Donation Laboratories

Division of Cellular Proteomics (BML)
細胞ゲノム動態解析(BML)寄付研究部門

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We analyze intracellular signaling pathways using proteomic approaches. Since resolution power of current proteomic technologies is not sufficient to analyze low abundance proteins such as components of signal transduction, we established the protocols to prefractionate phosphoproteins and proteins in lipid raft. Combining these prefractionation procedures with fluorescence difference two-dimensional gel electrophoresis (2-D DIGE), we identified many novel kinase substrates and raft proteins that are involved in signal transduction. We also analyze intracellular signaling pathways of kidney podocytes. The podocyte slit diaphragm (SD), which serves as a structural framework for filtration barrier in kidney glomerulus, also plays an essential role as a signaling platform. Nephrin and Neph1 that have been identified as products of nephrosis-causative genes are expressed in podocytes are tyrosine phosphorylated by Fyn and recruits a variety of signaling molecules including Grb2, phospholipase C-γ1, PI3-kinase.

Phosphoproteomic profiling of ERK MAP kinase signaling reveals a role of phosphorylation in the interaction of nucleoporins with transport factors.

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Many ERK MAP kinase substrates have been identified, but the diversity of ERK-mediated processes suggests the existence of additional targets. Here we present a phosphoproteomic approach to identify putative ERK substrates by combining the steroid receptor fusion system, immobilized metal affinity chromatography (IMAC), fluorescent two-dimensional difference gel electrophoresis (2D-DIGE), and phosphomotif-specific antibodies. Purification of phosphoproteins from whole cell lysates by IMAC enabled sensitive detection of minor phosphorylated signaling components that would otherwise be obscured by abundant cellular proteins. Changes in phosphoprotein profiles between selective activation and inhibition of the Raf-MEK-ERK pathway were globally analyzed by 2D-DIGE. Quantitative analysis detected 37 reproducibly changed protein spots, several of which were recognized by the ERK consensus motif-specific antibodies. Mass spectrometric analysis identified 38 proteins as ERK pathway-associated proteins. These included MEK1/2 and ERK1/2 as well as previously known ERK substrates such as RSK2, cPLA2, hnRNP K, caldesmon, cortactin, and vinexin,

demonstrating the feasibility of our approach. The remaining 24 proteins were considered candidates for novel ERK targets, which suggest as yet undefined roles for this signaling pathway in cytoskeletal regulation, mRNA processing, vesicle transport, proteolysis, and protein folding. We purified 14 proteins fused to GST, 13 of which were phosphorylated by ERK in vitro. Among them, cytoplasmic dynein intermediate chain 2 and the nucleoporin Nup50/Npap60 were shown to be phosphorylated by ERK in intact cells. ERK phosphorylation of the FG repeat region of Nup50 was found to reduce its affinity for importin-β. Moreover, the rate of nuclear transport of GFP-fused importin-β and transportin was reduced upon ERK activation. This approach is applicable to other protein kinases and may be useful for large-scale identification of cellular substrates.

Tyrosine Phosphorylation of the Kidney Slit Diaphragm Component Neph1 and its Modulation of Intracellular Signaling by Binding with Grb2.

Yutaka Harita[1,2], Hidetake Kurihara[3], Hidetaka Kosako[1], Takashi Sekine[2], Takashi Igarashi[2], Seisuke Hattori[1,4]: [1]Division of Cellular Proteomics (BML), Institute of Medical Science, The University of Tokyo, [2]Department of Pediatrics, Graduate School of Medicine, The University of Tokyo, [3]Department of Anatomy, Juntendo University School of Medicine, [4] School of Pharmaceutical Sciences, Kitasato University.

There are several lines of evidence that the podocyte slit diaphragm (SD), which serves as a structural framework for glomerular filtration barrier, has also an essential role as a signaling platform. Several SD components including nephrin and TRPC6 are known to be phosphorylated by a Src tyrosine kinase, Fyn. Here we have characterized Neph1 as a novel substrate of Fyn. Fyn interacted with and phosphorylated cytoplasmic domain of Neph1 in vitro and in intact cells. Peptide mass fingerprinting and site-directed mutagenesis identified several tyrosine phosphorylation sites. In pull-down analysis using rat glomerular lysates, Neph1 specifically bound to an adaptor protein Grb2 and a tyrosine kinase Csk in a phosphorylation-dependent manner. Both tyrosine 637 and 638 of Neph1 were crucial for Neph1-Grb2 binding. Phosphorylation of the tyrosine 637 was significantly upregulated in in vivo models of podocyte injury. Furthermore, Neph1 attenuated ERK activation elicited by Fyn, and this inhibitory effect required the intact binding motif for Grb2 SH2 domain. Our results shown here demonstrate that Neph1 is a novel in vivo substrate of tyrosine kinase phosphorylation likely by Fyn, and suggest that Neph1 modulates ERK signaling through phosphorylation-dependent binding with Grb2.

