib has shown promising activity in clinical trials for non–small cell lung cancer. We have now applied next-generation sequencing (NGS) to characterize FGFR alterations in LSCC patients as well as examined the antitumor activity of nintedanib in LSCC cell lines positive for FGFR1 copy number gain (CNG). The effects of nintedanib on the proliferation of and FGFR signaling in LSCC cell lines were examined in vitro, and its effects on tumor formation were examined in vivo. A total of 75 clinical LSCC specimens were screened for FGFR alterations by NGS. Nintedanib inhibited the proliferation of FGFR1 CNG positive LSCC cell lines in association with attenuation of the FGFR1-ERK signaling pathway in vitro and in vivo. FGFR1 CNG (10.7%), FGFR1 mutation (2.7%), FGFR2 mutation (2.7%), FGFR4 mutation (5.3%), and FGFR3 fusion (1.3%) were detected in LSCC specimens by NGS. Clinicopathologic features did not differ between LSCC patients positive or negative for FGFR alterations. However, among the 36 patients with disease recurrence after surgery, prognosis was significantly worse for those harboring FGFR alterations. Screening for FGFR alterations by NGS warrants further study as a means to identify patients with LSCC recurrence after surgery who might benefit from nintedanib therapy.

O-20

Antiepileptic drug use is associated with a decreased risk of cancer: Data mining of spontaneous reporting and claims

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Abstract

<Background> Voltage-gated sodium channels (VGSCs) are drug targets for the treatment of epilepsy. Recently, a decreased risk of cancer associated with VGSC-inhibiting drugs has become a research focus of much interest. The purpose of this study was to test the hypothesis that the use of sodium channel-blocking AEDs are inversely associated with cancer, using different methodologies, algorithms, and databases.

<Material and methods> A total of 65,146,507 drug-reaction pairs were downloaded from the US Food and Drug Administration Adverse Event Reporting System. The reporting odds ratio (ROR) and information component (IC) were used to detect an inverse association between AEDs and cancer. Upper limits of the 95% confidence interval (CI) of < 1 and < 0 for the ROR and IC, respectively, signified inverse associations. Furthermore, using a claims database, which contains 1.2 million insured persons, an event sequence symmetry analysis was performed to identify an inverse association between AEDs and cancer. The upper limit of the 95% CI of adjusted sequence ratio < 1 signified an inverse association.

<Results and Conclusions> Significant inverse associations were found between AEDs and various cancers, including colorectal cancer, lung cancer, gastric cancer, and hematological malignancies. Positive associations between AEDs and cancer were not found. The present study used multiple approaches involving different methodologies, algorithms, and databases to clearly demonstrate an inverse association between sodium channel-blocking AED use and the risk of cancer. These findings suggest that sodium channel-blocking AEDs are potential anticancer drug candidates.

O-21 Invited

Roles of protein phosphatases in cell polarity control

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Abstract

A molecular understanding of how cells define their own morphology in a spatiotemporal manner is one of the fundamental issues in biology and medical science. Rod shaped fission yeast *Schizosaccharomyces pombe* cells are highly polarised; cells grow only from cell tips with constant width. Interestingly, during G2 phase of the cell cycle, cells undergo a drastic polarity transition from monopolar to bipolar growth. This regulatory point is referred to as NETO, New-End-Take-Off.

We have identified Calcineurin (PP2B), and Casein kinase 1γ (Cki3) as critical determinants of NETO timing. Upon activation of the DNA replication checkpoint, a condition to delay NETO, *cki3*- or calcineurin mutant cells commit NETO prematurely. Intriguingly, *cki3* cells exhibit premature NETO even under unperturbed conditions. By contrast, PP1 is required for the execution of NETO. Subsequent analyses indicate that the kelch-repeat containing polarity factor Tea1 and the microtubule-associated protein Tip1 (CLIP170) are downstream factors, whose phosphorylation and dephosphorylation play a decisive role in NETO timing. In this talk, I will present our recent results in growth polarity control in fission yeast and discuss the general significance of these findings.

Kume K, Koyano T, Kanai M, Toda T, Hirata D (2011) Calcineurin ensures a link between the DNA replication checkpoint and microtubule-dependent polarized growth. Nat Cell Biol 13: 234-242.

Koyano T, Konishi M, Martin SG, Ohya Y, Hirata D, Toda T, Kume K (2015) Casein kinase 1γ ensures monopolar growth polarity under incomplete DNA replication downstream of Cds1 and calcineurin in fission yeast. Mol Cell Biol 35: 1533-1542.

Koyano T, Barnouin K, Snijders AP, Kume K, Hirata D, Toda T (2015) Casein kinase 1γ acts as a molecular switch for cell polarization through phosphorylation of the polarity factor Tea1 in fission yeast. Genes Cells 20: 1046–1058.

O-22 Invited

Identifying the human calcineurin signaling network

Author(s)

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Abstract

Systems-level analyses of phosphorylation-based signaling networks has transformed our understanding of kinase function, but knowledge of phosphatase signaling has lagged behind, primarily because global approaches to identify phosphatase substrates are lacking. Calcineurin, the conserved Ca^{2+}/calmodulin-dependent protein phosphatase and target of immunosuppressants, FK506 and Cyclosporin A, is ubiquitously expressed, and critically regulates Ca^{2+}-dependent processes in the immune system, heart, and brain. However, in the literature only ~30 human substrates are currently attributed to calcineurin.

We are using novel experimental and computational approaches to identify human proteins that contain "PxIxIT" and "LxVP" sequences. Calcineurin specifically recognizes its targets by binding to these short linear motifs (SLiMs), which occur preferentially in intrinsically disordered domains, and are challenging to identify due to sequence degeneracy and low affinity for calcineurin. Proteome peptide Phage Display (ProP-PD) was used to identify calcineurin-binding sequences of the PxIxIT and LxVP types experimentally from predicted disordered regions of the human proteome. These sequences directly identify novel and known calcineurin targets, and provide a large set of calcineurin-binding peptides for robust computational prediction of novel PxIxIT and LxVP-containing proteins in the human proteome. We also characterized the amino acid preference at each position of the PxIxIT and LxVP motifs using a novel technology that employs microfluidically-produced, spectraly-encoded beads, on which peptides are synthesized. Beads containing 96 distinct peptides, each with a unique spectral code, were incubated with calcineurin in a single volume and imaged to determine the amount of calcineurin that binds to each peptide.

Novel calcineurin-binding sequences that are identified either experimentally or computationally are validated using a high throughput calcineurin-binding assay, and their parent proteins tested for interaction with calcineurin in HEK-293 cells. These studies are identifying many new candidate substrates for calcineurin that include ion channels, kinases, transcription factors and receptors, and reveal new points of cross-talk between calcineurin and other signaling pathways in human cells. Furthermore, these approaches can be broadly applied to systematic characterization of any SLiM-based signaling network.

O-23 Invited

PTPRT is a tumor suppressor that regulates intestinal stem cell proliferation

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Abstract

Protein tyrosine phosphatase receptor-type T (PTPRT) is frequently mutated in a variety of human cancers including colon cancer. We demonstrated that PRPRT normally functions as a tumor suppressor using three different colon tumor models: (1) PTPRT knockout mice are highly susceptible to carcinogen azoxymethane-induced colon tumor; (2) PTPRT knockout increase incidence of AOM-DSS-induced colon tumors; and (3) PTPRT knockout increase the size of colon tumors in the Apc^{min} mouse genetic background. Recently, intestinal stem cells marked by Lgr5 are shown to be potential cell original of colon cancers. Interestingly, we found that Lgr5+ intestinal stem cells in PTPRT knockout mice are more proliferative than in the wild-type (WT) mice. Using a phospho-proteomics approach, we identified and validated STAT3 as a direct substrate of PTPRT. Moreover, phospho-STAT3 is up-regulated in the intestinal crypts of PTPRT knockout mice compare to WT mice. These studies suggest that PTPRT-regulated STAT3 signaling pathway that plays important roles in intestinal homeostasis and colorectal tumorigenesis.

O-24 Invited

Epigenetic Regulation by Notch Signaling in Glioma

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Abstract

In the neural stem cell, the Notch signaling pathway plays a dominant role in inhibiting differentiation through the activities of its downstream effectors, such as Hairy and enhancer of split 1/5 (Hes1/5), which repress the implementation of neurogenic programs. In the context of glioma tumorigenesis, Notch signaling has been shown to promote glioma stem cell (GSC) self-renewal and to suppress GSC differentiation. However, the mechanism by which Notch signaling and its downstream effectors maintains the stemness properties of GSCs through the function of a certain set of genes, such as SOX2, MYC and Nestin, remains unresolved. Here, we found that a specific Notch-regulated long non-coding RNA, TUG1, the expression of which is regulated by the Notch signaling pathway, was highly expressed in GSCs. TUG1 coordinately promotes self-renewal by sponging miR-145 in the cytoplasm and recruiting polycomb to repress differentiation genes by locus-specific methylation of histone H3K27 via YY1 binding activity in the nucleus. Furthermore, we developed new antisense oligonucleotides targeting TUG1 coupled with a potent drug delivery system, which can be used intravenously to provide efficient and selective delivery to glioma cells at sufficient concentrations to acquire anti-tumor effects. Our observations indicate that Notch-directed TUG1 is an effective epigenetic modulator that regulates the cancer stem cell population.

O-25

Modest attenuation of DNA damage repair delays therapy-related cancer in mouse model

Author(s)

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Abstract

Therapy-related myeloid neoplasms (t-MNs) including therapy-related myelodysplastic syndrome (t-MDS) and acute myeloid leukemia (t-AML) are serious complications for patients who received the treatment with ionizing radiation (IR) or DNA damaging agents. At present there is no active medical intervention available to reduce the risk of t-MNs. In addition, mouse models showing delay of t-MNs have not been described. We found that inactivation of the APLF-mediated non-homologous enjoining (NHEJ) pathway in mice moderately attenuated DNA damage repair and, unexpectedly, hampered t-MNs after exposure to IR. Systemic treatment of mice with IR showed increased p53-dependent cell death, fewer chromosomal translocations, and a delay in malignancy-induced mortality. Simultaneous inactivation of p53 abrogated IR-induced cell death and the benefit of impaired DNA repair on mortality. We therefore conclude that the classical NHEJ pathway plays a crucial role in producing genomic abnormalities when a number of DSBs are induced by IR. Manipulation of the efficiency in DNA damage repair may have potential in intervening the development of t-MNs.

O-26 Invited

A Surprising Role for PTP1B in Breast Cancer

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Abstract

Deletion of *Ptpn1*, which encodes Protein-Tyrosine Phosphatase-1B (PTP1B), delays the onset of *Her2/Neu*-driven breast cancers in mice, but the underlying mechanism(s) has been controversial. The role of PTP1B in *HER2*+ human breast cancer also is unresolved. We found that, unexpectedly, PTP1B protects *HER2*+ breast cancer (BC) cell lines and tumors from hypoxia-induced death. Although there was no consistent effect of *PTPN1* depletion or PTP1B inhibition on growth factor signaling or proliferation of *HER2*+ BC cells *in vitro*, PTP1B-deficient *HER2*+ xenografts showed increased hypoxia, necrosis and impaired growth. *PTPN1*-knockdown (1B-KD) also sensitized *HER2*+ BC lines to hypoxia-induced death *in vitro*. Remarkably, all known hypoxia response pathways appear normal or increased in PTP1B-deficient cells. Instead, biochemical and genetic analysis reveal a novel pathway for regulating tumor cell response to hypoxia, and a new function for PTP1B, acting via the Moyamoya disease gene *RNF213*, in the control of α-KG-dependent dioxygenases in *HER2*+ BC cells. Control of α-KG-dependent dioxygenase activity by this novel PTP1B/RNF213 hypoxia-regulatory pathway appears to be critical for the survival of breast cancer and possibly other malignant cells in the tumor microenvironment.

O-27

Applying Kinase-Phosphatase Interplay in Ca^{2+} signaling for Cancer Therapeutics: A pas de deux.

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Abstract

Cancer is caused by defects in the mechanisms underlying cell proliferation and cell death. Calcium signaling is central to both phenomena, serving as a major determinant for cell fate. Especially, calcineurin and Protein kinase C (PKC) are highly conserved key enzymes in calcium signaling mediating both cell proliferation and cell death. Calcineurin is a Ca^{2+}- and calmodulin-dependent protein phosphatase that regulates various physiological events such as cell growth, motility, secretion, and gene expression.

We have exploited the synthetic lethal genetic interactions utilizing the calcineurin inhibitor FK506, which lead to the identification of more than 100 genes that require calcineurin for growth and thus revealed physiological involvement of calcineurin in membrane trafficking, cell cycle and lipid signaling. Furthermore, the clear antagonism between calcineurin and PKC/MAPK signaling for growth has been demonstrated and thus utilized to identify numerous factors that regulate PKC-mediated MAPK signaling or substrates phosphorylated by MAPK, including Rho GTPases, SH3 adaptor protein, Hsp90, Ca^{2+} channels transcription factors and mRNA-binding proteins.

We have also applied the kinase-phosphatase antagonism to search for compounds that target calcium signaling and successfully identified several compounds as "Calcium signaling modulator". Notably, some of the compounds named "Sugiura Kagobutsu", including "Acremomannolipin A" and ACA derivatives displayed potent anti-tumorigenic properties with distinct mechanisms action that target calcium signaling. Notably, PKC-related enzyme or PKN is highly similar to Pcks in fission yeast both in structure and Rho-dependence. Here, we demonstrated the *in vivo* first evidence that PKN3 is involved in angiogenesis and metastasis in mice. Thus, the intimate kinase-phosphatase interplay is a strong indication that calcineurin and PKC/MAPK signaling share physiological functions in calcium signaling and that a delicate imbalance in this signaling regulation leads to aberrant cell proliferation and cancer.

O-28 Invited

Structural basis for PTPN3-p38gamma complex involved in colon cancer progression

Author(s)

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Abstract

The Ras signaling cascade acts as a key driver in human colon cancer progression. Among the modules in this pathway, p38gamma (MAPK12) and its specific phosphatase PTPN3 (PTPH1) are critical regulators responsible for Ras oncogenic activity. However, the molecular basis for their interaction is completely unknown. Here we report the unique architecture of the PTPN3-p38gamma complex by employing an advanced hybrid method integrating X-ray crystallography, small-angle X-ray scattering (SAXS) and chemical cross-linking/mass spectrometry (CX-MS). Our crystal structure of PTPN3 in complex with the p38gamma phosphopeptide presented a unique feature of the E-loop that defines the substrate specificity of PTPN3 towards fully activated p38gamma. The low-resolution structure demonstrated the formation of an active-state or a resting-state complex of PTPN3-p38gamma. We showed a regulatory function of PTPN3's PDZ domain, which stabilizes the active-state complex through interaction with the PDZ-binding motif of p38gamma. Using SAXS and CX-MS approaches, we found that binding of the PDZ domain to the PDZ-binding motif lifts the atypical auto-inhibitory constraint of PTPN3, enabling efficient tyrosine dephosphorylation of p38gamma to occur. Our findings emphasize the potential of structural approach for PTPN3-p38gamma complex that may deliver new therapeutic strategies against Ras-mediated oncogenesis in colon cancer.

O-29 Invited

Illuminating Cellular Phosphorylation Signaling by Kinase-Centric Phosphoproteomics

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Abstract

Rapid progress has been made in mass spectrometry-based phosphoproteomics with specific enrichment methods of phosphopeptides, and the current technology allows to identify thousands of phosphopeptides from complex biological samples. Further improvement will be achieved by increasing the resolution both in m/z and retention time in LCMS separation. We have employed meter-long monolithic silica C18 columns to improve LC separation, identifying more than 10,000 unique phosphopeptides from 0.1 mg of HeLa lysates. Together with 10-plex tandem mass tags, this approach allows high throughput quantitative phosphoproteome analysis of human samples for routine use. For most of the phosphosites, however, their biological functions have not been well characterized. So, kinase-centric approaches rather than phosphoproteomics approaches would be preferable to link the MS results to cellular signaling network. Here I would like to present a novel approach for phosphorylation network analysis by MS-based phosphoproteomics through kinome profiling.

In order to predict the sequence preference of phosphorylation by kinase, we experimentally obtained the kinase-substrate relationship by MS-based phosphoproteomics. Using position weight matrix and fold increase, we generated a new measure to predict the kinase substrate, named phosphorylation sequence preference (PSP) score. The PSP score allows us to convert the phosphoproteome profiles to pathway enrichment information through kinome profiles.

Our kinase-centric approaches based on phosphoproteomics have been successfully applied to quantitative studies in cellular phosphorylation networks controlled by kinases and phosphatases.

O-30

Identification of binding molecules for Ser/Thr phosphatases using structurally rigid libraries

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Abstract

Protein phosphorylation is the most widespread type of post-translational modification and disordered regulation of protein phosphorylation often causes serious diseases such as cancer. It is also known that the distribution of phosphorylated Ser/Thr and phosphorylated Tyr are around 96% and 4% respectively. These facts support the importance of identification of specific substrates and inhibitors for individual Ser/Thr phosphatases. These binding molecules give us insight for development of drugs and novel signal transduction by the Ser/Thr phosphatases. Here in order to identify potent and specific binding molecules for Ser/Thr phosphatases, we designed and prepared the structurally rigid libraries derived from cyclic peptide displayed phage and G-quadruplex aptamer. Cyclic peptide phage library was used for identification of substrate motif for FCP/SCP type phosphatase. After panning of the library with phosphate mimic molecules demonstrated that unique sequences were identified as FCP/SCP substrate motif and several clones contained similar sequence with the known substrate CTD of RNA polymerase II. We also identified specific inhibitors for PPM type phosphatase PPM1D from G-quadruplex DNA aptamer library. The most potent aptamer showed inhibitory activity not only in vitro but also in the cells. These data suggested that our designed structurally rigid libraries are useful for identification of substrates and inhibitors for Ser/Thr phosphatases.

