神戸大学グローバルCOEプログラム

次世代シグナル伝達医学の教育研究国際拠点

―基礎・臨床医学実質融合による Clinician-Scientist の育成―

Global Center of Excellence for Education and Research on Signal Transduction Medicine in the Coming Generation

― Bringing up Clinician-Scientists in the alliance between basic and clinical medicine ―

グローバルCOE最終成果報告書

神戸大学大学院医学研究科
Kobe University Graduate School of Medicine

ごあいさつ

平成20年度に採択されました本グローバルCOE「次世代シグナル伝達医学の教育研究国際拠点－基礎・臨床医学実質融合によるClinician-Scientistの育成－」も、はや5年が過ぎ、平成24年度をもって終了しました。これまで、神戸大学では、西塚泰美教授（元学長）によるリン脂質代謝とCキナーゼの発見を端緒にして、シグナル伝達医学に係る世界最高水準の研究が医学研究科とバイオシグナル研究センターを中心に展開され、この分野の世界的研究リーダーを多数輩出してきました。昨年には神戸大学医学研究科出身の山中伸弥教授がiPS細胞研究により、ノーベル生理学・医学賞を受賞されました。

「シグナル伝達医学」は生体の恒常性を維持する情報システムから疾患を捉え、疾患メカニズム、診断、治療、予防について研究する分野です。シグナル伝達医学の進歩は、個々の疾患の理解を格段に深める一方で、従来の疾患別・専門分野別のアプローチでは解明できない疾患の複雑性を浮き彫りにしました。本課題における拠点形成の目的は、本学におけるこれまでのシグナル伝達医学の成果を踏まえて、全て新しい視点とアプローチにより、疾患の病態を捉え、その本質を理解すること、革新的医療戦略を構築すること、さらに、次世代の医学・医療のリーダーとなる人材を育成することでした。そのために、基礎･臨床医学の実質的な融合を基にした分野横断的・統合的なアプローチにより、社会的に根本的な解決が急務となっているがん、代謝疾患、感染症、神経・筋疾患を対象とし、それらの疾患が互いに関わり合う核心メカニズムの解明、並びに画期的な診断・治療・予防法の確立を目指すと同時に、新分野を創成する能力を有するClinician-Scientist･医学研究者の育成を目指したシグナル伝達医学の教育研究国際拠点の形成を進めてきました。この5年間で、教授9名、准教授5名、助教2名（計16名）の基礎･臨床融合教官を配置するとともに、共同研究棟改修に伴い、基礎･臨床融合研究スペースを約2500平米確保し、研究者とスペースを一体化した実質的融合システムを構築することが出来ました。特記することとして、基礎・臨床融合教授である清野進拠点サブリーダーが、平成23年の秋の褒章で紫綬褒章を受章し、本拠点の若手研究者からテニュアポストに4名（教授1名、准教授2名、講師1名）を輩出しました。また、本拠点では、女性研究者の積極的な支援を進め、採用者の25.4％は女性を採用しました。成果発信も、Cell 1件、Science 2件、Nature 1件、Nature姉妹誌 5件、Proc Natl Acad Sci USA 6件、J Biol Chem 21件、Lancet 1件、J Clin Invest 4件等、基礎及び臨床の国際的一流学術誌に多くの論文発表が出来ました。

以上の実績により、平年22年度の中間評価ではA評価を頂くとともに、平成24年12月に開催された、国内外の著名なClinician-Scientistと海外の大学院教育専門化から構成される国際外部評価委員会（国外3名、国内4名）において、大学院教育体制と内容、若手研究者育成体制、ならびに、研究成果について世界最高水準の国際的拠点形成に向けて着実な成果を挙げているとの高い評価を得ました。この度、最終成果報告書を作成し、皆様に本拠点活動をご紹介すると同時に、私たち自身も、今回形成した、基礎・臨床医学実質融合による教育研究国際拠点を基に、今後も、Clinician-Scientist育成に全力を挙げて取り組む所存です。

神戸大学グローバルCOEプログラム
「次世代シグナル伝達医学の教育研究国際拠点」
拠点リーダー　東　　健

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1

プログラム概要

グローバルCOEプログラムの活動概要

拠点形成の目的

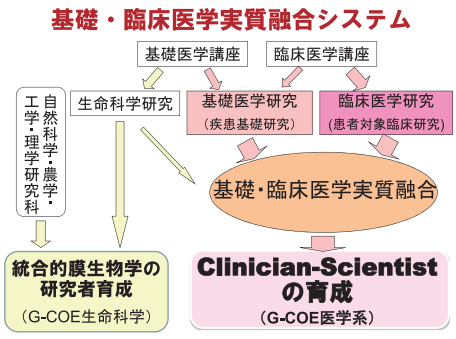
「シグナル伝達医学」は生体の恒常性を維持する情報システムから疾患を捉え、疾患メカニズム、診断、治療、予防について研究する分野である。シグナル伝達医学の進歩は個々の疾患の理解を格段に深める一方で、従来の疾患別・専門分野別のアプローチでは解明できない疾患の複雑性を浮き彫りにした。本拠点形成の目的は、本学におけるこれまでのシグナル伝達医学の成果を踏まえて、全く新しい視点とアプローチにより、疾患の病態を捉え、その本質を理解すること、革新的医療戦略を構築すること、さらに、次世代の医学・医療のリーダーとなる人材を育成することである。そのために、基礎・臨床医学の実質的な融合を基にした分野横断的・統合的なアプローチにより、社会的に根本的な解決が急務となっているがん、代謝疾患、感染症、神経･筋疾患を対象とし、それらの疾患が互いに関わり合う核心メカニズムの解明、並びに画期的な診断・治療・予防法の確立を目指すと同時に、新分野を創成する能力を有するClinician-Scientist・医学研究者の育成を目指した、シグナル伝達医学の教育研究国際拠点を形成する。

教育体制

従来の基礎･臨床医学講座の教員を主に、(a)生命科学研究、(b)基礎医学研究(実験を主とする疾患基礎研究)、(c)臨床医学研究(患者を対象とする臨床研究)を行う教員に分け、(b)と(c)の教員を同一講座に実質的に配置して教育研究を行う、基礎医学と臨床医学を融合させた、新しい分野横断的・統合的な教育研究体制を構築する。

大学院教育

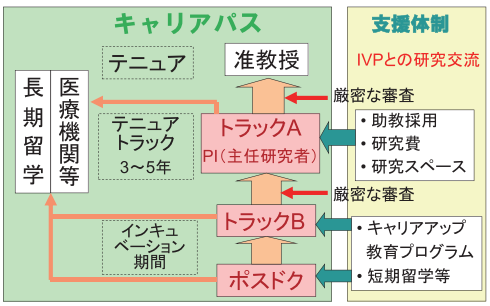
専攻･講座･分野横断型のClinician-Scientist・医学研究者リーダー育成コースを設置し、博士課程1、2年次学生から英文リサーチプロポーザルの厳正な審査を行い、毎年7名以内の優秀者を選抜し、自主的研究費の支給･経済的支援並びにコーディネーターを中心とした適切な教育研究指導を行うことにより、独創性を持った国際的活動能力を有するClinician-Scientist・医学研究者を育成する。グローバルな観点から大学院のレベルをより充実するために、国外から世界トップレベルのClinician-Scientistや医学研究者をInternational Visiting Professor (IVP)として定期的に招聘し、大学院セミナーの開催や大学院生と短期集中的に議論する機会を設ける。また、リサーチプロポーザルについても助言を受ける。



若手研究者独立支援

従来型ポスドク制度、及びポスドク終了後のClinician-Scientist・医学研究者を対象にしたトラックＡとトラックＢの3段階システムを並行して実施する。トラックＡは、3又は5年後に評価を経てテニュアポスト(准教授)へ移行するテニュアトラックであり、主任研究者(PI)として若手研究者(ポスドク)を採用し、研究面での完全な独立性を保障し、研究費とスペースを配分する。トラックＢは、Ａへ移行するインキュベーション期間として位置付け、主に所属するPIの研究に従事しつつ、将来PIとなるための萌芽的研究を展開する。いずれも採用は世界公募又は他薦とし厳正な審査を前提とするが、一部は拠点内の最優秀学生･ポスドクが本学で指導的活動を継続できるキャリアパスを設定する。各段階からの移行については数値目標を設定することによりキャリアパスとして明確に位置付け、流動性を十分配慮して運用する。本拠点における採用者の20%以上を女性とする。

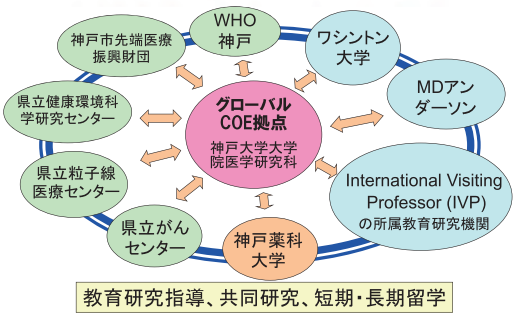
若手研究者(Clinician-Scientist)の育成システム

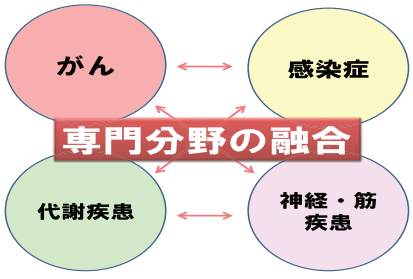


研究

がん、代謝疾患、感染症、神経･筋疾患を対象にして、全く新しい視点による疾患メカニズムの解明と医療戦略の構築を目指したシグナル伝達医学研究を展開する。そのために、分野横断的・統合的研究チームを編成し、独創的な発想に基づいた共同研究を推進する。病理診断･遺伝子診断･質量分析･画像解析を包括的に行う総合診断センターと密接に連携し、研究活動を推進、強化する。また、全研究期間を通じ、参加研究者による研究発表・交流会を定期的に開催し、横断的な知識及び方法論を共有すると共に地域・国際連携による共同研究を推進する。さらに、IVPの研究機関との共同研究の実施やポスドクや教員の双方向性交流を推進し、国際連携を強化する。

国内外の機関との連携





2

研究成果

事業推進担当者

テニュアトラック教員

グローバルCOE研究員（リサーチ・アソシエイト）

Achievements of individual member of Global COE Program for Education and Research on Signal Transduction Medicine in the Coming Generation(2008-2013)

Takeshi Azuma

Division of Gastroenterology, Department of Internal Medicine

1- Summary

I have been working on several projects related with gastrointestinal inflammation and cancer in these five years showing in the references. I show the recent progress in the gastric carcinogenesis induced by *Helicobacter pylori* infection in this progress report.

H. pylori is a group I carcinogen in human. CagA is a most important virulent factor of *H. pylori* associate with gastric cancer. CagA is directly injected from the *H. pylori* into the cells via the bacterial type IV secretion system and undergoes tyrosine phosphorylation in the host cells. We discovered that translocated CagA forms a physical complex with SHP-2, and stimulates phosphatase activity. SHP-2 is known to play an important positive role in mitogenic signal transduction. Deregulation of SHP-2 by CagA may induce abnormal proliferation and movement of gastric epithelial cells. The CagA protein is polymorphic. We discovered that predominant CagA proteins isolated in East Asia, where gastric cancer is prevalent, have a distinct sequence at the phosphorylation site of CagA. East Asian-specific sequence confers stronger SHP-2 binding and transforming activities to Western CagA. We examined the CagA diversity of *H. pylori* isolated from chronic gastritis and gastric cancer patients in Asian countries. The prevalence of East Asian CagA was significantly higher in patients with gastric cancer than in patients with chronic gastritis. The risk for gastric cancer associated with CagA-positive *H. pylori* infection was 6.0. In contrast, the risk associated with East Asian CagA-positive *H. pylori* infection was 11.8. The much greater magnitude of risk observed with East Asian CagA-positive *H. pylori* infection. Therefore, patients harboring East Asian CagA-positive *H. pylori* are at a higher risk for developing gastric cancer than those infected with Western CagA-positive strains. In addition, we sequenced whole genome of 4 Japanese strains. Phylogenetic analysis revealed greater divergence between the East Asian (hspEAsia) and the European (hpEurope) genomes in proteins in host interaction, specifically virulence factors, outer membrane proteins, and lipopolysaccharide synthesis (human Lewis antigen mimicry) enzymes. These results demonstrate dramatic genome evolution within a species, especially in likely host interaction genes, and provide essential information for understanding gastric carcinogenesis induced *H. pylori* infection and designing drugs and therapies that target them.

2- Selected publication list

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4) Masuda A, Yoshida M, Shiomi H, Morita Y, Kutsumi H, Inokuchi H, Mizuno S, Nakamura A, Takai T, Blumberg RS, Azuma T. Role of Fc Receptors as a therapeutic target. **Inflamm Allergy Drug Targets** 8(1):80-86, 2009.

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6) Furuta Y, Kawai M, Yahara K, Takahashi N, Handa N, Tsuru T, Oshima K, Yoshida M, Azuma T, Hattori M, Uchiyama I, Kobayashi I. Birth and death of genes linked to chromosomal inversion. **Proc Natl Acad Sci USA** 108(4):1501-1506, 2011.

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8) Shiomi Y, Nishiumi S, Ooi M, Hatano N, Shinohara M, Yoshie T, Kondo Y, Furumatsu K, Shiomi H, Kutsumi H, Azuma T, Yoshida M. GCMS-based metabolomic study in mice with colitis induced by dextran sulfate sodium. **Inflamm Bowel Dis** 17(11): 2261-2274, 2011.

9) Seleiman YB, Yoshida M, Nishiumi S, Tanaka H, Mimura T, Nobutani K, Yamamoto K, Takenaka M, Aoganghua A, Miki I, Ota H, Takahashi S, Matsui H, Nakamura M, Blumberg RS, Azuma T. Neonatal Fc receptor for IgG (FcRn) expressed in the gastric epithelium regulates bacterial infection in mice. **Mucosal Immunology** 5(1):87-98, 2012.

10) Nishiumi S, Kobayashi T, Ikeda A, Yoshie T, Kibi M, Izumi Y, Okuno T, Hayashi N, Kawano S, Takenawa T, Azuma T, Yoshida M. A novel serum metabolomics-based diagnostic approach for colorectal cancer. **PLoS ONE** 7: e40459, 2012.

11) Mimura M, Masuda A, Nishiumi S, Kawakami K, Fujishima Y, Yoshie T, Mizuno S, Miki I, Ohno H, Hase K, Minamoto T, Azuma T, Yoshida M. AP1B plays an important role in intestinal tumorigenesis with the truncating mutation of an APC gene. **Int J Cancer** 130(5):1011-1020, 2012.

12) Yahara K, Kawai M, Furuta Y, Takahashi N, Handa N, Tsuru T, Oshima K, Yoshida M, Azuma T, Hattori M, Uchiyama I, Kobayashi I.
Genome-wide survey of mutual homologous recombination in a highly sexual bacterial species. **Genome Biol Evol.** 4(5):628-40, 2012.

13) Yahara K, Furuta Y, Oshima K, Yoshida M, Azuma T, Hattori M, Uchiyama I, Kobayashi I.
Chromosome painting in silico in a bacterial species reveals fine population structure.
Mol Biol Evol. Jun;30(6):1454-64, 2013.

Achievements of individual member of Global COE Program for Education and Research on Signal Transduction Medicine in the Coming Generation(2008-2013)

Susumu Seino

Div. Cellular and Molecular Medicine, Dep. Physiology and Cell Biology,
and Div. Diabetes and Endocrinology, Dep. Internal Medicine

1- Summary

<Outline of the project>

The pancreatic β-cell plays a central role in glucose homeostasis by secreting insulin, a key hormone in the maintenance of glucose homeostasis; failure of β-cell functions causes diabetes mellitus, a disease that is rapidly increasing globally. In this global COE, we aim 1) to clarify how intracellular signals that regulate insulin secretion are spatially and temporally integrated in β-cells; 2) to identify novel metabolic signals that regulate insulin secretion; and 3) to clarify how insulin secretory function is acquired in the process of β-cell differentiation. These issues will be addressed at the molecular, cellular, and whole organism levels.

<Major achievements>

1. The cAMP sensor Epac2 is a direct target of anti-diabetic sulfonylurea drugs.

Epac2, a guanine-nucleotide-exchange factor for the small GTPase Rap1, is involved in cAMP-induced insulin secretion. By utilizing Epac2 FRET (fluorescence resonance energy transfer) sensor, we found that Epac2 is a direct target of SUs, widely used as anti-diabetic drugs. SUs activated Rap1 specifically through Epac2. SU-stimulated insulin secretion was reduced in mice lacking Epac2, and the glucose-lowering effect of the SU tolbutamide was decreased in these mice. Thus, in addition to closure of KATP channels, which is a well-known mechanism of SU action, activation of Epac2 is also required for SU-stimulated insulin secretion. (**Zhang et al., Science, 2009**).

2. Rim2α determines docking and priming states in insulin granule exocytosis.

Rim2α was originally identified as a Rab3A-interacting molecule. We found that the interaction of Rim2α and Rab3A is required for docking, which is considered a brake on fusion events. In addition, dissociation of the Rim2α/Munc13-1 complex by glucose stimulation activated Syntaxin1, indicating that Rim2α primes insulin granules for fusion. Since Rim2α$^{-/-}$ mice exhibited impaired secretion of various hormones stored as dense-core granules, Rim2α plays a critical role in exocytosis of dense-core granules (**Yasuda et al., Cell Metab, 2010**).

3. Progranulin is a potential therapeutic target for preventing high fat diet-induced insulin resistance, adipocyte hypertrophy, and obesity.

By differential proteome analysis, we identified progranulin (PGRN) as a novel adipokine. PGRN levels were markedly increased in obese mouse models and were normalized with treatment of an insulin-sensitizing agent. Ablation of PGRN prevented mice from high fat diet (HFD)-induced insulin resistance and obesity. PGRN deficiency blocked elevation of IL-6 induced by HFD in blood and adipose tissues. Insulin resistance induced by chronic administration of PGRN was suppressed by neutralizing IL-6 *in vivo*. Thus, PGRN is a key adipokine that mediates HFD-induced insulin resistance and obesity through production of IL-6 in adipose tissue (**Matsubara et al, Cell Metab, 2012**).

4. Role of cadherin-mediated cell-cell adhesion in pancreatic exocrine-to-endocrine transdifferentiation.

Although pancreatic exocrine acinar cells have the potential to transdifferentiate into pancreatic endocrine cells, the mechanisms are poorly understood. We found that MAPK and PI3-kinase are activated by enzymatic dissociation of acinar cells, and that spherical cell clusters are formed by cadherin-mediated cell-cell adhesion during transdifferentiation. We also show that loss of the adhesion induces and maintains a dedifferentiated state in

isolated pancreatic acinar cells. Thus, disruption and remodeling of cadherin-mediated cell-cell adhesion is critical in pancreatic exocrine-to-endocrine transdifferentiation, in which the PI3-kinase pathway plays an essential role (**Minami et al, JBC, 2008**).

2- Selected publication list

1) Minami K, Okano H, Okumachi A, Seino S. Role of cadherin-mediated cell-cell adhesion in pancreatic exocrine-to-endocrine transdifferentiation. ***J Biol Chem*** 283:13753-13761, 2008.
2) Niimura M, Miki T, Shibasaki T, Fujimoto W, Iwanaga T, Seino S. Critical role of the N-terminal cyclic AMP-binding domain of Epac2 in its subcellular localization and function. ***J Cell Physiol*** 219:652-658, 2009
3) Sugawara K, Shibasaki T, Mizoguchi A, Saito T, Seino S. Rab11 and its effector Rip11 participate in regulation of Insulin granule exocytosis. ***Genes to Cells*** 14:445-465, 2009
4) Fujimoto W, Miki T, Ogura T, Zhang M, Seino Y, Satin LS, Nakaya H, Seino S. Niflumic acid-sensitive ion channels play an important role in the induction of glucose-stimulated insulin secretion by cyclic AMP in mice. ***Diabetologia*** 52:863-872, 2009.
5) Zhang CL, Katoh M, Shibasaki T, Minami K, Sunaga Y, Takahashi H, Yokoi N, Iwasaki M, Miki T, Seino S. The cAMP sensor Epac2 is a direct target of antidiabetic sulfonylurea drugs. ***Science*** 325:607-610, 2009.
6) Granot Z, Swisa A, Magenheim J, Stolovich-Rain M, Fujimoto W, Manduchi E, Miki T, Lennerz JK, Stoeckert CJ Jr, Meyuhas O, Seino S, Permutt MA, Piwnica-Worms H, Bardeesy N, Dor Y. LKB1 regulates pancreatic beta cell size, polarity, and function. ***Cell Metab*** 10:296-308, 2009.
7) Iwasaki M, Minami K, Shibasaki T, Miki T, Miyazaki J-I, Seino S. Establishment of new clonal pancreatic β-cell lines (MIN6-K) useful for study of incretin/cAMP signaling. ***J Diabetes Invest*** 1:137-142, 2010.
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12) Kim M, Platt MJ, Shibasaki T, Quaggin SE, Backx PH, Seino S, Simpson JA, Drucker DJ.GLP-1 receptor activation and Epac2 link atrial natriuretic peptide secretion to control of blood pressure. ***Nat Med*** 19:510-518, 2013

Achievements of individual member of Global COE Program for Education and Research on Signal Transduction Medicine in the Coming Generation(2008-2013)

Yasuhiro Minami

Division of Cell Physiology, Department of Physiology and Cell Biology

1- Summary

We have previously shown that Wnt5a and its receptor, Ror-family of receptor tyrosine kinases (Ror1, Ror2) play crucial roles in developmental morphogenesis by regulating convergent extension (CE) movements and planar cell polarity (PCP), and that Wnt5a/Ror-signalings are involved in the regulation of cellular functions, including cell polarity, migration, proliferation and differentiation[6, 8]. In this program, we have studied the functions of Wnt5a/Ror-signalings under physiological and pathological conditions by using *in vitro* and *in vivo* analyse, and have obtained the following novel findings.

(A) Wnt5a/Ror2 signaling mediates polarized migration of fibroblasts by activating Wnt/JNK pathway via Filamin A, associated with Ror2, in *in vitro* wound-healing assay[1].

(B) Wnt5a/Ror2 signaling mediates phosphorylation and polymerization of Dishevelled (Dvl), that is required for Wnt5a-induced cell migration of fibroblasts[9].

(C) Wnt5a/Ror2 signaling is constitutively activated in osteosarcoma cell lines and plays critical roles in regulating their invasiveness by enhancing invadopodia formation and inducing matrix metalloproteinase 13 (MMP13)[3]. *MMP13* gene induction by Wnt5a/Ror2 signaling involves activation of Src/Dvl/Rac/JNK pathway, leading to the binding of phosphorylated c-Jun and ATF2 to the *AP-1* site within *MMP13* gene promoter[14]. Wnt5a/Ror2 signaling also mediates expression of intraflagellar transport 20 (IFT20; a critical component of ciliogenesis), which is required for the formation of invadopodia and localization of Ror2 and MMP-13 at invadopodia (ms in preparation).

(D) Both Wnt5a and Ror2 are expressed during epithelial-mesenchymal transition (EMT) of A431 carcinoma cell induced by ectopic expression of Snail, EMT-associated transcription factor[12]. Subsequently, constitutively activated Wnt5a/Ror2 signaling augments their invasiveness by inducing invadopodia formation and MMP2 expression.

(E) Both Wnt5a/Ror1- and Wnt5a/Ror2-signalings play an important role in regulating stemness (proliferation, self-renewal) of neural progenitor cells (NPCs) through phosphorylation of Dvl2[15], indicating a possible role of Wnt5a/Ror-signalings may also play a role in the regulation of stemness of tissue stem cells and cancer stem cells.

(F) Ror2 plays a role in establishing PCP during tissue-genesis of skeletons through phosphorylation of another PCP component, Vangl2[11].

(G) Wnt5a/Ror2 signaling plays an important role in osteoclastogenesis by enhancing RANKL-induced signaling via Wnt/JNK pathway[16].

(H) Ror2 plays important roles in midgut elongation through an epithelial CE mechanism and in palate formation through a directional cell migration mechanism[2, 7].

(I) Wnt5a/Ror2 signaling plays important roles in kidney morphogenesis by regulating GDNF/Ret signaling during ureteric bud formation (ms in preparation).

(J) Wnt5a/Ror-signalings are activated following EMT of tubular epithelial cells during inflammation of kidney (fibrosis), and may play a role in progression of fibrosis in a mouse model (UUO: unilateral urinary obstruction) (ms in preparation). [* (A)~(E) and (F)~(J) were obtained mainly by *in vitro* and *in vivo* analyses, respectively.]

In summary, aberrant Wnt5a/Ror-signalings are involved in anomalies, progression of cancers, and possibly in inflammations.

2- Selected publication list

1) Nomachi, A., Nishita, M., Inaba, D., Enomoto, M., Hamasaki, M., Minami, Y.: Receptor tyrosine kinase Ror2 mediates Wnt5a-induced polarized cell migration by activating c-Jun N-terminal kinase via actin-binding protein filamin A. **J. Biol. Chem.** 283: 27973-27981, 2008.

2) He, F., Xiong, W., Yu, X., Espinoza, R., Liu, C., Nishita, M., Yamada, G., Minami, Y., Chen, Y.: Wnt5a regulates directional cell migration and cell proliferation via the Ror2-mediated noncanonical pathway in mammalian palate development. **Development** 135: 3871-3879, 2008.

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4) Mikels, A., Minami, Y., Nusse, R.: The Ror2 receptor requires tyrosine kinase activity to mediate Wnt5a signaling. **J. Biol. Chem.** 284:30167-30176, 2009.

5) Yamagata, K., Fujiyama, S., Ito, S., Ueda, T., Murata, T., Naitou, M., Takeyama, K., Minami, Y., O'Malley, B. W., Kato, S.: Maturation of micro RNA is hormonally regulated by a nuclear receptor. **Mol. Cell** 36: 340-347, 2009.

6) Minami, Y., Oishi, I., Endo, M., Nishita, M.: The Ror-family receptor tyrosine kinases in non-canonical Wnt signaling: Their implications in developmental morphogenesis and human diseases. **Dev. Dyn.** 239: 1-15, 2010.

7) Yamada, M., Udagawa, J., Matsumoto, A., Hashimoto, R., Nishita, M., Minami, Y., Otani, H.: Ror2 is required for midgut elongation during mouse development. **Dev. Dyn.** 239: 941-953, 2010.

8) Nishita, M., Enomoto, M., Yamagata, K., Minami, Y.: Cell/tissue-tropic functions of Wnt5a signaling in normal and cancer cells. **Trends in Cell Biol.** 20: 346-354, 2010.

9) Nishita, M., Itsukushima, S., Nomachi, A., Endo, M., Wang, Z-C., Inaba, D., Qiao, S., Takada, S., Kikuchi, A., Minami, Y.: Ror2/Frizzled complex mediates Wnt5a-induced AP-1 activation by regulating Dishevelled polymerization. **Mol. Cell. Biol.** 30: 3610-3619, 2010.

10) Lyashenko, N., Weissenböck, M., Sharir, A., Erben, R. G., Minami, Y., Hartmann, C.: Mice lacking the orphan receptor Ror1 have distinct skeletal abnormalities and are growth retarded. **Dev. Dyn.** 239: 2266-2277, 2010.

11) Gao, B., Song, H., Bishop, K., Eliott, G., Garrett, L., English, M. A., Andre, P., Robinson, J., Sood, R., Minami, Y., Economedes, A. N., Yang, Y.: Wnt signaling gradients establish planar cell polarity by inducing Vangl2 phosphorylation through Ror2. **Dev. Cell** 20: 163-176, 2011.

12) Ren, D., Minami, Y.*, Nishita, M.* (corresponding authors).: Critical role of Wnt5a-Ror2 signaling in motility and invasiveness of epidermoid carcinoma cells following Snail-mediated epithelial-mesenchymal transition. **Genes Cells**, 16: 304-315, 2011.

13) Takahashi, S., Watanabe, T., Okada, M., Inoue, K., Ueda, T., Takada, I., Watabe, T., Yamamoto, Y., Fukuda, T., Nakamura, T., Akimoto, C., T., Fujimura, T., Hoshino, M., Imai, Y., Metzger, D., Miyazono, K., Minami, Y., Chambon, P., Kitamura, T., Matsumoto, T., Kato, S.: Non-canonical Wnt signaling mediates androgen-dependent tumor growth in a mouse model of prostate cancer. **Proc. Natl. Acad. Sci. USA** 108: 4938-4943, 2011.

14) Yamagata, K., Li, X., Ikegaki, S., Oneyama, C., Okada, M., Nishita, M., Minami, Y.: Dissection of Wnt5a-Ror2 signaling leading to *matrix metalloproteinase (MMP)-13* expression. **J. Biol. Chem.** 287: 1588-1599, 2012.

15) Endo, M., Doi, R., Nishita, M., Minami, Y.: Ror-family receptor tyrosine kinases regulate maintenance of neural progenitor cells in the developing neocortex. **J. Cell Sci.** 125: 2017-2029, 2012.

