Affiliated Facilities

Laboratory Animal Research Center
実験動物研究施設

Professor	Chieko Kai D.V.M., Ph.D	教　授　農学博士	甲　斐　知恵子
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Assistant Professor	Hiroki Sato Ph.D	助　教　理学博士	佐　藤　宏　樹
Assistant Professor	Tomoyuki Honda M.D., Ph.D	助　教　医学博士	本　田　知　之

Our major research interests are to elucidate molecular mechanisms of pathogenicity and species specificity of minus and single strand RNA viruses (Mononegavirales), and to control viral diseases. For these purposes, we are studying virus replication and identifying viral and host factors important for the expression of pathogenicity using a novel reverse genetics technique. We are also developing new virus vaccines and virus vectors by genetic engineering. In the animal research center, more than 30,000 mice, mainly transgenic or knockout, are kept for research of IMSUT, and the technical staff support their breeding, frozen storage of eggs and microbiological cleaning.

Comparative and mutational analyses of promoter regions of rinderpest virus.

Chieko Imai, Kentaro Fujita, Fusako Shimizu, Akihiro Sugai, Misako Yoneda, Chieko Kai.

Comparative and mutational analysis of promoter regions of rinderpest virus was conducted. Minigenomic RNAs harboring the genomic and antigenomic promoter of the lapinized virulent strain (Lv) or an attenuated vaccine strain (RBOK) were constructed, and the expression of the reporter gene was examined. The activities of the antigenomic promoters of these strains were similar, whereas the activity of the genomic promoter (GP) of the RBOK strain was significantly higher than that of the Lv strain, regardless of cell type and the source of the N, P and L proteins. Increased replication (and/or encapsidation) activities were observed in the minigenomes that contained RBOK GP. Mutational analysis revealed that the nucleotides specific to the RBOK strain are responsible for the strong GP activity of the strain. It was also demonstrated that other virulent strains of RPV (Kabete O, Saudi/81 and Kuwait 82/1) have weaker GPs than that of the RBOK strain.

Epitope mapping of Canine Distemper Virus phosphoprotein by monoclonal antibodies.

Akihiro Sugai, Takanori Kooriyama, Hiroki Sato, Misako Yoneda, Chieko Kai.

The gene for phosphoprotein (P) of canine disitemper virus (CDV) encodes three different proteins, P, V, and C. The P protein is involved in viral gene transcription and replication. In the present study, we produced monoclonal antibodies (MAbs) against a unique domain of the CDV-P protein, from aa 232 to 507, and determined their antigenic sites. By immunizing BALB/c mice with the recombinant P protein-specific fragment, we obtained six MAbs. Competitive binding inhibition assays revealed that they recognized two distinct regions of the P protein. Western blot analysis and immunofluorescence assays using deletion mutants of the

unique C-terminus of the CDV-P protein revealed that all MAbs recognized a central short region (aa 233-303) of the CDV-P protein. In addition, linear and conformational epitopes have been determined, and at least four antigenic sites exist in the P protein central region. Furthermore, four of the MAbs were found to react with the P protein of recent Japanese field isolates but not with that of the older CDV strains, including a vaccine strain. Thus, these MAbs could be clinically useful for quick diagnosis during the CDV outbreaks.

Pathological and phylogenetic features of prevalent canine distemper viruses in wild masked palm civets in Japan

Ikuyo Takayama, Masahito Kubo[1], Akiko Takenaka, Kentaro Fujita, Takaaki Sugiyama, Tetsuro Arai, Misako Yoneda, Hiroki Sato, Tokuma Yanai[1] and Chieko Kai: [1]Laboratories of Veterinary Pathology, Department of Veterinary Medicine, Faculty of Applied Biological Sciences, Gifu University.