O-31 Invited

Drugging the undruggable: exploiting PTP1B as a therapeutic target

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Abstract

The protein tyrosine phosphatases (PTPs) are important regulators of signal transduction. These cysteine-dependent phosphatases hydrolyze phosphoester bonds in proteins and non-protein substrates. Overall, the objective of the lab is to characterize the structure, regulation and function of PTPs, to define their role in critical tyrosine phosphorylation-dependent signaling events under normal and pathophysiological conditions, and to identify novel therapeutic targets and strategies based upon the PTPs themselves, or from components of the signaling pathways they regulate.

PTP1B plays a well-established role in down-regulating insulin and leptin signaling and is a validated therapeutic target for diabetes and obesity. Furthermore, PTP1B is a positive regulator of signaling by the HER2 oncoprotein tyrosine kinase, such that inhibition of the phosphatase also abrogates breast tumorigenesis and metastasis. Several potent, specific, reversible small molecule inhibitors of PTP1B have been developed, but they target the conserved, highly charged active site and exhibit poor oral bioavailability, which limits their drug development potential. This led industry to dismiss the members of the PTP family as "undruggable".

In contrast, I will illustrate how a detailed understanding of the structure, regulation and function of PTP1B, which has been generated in an academic setting, has revealed new approaches to the development of small molecule drug candidates that target this enzyme. For example, we are exploiting a physiological mechanism of regulation of PTP function by reversible oxidation and inactivation that is induced following stimulation of cells, such as with insulin or leptin. Our data illustrate that stabilization of the oxidized, inactive form of PTP1B with appropriate therapeutic molecules may offer a novel paradigm for phosphatase drug development. Furthermore, we have identified small molecule inhibitors that target a unique allosteric site in the regulatory, C-terminal segment of PTP1B. In addition to stimulating insulin signaling, we have demonstrated that such allosteric PTP1B inhibitors antagonize HER2 function, including abrogation of tumor metastasis in the NDL2 transgenic mouse model of HER2-positive breast cancer. This new approach to cancer therapy is currently the subject of a clinical trial. Finally, the application of such inhibitors is revealing new functions of PTP1B and suggesting new indications in which inhibition of PTP1B may be of therapeutic benefit, such as for treatment of the autism spectrum disorder Rett syndrome.

O-32

Skb5, an SH3 adaptor protein, regulates PKC/MAPK signaling via spatial regulation of MAPKKK

Author(s)

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Abstract

The MAPK cascade is a highly conserved signaling module composed of MAPK/MAPKK/MAPKKK. MAPKKK Mkh1 is an initiating kinase in Pmk1 MAPK signaling, which regulates cell integrity in fission yeast. Our genetic screen for regulators of Pmk1 signaling identified Skb5 (Shk1 kinase binding protein 5), an SH3 domain-containing adaptor protein. Here, we showed that Skb5 serves as an inhibitor of Pmk1 MAPK signaling activation by downregulating Mkh1 localization to cell tips via its interaction with the SH3 domain. Consistently, the Skb5YF2A mutant protein, with impaired Mkh1 binding, did not effect on Mkh1 localization, even when Skb5YF2A was overproduced. Intriguingly, Skb5 needs Mkh1 to localize to the cell tips as Mkh1 deletion and disruption of Mkh1 binding impairs Skb5 localization. Deletion of Pck2, an upstream activator of Mkh1, impaired the cell tip localization of Mkh1 and Skb5 as well as Mkh1/Skb5 interaction. Interestingly, both Pck2 and Mkh1 localized to the cell tips at the G1/S phase, which coincided with Pmk1 MAPK activation. Altogether, Mkh1 localization to cell tips is important for transmitting upstream signaling to Pmk1 and Skb5 spatially regulates this process.

O-33

CCR4 is involved in Th17 cell expansion and Th17-mediated induction of antitumor CTLs in mice bearing intradermal B16 melanoma

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Abstract

CCR4 is a major chemokine receptor expressed by Treg cells and Th17 cells. While Treg cells are known to suppress antitumor immunity, Th17 cells have recently been shown to enhance antitumor immunity through the induction of CD8+ cytotoxic T lymphocytes (CTLs). Here we examined the effect of CCR4 in antitumor immunity against murine melanoma. CCR4-deficient mice displayed enhanced tumor growth upon intradermal inoculation of B16-F10 melanoma cells. In CCR4-deficient mice, CTLs were decreased in tumor sites, while CTLs and Th17 cells were decreased in regional lymph nodes. In wild-type mice, CD4+IL-17A+ cells, which were identified as CCR4+CD44+ memory Th17 cells, were found to be clustered around dendritic cells expressing MDC/CCL22, a ligand for CCR4, in regional lymph nodes. Furthermore, these DC-Th17 cell interactions were reduced in CCR4-deficient mice. Compound 22, a CCR4 antagonist, also enhanced tumor growth and decreased Th17 cells in regional lymph nodes in tumor-bearing mice treated with Dacarbazine. In contrast, CCR6 deficiency did not affect the tumor growth and the numbers of Th17 cells in regional lymph nodes. These findings indicate that CCR4 is critically involved in regional lymph node DC-Th17 cell interactions that are necessary for Th17 expansion and induction of Th17 cell-mediated CTLs in mice bearing B16 melanoma. Thus, the infusion of CCR4 antagonists aiming at inhibition of Treg cell recruitment via CCR4 may not always enhance antitumor immunity but may rather promote tumor growth.

O-34

Anticancer-drug screening utilizing fission yeast genetics identified Acremomannolipin A, a Calcium signalling modulator with anti-tumor activity

Author(s)

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Abstract

Mitogen-activated protein kinase (MAPK) signaling and PI3K/AKT signaling pathways are highly conserved signaling pathways that regulate cell proliferation and cancer growth. Aberrant regulation of the MAPK and AKT signaling pathways contributes to cancer development and therefore, development of new therapeutics preventing tumor growth by targeting the ERK/AKT signaling is urgently needed. We have identified regulators of the Pmk1 MAPK signaling pathway by using the fission yeast model system based on our previous findings that Pmk1 MAPK signaling and calcineurin antagonistically regulate Cl^- homesotasis. These include farnesyltransferase (FTase), protein kinase C (PKC), MAPK kinase and MAPK phosphatase (Mol. Cell. Biol. 1996; EMBO J. 1998; Nature 1999; Mol. Biol. Cell 2006). Based on these results, we have further established genetic screening system to identify compounds that target Pmk1 MAPK signaling. As a consequence, we have successfully identified a number of compounds that have the potential to suppress MAPK signaling. Today, we focus on one of the compounds, Acremomannolipin A, a natural compound with glycolipid structure. Acremomannolipin A turned out to possess in vitro anti-tumor activities across several tumour types. Notably, the in vitro kinase assays unveiled that Acremomannolipin A inhibited activities of several isoforms of Ca^{2+}/Calmodulin-dependent protein kinases (CaMKs). Our findings demonstrated the first evidence of a role for Acremomannolipin A, a glycolipid in regulating cancer cell proliferation.

O-35

The coupling mechanism between translation and mRNA degradation mediated by RNA-binding protein HuD

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Abstract

In mammal, posttranscriptional gene expression is spatiotemporally fine-tuned by the cis-regulatory elements in mRNA sequences and trans-acting factors (e.g. RNA binding proteins). AU-rich element (ARE) is one of the well analyzed cis-regulatory elements and induces mRNA destabilization. Tristetraprolin (TTP) and Butyrate response factor 1 (BRF1) are ARE-binding proteins (ARE-BPs) and destabilize their target mRNAs through the recruitment of CCR4/NOT deadenylation complex. However, the coupling mechanism between translation and mRNA degradation is not well understood.

Hu proteins are also ARE-BPs and stabilize target mRNAs unlike TTP or BRF1. We have shown that the neuronal Hu protein HuD can stimulate cap dependent translation in a eIF4A- and poly(A)- dependent manner[1], and attenuate microRNA-mediated translation repression via eIF4A-binding[2]. Moreover, HuD directly binds to the active form of Akt1[3] that regulates mRNA stability through the phosphorylation of BRF1. We hypothesize that HuD-Akt1 complex on the target mRNA coordinates mRNA stability and translation activity via local signaling pathway from Akt1. Here we construct in vitro translation system derived from mammalian cell lines and can monitor both the translation activity and mRNA stability with deadenylation by ARE-BPs.

[1]Fukao *et al*., 2009, [2]Fukao *et al*., 2014, [3]Fujiwara and Fukao *et al*., 2011

O-36

Potential role of glycosylation in regulating biological function of PTPRA

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Abstract

Receptor-like protein tyrosine phosphatase-RA (PTPRA) is heavily glycosylated in its extracellular domain. However, the exact glycan pattern has never been explored. It also remains elusive how glycosylation may affect ligand binding, dimer formation or enzymatic activity of PTPRA. To tackle such challenging questions, we studied whether glycosylation plays a key role in regulation of PTPRA-mediated intracellular signaling. We detected two major variants of PTPRA expressed in HEK293 cells or fibroblast-like synoviocytes to be 110 kDa and 150 kDa, but not the 90 kDa species at the theoretic position in SDS-gel. Mass spectrometry-based analysis identified glycans appearing in PTPRA to be high mannose type N-glycosylation and HexNAc O-glycosylation in both 110 kDa and 150 kDa variants. The content of high mannose was then confirmed by diagnostic analysis using EndoH enzyme to react with immunoprecipitated PTPRA. To examine the potential role of N-glycosylation, we constructed the NQ mutant forms of PTPRA via site-directed mutagenesis. Our data suggested that all 7 predicted N-glycosylation sites within the extracellular domain of PTPRA were indeed modified. Moreover, we observed that PTPRA-promoted Src signaling is N-glycosylation-dependent. The interplay between glycosylation and phophotyrosine signaling for exerting biological function of PTPRA is under investigation.

O-37

Role of Src family kinases in regulation of intestinal epithelial homeostasis

Author(s)

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Abstract

Proper regulation of epithelial cell turnover is important for the structural integrity and homeostasis of various tissues including the intestine. Here we show that ablation of Csk, a negative regulator of Src family kinases (SFKs), specifically in intestinal epithelial cells (IECs) resulted in the development of hyperplasia throughout the intestinal epithelium of mice. Such conditional ablation of Csk also increased the proliferative activity and turnover of IECs, disturbed the differentiation of Paneth and goblet cells, reduced the number of intestinal stem cells, and attenuated the expression of Wnt target genes in the intestine. Moreover, the tyrosine phosphorylation of focal adhesion kinase (FAK) as well as the activity of both Rac and Yes-associated protein (YAP) were increased in intestinal crypts or organoids of the mutant mice, whereas inhibition of Rac or YAP activity rescued the mutant phenotypes. Our results thus suggest that SFKs promote the proliferation of IECs in intestinal crypts through activation of Rac or YAP, and that they thereby contribute to the proper regulation of IEC turnover and intestinal homeostasis.

O-38

Protein tyrosine phosphatase 1B deficiency in podocytes protects against hyperglycemia-induced renal injury

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Abstract

Diabetic nephropathy is one of the most devastating complications of diabetes, and growing evidence implicates podocyte dysfunction in disease pathogenesis. Protein tyrosine phosphatase 1B (PTP1B; encoded by PTPN1) is an established metabolic regulator in vivo but its metabolic functions in podocytes remains unexplored. To that end, we generated podocyte-specific PTP1B knockout (pod-PTP1B KO) mice and determined alterations under normoglycemia and streptozotocin (STZ)- and high fat diet (HFD)-induced hyperglycemia. pod-PTP1B KO mice displayed significant improvement in renal function and glucose homeostasis under STZ- and HFD-induced hyperglycemia. Consistent with these findings, podocyte PTP1B deficiency was associated with increased renal insulin signaling and enhanced autophagy with corresponding decrease in inflammation and fibrosis. These effects were recapitulated in E11 murine kidney podocytes with lentiviral-mediated PTP1B knockdown, consistent with being cell-autonomous. Moreover, reconstitution of PTP1B in knockdown cells reversed the improved insulin signaling and autophagy demonstrating that they were likely a consequence of PTP1B deficiency. Together, these findings identify PTP1B in podocytes as a significant contributor to signaling events following hyperglycemia-induced damage, and suggest that PTP1B inhibition in podocytes may be of value in combating podocytopathies.

O-39

Lacking of dopamine D2L receptor causes vulnerability against chronic stress in mice

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Abstract

Genetic studies show the association of dopamine D2 receptor (D2R), DRD2 polymorphisms with several mental disorders, including anxiety and schizophrenia. D2R exists as two alternatively spliced isoforms, D2R long isoform (D2LR) and D2R short isoform (D2SR), which are with or without a 29-amino acid insert in the third cytoplasmic loop, respectively. Recent evidence from human and animal studies indicates the functional diversity between the two isoforms. Although the emergence of neurological and psychiatric diseases is implicated in both genetic and environmental factors, the critical function of D2LR in mental illness is largely unknown. Here, we show that lack of D2LR causes the stress vulnerability through activation of serotonergic neurons in mouse brain. The exposure to forced swim (FS) stress increased anxiety-like behaviors in both D2R knockout (KO) and D2LR-KO mice compared to wild-type mice. DNA microarray analyses revealed that serotonin-related genes were up-regulated in brain stem of D2R-KO and D2LR-KO mice after FS stress exposure. Consistent with the results, FS-induced serotonin release assessed by microdialysis was significantly increased in the medial prefrontal cortex of D2LR-KO mice. The administration of 8-OH-DPAT, a serotonin 5-HT1A receptor (5-HT1AR) agonist ameliorated the FS-induced anxiety-like behaviors in wild-type mice, but not in D2LR-KO mice. Taken together, D2LR-KO mice showed vulnerability against FS stress through the increase in serotonin release, in which D2LR may regulate 5-HT1A autoreceptor functions in dorsal raphe nucleus.

O-40

Regulation of adipocyte differentiation and lipid droplet formation by PPM1D phosphatase

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Abstract

Adipocyte plays an important role in energy homeostasis. Triacylglycerols, a major energy reserve, are stored in lipid droplet of adipose tissue. Recently, one of third of adults in the world are considered to obesity, and this is a worldwide problem. The cause of obesity is hypertrophy of white adipocyte, and increase and enlargement of lipid droplets. PPM1D, also known as Wip1, is a member of PPM1 phosphatase family, and is originally identified as a protein induced by p53 in response to DNA damage. It is reported that PPM1D is involved in negative feedback regulation of p53 pathway. Protein overexpression and gene amplification of PPM1D are observed in many cancer cells. Also, it is shown that PPM1D controls in glucose homeostasis. Here, we report the effects of PPM1D on adipocyte differentiation and lipid droplet formation. PPM1D gene expression was upregulated during the adipose differentiation of 3T3-L1cells. Knock-down of PPM1D suppressed the expression of adipogenic genes including PPARγ and C/EBPα. These results suggested that PPM1D is involved in regulation of adipocyte differentiation. The treatment of a potent PPM1D inhibitor SL-176 dramatically reduced the formation of lipid droplets. Also, the inhibitor reduced gene expression related adipocyte differentiation and lipid droplet formation. Our study showed that PPM1D controls formation of lipid droplet through the regulation of PPARγ and C/EBPα.

O-41

The R3 receptor-like protein tyrosine phosphatase subfamily negatively regulates insulin signaling by dephosphorylating the insulin receptor at specific sites

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Abstract

The R3 subfamily (PTPRB, PTPRH, PTPRJ and PTPRO) of receptor-like protein tyrosine phosphatases (RPTPs) is characterized by an extracellular region with 6-17 fibronectin type III-like repeats and a cytoplasmic region with a single phosphatase domain. We previously demonstrated that the R3 RPTPs dephosphorylate several receptor protein tyrosine kinases (RPTKs) as substrates. Subsequently, we identified the insulin receptor (IR) as a substrate for R3 RPTPs by using the substrate-trapping mutants of R3 RPTPs. The autophosphorylation of specific tyrosine residues occurs in the cytoplasmic region of the IR upon insulin binding, and this in turn initiates signal transduction. The co-expression of R3 RPTPs with the IR in HEK293T cells suppressed insulin-induced tyrosine phosphorylation of the IR. In vitro assays using synthetic phosphopeptides revealed that R3 RPTPs preferentially dephosphorylated particular phosphorylation sites of the IR: Y960 in the juxtamembrane region and Y1146 in the activation loop. Among the four R3 members, only PTPRJ was co-expressed with the IR in major insulin target tissues, such as the skeletal muscle, live,r and adipose tissue. Importantly, activation of the IR and Akt by insulin was enhanced, and glucose and insulin tolerance was improved in Ptprj-deficient mice. These results demonstrate PTPRJ as a physiological enzyme that attenuates insulin signaling in vivo, and indicate that an inhibitor of PTPRJ may be an insulin-sensitizing agent.