16) Maeda, K., Kobayashi, Y., Udagawa, N., Uehara, S., Ishihara, A., Mizoguchi, T., Kikuchi, Y., Takada, I., Kato, S., Kani, S., Nishita, M., Marumo, K., Martin, T. J., Minami, Y., Takahashi, N.: Wnt5a-Ror2 signaling between osteoblasts and osteoclast precursors enhances osteoclastogenesis. **Nat. Med.** 18: 405-412, 2012.

Achievements of individual member of Global COE Program for Education and Research on Signal Transduction Medicine in the Coming Generation(2008-2013)

Yasuko Mori

Division of Clinical Virology, Department of Microbiology and Infectious Diseases

1- Summary

Human herpesvirus 6 (HHV-6) isolates have been classified into two variants, HHV-6A and HHV-6B, based on their genetic and antigenic differences, and their cell tropism. HHV-6B causes exanthem subitum in primary infections. The diseases caused by HHV-6A, are unknown. HHV-6B infects and remains as a life-long latent infection, in more than 90% of the general population. The homology between them is almost 90% over their entire genome. However, which HHV-6 genes are responsible for the differences in cell tropism between them remains unclear. Human CD46 has been shown to be a cellular receptor of HHV-6A, and we found that its viral ligand was a glycoprotein (g) complex of HHV-6A viral gH/gL/gQ1/gQ2.

We found that the HHV-6B gH/gL also associated with the gQ1/gQ2 complex; however, the HHV-6A gH/gL/gQ1/gQ2 complex bound to its human cellular receptor, CD46, while the corresponding complex in some HHV-6B strains seemed not to bind it.

When HHV-6A gH was replaced with HHV-6B gH in a recombinant virus, the HHV-6B gH was sufficient for normal viral growth, thus indicating that HHV-6 gH itself was not critical for HHV-6A's specific cell tropism. Therefore, We sequentially replaced each molecule of the HHV-6A complex, gH, gL, gQ1, or gQ2 with that of HHV-6B and asked first, whether the glycoprotein complex was able to form normally, and second, whether the recombinant complex could bind CD46. We found that the replacement of gH, gL, or gQ2 with the HHV-6B had no effect on complex formation or receptor binding. However, when HHV-6A gQ1 (AgQ1) was replaced with HHV-6B Q1 (BgQ1), the complex no longer bound CD46. Furthermore, the recombinant HHV-6A virus expressing BgQ1 could not be reconstituted from the genome. These results indicate that HHV-6 gQ1 is the key molecule for HHV-6A/B-specific cell tropism for the receptor recognition.

In GCOE program, I have educated one graduate student and one researcher who have been belonging to my laboratory, and also gave several advices and comments for research to two researchers and two graduate students belonging to the other departments. They have presented their data in several international conferences and their data have been published in international journals.

2- Selected publication list

1) A community-based survey of varicella-zoster virus-specific immune responses in the elderly. Tang H, Moriishi E, Okamoto S, Okuno Y, Iso H, Asada H, Yamanishi K, Mori Y. **J Clin Virol**. 55(1):46-50. 2012.

2) Complementation of the function of glycoprotein H of human herpesvirus-6 variant A by glycoprotein H of variant B in the viral life cycle. Oyaizu H, Tang H, Ota M, Takenaka N, Ozono K, Yamanishi K, Mori Y. **J Virol**. 86(16):8492-8. 2012.

3) Human herpesvirus 6 glycoprotein M is essential for virus growth and requires glycoprotein N for its maturation. Kawabata A, Jasirwan C, Yamanishi K, Mori Y. **Virology.** 429(1):21-8. 2012.

4) Poly-γ-Glutamic Acid Nanoparticles and Aluminum Adjuvant Used As an Adjuvant with a Single Dose of Japanese Encephalitis Virus-Like Particles Provides Effective Protection from Japanese Encephalitis Virus. Okamoto S, Yoshii H, Matsuura M, Kojima A, Ishikawa T, Akagi T, Akashi M, Takahashi M, Yamanishi K, Mori Y. **Clin Vaccine Immunol**. 19(1):17-22. 2012.

5) Analysis of a neutralizing antibody for human herpesvirus-6B reveals a role for glycoprotein Q1 in viral entry. Kawabata A, Oyaizu H, Maeki T, Tang H, Yamanishi K, Mori Y. **J Virol**. 85(24):12962-71. 2011.

6) Human Herpesvirus 6 Glycoprotein Complex Formation Is Required for Folding and Trafficking of the gH/gL/gQ1/gQ2 Complex and Its Cellular Receptor Binding. Tang H, Hayashi M, Maeki T, Yamanishi K, Mori Y. **J Virol**. 85(21):11121-30. 2011.

7) Human herpesvirus 6 major immediate early promoter has strong activity in T cells and is useful for heterologous gene expression. Matsuura M, Takemoto M, Yamanishi K, Mori Y. **Virol. J**. 8:9. 2011.

8) Human herpesvirus 6 encoded glycoprotein Q1 gene is essential for virus growth. Tang H, Kawabata A, Yoshida M, Oyaizu H, Maeki T, Yamanishi K, Mori Y. **Virology**. 407(2):360-7. 2010.

9) Human herpesvirus-6 entry into host cells. Tang H, Mori Y. **Future Microbiol**. 5(7):1015-23. 2010.

10) Characterization of varicella-zoster virus-encoded ORF0 gene-Comparison of parental and vaccine strains. Koshizuka T, Ota M, Yamanishi K, Mori Y. **Virology**. 405(2):280-288. 2010.

11) Rapid and efficient introduction of a foreign gene into bacterial artificial chromosome-cloned varicella vaccine by Tn7-mediated site-specific transposition. Somboonthum P, Koshizuka T, Okamoto S, Matsuura M, Gomi Y, Takahashi M, Yamanishi K, Mori Y. **Virology**. 402(1):215-21. 2010.

12) Characterization of the varicella-zoster virus ORF50 gene, which encodes glycoprotein M. Sadaoka T, Yanagi T, Yamanishi K, Mori Y. **J. Virol**. 84:3488-502. 2010.

Achievements of individual member of Global COE Program for Education and Research on Signal Transduction Medicine in the Coming Generation(2008-2013)

Mitsuhiro Ito

Division of Medical Biophysics, Department of Biophysics,
Graduate School of Health Sciences

1- Summary

Theme: Physiological role of Mediator and its implication in dysregulated signaling.

1. Background of Research: The Mediator master transcriptional coregulator, subcomplex of RNA polymerase II (pol II) holoenzyme, integrates signals of a variety of activators and intracellular signaling molecules, recruits pol II to the promoter, and eventually forms a preinitiation complex for transcription initiation (Ito et al. *Mol. Cell* 3:361-70, 1999; Ito & Roeder, *Trends Endocrinol. Metabolism* 12:127-34, 2001). We and others have found that dysfunction of any specific Mediator subunits may cause specific defects in signaling and human diseases. For example, MED1 is crucial for many biological events through the interaction with distinct activators such as nuclear receptors and GATA family activators (Ito et al. *Mol. Cell* 5:683-93, 2000; Ge, Guermah, Yuan, Ito et al. *Nature* 417:563-7, 2002, *etc*.). MED17 is responsible for signals of p53 and herpes viral activator VP16. MED23 is pivotal for signals of MAP kinase and adenoviral oncoprotein E1A, and its mutation causes intellectual disability (Ito et al. *Mol. Cell* 3:361-70, 1999; Ito et al. *EMBO J.* 21:3464-75, 2002, *etc*.). Mutation in MED12 is related to various tumors (such as uterine leiomyoma, prostate carcinoma and breast carcinoma), as well as neuropsychiatric diseases (FG and Opitz-Kaveggia syndromes). CDK8 is a colorectal cancer oncogene. In this study, we have focused on MED1 and analyzed its role in hematopoiesis, mammary gland development, and adipose metabolism.

2. Role for MED1 in erythropoiesis and hematopoietic niche: (i) We showed the dual mechanism of GATA1- and MED1-mediated transcription and erythroid differentiation. MED1 expression levels in erythroid cells paralleled the levels of GATA1-targeted gene transcription and erythroid differentiation, but the direct interaction between GATA1 and MED1 was partially redundant for this process. We found that the coactivator pair CCAR1 and CoCoA serves as a bridge between the C-terminal zinc-finger domain of GATA1 and the N-terminal domain of MED1 and bypasses the GATA1-mediated transcription and erythroid differentiation. Multiple modes of the GATA1-MED1 axis may help to fine-tune GATA1 function not only during erythropoiesis but likely during other GATA1-mediated homeostasis events (ms submitted). (ii) We showed evidence that MED1 in bone marrow (BM) niche cells is involved in supporting hematopoietic stem and/or progenitor cells (HSPCs) through osteopontin (OPN) expression. We found that the maintenance/proliferation of long-term culture-initiating cells (LTC-ICs) depends on MED1 in BM stromal cells and on the extracellular amount of OPN. MED1 in stromal cells was required for vitamin D receptor (VDR)- and Runx2-mediated expression of OPN. Consequently, MED1 in niche cells appears to play an important role in supporting HSPCs (ref. 3).

3. Role for MED1 in mammary gland development: To know in vivo functions of the LxxLL nuclear receptor recognition motifs in MED1, we have generated MED1 LxxLL motif-mutant knockin (MED1(LX) KI) mice. Although these mice did not exhibit any apparent gross abnormalities, they did exhibit severe defects in pubertal mammary gland development. These mammary epithelial cells no longer responded to estrogen stimulation. MED1 was differentially expressed in different types of mammary epithelial cells and its LxxLL motifs played a role in mammary luminal epithelial cell differentiation and progenitor/stem cell determination

(ref. 2). We also found a similar phenotype in MED1/MED24 double heterozygous knockout mice, where expression of estrogen receptor (ER)-targeted genes encoding cell cycle-related molecules E2F1 and cyclin D1 were repressed. MED1 and MED24 were abundantly expressed in various breast carcinoma cells and played a role in tumor cell growth. Thus, the MED24-containing submodule of Mediator functionally communicates specifically with MED1 and mediates ER functions and growth of both normal mammary epithelial cells and breast carcinoma cells (ref. 5).

3. Role for MED1 in adipose homeostasis: PPARγ is an important molecular target for management of obesity and diabetes. While the MED1 was essential for PPARγ 2-mediated adipogenesis, the N-terminal MED1 (MED1(1-530)) that cannot bind to PPARγ2 was sufficient for adipogenesis in cultured cells (ref. 1). To solve this discrepancy, we analyzed the MED1(LX) KI mice and showed that the receptor-binding ability of MED1 is physiologically essential for a full activity of PPARγ2 and induced adipocyte hypertrophy in a living animal. The MED1(LX) KI mice showed resistance to dietary and genetically induced obese stress with a markedly increased sensitivity to insulin, and attenuated PPARγ2-targeted gene expressions. An adaptor coactivator CCAR1 interacted both with PPARγ2 in a ligand-enhanced manner and with MED1(1-530), and CCAR1 enhanced PPARγ2-mediated transcription in a MED1(1-530)-dependent manner. Thus, dual pathways between PPARγ2 and MED1, namely, a direct interaction and a CCAR1-mediated bypass, appear to contribute to physiological adipose homeostasis, and might lead to diseased conditions. The interface for these interactions could be an attractive molecular target for treatment of obesity and diabetes (ms in preparation).

2- Selected publication list

1) K. Ge, Y.-W. Cho, H. Guo, T.B. Hong, M. Guermah, M. Ito, H. Yu, M. Kalkum, and R.G. Roeder. Alternative mechanisms by which Mediator subunit MED1/TRAP220 regulates PPARγ-stimulated adipogenesis and target gene expression. ***Mol. Cell. Biol.*** 28, 1081-1091, 2008.

2) P. Jiang, *Q. Hu, *M. Ito, S. Meyer, S. Waltz, S. Khan, R.G. Roeder, X. Zhang. Key roles for MED1 LxxLL motifs in pubertal mammary gland development and luminal-cell differentiation. ***Proc. Natl. Acad. Sci. USA*** 107, 6765-6770, 2010.
(* denotes equal contribution.)

3) A. Sumitomo, R. Ishino, N. Urahama, K. Inoue, K. Yonezawa, N. Hasegawa, O. Horie, H. Matsuoka, T. Kondo, R.G. Roeder, M. Ito. Transcriptional Mediator subunit MED1/TRAP220 in stromal cells is involved in hematopoietic stem/progenitor cell support through osteopontin expression. ***Mol. Cell. Biol.*** 30, 4818-4827, 2010.

4) T. Irino, M. Uemura, H. Yamane, S. Umemura, T. Utsumi, N. Kakazu, T. Shirakawa, M. Ito, T. Suzuki, K. Kinoshita. JAK2 V617F-dependent upregulation of PU.1 expression in the peripheral blood of myeloproliferative neoplasm patients. ***PLoS ONE*** 6, e22148, 2011.

5) N. Hasegawa, A. Sumitomo, A. Fujita, N. Aritome, S. Mizuta, K. Matsui, R. Ishino, K. Inoue, N. Urahama, J. Nose, T. Mukohara, S. Kamoshida, R. G. Roeder, M. Ito. Mediator subunits MED1 and MED24 cooperatively contribute to pubertal mammary gland development and growth of breast carcinoma cells. ***Mol. Cell. Biol.*** 32, 1483-1495, 2012.

Achievements of individual member of Global COE Program for Education and Research on Signal Transduction Medicine in the Coming Generation(2008-2013)

Kenichi Hirata

Division of Cardiovascular Medicine, Department of Internal Medicine

1- Summary

Disorder of HDL metabolism and inflammation is major risk factors for atherosclerosis. We have reported following results during the global COE program.

1) Role of Endothelial lipase (EL) in the HDL function and atherosclerosis.

We have cloned a new member of lipase, endothelial lipase (EL) produced by vascular endothelial cells. Our data in genetic modified mice of EL revealed that EL levels are inversely related to the plasma HDL cholesterol (HDL-C) levels. However, the role of EL in regulating plasma HDL-C concentrations and EL's potential involvement in atherosclerosis in humans has not been fully investigated due to the lack of reliable assays for EL mass. We developed an ELISA system for serum EL mass. The detection limit of the ELISA was 20 pg/ml. he serum EL mass in 645 human subjects was [mean (SE)] 344.4 (7.7) pg/mL (range 55.2-1387.7 pg/mL). Interestingly, serum EL mass was increased in patients with diagnosed cardiovascular disease and inversely correlated with serum HDL-C concentrations. There was no difference in EL mass between pre- and post-heparin plasma samples. This ELISA should be useful for clarifying the impact of EL on HDL metabolism and EL's potential role in atherosclerosis. These data indicate that EL is a determinant of HDL levels in humans.

2) Role of immune system in the atherosclerotic process.

We have been focusing on regulatory T cells (Tregs) and tolerogenic dendritic cells (tDCs) that are considered to down-regulate the activation of T cell responses, and to be actively generated or differentiated in the gut. We have shown that oral administration of anti-CD3 antibody or an active form of vitamin D_3 decreased atherosclerosis in mice by inducing Tregs and/or tDCs in the gut-associated lymphoid tissues. Systemic immunity was also affected and changed its phenotype. These research findings implied that the intestinal immune system might be a therapeutic target for preventing atherosclerosis. Further, we revealed that oral eicosapentaenoic acid (EPA) administration changed the phenotype of intestinal DCs to be tolerogenic and induced the regression of atherosclerosis in LDL receptor-deficient mice. In that study, we found that an enzyme associated with a tryptophan metabolism, indoleamine 2, 3-dioxygenase (IDO), was increased in intestinal DCs and inhibited the T cell proliferation. EPA also affected the maturation of intestinal DCs and reduced the expressions of co-stimulatory molecules, CD80 and CD86. These results indicated a new target molecule to induce atherosclerosis regression and the possibility to develop a new regression therapy. Taken together, modulation of the intestinal immune system could be a novel strategy for preventing systemic inflammatory diseases including CVDs.

Ultraviolet B (UVB) exposure is known to alter the function of epidermal Langerhans cells (LCs), immature dendritic cells residing in the skin, which may contribute to suppression of local and systemic immune reactions. UVB irradiation significantly reduced atherosclerosis development (aortic sinus plaque area: $1.84\pm0.20\times10^5$ vs. $2.94\pm0.31\times10^5$ μm^2, $p<0.01$) and plaque inflammation compared to controls. Interestingly, UVB-irradiated mice showed systemic expansion of $CD4^+Foxp3^+$ regulatory T cells (Tregs), which exhibited potent suppressor function, along with suppressed pathogenic T-cell immune responses. Depletion of $Foxp3^+$ Tregs in hyperlipidemic *foxp3*-diphtheria toxin receptor/*ApoE*$^{-/-}$ transgenic mice resulted in a loss of suppressed pathogenic T-cell immune responses and aortic inflammation by UVB treatment, arguing for a critical role of UVB-induced $Foxp3^+$ Tregs in the down-regulation of immune responses and possibly reduction of atherosclerosis. Furthermore, treatment of LC-depleted mice with UVB did not increase Treg numbers, indicating the critical involvement of LCs in Treg induction.

We would like to clarify the mechanisms how to interact the immune responses between the intestine, skin and atherosclerotic lesions, and hopefully apply this notion to clinical therapy.

2- Selected publication list

1) Oral anti-CD3 antibody treatment induces regulatory T cells and inhibits the development of atherosclerosis in mice. Sasaki N, Yamashita T, Takeda M, Shinohara M, Nakajima K, Tawa H, Usui T, Hirata K. ***Circulation***. 120(20):1996-2005. 2009.
2) Attenuation of Doxorubicin-induced cardiomyopathy by endothelin-converting enzyme-1 ablation through prevention of mitochondrial biogenesis impairment. Miyagawa K, Emoto N, Widyantoro B, Nakayama K, Yagi K, Rikitake Y, Suzuki T, Hirata K. ***Hypertension***. 55(3):738-46. 2010.
3) Endothelial cell-derived endothelin-1 promotes cardiac fibrosis in diabetic hearts through stimulation of endothelial-to-mesenchymal transition. Widyantoro B, Emoto N, Nakayama K, Adiarto S, Iwasa N, Yagi K, Miyagawa K, Rikitake Y, Yanagisawa M, Hirata K. ***Circulation***. 121(22):2407-18. 2010.
4) Pitavastatin decreases the expression of endothelial lipase both in vitro and in vivo.Kojima Y, Ishida T, Sun L, Yasuda T, Toh R, Rikitake Y, Fukuda A, Kume N, Koshiyama H, Taniguchi A, Hirata K. ***Cardiovasc Res***. 87(2):385-93. 2010.
5) Endothelial cell-selective adhesion molecule modulates atherosclerosis through plaque angiogenesis and monocyte-endothelial interaction. Inoue M, Ishida T, Yasuda T, Toh R, Hara T, Cangara HM, Rikitake Y, Taira K, Sun L, Kundu RK, Quertermous T, Hirata K. ***Microvasc Res***. 80(2):179-87, 2010.
6) Oral administration of an active form of vitamin D3 (calcitriol) decreases atherosclerosis in mice by inducing regulatory T cells and immature dendritic cells with tolerogenic functions. Takeda M, Yamashita T, Sasaki N, Ishida T, Hirata K. ***Arterioscler Thromb Vasc Biol***. (12):2495-503. 2010.
7) Targeted deletion of endothelial lipase increases HDL particles with anti-inflammatory properties both in vitro and in vivo. Hara T, Ishida T, Yasuda T, Toh R, Hirata K. ***J Lipid Res***. 52(1):57-67. 2011.
8) Orally administered eicosapentaenoic acid induces rapid regression of atherosclerosis via modulating the phenotype of dendritic cells in LDL receptor-deficient mice. Nakajima K, Yamashita T, Kita T, Takeda M, Sasaki N, Kasahara K, Shinohara M, Rikitake Y, Ishida T, Yokoyama M, Hirata K. ***Arterioscler Thromb Vasc Biol***. 31(9):1963-72. 2011.
9) Necl-5/poliovirus receptor interacts with VEGFR2 and regulates VEGF-induced angiogenesis. Kinugasa M, Amano H, Satomi-Kobayashi S, Nakayama K, Miyata M, Kubo Y, Nagamatsu Y, Kurogane Y, Kureha F, Yamana S, Hirata K, Miyoshi J, Takai Y, Rikitake Y. ***Circ Res***. 110(5):716-26. 2012.
10) Regulatory T cells in atherogenesis. Sasaki N, Yamashita T, Takeda M, Hirata K. ***J Atheroscler Thromb***. 19(6): 503-15. 2012.
11) FGD5 mediates proangiogenic action of vascular endothelial growth factor in human vascular endothelial cells. Kurogane Y, Miyata M, Kubo Y, Nagamatsu Y, Kundu RK, Uemura A, Ishida T, Quertermous T, Hirata K, Rikitake Y. ***Arterioscler Thromb Vasc Biol***. 32(4):988-96. 2012.
12) Osteoblast-like differentiation of cultured human coronary artery smooth muscle cells by bone morphogenetic protein endothelial cell precursor-derived regulator (BMPER). Satomi-Kobayashi S, Hatakeyama K, Asada Y, Takai Y, Hirata K, Rikitake Y. ***J Biol Chem***. 287(36):30336-45. 2012.
13) Dendritic Cells in Atherogenesis: Possible Novel Targets for Prevention of Atherosclerosis. Takeda M, Yamashita T, Sasaki N, Hirata K. ***J Atheroscler Thromb***. [Epub ahead of print] 2012.
14) Expression of Endothelial Lipase Correlates with the Size of Neointima in a Murine Model of Vascular Remodeling. Sun L, Ishida T, Okada T, Yasuda T, Hara T, Toh R, Shinohara M, Yamashita T, Rikitake Y, Hirata K. ***J Atheroscler Thromb***. [Epub ahead of print] 2012.

Achievements of individual member of Global COE Program for Education and Research on Signal Transduction Medicine in the Coming Generation(2008-2013)

Toshiaki Sakisaka

Division of Membrane Dynamics, Department of Physiology and Cell Biology

1- Summary

Title: Novel Process in Neurotransmitter Release

The main process of synaptic transmission form a neuron to another neuron is mediated by neurotransmitter release. Therefore, elucidating a series of molecular events during neurotransmitter release is important for understanding synaptic plasticity and memory. The entry of Ca^{2+} into nerve terminals initiates the series of molecular events that culminates with fusion of the synaptic vesicle and release of neurotransmitter into the synaptic cleft. SNARE proteins play essential roles in the synaptic vesicle fusion. SNARE proteins are also transported to the synaptic vesicles and the presynaptic plasma membrane as cargo proteins. Many regulatory proteins for SNARE proteins have been identified, but the molecular mechanism how SNARE proteins are posttranslationally inserted into the endoplasmic reticulum (ER) membrane remains elusive. In addition to investigating the roles of tomosyn, our originally identified SNARE regulatory protein, *in vivo* and in *vitro*, we have investigated the molecular mechanism for SNARE protein insertion into the ER membrane and obtained the results listed below.

1. We generated tomosyn-knockout mice (Sakisaka *et al*. ***J. Cell Biol.*** 2008).
2. Neurotransmitter release was enhanced in tomosyn-knockout mice.
3. Intra-molecular bindings balanced the opposite effects of N-terminal and C-terminal tomosyn on the SNARE complex formation (Yamamoto *et al*. ***J. Biol. Chem.*** 2009).
4. Interaction between tomosyn and synaptotagmin-1, a Ca^{2+}-sensor on the synaptic vesicle, controlled SNARE-mediated membrane fusion (Yamamoto *et al*. ***J. Biol. Chem.*** 2010).
5. We identified a novel protein complex (CAML complex) that drives SNARE protein insertion into the ER membrane (Yamamoto and Sakisaka. ***Molecular Cell*** 2012).

These results establish that tomosyn is a potent inhibitor of neurotransmitter release *in vivo*. The interplay between tomosyn and synaptotagmin-1 underlies inhibitory control of Ca^{2+}-dependent neurotransmitter release. In addition, we identify a novel protein complex for SNARE protein insertion into the ER membrane.

2- Selected publication list

1) Yamamoto, Y. and *Sakisaka, T. **(*correspondence)**
Molecular machinery for insertion of tail-anchored membrane proteins into the endoplasmic reticulum membrane in mammalian cells
(***Molecular Cell***, 48(3), 387-397, 2012)

2) Narita, H., Yamamoto, Y., Suzuki, M., Miyazaki, N., Yoshida, A., Kawai, K., Iwasaki, K., Nakagawa, A., Takai, Y., and *Sakisaka, T. **(*correspondence)**
Crystal Structure of the cis-Dimer of Nectin-1: implications for the architecture of cell-cell junctions
(***J. Biol. Chem.***, 286(14), 12659-12669, 2011)

3) Kurooka, T., Yamamoto, Y., Takai, Y., and *Sakisaka, T. ***(*correspondence)***
Dual regulation of RA-RhoGAP activity by phosphatidic acid and Rap1 during neurite outgrowth
(***J. Biol. Chem.***, 286(8), 6832-6843, 2011)

4) Yamamoto, Y., Mochida, S., Miyazaki, N., Kawai, K., Fujikura, K., Kurooka, T., Iwasaki, K., and *Sakisaka, T. **(*correspondence)**
Tomosyn inhibits synaptotagmin-1-mediated step of Ca^{2+}-dependent neurotransmitter release through its N-terminal WD40 repeats
(***J. Biol. Chem.***, 285(52), 40943-40955, 2010)

5) Yamamoto, Y., Fujikura, K., Sakaue, M., Okimura, K., Kobayashi, Y., Nakamura, T., and *Sakisaka, T. **(*correspondence)**
The tail domain of tomosyn controls membrane fusion through tomosyn displacement by VAMP2
(***Biochem. Biophys. Res. Commun.***, 399(1), 24-30, 2010)

6) Majima, T., Ogita, H., Yamada, T., Amano, H., Togashi, H., Sakisaka, T., Tanaka-Okamoto, M., Ishizaki, H., Miyoshi, J., and Takai, Y.
Involvement of afadin in the formation and remodeling of synapses in the hippocampus.
(***Biochem. Biophys. Res. Commun.***, 385(4), 539-544, 2009)

7) Togashi, H., Sakisaka T., and Takai, Y.
Cell Adhesion Molecules in the Central Nervous System.
(***Cell Adhesion &Migration***, 3(1), 29-35, 2009)

8) Yamamoto, Y., Mochida, S., Kurooka, T., and *Sakisaka, T. **(*correspondence)**
Reciprocal intramolecular interactions of tomosyn control its inhibitory activity on the SNARE complex formation.
(***J. Biol. Chem.***, 284(18), 12480-12490, 2009)

9) Sakisaka, T., Yamamoto, Y., Mochida, S., Nakamura, M., Nishikawa, K., Ishizaki, H., Okamoto-Tanaka, M., Miyoshi, J., Fujiyoshi, Y., Manabe, T., and Takai, Y.
Dual inhibition of SNARE complex formation by tomosyn ensures controlled neurotransmitter release.
(***J. Cell Biol.***, 183(2), 323-337, 2008)

Achievements of individual member of Global COE Program for Education and Research on Signal Transduction Medicine in the Coming Generation(2008-2013)

Yasuhiro Takeshima

Division of Pediatrics, Department of Internal Related

1- Summary

Development of molecular therapies for Duchenne muscular dystrophy.

Duchenne muscular dystrophy (DMD) is the most common form of inherited muscle disease and is characterized by progressive muscle wasting ultimately resulting in death of the patients in their twenties. DMD is characterized by a deficiency of the muscle dystrophin as a result of mutations in the dystrophin gene. Currently, no effective treatment for DMD is available. Promising molecular therapies have been developed specifically for correcting the mutations in the dystrophin gene.

Induction of exon skipping using antisense oligonucleotides is expected to correct the out-of-frame mutation into in-frame mutation of the translational reading frame of dystrophin mRNA. This strategy enables the production of truncated dystrophin protein in DMD patients with out-of-frame exon-deletion mutations in the dystrophin gene. A modified nucleic acid, 2'-O, 4'-C-ethylene-bridged nucleic acid (ENA), has high binding affinity for the complementary RNA strand and more nuclease resistance than unmodified nucleic acid. We have established antisense 2'-O-methyl RNA/ENA (RNA/ENA) chimeras that induced the skipping of exons of the dystrophin gene.

In the DMD cases with nonsense mutations, production of novel in-frame dystrophin mRNAs by skipping the exons containing the nonsense codon is one possible therapeutic approach. We identified TG003, a kinase inhibitor specific for Cdc-like kinases, which enhanced exon skipping and produced an internally deleted dystrophin protein in the cultured myotubes of a dystrophinopathy patient who has a nonsnese mutation.

Induction of the readthrough of nonsense mutations is expected to produce dystrophin in DMD patients with nonsense mutations. We have demonstrated that arbekacin-mediated readthrough can substantially ameliorate muscular dystrophy due to nonsense mutations by using *mdx* mice and DMD patient derived muscle cells.

We hope that these molecular therapies will contribute towards the treatment for DMD, and are planning the clinical trials.

2- Selected publication list

1) R.G,Malueka, Y.Takaoka, M.Yagi, H.Awano, T.Lee, E.K.Dwianingsih, A.Nishida, Y.Takeshima, M.Matsuo. Categorization of 77 dystrophin exons into 5 groups by a decision tree using indexes of splicing regulatory factors as decision markers. **BMC Genet**. 13: 23, 2012.