Thus, Fyn orchestrates a wide spectrum of protein-protein interactions at SD through tyrosine phosphorylation.

Phosphorylation of Nephrin Triggers Ca^{2+} Signaling by Recruitment and Activation of Phospholipase C-γ1.

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A specialized intercellular junction between podocytes, known as the slit diaphragm (SD), forms the essential structural framework for glomerular filtration in the kidney. In addition, mounting evidence demonstrates that SD also plays a crucial role as a signaling platform in physiological and pathological states. Nephrin, the major component of SD, is tyrosine phosphorylated by a Src-family tyrosine kinase, Fyn, in developing or injured podocytes, recruiting Nck to Nephrin via its SH2 domain to regulate dynamic actin remodeling. Dysregulated Ca^{2+} homeostasis has also been implicated in podocyte damage, but the mechanism of how podocytes respond to injury is largely unknown. Here we have identified phospholipase C-γ1 (PLC-γ1) as a novel phospho-Nephrin binding protein. When HEK293T cells expressing a chimeric protein consisting of CD8 and Nephrin cytoplasmic domain (CD) were treated with anti -CD8 and anti-mouse antibodies, clustering of Nephrin and phosphorylation of Nephrin-CD were induced. Upon this clustering, PLC-γ1 was bound to phosphorylated Nephrin Y1204, which induced translocation of PLC-γ1 from cytoplasm to CD8/Nephrin cluster on the plasma membrane. The recruitement of PLC-γ1 to Nephrin activated PLC-γ1, as detected by phosphorylation of PLC-γ1 Y783 and increase in inositol 1,4,5 -trisphosphate (IP3) level. We also found that Nephrin Y1204 phosphorylation triggers the Ca^{2+} response in a PLC-γ1- dependent fashion.

Furthermore, PLC-γ1 is significantly activated in injured podocytes *in vivo*. Given the profound effect of PLC-γ1 in diverse cellular functions, regulation of the Ca^{2+} signaling by Nephrin may be important in modulating the glomerular filtration barrier function.

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Donation Laboratories

Division of Stem Cell Engineering (Hitachi Plant Technologies, Denics, ArBlast)

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Our main project is to regenerate tooth using the methods of tissue engineering. To accomplish this goal, we are focusing on the following subjects; 1) identification and characterization of stem cells in epithelial or mesenchymal tissue from tooth germ, 2) search for molecules to affect the differentiation of the stem cells, 3) assembly of these stem cells on artificial scaffold.

Our division was established in July 2003 to accelerate the research on oral tissue regeneration, especially tooth regeneration, with the support of accumulated knowledge about genomic science and stem cell biology at IMSUT. We are trying to regenerate not only teeth but also other oral tissues including bone. There are three important elements in tissue regeneration; stem cells, signal molecules, and scaffolds We are focusing on the research of mesenchymal stem cell, bone morphogenetic protein (BMP), and synthetic polymer scaffold as an each element.

1. Clinical study

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Bone marrow stromal cells (BMSCs) have been widely used for bone tissue engineering. Although bone tissue engineering using BMSCs is considered feasible, little is known about the clinical efficacy of this novel treatment. We have developed a clinical trial to test if BMSCs will be clinically effective in regenerating bone in patients with severe maxillary and/or mandibular bone absorption who undergo dental implant surgery. Ten patients (2 males and 8 females, average age 52.3) were enrolled in this clinical study. Two of them were dropped because of possible contamination risk to autoserum or insufficient number of cultured cells. The remaining eight patients underwent cell transplantation and all of them had implant installation. The results from X-P, CT and histological analyses showed bone regeneration in all patients who underwent cell transplantation and the average bone area was 31% at 6 months after transplantation, though significant deviations were observed in the parameters such as cell numbers, ALP activities and regenerated bone area among individuals. Twenty nine dental implants were installed to the regenerated bone and overall survival rate was 93%. No side effect relating to

the cell transplantation was observed. The volume of the regenerated bone was kept 76% at two year after transplantation compared with that at 6 months. Bone regeneration using autologous BMSC-derived osteogenic cells was feasible and considered safe during the observation period.