O-42

Skeletal muscle enriched inositol polyphosphate phosphatase (SKIP) links ER stress to insulin resistance

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Abstract

Skeletal muscle enriched inositol polyphosphate phosphatase (SKIP) is a ER-localizing phosphoinositide 5-phosphatase that hydrolyzes 5-phosphate from PIP3 and PI(4,5)P2 to generate PI(3,4)P2 and PI(4)P, respectively. SKIP is a negative regulator of insulin-stimulated PI 3-kinase signaling in skeletal muscle and heterozygous knockout mice of SKIP show increased insulin sensitivity and are resistant to dietary-induced insulin resistance. Our recent results show that SKIP is a molecular link between ER stress and Type 2 diabetes. Expression of SKIP is induced by ER stress including treatment with thapsigargin and excess amount of non-esterified fatty acid. This induction is dependent on transcriptional factor XBP1, one of the sensor of ER stress. Mechanistically, SKIP binds to molecular chaperone GRP78 at the endoplasmic reticulum. This binding is necessary for insulin-dependent membrane localization of SKIP and for de-phosphorylation of phosphoinositides at the plasma membrane. SKIP may be a novel sensor of ER stress whose localization and activation are under control of GRP78. Furthermore, treatment of human myoblast cell with chemical or pharmaceutical chaperones, TUDCA and 4-PBA, decreased expression of SKIP and in turn resulted in increased insulin signaling. Therefore, SKIP is a novel promising pharmaceutical target for the intervention from insulin resistance in peripheral tissues.

O-43

Loss of NDRG2/PP2A complex induces global abnormalities in protein phosphorylation in cancer development and progression

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Abstract

Protein phosphatase 2A (PP2A) is a major protein serine/threonine phosphatase that regulates critical cellular processes by dephosphorylating many important cellular molecules and acts as a 'tumor suppressor'. N-myc downstream-regulated gene 2 (NDRG2) is a stress-responsive gene and its expression is induced by various stress conditions, which negatively regulates the activation of important signaling pathways including PI3K/AKT or NF-κB through interaction with PP2A. To clarify the global regulation by NDRG2/PP2A complex under stress conditions, we analyzed the phosphorylation status of NDRG2-binding proteins in various types of cancer cells by mass spectrometry. We found that phosphorylation of many signaling molecules such as 14-3-3, vimentin, STAT5 and others is affected by interaction with NDRG2. In particular, we identified that NDRG2 interacts with stress-responsive proteins, which results in the suppression of signaling pathways involved in cell growth and antiapoptosis in cancer cells. Therefore, the NDRG2/PP2A complex may play important roles in a wide range of vital regulatory processes, including mitogenic signal transduction, apoptotic cell death, and cell cycle control, and that loss of NDRG2 expression in cancer cells may enhance phosphorylation of stress-responsive proteins, rendering cells resistant to cellular stresses. Thus, we identified a novel target of cancer therapy and are now working on the establishment of novel concept for drug treatment and how to apply the novel drug candidates in cancers.

O-44

The protein phosphatase 6 catalytic subunit (Ppp6c) is indispensable for proper postimplantation embryogenesis

Author(s)

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Abstract

Ppp6c, which encodes the catalytic subunit of phosphatase 6（PP6）,is conserved among eukaryotes from yeast to humans. In mammalian cells, PP6 are reported to be involved in apoptosis, autophagy and DNA repair. Ppp6c depleted Hela cells do not form bipolar spindle with normal kinetics. This strongly suggests that PP6 activity is required for normal mitosis. Recently, Ppp6c mutations were identified as candidate drivers of melanoma and skin cancer. Nonetheless, little is known about the physiological roles of Ppp6c.

To investigate this function in vivo, we established mice lacking the Ppp6c phosphatase domain by crossing heterozygous mutants. No viable homozygous pups were born, indicative of a lethal mutation. Ppp6c homozygous mutant embryos were identified among blastocysts, which exhibited a normal appearance, but embryos degenerated by E7.5 and showed clear developmental defects at E8.5, suggesting that mutant embryos die after implantation. Accordingly, homozygous blastocysts showed significant growth failure on the inner cell mass （ICM）in in vitro blastocyst culture, and primary Ppp6c exon4-deficient MEFs showed greatly reduced proliferation. These results established for the first time that the Ppp6c phosphatase domain is in dispensable for mouse embryogenesis after implantation.

O-45

In vivo regulation of GSK3β activity, as revealed by quantitative measurements of its phosphoisotypes.

Author(s)

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Abstract

Glycogen synthase kinase 3β (GSK3β) is a multifunctional protein kinase involved in cell proliferation, survival, development and differentiation. GSK3β is also associated with diseases, such as diabetes and Alzheimer's disease. Therefore, it is important to understand the mechanisms that regulate its in vivo activity. GSK3β is thought to be constitutively activated by autophosphorylation of Tyr216 and inactivated by phosphorylation at Ser9. The kinase activity of GSK3β has been evaluated by inhibitory phosphorylation at Ser9, but it does not necessarily measure the kinase activity itself. Here, we applied the Phos-tag SDS-PAGE technique, which separates proteins according to phosphorylation states, to the analysis of GSK3β phosphoisotypes in cells and brains. There were three phosphoisotypes of GSK3β; double phosphorylation at Ser9 and Tyr216, single phosphorylation at Tyr216 and the nonphosphorylated isotype. Active GSK3β with phosphorylation at Tyr216 was the most abundant isotype and represented half or more of the total GSK3β. Although an increase in the phospho-Ser9 levels was observed in insulin-treated cells by immunoblotting with an anti-phospho-Ser9 antibody, Ser9 phosphorylation occurred only in a minor fraction of GSK3β and most GSK3β remained as an active phosphoisotype. In adult mouse brains, GSK3β was principally in the active form with little Ser9 phosphorylation, and the phosphoisotypes of GSK3β changed depending on the regions of the brain, age, sex and disease conditions. These results indicate that the Phos-tag SDS-PAGE method provides a simple and appropriate measurement of active GSK3β in vivo, and the activity is regulated by the mechanism other than phosphorylation on Ser9.

O-46

Protein kinase N (PKN) family-dependent regulation of hepatic cytochrome P450 2C and metabolic profile analysis in PKN mutant mice through targeted metabolomics by LC-MS/MS

Author(s)

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Abstract

Protein kinase N (PKN) is a serine/threonine protein kinase, which has a catalytic domain highly homologous to protein kinase C. There are three isoforms (PKN1, PKN2, and PKN3) in mammalian PKN. Although previous reports have suggested multiple layers of regulatory mechanisms of cytochrome P450 (Cyp) by several kinases, such as transcriptional regulation and protein degradation, whether PKN is involved in the regulation of Cyp activity at the transcriptional level remains unknown.

To clarify the role of PKN in Cyp regulation, we examined the mRNA and protein expression levels and the metabolic activities of several Cyps in liver of PKN1 and PKN3-mutated mice. Furthermore, in order to reveal the role of PKN in regulation of metabolic profile such as glycolysis and amino acids metabolism, we performed targeted metabolomics by LC-MS/MS, by using the LC system and a TSQ Endura Triple Quadrupole Mass Spectrometer with electrospray ionization.

PKN1, PKN3, and the double (D) mutant mice didn't exhibit altered expression levels of Cyp mRNA/protein as compared with the WT mice. However, Cyp2c activities in D mice were significantly decreased, suggesting the involvement of PKN1/PKN3 in the post-translational Cyp2c regulation. Neither PKN1 mutation nor PKN3 deletion alone affected Cyp2c activities. To clarify whether the enzymatic characteristics of Cyp2c were altered, the enzyme kinetics studies were conducted. The value of K_m but not V_{max} in D mice was larger than that in WT. These results suggest that both PKN1 and PKN3 collaboratively modulate the conformation of binding sites of Cyp2c for substrates. For targeted metabolomics, the measurement condition for determination of metabolic profile was established.

O-47

Down-regulation of ErbB2/ErbB3 heterodimer via ERK-mediated phosphorylation of ErbB2 Thr-677 in the juxtamembrane domain

Author(s)

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Abstract

We have previously reported ERK-mediated feedback regulation of EGFR homodimer in lung cancer cells, in which phosphorylation of EGFR at the juxtamembrane domain Thr-669 down-regulates its constitutive tyrosine phosphorylation (Cancer Sci, 2013). In breast cancer cells, ErbB2 and ErbB3 are highly expressed and ErbB2/ErbB3 heterodimer activates downstream growth and survival signals. To unveil the regulatory mechanisms of the ErbB family is critical for the molecular targeted therapy. In this study, we further analyzed feedback regulation of ErbB2/ErbB3 heterodimer. Constitutive tyrosine phosphorylation of both ErbB2 and ErbB3 were decreased in TPA-treated breast cancer cells and ErbB2/ErbB3 stably expressed HEK293 cells. However, Phos-tag western blot analysis showed the shift-upped bands, suggesting Ser/Thr phosphorylation. Pretreatment with vanadate, a non-selective tyrosine phosphatase inhibitor, suppressed the ErbB2/ErbB3 down-regulation, indicating the involvements of tyrosine phosphatases in this regulation. Amino acids sequence around the EGFR Thr-669 is highly conserved with ErbB2 and ErbB2 Thr-677 is thought to correspond to EGFR Thr-669. We generated a phospho ErbB2 Thr-677-specific rabbit monoclonal antibody and demonstrated phosphorylation of endogenous ErbB2 at Thr-677 in breast cancer cells. Furthermore, the substitution of ErbB2 Thr-677 to Ala diminished the down-regulation of tyrosine phosphorylation of ErbB2/ErbB3. These results revealed ERK-mediated phosphorylation of ErbB2 at Thr-677 is responsible for feedback regulation of ErbB2/ErbB3 heterodimer and suggested the conserved feedback regulatory mechanism among the ErbB family.

Award Lecture

O-48

Shp2 in Forebrain Neurons Regulates Synaptic Plasticity, Locomotion, and Memory Formation in Mice

Author(s)

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Abstract

A protein tyrosine phosphatase Shp2 is expressed ubiquitously, and regulates cell proliferation and differentiation downstream of growth factors or cytokines. Mutations of the human Shp2 gene (PTPN11) that result in constitutive activation or inactivation of the encoded protein cause Noonan syndrome (NS) and LEOPARD syndrome (LS), both of which are congenital malformation syndromes with similar pathologies characterized by facial dysmorphia and other developmental abnormalities, including learning difficulties and mental retardation. Shp2 is highly expressed in mature neurons, and it appears that its dysfunction in the adult brain contribute to insufficiency of higher brain function in NS and LS. To test our hypothesis, we generated conditional-knockout (cKO) mice lacking Shp2 specifically in postmitotic forebrain neurons, Shp2 cKO mice manifested abnormal behavior, including hyperactivity. Novelty-induced expression of immediate-early genes and activation of extracellular-signal-regulated kinase (Erk) were attenuated in the cerebral cortex and hippocampus of Shp2 cKO mice, suggestive of reduced neuronal activity. In contrast, ablation of Shp2 enhanced high-K^+-induced Erk activation in both cultured cortical neurons and synaptosomes, whereas it inhibited that induced by brain-derived growth factor in cultured neurons. Posttetanic potentiation and paired-pulse facilitation were attenuated and enhanced, respectively, in hippocampal slices from Shp2 cKO mice. The mutant mice also manifested transient impairment of memory formation in the Morris water maze. Our data suggest that Shp2 contributes to regulation of Erk activation and synaptic plasticity in postmitotic forebrain neurons and thereby controls locomotor activity and memory formation.

Poster Short Talk

Poster

PS-01 (P-01)

A novel A-kinase anchoring protein, BIG3, coordinates estrogen signalling in breast cancer cells

Author(s)

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Abstract

Approximately 70% of breast cancer cells express estrogen receptor alpha (ERalpha), and depend on estrogen (E2) for growth and survival. Recent experimental findings support that the Brefeldin A-inhibited guanine nucleotide-exchange protein 3–prohibitin 2 (BIG3-PHB2) complex plays a crucial role in the activation of E2/ERalpha signaling in these cells. However, it remains unclear how BIG3 regulates the inactivation of PHB2 suppressive activity. Here we demonstrate that BIG3 functions as an A-kinase anchoring protein that binds protein kinase A (PKA) and protein phosphatase 1alpha (PP1alpha) to enhance the PP1alpha activity in the presence of E2, thereby dephosphorylating and inactivating PHB2. The E2-induced PKA-mediated phosphorylation of BIG3-S305 and -S1208 enhances PP1alpha activity, resulting in the constitutive activation of E2/ERalpha signaling activation via PHB2 inactivation through S39 dephosphorylation. Furthermore, an analysis of independent cohorts of patients with ERalpha-positive breast cancers revealed that both BIG3 overexpression and PHB2-S39 dephosphorylation are strongly associated with poor prognosis. This is the first demonstration of the mechanism of E2/ERalpha signaling activation on the basis of "loss-of-function" of PHB2 via the BIG3-PKA-PP1alpha tri-complex in breast cancer cells. A better understanding of the precise pathophysiological roles of BIG3 in ERalpha-positive breast cancer may provide new approaches for therapeutic or diagnostic interventions in future preclinical and clinical studies.

PS-02 (P-02)

Phyllotaxis Patterns

Author(s)

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Abstract

There are some plants which have beautiful geometric patterns. To solve the mystery of this beauty, especially in the arrangement of leaves (phyllotaxis), I investigated from where the patterns originated and what the advantages are for plants. I devised a mathematical model of phyllotaxis patterns by analyzing the generation of leaves in shoots and the distribution of plant hormones and simulated phyllotaxis of general plants. As a result, it was observed that the phyllotaxis of plants is determined by the ratio between growth velocity and radius of shoot apical meristem.

PS-03 (P-03)

Protein tyrosine phosphatase Shp2 deficiency in the glomerular podocytes attenuates lipopolysaccharide-induced kidney injury

Author(s)

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Abstract

Effective glomerular filtration barrier is composed structurally of endothelium, glomerular basement membrane and podocytes, and is critical for normal renal function. Podocytes are specialized epithelial cells that play a significant role in filtration barrier integrity and in preventing urinary protein leakage. In this study we investigated the contribution of protein tyrosine phosphatase Shp2 to lipopolysaccharide (LPS)-induced renal injury. We report increased Shp2 protein expression in murine kidneys and cultured podocytes upon LPS challenge. To investigate the role of podocyte Shp2 in vivo we generated podocyte-specific Shp2 knockout (pod-Shp2 KO) mice by crossing Shp2 floxed mice to podocin-Cre transgenic mice. Following LPS treatment, pod-Shp2 KO mice exhibited lower proteinuria and blood nitrogen urea than controls indicative of preserved filter integrity and renal function. In addition, renal mRNA and serum concentrations of the inflammatory cytokines IL-1β, TNFα, INFγ and IL-12 p70 were dramatically decreased in LPS-treated pod-Shp2 KO mice compared with controls. The protective effects of podocyte Shp2 deficiency were associated with decreased LPS-induced NF-κB, MAPKs activation and endoplasmic reticulum stress. These effects were recapitulated in differentiated E11 murine podocytes with lentiviral-mediated Shp2 knockdown, consistent with this being cell autonomous. Moreover, reconstitution of Shp2 in knockdown podocytes reversed the effects demonstrating that they are likely a direct consequence of Shp2 deficiency. Further, Shp2 knockdown podocytes displayed reduced LPS-induced migration in a wound healing assay. Together, these findings identify Shp2 in podocytes as a significant contributor to the signaling events following LPS challenge, and suggest that Shp2 inhibition may be of potential value in combating podocytopathies.

PS-04 (P-04)

Inhibition of NF-kappaB by mangiferin increased the sensitivity of human multiple myeloma cells to anticancer drugs

Author(s)

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Abstract

<Purpose> Multiple myeloma (MM) is still an incurable hematological malignancy. The nuclear factor kappa B (NF-κB) pathway plays a crucial role in the pathogenesis of MM. Thus, inhibition of the NF-κB pathway is a potential target for the treatment of MM. In a previous study, we showed that mangiferin suppressed the nuclear translocation of NF-κB. However, the treatment of MM involves a combination of two or three drugs. In this study, we examined the effect of the combination of mangiferin and conventional anticancer drugs in an MM cell line.

<Methods>Cell viability was assessed by the trypan blue dye method. Signal molecules were determined by western blots.

<Results>We showed that the combination of mangiferin and an anticancer drug decreased the viability of MM cell lines in comparison with each of these drugs used separately. The decrease in the combination of mangiferin and an anticancer drug induced cell viability was attributed to increase the expression of p53 and Noxa and decreases the expression of XIAP, survivin, and Bcl-xL proteins via inhibition of NF-κB pathway.

<Discussion> Our findings suggest that the combination of mangiferin and an anticancer drug could be used as a new regime for the treatment of MM.

PS-05 (P-05)

Therapeutic application of anti-SIRPα antibody in cancer treatment

Author(s)

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Abstract

Tumor cells are thought to evade immune surveillance through interaction with immune cells. Much recent attention has focused on modification of immune responses as a basis for new cancer treatments. Signal regulatory protein α (SIRPα) is a transmembrane protein that binds to the protein tyrosine phosphatases SHP-1 and SHP-2 through its cytoplasmic region and especially abundant in myeloid cells such as macrophages and dendritic cells. SIRPα is known to inhibit phagocytosis in macrophages on interaction with its ligand CD47 expressed on the surface of target cells. Here we show that an anti-SIRP antibody (Ab), which blocks the binding of CD47 to SIRP, markedly enhances anti-tumor antigen Ab-mediated suppression of tumor growth in immunodeficient mice injected by human tumor cells. Consistently, such a blocking antibody to SIRPα promoted phagocytosis of anti-tumor antigen Ab-opsonized tumor cells by macrophages in vitro. In addition, we showed that SIRPα was highly expressed in tumor tissues from patients with melanoma and other carcinomas. The blocking anti-SIRP Ab alone markedly suppressed the in vivo tumor formation, as well as the antibody-dependent cellular phagocytosis by macrophages, of SIRP-expressing mouse tumor cells. These results thus suggest that SIRP is a promising target for cancer immunotherapy.