2) M.Ota, Y.Takeshima, A.Nishida, H.Awano, T.Lee, M.Yagi, M.Matsuo. A G-to-T Transversion at the Splice Acceptor Site of Dystrophin Exon 14 Shows Multiple Splicing Outcomes That Are Not Exemplified by Transition Mutations.**Genet Test Mol Biomarkers**.16: 3-8, 2012.

3) A.Nishida, N.Kataoka, Y.Takeshima, M.Yagi, H.Awano, M.Ota, K.Itoh, M.Hagiwara, M.Matsuo. Chemical treatment enhances skipping of a mutated exon in the dystrophin gene. **Nat Commun**. 2:308, 2011.

4) R.G,Malueka, M.Yagi, H.Awano, T.Lee, E.K.Dwianingsih, A.Nishida, Y.Takeshima, M.Matsuo. Antisense Oligonucleotide Induced Dystrophin Exon 45 Skipping at a Low Half-Maximal Effective Concentration in a Cell-Free Splicing System. **Nucleic Acid Ther**. 21(5): 347-53, 2011.

5) I.Kubokawa, Y.Takeshima, M.Ota, M.Enomoto, Y.Okizuka, T.Mori, N.Nishimura, H.Awano, M.Yagi, M.Matsuo. Molecular characterization of the 5'-UTR of retinal dystrophin reveals a cryptic intron that regulates translational activity. **Mol Vis**. 16:2590-7, 2010.

6) Y.Takeshima, M.Yagi, Y.Okizuka, H.Awano, Z.Zhang, Y.Yamauchi, H.Nishio, M.Matsuo. Mutation spectrum of the dystrophin gene in 442 Duchenne/Becker muscular dystrophy cases from one Japanese referral center. **J Hum Genet.** 55(6):379-88, 2010.

7) H.Awano, R.G,Malueka, M.Yagi, Y.Okizuka, Y.Takeshima, M.Matsuo. Contemporary retrotransposition of a novel non-coding gene induces exon-skipping in dystrophin mRNA. **J Hum Genet**. 55(12):785-90, 2010.

8) Y.Okizuka, Y.Takeshima, K.Itoh, Z.Zhang, H.Awano, K.Maruyama, T.Kumagai, M.Yagi, M.Matsuo. Low incidence of limb-girdle muscular dystrophy type 2C revealed by a mutation study in Japanese patients clinically diagnosed with DMD. **BMC Med Genet**. 11:49, 2010.

9) Y.Takami, Y.Takeshima, H.Awano, Y.Okizuka, M.Yagi, M.Matsuo. High incidence of electrocardiogram abnormalities in young patients with Duchenne muscular dystrophy. **Pediatr Neurol.** 39(6): 399-403, 2009.

10) Y.Okizuka, Y.Takeshima, H.Awano, Z.Zhang, M.Yagi, M.Matsuo. Small mutations detected by multiplex ligation-dependent probe amplification of the dystrophin gene. **Genet Test Mol Biomarkers.** 13(3): 427-31, 2009.

11) Z.Zhang, M.Yagi, Y.Okizuka, H.Awano, Y.Takeshima, M.Matsuo. Insertion of the IL1RAPL1 gene into the duplication junction of the dystrophin gene. **J Hum Genet.** 54(8): 466-73, 2009.

12) Y.Habara, Y.Takeshima, H.Awano, Y.Okizuka, Z.Zhang, K.Saiki, M.Yagi, M.Matsuo . In vitro splicing analysis showed that availability of a cryptic splice site is not a determinant for alternative splicing patterns caused by +1G-->A mutations in introns of the dystrophin gene. **J Med Genet.** 46(8): 542-7, 2009.

13) A.Nishiyama, Y.Takeshima, Z.Zhang, Y.Habara, TH.Tran, M.Yagi, M.Matsuo. Dystrophin Nonsense Mutations Can Generate Alternative Rescue Transcripts in Lymphocytes. **Ann Hum Genet.** 72(6): 717-24, 2008.

Achievements of individual member of Global COE Program for Education and Research on Signal Transduction Medicine in the Coming Generation(2008-2013)

Hak Hotta

Division of Microbiology,
Department of Microbiology and Infectious Diseases

1- Summary

Hepatitis C virus (HCV) infection is often associated with type 2 diabetes. However, the precise mechanism underlying this association is still unclear. We aimed to clarify the molecular mechanisms by which to render HCV-infected individuals prone to be diabetic, such as increased hepatic gluconeogenesis, which is a hallmark of type 2 diabetes. We have obtained the following two sets of results; HCV-mediated increase in hepatocytic gluconeogenesis and HCV-mediated inhibition of hepatocellular glucose uptake, both of which would lead to hyperglycemia and render the host prone to type 2 diabetes.

(1) HCV promotes hepatic gluconeogenesis through an NS5A-mediated, FoxO1-dependent pathway: Using Huh-7.5 cells either harboring HCV RNA replicons or infected with HCV, we showed that HCV transcriptionally upregulated the genes for phosphoenolpyruvate carboxykinase (PEPCK) and glucose 6-phosphatase (G6Pase), the rate-limiting enzymes for hepatic gluconeogenesis, and enhanced the cellular production of glucose 6-phosphate (G6P) and glucose. PEPCK and G6Pase gene expressions are controlled by the transcription factor forkhead box O1 (FoxO1). Although neither the mRNA nor the protein levels of FoxO1 expression were affected by HCV, phosphorylation of FoxO1 at Ser319 was markedly diminished in HCV-infected cells compared to the control cells, resulting in increased nuclear accumulation of FoxO1, which is essential for sustaining its transcriptional activity. It was unlikely that the decreased FoxO1 phosphorylation was mediated through Akt inactivation as we observed increased phosphorylation of Akt at Ser473 in HCV-infected cells compared to the control. By using specific inhibitors of c-Jun N-terminal kinase (JNK) and reactive oxygen species (ROS), we demonstrated that HCV infection induced JNK activation via increased mitochondrial ROS production, resulting in the decreased FoxO1 phosphorylation, FoxO1 nuclear accumulation and, eventually, increased glucose production. We also found that the NS5A protein of HCV was involved in the increased ROS production and JNK activation, which is directly linked with the FoxO1-dependent enhancement of gluconeogenesis. Furthermore, both mRNA and protein levels of MAP kinase phosphatase-3 (MKP3) were upregulated in HCV-infected cells compared to the mock-infected control. Overexpression of MKP3 resulted in decreased phosphorylation of FoxO1. These results collectively suggest that HCV promotes hepatic gluconeogenesis through an NS5A-mediated, MKP3- and FoxO1-dependent pathway.

(2) HCV down-regulates glucose transporter 2 (GLUT2) expression and cellular glucose uptake through transcriptional suppression of the hepatocyte nuclear factor (HNF)-1α gene expression and lysosomal degradation of the HNF-1α protein via interaction with the NS5A protein: We found that cell surface expression of GLUT2 and cellular glucose uptake were suppressed in HCV RNA replicon cells and HCV-infected cells. GLUT2 mRNA levels were reduced in those cells. GLUT2 gene expression is controlled by HNF-1α. We found that mRNA levels of HNF-1α, but not HNF-1β, were significantly reduced in HCV-infected cells. HNF-1α protein levels were also markedly reduced. It thus appears that HCV down-regulates HNF-1α at both transcriptional and posttranslational levels, leading to the suppression of GLUT2 promoter activities. The level of HNF-1α protein in HCV-infected cells was

restored by treatment of the cells with a lysosomal protease inhibitor, pepstatin A, and also by treatment of the cells with *N*-acetyl cysteine, an ROS inhibitor. Overexpression of the HCV NS5A protein, but not NS5B, enhanced lysosomal degradation of the HNF-1α protein. The NS5A protein physically interacted and colocalized with the HNF-1α protein. These results collectively suggest that HCV mediates transcriptional suppression of the HNF-1α gene expression and lysosomal degradation of the HNF-1α protein through interaction with the NS5A protein, leading to down-regulation of GLUT2 expression and cellular glucose uptake.

2- Selected publication list

1) Deng L, Adachi T, Kitayama K, Bungyoku Y, Kitazawa S, Ishido S, Shoji I, Hotta H. Hepatitis C virus infection induces apoptosis through a Bax-triggered, mitochondria-mediated, caspase-3-dependent pathway. **J Virol**, 82(21): 10375-10385, 2008.
2) El-Shamy A, Nagano-Fujii M, Sasase N, Imoto S, Kim SR, Hotta H. Sequence variation in the hepatitis C virus NS5A protein predicts clinical outcome of pegylated interferon/ribavirin combination therapy. **Hepatology,** 48: 38-47, 2008.
3) Inubushi S, Nagano-Fujii M, Kitayama K, Tanaka M, An C, Yokozaki H, Yamamura H, Nuriya H, Kohara M, Sada K, Hotta H. Hepatitis C virus NS5A protein interacts with and negatively regulates the non-receptor protein-tyrosine kinase Syk. J **Gen Virol**, 89(5): 1231-1242, 2008.
4) Kasai D, Adachi T, Deng L, Nagano-Fujii M, Sada K, Ikeda M, Kato N, Ide Y, Shoji I, Hotta H. HCV replication suppresses cellular glucose uptake through down-regulation of cell surface expression of glucose transporters. **J Hepatol**, 50: 883-894, 2009.
5) Mohd-Ismail NK, Deng L, Sukumaran SK, Yu VC, Hotta H, Tan YJ. The hepatitis C virus core protein contains a BH3 domain that regulates apoptosis through specific interaction with human MCL-1. **J Virol**, 83(19): 9993-10006, 2009.
6) Amako Y, Sarkeshik A, Hotta H, Yates J 3rd, Siddiqui A. Role of Oxysterol Binding Protein in Hepatitis C Virus infection. **J Virol**, 83(18): 9237-9246, 2009.
7) Bungyoku Y, Shoji I, Makine T, Adachi T, Hayashida K, Nagano-Fujii M, Ide Y, Deng L, Hotta H. Efficient production of infectious hepatitis C virus with adaptive mutations in cultured hepatoma. **J Gen Virol**, 90(7): 1681-1691, 2009.
8) Deng L, Shoji I, Ogawa W, Kaneda S, Soga T, Jiang DP, Ide YH, Hotta H. Hepatitis C virus infection promotes Hepatic gluconeogenesis through an NS5A-mediated, FoxO1-dependent pathway. **J Virol**. 85(17): 8556-8568, 2011.
9) Shoji I, Deng L, Hotta H. Molecular mechanism of hepatitis C virus-induced glucose metabolic disorders. **Front Microbiol**, 2: A278, 1-5. 2012.
10) Matsui C, Shoji I, Kaneda S, Sianipar IR, Deng L, Hotta H. Hepatitis C virus infection suppresses GLUT2 gene expression via down-regulation of hepatocyte nuclear factor 1α. **J Virol**, 86(23): 12903-12911, 2012.
11) Nakashima K, Takeuchi K, Chihara K, Horiguchi T, Sun X, Deng L, Shoji I, Hotta H, Sada K. HCV NS5A protein containing potential ligands for both Src homology 2 and 3 domains enhances autophosphorylation of Src family kinase Fyn in B cells. **PLoS ONE**, 7(10): e46634, 2012.
12) Kim SR, El-Shamy A, Imoto S, Kim KI, Ide YH, Deng L, Shoji I, Tanaka Y, Hasegawa Y, Ota M, Hotta H. Prediction of response to pegylated interferon/ribavirin combination therapy for chronic hepatitis C genotype 1b and high viral load. **J Gastroenterol**, 47(10): 1143-51, 2012.
13) Shimizu YK, Hijikata M, Oshima M, Shimizu K, Alter HJ, Purcell RH, Yoshikura H, Hotta H. Isolation of human monoclonal antibodies to the envelope E2 protein of hepatitis C virus and their characterization. **PLoS ONE**, 8(2): e55874, 2013.
14) El-Shamy A, Shindo M, Shoji I, Deng l, Hotta H. Polymorphisms of the core, NS3 and NS5A of hepatitis C virus genotype 1b associate with development of hepatocellular carcinoma. **Hepatology,** (in press. 2012 Dec 24. doi: 10.1002/hep.26205.)

Achievements of individual member of Global COE Program for Education and Research on Signal Transduction Medicine in the Coming Generation(2008-2013)

Toshio Imai

KAN Research Institute. Inc.

1- Summary

Migration and tissue localization of specific immune cell populations are critical for immune system homeostasis and inflammatory responses. The cell trafficking is finely regulated at multiple steps by chemokines and adhesion molecules. Selectins mediate rolling of leukocytes on the endothelial cell surface and then chemokines induce activation of integrins on the leukocytes, resulting in arrest and transmigration through the endothelial cells. We are investigating roles and mechanisms of action of chemokines/chemokine receptors and adhesion molecules in immune cell trafficking and inflammation.

Fractalkine (FKN)/CX3CL1 is a unique transmembrane chemokine with a chemokine/mucin hybrid structure. We have shown that fractalkine and its specific receptor CX3CR1, represent a novel type of leukocyte trafficking regulator that performs both adhesive and chemotactic functions. Membrane-bound fractalkine rapidly induces the firm adhesion of CX3CR1-expressing cells without requiring selectin-mediated rolling or the activation of integrins. In addition, soluble fractalkine released from the cell surface by proteolytic cleavage induces integrin activation and the migration of CX3CR1-expressing cells. We have shown that CX3CR1 is predominantly expressed on cytotoxic effector lymphocytes and mature monocytes/dendritic cells, suggesting involvement of fractalkine and its receptor CX3CR1 in the tissue accumulation and diapedesis of immune effector cells in inflammatory condition. We have generated neutralizing anti-fractalkine monoclonal antibody (mAb) and revealed that the antibody suppresses disease pathology in various inflammatory diseases such as hepatitis, rheumatoid arthritis and inflammatory bowel diseases (IBD). Furthermore, we have revealed that $CX3CR1^{+}CD4^{+}$ cells were increased in IBD patients and upregulation of CX3CR1 on CD4+ T cells was positively correlated with disease activity. Together, these results suggest contribution of fractalkine/CX3CR1 axis to the pathogenesis of IBD.

AMICA (adhesion molecule interacts with CXADR antigen 1) is expressed on cell surface of activated T cells and interacts with CAR (coxsackie virus and adenovirus receptor) expressed on endothelial cell-cell junctions. We have shown that interaction of AMICA and CAR facilitates T cell adhesion to endothelial cells and transendothelial migration. We have found that AMICA and integrin LFA-1 partly colocalizes in the ring-like structure at the lymphocyte-endothelial cell interface. Interestingly, AMICA-CAR interaction activates LFA-1-mediated cell adhesion and migration, and simultaneous stimulation with CAR and SDF-1 induces firm adhesion and cell spreading. These results suggest that cooperation between adhesion molecules and chemokines stops lymphocyte locomotion on endothelial surface and triggers transmigration. Furthermore, we have shown that neutralizing anti-AMICA mAb suppresses delayed-type hypersensitivity reaction in the skin.

To elucidate involvement of chemokines, chemokine receptors and adhesion molecules in inflammatory diseases, we are now analyzing these expressions in clinical samples. We are also generating humanized antibody against some of these molecules to develop antibody drugs for inflammatory diseases.

2- Selected publication list

1) Chemerin activates fibroblast-like synoviocytes in patients with rheumatoid arthritis. Kaneko K, Miyabe Y, Takayasu A, Fukuda S, Miyabe C, Ebisawa M, Yokoyama W, Watanabe K, Imai T, Muramoto K, Terashima Y, Sugihara T, Matsushima K, Miyasaka N, Nanki T. Arthritis Res Ther. 13 (5):R158, 2011.

2) Deficiency of chemokine receptor CCR1 causes osteopenia due to impaired functions of osteoclasts and osteoblasts. Hoshino A, Iimura T, Ueha S, Hanada S, Maruoka Y, Mayahara M, Suzuki K, Imai T, Ito M, Manome Y, Yasuhara M, Kirino T, Yamaguchi A, Matsushima K, Yamamoto K. J Biol Chem. 285 (37): 28826-37, 2010.

3) Role of CX3CL1/fractalkine in osteoclast differentiation and bone resorption. Koizumi K, Saitoh Y, Minami T, Takeno N, Tsuneyama K, Miyahara T, Sakurai H, Takano Y, Nishimura M, Imai T, Yoshie O, Saiki I. J Immunol. 183 (12): 7825-31, 2009.

4) Chemokines as novel therapeutic targets for inflammatory bowel disease. Nishimura M, Kuboi Y, Muramoto K, Kawano T, Imai T. Ann NY Acad Sci. 1173: 350-6, 2009.

5) Intra-neural administration of fractalkine attenuates neuropathic pain-related behaviour. Holmes FE, Arnott N, Vanderplank P, Kerr NC, Longbrake EE, Popovich PG, Imai T, Combadière C, Murphy PM, Wynick D. J Neurochem. 106 (2): 640-9, 2008.

6) Interaction of cancer cells with platelets mediated by Necl-5/poliovirus receptor enhances cancer cell metastasis to the lungs. Morimoto K, Satoh-Yamaguchi K, Hamaguchi A, Inoue Y, Takeuchi, M, Okada M, Ikeda W, Takai Y, Imai T. Oncogene. 27 (3): 264-73, 2008.

Achievements of individual member of Global COE Program for Education and Research on Signal Transduction Medicine in the Coming Generation(2008-2013)

Hironobu Minami

Division of Medical Oncology /Hematology, Department of Internal Medicine

1- Summary

For the last decade, clinical application of molecularly targeted agents emphasized importance of biomarker development for individualized therapy. Our department has been pursuing this strategy by creating research environment where preclinical studies and clinical phase I trials can be done as a continuum.

In clinic, our phase I team has conducted[12] phase I trials since 2008.[3, 5-10] In our laboratory, we mainly focus on revealing the mechanisms of resistance to targeted agents and the discovery of new therapeutic targets. As an example of the former, we found that presence of *PIK3CA* gain-of-function mutations in *HER2*-amplified breast cancer cell lines was associated with resistance not only to anti-HER2 antibody, trastuzumab, but also to small molecule HER2 inhibitors.[11] In efforts to explore mechanisms of acquired resistance, we have developed several acquired resistant models by continuously exposing cancer cell lines sensitive to targeted drugs to corresponding drugs; trastuzumab resistant *HER2*-amplified BT474 breast cancer cell line (BT474-TR), Insulin-like Growth Factor-1 Receptor (IGF-1R) inhibitor (NVP-AEW541) resistant MCF-7 breast cancer cell line (MCF-7NR), and MET inhibitors (PHA665752 and GSK1363089) resistant *MET*-amplified MKN45 gastric cancer cell line (MKN45-PR and –GR).[1,2,4,11,12] In a study using MCF-7-NR, we found that a novel receptor tyrosine kinase (Tyro3) was up-regulated in MCF-7-NR as compared to parental MCF-7, and control of cyclin D1-dependent cell proliferation was maintained in a Tyro3-dependent and IGF-1R-indepent manner in MCF-7-NR, resulting in resistance to NVP-AEW541.[1] Furthermore, Tyro3 knock-down inhibited the growth of both MCF-7 and MCF-7NR, suggesting that Tyro3 might be a new therapeutic target. In addition, we found that the expression level of insulin substrate 1 (IRS-1), but not of IGF-1R, might be predictive for sensitivity to NVP-AEW541, which should be verified in future clinical studies.[12] In our recent study using the MKN45-PR and –GR, we found point mutations and increased copy number of already amplified *MET* gene appeared to cause resistance to the MET inhibitors.[4] Interestingly, MKN45-PR or –GR could not grow in the absence of MET inhibitors, suggesting "addiction" to the inhibitors. We found that this novel phenomenon can be explained by excessive MET signaling, subsequent excessive replication stress, and resultant S-phase arrest in the absence of MET inhibitors. Clinical relevance of this phenomenon will be evaluated in clinical setting.

In efforts to discover new therapeutic targets, we began to utilize omics technologies. We are conducting exome analysis for relatively rare types of cancer such as salivary gland cancers. And also, we are utilizing proteomics and metabolomics technologies to explore true targets of molecularly targeted drugs. As the first attempt, we are focusing on iniparib (BSI-201), which was once believed to be a PARP inhibitor but later turned out to be unable to inhibit PARP at clinically achievable concentrations. We are performing proteomic and metabolomic analysis for cellular extract obtained from iniparib-untreated and –treated breast cancer cell lines. We will also be performing the similar analysis by using mononuclear cells obtained from patients who treated with iniparib in a phase I study we conducted at our institution.

2- Selected publication list

1) Ekyalongo RC, Mukohara T, Kataoka Y, Kiyota N, Fujiwara Y, and Minami H. Mechanisms of acquired resistance to insulin-like growth factor 1 receptor inhibitor in MCF-7 breast cancer cell line. Invest New Drugs 2012 (Epub ahead of print, July 25, 2012)
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4) Kataoka Y, Mukohara T, Tomioka H, Kiyota N, Fujiwara Y, Yashiro M, Hirakawa K, Hirai M, and Minami H. Foretinib (GSK1363089), a multi-kinase inhibitor of MET and VEGFRs, inhibits growth of gastric cancer cell lines by blocking inter-receptor tyrosine kinase networks. Invest New Drugs 30: 1352-1360, 2012
5) Fujiwara Y, Kiyota N, Chayahara N, Suzuki A, Umeyama Y, Mukohara T, Minami H. Management of axitinib (AG-013736)-induced fatigue and thyroid function, and predictive biomarkers of axitinib exposure: Results from phase I studies in Japanese patients. Invest New Drugs 30 (3): 1055-1064, 2012
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10) Mukohara T, Nakajima H, Mukai H, Nagai S, Itoh K, Yoshiko Umeyama Y, Hashimoto J, Minami H. Effect of axitinib (AG-013736) on fatigue, thyroid-stimulating hormone, and biomarkers: A phase I study in Japanese patients. Cancer Sci 101: 963-8, 2010
11) Kataoka Y, Mukohara T, Shimada H, Saijo N, Hirai M, Minami H. Gain of function mutations of PIK3CA is associated with resistance to HER2-targeted agents in HER2-amplified breast cancer cell lines. Ann Oncol 21: 255-262, 2010
12) Mukohara T, Shimada H, Ogasawara N, Wanikawa R, Shimomura M, Nakatsura T, Ishii G, Park JO, Jänne PA, Saijo N, Minami H. Sensitivity of breast cancer cell lines to the novel Insuline-like Growth Factor-1 Receptor (IGF-1R) inhibitor NVP-AEW541 is dependent on IRS-1 expression. Cancer Lett 282: 14-24, 2009

Achievements of individual member of Global COE Program for Education and Research on Signal Transduction Medicine in the Coming Generation(2008-2013)

Hiroshi Kitagawa

Division of Clinical Pharmacokinetics, Department of Biochemistry,
Kobe Pharmaceutical University

1- Summary

Chondroitin sulfate (CS) is a representative sulfated glycosaminoglycan (GAG), which is covalently attached to a panel of core proteins to form proteoglycans (CSPGs), and is ubiquitously located in extracellular matrices and on cell surfaces in various tissues. CSPGs regulate diverse physiological phenomena such as cytokinesis, morphogenesis, and infections with viruses and bacteria. In particular, the pathologic functions of CS moieties of CSPGs as major axon growth-inhibitory molecules in the injured adult central nervous system (CNS) have attracted widespread attention, and prompted research aimed at overcoming their barrier effects on neuronal regeneration processes. Although axonal regeneration is indeed improved by the removal of CS moieties around lesion sites, CS does not always impede neurite outgrowth. For example, several CS preparations serve as stimulatory substrata for neurite outgrowth of cultured primary neurons.

The apparently contradictory actions of CS in the CNS are thought to be attributable to its structural diversity. CS is a linear polysaccharide that contains repeating disaccharide units consisting of glucuronic acid (GlcUA) and *N*-acetyl-D-galactosamine (GalNAc). The building blocks can be substituted with sulfate groups at various positions, thereby producing characteristic "sulfation codes". CS polysaccharides are divided into subclasses based on their disaccharide composition. The major CS subclasses found in mammalian tissues contain monosulfated disaccharide units, A [GlcUA-GalNAc(4-*O*-sulfate)] and C [GlcUA-GalNAc(6-*O*-sulfate)]. CS polysaccharides rich in A and C units (CS-A and CS-C, respectively) are poorly permissive for neurite extension, probably reflecting the inhibitory nature of typical mammalian CS. In contrast, squid cartilage-derived CS-E polysaccharide possesses strong neuritogenic activity toward primary hippocampal neurons. CS-E is characterized by the predominant disulfated disaccharide E unit, [GlcUA-GalNAc(4,6-*O*-disulfate)]. Recent studies on chemically synthesized CS-E tetrasaccharides also support the structural importance of E unit for neurite outgrowth-promoting activity.

The inherent potential of CS-E is of special interest for therapeutic application to CNS injury. We found that CS-E binds to several humeral factors, such as midkine (MK) and Wnt-3a, and regulates neurite outgrowth through the activation of MK and Wnt-3a signaling inputs to primary neurons, suggesting the possible roles of CS-E as a co-receptor and/or reservoir for neuritogenic factors (1, 3). In contrast, while it has been postulated that functional receptor molecules receiving a "sulfation code" of CS reside on the neuronal membrane surface, identification of such potential molecules remains challenging.

Focusing on neuroregulatory roles of CS, we showed the involvement of a cell adhesion molecule, contactin-1 (CNTN-1), in CS-E-mediated neuritogenesis in a neuroblastoma cell line and primary hippocampal neurons (2). CS-E engaged CNTN-1 and induced intracellular signaling downstream of CNTN-1, indicating that CS-E is a ligand for a potential CS receptor, CNTN-1. Our data provide the first evidence for functional expression of CS through the CS receptor-mediated signaling pathway(s).

Cortical plasticity is most evident during a critical period in early life, but the mechanisms that restrict plasticity after the critical period are poorly understood. We recently showed that a developmental increase in the

4-sulfation/6-sulfation (4S/6S) ratio of CSPGs, components of the brain extracellular matrix, leads to the termination of the critical period for ocular dominance plasticity in the mouse visual cortex (4). Condensation of CSPGs into perineuronal nets that enwrapped synaptic contacts on parvalbumin-expressing interneurons (PV-cells) was prevented by cell-autonomous overexpression of chondroitin 6-sulfation, which maintains a low 4S/6S ratio. Furthermore, the increase in the 4S/6S ratio was required for accumulation of Otx2, a homeoprotein that activates development of PV-cells, and for functional maturation of electrophysiological properties of these cells. Our results propose that the critical period for cortical plasticity is regulated by the 4S/6S ratio of CSPGs, which determines the maturation of PV-cells.

2- Selected publication list

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J. Biol. Chem. 283 (40) 27333-27343 (2008)

2) Mikami, T., Yasunaga, D., and Kitagawa, H.
Contactin-1 Is a Functional Receptor for Neuroregulatory Chondroitin Sulfate-E.
J. Biol. Chem. 284 (7), 4494-4499 (2009)

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Down-regulation if Chondroitin 4-*O*-Sulfotransferase-1 by Wnt Signaling Triggers Diffusion of Wnt-3a.
J. Biol. Chem. 286 (6), 4199-4208 (2011)

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Persistent Cortical Plasticity by Upregulation of Chondroitin 6-sulfation.
Nature Neurosci. 15 (3), 414-422 (2012)

Achievements of individual member of Global COE Program for Education and Research on Signal Transduction Medicine in the Coming Generation(2008-2013)

Kaoru Sugasawa

Biosignal Research Center, and Div. Genomic Physiology,
Dep. Biochemistry and Molecular Biology

1- Summary

Genomic DNA constantly suffers from insults caused by various agents, such as reactive oxygen species, radiations and chemicals. To maintain genomic stability, it is crucial for cells to detect and repair such DNA damage, while induction of apoptosis and/or senescence is another important defense system, especially in the presence of the very high burden of injuries. The perturbed balance between these two options (*to be, or not to be*) can lead to different clinical consequences, such as carcinogenesis, neurological degeneration, premature ageing, and so on. To understand the mechanism underlying the possible molecular switch that determines the cell fates, the following studies have been accomplished in this GCOE program.

1) Roles for the FANCD2 protein in cellular DNA damage responses

Fanconi anemia (FA) is a human genetic disorder related to perturbed DNA damage responses (DDR), and cells from FA patients exhibit extremely high sensitivity to DNA crosslinking agents including a cancer chemotherapeutic agent, cisplatin. Among the protein products encoded by the 15 identified FA responsible genes, FANCD2 is supposed to play key roles in DNA repair as well as cellular DDR, undergoing phosphorylation and mono-ubiquitination in response to DNA damage. However, its precise functions still remain to be elucidated.

In analyzing in vivo behaviors of FANCD2, we unexpectedly found that this protein is cleaved into several smaller fragments, particularly in the presence of relatively high levels of DNA damage. By using specific inhibitors, involvement of caspases was indicated, whereas proteasome inhibitors resulted in accumulation of the cleaved FANCD2 fragments. *In vitro* assays with recombinant caspases as well as the experiments with MCF-7 cells (caspase-3 deficient) revealed that the DNA damage-induced cleavage of FANCD2 depends on caspase-3.