2. Effect of ischemic culture conditions on the survival and differentiation of porcine dental pulp-derived cells

Agata H, Kagami H, Watanabe N[1], Ueda M: [1]FACS Core Laboratory

Although differentiated and undifferentiated cells can be exposed to ischemic conditions in cases of injury or inflammation, the effects of ischemia on cell survival and differentiation have not been well characterized. In this study, the response of porcine dental pulp-derived cells (pDPCs) to culture conditions that approximate ischemia was characterized. Anoxia was the most detrimental factor to cell viability, whereas hypoxia did not significantly affect survival. Glucose concentrations had a significant, mechanism-dependent effect on cell death. The presence of glucose correlated with caspase-dependent cell death, whereas the absence of glucose was linked to caspase-independent cell death. In contrast, differentiation status (i.e. induced vs. non-induced pDPCs) did not affect the degree or mechanism of cell death. Finding depletion of specific markers by RT-PCR in both induced and non-induced cells suggests that the cells are de-differentiating under anoxia. Non-induced pDPCs were susceptible to anoxic induction of Oct-4, Sox-2, and hypoxia inducible factor-2α, while these genes did not change in induced pDPCs. Re-differentiation analysis revealed that the surviving cells from non-induced pDPCs showed 2-fold higher alkaline phosphatase activity as compared to induced pDPCs, which suggest greater plasticity among the surviving fraction of non-induced pDPCs. These data showed that the ischemic conditions have similar detrimental influence on both undifferentiated and differentiated pDPCs, and affect differentiation status of pDPCs. Furthermore, ischemic conditions may influence the plasticity of undifferentiated pDPCs.

3. A novel serum-free culture method of mouse submandibular gland epithelial cells

Aoki R[1], Agata H, Imajoh-Ohmi S[1], Kagami H: [1]Laboratory Center for Proteomics Research

Although the presence of somatic stem/progenitor cells from various organs has been reported, the character of those cells is largely unknown. One of the reasons for this discrepancy might be due to the lack of adequate culture system for those somatic stem/progenitor cells. In this study, a novel serum-free culture condition for the primary culture of mouse submandibular gland epithelial cells was developed, which can maintain putative stem/progenitor cells for relatively longer period. The submandibular glands was minced with blades, digested, and washed several times, which were resuspended in the serum-free culture medium containing bFGF and EGF. Almost pure and uniform epithelial cell clusters were observed after two weeks. The culture was maintained up to 4 to 6 weeks when dome- and nodule-like structures were appeared, which correspond to duct and acinar of salivary gland, respectively. The epithelial cells cultured in this system maintained the constant proliferative capability. The cells cultured under this condition showed myoepithelial and ductal cell markers such as α-SMA and ZO-1. The isolated cells from this culture system were able to undergo complex ductal formation when embedded in a gel. These results suggest the presence of putative epithelial stem/progenitor cells in this culture system, which might be used for future analyses of somatic stem cells in salivary gland.

4. Tooth-tissue engineering

4-1. Quiescent epithelial cell rests of Malassez can differentiate into ameloblast

Shinmura Y, Tsuchiya S, Hata KI[1], Honda MJ: [1]Japan Tissue Engineering Co., Ltd.

Epithelial cell rests of Malassez (ERM) are quiescent epithelial remnants of Hertwig's epithelial root sheath (HERS). This study demonstrated that subcultured ERM can differentiate into ameloblast and generate enamel in combination with dental pulp cells. Porcine ERM were obtained from periodontal ligaments and were subcultured with non-serum medium. Thereafter, subcultured ERM were expanded on 3T3-J2 feeder layers. The *in vitro* mRNA expression pattern of the ERM was found to be different from that of enamel organ epithelial cells and oral gingival epithelial cells. When subcultured ERM were combined with subcultured dental pulp cells, ERM expressed cytokeratin14 and amelogenin proteins *in vitro*. In addition, subcultured ERM combined with dental pulp cells seeded onto scaffolds showed enamel at eight

weeks post-implantation. Moreover, positive staining for amelogenin was observed in the enamel, indicating the presence of well-developed ameloblasts in the implants. These results suggest that ERM can differentiate into ameloblast.

4-2. Collagen type I matrix affects the molecular and cellular behavior of purified porcine dental follicle cells

Tsuchiya S, Honda MJ, Shinohara Y, Saito M[1], Ueda M: Department of Molecular and Cellular Biochemistry, Osaka University Graduate School of Dentistry

This study investigated porcine dental follicle cells at the early crown-formation stage and examined the behavior of cells proliferation under collagen type I matrix (Col-I). Clone dental follicle cells (DFC-I) and controls (dental follicle, nonclone-dental follicle cells (DFC), periodontal ligament cells (PDLC), and bone marrow stromal cells (BMSC) were obtained from 6-month-old pigs. The DFC-I showed different gene expression patterns from controls by RT-PCR analysis. Furthermore, Col-I treatment enhanced DFC-I proliferation and increased the ALP activity compared to non-treated DFC-I. The expressions of periostin, biglycan, and osteocalcin (OCN) in cells growing on collagen were upregulated similar to the pattern seen in the PDLC. DFC-I with and without Col-I treatment were combined with TCP particles and implanted into mice. There were significant differences in the gene expression patterns of bone sialoprotein (BSP), OCN, and periostin in both treated and non-treated implants at 2 and/or 4 weeks. The results showed that Col-I induces the mineralization pathway in these cells. Hard tissue formation was observed in both implants at 8 weeks. These results suggested that Col-I facilitate the differentiation of DFC-I into the mineralization process.