PS-06 (P-06)

Mutation and Inhibition of Hsp90 affects stress granule assembly and MAPK signaling ~Implications of anti-cancer mechanisms of Geldanamycin~

Author(s)

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Abstract

Heat shock protein 90 (Hsp90) is a highly conserved molecular chaperone, which is overproduced when exposed to various stresses, including heat shock, thereby regulating proteolytic turnover of many key regulators of cell growth, differentiation, and survival. Furthermore, Hsp90 plays key roles in oncogenesis and thus serves as a critical therapeutic target for human cancers.

Stress granule (SG) is a macromolecular RNA-protein complex, containing RNA, ribosome and mRNA-binding proteins, that regulates cell fate and stress responses by controlling gene expression and various signaling pathways. We have demonstrated that an RRM-type RNA binding protein Nrd1 is a key regulator of SG assembly and that Nrd1 localization to SG is dependent on its phosphorylation mediated by Pmk1 MAPK.

Here, we showed that Hsp90 is a component of the fission yeast Pmk1 MAPK signaling pathway and a potential regulator of SG assembly. Our genetic studies identified the hsp90 mutant swo1 as a vic mutant, which represents MAPK signaling inhibition. In addition, our chemical genetic screen aiming to isolate MAPK signaling inhibitor also identified Geldanamycin (GA), which is known to possess potent anti-cancer activity through the inhibition of HSP90-chaperone function.

Notably, the Hsp90 mutant swo1-26 and Geldanamycin (GA) treatment similarly impaired Nrd1 translocation to SG as well as its phosphorylation, suggesting that inhibition of Hsp90 function affects MAPK signaling thereby perturbing SG assembly. We will discuss the mechanisms underlying the functional connection between Hsp90 and MAPK phosphorylation mediated SG assembly, as well as its implication for anti-cancer property exerted by Hsp90 inhibitor.

PS-07 (P-07)

ERK-RSK mediated phosphorylation of FilGAP regulates cell migration by promoting the conversion from lamellipodia to membrane blebbing downstream of epidermal growth factor signaling

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Abstract

Cell migration is a fundamental process involved in embryonic development, immune defense, and cancer cell metastasis. On flat 2D surface, cells migrate using lamellipodia, flat membrane protrusions generated by actin polymerization. In 3D environment, e.g. in extracellular matrices like collagen, some type of cancer cells migrate using membrane blebbing, spherical membrane protrusions generated by contractions of cortical actomyosin. Rac, a member of Rho family small GTPases, generates lamellipodia in front of the migrating cells. Activation pathway of Rac downstream of growth factor signaling has been extensively studied, but its negative regulatory pathway is not well understood. We identified FilGAP, a Rac-specific GAP, as a lamellae suppressor. In this study, we revealed that FilGAP is phosphorylated downstream of EGF signaling. FilGAP was sequentially phosphorylated at Ser625 through ERK-RSK pathway and then phosphorylated at Ser621 by GSK3. Phosphorylation of serine 621 and 625 was required for the suppression of lamellae mediated by FilGAP. Interestingly, EGF treatment increased membrane blebbing in FilGAP expressing cells, but not in nonphosphorylatable FilGAP expressing cells. FilGAP expressing cells showed slower motility in two-dimensional surface but faster motility on thick collagen gel than unphosphorylatable FilGAP expressing cells, suggesting that phosphorylation of FilGAP is required for effective cell migration on collagen gel. These data suggest that FilGAP was phosphorylated through the ERK-RSK pathway and this phosphorylation regulates cell migration by promoting the conversion from lamellipodia to membrane blebbing in response to EGF.

PS-08 (P-08)

Skb5, an SH3 domain adaptor protein, plays a regulatory role in the PKC/MAPK signaling pathway by controlling the intracellular localization of the MAPKKK Mkh1

Author(s)

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Abstract

The pathway from Ras through Raf and MEK to ERK/MAPK regulates many fundamental cellular processes. MAPKKK Mkh1 is an initiating kinase in Pmk1 MAPK signaling, which regulates cell integrity in fission yeast. Our genetic screen for regulators of Pmk1 signaling identified phosphatases that inactivate MAPK signaling, including the Pmp1 dual specificity phosphatase, and the PP2C serine/threonine protein phosphatase. Here, we identified Skb5 (Shk1 kinase binding protein 5), an SH3 domain-containing adaptor protein as a novel regulator of PKC/MAPK signaling. We showed that Skb5 overproduction negatively regulates the Pck2/Pmk1 MAPK signaling activity by binding to Mkh1 thereby affecting Mkh1 localization at the growing ends. Consistently, the Mkh13PA mutant protein, with impaired Skb5 binding, remained in the growing ends, even when Skb5 was overproduced. Intriguingly, Skb5 needs Mkh1 to localize to the growing ends as Mkh1 deletion and disruption of Mkh1 binding impairs Skb5 localization. Pck2 influences Mkh1/Skb5 localization to the growing ends and their interaction. Deletion of Pck2 impaired the growing ends localization of Mkh1 and Skb5. Altogether, Skb5 spatially regulates PKC/Mkh1/MAPK signaling by controlling the Mkh1 localization to growing ends, which is critical for transmitting upstream signaling to Pmk1 MAPK.

PS-09 (P-09)

Role of SIRPα in the homeostasis of fibroblastic reticular cells by dendritic cells in the spleen

Author(s)

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Abstract

Signal regulatory protein α (SIRPα) is a transmembrane protein that binds the protein tyrosine phosphatases SHP-1 and SHP-2 through its cytoplasmic region. SIRPα is predominantly expressed in dendritic cells (DCs) that play important roles in innate and adaptive immune responses. We previously demonstrated that SIRPα is important for homeostasis of CD4+ DCs, a major subset of DCs, as well as organization of T cell zone in the steady-state of the spleen. Here we showed that the mice, in which SIRPα is specifically ablated in DCs (SirpaΔDC), manifest a significant reduction of CD4+ DCs and T cells compared with control SIRPα flox (Sirpaf/f) mice in the spleen. SirpaΔDC mice showed a marked reduction of fibroblastic reticular cells (FRCs) associated with lower production of homeostatic chemokines and cytokine for T cells, such as CCL19, CCL21 and IL-7, in the spleen. In addition, lack of SIRPα in DCs induced apoptosis and increased the turnover rate of FRCs in the spleen, suggesting that SIRPα in DCs is essential for the homeostasis of FRCs. Co-culture of splenic FRCs with wild-type DCs, but not SirpaΔDC, promoted proliferation and survival of FRCs. In addition, neutralization of soluble tumor necrosis factor receptor (TNFR) ligands, such as TNFα and lymphotoxin $\alpha 3$, inhibits such proliferation of FRCs by DCs in vitro. Furthermore, we demonstrated that in vivo neutralization of TNFR ligands results in a significant reduction of FRCs in the spleen, suggesting that the homeostasis of splenic FRCs is dependent on TNFR ligands in the spleen. Our data thus suggest that SIRPα is essential for the steady-state homeostasis of FRCs through regulation of TNFR ligand production from DCs in the spleen.

PS-10 (P-10)

Global gene expression profiling reveals unexpected spectrum of effects of a novel immune modulator FTY720 ~Possible involvement of iron homeostasis as an antitumor property of FTY720~

Author(s)

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Abstract

Our laboratory has exploited combined chemical genetic approaches to delineate the complex signalling networks and drug target pathways relevant to human diseases. These include MAPK and calcineurin signalling pathways, of which aberrant control has been implicated in the development of immune diseases, inflammatory diseases, Alzheimer's disease (AD) and cancers. We have studied the signaling pathways mediated by Fingolimod (FTY720), an immune modulator approved to treat multiple sclerosis. Although FTY720 is known as sphingosine1-phosphate (S1P) receptor modulator, how FTY720 exerts its potent anti-tumor activity remains unknown. We have previously demonstrated that FTY720 activates calcineurin (CN)/Prz1 signaling by stimulating Ca^{2+} influx (Hagihara *et al*., 2013), and that FTY720 treatment stimulated the accumulation of ROS and subsequent activation of the stress-activated MAPK/Atf1 signaling pathway (Hagihara *et al*., 2014). To obtain a comprehensive view of the mechanism of actions of FTY720, we performed global gene expression profiling by DNA microarray and drug sensitivity assays. The transcriptional profiles revealed that the expression of more than 70 genes was up-regulated upon FTY720 treatment. Notably, in addition to stimulation of Calcineurin and SAPK signaling, upregulation of Pap1 and Rst2, transcription factors involved in oxidative stress and sexual development, respectively was observed. Furthermore, addition of FTY720 affected gene expression involved in iron metabolism. Consistently, cells treated with FTY720 displayed altered iron homeostasis. We will discuss the involvement of iron homeostasis as possible mechanisms of action of FTY720 in terms of its anti-tumor property.

PS-11 (P-11)

Mangiferin induced the apoptosis via suppression of NIK/NF-κB pathway in human multiple myeloma cells

Author(s)

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Abstract

<Purpose> NF-κB-inducing kinase (NIK) plays a key role in the nuclear factor kappa B (NF-κB) pathway. Dysregulation of NIK has emerged as one of the major ways in which NF-κB is subverted in multiple myeloma (MM). Thus, inhibition of NIK may be an effective therapeutic target for MM. Our previous study, we found that mangiferin inhibited NIK. In this study, we examined the effect of mangiferin in MM cell lines.

<Methods> The cell viability was used the trypan blue exclusion assay. Western blotting was used to evaluate protein expression.

<Results> We found that mangiferin decreased the viability of MM cell lines. We observed that mangiferin inhibited nuclear translocation of NF-κB by suppressing NIK activation. Moreover, mangiferin decreased the expression of XIAP, survivin, and Bcl-xL proteins.

<Discussion> Our results suggest that mangiferin induces apoptosis through the inhibition of nuclear translocation of NF-κB by suppressing NIK activation in MM cell lines. Importantly, since the number of reported NIK inhibitors is limited, mangiferin, which targets NIK, may be a potential anticancer agent for the treatment of MM.

PS-12 (P-12)

Anti-cancer drug discovery using fission yeast genetics identified a novel analog of 1'-Acetoxychavicol Acetate (ACA) with a potent anti-tumor activity against human melanoma cells

Author(s)

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Abstract

Malignant Melanoma (MM) remains incurable despite the use of conventional chemotherapy. However, novel agents targeting the MAPK pathway have generated striking clinical response in melanoma therapy because the Ras-Raf-MEK-ERK signaling pathway is one of the most commonly altered oncogenic pathway in MM. Unfortunately, acquired drug resistance is triggered by administration of MAPK signaling inhibitors in clinical settings. Therefore, development of new therapeutics is urgently needed to overcome acquired tolerance and improve clinical outcomes in MM.

Model organisms provide simplified but biologically intact systems in which to test specific hypotheses, and the insights gained using yeasts, worms and flies can be extrapolated to mammalian systems. We have established a chemical genetic screening to identify compounds that target MAPK signaling, based on our previous data that calcineurin and MAPK play antagonistic roles in Ca^{2+} signaling.

As a consequence, we have successfully identified a novel analog of 1'-Acetoxychavicol Acetate (ACA), ACA-#28. ACA-#28 inhibited cell proliferation and induced cell death in several human melanoma cell lines in a dose-dependent manner. In addition, *in vitro* transformation was assessed by growth in soft agar, and ACA-#28 inhibited anchorage-dependent cell growth. We will discuss the possible signaling pathways targeted by ACA-#28. Further studies investigating clinical applications are necessary.

PS-13 (P-13)

Mangiferin, a novel nuclear factor kappa B-inducing kinase inhibitor, suppresses metastasis in a mouse metastatic melanoma model

Author(s)

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Abstract

<Purpose> Advanced metastatic melanoma, one of the most aggressive malignancies, is currently without reliable therapy. Therefore, new therapies are urgently needed. Mangiferin is a naturally occurring glucosylxanthone and exerts many beneficial biological activities. However, the effect of mangiferin on metastasis of metastatic melanoma remains unclear. In this study, we evaluated the effect of mangiferin on metastasis in a mouse metastatic melanoma model.

<Methods> B16BL6 cells were subcutaneously injected in C57BL/6 mice. For spontaneous metastasis, primary tumors were removed and animals were monitored for another 3 weeks. Effects of mangiferin on signal molecules were determined by western blots.

<Results> We found that mangiferin inhibited spontaneous metastasis. Furthermore, mangiferin suppressed the NIK/IKK/NF-κB pathway in vivo. In addition, we found that mangiferin inhibited the expression of matrix metalloproteinases (MMPs) and very late antigens (VLAs) in vivo.

<Discussion> These results indicate that mangiferin selectivity suppresses the NF-κB pathway via inhibition of NIK activation, thereby inhibiting metastasis and tumor growth. Importantly, the number of reported NIK selective inhibitors is limited. Taken together, our data suggest that mangiferin may be a potential therapeutic agent with a new mechanism of targeting NIK for the treatment of metastatic melanoma.

SET/I2PP2A Is a Prognostic Marker and a Potential Therapeutic Target for Gastric Cancer

Author(s)

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Abstract

Increased kinase and decreased phosphatase activity are hallmarks of oncogenic signaling. Protein phosphatase 2A (PP2A) is a conserved serine/threonine phosphatase that functions as a tumor suppressor. SET/I2PP2A, the endogenous PP2A inhibitor, binds PP2A and suppresses its activity. Elevated SET levels are correlated with poor prognosis of several human cancers; however, the role of SET in gastric cancer remains unknown. This study investigated the role of SET in the tumorigenic potential of gastric cancer and to access the effects of SET-targeting drugs. METHODS: Gan mice (transgenic model of gastric cancer) and patient-derived gastric cancer tissues were analyzed by immunohistochemistry to measure SET levels. SET was knocked-down by expression of small hairpin RNAs or inhibited by SET-targeting drug OP449 in human gastric cancer cell lines. Cells were analyzed by proliferation assay, colony formation assay, and a xenograft model. The effects of SET knockdown on cell signaling were analyzed by immunoblotting, DNA microarray, and quantitative reverse-transcription polymerase chain reaction.

SET expression was higher in tumor tissues from Gan mice than in paired non-tumor tissues. Kaplan-Meier analysis of patients with survival data revealed that higher SET expression was correlated with poor survival. SET knockdown decreased E2F1 levels and suppressed the tumorigenic potential of gastric cancer cells. SET specifically associated with the PP2A-B56 complex, and B56α bound to E2F1. Treatment of gastric cancer cells with OP449 increased PP2A activity, decreased E2F1 levels, and exerted anti-cancer effects.

SET/I2PP2A plays important roles in the tumorigenic potential of gastric cancer, and the SET/PP2A/E2F1 axis is a potential target for gastric cancer therapy.

PS-15 (P-15)

Functional Analysis of the Puf family RNA-binding protein Pumilio in stress responses and the inositol phospholipid signaling pathway

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Abstract

Inositol phospholipid (IP) signaling is evolutionarily conserved from yeast to humans. IP species are important signaling molecules that have been implicated in almost all aspects of cellular physiology, including cellular growth, metabolism, proliferation, and survival. Therefore, disruptions in this key signaling pathway have been linked to conditions as diverse as cancer, obesity and diabetes. In particular, phosphatidylinositol-4-phoshate 5-kinase (PI4P5K) phosphorylates PI(4)P to synthesize PI(4,5)P2 and plays key roles in cell proliferation, morphology and intracellular transport. We have identified the essential PI4P5K Its3 and its loss-of mutants in fission yeast and demonstrated that Its3 regulates cytokinesis and membrane trafficking in fission yeast. Its3 mutant cells showed sensitivity to the calcineurin inhibitor FK506 and high temperatures. Here, we identified Puf4, a member of the Pumilio RNA-binding protein family as a gene-dosage suppressor of the temperature-sensitive growth defect of the *its3* mutant cells. Interestingly, Puf4 deletion cells showed sensitivity to H_2O_2 and a phospho-proteomic analysis demonstrated that Puf4 is phosphorylated in response to H_2O_2 stimuli, strongly suggesting that Puf4 function is closely related to oxidative stress responses. We will discuss the possible roles of Puf4 in the inositol phospholipid signaling pathway and oxidative stress response.

PS-16 (P-16)

Modification of PP2A Methylation Status Assay and Implication for Protein Phosphatase Methylesterase-1 (PME-1) as a Therapeutic Target for a Subset of Melanoma

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Abstract

Introduction

Protein Phosphatase 2A (PP2A) is a conserved Ser/Thr protein phosphatase that regulates multiple cellular processes and functions as a tumor suppressor. Protein Phosphatase Methylesterase-1 (PME-1) catalyzes specifically the demethylation of the C-terminal Leu309 residue of PP2A. Although the expression of PME-1 is correlated with the malignancy of several tumors, the molecular mechanism of PME-1-mediated malignancy has not been fully understood. Here, we investigated the role of PME-1 on tumor growth and the effect of PME-1 inhibition in melanoma.