Ten wild masked palm civets infected with CDV, captured in Japan from 2005 to 2007, were histopathologically and phylogenetically analyzed. Phylogenetic analysis based on the amino acid sequences of the H protein of two CDV isolates from masked palm civets revealed that the two isolates were classified into the clade of recent isolates in Japan. Histopathologically marked lesions of virus encephalitis were present in the brain, whereas gastrointestinal lesions were absent or at a mild degree. The distribution of the lesions resembles that of recent CDV cases in dogs. Therefore, recent CDV infections in masked palm civets could be caused by recently prevalent CDV in dogs. The possibility of the masked palm civet as a spreader of CDV among wildlife is also discussed.

Inhibition of host protein synthesis in B95a cells infected with the HL strain of measles virus

Yoshihisa Inoue, Kyoko Tsukiyama-Kohara[1], Misako Yoneda, Hiroki Sato and Chieko Kai: [1]Faculty of Medical and Pharmaceutical Sciences, Kumamoto University

The shut-off of host protein synthesis in virus-infected cells is one of the important mechanisms for viral replication. In this report, we showed that the HL strain of measles virus (MV-HL) as well as other field isolates, which were isolated from human blood lymphocytes using B95a cells, induce the shut-off in B95a cells. Since the Edmonston strain of MV failed to induce the shut-off in B95a cells, the ability to induce the shut-off was considered to be dependent on virus strains. Although the modification of eukaryotic translation initiation factors (eIF) including eIF4G, eIF4E, and 4E-BP1 was reported for shut-off by various viruses, the involvement of these eIFs was not observed in MV-HL-infected B95a cells. Instead, the accumulation of phosphorylated eIF2α was found to coincide to the decrease of host protein synthesis, suggesting the involvement of phosphorylation of eIF2α in inhibition of translation as one of the mechanisms of the shut-off.

Publications

Watanabe A, Yoneda M, Ikeda F, Terao-Muto Y, Sato H, Kai C. CD147/EMMPRIN Acts as a Functional Entry Receptor for Measles Virus on Epithelial Cells. J Virol. (in press)

Imai C, Fujita K, Shimizu F, Sugai A, Yoneda M, Kai C. Comparative and mutational analyses of promoter regions of rinderpest virus. Virology. 396(2): 169-77, 2010

Sugai A, Kooriyama T, Sato H, Yoneda M, Kai C. Epitope mapping of Canine Distemper Virus phosphoprotein by monoclonal antibodies. Microbiol Immunol. 53(12): 667-74, 2009

Takayama I, Kubo M, Takenaka A, Fujita K, Sugiyama T, Arai T, Yoneda M, Sato H, Yanai T, Kai C. Pathological and phylogenetic features of prevalent canine distemper viruses in wild masked palm civets in Japan. Comp Immunol Microbiol. 32(6): 539-49, 2009

Inoue Y, Tsukiyama-Kohara K, Yoneda M, Sato H, Kai C. Inhibition of host protein synthesis in B95a cells infected with the HL strain of measles virus. Comp Immunol Microbiol. 32 (1): 29-41, 2009

Yoneda M, Fujita K, Sato H, Kai C. Reverse genetics of Nipah virus to probe viral pathogenicity. Methods Mol Biol. 515: 329-37, 2009

佐藤宏樹・甲斐知恵子　ジステンパーウイルス（特集：動物のウイルス感染症）　Virus Report 6(1)：72–80，2009

Affiliated Facilities

Amami Laboratory of Injurious Animals
奄美病害動物研究施設

Professor	Chieko Kai, D.V.M., Ph.D.	教　授　農学博士	甲　斐　知恵子
Associate Professor	Shosaku Hattori, D.V.M., Ph.D.	准教授　農学博士	服　部　正　策

The Amami Laboratory of Injurious Animals was established in 1965 at Setouchi-cho in Amami-oshima Island in order to study on endemic diseases involving parasite, arthropods, and venomous snakes in the tropics or subtropics.
The Amami-oshima Island belongs to the Nansei (Southwest) Islands and the fauna is quite different from that in other islands of Japan. Since establishment of the laboratory, trials have been carried out to utilize small mammals found unique in the Amami islands as experimental animals in addition to studies on prevention of Habu bites. As well known, successful eradication of filariasis from this island is one of the monumental works of the laboratory. Our present works are as follows:

1. Research on the Habu control

Shosaku Hattori, Takeshi Kuraishi, Motonori Ohno[1], Naoko Ueda[1], Takahito Chijiwa[1], Aichi Yoshida[2], Yoshihiro Hayashi[3], Michihisa Toriba[4] and Tomohisa Ogawa[5],: [1]Department of Applied Life Science, Faculty of Bioscience, Sojo University, [2]School of Health Science, Faculty of Medicine, Kagoshima University, [3]Department of Veterinary Anatomy, Faculty of Agriculture, University of Tokyo, [4]The Japan Snake Institute, [5]Faculty of Agriculture, Tohoku university

Snake bites by the venomous snake Habu, *Protobothrops flavoviridis*, have been reported annually about 60 cases in the population of 100,000 in the Amami Islands. Moreover, there is no indication that the population of the Habu itself has decreased, despite a campaign for capture of snakes by the Kagoshima Prefectural Government. Rat-baited box traps have been introduced to catch the snakes and found to be quite effective. However, maintenance of live rats requires man power and its cost is expensive. Therefore, our effort has been focused on the development of attractant for Habu. The attractant extracted from rats seems ineffective if compared with use of live rats.

It was known that the Habu survived the injection of the Habu venom since early times, because some proteins in the serum of the Habu blood combine to the elements of the Habu venom. The research of these binding proteins has been initiated with an objective of clinical trials. Phospholipase A_2 and its isozymes isolated from Habu venom have myonecrotic activity and hemorrhagic activity, and metal protease has hemorahagic activity. The binding proteins isolated from serum of Habu inhibit myonecrotic activity of phospholipase A_2 and its isozymes. We found that protein-HSF and peptide-AHP isolated from the Habu serum effectively control the hemorrhage caused by venom of the Habu, *Ovophis okinavensis, Agkistrodon blomhoffi brevicaudus, Calloselasma rhodostoma, Bitis arietans, Bothrops asper,* and, *Trimeresurus stejnegeri.*

Further, a statistics analysis and the simulation were done with the snakes captured by the Government, and the analysis of population dynamics of Habu was attempted. As a result of

investigating the individual measurement data of the captured Habu over 9 years, we were able to obtain the generous age composition of the Habu. From analyzing of the age pyramid of the Habu and the result of questionnaire surveys for the inhabitant in the Amami-oshima Island, the total population of the Habu which lives in this island was estimated at about 80,000. By the analysis of the measured data of last nine years, the snake sizes were miniaturized, and the population of young snakes decreased. According to these investigations, the population of the Habu is expected to decrease in the near future.

These studies are supported by grants from the Ministry of Land, Infrastructure and Transport and the Kagoshima Prefectural Government.

2. Island specific expression of a novel [Lys^{49}] phospholipase A_2 (BPIII) in *Protobothrops flavoviridis* venom in Amami-Oshima

Tatsuo Murakami[1], Shosaku Hattori, Takahito Chijiwa[1], Motonori Ohno[1] and Naoko Ueda[1]:

In search of the transcripts expressed in *Protobothrops flavoviridis* venom gland, 466 expressed sequence tags (ESTs) were generated from the venom gland cDNA library of *P. flavoviridis* in Amami-Oshima, Japan. The sequencing of randomly selected cDNA clones followed by identification in similarity search against existing databases led to the finding of a novel lysine-49-phospholipase A_2 ([Lys^{49}]PLA_2) clone. It coded for one amino acid-substituted BPII homologue or two amino acids-substituted BPI homologue in which BPII and BPI are [Lys^{49}]PLA_2s contained in Amami-Oshima and Tokunoshima *P. flavoviridis* venoms. This isozyme, named BPIII, was isolated from Amami-Oshima *P. flavoviridis* venom. BPIII gave a specific $[M+2H]^{2+}$ peak of m/z 736.3 on mass spectrometry (MS) analysis after S-carboxamidomethylation and trypsin digestion when compared with BPII. It became evident from MS analysis after S-carboxamidomethylation and trypsin digestion of the mixed protein peaks ranging from BPI to BPII obtained by fractionation on a carboxymethyl cellulose column of Amami-Oshima and Tokunoshima *P. flavoviridis* venoms that BPIII protein is contained in Amami-Oshima *P. flavoviridis* venom but not in Tokunoshima *P. flavoviridis* venom. It is for the first time that a protein present in Amami-Oshima *P. flavoviridis* venom is not found in Tokunoshima *P. flavoviridis* venom.