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Donation Laboratories

Division of Molecular and Developmental Biology
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Our long-term goal is to understand the molecular mechanisms which coordinately regulate growth and differentiation of stem cells as well as differentiated cells with emphasis on intracellular signal transduction. For this purpose, we are using models ranging from culture cells, zebrafish, mouse, to monkey. Based on our research background on the area of haematology and cytokine signals, we now focuse on analysis of development of neural retina from retinal stem cells and application of these knowledge for retinal regeneration.

The neural retina is a part of the central nervous system (CNS), and regeneration of the retina from retinal stem cells or other sources by transplantation is a critical issue from both clinical and neurobiological points of view. Although reports of successful regeneration of the CNS from neural stem cells (NSC) have appeared in the literature, such has not been the case for the vertebrate neural retina. Furthermore, the nature of retinal stem cells has not been clarified, making it difficult to attempt regeneration of the retina. Based on the technique and knowledge that have been accumulated through works of haematopoietic systems in our laboratory, we attempt to identify mammalian retinal stem cells and following developmental process. Various signaling molecules and transcriptional factors are under investing for their roles in retinal development. For developmental biological analyses, we use zebrafish in addition to mouse as model animals. We also continue to work on haematological projects, and bidirectional cooperative progress between neurological and haematological works is one of unique features of our laboratory. Projects which gave major findings during 2008 are as follows.

The spatial patterning of mouse cone opsin expression is regulated by BMP signaling through downstream effector COUP-TF nuclear receptors

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Mouse cone cells express two types of color photopigment, which are sensitive for short wavelength (S-opsin) and middle wavelength (M-opsin) of lights. Their expression has unique dorsoventral gradient in the retina along with dorso-ventral axis. However, mechanism of spatial patterning of opsin expression in the retina has not been understood. By using retina-specific BMP receptor mutant mice, we found that dorsoventral patterning of mouse cone opsins is regulated by BMP signaling. Furthermore, orphan nuclear receptors, COUP-TFI and COUP-TFII, which are expressed in unique patterns along the dorsoventral axis in the developing retina, were appeared to work downstream of BMP to regulate opsin gene spatial pattern-

ing. The expression pattern of both COUP-TFI and II in dorsal retina of retina specific BMP receptor mutant mice was perturbed. Furthermore, in COUP-TFI KO mice, S-opsin was expressed at nearly equal levels on the dorsal and ventral sides, while the expression of M-opsin was up-regulated on the ventral side, resulting in a loss of the dorsoventral expression gradient. In the retina-specific COUP-TFII conditional KO mice, up-regulation of S-opsin expression on the dorsal side was observed, whereas the M-opsin expression pattern appeared to be normal. These results indicate that COUP-TFI and -TFII are necessary for the suppression of S-opsin expression in the dorsal cones, whereas COUP-TFI, but not COUP-TFII, is required for the suppression of M-opsin expression in the ventral cones. Importance of COUP-TFs' function for the regulation of S-opsin expression was also confirmed by using Y79 retinoblastoma cell line. Taken together, we propose a new molecular cascade involving BMP and COUP-TFs that conveys dorsoventral information to direct the expression of cone opsins during retinal development.

Identification of cell surface markers that define temporally and spatially distinct retinal progenitor subsets I

Hideto Koso, Atsumi Iida, Yoko Tabata, Yukihiro Baba, Makoto M. Taketo[4], and Sumiko Watanabe: [4]Department of Pharmacology, Graduate School of Medicine, Kyoto University

Although neural stem cells has been extensively studied, the character of the immature retinal cells has not been elucidated because of the lack of prospective approach to identify retinal progenitor cells. We aimed to identify cell surface markers of retinal stem or progenitor cells by using cell sorter. For that purpose, we screened the expression pattern of cell surface proteins (CD antigens) in mouse immature retina by flow cytometer using a large number of antibodies against different membrane proteins. Among them, 25 antibodies recognized subpopulations of immature retina, and we examined the proliferation and differentiation abilities of purified those sub-populations of retina by various in vitro assay systems. Until now, we found c-kit and SSEA-1 to be spatiotemporal markers of distinct populations of retinal progenitor cells, and these cells dramatically changed their expression profiles of c-kit and SSEA-1 during development. Furthermore, CD73 was appeared to be a marker for photoreceptor. We continue to characterize retinal cell subsets which are labeled by certain antibodies anti CD antigens.