Result and conclusion

First, we modified the method for measuring PP2A methylation level using anti-demethylated PP2A antibody and strong base. We found that addition of PP2A inhibitor okadaic acid in cell lysate, which is the original protocol, is not enough to stop PP2A demethylation in vitro, and addition of PME-1 inhibitor ABL127 is effective. By using this modified method, we analyzed the methylation level of PP2A in various human melanoma cell lines, and found the difference between melanoma cell lines. We found knockdown of PME-1 expression decreased proliferation of cell lines with low-PP2A methylation level, while PME-1 did not affect on proliferation of cell lines with high-PP2A methylation level. These data suggests that PME-1 can be a potential therapeutic target for a subset of melanoma with low-PP2A methylation level.

PS-17 (P-17)

Cross-species reaction of anti-human LAT1 with LAT1 of crab-eating monkey

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Abstract

L-type amino-acid transporter 1 (LAT1), also referred to solute carrier (SLC) 7A5, is the first identified light chain (lc) of CD98, disulfide-linked to a heavy chain (hc; SLC3A2) of CD98 (CD98hc). In six CD98hc-CD98lc (SLC7A5-8, 7A10 and 7A11) complexes, CD98hc-LAT1 complex (CD98C5) has been recently identified as an oncogenic complex by our targeted disruption of LAT1 gene, and is now considered to be a promising target for human cancer therapy. We have successfully prepared novel rat monoclonal antibodies (mAb), reshaped them to human-rat chimeric mAb, and demonstrated in vitro ADCC and in vivo anti-tumor effect in immunodeficient mice, towards mAb-based cancer therapy. To evaluate possible side effects in clinical trials, we examined the reactivity of mAb with LAT1 of crab-eating monkey tissues and cultured cells. Although reactivity of mAb was negligible in various normal monkey tissues, our anti-LAT1 mAb definitely reacted with MK.P3 kidney-derived cells of crab-eating monkey. This reactivity was significantly decreased in MK.P3 cells treated with siRNA, suggesting that crab-eating monkeys can be used in pre-clinical study with anti-human LAT1 mAb.

PS-18 (P-18)

Identification of specific inhibitors for oncogenic protein phosphatase PPM1D from G-quadruplex DNA aptamer library

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Abstract

PPM1D is a member of the PPM type Ser/Thr phosphatase family and known as negative regulator of p53 pathway and oncogenic protein. In many human tumors, gene amplification and overexpression of PPM1D are observed. Recently, we have reported that PPM1D contains a characteristic basic rich loop (B-loop) in the catalytic domain. Specific inhibitors targeting B-loop suggest to confer selectivity for PPM1D over other phosphatases as anti-cancer agents. DNA aptamers are known to bind to target protein and inhibit protein activity like antibodies. Negatively charged DNA aptamers are suggested to play attractive molecules targeting B-loop of PPM1D. Here we designed stimuli-responsive G-quadruplex DNA aptamer library which change the structure in the response to ionic cations. Screening of DNA aptamers was performed against PPM1D430, an alternative splicing variant and it shows similar structure to other C-terminal truncated PPM1D mutants. After 12 SELEX rounds, we isolated several homologous aptamer clones. In vitro phosphatase assay showed that one of identified aptamers, namely Q5F, inhibited PPM1D activity specifically. ELISA assay also exhibited that the aptamer recognized B-loop. Furthermore, the aptamer inhibited PPM1D activity in breast cancer MCF7 cells. These data suggested that obtained G-quadruplex aptamer may be ion-responsive specific inhibitor for PPM1D.

PS-19 (P-19)

Influence of radixin knockdown on drug efflux transporters of cancer cells

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Abstract

ATP-binding cassette (ABC) transporters such as P-glycoprotein (P-gp), multidrug resistance-associated protein (MRP) and breast cancer resistance protein (BCRP) in tumor are involved in anticancer multidrug resistance. The effective strategy to overcome anticancer multidrug resistance is needed. The knockdown of ezrin/radixin/moesin (ERM) proteins such as scaffold proteins could simultaneously affect the efflux activities of ABC transporters in cancer cells. The aim of this study is to evaluate the effect of radixin knockdown on the activity of ABC transporters in cancer cells. HepG2, A549 and MDA-MB-453 cells were transfected with siRNA for radixin. After 48 hr, the mRNA and protein expression levels of radixin were significantly decreased in all cells. Additionally, the intracellular accumulation of 5 (and 6)-carboxy-2',7'-dichlorofluorescein diacetate (a MRP2 substrate), rhodamine-123 (a P-gp substrate) in HepG2 was increased by radixin siRNA treatment, indicating the decreased activity of MRP2 and P-gp, although the latter decrease was not significant. In MTT assay, the cytotoxicity of methotrexate and cisplatin as substrates of MRP2 in HepG2 treated with radixin siRNA didn't change compared with those in no treatment and negative siRNA treated cells. The reason why the improvements of anticancer effects were not obtained may be due to lower expression levels of ABC transporters in HepG2. Further study is needed using the multi-drug resistance cancer cells. These results showed that the improvement of the cytotoxicity couldn't be obtained. However, the decreased levels of radixin reduced the ABC transporter activities.

PS-20 (P-20)

Inhibition of p53-inducible Ser/Thr phosphatase PPM1D induces differentiation of human testicular embryonal carcinoma cell line

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Abstract

Serine/threonine phosphatase PPM1D, a member of PPM1 family, is known as a negative regulator of genotoxic stress response of p53-dependent pathways. PPM1D has two alternative splice variants, PPM1D605 and PPM1D430. PPM1D605 ubiquitously expresses in human tissues, and PPM1D430 specifically expresses in testis and leukocyte. It is reported that PPM1D-deficient mice exhibit defective immune response. In this study, we analyzed the role of PPM1D in neutrophil-differentiation. Here we report that inhibition of PPM1D enhanced differentiation of human testicular embryonal carcinoma cell line (NT2/D1 cells). NT2/D1 cells can be differentiated into neuronal cells by treatment with all-trans retinoic acid. We showed that PPM1D605 and PPM1D430 mRNA and protein levels were increased by ATRA-induced differentiation of NT2/D1 cells. We also showed that treatment of NT2/D1 cells with PPM1D specific inhibitor, SL-176, induced the typical neuronal morphology of the cells. Furthermore, PPM1D knockdown induced differentiation of NT2/D1 cells. These results suggested that PPM1D regulates pluripotency in NT2/D1 cells.

PS-21 (P-21)

A CC3 variant of lymphotactin/XCL1 (XCL1- CC3) is an effective CTL-inducing adjuvant for cancer immunotherapy

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Abstract

Current vaccines are capable to induce B cell responses and neutralizing antibodies, but incapable to prime antigen-specific cytotoxic T lymphocyte (CTL) responses which are needed for cancer immunotherapy. Recently, the chemokine receptor XCR1 has shown to be exclusively expressed by murine and human cross-presenting dendritic cells. Therefore, targeting antigens to XCR1 by using antigen fused to lymphotactin/XCL1, an XCR1 ligand, represents an effective induction of CTL responses. In the present study, we generated a murine CC3 variant of XCL1 (XCL1-CC3), a highly active form, and investigated in vivo adjuvant efficacy by the induction of CTL responses. Wild-type XCL1 (XCL1-WT) and XCL1-CC3 derived from human 293-F cells showed higher cell migration activity and calcium mobilization activity than those derived from hamster CHO-F cells. The XCL1s derived from human 293-F cells were highly glycosylated compared with those derived from hamster CHO-F cells, which may explain the different chemokine activity. Intradermal injection with ovalbumin, as a model antigen, and XCL1-WT or XCL1-CC3 induced potent CTL responses, and XCL1-CC3 showed more effective prophylactic and therapeutic antitumor immunity than XCL1-WT. In addition, XCL1-CC3, but not XCL1-WT, significantly enhanced an accumulation of CD103+XCR1+ cross-presenting dendritic cells in the injection sites. The present results indicate that XCL1-CC3 functions a potent CTL-inducing vaccine adjuvant for cancer immunotherapy than XCL1-WT.

PS-22 (P-22)

Effect of inhibition of p53-inducible Ser/Thr phosphatase PPM1D on neutrophil differentiation

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Abstract

The p53-inducible serine/threonine phosphatase PPM1D, a member of PPM1 family, is known as a negative regulator of genotoxic stress response of p53-dependent pathways. PPM1D has two alternative splice variants, PPM1D605 and PPM1D430. PPM1D605 ubiquitously expresses in human tissues, and PPM1D430 specifically expresses in leukocyte and testis. Gene amplification, and mRNA and protein overexpression of PPM1D have been reported in various human tumors, including leukemia, breast cancer and neuroblastoma. It is reported that PPM1D knockout mice exhibited neutrophilia in blood and showed defective immune response. In this study, we analyzed the role of PPM1D in neutrophil-differentiation.

Here we report that inhibition of PPM1D enhanced neutrophil differentiation of human promyelocytic leukemia (HL-60) cells. HL-60 cells can be differentiated into neutrophil-like cells by treatment with all-trans retinoic acid (ATRA). We showed that treatment of HL-60 cells with PPM1D specific inhibitor, SL-176, induced neutrophil-differentiation of HL-60 cells. Furthermore, co-treatment of PPM1D inhibitor and ATRA significantly enhanced the efficiency of neutrophil-differentiation. Immunocytochemistry of PPM1D revealed that subcellular localization of PPM1D was changed before and after differentiation in HL-60 cells. We also showed that PPM1D mRNA level was increased by neutrophil-differentiation. These results suggested that PPM1D regulates neutrophil-differentiation of HL-60 cells.

PS-23 (P-23)

The BCR crosslinking-induced phosphorylation of Bcl-xL and apoptosis are controlled by alpha4 in immature B cell

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Abstract

Our previous study showed that c-Jun N-terminal kinase (JNK) phosphorylates serine 62 of Bcl-xL to induce the degradation of Bcl-xL and apoptosis in an immature B cell line WEHI-231. To elucidate the regulatory mechanisms underlying the phosphorylation of Bcl-xL, we prepared an assay system in which JNK phosphorylated Bcl-xL in HEK293T cells. We found that a phosphatase regulatory molecule, alpha4, enhanced the phosphorylation of Bcl-xL by JNK. Furthermore, co-expression of C-terminal alpha4 (220-340) diminished the phosphorylation of Bcl-xL induced by JNK. Full-length alpha4 associated with both JNK and Bcl-xL, whereas C-terminal alpha4 (220-340) associated only with Bcl-xL, not JNK. WEHI-231 cells transfected with the cDNA of C-terminal alpha4 (220-340) exhibited decreased phosphorylation of Bcl-xL and increased resistance to apoptosis induced by BCR crosslinking. These results indicate that alpha4 is an important regulatory molecule of apoptosis induced by BCR crosslinking and that C-terminal alpha4 (220-340) functions as a dominant negative form.

PS-24 (P-24)

Identification of aldolase A as a novel diagnosis biomarker for colorectal cancer based on proteomic analysis using formalin-fixed paraffin-embedded tissue

Author(s)

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Abstract

Colorectal cancer (CRC) is one of the most common cancers worldwide and many patients are already at an advanced stage when they were diagnosed. Carcinoembryonic antigen is widely used as a biomarker for CRC detection; however, its sensitivity is low in early stage cancer. Therefore, novel biomarkers for early detection of CRC are required. In this study, we performed a global shotgun proteomic analysis using formalin-fixed and paraffin-embedded CRC tissue to identify candidate proteins for use as possible early detection biomarkers. Using semi-quantification based on spectral counting, we identified 84 proteins differentially-expressed in the cancer region. We focused on 21 proteins, which were classified in the extracellular region by gene ontology analysis, as potential candidates for biomarker detection in blood. Then, we performed immunohistochemical analysis to validate the result of the proteomic analysis. We found aldolase A expression levels were significantly higher in the cancer regions than the non-cancer regions. Finally, we found aldolase A secretion was clearly blocked in CRC cells as compared to normal colon epithelium. This finding suggested decreased expression of aldolase A in blood might be novel biomarkers for the early detection of CRC.

P-25

Cav3.2 T-type calcium channels as therapeutic targets for the oxaliplatin-induced peripheral neuropathy

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Abstract

Oxaliplatin is used for the treatment of advanced colorectal cancer, whereas it frequently induces peripheral neuropathy. We have shown that the Cav3.2 isoform of T-type calcium channels (T-channels) participates in the neuropathic pain induced by spinal nerve injury or repeated administration of paclitaxel, an anticancer drug. In the present study, we asked if Cav3.2 T-channels are involved in the oxaliplatin-induced neuropathic pain in mice. The mechanical nociceptive threshold assessed by von Frey test significantly decreased 4 h after a single i.p. administration of oxaliplatin (acute phase), and the evoked allodynia lasted 8 days or more after oxaliplatin challenge (chronic phase). The oxaliplatin-induced acute allodynia was reduced by T-channel blockers, such as NNC55-0396 or Z-944, but not by mibefradil or RQ-00311651. On the other hand, the oxaliplatin-induced neuropathic allodynia in the chronic phase was markedly reversed by the same dose of mibefradil, RQ-00311651, NNC55-0396 or Z-944, and also by TTA-A2, another T-channel blocker. Further, the neuropathic allodynia in the chronic, but not acute phase was also suppressed by i.p. administration of ascorbic acid, known to inhibit Cav3.2, but not other T-channel isoforms. Finally, knockdown of Cav3.2 by intrathecal administration of the antisense oligodeoxynucleotides abolished the oxaliplatin-induced chronic allodynia. These data strongly suggest that Cav3.2 T-channels contribute to the oxaliplatin-induced peripheral neuropathy in the chronic phase and serve as the therapeutic target, although the role of T-channels in the acute allodynia is still open to question.

P-26

Regulation of Beclin 1 Phosphorylation and Autophagy by PP2A and DAPK3

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Abstract

Autophagy is an evolutionarily conserved intracellular degradation system that is involved in cell survival and is activated in various diseases, including cancer. Beclin 1 is a central scaffold protein that assembles components for promoting or inhibiting autophagy. Association of Beclin 1 with its interacting proteins is regulated by the phosphorylation of Beclin 1 by various Ser/Thr kinases, but the Ser/Thr phosphatases that regulate these phosphorylation events remain unknown. We utilized Phos-tag Biotin HRP to detect enhanced phosphorylation level of Beclin 1 in cells treated with type 2A phosphatase inhibitor okadaic acid (OA), but not with type 1 phosphatase inhibitor tautomycin or type 2B phosphatase inhibitor cyclosporine A. By using non-phosphorylateble mutants, we identified Ser90 as the OA-induced phospho-site. We generated the specific antibody against phospho-Ser90 Beclin 1, and showed Beclin 1 Ser90 is phosphorylated by starvation in mouse skeletal muscle tissues and cultured cells. Furthermore, autophagic activity was diminished in cells expressing Beclin 1 S90A mutant.

We found PP2A B55α associates with Beclin 1 and shRNA targeting PP2A enhances starvation induced Beclin 1 Ser90 phosphorylation. We also found that death-associated protein kinase 3 (DAPK3) directly phosphorylates Beclin 1 Ser90. We propose that physiological regulation of Beclin 1 Ser90 phosphorylation by PP2A and DAPK3 controls autophagy.

P-27

Interleukin-6-induced neuroendocrine-like differentiation of human prostate cancer cells: cell signaling and upregulation of Cav3.2 T-type calcium channels

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Abstract

Neuroendocine (NE)-like differentiation of prostate cancer cells occurs in response to stimulation with interleukin-6 (IL-6), an elevation of intracellular cyclic AMP levels, and androgen deprivation. The NE-like prostate cancer cells secrete proliferative neuropeptides, which promote androgen-independent proliferation of surrounding cancer cells. Cav3.2 T-type Ca^{2+} channels are overexpressed and mediate Ca2+-dependent secretion in NE-like prostate cancer cells. Thus, we examined cell signals for the IL-6-induced NE differentiation and Cav3.2 upregulation in human prostate cancer LNCaP cells. Stimulation with IL-6 caused neurite outgrowth accompanied by overexpression of Cav3.2 channels and T-type Ca2+-dependent enhancement of secretory function, when determined as spontaneous release of prostatic acid phosphatase. Pyridone 6 and rapamycin, inhibitors of JAK and mTOR, respectively, abolished the IL-6-induced neuritogenesis and Cav3.2 upregulation. IL-6 did not alter levels of Cav3.2 mRNA and Egr-1 protein, known to activate the Cav3.2 promoter, but reduced levels of REST protein, known to counteract the action of Egr-1 on the Cav3.2 promoter. LNCaP cells, when co-cultured with human monocyte-like THP-1 cells, exhibited Cav3.2 upregulation. Our data thus suggest that JAK and mTOR are involved in the IL-6-induced neuritogenesis and upregulation of Cav3.2 channels, essential for Ca2+-dependent secretion, in LNCaP cells. Monocytes/macrophages capable of secreting IL-6 might participate in the NE-like differentiation of prostate cancer cells, which is associated with acquisition of androgen-independent pathology.