3. Reproduction of squirrel monkeys.

Shosaku Hattori, Takeshi Kuraishi, Kumiko Ikeda, Hazuki Yoshimura and Chieko Kai

The squirrel monkey, *Saimiri sciurea*, is widely distributed in the tropical rainforest in Central and South America between 10 degrees N and 17 degrees S of latitudes. The advantage of using this species for medical researches resides in its small size and gentle behavior. In this laboratory, about 3 newborns are given annually by 25 adult females.

The aim is to optimize the use of the non-human primate model in future the Amami Laboratory research activities. The laboratory newly established experimental infection systems which require or can be adapted to the squirrel monkey model, particularly the study of human falciparum malaria. Development of parasites, immune response to malaria parasites and pathological changes were investigated in in-vivo condition, further more, in vitro analysis of cell and molecular level was performed. It is also investigating the mechanisms of infection in immunology, vector development, a vaccine production program, and a clinical trials program.

Publications

Murakami T, Kariu T, Takazaki S, Hattori S, Chijiwa T, Ohno M, Oda-Ueda N. Island specific expression of a novel [Lys^{49}]phospholipase A_2 (BPIII) in *Protobothrops flavoviridis* venom in Amami-Oshima, Japan. *Toxicon*, 54 (4): 399-407, 2009.

服部正策，倉石武．病理実験部会報告．平成20年度奄美ハブ毒免疫機序研究報告書．（鹿児島県）．pp. 40–46，2009.

倉石武．間接ELISA法を用いたハブ毒特異的IgEの測定．平成20年度奄美ハブ毒免疫機序研究報告書．（鹿児島県）．pp. 58–60，2009.

服部正策，三島章義．野外調査部会報告．平成20年度ハブ動態制御研究報告書．（鹿児島県）．pp. 11–44，2009.

服部正策，倉石武．ハブ低密度地域の環境調査．平成20年度ハブ動態制御研究報告書．（鹿児島県）．pp. 57–62，2009.

Affiliated Facilities

Laboratory of Molecular Genetics
遺伝子解析施設

Professor	Izumu Saito, M.D., D.M.Sc.	教　授　医学博士	斎　藤　　泉
Assistant Professor	Yumi Kanegae, D.M.Sc.	助　教　医学博士	鐘ヶ江　裕　美
Assistant Professor	Saki Kondo, D.M.Sc.	助　教　医学博士	近　藤　小　貴

This laboratory has two main activities, development of efficient expression vectors for gene therapy, especially for anti-cancer, and supporting the researchers by advising on recombinant DNA technology and on biohazards under the safety guidelines.