This year, we focused on CD138/syndecan-1 and found that CD138 and SSEA-1 mark distinct populations of developing ciliary epithelium that are regulated differentially by Wnt signal. Ciliary epithelium (CE), which consists of non-pigmented and pigmented layers, develops from the optic vesicle. However, the molecular mechanisms underlying CE development have not been closely examined, in part because cell-surface markers suitable for specific labeling of sub-regions of the retina were unknown. We identified CD138 and SSEA-1 as cell-surface antigens marking non-pigmented and pigmented CE, respectively. During retinal development, both CD138 and SSEA-1 were expressed in the early stage, and segregation of these markers in the tissue began at around E10. As a result, CD 138-positive (CD138+) cells were found at the most distal tip of the retina, and SSEA-1+ cells were found in the periphery adjacent to the area of CD138 expression. In vitro characterization of isolated CD138+ or SSEA-1+ cell subpopulations revealed that CD138+ cells lose their retinal progenitor characteristics between E13 and E 16, suggesting that they commit to becoming non-pigmented CE cells within this period. By in vivo mouse models, we found that stabilized β-catenin expanded the area of CD138+ non-pigmented CE and that elimination of β-catenin inhibited development of non-pigmented CE cells. These findings are the first to use cell-surface markers to ascertain the spatial and temporal transition that occur in developing CE.

Identification of cell surface markers that define temporally and spatially distinct retinal progenitor subsets II

Jing Zhao, Atsumi Iida, Yasuo Ouchi, Shinya Satoh and Sumiko Watanabe

We also used proteomics to examine the comprehensive expression profile of total membrane proteins from embryonic and adult mouse retina. To establish such a database, we used shotgun analysis and a nanoflow LC-MS/MS system to examine total protein expression in purified membrane fractions and identified several membrane associated proteins which expressed embryonic retina. Among the proteins, we focused on glycoprotein m6a (M6a) and found that M6a is expressed in early developmental period of the murine neural retina and regulates neurite extension. M6a is a cell-surface glycoprotein that belongs to the myelin proteolipid protein family. M6a is expressed mainly in the nervous system, and its expression and function in mammalian retina have not been described. Using proteomics analysis of mouse retinal membrane frac-

tions, we identified M6a as a retinal membrane protein that is strongly expressed at embryonic stages. M6a transcripts were strongly expressed in embryonic retina and after completion of the differentiation of retinal subpopulations, the level of expression decreased as mouse development progressed. Immunohistochemistry showed that in the immature mouse retina, M6a was strongly expressed in the axons of retinal ganglion cells. After birth, M6a expression was confined to the inner plexiform layer, and finally, in the adult retina, it was still expressed in the plexiform layers. M6a expression was completely paralleled by that of the synaptic marker synaptophysin. Mouse retinal progenitor cells that overexpressed M6a following retrovirus-mediated gene transfer were subjected to in vitro explant or monolayer cultures. The neurite outgrowth of M6a-overexpressing retinal cells was strikingly enhanced, although M6a did not affect differentiation and proliferation. These results suggest that M6a plays a role in retinal development by regulating neurites, and it may also function to modulate synaptic activities in the adult retina.

TGIF, a homeodomain transcription factor, regulates retinal progenitor cell differentiation

Shinya Satoh and Sumiko Watanabe

TG-interacting factor (TGIF) is a TALE homeodomain protein expressed predominantly in the central nervous system, that functions as a transcriptional repressor. Several mutations in TGIF have been identified in patients with holoprosencephaly, the most common congenital malformation of the developing human forebrain. However, the precise role of TGIF in neural development is not well understood. We found that TGIF was expressed strongly in the mouse retina during early stages of development, and that its expression gradually decreased as retinal development progressed. *In vitro* explant cultures of mouse retina mimic the *in vivo* development of retinal subtypes. Forced expression of TGIF using a retrovirus in explant culture induced the differentiation of amacrine cells from retinal progenitor cells. A *TGIF* paralog, *TGIF2*, showed a similar transition in expression during retinal development, and TGIF2 also promoted amacrine cell differentiation in a retinal explant culture system. However, no apparent difference between wild-type and TGIF-knockout mouse retina was observed, suggesting that TGIF and TGIF2 function redundantly in that tissue. Forced expression of TGIF homeodomain (HD)-EnR (repressing) rather than TGIF HD-VP16 (activating) resulted in a phenotype similar to that induced by wild-type TGIF, suggesting that TGIFs may act as transcriptional repressors to induce amacrine genesis.