P-28

Analysis of a direct cell-cell communication signal that regulates glial activation in the brain

Author(s)

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Abstract

SIRPα (Signal regulatory protein α) is a member of immunoglobulin superfamily membrane proteins. Preceding studies suggest that the interaction of SIRPα on phagocytes, such as macrophages, with its ligand CD47 on phagocytic targets negatively regulates phagocytosis. In the central nervous system, SIRPα and CD47 are dominantly expressed in matured neurons. SIRPα is also expressed in microglia, the tissue macrophages of the brain, which play a crucial role in phagocytic clearance of damaged brain tissue, physiological role of CD47-SIRPα signal in regulation of the brain microglia has not been fully understood, however. Here we analyzed the effect of genetic ablation of SIRPα on activation of microglia in mouse brain. Microglia specific knockout of SIRPα resulted in an increase in the number of a subpopulation of activated microglia as well as of reactive astrocytes in the brain. Our data suggest that CD47-SIRPα signal is important for the proper control of glial activation in the brain.

P-29

A role of macrophage-derived HMGB1 in paclitaxel-induced peripheral neuropathy in mice

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Abstract

Chemotherapy-induced peripheral neuropathy reduces "quality of life" and is a dose limiting factor in cancer patients. Most recently, we have reported that the neuropathic pain induced by paclitaxel (PTX) or vincristine, anti-cancer drugs, involves high mobility group box 1 (HMGB1), a nuclear protein, that is released from necrotic cells or secreted by certain cells including macrophages (Mφ) in rats. Here, we evaluated and characterized the involvement of Mφ-derived HMGB1 in the PTX-induced neuropathic pain in mice. Repeated i.p. administration of PTX caused delayed neuropathic hyperalgesia, which was inhibited by an anti-HMGB1 neutralizing antibody, ethyl pyruvate (EP), known to inhibit HMGB1 release from Mφ, liposomal clodronate that depletes Mφ, or pyrrolidine dithiocarbamate (PDTC), an NF-κB inhibitor. PTX treatment caused Mφ accumulation in the sciatic nerve. The antagonists of RAGE and CXCR4, but not TLR4, among the three pronociceptive well-known membrane targets for HMGB1, prevented PTX-induced hyperalgesia. In Mφ-like RAW264.7 cells, PTX or lipopolysaccharide (LPS) caused translocation of the nuclear HMGB1 to the cytoplasm followed by its secretion. EP or PDTC inhibited the HMGB1 release induced by PTX or LPS. Pyridone 6, a JAK inhibitor, or STO609, a CaMKK inhibitor, inhibited the HMGB1 release by LPS, but not by PTX. Our data suggest that the PTX-induced peripheral neuropathy involves NF-κB-dependent secretion of HMGB1 by Mφ, followed by the activation of RAGE and CXCR4.

P-30

Small-molecule inhibition of PTPRZ reduces tumor growth in a rat model of glioblastoma

Author(s)

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Abstract

Protein tyrosine phosphatase receptor-type Z (PTPRZ) is aberrantly over-expressed in glioblastoma and a causative factor for its malignancy. However, small molecules that selectively inhibit the catalytic activity of PTPRZ have not been discovered. We herein performed an in vitro screening of a chemical library, and identified SCB4380 as the first potent inhibitor for PTPRZ. The stoichiometric binding of SCB4380 to the catalytic pocket was demonstrated by biochemical and mass spectrometric analyses. We determined the crystal structure of the catalytic domain of PTPRZ, and the structural basis of the binding of SCB4380 elucidated by a molecular docking method was validated by site-directed mutagenesis studies. The intracellular delivery of SCB4380 by liposome carriers inhibited PTPRZ activity in C6 glioblastoma cells, and thereby suppressed their migration and proliferation in vitro and tumor growth in a rat allograft model. Therefore, selective inhibition of PTPRZ represents a promising approach for glioma therapy.

P-31

Evaluation of the measurement method of intracellular calcium ion concentration in fission yeast

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Abstract

Elucidating molecular mechanisms that underlie the effects of certain metals or metalloids on human health is very important. Intracellular calcium ions affect protein functions and structures, which is related to the activation of transcription factors and enzymes. Particularly, calcium ions play a major role in intracellular signal transduction cascade by activating various intracellular signaling molecules (*e.g.* protein kinase C). Therefore, we developed the method to quantify intracellular calcium ion in fission yeast. The effects of HNO_3, zymolyase, and westase treatment on cytolysis in fission yeast were investigated. Moreover, we investigated the changes in the intracellular calcium ion concentrations in fission yeast treated with/without micafungin. The lysis of fission yeast was achieved by treatment with 36 % HNO_3. The treatment is very simple and cheaper than other similar treatments. In addition, the intracellular calcium ion concentration in fission yeast treated with 36 % HNO_3 was directly measured by inductively coupled plasma atomic emission spectrometry (ICP-AES). This study provides important information required for the measurement of intracellular calcium ion concentration in fission yeast, which is useful for elucidating the physiological or pathological roles and functions of calcium ion in the biological systems.

P-32

A simple method for preparing nonphosphorylated protein kinases using E. coli strain BL21(DE3, pλPP) which constitutively expresses λPPase

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Abstract

The E. coli expression system is very useful for a large-scale preparation of recombinant proteins. In some cases, however, protein kinases are often (auto)phosphorylated in E. coli cells, and thus prepared as phosphorylated forms, which can be artifacts associated with overexpression in prokaryotic cells. The unfavorable phosphorylation sometimes hampers enzymatic characterization or crystallographic studies of the kinases. To circumvent this problem, here, we established an expression system using E. coli strain BL21(DE3, pλPP), in which λPPase is constitutively expressed, but can be completely removed by an affinity chromatography. First, we examined whether PKL01, a plant Ndr kinase reported to be autophosphorylated in E. coli, could be readily prepared as a dephosphorylated form using this system. Because of autophosphorylation, PKL01 prepared from BL21(DE3, pLysE) was shifted up on SDS-PAGE, and it was also detected by an anti-phosphotyrosine antibody. On the other hand, no shift-up of PKL01 band was observed on SDS-PAGE, and it was not detected by an anti-phosphotyrosine antibody, when it was prepared using BL21(DE3, pλPP). These data indicate that λPPase expressed in E. coli can act on the coexpressed PKL01 to prevent its autophosphorylation. Next, we examined whether BL21(DE3, pλPP) was effective to prepare other protein kinases (CaMKIδ, DYRK1A, CoPK02) as dephosphorylated forms. These kinases were expressed as highly autophosphorylated forms in BL21(DE3) or BL21(DE3, pLysE), whereas they were readily prepared as dephosphorylated forms using BL21(DE3, pλPP). Thus, this simple method using BL21(DE3, pλPP) to prepare nonphosphorylated protein kinases would be valuable for biochemical analysis of various protein kinases.

P-33

Extra-mitochondrial function of cleaved PGAM5

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Abstract

Phosphoglycerate mutase family member 5 (PGAM5) is a new type of protein phosphatase that exists in mitochondria. We have been focusing on its role as a molecule involved in the sensing system of mitochondrial stress and dysfunctions. PGAM5 exists in the inner mitochondrial membrane through its N-terminal transmembrane (TM) domain and is cleaved within the TM domain in response to the loss of mitochondrial membrane potential. It has recently been shown that the cleaved PGAM5 is released from mitochondria depending on the cellular context. However, the motion and function of cleaved PGAM5 have been largely unknown. Thus, we examined them using PGAM5 mutants lacking the TM domain (PGAM5-dTM) as models of cleaved PGAM5 in this study. PGAM5-dTM no longer existed in mitochondria and was co-localized mainly with the microtubules in the cytosol. In accordance with this localization, PGAM5-dTM, but not phosphatase-inactive PGAM5-dTM-H105A, induced stabilization of microtubules. PGAM5-dTM also existed in the nucleus to some extent, and particularly nuclear PGAM5-dTM-H105A exhibited a speckle-like pattern. We accordingly found that PGAM5 was associated with SRm160/SRRM1, a nuclear speckle protein that regulates processing and transport of mRNA. PGAM5 dephosphorylated SRm160, which was basally highly phosphorylated, and inhibited the role of SRm160 in pre-mRNA alternative splicing. These results suggest that cleaved PGAM5, depending on its phosphatase activity, mediates cellular response to mitochondrial stress by modulating the state of cytoskeleton and gene expression.

P-34

Role of Chondroitin Sulfate (CS) Modification in the Regulation of Proteintyrosine Phosphatase Receptor Type Z (PTPRZ) Activity: PLEIOTROPHIN-PTPRZ-A SIGNALING IS INVOLVED IN OLIGODENDROCYTE DIFFERENTIATION

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Abstract

Protein-tyrosine phosphatase receptor typeZ (PTPRZ) is predominantly expressed in the developing brain as a chondroitin sulfate (CS) proteoglycan. PTPRZ has long (PTPRZ-A) and short type (PTPRZ-B) receptor forms by alternative splicing. The extracellular CS moiety of PTPRZ is required for high-affinity binding to inhibitory ligands, such as pleiotrophin (PTN), midkine, and interleukin-34; however, its functional significance in regulating PTPRZ activity remains obscure. We herein found that protein expression of CS-modified PTPRZ-A began earlier, peaking at approximately postnatal days 5–10 (P5–P10), and then that of PTN peaked at P10 at the developmental stage corresponding to myelination onset in the mouse brain. Ptn-deficient mice consistently showed a later onset of the expression of myelin basic protein, a major component of the myelin sheath, than wild-type mice. Upon ligand application, PTPRZA/B in cultured oligodendrocyte precursor cells exhibited punctate localization on the cell surface instead of diffuse distribution, causing the inactivation of PTPRZ and oligodendrocyte differentiation. The same effect was observed with the removal of CS chains with chondroitinase ABC but not polyclonal antibodies against the extracellular domain of PTPRZ. These results indicate that the negatively charged CS moiety prevents PTPRZ from spontaneously clustering and that the positively charged ligand PTN induces PTPRZ clustering, potentially by neutralizing electrostatic repulsion betweenCSchains. Taken altogether, these data indicate that PTN-PTPRZ-A signaling controls the timing of oligodendrocyte precursor cell differentiation in vivo, in which the CS moiety of PTPRZ receptors maintains them in a monomeric active state until its ligand binding.

P-35

Ca^{2+}/calmodulin-dependent protein kinase phosphatase (CaMKP/PPM1F) interacts with neurofilament L and inhibits its filament association

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Abstract

Ca^{2+}/calmodulin-dependent protein kinase phosphatase (CaMKP/PPM1F) is a Ser/Thr phosphatase that belongs to the PPM family. Growing evidence suggests that PPM phosphatases including CaMKP act as complexes with other proteins to regulate cellular functions. In this study, using the two-dimensional far-western blotting technique with digoxigenin-labeled CaMKP as a probe, in conjunction with peptide mass fingerprinting analysis, we identified neurofilament L (NFL) as a CaMKP-binding protein in a Triton-insoluble fraction of rat brain. We confirmed binding of fluorescein-labeled CaMKP (F-CaMKP) to NFL in solution by fluorescence polarization. The analysis showed that the dissociation constant of F-CaMKP for NFL is 73 ± 17 nM. Co-immunoprecipitation assay using a cytosolic fraction of NGF-differentiated PC12 cells showed that endogenous CaMKP and NFL form a complex in cells. Furthermore, the effect of CaMKP on self-assembly of NFL was examined. Electron microscopy revealed that CaMKP markedly prevented NFL from forming large filamentous aggregates, suggesting that CaMKP-binding to NFL inhibits its filament association. These findings may provide new insights into a novel mechanism for regulating network formation of neurofilaments during neuronal differentiation.

P-36

Disease-associated EED Ile363Met mutation increases susceptibility to hematologic malignancies

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Abstract

EED is required for the robust activity of polycomb repressive complex 2 (PRC2) through the direct interaction with histone methyltransferase EZH2, and the binding to trimethylated H3K27 (H3K27me3). We previously reported mutated forms of EED in 3.1% with myelodysplastic syndrome and related diseases. Among these mutants, EED Ile363Met (I363M) was found to possess impaired binding ability to H3K27me3. Overexpression of I363M in cultured cells significantly decreased global H3K27me3 levels, indicating that this mutant attenuates the propagation of histone methylation repressive marks by PRC2. To investigate the role of I363M on disease pathogenesis, we generated and analyzed Eed I363M knock-in mice. The homozygotes exhibited embryonic lethality coupled with dramatically reduced global H3K27me3 levels but normal levels of H3K27me1 and K27me2. The heterozygotes increased the clonogenic capacity and bone marrow repopulating activity of hematopoietic stem/progenitor cells, indicating a cell-autonomous growth advantage of the mutant cells. In addition, murine leukemia virus-infected heterozygous mice developed malignancies at a significantly higher frequency compared to the control mice. We thus establish the link between defective EED-H3K27me3 interaction and hematologic malignancies.

P-37

Evidence that Warburg effect functions as anti-cancer barrier

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Abstract

Switching two isoform of pyruvate kinase M (Pkm), Pkm1 and Pkm2, by alternative splicing shapes the framework of cellular glucose metabolism. Pkm1 facilitates glucose oxidation rather than fermentation while Pkm2 does opposite. Cancer cells generally express Pkm2, thereby fails to maximally oxidize glucose. Using novel mouse models, here we show that such a trait of cancer is legacy from their cells of origin wherein Pkm2 is major, and the Pkm2 of normal cells functions as intrinsic barrier against cancer. In normal tissues, Pkm1 expression was licensed only in specific lineages such as neuron and muscle. Ectopic expression of Pkm1, rather than that of Pkm2, benefited tumor cell's growth in vitro, under the metabolic stress, as well as in allograft model, and also promoted tumorigenesis in mice. Small cell lung cancer (SCLC), arising from bronchial neuroendocrine cells expressing Pkm1, were highly Pkm1-positive, further supporting a concept that isoform selection of Pkm in cancer is defined by their origin. Functional analysis revealed that Pkm1, but not Pkm2, can fully support proliferation and chemo-resistance of SCLC cell-lines. These results challenge a previous notion that the Warburg effect is cancer abetting. We propose limitation of oxidative glucose metabolism in proliferative normal cells attenuates their risk for malignant transformation. Pkm1 and its effectors may be novel therapeutic target(s) for certain types of cancer such as SCLC.

P-38

Elucidation of adipogenesis by the H3K27 histone demethylase Utx

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Abstract

Understanding the molecular mechanisms that regulate adipogenesis is important for research of obesity and diabetes. Epigenetic regulations direct the capacity of adipogenesis. In this study, we examined the role of a histone H3 lysine 27 demethylase, Utx, in the differentiation of mouse embryonic stem cells (mESCs) to adipocytes. Using gene trapping, we generated Utx-deficient male mESCs to determine whether loss of Utx would impact the differentiation of mESCs to adipocytes. We discuss the roles of Utx depend on the stages of adipocyte differentiation.

P-39

Interaction of SHP2 with ALK regulates oncogenicity of neuroblastoma cells

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Abstract

Recently, oncogenic gene alterations of src homology 2 domain-containing phosphatase 2 (SHP2) as well as anaplastic lymphoma kinase (ALK) were frequently found by genome wide screening of clinical neuroblastoma samples. We have been analyzing the oncogenic signaling mediated by ALK in neuroblastoma through the phosphoproteomic approach. During the screening of phosphotyrosine-containing proteins associated with ALK in neuroblastoma cells, we identified SHP2 as one of the binding partners of ALK. Immunoprecipitation study revealed binding of SHP2 with ALK by ALK activity-dependent manner, and knockdown of ALK or inhibition of kinase activity of ALK by ALK inhibitors suppressed phosphorylation of SHP2 at Tyr540 and Tyr580 in NB-39-nu neuroblastoma cells which have ALK addiction. In addition, knockdown of ALK-binding docking protein ShcC resulted in decrease of ALK-SHP2 interaction. On the other hands, treatment of SHP2 inhibitor PHPS1 or knockdown of SHP2 resulted in down-regulation of ERK1/2 activation, proliferation and migration of NB-39-nu cell. However, phosphorylation of ALK was up-regulated by inhibition or knockdown of SHP2. From these results, very complicated interplay between ALK and SHP2 during the regulation of oncogenesis of the neuroblastoma cells is suggested.

P-40

IL-18 amplifies macrophage M2 polarization, leading to enhancement of angiogenesis via up-regulation of osteopontin

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Abstract

Depending on micro-environmental stimuli, macrophage (Mφ) can generally be classified into M1 and M2 phenotypes. In clinical specimens, increases in the number of M2 Mφ and angiogenesis in tumor tissue are both positively correlated with poor prognosis of cancer patients. Interleukin (IL)-18, predominantly produced by Mφ, promotes angiogenesis and deteriorates a variety of solid cancer in which angiogenesis is a complicating factor. Osteopontin (OPN) is an extracellular matrix glycoprotein secreted by various cell types including Mφ and is overexpressed in various cancer, inducing tumor angiogenesis. However, there are no reports showing a set of relationship between Mφ, IL-18 and OPN in tumor angiogenesis. The aim of this study is to examine the role of IL-18 and OPN in Mφ polarization and its angiogenic capacity. Treatment of mouse monocyte/Mφ cell line, RAW264.7 cells, with IL-10 significantly increased the protein expressions of Mφ M2 marker and OPN, both of which are facilitated by the combination use of IL-18. Furthermore, co-culture of IL-10-induced M2 Mφ with mouse endothelial cell line, b.End5 cells, on the matrigel significantly increased the degree of tube formation, which is also enhanced by simultaneous treatment with IL-18. Interestingly, neutralizing antibody against OPN strongly suppressed increases in the surface expression of Mφ M2 marker and in the angiogenic capacity irrespective of the presence of IL-18.