Next we tried to determine the cleavage sites in FANCD2, by introducing point mutations at putative caspase target sites. Consequently, four cleavage sites could be identified, so that the mutant FANCD2 protein, in which the four aspartates were changed to alanines simultaneously (4DA mutant), was found resistant to the DNA damage-induced cleavage. Intriguingly, when the FANCD2-4DA mutant was stably expressed in a FANCD2-deficient cell line, those cells were more resistant to killing by DNA damaging agents compared to the control cells expressing wild-type FANCD2. Thus the non-cleavable version of FANCD2 seems to have some anti-apoptotic effects against DNA damage, implicating its novel function in cellular DDR.

2) Post-translational modifications regulating nucleotide excision repair

Xeroderma pigmentosum (XP) is an autosomal recessive disorder, which is associated with defects in a major DNA repair pathway, nucleotide excision repair (NER). Patients with XP are clinically characterized by a marked predisposition to skin cancer and, in some cases, progressive neurological abnormalities. In human NER, the XPC protein plays an essential role in DNA damage recognition and initiation of the repair reaction.

We have recently shown that XPC undergoes modification by a small ubiquitin-like modifier (SUMO). At least three modification sites were identified, and the cell line stably expressing the mutant XPC lacking those sumoylation sites (XPC-3KR) exhibited significantly slower repair of UV-induced DNA damage. In vitro

pull-down assays revealed that the sumoylation augments physical interaction between XPC and UV-DDB. It has been shown that UV-DDB, identified as the DDB1-DDB2 (XPE) heterodimer, efficiently recognizes and binds to UV-induced DNA photolesions and then vitally recruits XPC. Intriguingly, suppression of DDB2 expression by siRNA partially alleviated the retarded repair in the XPC-3KR expressing cells, suggesting that, in the absence of sumoylation, damage handover from UV-DDB to XPC may be compromised, so that UV-DDB itself may adversely block initiation of NER.

2- Selected publication list

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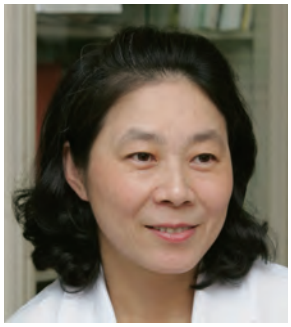

Achievements of individual member of Global COE Program for Education and Research on Signal Transduction Medicine in the Coming Generation(2008-2013)

Chikako Nishigori

Division of Dermatology, Department of Internal Related

1- Summary

Many of cancer related genes were found out to be key molecules involved in proliferation, apoptosis or transcription factors. A cancer prone disease, xeroderma pigmentosum, is a DNA repair disorder and some of the patients develop severe neurological abnormalities. Dysfunction in transcription is indicated as one of the causes of its neurological abnormalities. Thus, we aimed at approaching the common mechanisms for cancers and neurological dysfunction, hoping a possible targeted therapy, focusing on the investigation of transcription factors.

Many melanoma cells constitutively produce interleukin-8 (IL-8), being closely related to its invasion and metastasis. We found that the levels of IL-8 production correlated well with the level of phosphorylated (activated) a transcription factor, signal transducer and activator of transcription 3 (STAT3), in human melanoma cell lines. Introduction of the constitutively activated form of STAT3 into melanoma cells, WM35, that show low levels of IL-8 production and phosphorylated STAT3, enhanced IL-8 production. On the other hand, siRNA-mediated knockdown of STAT3 suppressed IL-8 production and its growth in vitro in melanoma cells, WM1205Lu, which produce a high level of IL-8 accompanying STAT3 activation. STAT3 has two important activation sites, Tyr705 and Ser727, for its activation. It has been generally thought that Tyr705 activation is a hallmark of STAT3 activations whereas Ser727 activation is a secondary event after Tyr705 activation required for the maximal transcriptional activity of STAT3. In normal cells, the duration of STAT3 activation is temporary, lasting from a few minutes to several hours. However, constitutive activation of STAT3, in terms of Tyr705 activation, has been observed in melanomas. In addition, we found that Ser727 was constitutively activated both in normal melanocytes and melanoma cells irrespective of Tyr705 phosphorylation. Increase in cell survival and nuclear translocation of STAT3 induced by TPA was associated with Ser727 activation. The constitutive Ser727 activation in melanoma cells was partially mediated by the B-Raf-MEK-ERK1/2 pathway. Immunohistochemical studies on specimens of primary lesions of acral lentiginous melanoma revealed that Ser727 activation precedes Tyr705 activation in the early stages of melanoma progression. Our results indicate that Ser727 activation on STAT3 is not necessarily a secondary event after Tyr705 activation and suggest that it has Tyr705 and Ser727 has its differential role in the regulation of cell survival activity and nuclear translocation of STAT3 in melanocytic cells.

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7) Nagai H, Oniki S, Fujiwara S, Yoshimoto T, Nishigori C : Antimelanoma immunotherapy: Clinical and preclinical applications of IL-12 family members. ***Immunotherapy***, 2 : 697-709, 2010.

8) Oka M, Edamatsu H, Kunisada M, Hu L, Takenaka N, Sakaguchi M, Kataoka T, Nishigori C : Phospholipase Ce has a crucial role in ultraviolet B-induced neutrophil-associated skin inflammation by regulating the expression of CXCL1/KC. ***Lab Invest***, 91 : 711-718, 2011.

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Achievements of individual member of Global COE Program for Education and Research on Signal Transduction Medicine in the Coming Generation(2008-2013)

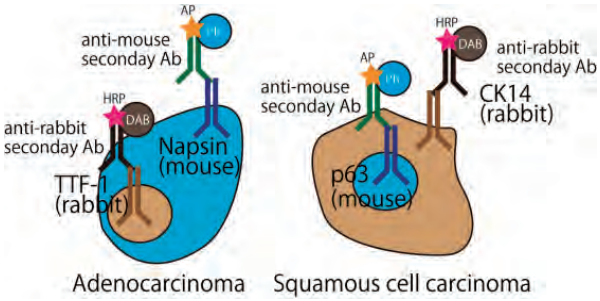
Tomoo Itoh

Division of Pathology Diangnostic, Department of Pathology

1- Summary

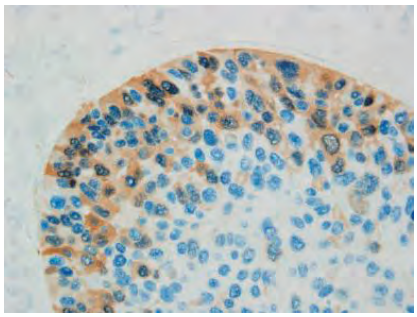
In 2008, we established a specialized section, Kobe University Advanced Staining Center (KATS), in our department as for this G-COE program. This center had been providing highly qualified immunohistochemistry performed by specialized technician for the G-COE teams. The morphological assessments were supported by pathologists belonging this center, and the quality of the researches of the teams was ensured.

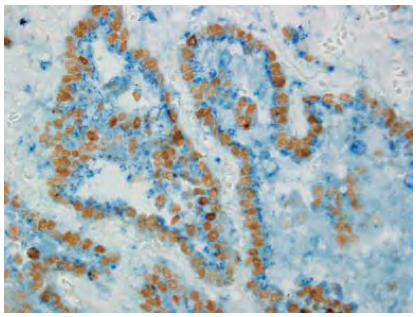
The development of novel immunohistochemical methods is one of the most important missions of us. Recently, we regarded multiplex immunohistochemistry as important methodology.

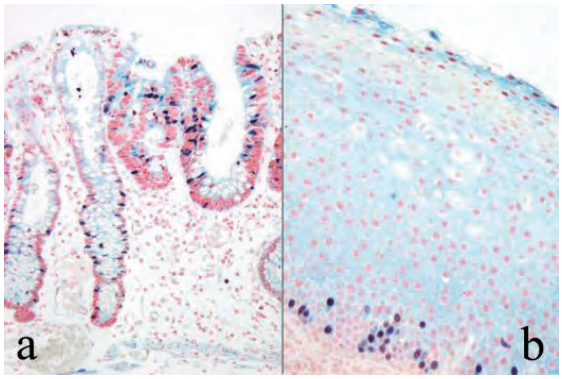


We designed a rapid multiplex immunostaining method using a novel 4-antibody cocktail, **YANA-4**. This method labels the nuclei of adenocarcinomas as brown with TTF-1, and cytoplasm as blue with napsin A. Squamous cell carcinomas could be differentiated from adenocarcinomas with an inverse staining pattern: blue nuclei with p63 and brown cytoplasm with CK14. This rapid immunohistochemical method can thus be considered highly specific and sensitive for differentiating adenocarcinomas and squamous cell carcinomas. Furthermore, we had already developed a improved method "YANA-5", which composed of five antibodies and can be stained by autoimmunostainers.

We developed a multiplex immunohistochemical method , **iCCD**, that can simultaneously stain cells in the G1 and S/G2/M phases and those undergoing apoptosis with the 3 markers Cdt1, geminin, and gamma H2A.X. The staining procedure can be performed using an autoimmunostainer. The nuclei of cells in the G1 phase stain red with the antibody for Cdt1, those in the S/G2/M phases stain blue with the antibody for geminin, and the nuclei of cells undergoing apoptosis stain brown with the antibody for H2A.X. The present method enables accurate cell cycle assessments using paraffin-embedded tissue specimens, which are superior to other forms of specimens in terms of morphologic observation.







2- Selected publication list

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2) Distinctive expression of CD133 between intraductal papillary neoplasms of the bile duct and bile duct adenocarcinomas. Ohtsubo I, Ajiki T, Hori Y, Murakami S, Shimizu K, Itoh T, Shimizu M, Fukumoto T, Ku Y. Hepatol Res. 2012 Jun; 42(6): 574-82.doi:10.1111/j.1872-034X.2011.00954.x. Epub 2012 Jan 6.

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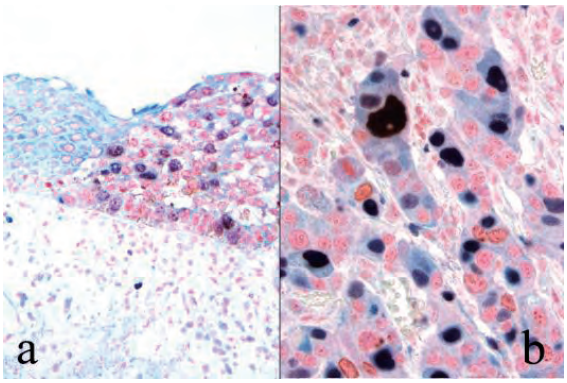

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Achievements of individual member of Global COE Program for Education and Research on Signal Transduction Medicine in the Coming Generation(2008-2013)

Kazuro Sugimura

Division of Radiology, Department of Internal Related

1- Summary

Radiological examination in patients with cancer is fundamental and important for treatment planning, improving the quality of life and saving the patient life. Currently, advancement of imaging technique and development of new clinical application and software of computed tomography (CT), magnetic resonance imaging (MRI), whole-body positron emission tomography with [18F] fluoro-2-D-glucose (FDG-PET), and FDG-PET image fused with CT (PET/CT) make it possible to detect small cancers, provide morphological, physiologic and physiopathologic information based on microstructural changes, physical parameters such as relaxation time, proton density, molecular diffusion, physiologic parameters such as perfusion, ventilation, oxygen-diffusion, and metabolic information. In addition, pharmacokinetical, morphological, functional, and other basic science based analyses provide various information in routine clinical practice. Therefore, radiological method would be better to consider the new and clinically available method for translational research from basic science to clinical implementation.

In this GCOE programs, our mission in this program is to develop the new diagnostic imaging techniques for translational research of signal transduction medicine, and educate the clinicians, especially radiologists and radiation oncologists, as clinician-scientists in the alliance between basic and clinical medicine. To achieve the above-mentioned mission, Kobe University Graduate School of Medicine established the Division of Functional and Diagnostic Imaging Research and Advanced Biomedical Imaging Research Center as well as the Division of Radiation Oncology and Department of Endovascular Therapy since 2008. Although the latter were mainly work as clinical research field, the former were continuously studying and working with a few companies, achieve several patents in Japan and other countries such as USA, EU, China, etc., and developed several software for computer-aided diagnosis, image-based analysis for diagnosis or management of lung cancer, hepatocellar carcinoma, COPD, asthma, etc. Moreover, they established new radiological examination for pharmacokinetical, functional and metabolic analysis on CT and MRI. We also trained and educated the doctoral course students and postdoctoral course staffs about clinical and basic studies to perform translational research, and published papers during this GCOE projects.

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Achievements of individual member of Global COE Program for Education and Research on Signal Transduction Medicine in the Coming Generation(2008-2013)

Yonson Ku

Division of Hepato-Biliary-Pancreatic Surgery, Department of Surgery

1- Summary

The incidence of hepato-biliary-pancreatic cancer is still increasing worldwide. Although surgical resection remains the best therapeutic option that may offer a chance for improved survival, several factors such as major vascular invasion and metastasis, contribute to the dismal prognosis of advanced cancer. Recent progress of chemotherapy for pancreatic and biliary cancers have improved survival, however, its efficacy still remains poor.

Cancer stem cells (CSCs) have a critical role for tumor generation and maintenance. Recent advances in stem cell biology reveal that CSCs seem to be protected against chemotherapeutic agents. We have already isolated and explored pancreatic cancer stem cells from ascites of a chemotherapy-resistant patient. We revealed that expression of CD133, a cancer stem cell marker, could distinguish pancreatic intraductal papillary mucinous neoplasm (IPMN) from pancreatic ductal adenocarcinoma (Shimizu et al, 2009). We next tried to examine the mechanism of chemoresistance in bile duct cancers by focusing expression of CD133. While no CD133 expression was observed in intraductal papillary neoplasm of the bile duct (IPNB), low malignant bile duct tumor like pancreatic IPMN, 16-7% of cancer cells in bile duct cancer expressed CD133 (Ohtsubo et al, 2012).

In addition, using the same methods as isolation of pancreatic CSCs, we could isolate and examine CSCs from patients with biliary tract cancers. We could isolate 2 kinds of CSCs from 2 patients, respectively. Orthotopic implantation of these cells into subcutaneous of nude mice resulted in formation of ductal adenocarcinoma. We continue to examine characteristics of these cells including mucin expression and CD133 expression.

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Achievements of individual member of Global COE Program for Education and Research on Signal Transduction Medicine in the Coming Generation(2008-2013)

Wataru Ogawa

Division of Diabetes and Endocrinology, Department of Internal Medicine

1- Summary

Type 2 diabetes mellitus, a rapidly increasing health burden across the globe, is characterized by the dysfunction of pancreatic beta-cells as well as the impairment of insulin action (insulin resistance). To understand the pathogenesis of Type 2 diabetes mellitus and develop novel drugs for this condition, we have been working on the molecular mechanism of insulin action and insulin resistance. We have investigated the possible role of the transcription factor KLF15 in the regulation of gluconeogenesis and whether KLF15 participates in the antidiabetes effect of metformin (4). We have found that KLF15 plays an important role in regulation of the expression of genes for gluconeogenic and amino acid–degrading enzymes, and that the inhibitory effect of metformin on gluconeogenesis is mediated by down-regulation of KLF15 and consequent attenuation of the expression of such genes (4). We also investigated the role of the E3 ubiquitin ligase GRAIL in glucose and lipid metabolism in the liver. The knockdown of GRAIL in the liver resulted in the dysregulation of metabolism-related genes, indicating the possible role of this protein in hepatic energy metabolism (3). PDK1 is a key regulator of metabolic action of insulin. We have previously shown the important roles of this protein in metabolic control in various organs (Cell Metab 3: 267-275, 2006; Nat Genet 38: 589-593, 2006; Diabetes 56: 1000-1009, 2007). In this program, we further revealed the role of PDK1 in vascular endothelial cells and its relationship between glucose metabolism (3). Hepatic steatosis is often associated with type 2 diabetes and insulin resistant individuals. We also have found that the mTORC1/S6K1 pathway is activated in the liver of insulin resistant diabetic animals by nutrient signals and that the activation of this pathway contributes to the development of hepatic steatosis and dyslipidemia (2). PGC-1α is a transcriptional coactivator that regulates various metabolic processes, including mitochondrial biogenesis and thermogenesis. We have identified novel PGC-1α variants transcribed from an alternative promoter and induced by exercise in skeletal muscle (7). The robust increase in PGC-1α in human skeletal muscle by exercise (5) suggests that that this protein is involved in the metabolic adaptation of skeletal muscle by acute exercise. We have generated mice lacking these PGC-1α variants and found that these mutant mice develop age-dependent obesity and insulin resistance due to the impaired energy expenditure during physical activity. These results suggest that PGC-1α variants play an important role in controlling fat mass and insulin sensitivity via the regulation of energy expenditure during exercise.

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Achievements of individual member of Global COE Program for Education and Research on Signal Transduction Medicine in the Coming Generation(2008-2013)

Kyoko Shinya

Division of Zoonosis Infection, Department of Microbiology and Infectious Diseases

1- Summary

We are belonging to this program from April 2009. Influenza virus is an enveloped virus belonging to the *Orthomyxoviridae* family. The virus binds to sialic acid receptors of host cells to enter an endosome, and then fuses with the host endosome membrane to release the viral genomes into host cells for its replication. Hemagglutinin (HA), which is the main antigenic glycoplotein on the surface of the virus, plays a pivotal role in the receptor-binding and the fusion between the viral envelope and the endosome membrane.

During 2009-2011, we had tested the binding of the HA to some receptor molecules of the host cell, and then evaluated its signal transduction. Our research revealed that direct association between HA molecule and Toll like receptor (TLR) 4. And the innate immune stimulation through the TLR 4 provided some extent of resistance, although not perfect, against lethal H5N1 infection *in vivo*. TLR4 pre-stimulation by LPS also reduced influenza virus replication at the *in vitro* level, and we demonstrated that the TLR4-TRIF pathway was required for this LPS-mediated virus reduction. During those LPS-mediated protections, strong upregulation of antiviral molecules was observed both *in vitro* and *in vivo* levels. Our results indicated that innate immune stimulation through TLR4 could potentially prevent lethal influenza infection (Virol. J. 2011 and J. Virol. 2012).

We are next focusing on the membrane fusion between the virus and host cells which is mediated by HA molecule. To obtain further insight into the fusion mechanisms, we have started the new research for establishing the experimental system using substrate-supported lipid membranes (i.e., model membranes). The experimental system enabled us to microscopically observe the binding of virus and the subsequent fusion to the model membrane, which is mediated by the conformational change of HA at low pH, a condition known to induce membrane fusion. This model system could be applied to establish the methods to examine anti-fusion drugs.

2- Selected publication list

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Achievements of individual member of Global COE Program for Education and Research on Signal Transduction Medicine in the Coming Generation(2008-2013)

Yoshiyuki Rikitake

Division of Signal Transduction, Department of Biochemisty and Molecular Biology

1- Summary

In the Global COE program, we have investigated mainly the role of nectins and their related molecules, afadin and Necl-5, in the pathogenesis of atherosclerosis and neurodegenerative diseases. Intimal thickening is an early event of atherosclerosis and is considered to result from dedifferentiation of medial smooth muscle cells (SMCs) from a contractile to a synthetic phenotype and their subsequent migration and proliferation. We found that Necl-5 was upregulated in dedifferentiated SMCs and could augment dedifferentiation, migration and proliferation of SMCs. Intimal thickening after carotid artery ligation was milder in Necl-5 knockout mice compared with wild-type mice.

Angiogenesis plays an important role in the development of atherosclerosis. We reported that Necl-5 and afadin regulated angiogenesis in response to vascular endothelial growth factor (VEGF). Necl-5-knockout or endothelial cell-specific conditional afadin-knockout mice exhibited impaired adaptive angiogenesis following hind limb ischemia. VEGF-induced network formation, migration, proliferation and survival were significantly decreased in cultured Necl-5- or afadin-knockdown endothelial cells, accompanied by inhibition of activation of the phosphatidylinositol-3 kinase–Akt signaling pathway.

Diabetes mellitus is an important risk factor for neurodegenerative diseases. We found that neurodegeneration was enhanced by the lack of Necl-5 in streptozotocin-induced diabetic mice. Taken together, these results suggest that Necl-5 is critical for the development of atherosclerosis and neurodegenerative diseases.

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Achievements of individual member of Global COE Program for Education and Research on Signal Transduction Medicine in the Coming Generation(2008-2013)

Masaru Yoshida

Division of Metabolomics Research and Gastroenterology, and The Integrated Center for Mass Spectrometry

1- Summary

Research Title; **Establishment of Clinical Metabolomics**
Background and Aims: In the post-genomic era, disease-specific biomarkers have been searched by "Omics technologies," especially genomics and proteomics, for preventing or detecting diseases at an early stage. The metabolome is thought to be closely connected to the genotype of an organism and its physiology. Therefore, the metabolome analysis (metabolomics) using mass spectrometry should be useful for discovery of disease-specific biomarkers and elucidation of pathological conditions. **Methods**: In metabolomics using gas chromatography-mass spectrometry (GC-MS), the serum metabolites were collected via liquid-phase extraction with methanol/chloroform/water, and then trimethylsilyl-derivatized and measured with GC-MS. **Results**: The non-targeted metabolome analysis system using GC-MS (8) was also applied to (A) clinical cases and (B) *in vivo* experiments using experimental animals. (A) Clinical cases; Various diseases (1, 5, 10, 12) such as colorectal cancer, gastric cancer, esophageal cancer, pancreatic cancer and inflammatory bowel disease (9), lung cancer (6) and cardiovascular disease (2), were examined, and specific changed metabolites were identified. Especially, in colorectal cancer (1), first, the accuracy of our GC/MS-based serum metabolomic analytical method was evaluated by calculating the RSD% values of serum levels of various metabolites. Second, the intra-day (morning, daytime, and night) and inter-day (among 3 days) variances of serum metabolite levels were examined. Then, serum metabolite levels were compared between colorectal cancer patients (N=60; N=12 for each stage from 0 to 4) and age- and sex-matched healthy volunteers (N=60) as a training set. The metabolites whose levels displayed significant changes were subjected to multiple logistic regression analysis with the stepwise variable selection method, and a colorectal cancer prediction model was established. The validity of the prediction model was confirmed using colorectal cancer patients (N=59) and healthy volunteers (N=63) as a validation set. The prediction model was composed of the 4 metabolites selected, and its AUC, sensitivity, specificity, and accuracy were 0.9097, 85.0%, 85.0%, and 85.0%, respectively, according to the training set data. In contrast, the sensitivity, specificity, and accuracy of CEA were 35.0%, 96.7%, and 65.8%, respectively, and those of CA19-9 were 16.7%, 100%, and 58.3%, respectively. At the validation set, the sensitivity, specificity, and accuracy of the prediction model were 83.1%, 81.0%, and 82.0%, respectively, and these values were almost the same as those obtained with the training set. In addition, the model displayed high sensitivity for detecting stage 0-2 colorectal cancer (82.8%). Our prediction model established via GC/MS-based serum metabolome analysis is valuable for early detection of colorectal cancer and has the potential to become a novel screening test for colorectal cancer. (B) *in vivo* experiments; The mouse models for Inflammatory bowel diseases (7) and acute pancreatitis (3), colon cancer (4) and nonalcoholic fatty liver disease (11) were examined, and therapeutic metabolites and/or the metabolites related to pathogenesis were identified. **Discussion**: In conclusion, we have established metabolomics. For discovery of novel disease-specific biomarkers, the metabolome on a variety of diseases need to be compared with each other's, and the metabolite database for various diseases should be established for the clinical application.

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（*は、correspondenceを示す）

Achievements of individual member of Global COE Program for Education and Research on Signal Transduction Medicine in the Coming Generation(2008-2013)

Hiroshi Kaji

Department of Physiology and Regenerative Medicine,
Kinki University Faculty of Medicine

1- Summary

Numerous studies indicate that muscle mass is closely related to higher bone mass and a decrease in fractures, suggesting that there might be some interactions between muscle tissues and bone metabolism. Fibrodysplasia ossificance progressiva (FOP) is a genetic disease that exhibits progressive ossification in muscle tissues, which is caused by an activating mutation (R206H) of a BMP receptor, ALK2. This disease was considered to be a clue for studying the interaction between muscle and bone.

1. Search for local regulators of muscle ossification.

We performed a comparative DNA microarray analysis between stable empty vector- and ALK2(R206H)-transfected mouse myoblastic C2C12 cells. Tmem119 was identified whose expression was increased in the experimental group versus the control. Osteoblast differentiation markers and mineralization were enhanced in C2C12 cells expressing Tmem119. Transcriptional activity of the BMP-2 signaling molecule was increased even in the absence of exogenous BMP-2. Tmem119 promoted the differentiation of myoblasts into osteoblasts and the interaction with the BMP signaling pathway likely occurs downstream of Runx2 and Osterix in myoblasts. These data suggest that Tmem119 may play a critical role in the commitment of myoprogenitor cells to the osteoblast lineage.

2. Search for the systemic bone anabolic factors produced in muscle tissues.

We hypothesized that there might be some humoral factors that are produced in muscle tissues and exhibit bone anabolic activity. Twenty-five genes, whose expression was decreased to <1/4, were identified from the above mentioned DNA microarray to search for muscle-derived bone anabolic factors; these included osteoglycin (OGN) and FAM5C. Overexpression of OGN or FAM5C significantly enhanced the levels of alkaline phosphatase, type I collagen and osteocalcin mRNA as well as β-catenin and mineralization in mouse osteoblastic MC3T3-E1 cells. The conditioned medium from OGN-overexpressed and OGN or FAM5C-suppressed myoblastic cells enhanced and decreased the levels of ALP, Col1 and β-catenin in MC3T3-E1 cells, respectively. Taken together, our data suggests that OGN and FAM5C may be a crucial humoral bone anabolic factor that is produced by muscle tissues.

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8) Canaff L, Vanbellinghen JF, Kaji H, Goltzman D, Hendy GN. Impaired transforming growth factor-β transcriptional activity and cell proliferation control of a menin in-frame deletion mutant associated with multiple endocrine neoplasia type 1 (MEN1).**J Biol Chem** 287: 8584-8597,2012.
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Achievements of individual member of Global COE Program for Education and Research on Signal Transduction Medicine in the Coming Generation(2008-2013)

Akiyoshi Uemura

Division of Vascular Biology, Department of Physiology and Cell Biology

1- Summary

In diabetic retinopathy, ischemia following retinal capillary obstruction evokes aberrant angiogenesis which directly causes vision-threatening hemorrhage and retinal detachment. In order to fundamentally resolve the disease pathogenesis, regeneration of functional vessels in ischemic retinas would be of great benefit. For this purpose, it is desired to develop a modality which can selectively inhibit abnormal angiogenesis without affecting vascular regeneration.

In this GCOE program, we found that endothelial cells (ECs) of angiogenic retinal vessels intensively express a small GTPase RhoJ, that belongs to the Cdc42 subfamily (J Clin Invest. 2011). In cultured ECs, RhoJ overexpression induced cell contraction, whereas RhoJ deficiency abrogated Sema3E-induced cell contraction. Because RhoJ was activated by Sema3E and inactivated by VEGF, we proposed that RhoJ is an EC-autonomous molecular switch that determines cell motility by counteracting Cdc42. Indeed, RhoJ overexpression facilitates EC retraction in sprouting vessels, which led to disorganized patterning of retinal vasculature. These results indicated a therapeutic potency of RhoJ as a drug target.

To further search for intracellular signaling molecules which regulate the activation status of RhoJ, we performed DNA microarray analysis exploiting ECs and non-ECs sorted from living mouse retinas. While a number of Rho guanine nucleotide exchange factors (GEFs) and Rho GTPase activating proteins (GAPs) were expressed in angiogenic ECs, we uncovered that Arhgef15 acts as an endothelial-specific RhoGEF to mediate VEGF-induced Cdc42 activation and potentiate RhoJ inactivation, thereby facilitating EC migration (PLoS ONE 2012).

We are currently elucidating the entire signaling network that regulates dynamic behavior of angiogenic ECs (Fig 1), and we believe that our work will contribute to the development of selective anti-angiogenic therapy; not only in eyes but also in various disease settings such as cancer.

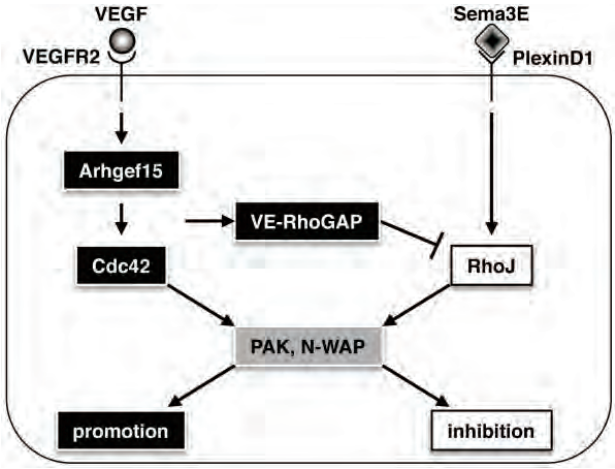

Fig 1. Signal transduction for the regulation of EC motility. Cdc42 and RhoJ commonly bind to PAK and N-WASP, and inversely regulate cytoskeletal rearrangement.

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3) Toyofuku T, Nojima S, Ishikawa T, Takamatsu H, Tsujimura T, Uemura A, Matsuda J, Seki T, Kumanogoh A. Endosomal sorting by Semaphorin 4A in retinal pigment epithelium supports photoreceptor survival. ***Genes Dev.*** 26:816-829, 2012.