The forkhead transcription factor foxe1 regulates chondrogenesis in zebrafish

Chisako Nakada, Yoko Tabata and Sumiko Watanabe

Forkhead genes (Fox) are a family of transcription factors that play important roles during embryonic development. Among them, Foxe1 is causative gene for Bamforth-Lazarus syndrome, which is characterized by hypothyroidism and cleft palate. Applying degenerate PCR using primers specific for the conserved forkhead domain, we identified zebrafish *foxe1*. Foxe1 is expressed in the thyroid, pharynx, and pharyngeal skeleton during development; strongly expressed in the gill and weakly expressed in the brain, eye, and heart in adult zebrafish. A loss of the function of foxe1 by morpholino antisense oligo (MO) exhibited abnormal craniofacial development, shortening of Meckel's cartilage and the ceratohyals, and suppressed chondrycytic proliferation. However, at 27 hpf, the *foxe1* MO-injected embryos showed normal *dlx2*, *hoxa2*, and *hoxb2* expression, suggesting that the initial steps of pharyngeal skeletal development, including neural crest migration and specification of the pharyngeal arch occurred normally. In contrast, at 2 dpf, a severe reduction in the expression of *sox9a*, *colIIaI*, and *runx2b*, which play roles in condrocytic proliferation and differentiation, was observed. Interestingly, *fgfr2* was strongly up-regulated in the branchial arches of the *foxe1* MO-injected embryos. Unlike Foxe1-null mice, normal thyroid development in terms of morphology and thyroid-specific marker expression was observed in foxe1 MO-injected zebrafish embryos. Taken together, our results indicate that foxe1 plays an important role in chondrogenesis during development of the pharyngeal skeleton in zebrafish, probably through regulation of *fgfr2* expression. Furthermore, the roles reported for Foxe1 in mammalian thyroid development may have been acquired during evolution.

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渡辺すみ子　再生医療と幹細胞「再生医療入門～夢の実現をめざして～」東洋経済オンラインマガジン全８回，2008年

http://www.toyokeizai.net/online/magazine/story05/?kiji_no＝30

Donation Laboratories

Division of Exploratory Research (Ain Pharmaciez) Division of Social Communication System for Advanced Clinical Research

探索医療ヒューマンネットワークスステム寄付研究部門 先端医療社会コミュニケーションシステム社会連携研究部門

Project Associate Professor	Masahiro Kami, M.D., Ph.D.		特任准教授	医学博士	上　昌広
Project Assistant Professor	Yuji Tanaka, M.D., Ph.D.		特任助教	医学博士	田中祐次
Project Assistant Professor	Tomoko Matumura, M.D., Ph.D.		特任助教	医学博士	松村有子

The aim of our division is to establish and popularize state-of-the-art medicine and to promote translational research (TR). We develop clinical research infrastructures by unveiling bottlenecks for such establishment and popularization and exploring solutions.

1. Clinical Research

Tokomo Matsumura, Masahiro Kami

After various multiple-center clinical researches, we have reported on adverse events, effectiveness, and safety of state-of-the-art medicine and case studies.

2. Patient Recruitment

Masahiro Kami

Since medical practice is rooted in a regional community, investigating patient migration is one of the key factors to promote state-of-the-art practice and clinical researches.

We surveyed hematology practice in Some Prefectures as a model region to investigate optimal coordination among regional medical associations, public administration and regional media, and patient migration.

3. Structure for Medical Provision

Tomoko Matsumura, Masahiro Kami

We studied the structure for medical provision which can respond to the people's demand with increasing diversity. Our collaborative study with home medical care staff demonstrated that home medical care of an elderly patient with cancer is possible. In our collaborative study with clinics, we identified significant medical needs of young adults with growing health concerns and demands for medical care located on pathways of their daily living such as railway stations.

4. Information Provision for the People

Tomoko Matsumura

We investigate the media effects on the people to improve infrastructures which provide medical information to the people.

In our study on NHK Special released in 2005, we reported the possibility of harmful media effects on patients which increased physicians' prescription and led to significant number of deaths due to adverse drug effects.

We investigated websites containing cancer information. The website of the National Cancer Center Hospital was easily accessible by Internet search, which covered large amount of general cancer information and was linked to websites of many hospitals. Yet, user friendliness can be improved.

5. Drug Approval and Safety Management

Masahiro Kami

A proteasome inhibitor for multiple myeloma (Velcade) was used before approval in Japan, and some patients died of adverse drug effects. We reported the lack of safety management of unapproved drugs. We continue collaborative studies with Pharmaceuticals and Medical Devices Agency to investigate the structures of clinical trials and drug approval, including distribution systems.

6. Medicolegal Issues

Tokomo Matsumura, Masahiro Kami

After an obstetrician was arrested at Fukushima Prefectural Ohno Hospital, Article 21 of Physician Law and introduction of criminal penalty to medical practice induced shrinkage of medical practice and became a nationwide concern. Research articles reporting adverse events, complications, safety and effectiveness dramatically decreased in 2007, suggesting researchers' fear of legal reference. We collaborate with law schools to propose drafts for creation of constructive relationship between legal issues and medical practice/researches.