In conclusion, IL-18 amplified Mφ M2 polarization, leading to the augmentation of angiogenic capacity via up-regulation of OPN.

P-41

Down-regulation of ErbB2/ErbB3 heterodimer via ERK-mediated phosphorylation of ErbB2 Thr-677 in the juxtamembrane domain

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Abstract

We have previously reported ERK-mediated feedback regulation of EGFR homodimer in lung cancer cells, in which phosphorylation of EGFR at the juxtamembrane domain Thr-669 down-regulates its constitutive tyrosine phosphorylation (Cancer Sci, 2013). In breast cancer cells, ErbB2 and ErbB3 are highly expressed and ErbB2/ErbB3 heterodimer activates downstream growth and survival signals. To unveil the regulatory mechanisms of the ErbB family is critical for the molecular targeted therapy. In this study, we further analyzed feedback regulation of ErbB2/ErbB3 heterodimer.

Constitutive tyrosine phosphorylation of both ErbB2 and ErbB3 were decreased in TPA-treated breast cancer cells and ErbB2/ErbB3 stably expressed HEK293 cells. However, Phos-tag western blot analysis showed the shift-upped bands, suggesting Ser/Thr phosphorylation. Pretreatment with vanadate, a non-selective tyrosine phosphatase inhibitor, suppressed the ErbB2/ErbB3 down-regulation, indicating the involvements of tyrosine phosphatases in this regulation. Amino acids sequence around the EGFR Thr-669 is highly conserved with ErbB2 and ErbB2 Thr-677 is thought to correspond to EGFR Thr-669. We generated a phospho ErbB2 Thr-677-specific rabbit monoclonal antibody and demonstrated phosphorylation of endogenous ErbB2 at Thr-677 in breast cancer cells. Furthermore, the substitution of ErbB2 Thr-677 to Ala diminished the down-regulation of tyrosine phosphorylation of ErbB2/ErbB3.

These results revealed ERK-mediated phosphorylation of ErbB2 at Thr-677 is responsible for feedback regulation of ErbB2/ErbB3 heterodimer and suggested the conserved feedback regulatory mechanism among the ErbB family.

P-42

The role of histone demethylase KDM4b in breast cancer stem cell

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Abstract

KDM4B is histone demethylase that targets histone H3 lysine 9 and promotes transcriptional activation. In breast cancer, hypoxia induces transcription of the KDM4b genes and the expression of KDM4b is correlate with prognosis of breast cancer. Furthermore, KDM4b induces the expression of GATA-3 which regulates tumor differentiation. KDM4b may regulate the maintenance of stemness of breast cancer stem cell. MDA-MB-175VII which is luminal type breast cancer cell line, highly expresses the cancer stem cell marker of ALDH1A1. MDA-MB-468 which is Basal type cell line, expresses CD133. siKDM4b reduced the expression of both ALDH1A1 and CD133. siKDM4b also suppresses the sphere formation of both MDA-MB-175VII and MDA-MB-468. The inhibitor of KDM4b strongly suppressed the sphere forming ability of MDA-MB-175VII and MDA-MB-468. Consequently, KDM4b may related the growth and maintenance of stemness of breast cancer stem cell, and siKDM4b or the inhibitor of KDM4b may have the therapeutic effect on breast cancer.

P-43

Analyses of the molecular function of PABP interacting protein 1 (PAIP-1) in translational regulation

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Abstract

Translation initiation is a rate-limiting step of translation and a major target for translational control in eukaryotes. Cellular mRNAs have the cap structure at the 5' end and the poly(A) tail at the 3' end. The cap binding protein, eIF4E, the RNA helicase, eIF4A, and the scaffolding protein, eIF4G, form the cap-binding eIF4F complex, which associates with the cap, leading to translation initiation by facilitating recruitment of the 40S ribosomal subunit to mRNAs. Poly(A) binding protein (PABP), an essential factor in translation, interacts with the poly(A) tail and plays a key role in mRNA stability. The interaction between PABP and eIF4G results in circularization of the mRNA to facilitate translation. PABP interacting protein 1 (PAIP1), a binding partner of PABP, is considered a positive regulator of translation by supporting the formation of circularized mRNA.

We have shown that HuD, a member of the neuronal Hu family of protein, accelerates translation of capped and polyadenylated mRNA (Fukao *et al*., 2009). This stimulatory effect of HuD on translation depends on the interaction of HuD with eIF4A and the poly(A) tail. However, the molecular mechanism underlying translational activation caused by HuD is still obscure.

Here, we focus on the molecular mechanism by which PAIP1 activates translation. The interaction between HuD and eIF4A exhibits some similar properties to those of PAIP1, which binds eIF4A and PABP. HuD does not bind to PABP directly, but binds directly to the poly(A) tail. In this study, we attempt to elucidate whether HuD and PAIP-1 stimulate translation via a similar molecular mechanism by using an in vitro translation system.

P-44

A Genome-wide Screen Reveals Genes Involved in Calcium Signaling and Glycosylation for Tolerance to SKB (Sugiura Kagobutsu B), a Novel Glycolipid with Potent Anti-tumor Activity

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Abstract

Calcineurin is a Ca^{2+}/calmodulin-dependent serine/threonine phosphatase which regulates Ca^{2+} signaling in eukaryotes. Calcineurin and MAPKs (mitogen-activated protein kinases)are highly conserved enzymes, which play central roles in various biological processes.

We demonstrated that calcineurin and MAPK act antagonistically in the Cl^- homeostasis in fission yeast, and identified various genes involved in MAPK signaling, including *pmp1*$^+$ encoding dual-specificity MAPK phosphatase (Sugiura *et al*., EMBO J. 1998), *rnc1*$^+$ encoding RNA-binding protein (Sugiura *et al*., Nature, 2003), and *rho2*$^+$ encoding Rho family GTPase (Ma, Sugiura *et al*., Mol. Biol. Cell, 2006). We further developed a chemical genetic screen to identify compounds that target MAPK signaling by utilizing a counteractive interaction between calcineurin and MAPK. One of the compounds SK (Sugiura Kagobutsu) B has a characteristic glycolipid structure and a potent anti-tumor activity against several cancer cell lines.

Here, we identified genes that modulate the tolerance to SKB. For this purpose, we screened a collection of haploid deletion mutant cells, of which 6 were sensitive to SKB. The genes mutated in these sensitive clones were involved in a number of cellular roles, such as calcium signaling and glycosylation. These include the *gmh4*$^+$ and *pvg3*$^+$ encoding galactosyltransferase, *pvg1*$^+$ encoding pyruvyltransferase, and *cta5*$^+$ encoding Ca^{2+}/Mn^{2+} transporting p-type ATPase. We will discuss the functional relationship between these genes and mechanisms of action of SKB.

P-45

Elucidation of elementary processes in which RNA-binding protein HuD stimulates the cap- poly(A) dependent translation

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Abstract

Hu proteins are RNA-binding proteins that bind to adenosine-uridine (AU)-rich elements (AREs). Hu proteins affect almost every posttranscriptional aspect of RNA metabolism, including the control of mRNA translation. HuD is one member of the neuronal Hu family of proteins and promotes neuronal differentiation. We have previously shown that HuD enhances cap-dependent translation through a direct interaction with eIF4A and poly(A). We have also shown that the stimulatory effect of HuD on neurite outgrowth depends on the interaction of HuD with eIF4A- and poly(A). However, the underlying molecular mechanism(s) and interactions are poorly understood. In particular, little is known about the relationship between HuD and PABP on mRNA. The strategy of HuD to interact with eIF4As has some similarity to that of the PAIP-1, which binds eIF4A and stimulates cap-dependent translation. However, HuD contributes to cap dependent translation in a distinct way and not by stabilizing the interaction between eIF4G and PABP because HuD dose not bind to PABP directly.

Here, we depleted PABP from the translation extracts with or without HuD by PAIP-2 mediated affinity chromatography to directly evaluate the role of PABP for HuD-mediated translation stimulation. We observe that this leads to decreased levels of translation from cap – poly(A) mRNA but in the presence of HuD suggesting that HuD stimulates cap dependent translation independently of PABP. To address this hypothesis, we are currently performing in vitro translation and mRNA pull-down assays using HeLa cell extracts that are depleted of PABP. The status of these experiments will be discussed.

P-46

Lung epithelial cell apoptosis induced by increased ectodomain shedding of cell adhesion molecule 1 in the lungs of emphysema and idiopathic interstitial pneumonia

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Abstract

Emphysema and interstitial pneumonia are potential risk factors for lung cancer; however, the underlying mechanism remains unknown. Cell adhesion molecule 1 (CADM1) is lung epithelial adhesion molecule, which belongs to immunoglobulin superfamily. It is enzymatically shed at its juxtamembranous ectodomain to produce three types of membrane-bound C-terminal fragments (alpha, beta, gamma-CTFs). In the present study, we examined CADM1 expression in the lungs of emphysema and idiopathic interstitial pneumonia, and found that CADM1 ectodomain shedding increased in both cases. In emphysematous lung, the expression levels of alpha, beta, gamma-CTFs increased. alpha or gamma-CTFs internalized into lung epithelial cells, accumulated in mitochondria, and led apoptosis. We also demonstrated that accumulated alpha-CTF depolarizes the mitochondrial outer membrane potential to activate mitochondrial apoptotic pathway. Through these events, CADM1 is thought to be involved in the progression of peripheral airspace enlargement, a hallmark of emphysema. In idiopathic interstitial pneumonia lungs, the increase of CADM1 alpha-shedding resulted in the decrease of full length CADM1. Silencing of full length CADM1 led lung epithelial cell apoptosis, which assumed to be an early pathogenic event in the development of this idiopathic disease. Interestingly, in these two diseases, an imbalanced protease activity has been reported. Since CADM1 is a tumour suppressor in lung cancer, our studies suggested that the protease-mediated shedding of CADM1 is an important process for alveolar epithelial cell degeneration and tumorigenesis in emphysema and interstitial pneumonia.

P-47

High affinity RNA for capping enzyme of Saccharomyces cerevisiae

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Abstract

The m7GpppN 5' cap of eukaryotic messenger RNA (mRNA) is essential for cell viability from yeast to mammals, and is the first cotranscriptional modification of cellular pre-mRNA. The cap structure plays a critical role in mRNA maturation, nuclear export of mRNAs, and efficient translation of the majority of cellular mRNAs. The mRNA cap is formed by a series of three essential enzymatic activities. The first step is the hydrolysis of the γ-phosphate from the 5'-triphosphate end of the nascent transcript by RNA 5'-triphosphatase (RTase). The second step is the transfer of the GMP portion of GTP to the diphosphate end of the RNA by RNA guanylyltransferase (GTase). The third step is the methylation of the N7 position of the guanine base by RNA (guanine-7)-methyltransferase to produce m7GpppN. These three enzymes are encoded by separate genes in fungals whereas the first two steps are catalyzed by a single polypeptide chain in metazoans. In Saccharomyces cerevisiae, the first two steps of mRNA capping are catalyzed by the RNA triphosphatase (Cet1p) and the RNA guanylyltransferase (Ceg1p). Recently, we reported that the interaction of Cet1p and Ceg1p is critical for both nuclear localization and activity of the mRNA capping enzyme complex in budding yeast. This has prompted us to develop new antifungal drugs targeted to the Cet1p-Ceg1p interaction, which plays essential roles in the cell. In this study, to create a novel molecule that inhibits or modulates the activity of Cet1p, we chose to generate RNA aptamers with high affinity for Cet1p by in vitro RNA selection-amplification. Using these RNAs, we investigated the sequence and conformational requirements in both the RNA aptamers and Cet1p for their high affinity interactions, and a possible constraint by the RNA on the RTase of Cet1p.

P-48

Expression of amino-acid transporters, adhesion molecules and oncogene products in human cancers revealed by novel mAb

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Abstract

Overcome of cancer heterogeneity is important in both cancer diagnosis and therapy. We have focused on monoclonal antibody (mAb)-defined cancer cell-surface molecules containing amino-acid transporters (LAT1 and ASCT2), adhesion molecules (EpCAM) and oncogene products (HER1, HER2, HER3 and MET) expressed on various human epithelial cancers. Flow cytometry (FCM) analysis of various cancer cell lines with mAb showed coordinated expression between LAT1 and ASCT2 co-transporters. FCM analysis of mAb with breast cancer cell lines showed reciprocal expression between MET and HER3, but positive correlation of protein expression between HER1 and MET in these breast cancers. Furthermore, breast cancers could be classified into HER3-low, MET-high basal-like subtype and HER3-high, MET-low luminal-like subtype, and this trend could be substantiated by mRNA expression by microarray analyses. EpCAM was detectable in various basal and luminal breast cancer subtypes, but not in claudin-low-type subtype. Taken together, combinations of mAb can break antigenic heterogeneity in a given cancer, in conjunction with existing anti-cancer drugs.

P-49

Butyrate response factor 1 induces translation repression independently of ARE-mediated mRNA decay

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Abstract

In mammal, posttranscriptional gene expression is spatiotemporally fine-tuned by the cis-regulatory elements in mRNA sequences and trans-acting factors (e.g. RNA binding proteins). AU-rich element (ARE) is one of the well analyzed cis-regulatory elements and induces mRNA destabilization. Tristetraprolin (TTP) and Butyrate response factor 1 (BRF1) are ARE-binding proteins (ARE-BPs) and destabilize their target mRNAs through the recruitment of CCR4/NOT deadenylation complex. However, the coupling mechanism between translation and mRNA degradation is not well understood. We have previously developed the cell-free system derived from mammalian cell lines for monitoring translation and mRNA stability simultaneously. Using this system, we have shown that the neuronal Hu protein HuD can stimulate cap dependent translation in a eIF4A- and poly(A)- dependent manner[1], and attenuate microRNA-mediated translation repression via eIF4A-binding[2].

Here, we showed that BRF1 represses translation independently of ARE-mediated mRNA decay induced by CCR4/NOT deadenylation complex. Interestingly, the BRF1 mediated translational repression was canceled by HuD. The molecular mechanism underlying translational repression caused by BRF1 would be discussed.

[1]Fukao *et al*.,2009, [2]Fukao *et al*.,2014,

P-50

The diagnosis of an innovation cancer by the antibody secretion hybridoma transplantation and establishment of the treatment system

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Abstract

Heavy chain (hc) of CD98 (CD98hc; SLC3A2) disulfide-links to one of six light chains (CD98lcs; SLC7A5-8, 7A10 and 7A11). L-type amino-acid transporter 1 (LAT1; SLC7A5) is the first identified CD98lc, and CD98hc-LAT1 complex (CD98C5) has been recently identified as an oncogenic complex. CD98hc-xCT complex (CD98C11) has also been identified as a responsible molecule for the survival of cancer stem cells (CSC) expressing variant forms of CD44 (CD44v). They are now considered to be a promising target for cancer treatment, therefore, we analyzed therapeutic and diagnostic potential of anti-mouse CD98hc monoclonal antibodies (mAb) reacting with both CD98C5 and CD98C11. We have recently developed hybridoma-therapy model system enabling rapid evaluation of in vivo anti-tumor effect of mAb by the inoculation of relevant hybridoma cells to immunodeficient mice. In connection with this strategy, we have immunohistochemically evaluated in vivo targeting of mAb in mice grafted with tumors followed by hybridoma inoculation. Our study will contribute to rapid analyses of anti-tumor effect, side effect and localization to normal and tumor tissues by anti-tumor mAb.

P-51

Analyses of cell type specific translation from IRES mRNA derived from two different poliovirus strains

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Abstract

Protein synthesis of Poliovirus (PV) is governed by the internal ribosome entry site (IRES) locating in 5' untranslated region (5' UTR) of its RNA genome. During the PV infection, viral proteases cleave eIF4G, followed by inhibition of host protein synthesis. PV IRES binds to a complex composed of eIF4A and eIF4G and recruits 40S ribosome subunit. Mahoney strain can propagate in the gut epithelial cells but also in neuronal cells, followed by degeneration and lysis of cells leading to paralytic poliomyelitis. In contrast, Sabin strain also propagates in gut but not in neuronal cells. It is conceivable that the mutations with IRES of Sabin strain reduce its propagation in neuronal cells. This means that translation from Sabin IRES mRNA is attenuated in neural cell. Therefore, we aimed to test the hypothesis of whether mutations in Sabin IRES suppress translation in neuronal cells by disturbing translation initiation complex conformation. To test this possibility, we first constructed in vitro translation system utilizing HeLa and SK-N-SH cell extracts. Secondly, we monitored the translation of both PV IRES mRNAs utilizing their extracts. Moreover, to check the presence of accelerator or inhibitor in SK-N-SH cell extract, we monitored the translation of both PV IRES mRNAs in mixture of HeLa and SK-N-SH cell extracts. The status of these experiments will be discussed.

P-52

Aiming at establishment of discrimination method of a male and female of pistachio

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Abstract

When pistachio and asparagus don't grow, a distinction of the male and female doesn't stick. So a DNA was picked out from a plant and distinction of a male and female was tried from a DNA. DNA abstraction from a plant could be done now in stable way. And it was possible to amplify DNA fragment for distinction of a male and female of asparagus by PCR method. The applicability to the pistachio is being tried.