The purposes of our laboratory are concerned about not only research but also support for all researchers in this institute. Our supporting activity is involved in advising service on gene-manipulation experiments and on biohazards under the safety guidelines. For the research part, we intend to develop novel methods or new experimental systems leading in the field of gene expression and its regulation. We are concentrating mainly on developing efficient adenovirus expression vectors aiming at gene therapy. We are maintaining more than 20 collaborations within and outside of this institute. In these collaborations, we offer and supply our efficient method to construct adenovirus vector (AdV) expressing various genes efficiently. And recently we developed the new cosmid cassette for AdV construction, which can choose not only very efficient COS-TPC method (Miyake *et al*., PNAS 93: 1320-1324, 1996) but also an easier method using a full-length viral genome with intact viral termini (Fukuda *et al*., Microbiol. Immunol. 50: 643-654, 2006). This new cassette is available from Takara Bio and Nippon Gene. We have also developed a method for ON/OFF switching of gene expression in mammalian cells using a combination of adenovirus vector and Cre/*loxP* system (Kanegae *et al*., Nucleic Acids Res. 23: 3816-3821, 1995; Kanegae *et al*., Gene 181: 207-212, 1996) as well as FLP/FRT system (Nakano *et al*., Nucleic Acids Res. 29: e40, 2001; Kondo *et al*., Nucleic Acids Res. 31: e76, 2003; Kondo *et al*., Microbiol. Immunol., 50: 831-843, 2006). The methods will promote studies of various fields of molecular biology and medicine and may open a new field of "intracellular gene manipulation". We recently identified adenovirus pIX gene as a main cause of inflammation observed in AdV infection (Nakai *et al*., Hum. Gene Ther. 18: 925-936, 2007). The research activities in 2009 were shown below.

1. Improvement of "double-unit" adenovirus vector for enhanced and cell-specific expression

Yumi Kanegae, Miho Terashima, Saki Kondo, Aya Maekawa, Zheng Pei and Izumu Saito

Adenovirus vectors (AdV) are valuable because of high transduction efficiency to broad range of cell types but, conversely, do not show cell specificity. Therefore, for cell-specific expression this vector must be used together with an-

other technique, such as use of cell-specific promoter. However, expression level of a cell-specific promoter is much lower, about several hundred times less, than a versatile potent promoter such as CAG and EF1α promoters and, if this problem could be solved, cell-specific promoters must become much more useful in many *in vivo* studies. Here, we provide a new strategy using a novel "excisional-expression" system on a single AdV genome. This AdV, named "double-unit" vector, uses a "switch" unit under control of cancer-specific, α-fetoprotein (AFP) promoter as an example of a cell-specific promoter, and a "target" unit, which consists of a line as loxP, purpose gene, polyA signal, EF1α promoter and second loxP in that order. In normal cells, Cre can not be expressed because of strict specificity of AFP promoter, however in the hepatocellular carcinoma cells, Cre expression is induced and a target unit is excised as a circle, where the purpose gene can be connected with EF1α promoter and at last expressed.

However, during preparation of the vector, a deleted AdV lacking the target unit was generated because of a leak expression of Cre in *E. coli* and in 293 cells. To overcome this problem in 293 cells, we have constructed a dominant-negative Cre (dnCreRY), which efficiently suppressed the leak expression of Cre. We generated a cell line which constitutively express dnCreRY and found that improved preparations of this vector were able to be achieved using this 293 cell line. In 2009, we were able to solve the problem of Cre-leak expression in *E. coli* by constructing an AdV cosmid cassette expressing dnCreRY under the control of *trc* promoter. In the preparation of AdV cassette, Cre-processed molecule was not detected at all. We also construct a short-hairpin RNA against Cre, shCreD, and established a 293 cell line constitutively expressing shCreD (shCreD13). The shCreD13 suppressed the leak expression of Cre more efficiently than any cell line expressing dnCreRY, and enabled us to prepare "double-unit" AdV without trouble. This established method will offer high-level expression while maintaining very high specificity and will not only provide a novel strategy of suicide gene therapy using AdV, but also be very valuable for many studies requiring cell-specific expression.