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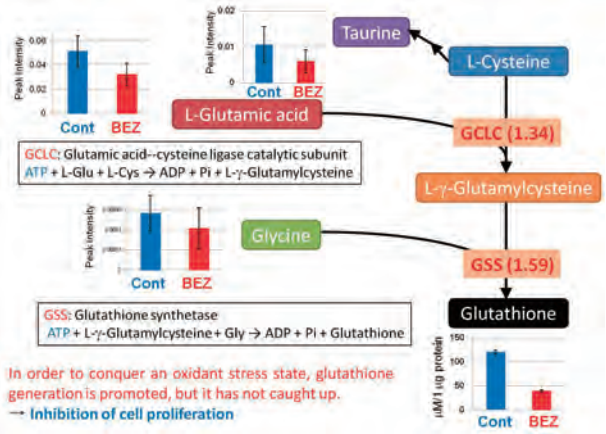
Achievements of individual member of Global COE Program for Education and Research on Signal Transduction Medicine in the Coming Generation(2008-2013)

Naoya Hatano

The Integrated Center for Mass Spectrometry

1- Summary

Metabolic disorders are seen in many diseases. Especially in cancers, the abnormalities are seen in the metabolic pathway. Its discovery goes back to the 1920's. According to the recent research findings, it is revealed that cancer cells build environment to ensure their own survival by changing the metabolism of a cell. Furthermore, the metabolome changes are regulating the expression of the genes, and the concept "cancer is a metabolic disease" become the paradigm in the latest cancer research. In recent years, the metabolome analysis, which can compare metabolites comprehensively at once, is established using a mass spectrometer. The research, which applied the metabolome analysis to medical diagnosis of cancer at an early stage, is advancing and I also contributed to the construction of metabolome analysis system with GC-MS. Although it has demonstrated that there are significant differences in circulating metabolites between the cancer patient and healthy person, the underlying molecular mechanism mostly remains unknown. In order to show clearly how a metabolic disorder causes cancer, I have planned to establish the comparison proteomics method, which targeted the important metabolic enzymes. Human breast cancer cell line (MCF-7) with or without PI3K inhibitor (BEZ235) administration was used as experimental. The number of the cells was reduced by half after 24 hrs. Metabolic profiles of MCF7 and T47D cells were changed by BEZ235, but not changed by MEK inhibitor. There were some metabolites that contributed to the grouping of samples. Glutamic acid and taurine were decreased by half. Therefore, the quantitative proteome analysis by SILAC (stable isotope labeling by amino acids in cell culture) was performed. As a result, after treatment with BEZ235, in order to survive, MCF7 cells actively produce the several subunits of ATP synthase and the glutathione synthase enzymes (GCLC and GSS), which require ATP. However, glutathione was decreased by BEZ235 treatment. According to the metabolome data, glutamic acid and glycine were decreased by BEZ235. They may be used to produce glutathione. In order to conquer an oxidant stress state, glutathione generation is promoted, but it has not caught up. As a result, inhibition of cell proliferation was caused. These results suggest that metabolic proteome analysis is useful for explaining the metabolome changes.



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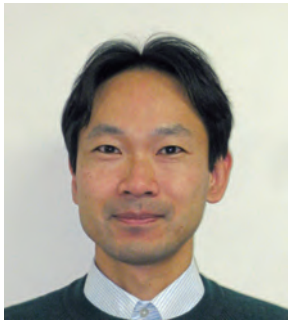

Achievements of individual member of Global COE Program for Education and Research on Signal Transduction Medicine in the Coming Generation(2008-2013)

Makoto Kunisada

Department/Division：Dermatology

1- Summary

I joined the program of GCOE on April in 2009 after coming back to the department of Dermatology of Kobe university of Medicine from National Institute on Aging, US. Firstly I have started the study what genes are involved in *Ogg1* knockout mice, which is defective of repair of reactive species induced (ROS) DNA damage, showing highly skin-carcinogenic phenotype with long term UVB exposure. We performed microarray for the *Ogg1* knockout mice and wild-type counterpart, comparing gene profiling following UVB exposure. The test showed that most significantly changed pathway genes are inflammatory response associating genes, indicating that highly inflammatory status in *Ogg1* knockout mice is associating with the susceptibility for developing skin cancer after UVB irradiation. We also found that *versican*, coding one of proteoglycan, is significantly up-regulated in knockout mice and also highly express in the skin tumors developed from knockout mice. Furthermore, versican is also highly expressed in human skin tumors that is thought to be long term sun exposed related. It was novel findings, because inflammatory response is related with skin carcinogenesis by UVB/ROS induced DNA damage, which never been reported before. We published those findings in *Am J Pathol*, 2011. After that, we moved our projects onto the attempts to elucidate the molecular mechanism between inflammatory pathway gene and versican following UVB using mouse embryonic fibroblasts (MEFs). We have already identified some important key inflammatory related genes which are closely regulating *versican* in MEFs. Currently we are engaged to investigate the role of signal protein regulating the important gene to clarify the functional point of the gene. In other progress, we performed the study comparing mutation patterns between the mice skin tumors developed with conventional broad band UVB (BB-UVB) and newly developed narrow band UVB device (NB-UVB), because NB-UVB previously showed higher carcinogenic mice skin tumors than BB-UVB. We found that the tumors developed with NB-UVB harbored much more CC→TT transition *p53* mutation, indicating that NB-UVB induced more cyclobutane pyrimidine dimes (CPD), most commonly formed DNA damage with UVB, lead to the highly carcinogenic skin tumors. We published those data on *Mutagenesis* in 2012. Finally, we studied whether prescribed drugs could enhance the formation of CPD with UVA because that drug is the possibility to be capable to develop skin tumor with its long term usage. We found diuretic agent hydrochlorothiazide does enhance the formation of CPD of isolated DNA measuring with HPCL-MS. We published those findings on *Photochem Photobiol* in 2013, which is currently in press.

2- Selected publication list

1) Kunisada M, Yogianti F, Sakumi K, Ono R, Nakabeppu Y, Nishigori C *Am J Pathol* 179(6); 3056-65, 2011

2) Yogianti F, Kunisada M, Ono R, Sakumi K, Nakabeppu Y, Nishigori C Skin Tumours Induced by Narrowband UVB Have Higher Frequency of p53 Mutations than Tumours Induced by Broadband UVB Independent of *Ogg1* Genotype *Mutagenesis* 27(6); 637-43, 2012

3) Kunisada M, Masaki T, Ono R, Morinaga H, Nakano E, Yogianti F, Sugiyama H, Nishigori C Hydrochlorothiazide enhances UVA-induced DNA damage *Photochem Photobiol in press*.

Achievements of individual member of Global COE Program for Education and Research on Signal Transduction Medicine in the Coming Generation(2008-2013)

Kaoru Yamagata

Division of Cell Physiology, Department of Physiology and Cell Biology

1- Summary

It has been shown that constitutively active Wnt5a-Ror2 signalingin osteosarcoma cells plays crucial roles in induced expression of *matrix metalloproteinase-13* (*MMP-13*), required for their invasiveness. However, it remains largely unclear about the molecular basis of *MMP-13* gene induction by Wnt5a-Ror2 signaling. Here we show by reporter assay that the activator protein 1 (AP1) (binding site in the promoter region of *MMP-13* gene is primarily responsible for its transcriptional activation by Wnt5a-Ror2 signaling in osteosarcoma cell lines SaOS-2 and U2OS. Chromatin immunoprecipitation assays revealed that c-Jun and ATF2 are crucial transcription factors recruited to the AP1-binding site in the *MMP-13* gene promoter during Wnt5a-Ror2 signaling in SaOS-2 cells. Using siRNA-mediated suppression or specific inhibitors, we also show that Dishevelled2 (Dvl2) and c-Jun N-terminal kinase are required for *MMP-13* induction presumably via phosphorylation of c-Jun and ATF2 during Wnt5a-Ror2 signaling in SaOS-2 cells. Interestingly, Dvl2 and Rac1, but not Dvl3, are required for *MMP-13* expression in SaOS-2 cells, whereas Dvl3, but not Dvl2 and Rac1, is required for its expression in U2OS cells, indicating the presence of distinct intracellular signaling machineries leading to expression of the same gene, in this case *MMP-13* gene in different osteosarcoma cell lines. Moreover, we provide evidence suggesting that Wnt5a-Ror2 signaling might also be required for expression of *MMP-13* gene during the development of the cartilaginous tissue.

More recently, Wnt5a-Ror2 signaling has been shown to be critically involved in epithelial to mesenchymal transition (EMT) through induction of *MMP-2* in Snail-expressing epidermoid carcinoma cells. Although EMT have been also implicated in the progression of tissue fibrosis usually found in severely damaged tissues, it remains largely unclear about a possible involvement of Wnt5a-Ror2 signaling in EMT triggered during sustained fibrosis of multiple tissues, including renal fibrosis. Here we show by using a mouse model of renal fibrosis that expression of both Wnt5a and Ror2 is induced in injured kidney after unilateral ureteral obstruction (UUO). Immunofluorescent analysis revealed that the expression of Ror2 is clearly induced in tubular epithelial cells during renal fibrosis, and these Ror2-expressing cells also express Snail and vimentin, a marker of mesenchymal cells, suggesting that Ror2 might be induced in epithelial cells undergoing EMT. We also show that MMP-2 expression is induced at Ror2-positive epithelium adjacent to significantly disrupted tubular basement membrane (TBM). Interestingly, reduced expression of MMP-2 is detected at tubular epithelium in obstructed kidneys of *Ror2*$^{+/-}$ mice compared with *wild-type Ror2*$^{+/+}$ mice. Importantly, significant disruption of TBM is detected adjacent to Ror2-positive tubular epithelial cells in obstructed kidneys of *wild-type* mice, and such disruption of TBM is apparently reduced in *Ror2*$^{+/-}$ mice. Collectively, these findings reveal that induced expression of Ror2 in epithelial cells with mesenchymal phenotypes plays an important role in disrupting TBM via MMP-2 induction during renal fibrosis accompanied with EMT.

2- Selected publication list

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3

研究者育成

グローバルCOE研究員を経て

グローバルCOEリサーチアシスタント（RA）

グローバルCOE研究員を経て

本研究拠点の活動を充実させるために、必要な人材雇用を行いました。トラックB（リサーチ・アソシエイト）およびポスドクは、主に所属するPIの研究に従事しつつ、将来PIとなるための萌芽的研究を展開し、キャリアアップにつなげる機会も提供しています。また、本拠点における採用者の20%以上を女性とし、女性研究者支援にも力を入れています。

実施実績

年　　度	各年度雇用数
平成20年度	3名
平成21年度	5名
平成22年度	8名
平成23年度	9名
平成24年度	10名

「神経可塑性におけるコンドロイチン硫酸の役割」

薬物動態学（神戸薬科大学生化学研究室）　**宮田　真路**

私は、2008年から本グルーバルCOEプログラムの研究員として、神経可塑性おけるコンドロイチン硫酸の機能解析を進めてきた。可塑性は生後初期の一定期間に最も高く、その後成体になると低下する。コンドロイチン硫酸を分解することで、可塑性が回復することから、コンドロイチン硫酸は可塑性を非特異的に阻害する物理的障壁だと考えられていた。私は、発生に伴いコンドロイチン硫酸の構造が変動することで、神経細胞周囲の細胞外マトリクスが変化し、可塑性の低下を引き起こすという、これまでに知られていなかった制御機構を明らかにし、国際的にもインパクトの高いNature Neuroscience誌に報告した。本プログラムで、５年間という比較的長期間に渡り安定した支援を受け、さらに、プログラム内の多くの先生方から貴重なアドバイスを頂いたおかげで、このような成果を挙げることができ、非常に感謝している。この成果が認められ、名古屋大学高等研究院の特任助教に採用されたため、今後は、本プログラムで培った経験をもとに、より独立した研究者として研究を発展させたい。

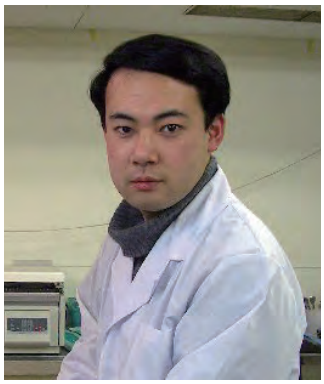
「C型肝炎ウイルスによる糖代謝異常の分子機序の解明」

微生物学　**DENG Lin**

C型肝炎ウイルス（HCV）は慢性肝炎や肝硬変、肝細胞癌を引き起こすのみならず、２型糖尿病や脂肪肝と密接な関連があることが臨床的に知られています。私は、グローバルCOE研究員採用中、糖代謝に重要な役割を果たす転写因子FoxO1に焦点を当て、HCV感染による糖代謝異常に関わるFoxO1の役割及びその制御における上流因子について精力的に研究に取り組んできました。その結果、HCV感染が酸化ストレスの誘導（ROS産生の亢進）を介してJNKを活性化し、これがFoxO1のリン酸化を抑制し核内に留め、その転写活性を亢進させ、糖新生律速酵素遺伝子群の転写促進を介して糖新生を亢進させることを明らかにしました。

採用期間中、年４回の研究報告会に参加し、様々な先生方から研究の方向性や不足する部分についてsuggestionを頂き、順調に研究を進めることが出来ました。また、グローバルCOEのサポートを頂き、HCVの国際学会に参加することができ、世界各国の研究者による講演や活発な議論を聞くことは、自分にとって非常に刺激になりました。グローバルCOEという恵まれた環境で研究できることに常に喜びを感じていました。

今後、これまでの経験を生かし、自分自身さらに精進し、自立した研究者への道を目指したいと思います。



「本グローバルCOEプログラムに参加して」

シグナル伝達学　**宮田　宗明**

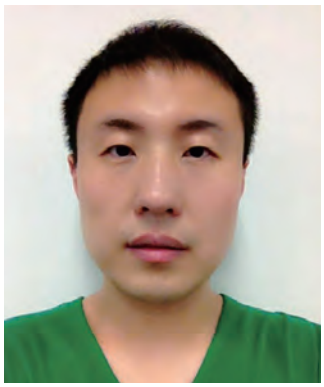
本グローバルCOEプログラムにおいて、私は代謝疾患と神経疾患を結ぶメカニズムの解明を目的として研究をおこないました。特に代表的代謝異常である糖尿病の中枢性神経障害における免疫グロブリン様細胞接着分子Necl-5の役割について主に免疫組織学的手法を用いて検討しました。ストレプトゾトシン投与I型糖尿病をNecl-5ノックアウトマウスで作成すると、野生型マウスの場合と比較して神経変性が亢進しており、Necl-5が糖尿病で併発する神経変性に対して抑制的にはたらいていることを見出しました。今後は継時的、統計学的な解析を進めるとともに、生化学、分子生物学的手法を用いてNecl-5が神経変性を抑制する分子メカニズムを解明したいと考えています。

また、本グローバルCOEプログラムでは研究報告会、研究討論会、ワシントン大学との合同国際シンポジウムなどを通じて研究発表をおこなう機会が数多くあり、多数の指導者やその他のグローバルCOEリサーチアシスタントと基礎医学と臨床医学を融合した分野横断的な観点から建設的なディスカッションができました。これらの機会を通じて幅広い知識の習得と私自信のプレゼンテーション技術の向上を図れたことは大きな成果でした。

「ヒトヘルペスウイルス6の感染機構の解析」

臨床ウイルス学　**河端　暁子**

私は2010年４月に臨床ウイルス学分野グローバルCOE研究員として採用して頂き、ヒトヘルペスウイルス６ (HHV-6) の感染機構についての解析を行ってきました。HHV-6、特にHHV-6Bは乳幼児の突発性発疹の原因ウイルスであり、ほぼ100%の成人がその抗体を保有していますが、移植等の免疫抑制状態で頻繁に再活性化し、脳炎など重篤な病気を引き起こすことが知られています。しかしながらHHV-6Bの感染機構に関しては不明な点が多く、その早期解明が望まれています。私は主にHHV-6Bの宿主細胞への侵入機構に焦点を当てた研究を行い、その侵入にはウイルス表面に存在するエンベロープ糖タンパクであるglycoprotein Q1 (gQ1) が重要であることを見出し、成果をJournal of Virology誌に発表することができました (Kawabata et al, J Virol. 2011 Dec;85(24):12962-71.)。また、HHV-6の別の糖タンパクであるglycoprotein Mについても解析を行い、こちらも成果をVirology誌に発表することができました (Kawabata et al, Virology. 2012 Jul 20;429(1):21-8)。この３年間で得た結果をもとに、今後は前述のgQ1の構造解析を行い、HHV-6の侵入機構の詳細をさらに明らかにしていきたいと考えております。本研究にあたりご指導頂きました臨床ウイルス学分野教授、森　康子先生に心より感謝申し上げます。

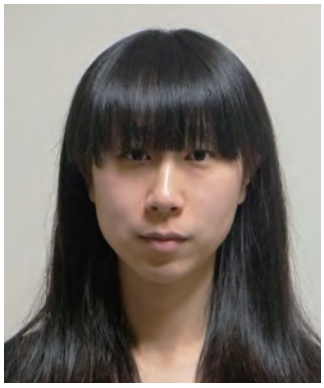


「C型肝炎ウイルスに対する予防および治療ワクチン開発に関する研究

微生物学　**姜　大鵬**

私はC型肝炎ウイルス(HCV)に対する予防ワクチンと治療ワクチンを開発する目的で、HCV(1b型)のエンベロープタンパク質及び非構造タンパク質の一部をコードする遺伝子領域を組み込んだ発現プラスミドを数種類作製しました。非構造タンパク質発現プラスミドをDNAワクチンとしてマウスに接種して、細胞性免疫の誘導指標インターフェロンγ誘導活性とCTL活性を調べました。その結果、ワイルドタイプNS3とプロテアーゼ失活NS3(S139A)がマウスに効率良く細胞性免疫を誘導することを確認しました。また、数種類のエンベロープタンパク質発現プラスミドをDNAワクチンとして及び培養細胞に発現させ、マウスに接種して、抗HCV中和抗体誘導能について調べました。その結果、より安全性高い、そして免疫誘導に効果的なエンベロープタンパク質の領域を同定しました。

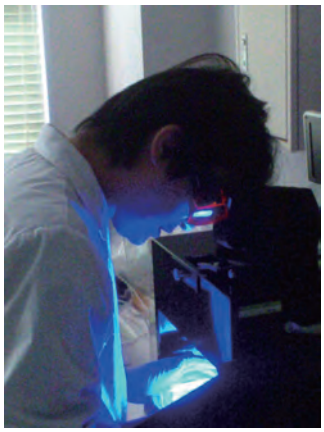
グローバルCOE研究員に採用された期間、GCOE海外研究発表援助により、HCV国際学会に参加し、発表することができました。その場で海外の研究者と情報交換を行い、自分の研究と成長にとって大きな収穫になったと思います。今後、同定されたHCV のエンベロープタンパク質及び非構造タンパク質領域を基にした組換え水痘生ワクチンを作製し、ヒトに実用化が可能なHCV に対する予防と治療ワクチンの開発に頑張りたいと思っています。



「血管内皮細胞運動を制御する分子機構の解明」

血管生物学　**福嶋　葉子**

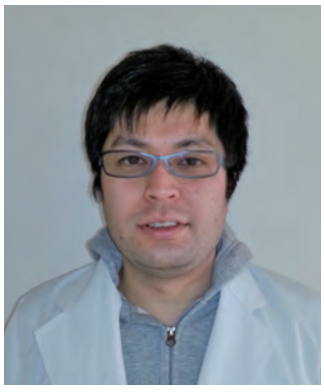
2012年4月1日より血管生物学分野　植村明嘉先生の指導のもと、血管内皮細胞の糸状仮足形成を制御する分子機構の研究に従事してまいりました。特に糸状仮足を構成するアクチン線維の再構成に関わる低分子量Gタンパク質に着目して研究を進めています。これまでに血管内皮細胞に特異的に発現するRhoJを同定し、血管新生を抑制するSema3Eシグナルの下流で活性化されることを明らかにしました。一方、血管新生を促進するVEGFシグナルにおいては血管内皮特異的に発現するArhgef15によりCdc42の活性化およびRhoJの不活化が誘導され、アクチン重合による糸状仮足が形成されることを報告しました (PLoS ONE, 2012)。現在、マウス網膜を用いて生体における血管網の形成でのRhoJの機能を検討しています。生体では血管新生を促進する因子、抑制する因子が同時に内皮細胞に作用することが予想されます。相反するシグナルに対し内皮細胞がどのように応答し、正常血管網のパターンがいかにして構築されるのかを明らかにし、正常血管形成の理解とともに病的な血管新生の制御に寄与できるような研究を継続していきたいと考えています。



「ヌクレオチド除去修復における DNA損傷認識機構とSUMO化修飾の意義」

バイオシグナル研究センター　**秋田　眞季**

哺乳類のヌクレオチド除去修復 (NER) において、C群色素性乾皮症 (XPC) タンパク質がDNA損傷の認識と修復反応の開始に必須な役割を果たしている。紫外線損傷に対しては、DDB1-DDB2複合体 (UV-DDB) と共に協調的に働くことによって効率な損傷の検出を可能にしている。我々は、XPCが細胞内でユビキチン様タンパク質SUMOによる修飾を受けること明らかにし、本プログラムではXPCのSUMO化修飾の役割についての研究をおこなった。SUMO修飾部位を置換した変異XPCを安定発現する細胞株では、紫外線により生じる(6-4)光産物の修復に遅延が認められた。さらにXPCのSUMO化がUV-DDBとの物理的相互作用を増強することを見出し、DDB2の発現抑制により変異XPC発現細胞における修復の遅延が回復することを見出した。このことは、XPCのSUMO化がCRL4DDB2との機能的連携において重要な役割を果たしており、効率よくDNA損傷の認識を保証するメカニズムにSUMO化修飾が関与する可能性を示唆する。



「Anti-CXCL13 antibody can protect against gastric lymphoid follicles induced by *Helicobacter* infection」

消化器内科学　**山本　幸司**

Helicobacter suis (H. suis) は、グラム陰性細菌で、イヌやネコ、ブタ、ヒトを含む多くの動物種の胃に感染し、胃病変の発症につながることが示唆されている。

近年、*H. suis* 感染により、マウス胃粘膜に、胃MALTリンパ腫が誘導され、本細菌は、胃MALTリンパ腫の原因菌であることが明らかにされた。

これまでに我々は、H. suisをマウスに経口感染させることで、マウス胃粘膜にリンパ濾胞の形成を認め、B細胞の走化性に関与するCXCL13の発現が上昇することを報告した。

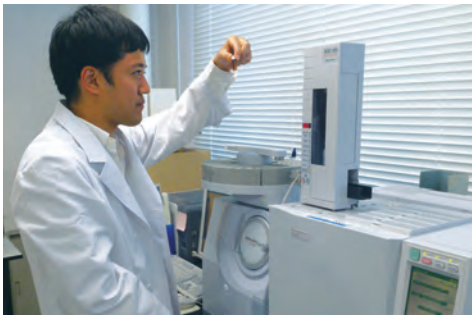
そこで我々は、*H. suis* 感染により発現誘導されるCXCL13に着目し、*H. suis* 感染マウスに抗CXCL13抗体を投与して、胃リンパ濾胞形成の抑制効果について検討した。その結果、抗CXCL13抗体投与後の *H. suis* 感染マウスの胃粘膜おいて、リンパ濾胞形成が抑制されており、CXCL13発現誘導に関与するNF-κB2の活性化も抑制されていた。さらに、リンパ濾胞形成に関与するLTA、LTB、CXCR5、THFR1、ならびに、LTBRの発現も、同様に低下していた。

これらの結果は、*H. suis* 感染によって誘導されたCXCL13が、胃リンパ濾胞形成に関与していることを支持しており、今後、抗CXCL13抗体を用いたヒト胃MALTリンパ腫発症の治療効果が期待される。

「インフルエンザウイルスの研究を始めて」

人獣共通感染症学　**笹原 健二**

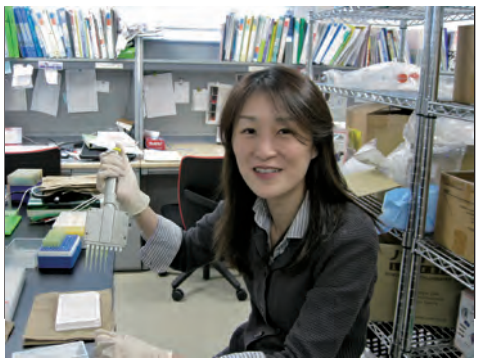
シグナル伝達グローバルCOEプログラムは2008年からスタートしましたが、私は最終年度の2012年5月からGCOE研究員として本プログラムに参加しました。それに伴って、タンパク質の研究室からインフルエンザウイルスの研究室（人獣共通感染症学分野）に移り、ウイルスを用いた新たな研究を始めました。インフルエンザウイルスは、タンパク質、脂質膜、そして核酸からなるので、これまで行ってきたタンパク質と脂質膜の物理化学的な研究から、核酸という生体物質が新たに研究材料に加わることで大きく研究内容が広がりました。その結果、これまであまり身近な分野ではなかったインフルエンザウイルスを病原とする感染症について多くのことを学ぶ機会を得ました。研究のほうも短期間でしたが、研究室のメンバーの協力のおかげで学会発表（平成24年度日本生化学会）ができるまで進展しました。今後は、本プログラムで行ったことを研究としてまとめ、更にインフルエンザウイルス研究を物理化学的な側面から発展させていきたいと考えています。



「膵がん早期発見を目指して」

消化器内科学（グローバルCOE特別研究員）　**小林　隆**

膵がんは、がんの中でも特に早期発見が難しく、治療にも難渋する予後不良の疾患である。私は、グローバルCOEのRA、特別研究員として、膵がんの早期発見を目指して日々研究に取り組んできた。私が行ってきたのは、ヒト血液を用いて、メタボロミクスで膵がんを診断しようとするtranslational researchである。研究を進める中で、特に重要であったのは、安定した分析系の構築することと、ヒト血液検体を収集することであった。前者に当たっては、100種類以上の標品分析によるデータベースの構築、最適な測定条件を見出すための試行錯誤、多検体処理における課題克服などと、地道な努力が必要であった。後者に当たっては、100人以上の検体が必要であったことから、所属施設のみならず、複数の他施設に足を運んで協力を依頼し、対象者には一人一人丁寧に研究の説明を直接行い検体提供の同意を得た。このような、研究室内での作業にとどまらない研究活動を経験することで、結果的に、視野が広く客観性のある研究を行うことができたのではないかと自負している。これまでに、臨床応用に向けて有望な結果を得ていることから、今後もこの研究を継続発展し、社会に貢献したいと考えている。



「冠動脈石灰化とBMP-binding endothelial cell precursor-derived regulator（BMPER）」

循環器内科学　**小林　成美**

私は循環器内科において心肥大・心不全についての研究を行ってきましたが、グローバルCOE研究員に任用されてからの5年間では、新たに動脈硬化の成因機序についての研究を行うことが出来、私にとっては循環器内科医としての視野を広げる良い機会となりました。BMPERという新規分子に着目し、BMPERの冠動脈石灰化の促進作用を見出しました。冠動脈石灰化は急性冠症候群を引き起す重要な危険因子であり、今回の研究成果はその治療戦略に寄与するものと考えています。さらに現在はBMPERの患者血中濃度測定を行い、冠動脈疾患に対するバイオマーカーとしての可能性を検討しています。2013年4月からは循環器内科の特定助教として任用されることとなり、グローバルCOE研究員としての経験を臨床と基礎研究を繋ぐ仕事に活かしたいと思っております。グローバルCOEでは、様々な講座の先生方にご指導を賜り、広く医学、基礎科学の考え方を学ばせて頂きました。また、同輩の研究員の皆様からは励ましやアドバイスを頂き、ともに頑張ることが出来ました。いずれも素晴らしい経験でした。事務局の皆様には大変お世話になり、深く御礼を申し上げます。本当に有難うございました。

グローバル COE リサーチアシスタント（RA）

本拠点基礎･臨床医学融合による分野横断型のリサーチリーダー育成コースを設置し、博士課程1、2年次学生5 ～7/年の優秀者を選抜し、自主的研究費の支給・経済的支援並びにコーディネーターを中心とした適切な教育研究指導を行うことにより、独創性を持った国際的活動能力を有するClinician-Scientist医学研究者を育成しています。

実施実績

年　　度	各年度雇用数
平成20年度	7 名
平成21年度	7 名
平成22年度	7 名
平成23年度	7 名
平成24年度	5 名

「本グローバルCOEプログラムに参加して」

遺伝学分野　**國政　啓**

私は2012年度に神戸大学大学院医学研究科に入学し、遺伝学分野にて井垣達吏先生の指導のもと、研究を開始した。呼吸器内科医として臨床に従事するなかで、肺癌を中心とした癌診療に強く惹かれ、新しい治療薬の作用点につながる癌生物学の研究をしたくて、細胞競合という現象を研究している現研究室の門をたたくに至った。

研究生活はまだ1年足らずであるが、グローバルCOE研究員の資格を頂くという幸運にも恵まれ、充実した研究生活を送らせて頂いている。グローバルCOEでは、定期的な研究進捗報告会があり、多くの先生方より、普段の研究室内での討議では見落としてしまうようなことについてコメントを頂いたり、新たな展開を考えるきっかけを頂き、研究を進めるにあたって大きな支えとなった。

「Interferon-γ induces the formation of gastric lymphoid follicles after Helicobacter suis infection」

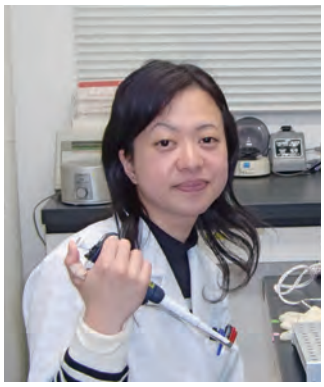
消化器内科学分野　**楊　林**

Helicobacter (H.) suis, which belongs to the Helicobacter family just like H. pylori, is a relatively larger, spiral-shaped gram-negative bacterium, and is found in the stomachs of various animals including cats, dogs, pigs, and furthermore humans. H. suis infection causes various diseases, and especially has a greater tendency to induce gastric mucosa-associated lymphoid tissue (MALT) lymphoma compared with H. pylori infection, suggesting their different pathogenic mechanisms in the gastric disease.