7. Patient Literacy

Yuji Tanaka

a) Patient Associations

We continue researches on patient feelings with patient associations. We collaborate with the Liaison Council of Inhospital Patient Association Representatives which coordinates 20 inhospital patient associations all over Japan and the Research Division led by Professor Ohsawa at Faculty of Engineering, the University of Tokyo.

b) Pancreatic Islet Transplantation

In our collaborative research with Baylor University in Texas, USA, we study for improvement in quality of life of patients with type I diabetes before and after pancreatic islet transplantation. We aim to develop an informed consent format for pancreatic islet transplantation which improves patient understanding and better convince them, doing patient surveys to evaluate the current version.

8. Collaborative Studies

Masahiro Kami

a) Regenerative Medicine

In the field of regenerative medicine, we develop clinical trial protocols for regeneration of corneal endothelium in collaboration with Department of Ophthalmology, the University of Tohoku. After accumulation of clinical cases in concordance with guidelines at each institute, we plan to collect information on system development at each institute and to standardize necessary steps for clinical application into a database. We also review the safety of each protocol by investigating individual cases.

b) Fungal Infections

We investigate the dynamics of infections introduced from abroad in a research project granted by the Ministry of Health, Labour and Welfare. At present, we conduct nationwide surveys on pathways of communication on fungal infections and on the people's awareness.

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Collaborative Research Unit

Division of Systems Biomedical Technology (Konika Minolta Technology Center)

システム生命医科学技術開発共同研究ユニット (コニカミノルタテクノロジーセンター)

Project Associate Professor Noriko Gotoh, M.D., Ph.D ｜ 特任准教授 医学博士 後 藤 典 子

Our major research interest is to elucidate the molecular mechanisms regulating cancer cells, stem cells, cancer stem cells and development. Our team has two important research directions: One is to clarify the basic principles underlying biology and the other is to apply the knowledge extracted from the basic principles to translational medicine. In particular, we are focusing on growth factor signaling, such as fibroblast growth factor (FGF) and epidermal growth factor (EGF). In order to achieve the goal, we take a challenging combinatorial approaches of molecular biology and systems biology, in addition to conventional methods of molecular biology.

1. FRS2α regulates Erk levels to control a self-renewal target Hes1 and proliferation of FGF-responsive neural stem/progenitor cells

Takuya Sato, Takuya Shimazaki[1], Hayato Naka[1], Hideyuki Okano[1], and Noriko Gotoh: [1]Keio University School of Medicine, Tokyo, Japan

FGF is among the most common growth factors used in culture to maintain self-renewal and proliferative capabilities of a variety of stem cells, including neural stem cells (NSCs). However, the molecular mechanisms underlying the control by FGF have remained elusive. Studies on mutant mice of FRS2α, a central mediator for FGF signaling, combined with FRS2α knock-down or gain-of-function experiments, allowed us to dissect the role of FGF signaling in the self-renewal and proliferation of NSCs. We found that Hes1 is a self-renewal target of FGF signaling in NSCs and that low levels of Erk activated by FRS2α are sufficient to induce Hes1 expression and promote the self-renewal. Moreover, knock-down of FRS2α in the embryonic telencephalon reduced stemness, a phenotype that was rescued by enforced expression of Hes 1. Thus, FRS2α fine-tunes the FGF-Erk axis to control self-renewal at least partly via Hes1 and proliferation of stem cells.

2. Prominent expression of FRS2α protein in neural cells and its association with intracellular vesicles

Yuriko Minegishi, Hiroko Iwanari[2], Takako Horii, Tomoko Hoshino, Takao Hamakubo[2], and Noriko Gotoh: [2]Laboratory for systems biology and Medicine, Research Center for Advanced Science and Technology, The University of Tokyo

The fibroblast growth factor receptor substrate (FRS)-2 protein family comprises FRS2α a well-known central mediator for fibroblast growth

factor signaling, and FRS2β, whose endogenous expression pattern and function are not yet defined. Immunohistochemical analysis revealed that expression of FRS2β was restricted to neural tissues and it colocalized with Tuj1, a neuronal marker. There are 2 distinct patterns of FRS2 β expression in neural cells: punctate and cup/ring-shaped; moreover, some particles colocalized with lysosomes. Stimulation with brain-derived neurotrophic factor enhanced FRS2β phosphorylation and the cup/ring-shaped pattern. These results suggest a probable role of FRS2β in the intracellular degradation systems of neural cells, which involves lysosomes.

3. Inhibitors of EGFR signaling pathways

Noriko Gotoh

The epidermal growth factor receptor (EGFR) family of tyrosine kinases transduce signals for cell proliferation and migration and contribute to tumorigenesis. The signaling networks of family members are very complex with positive and negative regulators acting to fine-tune signaling. In the past decade, extensive research about negative regulators has revealed major roles in signal transduction. Mig-6/RALT/Gene 33, FRS2α/SNT-2/FRS3, SOCS3/4/5 and LRIG1 are all feedback inhibitors that target EGFRs. The first three are cytoplasmic adaptors while the last is a transmembrane protein. They inhibit EGFR family members via multiple modes of action. Although evidence is still fragmentary, these inhibitors might be useful as cancer biomarkers and the development of drugs targeting them would certainly advance personalized medicine in the near future.