P-53

Effect of interaction between RNA binding protein HuD and SMN protein on protein synthesis

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Abstract

The RNA binding protein HuD plays essential roles in neuronal development and plasticity. We have previously shown that HuD stimulates cap- poly(A) mRNA translation. Moreover, we recently showed that HuD impairs miRNA-mediated translational silencing. However, how HuD regulates spatiotemporal translation is little known. It has recently been shown that HuD binds to SMN protein to form mRNP complex with mRNAs of neural specific proteins that play crucial role in neuronal development, followed by contribution to their local translation in axonal processes. Here, to address the role of SMN protein in HuD-containing mRNP complexes, we assessed the effect of HuD on cap dependent translation in the presence or absence of SMN protein. The molecular mechanism underlying local translation regulated by RNA binding proteins in mRNP would be discussed.

P-54

Hepatoprotective triterpene saponin constituents from roots of Bupleurum falcatum

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Abstract

An Umbelliferae plant, Bupleurum falcatum L., is cultivated in Asia, and its root part, Bupleuri Radix ("Saiko" in Japanese), is one of the most important natural medicines in Japan. In the Japanese Pharmacopoeia XVII, the root has been used for antiinflammatory, anti-pyretic, and anti-hepatotoxic effects in the treatments of common cold, fever, and hepatitis. We have found that MeOH extract of the roots of B. falcatum showed inhibitory activity in D-galactisamine (D-GalN)-induced cell damage in hepatocytes. Through bioassay-guided separation, several triterpene saponins were identified as the bioactive constituents. Furthermore, hepatoprotective effects of major active saponins against D-GalN/lipopolysaccharide (LPS)-induced liver injury in mice were also examined.

From the MeOH extract of the roots of B. falcatum cultivated in Sichuan province, China, a new and 20 known saponins were isolated. The structure of a new saponin was determined on the basis of spectroscopic properties and chemical evidence. Among the isolates, saikosaponins b3 and b4, and bupleuroside IX significantly inhibited at 1.0-3.0 μg/mL in D-GalN-induced cytotoxicity. In addition, major saikosaponins, such as saikosaponins a, c, and d, were found to significantly inhibit the liver injury.

The data demonstrated that saponin constituents from B. falcatum roots could protect against D-GalN and D-GalN/LPS-induced liver injury.

P-55

Inhibitory Effects of Oligostilbenoids from the Bark of Shorea roxburghii on Malignant Melanoma Cell Growth: Implications for Novel Topical Anticancer Candidates

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Abstract

Human malignant melanomas remain associated with dismal prognosis due to their resistance to apoptosis and chemotherapy. There is growing interest in plant oligostilbenoids owing to their pleiotropic biological activities, including anti-inflammatory, antioxidant, and anticancer effects. Recent studies have demonstrated that resveratrol, a well-known stilbenoid from red wine, exhibits cell cycle-disrupting and apoptosis-inducing activities on melanoma cells. The objective of our study was to evaluate the anti-melanoma effect of oligostilbenoids isolated from the bark of Shorea roxburghii.

Among the isolates, four resveratrol oligomers, i.e., (–)-hopeaphenol, vaticanol B, hemsleyanol D, and (+)-α-viniferin, possessed more potent antiproliferative action than did resveratrol against SK-MEL-28 melanoma cells. Cell cycle analysis revealed that (–)-hopeaphenol, hemsleyanol D, and (+)-α-viniferin arrested cell division cycle at the G1 phase, whereas vaticanol B had little effect on the cell cycle. In addition, cell proliferation assay also revealed that (+)-α-viniferin induced apoptosis in SK-MEL-28 cells, which was confirmed by an increased expression of cleaved caspase-3. The compounds vaticanol B, hemsleyanol D, and resveratrol significantly increased the expression of p21, suggesting that they are able to block cell cycle progression. Moreover, these oligostilbenoids downmodulated cylin D1 expression and extracellular signal-regulated kinase (ERK) activation. Furthermore, hemsleyanol D, (+)-α-viniferin, and resveratrol significantly decreased the expression of cyclin B1, which could also suppress cell cycle progression.

The present study thus suggests that these plant oligostilbenoids are effective as therapeutic or chemopreventive agents against melanoma.

P-56

Limonoids from Brazilian folk medicine, Andiroba, with fat metabolizing activity in hepatocytes

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Abstract

Limonoids, a series of structurally diverse and highly oxygenated tetranortriterpenes, are mainly found in the Meliaceae family and have attracted attention from biogenetic and synthetic perspectives. Carapa guianensis Aublet (Meliaceae), known locally as andiroba, is distributed in the tropical rainforests of countries such as Brazil and Colombia. Extracts of the bark, flowers, and seeds have been used for centuries by the Amazonian people and exhibit various effects: anti-bacterial, anti-cancerous, anti-tumor, anti-malarial, anti-inflammatory, and so on. In the course of our studies on the chemical constituents from C. guianensis, we have isolated several limonoids from flower oil and seed oil.

Fatty liver is recognized as a significant risk factor for serious liver disease. There is a strong causal linkage between fatty liver disease and hyperinsulinemic insulin resistance. Thus, fatty liver is considered to be highly associated with obesity and type 2 diabetes. We further evaluated the principal gedunin-type limonoids from the oil of C. guianensis. In results, gedunin and 7-deacetoxy-7-hydroxygedunin showed to reduce triglyceride content markedly. Furthermore, structure-activity relationships on the reduction of triglyceride content in high glucose-pretreated HepG2 were revealed partly. The relationships indicated that functional groups of 6 and 7 position of the limonoids affect in the activity.

P-57

Mysterious eukaryotic translation initiation factor eIF4H

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Abstract

The assembling of eIF4F complex is the rate limiting step of translation initiation in eukaryotes. eIF4F complex is composed of the cap-binding protein eIF4E, the scaffold protein eIF4G and the RNA helicase eIF4A. Once eIF4F complex has been recruited to mRNA, eIF4A unwinds mRNA secondary structures within the 5'UTR. Then, 43S PIC recruits to mRNA efficiently. ATPase-dependent helicase activity of eIF4A is low by itself. In order to obtain sufficient helicase activity, eIF4A needs interactions with either eIF4H or eIF4B. eIF4H and eIF4B are expressed ubiquitously, and their expression level varies dependent of tissue. eIF4H has two splicing variants, eIF4H1 (27kDa) and eIF4H2 (25kDa). eIF4H2 lacks 20 residues compared with eIF4H1. eIF4Hs and eIF4B have highly conserved amino acid sequence, for example RNA recognition motif (RRM) within the N-terminal region. Although eIF4B is stimulated by phosphorylation via Akt/mTOR pathway, eIF4Hs do not require phosphorylation for their activity. Little is known how eIF4Hs stimulate unwinding activity of eIF4A differently by eIF4B. We performed in vitro cap-dependent translation assays and analyzed the functional difference between eIF4Hs and eIF4B. The status of these experiments will be discussed.

P-58

Diterpenoids from the Aerial Part of Isodon trichocapus with Melanogenesis Inhibitory Activity

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Abstract

Melanin is one of the most widely distributed pigments and is observed in bacteria, fungi, plants, and animals. It is a heterogeneous, polyphenol-like biopolymer with a complex structure and color varying from yellow to black. Mammalian skin and hair color is determined by several factors. The degree and distribution of melanin pigmentation are recognized most important matter for the color. The role of melanin is to protect the skin from ultraviolet (UV) damage by absorbing UV light and removing reactive oxygen species. On the other hand, excessive production of melanin due to a prolonged exposure to sunlight causes dermatological disorders such as melisma, freckles, post-inflammatory melanoderma, and solar lentigines. Melanin is secreted by melanocytes distributed in the basal layer of the dermis. Melanocytes are known to be stimulated by a variety of factors including UV radiation, -melanocyte-stimulating hormone (-MSH), and a phosphodiesterase inhibitor, theophylline. In our extensive screening for agents of inhibitor of melanin production in plant materials, the methanol extract from the aerial part of Isodon trichocapus showed inhibitory activity in theophylline-induced melanogenesis in B16 4A5 mouse melanoma cells. By bioassay-guided separation, 10 diterpenoids were isolated from the extract. Among the isolates, enmein, nodosin, isodocarpin and oridonin were identified as the active constituents.

P-59

Novel target molecules for treatment of cancer of unknown primary

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Abstract

Patients with cancer of unknown primary (CUP) present with metastatic disease for which the primary site cannot be found. The prognosis of patients with CUP is usually poor for those receiving empiric treatments. However, the survival of patients can be improved if the primary site is identified and a site-specific therapy is applied. Clinically, CUPs exhibit common characteristics, such as rapid progression and a silent primary tumor, with signs and symptoms related to the metastatic site(s). Existence of such common properties prompts us to hypothesize that there may be potential biological markers that elucidate CUP as a whole.

We were previously involved in a multicenter clinical study to predict the primary site of CUP for a site-specific therapy. Based on the microarray analysis of gene expression pattern, we not only predicted the primary site for each CUP patient but also extracted 44 up-regulated genes common to each CUP showing 2.5-fold change compared to normal tissue (lymph node). To identify genes related to CUP development among these candidate genes, we performed cell-based siRNA screening.and estimated how far A549 cells change the ability of migration, and found 4 genes, GRN, MIF, PRKDC, PSMB4. Konckdown of each of these genes suppressed metastasis of implanted cells from a footpad to a popliteal lymph node in vivo.

As there is no standard treatment for CUP, drugs targeting programmed cell death-1 (PD-1) may reveal promising antitumor activity for CUP. Recent studies suggest that expression levels of PD-1's ligand, PD-L1, may be a biomarker of patient response to anti-PD-1 therapy. We also analyzed the expression of PD-L1 for the CUP tissues and found several patients with high PD-L1 expression who may be most likely respond to this novel immunotherapy.

P-60

In situ photopolymerization of polyacryrlamide gel for specific entrapment and analysis of a phosphate compounds using microchip electrophoresis

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Abstract

A method for the simultaneous concentration and separation of phosphate compounds using an Phos-tag polyacrylamide gel, fabricated in the microfluidic channel of a commercial poly(methyl methacrylate)-made microchip, is reported. This approach is based on simple photochemical copolymerization for the fabrication of a Phos-tag preconcentrator. The intersection of the poly(methyl methacrylate)-made microchip was filled with a gel solution comprising acrylamide, N,N-methylene-bis-acrylamide, and Phos-tag acrylamide, with 2,2-azobis(2-methyl-N-(2-hydroxyethyl) propionamide) as a photocatalytic initiator. In situ polymerization, near the cross of the sample outlet channel, was performed by irradiation with an LED laser beam. The Phos-tag was used to trap up to a few tens of femtomoles of phosphate compounds labeled with FITC, and the amount trapped corresponded to the amount of Phos-tag in the gel. The trapped phosphate compounds were released from the gel by high concentration of phosphate and EDTA in background electrolyte. The stacked sample components were then separated and fluorometrically detected at the end of the separation channel. Under the optimized conditions, resolution of the phosphate compounds was good, and was similar to that obtained by pinched injection. The method was applied to preconcentration and analysis of phosphorylated autacoid and peptides.

P-61

Evaluation of binding structures predicted by SDO-VS method

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Abstract

Protein kinase CK2 (CK2), a serine/threonine protein kinase, is a drug target for potentially treating a wide variety of tumors. Novel CK2 inhibitors with diverse chemical scaffolds have been identified using the solvent dipole ordering virtual screening methodand their binding structures are also predicted. For hit to seed/lead modification studybystructure-based drug design, accuracy of the predicted binding structures are very important and should be validated. Generally, to estimate the true protein-ligand complex structure computationally, lots of candidate binding structures are generatedand evaluated by a docking study. Generated poses are ranked by various kinds of scoring functions for ligand affinity estimation, and some plausible binding structures are subject to further rigorous evaluation to deduce the true binding structure. However, more convenient method are required since it is very time consuming process. Formation of protein-ligand complex is a state of equilibrium of two processes, association and dissociation. Ligands with faster association and slower dissociation velocities have higher affinity to the protein. On the other hand, the true binding structure of a ligand should show higher affinity than false binding structures. Therefore, it can be said that the true binding structure could be find among false structures by simulating association and dissociation process. In this study, we have evaluated the stabilities of binding structures by molecular dynamics simulation monitoring positional root mean squares deviation from an initial structure. Stability of binding structure obtained by the SDO-VS method are compared with those arbitrary generated by docking study.

P-62

The role of cyclophilin A as a novel therapeutic target for colorectal cancer

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Abstract

Colorectal cancer (CRC) is one of the most common cancers worldwide and many patients are already at an advanced stage when they were diagnosed. Therefore, effective novel therapeutic target for colorectal cancer treatment are required. In this study, we performed a global shotgun proteomic analysis using formalin–fixed and paraffin embedded (FFPE) colorectal cancer tissue to identify candidate proteins as therapeutic targets, and we identified 84 proteins differentially-expressed in the cancer region using semi-quantification based on spectral counting. We found cyclophilin A expression levels in the cancer regions were significantly higher than that in the non-cancer regions. Then, we examined cell proliferation, migration and invasion assays using cyclophilin A down-regulated CRC cell line to clarify the role of cyclophilin A in CRC cells. As a result of these assays, down-regulation of cyclophilin A significantly inhibited CRC cell proliferation, migration and inversion. These findings suggested that cyclophilin A might play an important role in not only cell proliferation but cell metastasis, and might be a novel therapeutic target for CRC patients.

P-63

Potential role of glycosylation in regulating biological function of PTPRA

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Abstract

Receptor-like protein tyrosine phosphatase-RA (PTPRA) is heavily glycosylated in its extracellular domain. However, the exact glycan pattern has never been explored. It also remains elusive how glycosylation may affect ligand binding, dimer formation or enzymatic activity of PTPRA. To tackle such challenging questions, we studied whether glycosylation plays a key role in regulation of PTPRA-mediated intracellular signaling. We detected two major variants of PTPRA expressed in HEK293 cells or fibroblast-like synoviocytes to be 110 kDa and 150 kDa, but not the 90 kDa species at the theoretic position in SDS-gel. Mass spectrometry-based analysis identified glycans appearing in PTPRA to be high mannose type N-glycosylation and HexNAc O-glycosylation in both 110 kDa and 150 kDa variants. The content of high mannose was then confirmed by diagnostic analysis using EndoH enzyme to react with immunoprecipitated PTPRA. To examine the potential role of N-glycosylation, we constructed the NQ mutant forms of PTPRA via site-directed mutagenesis. Our data suggested that all 7 predicted N-glycosylation sites within the extracellular domain of PTPRA were indeed modified. Moreover, we observed that PTPRA-promoted Src signaling is N-glycosylation-dependent. The interplay between glycosylation and phophotyrosine signaling for exerting biological function of PTPRA is under investigation.

P-64

Sulfonic acid formation of the active-site cysteine directs ubiquitin proteasome system-mediated degradation of myocardial protein tyrosine phosphatases

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Abstract

Protein oxidation is a natural consequence of aerobic metabolism in cells. However, oxidative modification of amino acids may lead to loss of protein function. Such unwanted protein should be removed through intrinsic machinery, presumably mediated by degradation pathways which act to maintain the quality control of cellular proteins. One such removable protein might be induced by oxidation on cysteine (Cys) residues with a low pKa characteristic. However, to date it remains unclear whether irreversible Cys oxidation, if happens under pathophysiological conditions, can drive degradation of the targeted protein. In the present study, we have demonstrated that protein tyrosine phosphatases (PTPs) including PTP1B are constitutively oxidized to the sulfonic acid state (Cys-SO3H) in normal cardiomyocytes. We examined whether such highly oxidized PTPs are undergoing ubiquitin proteasome system (UPS)-mediated degradation. Interestingly, in the presence of proteasome inhibitors, we showed that proteolysis of PTP1B was inhibited. Irreversible oxidation of the active-site Cys215 was essential for degradation of PTP1B through the UPS pathway in cardiomyocytes. We further demonstrated the underlying mechanism of stepwise Cys215 oxidation followed by ubiquitination of PTP1B using an in vitro assay. Our findings thus provide new insights into a process through which Cys oxidation directs myocardial protein turnover via UPS-mediated degradation. Identification of a specific E3 ligase that targets sulfonic acid-modified PTP1B for ubiquitination is now under study.

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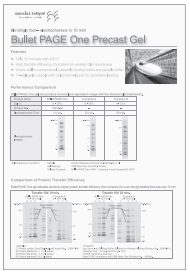

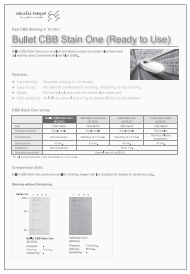

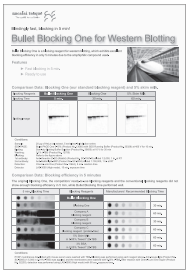

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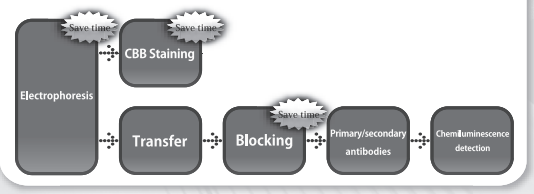

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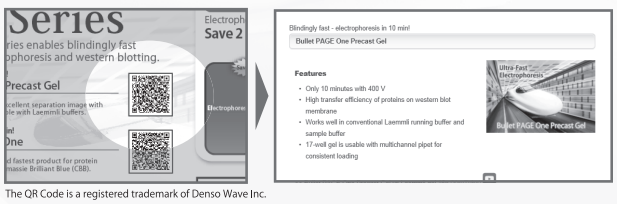

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