2. Advance for efficient generation of cancer/tissue-specific helper-dependent adenovirus vector

Saki Kondo, Miho Terashima, Yumi Kanegae and Izumu Saito

We previously reported a "double infection" method for adenovirus vector (AdV): we solved a problem of the low activity of cell-specific promoter by using a combination of a "switch vector" utilizing Cre, and "target vector" possessing a Cre-inducing potent expression unit. However, both vectors must be infected simultaneously into the same cell. To improve its expression level *in vivo*, we are developing a helper-dependent AdV (HD-AdV), containing these two vector functions in one AdV genome. Firstly, we have established 293 cells containing improved-FLP for efficient HD-AdV generation, and secondly identified novel stuffer regions not only fit for the AdV genome size but for efficient transgene expression. Like the "double-unit" vector described above, we observed a leak expression of Cre during vector preparation; Cre enzyme can excise the stuffer of its target unit of HD-AdV efficiently, causing a contamination and eventual loss of cancer specificity. To overcome this problem, we newly generated a new helper virus, which carried an siRNA against Cre, shCreD, described above, and we obtained more efficient and strict HD-AdV preparation; the HD-AdV with cancer/tissue specificity will be a useful tool for cancer gene therapy in a recent future.

3. Development of rapid titeration method for adenoviral vectors using real-time PCR

Zheng Pei, Miho Terashima, Saki Kondo, Izumu Saito and Yumi Kanegae

The first-generation adenoviral vectors substituting its E1 region for a target gene were widely applied not only in the gene therapy but also in basic studies. However, measuring its titer is very time-consuming and it took for at least two weeks, though it is a basic and popular technology. To solve this problem, several new methods have been developed using an expensive immunostaining, for example. However, measured titers using standard $TCID_{50}$ methods frequently show much higher or lower numbers, because the target protein expressed by the AdV influences on the viral growth of 293 cells. For the AdV, the number we want to know is the ability to transfer copy numbers of a viral genome to target cells and is not that to grow in 293 cells.

In this study, we propose a quick and reliable method measuring actually-infectious virus titer, taking only for two days using real-time PCR (RT-PCR). To establish the method, CV-1 cells, derived from monkey kidney, were infected with AdV expressing GFP and adsorbed with the virus for one hour. Three days later, total cell DNA was extracted and subjected to RT-

PCR to measure copy numbers of introduced viral genome. The result confirmed linearity up to 100-fold. Then, AdV was infected either to adherent cells or to floating cells. The result showed that both methods gave almost same copy number of AdV genome transferred. Then, titers were measured in several AdVs using methods of the standard $TCID_{50}$ and of RT-PCR. The results showed that there were linear relation between these two methods and that, although the titer measured by RT-PCR method was fluctuated by cell numbers, etc, it can be corrected by using a standard AdV. Further experiments showed that the infection time and the culturing days were sufficient for only five minus and for two days, respectively. This method gave a correct titer only for two days and was found very practical. Probably the current method used in the world will be replaced by this method.

Publications

Kondo, S., Takata, Y., Nakano, M., Saito, I. and Kanegae, Y. Activities of various FLP recombinases expressed by adenovirus in mammalian cells. J. Molec. Biol. 390: 221-230, 2009.

Fukamachi, K., Tanaka, H., Hagiwara, Y., Ohara, H., Joh, T., Iigo, M., Alexander, D.B., Xu, J., Long, N., Takigahira, M., Yanagihara, K., Hino, O., Saito, I. and Tsuda, H. An animal model of preclinical diagnosis of pancreatic ductal adenocarcinomas. Biochem. Biophys. Res. Commun. 390: 636-641, 2009.

Affiliated Facilities

Medical Proteomics Laboratory
疾患プロテオミクスラボラトリー

Professor	Jun-ichiro Inoue, Ph.D.		教　授	薬学博士	井　上　純一郎
Associate Professor	Shinobu Imajoh-Ohmi, D. Sc.		准教授	理学博士	大　海　　忍
Assistant Professor	Hiroshi Sagara, Ph.D.		助　教	医学博士	相　良　　洋
Project Assistant Professor	Masaaki Oyama, Ph.D.		特任助教	医学博士	尾　山　大　明

The mission of our laboratory is to develop technologies for protein research that enable us to analyze complex cellular systems leading to a variety of diseases such as cancer and infection. We mainly focus on the researches based on advanced technologies regarding mass spectrometry and electron microscopy for precise measurement of dynamic behaviors of functional protein networks.
We are also engaged in collaborative researches regarding electron microscopy, mass spectrometry, peptide synthesis and purification of proteins and their functional analyses and have made a substantial contribution to many scientific achievements.