4. Control of stemness by fibroblast growth factor signaling in stem cells and cancer stem cells

Noriko Gotoh

Since the discovery of stem cells, scientists have invested tremendous effort in establishing in vitro culture conditions in order to maintain the self-renewal and efficient proliferative capabilities of stem cells by manipulating a variety of growth factors. FGF is one of the most common growth factors used to expand stem cells, including human embryonic stem (hES) cells and several tissue type-specific stem cells. Moreover, it has been recently recognized that FGF is useful for culturing cancer stem cells derived from various types of human tumor tissues, such as brain and breast tumors. The molecular mechanisms underlying the control of stemness by FGF have remained elusive for a long time. The main signal transduction pathway initiated at the FGF receptors leads to the activation of Ras/ERK pathways via the control center FRS2 α. Recent emerging evidence suggests that the FGF-ERK axis controls stemness via multiple modes of action.

5. A key role of NF-κB pathways in breast cancer stem cells for tumorigenesis

Michiko Murohashi, Masahiko Kuroda[3], Takayuki Isagawa[4], Shingo Tsuji[4], Seiichiro Kobayashi[5], Kazuo Umezawa[6], Arinobu Tojo[5], Hiroyuki Aburatani[4] and Noriko Gotoh: [3]Department of Pathology, Tokyo Medical University, [4]Genome Science Division, Research Center of Advanced Science and Technology, University of Tokyo, [5]Division of Molecular Therapy, Institute of Medical Science, University of Tokyo, [6]Department of Applied Chemistry, Faculty of Science and Technology, Keio University

Tumor-initiating cells (TICs) or cancer stem cells can exist as a small population in malignant tissues. However, the contribution of TICs to tumorigenesis remains unclear. We found that several breast cancer cell lines have a small population of $CD24^{-/low}/CD44^{+}$ in which TICs may be enriched and this population generated tumors when fewer than 100 cells were transplanted. Furthermore, the tumors from $CD24^{-/low}/CD44^{+}$ populations showed invasive histology associated with cells positive for cytokeratin (CK)-14, a basal marker. Gene set enrichment analysis (GSEA) revealed that gene sets associated with transforming growth factor (TGF)-β, oncogenic Ras, tumor necrosis factor (TNF) and interferon (IFN) response pathways were enriched in $CD24^{-/low}/CD44^{+}$ populations. These results suggest that TICs have strong epithelial-mesenchymal transition (EMT) activity, oncogenesis, and stroma-like properties. Moreover, nuclear factor kappa B (NF-κB) activity was higher in $CD24^{-/low}/CD44^{+}$ cells than in control cells and NF-κB inhibitor dehydroxymethylepoxyquinomicin (DHMEQ) prevented tumorigenesis of $CD24^{-/low}/CD44^{+}$ cells in vivo. Thus, we propose an intriguing possibility: TICs actively contribute to a cancer stem cell niche via NF-κB-related paracrine mechanisms that facilitate tumorigenesis.

6. Growth factor-signaling systems identify critical genes for survival prediction in lung adenocarcinoma

Mai Yamauchi, Rui Yamaguchi[7], Masao Nagasaki[7], Teppei Shimamura[7], Seiya Imoto[7],

Ayumu Saito[7], Kazuko Ueno[7], Yousuke Hatanaka[7], Ryo Yoshida[8], Tomoyuki Higuchi[8], Satoru Miyano[7] and Noriko Gotoh: [7]Human Genome Center, Institute of Medical Science, University of Tokyo, [8]Institute of Statistical Mathematics, Tokyo, Japan

Effective prognostic molecular markers of lung adenocarcinoma have been an urgent need to help patients get the maximal benefits of treatment. However, cancer diversity has prevented us to find key regulators for prognosis and treatment target. By avoiding complexity caused by cancer, here we found such molecules by analyzing EGF signaling in normal lung epithelial cells using systems biological strategy. We treated cells with EGF or/and gefitinib, a specific inhibitor for EGF receptor tyrosine kinase (RTK), and performed time-series gene expression profiling with microarray. Moreover, by applying mathematical method called a state space model (SSM) to infer dynamic gene regulatory systems, we dissected the EGF-signaling into sensitive system and insensitive/robust system to gefitinib treatment. Surprisingly, from the genes belonging to the former system we were able to extract 139 key genes for good prediction of survival among stage I patients in lung adenocarcinoma. Thus, systems sensitive to EGF RTK in normal cells reflect aggressiveness of lung adenocarcinoma.

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