Recently, we reported that Th1 cytokine Interferon (IFN)-γ was strongly induced in H. suis-infected stomachs of mice compared with Th2 cytokines IL-4. Six months after H. suis infection, the formation of gastric lymphoid follicles was consisted of B cells, CD4+T cells, dendritic cells (DCs), and follicular dendritic cells (FDCs), which was detected in wild type (WT) mice but not in IFN-γ knockout (KO) mice. Interestingly, the formation of gastric lymphoid follicles in the stomach of H. suis-infected T cell receptor (TCR) KO mice as same as WT mice was also observed, suggesting that T cells were not required for the formation of gastric lymphoid follicles after H. suis infection. So we purified B cells, DCs and FDCs except for T cells from non-infected WT mice by FACS and transferred these cells into H.suis-infected IFN-γ KO mice respectively to identify IFN-γ-producing cells.

In B cell transferred IFN-γ KO mice at 3 months after H.suis infection, the formation of gastric lymphoid follicles and the induction of IFN-γ were detected. On the other hand, we recently revealed that chemokine CXCL13 played an important role in the formation of gastric lymphoid follicles after H. suis infection (in submission). In this study, the expression level of CXCL13 was restored in the stomach of B cell transferred IFN-γ KO mice after H.suis infection as same as that in WT mice. Moreover, it has been reported that recombinant IFN-γ significantly induced the activation of CXCL13 in immune-related cells. Therefore, we considered that the up-regulation of IFN-γ from B cells induced the formation of gastric lymphoid follicles along with the activation of CXCL13 after H. suis infection.

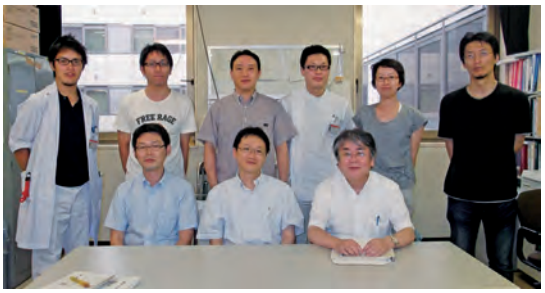
In future, we will detect the lymphoid follicles formation and IFN-γ expression level in the stomach of DCs and FDCs transferred IFN-γ KO mice. In addition, we plan to isolate B cells, DCs and FDCs from H.suis-infectd stomachs by FACS and detect the expression level of IFN-γ mRNA to confirm IFN-γ producing cells in the stomach, which was expected to elucidate the influence of H. suis infection on host functions and establish the feasible therapeutic strategy for H. suis infection-related diseases in humans.



「C型肝炎ウイルスによるGLUT2遺伝子発現抑制の分子機構」

微生物学分野　**松井　千絵子**

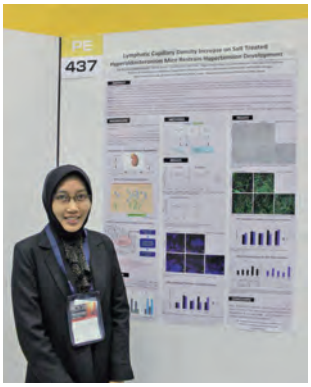
C型肝炎ウイルス(HCV)の増殖や肝発癌に糖・脂質代謝異常が重要な役割を果たしていることが明らかになってきています。私はHCVによる糖代謝異常の分子機構を明らかにするため、HCV感染に伴うGLUT2遺伝子発現抑制機構を解析しています。ヒト肝癌細胞株にHCVを感染させると細胞内転写因子HNF-1αがライソソーム依存性蛋白分解系を介して顕著に減少しGLUT2遺伝子の転写が抑制されること、その分解にはHCV NS5A蛋白質とHNF-1αの結合が重要であることをJournal of Virologyに報告しました。G-COE RAに採用され、ヴェネツィアでのHCV国際学会にてポスター発表を行う機会に恵まれ、世界中のHCV研究者とディスカッションし、とても刺激を受けました。G-COEを通じて英語発表の機会が国内でも増え、他分野の研究者とも交流し、多くの研究上のヒントを得ました。今後はG-COE RAで得た経験を活かして更に研究に邁進し、NS5A蛋白質がいかにHNF-1αを特異的に標的にするのか、HCVによるHNF-1α分解が脂質代謝異常に与える影響などを明らかにしていきたいと考えています。



「動脈硬化症における新規の抗炎症治療法の開発に向けて」

循環器内科学分野　**笠原　和之**

近年、動脈硬化症は慢性の炎症性疾患であるという概念が定着した。我々は、免疫担当細胞の中でも、とくに炎症を負に制御する細胞である制御性T細胞に着目し、動脈硬化症と制御性T細胞の関わりに関して、マウスを用いた研究を進めている。私の当初のGCOE-RAとしての研究テーマは、制御性T細胞をin vivoで増殖させることができる治療薬IL-2 complexにおける抗動脈硬化作用の検討であった。研究成果を学会発表行うなど順調に進んでいたが、海外のグループから先に同様の報告が2報相次ぎ、私の研究結果は彼らの報告とほとんど違いを示せないことから、この研究は中断せざるを得なかった。次の研究テーマとして、制御性T細胞を特異的に除去できるマウスを用いて、動脈硬化と制御性T細胞の関わりを明らかにしようと試みた。この研究は、仮説通りにはうまく進まず、多くの問題点が生じた。さらに最近この分野で著名なグループから、同じマウスを用いた研究が報告された。先んじて報告されたものの、我々の結果と彼らの結果で相違点もあり、その違いも含めてなんとか形にしたいと考えている。また、腸管免疫や腸内細菌など、腸管にfocusをあてた研究も進めており、腸管と動脈硬化の関わりについても明らかにしていきたい。

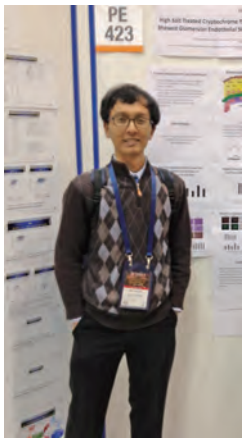


「The Novel Role of Aldosterone on Salt sensitive Hypertension」

循環器内科学分野　**Dwi Aris Agung Nugrahaningsih**

The novel study about sodium homeostasis reveals that despite the kidney, skin also has a role on blood pressure regulation through sodium homeostasis regulation. Based on that study, during high salt intake, sodium will be deposited in the skin and stimulates macrophage to secrete VEGF-C. It will cause lymphatic capillary density increase which provides buffering space for water retention, resulting in maintaining the blood pressure. Although aldosterone causes hypertension partly through its effect on kidney sodium re-absorption, it is not known whether aldosterone affects sodium regulation in skin. Objective: Investigate the skin sodium regulation role on hyperaldosteronism condition. Method: Cryptochrome null mice which show hyperaldosteronism were treated with high salt diet for 2 week and 32 weeks. Blood pressure was measured weekly. Macrophage and lymphatic capillary in the skin were stained using F4/80 and LYVE-1 immunostaining respectively. Investigation of macrophage polarization were done using CD40 and CD206 immunofluoresecent staining and real time of M1 and M2 macrophage marker mRNA. Result: Blood pressure measurement showed the same blood pressure between groups on 2 week of treatment but after 32 week of treatment the high salt treated Cryptochrome null mice showed higher blood pressure compare with the one on other groups. The 2 weeks high salt treatment on Cryptochrome null mice also result in higher macrophage number and higher lymphatic capillary density compare with the one on Cryptochrome null mice under normal salt intake. Whereas, 32 weeks high salt diet treated Cryptochrome null mice showed the same macrophage number and lymphatic capillary density compare with those on Cryptochrome null mice under normal salt diet. Staining result of CD40 (M1 macrophage marker) and CD206 (M2 macrophage marker) showed the dominance of CD206 in the skin samples. Real time PCR result also showed that M2 macrophage marker mRNA increase in wild type mice under high salt treatment but not the M1 macrophage marker mRNA. Conclusion: Chronic hyperaldosteronism and high salt treatment might affect the macrophage and lymphatic capillary in the skin and play at least in part on the blood pressure maintenance. Investigation of macrophage in the skin showed that M2 macrophage is the macrophage involve in the skin lymphatic capillary development during high salt diet.

Future plan: isolate monocyte from cryptochrome nullmice and also from wild type mice both uder normal and high salt treatment. Further, we will treat the isolated monocytes and treat them with IL-4 to stimulate the M2 macrophage polarization. We will compare the M1 and M2 macrophage marker expression on onocytes of wild type and cryptochrome null mice under treatment. Therefore we can evaluate whether there are any difference on the monocytes ability to polariza into M2 macrophage between wild type mice and cryptochrome null mice monocytes.



「The Role of Heparan Sulfate Proteoglycan in the Vascular Calcification Development」

循環器内科学分野　**Eko Purnomo**

I study in Cardiovascular Division, Internal Medicine Department of Kobe University Graduate School of Medicine. My research topic is vascular calcification. Vascular calcification increases the mortality of end stage renal disease patients due to cardiovascular diseases. Vascular calcification is known as an actively regulated process partially through enhanced dedifferentiated and apoptotic vascular smooth muscle cells (VSMCs). In other hand, heparan sulfate expressed in the cell surface and extracellular matrix of VSMCs highly contributes to the vascular calcification, although precise mechanisms are unknown. We hypothesized that altering the fine structure of HS would impact phagocytosis of apoptotic cells by vascular smooth muscle cells (VSMCs) and induce vascular calcification. We create a chronic kidney disease model in mice by performing sub total nephrectomy and with high phosphate diet to induce vascular calcification. By using the EXTL2 deficient mice, which have higher heparan sulfate amount in their aorta, we found that aortic calcification was severely occurred in these mice than in wild type littermates. Several osteoblastic markers were expressed in the calcified aorta but not in uncalcified aorta. Supporting this result, we explanted the aortic ring with high phosphate medium and compare with normal medium. High phosphate treatment augmented calcium deposition in both genotype which significantly higher in extl2 deficient mice. In the in vitro study, heparan sulfate expression was augmented in the human aortic smooth muscle cells under high phosphate treatment. Attenuation of heparan sulfate with heparitinase, decreases the calcium deposition in high phosphate condition. In addition, psmad 1/5/8 expression as a downstream signaling of BMP2 was augmented in the in vitro and in vivo result. From those evidences, we suggest that heparan sulfate expression may regulate the vascular calcification progression in part through BMP2 signaling pathway. Blocking the heparan sulfate synthesis or its function is needed to inhibit the progression of vascular calcification in end stage renal disease. We plan to investigate the reason of higher blood pressure, which is presence in EXTL2 deficient mice with kidney disease. We hypothesize that heparan sulfate expression may related with trans activated of endothelin and/or renin angiotensin aldosterone system which farther induced high blood pressure in uremic condition then propagate the kidney disease condition.

「GCOEと研究室の皆様のサポートを受けて」

分子細胞生物学　**福田　旭伸**

私は、医学部卒業後、医師として５年間臨床経験を積んだ後に、母校に戻ってまいりました。臨床では、EBMに基づいた診療が行われ、診断から治療に至るまでマニュアル化しつつあります。一方で、現在の医療は、過去の先駆者らの基礎実験を基に構築されていたものであり、医療の進歩の歴史を知れば知る程、基礎研究の重要性を理解するようになり、基礎研究をするために大学院に進学することを決意しました。とはいえ、ミクロな話は、学生以来であり、実験手技を獲得し、理論の構築ができるようになるのは容易ではありません。

GCOE RAに採用されるにあたり、研究活動に集中できただけでなく、GCOEが主催するシンポジウムや講演会で、著名な研究者の講演を聞くことで学力やモチベーションの向上にもつながり、大変有意義でありました。私は、耳感覚上皮細胞における細胞間接着と細胞極性の関連をテーマに実験をすすめ、現在、論文を投稿中です。

高井義美教授をはじめ、親切丁寧に実験手技を教えていただいた研究室の皆様、そしてGCOEによるサポートがあったからこそ投稿できたといえます。いずれ臨床に還元できるように、今後も頑張っていきたいとおもいます。

「C型肝炎ウイルスNS5A蛋白質の新規結合因子 ヒストンメチル基転移酵素SMYD3の同定と機能解析」

微生物学　**陳　明**

C型肝炎ウイルス（HCV）は肝臓に高率に慢性の炎症を起こし、数十年を経て肝細胞癌を引き起こしますが、その詳細な分子機序は未だ明らかにされていません。私はHCVの非構造蛋白質の一つであるNS5Aに着目し、NS5Aと結合する新規宿主因子を同定し、NS5Aによる新たな病原性発現機構を明らかにすることを目的とした。その結果、新規NS5A結合因子として、ヒストンメチル基転移酵素SET- and MYND-domain containing 3 (SMYD3)を同定しました。NS5AがSMYD3の細胞内局在に影響を与えました。また、HCV感染によりSMYD3蛋白質量が減少し、ヒストンH3の発現量及びヒストンH3-K4のメチル化が低下しました。HCV感染によるSMYD3発現抑制がヒストンH3の発現量およびヒストンH3-K4のメチル化を低下させ、標的遺伝子の発現に影響する可能性が示されました。

グローバルCOE　RAの採用期間中、いろいろな発表を通じて、他の研究者達と直接議論し、私の今後の研究方針について有意義な検討を行うことができました。実験も順調にすすめていました。

今後の研究計画はNS5AがSMYD3の発現レベル、活性、標的遺伝子及び細胞内局在に影響を与え、肝細胞の癌化あるいはその他の病原性に関与する分子機序を解析したいと思います。

「この1年を振り返って」

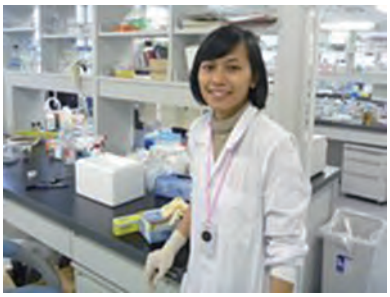
循環器内科学　**淀井　景子**

臨床では、基本的にエビデンスに基づいた治療法を選択します。その大筋に加え、あとは本人の体力や抵抗力を信じ、対症療法で回復を待つこともしばしば。たいていの患者さんが良くなる一方でそうでない人がいる―その違いは何か。元気に退院したように見えた人は本当によくなったのか。

卒後数年の臨床現場において、このような漠然とした疑問を抱きつつ、診療に追われる日々でした。まずこの疑問をはっきりさせ、理解できればと思い大学院に入りました。そして自分の知らない基礎研究の深さに圧倒されながらも、特にこの１年はGCOEの研究員として、非常に貴重な経験をさせて頂きました。

私達の研究室では、動脈硬化と免疫をテーマに様々な課題に取り組んでいます。病気の発症様式、経過が個々により異なる原因は様々ですが、その一因として、免疫の役割も重要であり、現在私は大動脈瘤モデルマウスを用いてその研究を行っています。

GCOEを通じて同世代の研究者の成果を身近に知ることができ、とても刺激になりました。関係の先生方や研究支援の方々には大変感謝しております。まだ始めたばかりですが、今後も少しでも日々進歩のある研究生活を続けていきたいです。



「Roles of vascular endothelial protein tyrosine phosphatase (VE-PTP) in endothelial cells」

シグナル統合学　**Kemala Isnainiasih Mantilidewi**

Currently, I am a 3rd year Graduate Student of Faculty of Medicine, and I have been enrolled as a Research Assistant (RA) in the Signal Transduction Medicine since 2011. My project entitled "Roles of vascular endothelial protein phosphatase (VE-PTP) in endothelial cells", mainly concentrates on the mechanism and functional role of localization of VE-PTP in response to a stimulus called shear stress in vitro. I have gained many benefits through this program, since this project has programs that encourage the students to be always productive and show their progress during routine progress report. Through this, I have the media to train my presentation skill, since I have many chances to practice deliberating my idea and work to others. A more global benefit is the opportunity to meet not only local, but also international young researchers, as well as to listen to experts came worldwide during symposium. In this way I could broaden my knowledge about many different research themes. This is very beneficial compare to others which may have only rare chances to participate in symposium or seminars. For my future research, I would reveal the functional role of VE-PTP in response to shear stress in vivo, using the conditional knock out of VE-PTP.

「Expression and Function of Chondroitin Sulfate N-acetylgalactosaminyltransferase 2 in the Development of Atherosclerosis」

循環器内科学　**Dyah Samti Mayasari**

Until now, atherosclerosis still becomes the major underlying pathology in mortality caused by cardiovascular disease. For several decades, the treatment of atherosclerosis was already focused on the modification of its risk factors. However, through this approach, the efficacy of such therapies is less than optimal and the number of atherosclerosis case still remains high. Therefore, the totally new approach in the treatment or prevention of atherosclerosis is urgently needed. Our study emphasizes the early stage in the development of atherosclerosis as an important phase that can be targeted as a new approach in the prevention of severe atherosclerosis. Previous basic research about early atherosclerosis revealed the role of proteoglycan, particularly the modification of glycosaminoglycan (GAG), in the retention of lipoprotein in arterial wall. Although several enzymes known important in the biosynthesis and modification of GAG, the cooperation of two enzymes, chondroitin 4-O-sulphotransferase-1 (C4ST-1) and chondroitin sulfate N-acetylgalactosaminyltransferase-2 (ChGn-2), is needed in the elongation of GAG. This elongation of GAG and modification of sulfation pattern increase the binding of GAG chain with low density lipoprotein (LDL). Therefore, this modification of GAG promotes the retention of lipoprotein in arterial wall. Previous studies also reported that the GAG produced by macrophage is longer compared to which is produced by monocyte, suggesting that GAG elongation also occur in monocyte to macrophage differentiation, and it may important in foam cell formation.

This study was designed to elucidate the role of ChGn-2 in the development of atherosclerosis, particularly in the trapping of LDL and in foam cell formation. We used mice lacking of ChGn-2 (ChGn-2-/-), bred with LDL receptor knockout (LDLr-/-) mice and used the littermates. Disaccharide composition analysis showed that total chondroitin sulfate (CS) was less in ChGn-2-/-/LDLr-/- mice after treatment of western diet (21gm% fat, 0.21% cholesterol) for 8 weeks (2949±144 vs 3354±149 pmol/mg), suggesting that elongation of GAG was prevented in ChGn-2 deficiency mice. The atherosclerotic plaque progression was also significantly attenuated in ChGn-2-/-/LDLr-/- mice compared to LDLr-/- mice (115800±11120 vs 209500±22480 μm2; $p<0.05$). Injection of rhodamine-labeled LDL into ligated-carotid mice showed that the retention of LDL was diminished in ChGn-2-/-/LDLr-/- mice, indicating that ChGn-2 has a role in the development of atherosclerosis through the modification of lipoprotein trapping. Differentiation of monocyte to macrophage by phorbol 12-myristate 13-acetate (PMA) treatment in THP-1 cells induced the increasing of ChGn-2 and C4ST-1 expression significantly ($p<0.0001$). Transfection of ChGn-2 into THP-1 cells and its differentiation into macrophage and foam cell also indicated the higher uptake of rhodamine-labeled oxidized LDL ($p<0.01$) through the upregulation of CD36 receptor ($p<0.05$). In conclusion, ChGn-2 takes part in the development of atherosclerosis through the modification of lipoprotein trapping and foam cell receptor. Thus, ChGn-2 may be a plausible target in the prevention of atherosclerosis.

For the future plan, we want to observe the relation of GAG chain length elongation and inflammation. We will also confirm the cell culture result using human monocyte primary culture.

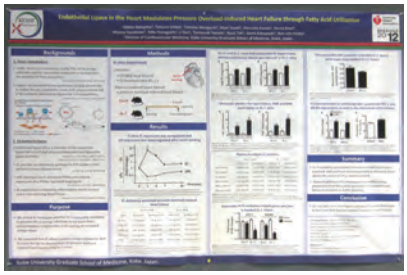
「Analysis of the effects of Spirulina platensis on UVB-induced skin carcinogenesis」

皮膚科学　**Flandiana Yogianti**

I first came to Kobe University on April 2009 as a Research Student and I officially started to become a Graduate Student on October 2009. I belong to Division of Dermatology and worked under the supervision of Prof. Chikako Nishigori and Dr. Makoto Kunisada. My main research theme is about Photobiology. We need sunlight for our life, and we also use ultraviolet as treatment for various skin diseases. However, chronic exposure to ultraviolet may cause damage to skin and cause skin cancer. Here, I conduct several studies about Photo carcinogenesis. First, I investigated the p53 mutations in skin tumors which were obtained from chronic exposure of two different wavelengths of UVB in C5Bl6/J wild-type and Ogg1 knockout mice. I reported that narrow band UVB-induced skin tumors have higher frequency of p53 mutations than those which were induced by conventional broad band UVB, where the Ogg1 genotype did not affect the patterns of the mutation (Yogianti F et al, Mutagenesis 2012).

I also worked on project to study the effect of blue-green algae Spirulina platensis, which has been used as a human food supplement because it contains abundant nutritional components, on UV response using mouse model. I found that daily dietary of 10% Spirulina platensis against the development of UVB-induced skin tumor in both wild type and Ogg1 knockout mice. Our other findings also showed that Spirulina platensis has an antioxidant effect as it had reduced the formation of 8-oxoG. We also conclude that Spirulina platensis has anti-inflammatory effects as it significantly decreased the pro-inflammatory cytokines expressions and in hairless mice which were fed with Spirulina platensis diet, single shot minimal erythema dose of UVB irradiation resulted in less erythema compared that mice which were fed with normal diet. These results indicate Spirulina platensis exert anti tumor effect on UVB-induced skin cancer through antioxidant and anti-inflammatory effects. Moving from these in-vivo results, I investigated the role of Phycocyanin, one of the major constituents of Spirulina platensis, in UVB-induced signaling pathway by using mice keratinocytes, PAM212. I found that keratinocytes treated with Phycocyanin showed lower phosphorylation of p38 MAPK, JNK and ERK 30 min after UVB irradiation. Furthermore, when I investigated the effect of Phycocyanobilin, an open-chain tetrapyrrole chromophore of Phycocyanin, in mice embryonal fibroblast (MEF) which were obtained from Ogg1 knockout mice, a significant lower phosphorylation of p38 MAPK, JNK and ERK prior to UVB irradiation was showed by MEF which were treated with Phycocyanobilin.

I joined the Global Center of Excellence for Education and Research on Signal Transduction Medicine in the Coming Generation as a research assistant (RA) from May 2011. During this period, I received supports and got opportunities to present my research work on some international conferences and also had fruitful discussion about my research progress with other experts in and outside Japan. My first presentation was at Asia and Oceania Conference for Photobiology which was held on July 2011. Later on December 2011, I also present my work on The 36th Meeting of The Japanese Society for Investigative Dermatology. And on June 2012, I got opportunity to talk about my research on the 36th Annual Meeting of American Society for Photobiology at Montreal, Canada. On December 2012, I got an honor to receive the Diploma of Dermatological Scientist award from The Japanese Society for Investigative Dermatology.



「血管内皮リパーゼは心筋の脂肪酸取込みの副経路として圧負荷心不全の進展に関与する」

循環器内科学　**中島 英人**

私はグローバルCOEリサーチアシスタントに採用され、心不全に関する基礎研究を行った。心筋は収縮と弛緩を絶えず繰り返しており、そのエネルギー基質の約70%を脂肪酸に依存している。この脂肪酸の多くは、トリグリセリドを豊富に含んだリポ蛋白をリポ蛋白リパーゼ(Lipoprotein lipase, LPL)が分解することによって産生され、心筋に供給される。血管内皮リパーゼ (Endothelial lipase, EL) はLPLと同じトリグリセリドリパーゼファミリーに属するが、リン脂質に基質特異性が高いホスホリパーゼA1分子で、高比重リポ蛋白 (High-density lipoprotein, HDL) などに含まれるリン脂質を分解することで脂肪酸を産生する。我々は、ELがHDLを基質として心筋の脂肪酸取込みの副経路として機能するという仮説を立て、大動脈縮窄による圧負荷心不全モデルを用いて、圧負荷心不全の発生におけるELの役割について検討した。その結果、ELは心臓に脂肪酸を供給するための副経路として働き、圧負荷心不全の病態で心機能を制御している可能性が示唆された。

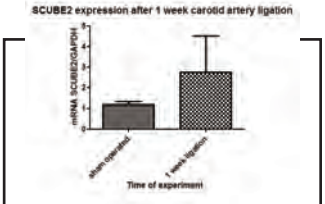
「Analysis of human herpesvirus-6-induced cell signaling and immune suppression」

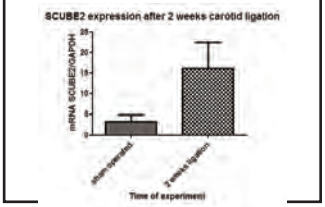
臨床ウイルス学分野　**林　麻佑子**

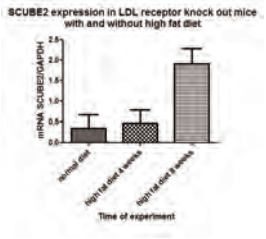
当研究室では、ヒトヘルペスウイルス6(以下HHV-6)の感染及び再活性化と病原性発現のメカニズムを解明することを目指し研究を行っている。我々の研究により、HHV-6が主に感染増殖するT細胞において、免疫学的シナプス構成因子の1つであり、T細胞の活性化と分化を担うある種のスカベンジャーレセプターの発現がHHV-6感染細胞膜表面において低下することが明らかとなった。この新たな知見により、HHV-6感染による免疫抑制機構を明らかにすることができる可能性が示唆された。そこで、我々はHHV-6感染によるスカベンジャーレセプターの細胞膜表面からの発現減少のメカニズムを解明するために、このスカベンジャーレセプターの細胞膜表面からの発現低下に関わるウイルス因子を同定することを目指し研究を行っている。当該グローバルCOEの報告会においても、その進捗状況ならびに成果を報告してきた。今後も、その詳細な機序を解明する事を目指し研究を継続している。

「Isolation and characterization of novel secreted protein, SCUBE2 in neointimal formation」

循環器内科学 **Hirowati Ali**







Neointimal formation, also known as diffuse intimal thickening (DIT) is a thickened intima mainly composed of smooth muscle cells, extracellular matrix, and lipid deposition in atherosclerosis-prone arteries such as coronary artery, iliac artery, and abdominal aorta. DIT is believed to play an important role in atherogenesis and has been considered as an early stage of atherosclerosis. Smooth muscle cells proliferation has been postulated to play a role in intimal thickening process. Signal peptide CUB domain EGF-like repeat (SCUBE), a novel secreted and membrane-anchored protein, is found to be expressed in broad spectrum of tissue and cells. SCUBE1 and SCUBE3 has been reported to play a role in cardiovascular disease. To date, SCUBE2 is reported to express in major blood vessels of the heart in mouse embryogenesis but its role in cardiovascular disease remains to be elucidated. The aim of this study was to observe the SCUBE2 expression and localization in atherogenesis and to examine SCUBE2 involvement in TGF-β pathway.

Carotid artery ligation in C57/BL6 mice was performed to induce intima thickening, mimicking diffuse intima thickening in human coronary artery. After 2 weeks of ligation, mRNA level of SCUBE2 increased significantly compared to sham operated control, and co-localized with α-actin smooth muscle cells in the intima layer and small amount in medial layer of carotid artery.

In LDLr-/- mice fed with high fat diet, a mice model of human atherosclerosis, mRNA level of SCUBE2 expression increased in 4 weeks high fat diet and markedly increased after 8 weeks high fat diet feeding where α-actin positive smooth muscle cells and macrophage marker positive cells were found to be co-localized with SCUBE2 in 4 weeks and 8 weeks of feeding.

Finally, in human coronary artery consisting DIT, SCUBE2 co-localized with α-actin positive smooth muscle cells, not with macrophage. As lesion progressed, SCUBE2 is found to be expressed in both smooth muscle cells and CD68 positive cells. In human cultured cells, SCUBE2 expressed ubiquitously in endothelial cells, smooth muscle cells, Jurkat cells, and HEK-293 cells, faint expression in Meg-01 cells. As smooth muscle cells mainly composed in DIT, we treated human coronary artery smooth muscle cells with TGF-β 5 ng/ml time dependent. SCUBE2 expression increased after 24 hours TGF-β stimulation. Thus indicated SCUBE2 involvement in TGF-β signaling pathway. Monitored WST-1 assay, SCUBE2 stable transfected CHO-K1 cells showed higher proliferation assay compared to CHO-K1 cells only, implying for SCUBE2 role in cell proliferation.

Our results suggested that SCUBE2 plays an important role during atherogenesis and it may involve the smooth muscle cells proliferation during neointima formation, last to advanced lesion development. Thus, SCUBE2 can be a novel protein in a thickening of intima and atherosclerotic plaque development.

「誘導性Cre-loxPシステムを用いた膵β細胞の運命追跡」

細胞分子医学 **田村　香楠子**

私は、2011年の6月からGCOEリサーチアシスタントとして大学院生生活をさせていただくことが出来ました。研究費や海外での口頭発表のための援助だけでなく、GCOEの先生方にはたくさんのご指導をして頂きました。また、他のリサーチアシスタントの皆様にも発表に関するアドバイス、優しいお言葉をかけて頂き、本当に恵まれた環境の中で、自分の実験を進めることができたととても感謝しています。

私の研究テーマは、「誘導性Cre-loxPシステムを用いた膵β細胞の運命追跡」であり、本研究において、膵β細胞を選択的に蛍光標識できるマウス(Ins2-CreER/R26R-YFP)を用いて、生理的条件下では、新生膵β細胞はP14からP28の間にだけ生じ、その後は既存の膵島と融合する可能性を示しました。また、糖尿病薬の一つであるインクレチン関連薬のリラグルチドが、アロキサン処置の膵β細胞傷害時において血糖値の改善だけでなくβ細胞のmassを増加させることを明らかとしました。今後は、その増殖、新生、あるいはアポトーシスの抑制にどのような作用があるかを検討するために、組織学的に標識されたβ細胞を観察し、標識率の割合の変化をみるとともに、転写因子の発現、他の内分泌細胞の発現を見ていく必要があります。

今までにリサーチアシスタントとして研究活動できたのは、吉田優先生をはじめとする先生方、そして他のRAの皆様のおかげです。深く御礼を申し上げます。

4

事業一覧

4-1基礎・臨床医学の融合

研究進捗報告会（2008 ～ 2012 開催）

次世代シグナル伝達医学グローバルCOE研究討論会

各種研究会、市民公開講座等の開催・共催支援

グローバルCOE学術講演会一覧

4-2国際教育プログラム

グローバルCOE国際シンポジウム、国際外部評価委員会

海外派遣援助プログラム

研究進捗報告会（2008〜2012 開催）

年に4回（研究討論会を含む）開催。GCOE研究員および次世代シグナル伝達医学リサーチリーダー育成コースに所属するRAは、教育研究担当コーディネーターを中心に事業推進担当者より個別研究指導を受け、研究情報の交換と問題点の共有、ならびに異分野間の相互技術指導を行いました。


グループごとの研究発表の様子


研究会での口頭発表

ポスターセッションによる研究発表の様子

英語クラス

国際推進の一環としてH20年度より大学院博士課程の学生を対象に少人数制の英語クラスを開催しました。特に研究発表や論文作成に重点を置いた内容となっており、実践的な英語力の獲得の場となっています。

次世代シグナル伝達医学グローバルCOE研究討論会（2009～2012 開催）

本プログラムの主体となるがん、代謝疾患、感染症、神経・筋疾患を対象とした5つの研究WGグループの成果発表ならびに、各分野で世界をリードする国内研究者を招へいし、討論会を開催。年1回の合宿形式の研究交流会として本グローバルＣＯＥプログラムの関係者が一同に会する貴重な機会となりました。また、大学院生、ポスドクなどの若手研究者によるポスターセッションを行い、終了後には、講師を囲んでのディスカッションや歓談の時間を設け、普段は接する機会の少ない異分野の研究者間の交流を行い、リトリートの醍醐味を存分に生かした大変有意義な場となりました。

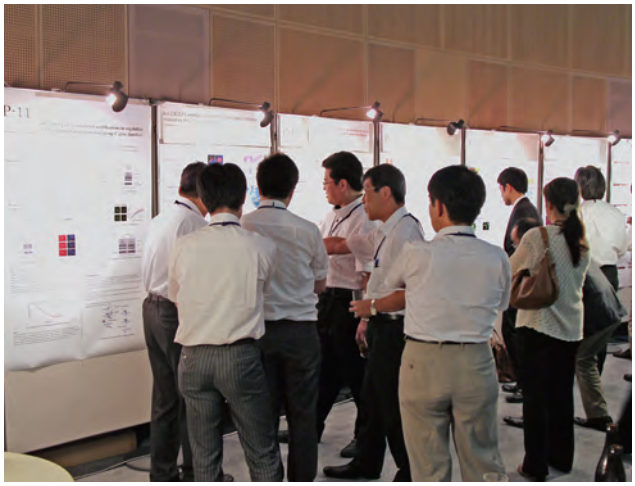
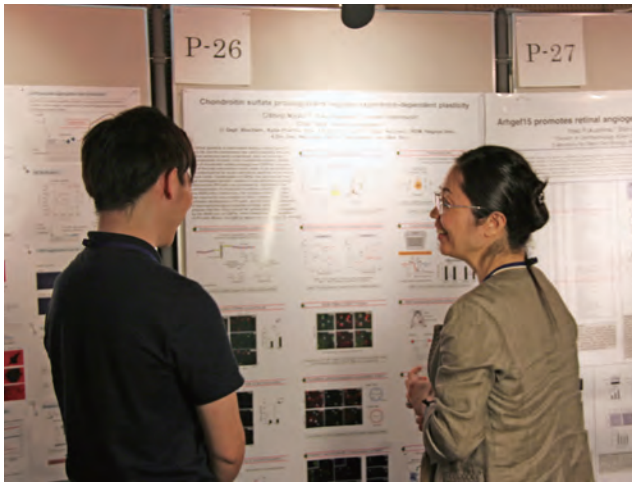
淡路夢舞台国際会議場にて（2009～2012）

次世代シグナル伝達医学グローバルCOEプログラム研究討論会　開催記録　（2009～2012）

	特別講演招へい者	所属	演題名
2009	的崎　尚	群馬大学生体調節研究所バイオシグナル分野	細胞間シグナルCD47–SIRPα系の機能と病態
	加藤　茂明	東京大学分子細胞生物学研究所 核内情報研究分野	核内受容体によるepigenetic制御の分子機構
2010	前田　慎	横浜市立大学医学部消化器内科　教授	消化器癌発生におけるIKKβ/NF–κB 活性化経路の関与
	秋吉　一成	東京医科歯科大学生体材料工学研究所 有機材料分野　教授	ナノゲル工学による新規タンパク質DDSの開発
	野田　哲生	財団法人癌研究会　癌研究所　所長	ヒト発がんモデルマウス解析による新規分子標的の探索
2011	小安　重夫	慶應義塾大学 医学部微生物学・免疫学教室	ナチュラルヘルパー細胞 –Th2型の自然免疫反応にかかわる新しいリンパ球–
	山本　雅	東京大学医科学研究所　癌細胞シグナル分野	シグナル伝達とmRNA代謝制御
	鍋島　陽一	財団法人先端医療振興財団　先端医療センター	代謝を制御するシグナル伝達におけるKlothoの機能
2012	大隅　典子	東北大学大学院医学系研究科 発生発達神経科学分野	心の病の動物モデル
	小室　一成	大阪大学大学院医学系研究科　循環器内科学	心不全の新しい発生機序について–虚血、炎症、老化
	畠山　昌則	東京大学大学院医学系研究科　医学部 病因・病理学専攻　微生物学講座　微生物学教室	ピロリ菌がんタンパク質CagAの構造依存的機能制御

WG グループによる口頭発表

第４回研究討論会（2012）

ポスターセッションでは活発な討論が行われます。



外部からの招へい者による特別講演

(2012)

グローバルCOE学術講演会、研究会、公開講座等の開催・共催支援

本研究科の教員や若手研究者が国内外の講師を招へいし、グローバルCOE学術講演会を開催しました。中でも、大学院特別セミナーは、国外から世界トップレベルのClinician-Scientistや医学研究者を招へいし、大学院生と短期集中的に議論する機会を設けました。終了後の懇話会では、英語でのコミュニケーションの向上が図られました。また、異分野間融合研究の推進、若手研究者育成を目的として、若手研究者が主催する研究会などの開催を支援・共催しました。年に一度開催の市民公開講座は、本拠点の研究活動や関連学問分野の動向を広く一般の方々に知って頂く機会となり、大変好評を得ました。

グローバルCOE学術講演会・特別セミナー（2009〜2013 開催）

各分野で世界的な実績を持つ一流研究者を招へいしてセミナーを開催。最新の研究成果だけではなく、研究者としてのビジョンやキャリアパス、人材育成や大学院教育についてなど、幅広い観点での内容を聞くことにより、多くの価値ある情報を得ることができました。

（女性研究者交流会・2009）

（第29回・2010）

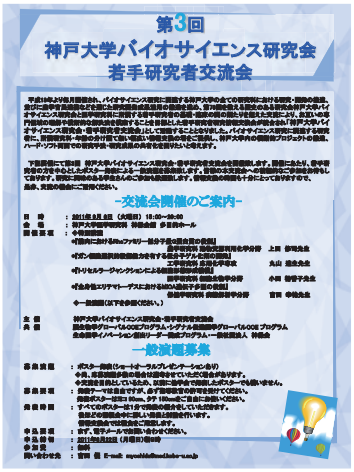
（第62回・2012）

（第74回・2013）

臨床メタボロミクス研究会（2009~2011 開催）

本研究会では国内におけるプロテオミクス･メタボロミクスにおける第一線の研究者を招聘し、最先端の研究をご紹介いただきました。ポストゲノムの新しいオミックスであるプロテオミクス･メタボロミクスの可能性･発展性を実感する貴重な機会となりました。

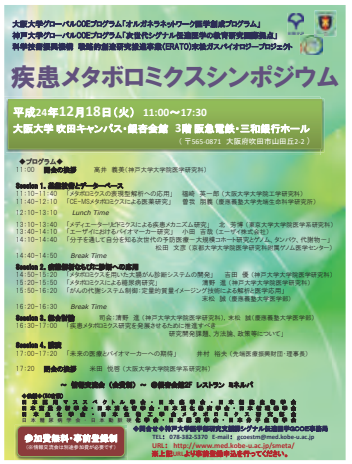
神戸大学バイオサイエンス研究会・若手研究者交流会（2010・2011 開催）



バイオサイエンス研究に関連する研究者に、所属研究科･年齢の分け隔て無い幅広い情報交換の場を提供し、神戸大学内の横断的プロジェクトの推進、ハード･ソフト両面での研究手法･研究成果の共有化を図っています。前半は若手研究者による口頭発表、後半は学生やポスドクを中心にポスター会場での交流会で活発な情報交換の機会をつくっています。

疾患メタボロミクスシンポジウム（2012.12.18 開催）

大阪大学吹田キャンパス･銀杏会館にて、神戸大学と大阪大学のグローバルCOEプログラム、並びに慶応大学戦略的創造研究推進事業の共催、また13の学会の後援により「疾患メタボロミクスシンポジウム」を開催。メタボロミクス研究において第一線で活躍されている先生方の最新の研究成果発表や特別講演が行われ、総合討論では、疾患メタボロミクス研究の世界と国内での現況や期待される成果についても活発な意見交換が行われました。



市民公開講座（2009～2012 開催）

本研究科の研究活動やその成果を広く一般の方々に知って頂くために、年一回、本研究科の教員や研究者、若手医師研究員による公開講座を開催しました。毎回、100名を超える一般市民の方の参加があり、大変好評を得ました。

（2011）

（2011）

（2011）

（2013）

（2013）

グローバルCOE学術講演会開催一覧

第1回　平成19年10月29日（水）神戸大学医学部臨床研究棟 6階 大講義室

『Signaling at the T cell antigen receptor』

Dr. Lawrence E. Samelson

Chief, Laboratory Cellular and Molecular Biology and Deputy Director Center for Cancer Research, National Cancer Institute National Institutes of Health, U.S.A

第2回　平成20年12月3日（水）神戸大学医学部臨床研究棟 6階 大講義室

『Cytokine signaling and T cell differentiation』

Dr. John J. O' Shea

Branch Chief, Molecular Immunology and Inflammation Branch, Scientific Director, NIAMS, NIH

第3回　平成21年1月27日（火）神戸大学医学部付属病院 4階 第二会議室

『Credible speking skills are essential to most successful physicians』

Dr. Doric Little

ホノルルコミュニティーカレッジ 名誉教授

第4回　平成21年2月16日（月）　神戸大学医学部臨床研究棟 6階 大講義室

『Pharmacogenetics or Pharmacogenomics : Germline or Tumor?』

Dr. Mark J. Ratain

Leon O. Jacobson Professor of Medicine Chairman, Committee on Clinical Pharmacology and Pharmacogenomics, Associate Director for Clinical Sciences, Cancer Research Center, The University of Chicago

第5回　平成21年2月20日（金）　神戸大学医学部臨床研究棟 5階 B講義室

『新規の骨粗鬆症治療薬開発を目指した破骨細胞研究』

宇田川 信之先生

松本歯科大学 歯学部口腔生化学 大学院歯学独立研究科硬組織疾患制御再建学 教授

第6回　平成21年3月4日（水）　神戸大学医学部臨床研究棟 6階 大講義室

『Modulation of p53 Activity by Post-Translational Modifications : The Yind and Yang of Kinase and Phosphatase Control』

Dr. Ettore Appella

Chief, Chemisty Section, Laboratory of Cell Biology, National Cancer Institute, U.S.A.

第7回　平成21年3月9日（月）　神戸大学医学部臨床研究棟 5階 B講義室

『IRCAD training course, Hands-on training course for NOTES and advanced therapeutic endoscopy』

Dr. Dimitri Coumaros

University of Strasbourg, France

第8回　平成21年4月14日（月）　神戸大学医学部臨床研究棟 4階 A講義室

『腫瘍の特異的標的化を目指した遺伝子治療法の開発』

濱田 洋文先生

札幌医科大学分子医学研究部門 教授

第9回　平成21年5月29日（金）　神戸大学医学部臨床研究棟 5階 B講義室

『動物モデルを用いた癌幹細胞の解析と応用』

佐谷 秀行先生

慶應義塾大学医学部先端医科学研究所 遺伝子制御研究部門 教授

『消化器病研究の進歩」

千葉 勉先生

京都大学大学院医学研究科消化器内科学講座　教授

第10回　平成21年7月22日（水）　神戸大学医学部臨床研究棟 5階 B講義室

『The role of regulatory T cells during ultraviolet (UV) irradiation-induced skin cancer.Signaling at the T cell antigen receptor.』

Dr. Stefan Beissert

Department of Dermatology University, of Münster

第11回　平成21年7月24日（金）　神戸大学医学部付属病院 第一病棟 2階 カンファレンス室

『Behcet's Disease as an Autoinflammatory Disorder』

Dr. Ahmet GÜL

Istanbul Univ./Istanbul Faculty of Medicine, Division of Rheumatology

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第12回　平成21年7月29日（水）　神戸大学医学部臨床研究棟 6階 大講義室

『K^{ATP} channels and neonatal diabetes : from molecule to disease』

Dr. Frances M. Ashcroft

Rpyal Society Research Professor, University Laboratory of Physiology,
The University of Oxford

第13回　平成21年8月4日（火）　神戸大学医学部付属病院 第一病棟 2階 共通カンファレンス室

『大量ゲノム情報に基づく微生物の比較ゲノミクス』

内山 郁夫先生

自然科学研究機構基礎生物学研究所 助教

第14回　平成21年10月2日（金）　神戸大学医学部神緑会館多目的ホール

『大量ゲノム情報に基づく微生物の比較ゲノミクス』

田中 良哉先生

産業医科大学医学部 第1内科学講座 産業医科大学病院・副院長

第15回　平成21年10月28日（水）　神戸大学医学部臨床研究棟 6階 大講義室

『Human Papillomaviruses and Cancer : Mechanistic Insights』

Dr. Peter M. Howley

Shattuck Professor of Pathological Anatomy Chair, Department of Pathology,
Harvard Medical School,USA

第16回　平成21年12月18日（金）　神戸大学医学部神緑会館多目的ホール

『Theroleofinsulingeneindiabetes』

Dr. Graeme I. Bell

Louis Block Distinguished Service Professor Of Medicineand Human Genetics Director, Diabetes Researchand Training Center, The University of Chicago

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第17回　平成20年1月12日（火）　神戸大学医学部管理棟 3階 共同会議室

『Regulation of VEGF-induced angiogenesis by receptor endocytosis』

Dr. Masanori Nakayama

Max Planck Institute for molecular biomedicine, Department of tissue morphogenesis

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第18回　平成22年1月26日（火）　神戸大学医学部管理棟 3階 共同会議室

『臨床グライコミクスと創薬研究』

西村 紳一郎先生

北海道大学大学院 先端生命科学研究院 先端生命科学部門 先端生体制御科学部門 教授

第19回　平成22年2月8日（月）　神戸大学医学部管理棟 3階 共同会議室

『米国での幹細胞研究：Stem cell research in US』

大谷 顕史先生

Department of Medicine, Stanford University School of Medicine

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第20回　平成22年2月9日（火）　神戸大学医学部管理棟 3階 共同会議室

『悪性腫瘍に対する工学的アプローチ：マクロ・ミクロの先端外科治療』

大平 猛先生

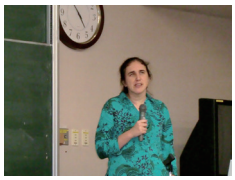
九州大学 先端医工学診療部 教授

第21回　平成22年3月2日（火）　神戸大学医学部臨床研究棟 6階 大講義室

『Imaging leukocyte cell migration in vivo : implications to human disease』

Dr. Anna Huttenlocher

Professor of Pediatrics, Pharmacology, and Medical Microbiology and Immunology, UW-Madison School of Medicine and Public Health, University of Wisconsin



第22回　平成22年3月16日（火）　神戸大学医学部管理棟 3階 共同会議室

『超臨界流体利用技術のメタボロミクスへの応用発表者』

馬場 健史先生

大阪大学大学院工学研究科生命先端工学専攻 准教授

第23回　平成22年3月30日（火）　神戸大学医学部臨床研究棟 6階 大講義室

『Function of Diverse Transcriptional Coactivatorsin Animal Cells』

Dr. Robert G. Roeder

Arnold O. and Mabel S. Beckman Professor Laboratory of Biochemistry and Molecular Biology, The Rockefeller University

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第24回　平成22年4月30日（金）　神戸大学医学部附属病院外来診療棟 5階 B講義室

『CD44の癌幹細胞における機能』

佐谷 秀行先生

慶應義塾大学医学部 先端医科学研究所遺伝子制御研究部門 教授

第25回　平成22年5月17日（月）　神戸大学医学部神緑会館多目的ホール

『Antisense oligonucleotide treatment of Duchenne muscular dystrophy by inducing exon skipping』

Dr. Yasuhiro Takeshima

Kobe University

『Genomewide analysis for Parkinson's disease』

Dr. Tatsushi Toda

Kobe University

『Rett Syndrome and MECP2-Status of Knowledge 10 Years after the Gene』

Dr. Uta Francke

Departments of Genetics and Pediatrics, Stanford University School of Medicine

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第26回　平成22年6月29日（火）　神戸大学医学部臨床研究棟 6階 大講義室

『Ubiquitin-proteolytic control of DNA repair and carcinogenesis』

Dr. Pengbo Zhou

Associate Professor, Department of Pathology and Laboratory Medicine,
Weill Cornell Medical College, Cornell University, NY, USA

第27回　平成22年8月23日（月）　神戸大学医学部臨床研究棟 6階 大講義室

『The role of Stats in the changing epigenetic and transcriptional landscape of helper T cells』

Dr. John J. O' Shea

Chief, Molecular Immunology and Inflammation Branch, Scientific Director,
NIAMS, NIH

第28回　平成22年8月24日（火）　神戸大学医学部臨床研究棟 4階 A講義室

『The immunobologyof the (not so) neonatal FcRfor IgG』

Dr. Richard S. Blumberg

Chief, Division of Gastroenterology, Hepatology and Endoscopy, Brigham
and Women's Hospital,and Professor of Medicine, Harvard Medical School

第29回　平成22年9月9日（木）　神戸大学医学部臨床研究棟 6階 大講義室

『UVB and UVA radiation reactions of DNA in cells and human skin:photoproduct formation and repair』

Dr. Jean Cadet

Scientific Adviser, Direction des Sciences de la Matière, Institut Nanosciences & Cryogénie,
CEA/Grenoble, Adjunct Professor, University of Sherbrooke, Canada

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第30回　平成22年10月19日（火）　神戸大学医学部管理棟 3階 共同会議室

『極長鎖脂肪酸の代謝経路と調節機構』

木原 章雄先生

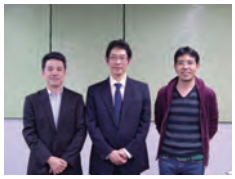
北海道大学大学院薬学研究院 生化学研究室 教授

第31回　平成22年11月15日（月）　神戸大学医学部管理棟 3階 共同会議室

『RasとPI3Kによる方向探知と細胞移動制御-Regulation of Directional Sensing and Cellular Motility by Rasand PI3K-』

佐々木 敦朗先生

Beth Israel Deaconess Medical Center Department of Systems Biology
Harvard Medical School



第32回　平成22年11月29日（月）　神戸大学医学部臨床研究棟 6階 大講義室

『Breakthroughs in imaging using photoactivatable fluorescent proteins』

Dr. Jennifer Lippincott-Schwartz

Head, Distinguished NIH Investigator Section on Organelle Biology, Cell Biology and
Metabolism Branch, NICHD, National Institutes of Health

第33回　平成22年12月9日（木）　神戸大学医学部外来診療棟 5階 B講義室

『酸化ストレス誘発発がんの抑制に関与する分子機構の解明-Mutyh遺伝子欠損マウスでの消化管発がんの解析を中心として-』

續 輝久先生

九州大学大学院医学研究院 基礎放射線医学分野 教授 / 医学研究院附属動物実験施設長

第34回　平成23年2月7日（月）　神戸大学医学部臨床研究棟 4階 第二会議室

『蛍光誘導体化-LC-MS/MSプロテオーム解析法』

今井 一洋先生

武蔵野大学薬学部長 教授

第35回　平成23年2月14日（月）　神戸大学医学部臨床研究棟 4階 第二会議室

『ゲノム・プロテオーム解析によるがんのバイオマーカーと創薬標的分子の開発』

山田 哲司先生

国立がん研究センター研究所副所長・創薬臨床研究分野長

第36回　平成23年2月28日（月）　神戸大学医学部管理棟 3階 共同会議室

『ビタミンKの体内変換とその生物学的意義』

岡野 登志夫先生

学校法人神戸薬科大学理事 / 神戸薬科大学衛生化学研究室 教授

第37回　平成23年3月1日（火）　神戸大学医学部臨床研究棟 6階 大講義室

『Signaling at the T cell antigen receptor』

Dr. Lawrence E. Samelson

Chief, Laboratory of Cellular and Molecular Biology, Deputy Director, Center for Cancer Research, National Cancer Institute National Institutes of Health

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第38回　平成23年3月10日（木）　神戸大学医学部研究棟B 2階 第2講堂

『細胞のストレスシグナルと疾患～小胞体ストレス応答から見出されたALSの全く新しい診断治療法～』

一條 秀憲先生

東京大学大学院薬学系研究科 細胞情報学教室　教授

第39回　平成23年4月14日（木）　神戸大学医学部神緑会館多目的ホール

『Genetic Studies of Diabetes: The Beginning of the End』

Dr. Graeme I. Bell

Louis Block Distinguished Service Professor in Medicine and Human Genetics, The University of Chicago Director, university of Chicago Diabetes Research and Training Center

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第40回　平成23年5月24日（火）　神戸大学医学部臨床研究棟 4階 A講義室

『Differential requirement for the dual functions of β-catenin-a lesson learned from mESCs』

Dr. Christine Hartmann

Group Leader Institute of Molecular Pathology Austria

第41回　平成23年6月13日（月）　神戸大学医学部外来診療棟4階 第二会議室

『膵癌におけるIPMNの位置づけ-何故IPMNか? -』

柳澤 昭夫先生

京都府立医科大学大学院 医学研究科人体病理学 教授

第42回　平成23年6月23日（木）　神戸大学医学部臨床研究棟 4階 A講義室

『Exploring the Roles of Type 2 Diabetes Risk Genes "Zn2+ and the regulation of insulin secretion"』

Dr. Merewyn Loder

Section of Cell Biology, Faculty of Medicine, Imperial College, London, UK

第43回　平成23年7月26日（火）　神戸大学医学部臨床研究棟 4階 A講義室

『がんの発生・進展におけるマイクロRNAの制御異常』

中釜 斉先生

独立行政法人 国立がん研究センター研究所長 / 同 発がんシステム研究分野長（併任）

第44回　平成23年7月29日（金）　神戸大学医学部臨床研究棟 5階 B講義室

『UV induced immune suppression in humans, a critical role for energy balance and complement』

Dr. G.M. Halliday

Professor, Departments of Dermatology, Bosch Institute and Sydney Cancer Centre, Central Clinical School, University of Sydney

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第45回　平成23年8月2日（火）　神戸大学医学部臨床研究棟 5階 B講義室

『Gender-bias and the acute UVB response in a Xiphophorus hybrid fish melanoma model』

Dr. David Mitchell

Professor, Department of Molecular Carcinogenesis, The University of Texas M.D. Anderson Cancer Center

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第46回　平成23年8月8日（月）　神戸大学医学部外来診療棟 4階 第二会議室

『Immature dentate gyrus as a candidate endophenotype ofpsychiatric disorders』

宮川 剛先生

Professor, Division of Systems Medical Science Institute for Comprehensive Medical Science Fujita Health University

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第47回　平成23年8月12日（金）　神戸大学医学部外来診療棟 4階 A講義室

『Signaling at the T cell antigen receptor』

滝川 修先生

（独）国立長寿医療研究センター 認知症先進治療開発センター　治療薬探索研究部 リード分子探索研究室・室長 / ラジオアイソトープ管理室・室長（兼任）

第48回　平成23年9月97（水）　神戸大学医学部外来診療棟 5階 B講義室

『脂質メタボロミクスから理解する炎症性疾患』

中西 広樹先生

秋田大学・バイオサイエンス教育研究センター

第49回　平成23年10月7日（金）　神戸大学医学部外来診療棟 6階 小児科医局内遠隔医療室

『米国における抗がん剤開発』

小島 研介先生

Visiting Associate Professor Section of Molecular Hematology and Therapy, Department of Leukemia, The University of Texas M.D. Anderson Cancer Center

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第50回　平成23年10月12日（水）　神戸大学医学部臨床研究棟 6階 大講義室

『Nanocarriers as an emerging platform for new molecular imaging and therapy』

西村 紳一郎先生

北海道大学 大学院先端生命科学研究院 先端融合科学研究部門　新薬探索研究分野 教授

第51回　平成23年12月5日（月）　神戸大学医学部外来診療棟 4階 第二会議室

『トランスクリプトーム・ネットワーク解析の展望』

林崎 良英先生

独立行政法人 理化学研究所 オミックス基盤研究領域 領域長

第52回　平成24年1月23日（月）　神戸大学医学部研究棟B 2階 共同会議室

『microRNAs that suppress cancer metastasis』

吉田 光邦先生

Research Assistant, Rockefeller University M.D. PhD. Candidate, Washington University

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第53回　平成24年2月1日（水）　神戸大学医学部研究棟B 2階 共同会議室

『ミトコンドリアとCommon Disease』

康 東天先生

九州大学大学院医学研究院　臨床検査医学　教授

第54回　平成24年2月8日（水）　神戸大学医学部外来診療棟 5階 B講義室

『Small Molecule Inhibition of Phosphatidylcholine Transfer Protein/StARD2 Ameliorates Diet-Induced Diabetes in the Mouse』

Dr. David E. Cohen

Professor, Department of Medicine, Division of Gastroenterology Brigham and Women's Hospital, Harvard Medical School

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第55回　平成24年2月24日（金）　神戸大学医学部神緑会館多目的ホール

『Molecular signal transduction by the H. pylori type-IV secretion system and injected CagA』

Dr. Steffen Backert

Professor, Cellular Microbiology, University College Dublin, Ireland

『Connecting chronic H.pylori infection and gastric cancer : a role for IL-11/STAT3 signaling』

Dr. Andrew Giraud

Research Director, Infection & Immunity Division,Murdoch Childrens Research Institute
Professorial Fellow, Department of Paediatrics,University of Melbourne

『ESD of Barrett's in the US – Challenges and Potential Solutions』

Dr. Roy Soetikno

Chief of GI Endoscopy, Veterans Affairs Palo Alto Health Care System, USA

第56回　平成24年12月28日（火）　神戸大学医学部研究棟B 2階 共同会議室

『Apolipoprotein Eの脳内脂質輸送ならびにアルツハイマー病発症に関与する役割』

道川 誠先生

独立行政法人 国立長寿医療研究センター アルツハイマー病研究部・部長

第57回　平成24年3月22日（木）　神戸大学医学部研究棟B 2階 共同会議室

『ダウン症因子 DSCR-1 の 抗血管疾患活性と網羅解析アプローチ』

南　敬先生

東京大学先端科学技術研究センター　血管生物学分野 特任教授

第58回　平成24年4月20日（金）　神戸大学医学部外来診療棟 4階 二会議室

『質量分析による次世代タンパク質定量システムの開発と創薬・臨床研究への応用』

大槻 純男先生

熊本大学大学院生命科学研究部　学微生物学分野 教授

第59回　平成24年4月23日（月）　神戸大学医学部外来診療棟 5階 B講義室

『一次繊毛形成における膜輸送の制御機構』

水野 健作先生

東北大学大学院生眼科学研究科 情報伝達分子解析講座 教授

第60回　平成24年5月30日（水）　神戸大学医学部外来診療棟 5階 B講義室

『Mechanisms of Wnt signaling: signalosomes and degradasomes』

Dr. Mariann Bienz

Divisional Head MRC Laboratory of Molecular Biology

第61回　平成24年6月4日（月）　神戸大学医学部外来診療棟 6階 大講義室

『Regulatory T cells induced by ultraviolet radiation. Facts and perspectives』

Dr. Thomas Schwarz

Professor of Dermatology, Department of Dermatology and Allergology, University Kiel, Kiel Germany

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第62回　平成24年6月14日（木）　神戸大学医学部外来診療棟 B講義室

『The unfolded protein response in intestinal epithelial cell homeostasis and disease』

Dr. Richard S. Blumberg

Professor, Department of Medicine, Harvard Medical School Chief, Division of Gastroenterology, Hepatology and Endoscopy, Brigham And Women's Hospital.

第63回　平成24年8月1日（水）　神戸大学医学部外来診療棟 4階 A講義室

『アルツハイマー病：分子病態解明から疾患修飾療法に向けて』

岩坪 威先生

東京大学大学院医学系研究科 基礎神経医学講座神経病理学 教授

第64回　平成24年8月9日（木）　神戸大学医学部研究棟B 2階 共同会議室

『統合失調症の疾患酵素学：D-アミノ酸酸化酵素の活性制御による新規治療』

福井 清先生

徳島大学疾患酵素学研究センターセンター長 / 病態システム酵素学研究部門 教授

第65回　平成24年9月3日（月）　神戸大学医学部研究棟B 2階 共同会議室

『Global Pharma Innovator：第一三共の挑戦』

廣川 和憲先生

第一三共株式会社 取締役 専務執行役員 戦略本部長

第66回　平成24年10月16日（火）　神戸大学医学部研究棟B 2階 共同会議室

『シナプス形成・リモデリングの分子機構』

岡部 繁男先生

東京大学大学院医学系研究科神経細胞生物学分野 教授

第67回　平成24年10月17日（水）　神戸大学医学部研究棟B 2階 第二講堂

『将来構想に基づく博士課程教育リーディングプログラム』

徳久 剛史先生

千葉大学 理事（研究・国際担当）

第68回　平成24年11月5日（月）　神戸大学医学部外来診療棟 5階 B講義室

『ヒト及びマウスの網羅的プロモーター解析』

河合 純先生

独立行政法人 理化学研究所 オミックス基盤研究領域 副領域長

『プロテオーム解析によるがんの個別化医療のためのバイオマーカー開発』

近藤 格先生

国立がん研究センター研究所 創薬プロテオーム研究分野 分野長

第69回　平成24年11月22日（木）　神戸大学医学部研究棟B 8階 セミナー室

『嗅覚神経回路の再編と食後睡眠』

森 憲作先生

東京大学大学院医学系研究科　細胞分子生理学分野 教授

第70回　平成24年11月26日（月）　神戸大学医学部外来診療棟 4階 第二会議室

『Mechanisms of control of skin pigmentation』

Dr. Mauro Picardo

San Gallicano Dermatology Institute – Laboratory of Cutaneous Physiopathology and Metabolomic Center(Roma)

第71回　平成24年11月30日（金）　神戸大学医学部研究棟B 8階 セミナー室

『プロテインホスファターゼ2Cによる細胞機能の制御』

田村 眞理先生

東北大学加齢医学研究所 遺伝子情報研究分野 教授

第72回　平成24年12月12日（水）　神戸大学医学部神緑会館多目的ホール

『Diabetes Vignettes from Chicago』

Dr. Graeme I. Bell

Louis Block Distinguished Service Professor of Medicine and Human Genetics, The University of Chicago

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第73回　平成25年1月30日（水）　神戸大学医学部外来診療棟 4階 第二会議室

『Klothoが紡いだ生命の糸を解きほぐす』

鍋島 陽一先生

公益財団法人先端医療振興財団 先端医療センター センター長

第74回　平成25年2月26日（火）　神戸大学医学部神緑会館多目的ホール

『Endoplasmic reticulum stress, inflammation,and tumorigenesis in the intestine』

Dr. Arthur Kaser

Professor, Division of Gastroenterology and Hepatology, Department of Medicine, University of Cambridge

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第75回　平成25年3月6日（水）　神戸大学医学部神緑会館多目的ホール

『B cell pathology from clinical aspects in SLE』

Dr. Yoshiya Tanaka

University of Occupational and Envionmental Health, Japan

『Signaling at the T cell antigen receptor』

Dr. Lawrence E. Samelson

Laboratory of Cellular and Molecular Biology, Center for Cancer Research, NCI, NIH

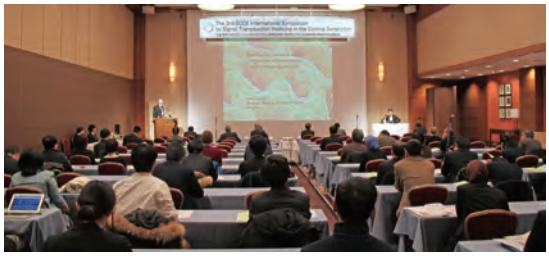
グローバルCOE国際シンポジウム（2009〜2012 開催）

シグナル伝達医学の研究で世界をリードする研究者を招へいしてシンポジウムを開催。最新の研究成果や情報を得る良い機会となりました。特に若手研究者や大学院生を交えた異分野間の交流については招へい者からも非常に高い評価を受けました。

第３回国際シンポジウム（2012）







また、2011年12月に、神戸大学グローバルCOE「統合的膜生物学の国際教育研究拠点」と「次世代シグナル伝達医学の教育研究国際拠点」の2拠点の事業推進担当者が中心となって企画・開催したワシントン大学医学部との合同シンポジウムでは、ワシントン大学の研究者・ポスドク・大学院生を招へいし、国際交流や英語によるコミュニケーション力の向上に良い影響がありました。今後も、定期的に同様の若手研究者・大学院生の交流活動を含むシンポジウムが開催されることを期待しています。

UW合同国際シンポジウム（2011）





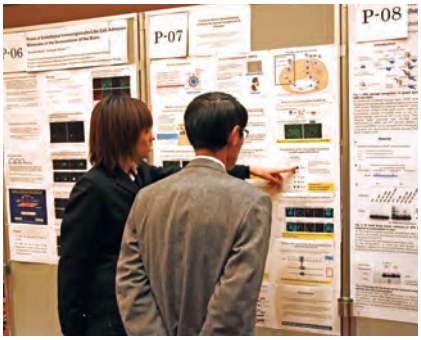


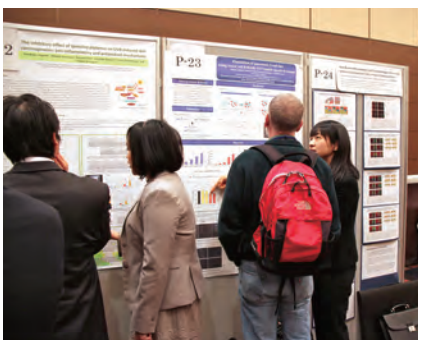


国際外部評価委員会（2010, 2012 開催）

本拠点の概要と大学院教育プログラムについての意見交換ならびに、事業推進担当者とトラックAの英語による研究成果のプレゼンテーションが行われ、10名で構成された国内外の評価委員により事前に提出されたレポートと併せて個人評価をしていただきました。

プログラムの達成と理解を促すとともに、次世代の医学･医療のリーダーとなる人材、また研究開発能力と国際的活動能力を合わせもつ若手研究者を育成するための貴重な意見交換が行われました。





海外派遣援助プログラム

若手研究者育成と国際交流を目的として、若手研究員や大学院生を対象に海外での活動を支援するプログラム。長期研究留学や学会発表、共同研究、医療技術研修への参加など、個々のレベルアップを図り、国際色豊かな研究者として育っています。

海外研究発表援助プログラム　－ 体験記 1 －

グローバルCOE研究員 **Deng Lin**

私は2011年9月8日から12日にアメリカ·シアトルで開催された「18th International Symposium on Hepatitis C Virus and Related Viruses」学会にて、「Hepatitis C virus infection promotes hepatic gluconeogenesis through an NS5A- mediated, FoxO1-dependent pathway」と題してポスター発表を行いました。その後引き続いて、札幌で行われた「XV International Congress of Virology」学会において、「Molecular mechanisms involved in HCV infection-induced hepatic gluconeogenesis」という研究課題で同じくポスター発表を行いました。発表に興味を持っていただけた多くの人との議論から、自分の実験での見落としていた部分を指摘されたり、また数々の有益なアドバイスを頂いたことで、さらに実験·研究の意欲が湧いてきました。国際学会で多くの知見を得られただけでなく、HCV研究の最前線を行く世界の研究者とも交流ができ、大変有意義な学会でした。

今回の国際学会に参加し、講演·ディスカッションを通じて世界中の人々が同じように頑張っているのを肌で感じ、それが今日の自分のモチベーションにもなっています。これからもさらに精進し頑張って行こうと思います。

海外研究発表援助プログラム　－ 体験記 2 －

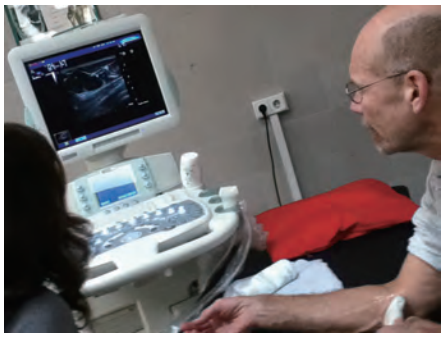
グローバルCOE研究員 **姜　大鵬**

私は2012年10月3日から10月10日までイタリアで開催された第19回HCV国際学会に参加させていただき、“Development of therapeutic and preventive vaccines against hepatitis C virus”と題してポスター発表を行いました。この学会は世界中から多数の著名なHCV研究者が招待講演者として来られており、その発表を聞くことを通してHCVに関する最新の研究成果を知ることができました。特に、米国Rockefeller UniversityのDr. Marcus Dornerによる”Engineering protective immunity against hepatitis C Virus infection”の発表を聞いて、HCVのワクチン開発と免疫の最新進展についてより深く理解することができ、それは大きな収穫になったと思います。また各研究における方法論なども今後自分の研究を進めていく上で参考になりました。
ポスター会場においては、自分が興味を持った研究をされている方に対して、積極的に英語でディスカッションするよう努めました。研究発表を通して様々なHCVワクチンの研究開発に関する質問やコメントを受け、その場で海外の研究者と情報交換や検討を行うことができました。これらを通して、研究方法や実験の問題点に関するさまざまなアイデアを得ることができたので、人に実用化が可能なHCVに対する治療と予防ワクチンの開発に力を注ぎたいと思います。
最後に、このような貴重な海外発表体験をさせていただいたプログラムに深く感謝いたします。

短期研究留学プログラム　－ 体験記 1 －

グローバルCOE RA（博士課程2年）　三崎　健太

2011年2月16日～2011年3月7日までスペインはバルセロナへ短期留学をさせて頂きました。今回の留学の目的は、リウマチ性疾患の細胞レベルでの病態生理の研究及びヨーロッパ各国で盛んに行われている関節超音波の技術習得でありました。関西国際空港から飛行機を乗り継いで約18時間。地中海に臨むバルセロナ市に到着したのはすっかり夜でしたが、神戸と同様に夜景が非常に綺麗な街でありました。1992年に夏のオリンピックが開催された都市ともあって、空港は非常に設備が素晴らしく、交通の便もよかったため到着が夜ではありましたが迷うことなく目的地へ辿り着くことができました。



2月17～19日まではバルセロナ大学医学部で行われた関節超音波学会に参加しました。日本の学会では経験したことのない解剖献体を用いての関節エコーの講義・実習と付着部炎、滑膜炎等のリウマチ性疾患の細胞レベルでの追及が大変印象的でした。この学会にはヨーロッパ各国、アメリカ合衆国、カナダ、中東、ロシアのリウマチ内科専門医、整形外科医、将来リウマチ内科を志望する研修医が参加しており各国のリウマチ医の診療体制や研究体制等も含め多くの討論ができたことが非常に勉強になりました。何より一番驚いたことはヨーロッパ各国の関節超音波の技術とリウマチ専門医の解剖学の知識の豊富さでした。私も2006年頃より関節超音波に関心を持ち、関節リウマチの症例はある一定の症例数を経験しこの学会に臨みました。しかし、彼らのレベルは桁違いでした。リウマチ専門医は内科であることが多いですが、まるで整形外科医のようにあらゆる筋骨格系の内容に精通しており、関節超音波ガイド下にリウマチ内科医自らあらゆる関節へ関節注射を施行している現状はまさに目から鱗でありました。今後日本のリウマチ内科医にも求められる重要な課題であると実感しました。

関節超音波学会後はInstituto Poal de Reumatologia、Hospital de Platoにおいて関節超音波のトレーニングとリウマチ専門外来での研修を約2週間行いました。直接指導して下さったのはInstituto Poal de ReumatologiaのProf.Ingrid Möller, Dr.David Bong, Hospital de Plato整形外科のDr.Albert Armanの3人で、朝8時から夜9時まで連日、熱血的な指導をして頂きました。来院する患者数は1日15～20人程度ですが、聴診と同様に必ず全員に関節超音波を行い一人30分程かけて診療をしていました。また、整形外科医と合同で行う外来も週に数回あり治療方針等の決定や関節超音波の動的評価の際には大変勉強になりました。しかし、神経・腱・靭帯といった関節包以外の評価もかなり多かったので時差ボケと闘いながら帰宅してひたすら解剖書と向き合う日々が続きました。その成果、約200症例の関節超音波を留学中に経験させて頂きました。

せっかくバルセロナに赴いたので休日は自転車を借りてバルセロナ市内を周りました。その中でもサクラダ・ファミリア、オリンピック記念館、本場ヨーロッパのサッカー公式戦が大変魅力的でありました。また、スペインの人々の陽気な性格と親切心は今でも脳裏に焼き付いており、片言ではありますがスペイン語を現地で勉強できたのも彼らの温かい指導によるものでした。

3週間という短期間ではありましたが内容の充実した留学であり、今後日本にも必ず我々の技術を普及してほしいとの使命を与えられ帰国しました。海外の「褒めて伸ばす」の精神に基づいた教育方法も身を持って実感し、辛かった日々もありましたが指導してくれたスタッフに助けてもらいながら何とかやり遂げることができました。現地にて「You are SAMURAI!」というフレーズでよく呼ばれましたが、異国にて侍の根性で頑張れたのも英語発表を始めとしたグローバルCOEのプログラムで鍛えて頂いた賜物であると感謝しております。今後もこのプログラムで後輩達が海外留学にて飛躍できることを願っております。本当によい機会を与えて頂きありがとうございました (Muchas Gracias!!)。

短期研究留学プログラム　－ 体験記 2 －

グローバルCOE RA（博士課程2年）　Yang Lin

In January 2013, I was funded by Global COE program of Kobe University to go to U.S.A. for short-term study abroad. On Jan 6th, I arrived in Boston and visited Harvard Medical School and Brigham and Women's Hospital, which is a 793-bed teaching affiliate of Harvard Medical School located in the heart of Boston's renowned Longwood Medical Area. Its departments and divisions are reputed for their excellence in research. The research programs of the Division of Gastroenterology, Hepatology, and Endoscopy aim to create new knowledge and expertise that will promote understanding and treatment of digestive diseases, especially in the areas of inflammatory bowel disease, hepatobiliary disease, pancreatic disease, gastrointestinal oncology and developmental endoscopy. In the conference room of Gastroenterology department, I made a presentation about mucosal immunology for Helicobacter species and consulted Prof. David Cohen for my present research.

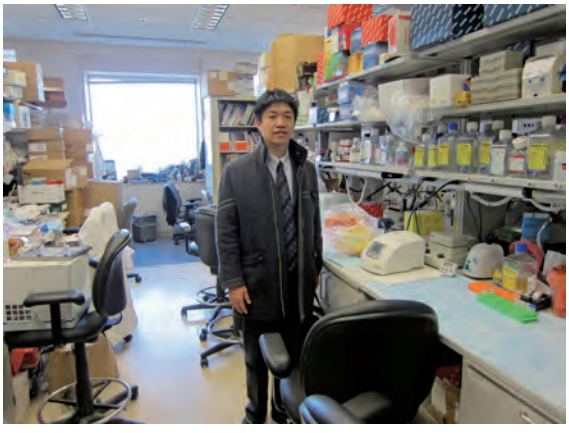
On Jan 7th, I visited the laboratory of Gastroenterology department, which studies mucosal immunity and focuses on several pathways that are especially relevant to the physiologic processes and diseases related to these compartments. The members of the laboratory introduced their own research to me, which had four major areas of interest:(1) characterization of the neonatal MHC class I-related Fc receptor (FcRn) in epithelial cells and dendritic cells; (2) understanding carcinoembryonic antigen cell adhesion molecule 1 (CEACAM1) as a regulatory molecule on T and NK cells; (3) determining the role of the nonclassical MHC class I-related molecule, CD1d, and microsomal triglyceride transfer protein in mucosal immunity; and (4) evaluation of ER stress pathways in intestinal inflammation. I also made a presentation and consulted Prof. Richard S.Blumberg for my research. After discussion, I had lunch with several professors and Japanese doctors who studied there.

On Jan 8th, I went to Bethesda and visited National Institute of Allergy and Infectious Diseases in National Institutes of Health. I made a presentation about my research and communicated with Prof. Warren Strober. He is the chief of Mucosal Immunity Section and also a leader in the study of mucosal antibody responses, oral tolerance, and gastroenterological diseases caused by immunologic abnormalities. Prof. Warren was interested in my research and gave me some good advice. On January 9th and 10th, I stayed in the laboratory, watched and learned some experimental techniques of mucosal immunology with Dr. Takagawa who had studied there over 4 years.

On January 11th, I finished my study abroad and returned to Kobe. I think this valuable experience of study is very helpful for my research progress.

Visit Harvard Medical School


Prof.Warren Strober and Dr.Takagawa in NIAID


Visit the laboratory of NIAID

長期研究留学プログラム

『腸管炎症と脂質メディエーターの関係の解析』
-Analysis of the relationship between lipid mediators and intestinal inflammation-

グローバルCOE RA（博士課程3年） **氣比 恵**

留学先機関　Center for Experimental Therapeutics and Reperfusion Injury
Brigham and Women's Hospital
Harvard Medical School
受入研究室　Dr. Charles N. Serhan 研究室
研究機関　2012年6月30日～ 2013年3月31日（4月以降も継続中）

2010年、神戸大学大学院医学研究科消化器内科学講座の博士課程に入学し、ガスクロマトグラフ質量分析計(GC/MS)を用いた臨床メタボロミクス研究に取り組んだ。消化器内科、外科、検査部門の協力のもと、消化管癌患者および健常者から得られた血清を解析対象とし、水溶性代謝物を中心としたメタボリックプロファイリングを進めていた。解析の中で、消化管癌患者は健常者と比較して、有意に異なる代謝産物を有することが判明した。さらに癌臓器別に検討した場合、消化管癌に特徴的な代謝プロファイルを定義することも可能であり、メタボロミクス解析にて消化管癌を早期発見できるという可能性を見いだした。これらの研究の過程で、疾患と代謝物プロファイルとの強い相関を知ることができたが、その機序を理解するための生化学的·分析科学的研究の経験が浅いことを痛感していた。消化管臓器においては慢性炎症が発癌に関与する事が知られている。代謝物プロファイルをより深く理解するための専門的知識と経験を得るために、メタボロミクス研究を遂行している研究室への留学を 考えるようになり、２０１１年に Global COE program「次世代シグナル伝達医学の教育研究国際拠点」での留学助成プログラムに『腸管炎症と脂質メディエーターの関係の解析』というテーマで応募し採択となった。

炎症反応の収束期には、抗炎症性脂質メディエー ターが産生され、生体は炎症を能動的に収束し、ホメオスタシスを保つということが報告されている。これら抗炎症性脂質メディエー ターを世界に先駆けて発見し、先進的に研究を進めている ハーバード大学 Brigham and Women' s Hospital の麻酔科学講座 Charles N. Serhan 教授の研究室へ2011年、Global COE programの助成により短期留学をする機会に恵まれた。同研究室での代謝物プロファイルの解析、抗炎症性脂質メディエー ターの作用機序の分析手法について学ぶことを目的に、大学院在学３年目の１年間を研究留学することが決定し、2012年６月末より渡米した。

渡米後は、抗炎症性脂質メディエー ターの 炎症収束作用について、マウスの虚血再還流モデル、盲腸結紮鮮穿孔モデルを用い、詳細な検討を進めている 段階である。

同研究室は様々な研究的背景をもつ専門家が集結し研究を進めており、構成メンバーの多様性が大きな特徴である。

現在のメンバーは循環器内科学、消化器内科学、腫瘍学、歯学、薬理学、分析科学、栄養学、有機合成化学、タンパク工学を専門とする博士研究員で構成されており、基礎から極めて専門的な内容にいたるまで多岐にわたる指導を受け、さらに様々なセミナーに参加するなどし、世界的に著名な研究者や臨床家の講演を聴く機会を得るなど 大変な知的刺激をうけている。

他方で、夏はビーチデーと称し、大西洋を目の前にしての論文抄読を行い、冬はIsabella Stewart Gardner Museumを貸し切ってのパーティーなど、同研究室ならでは様々なイベントに参加して、人脈を広げる事ができた。

予想していた事ではあるが、渡米後専門的な知識に関する英語力に対する壁の高さを痛感しBrigham and Women's Hospital が提供する English as a second language (ESL) for scientists class にも現在参加中である。

今回の長期留学においては、個人的には初めてのルームシェアや長期海外生活の立ち上げから、気候、文化の違いなど、いろいろな苦労も経験した。しかしながら研究に関する事も含め、これまでにない他分野の方との広い交流関係を得た。

ほかでは得難いこれらの経験は一生の財産になると考えている。

このGlobal COE programプログラムを立ち上げ、大学院生としての留学に協力してくださった拠点リーダーの東健教授、受け入れてくださった同研究室のCharles N. Serhan教授、留学に関して研究室の紹介や手続きを全面的に補佐してくださった吉田優先生、事務手続きや留学後も様々なサポートをしてくださった職員の皆様に感謝申し上げます。


After journal club at Good Harbar Beach


lunch with all lab members


mice experiment using specialised proinflammatory mediator

5

受　賞

受賞 AWARDS

・2010年5月15日 清野 進教授(細胞分子医学の「インスリン分泌におけるシグナル伝達」の研究における業績が認められ、5月15日、シカゴ大学主催のAnnual Diabetes Dayにおいて授賞式および清野教授による受賞記念講演が行われました。


Donald Steiner Award,May 15, 2010

・2010年9月21日 スウェーデン、ストックホルムにて開催された第46回欧州糖尿病学会(European Association for the Study of Diabetes: EASD)において医学研究科内科学講座糖尿病・内分泌内科学、生理学・細胞生物学講座細胞分子医学の清野進教授がThe Albert Renold Prizeを受賞しました。

・2011年11月15日付で、政府は平成23年「秋の褒章」を発表し、本学関係者としては糖尿病・内分泌内科学分野および細胞分子医学分野の清野進教授が紫綬褒章を受章しました。清野教授は糖尿病・内分泌内科学分野で、分子レベルでのインスリン分泌機構の解明や、新たなインスリン分泌増強メカニズムの発見など優れた業績を挙げています。


紫綬褒章, 2011年11月

・2012年5月4日 The Stefan S. Fajans Lectureship in Diabetes (University of Michigan, Ann Arbor, USA)
・2012年5月28日 The Kroc Lectureship (Uppsala University, Uppsala, Sweden) Kroc Lecture Award

・2008年10月27日 匂坂 敏朗教授(膜動態学)が、「小胞輸送による神経軸索形成機構」のテーマで、平成20年度日本生化学会奨励賞を受賞しました。

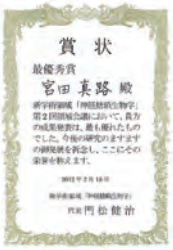
・グローバルCOE RA 田村 香楠子さん(細胞分子医学 博士課程1年)が、日本糖尿病協会 国際交流研究奨励賞を受賞しました。

英語表記名:「JADEC International Research Promotion Award」
August 2011
研究テーマ:「Elucidation of pancreatic beta-cell fate using the inducible Cre/loxP system」

・グローバルCOE RA Kemala Isnainiasih Mantilidewiさん(シグナル統合学 博士課程3年)が、2012年10月6日 第2回バンドン生体分子医学カンファレンスにおいて「血管内皮細胞におけるチロシンホスファターゼVE-PTPの役割」のテーマで最優秀口頭発表賞を受賞しました。

・堀田 博教授(微生物学)が2009年2月27日に香港で開催された第8回Asia-Pacific Congress of Medical Virologyにおいて、APSMV Excellence Awardを受賞しました。

・梶 博史客員教授(糖尿病・内分泌内科学)が2012年7月28日～30日 大阪国際会議場にて開催された、第29回日本骨代謝学会学術集会において学術賞を受賞しました。

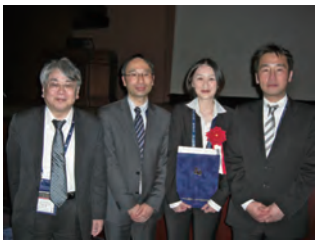


・グローバルCOE研究員 宮田 真路先生(薬物動態学分野(神戸薬科大学))が、2012年5月19日 日本生化学会近畿支部例会にて、「コンドロイチン硫酸の硫酸化パターンによる神経可塑性の制御」のテーマで最優秀発表賞を受賞しました。
・2012年2月18日 新学術領域「神経糖鎖生物学」第2回領域会議において「6-硫酸化コンドロイチンの過剰発現によって大脳皮質の神経可塑性が維持される」のテーマで最優秀賞を受賞しました。

植村 明嘉特命助教(血管生物学)による網膜血管新生の研究に対して、下記の賞が授与されました。

・2012年12月1日 日本網膜硝子体学会 第5回田野 Young Investigator's Award
・2011年9月6日 成人血管病研究振興財団 第26回岡本研究奨励賞
・2009年8月21日 Korea-Japan Joint Symposium on Vascular Biology Young Scientist Award

・グローバルCOE研究員 Deng Lin先生(微生物学)が、2011年10月20日～ 21日に福岡で開催された第15回日本肝臓学会大会において、社団法人日本肝臓学会第10回MSD Awardを「C型肝炎ウイルスによる糖代謝異常の分子機序の解明」のテーマで受賞しました。
・2011年6月2日～3日に東京で開催された第47回日本肝臓学会総会において「C型肝炎ウイルスは酸化ストレスを介して糖新生を亢進し糖尿病発症に関与する」のテーマで優秀演題賞に選ばれました。



・グローバルCOE研究員 小林 成美先生(循環器内科学)が、2013年3月15日～17日 日本循環器学会総会学術集会において「Osteoblast-like Differentiation of Cultured Human Coronary Artery Smooth Muscle Cells by Bone Morphogenetic Protein Endothelial Cell Precursor-derived Regulator (BMPER).」のテーマで日本循環器学会女性研究者奨励賞を受賞しました。

・グローバルCOE RA Flandiana Yogiantiさん(皮膚科学 博士課程4年)が、2012年12月7日～9日 ロワジールホテル那覇行われた 日本研究皮膚科学会 第37回年次学術大会・総会において、外国人留学生を育成・援助することを目的とした学会賞である Diploma of Dermatological Scientist を受賞しました。

Flandiana Yogiantiリサーチアシスタントは皮膚科の治療現場で多用されるナローバンド波長UVB照射装置においては従来型のブロードバンド波長型装置に比べてその長期照射において悪性皮膚腫瘍の発生が多く認められることが動物実験で報告されていました。しかし、今回初めてそのナローバンドとブロードバンドで生じてきたマウス皮膚腫瘍を用いてがん抑制遺伝子であるp53の変異の違いを検討し、ナローバンドにおける同遺伝子の変異は有意にブロードバンドと比して高率に認められることを発見しました。これは、ナローバンド型の皮膚悪性腫瘍発生率が動物実験において高率に認められる原因として皮膚におけるピリミジン二量体形成が多く形成されることと推測されていましたが、それを証明しました。

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「次世代シグナル伝達医学の教育研究国際拠点」

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発　　行　シグナル伝達医学グローバルCOE事務局
〒650-0017　神戸市中央区楠町7-5-1　神戸大学医学部研究支援課内
TEL (078)382-5370　　FAX (078)382-5371
e-mail　gcoestm@med.kobe-u.ac.jp
URL　　http://gcoe.med.kobe-u.ac.jp/


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