‹Group I›

1. Development of novel antibodies as tools available for in situ analyses of post-translational modification of proteins

After biosynthesis proteins undergo various post-translational modifications, and their functions are modulated. In order to understand such biochemical reactions in a single cell, we have been making modification-specific antibodies as probes for such in situ analyses; cleavage-site-directed antibodies for proteolysis, phosphorylation-site-specfic antibodies, myristoilated peptide-specific antibodies, ubiquitination-specific antibodies, inhibitor-bound enzyme-specific antibodies etc. These antibodies should be useful tools for research in cellular biochemistry.

a. Evaluation of polyclonal cleavage-site directed antibodies and their fractionation into more easy-to use probes

Tsuyoshi Katagiri, Chizuko Takamura, Nozomi Ichikawa and Shinobu Imajoh-Ohmi

Cleavage-site directed antibodies are convenient tool for in situ analysis of proteolysis, since they do not bind unproteolyzed native proteins that retain the same sequence internally. To obtain such antibodies, peptides corresponding to the terminal regions around the cleavage are chemically synthesized and used for haptens, where molecular design of the peptides is critical for quality of the antibodies. Too short peptide results in generation of useless antibodies recognizing the short peptide but not the terminus of cleaved proteins. On the other hand, when a longer sequence is selected for immunogenic peptide, antibodies raised bind unproteolyzed proeins as well as the cleaved ones. Thus, an evaluation system is necessary for cleavage-site directed antibodies. Phage display liblaries were used for evaluation of antigenic specificity of cleavage-site directed antibodies. Randomized sequences of synthetic oligonucleotide were introduced into phage DNA in order that a fusion protein with randomized se-

quences of amino- or carboxyl-terminal region. A library was applied to immobilized antibodies, and phages bound were subjected to sequence analysis for terminal regions. When antigenic specificity of a cleavage-site derected antibody was examined by this method, the antibody was found to be a mixture of three or more types of antibodies that bind to terminal and internal regions of the peptide used for immunogen. Quality of the antibody was successfully improved by affinity chromatography immobilized three peptides according to the evaluation method.

b. A novel method for hunting substrates of caspases in apoptotic cells

Maiko Okada, Chizuko Takamura, Hiroyuki Fukuda, Masahiko Kato and Shinobu Imajoh-Ohmi

Caspases catalyze limited proteolysis of many proteins in apoptotic cells. Hundreds of substrates have been identified as targets of caspases so far. Previously, nonmuscle myosin heavy chain-A and a component of DNA-dependent proein kinase, Ku80, are found to be cleaved during apoptosis in human Jurkat T cells. We used first a cleavage-site derected antibody against the amino-terminal fragment of caspase 3/7-catalyzed calpastatin. Carboxyl-terminal region of caspase-proteolyzed fragments resemble each other, and such antibodies are expected to misrecognize the target molecules. We further investigated the apoptotic Jurkat cells for the anitobody-stained polypeptides. Cells were selectively extracted with salt- and denaturant-containing buffers, and extracts were subjected to two-dimensional gel electrophoresis/immunoblotting. Candidate polypeptides stained with antibodies were digested with trypsin and analyzed by mass spetrometer. Isoforms of ribonucleoprotein were thus identified.

c. Identification of cysteine proteases in *Caenorhabditis elegans*

Yohei Kato and Shinobu Imajoh-Ohmi

E64c, [L-3-trans-carbonyloxirane-2-carbonyl]-L-leucine(3-methylbutyl)amide, is a synthetic inhibitor for cysteine proteases such as cathepsins B, H, L and calpain. To inhibit intracellular cysteine proteases E64d, [L-3-trans-ethoxycarbonyloxirane-2-carbonyl]-L-leucine(3-methylbutyl)amide, a membrane-permeable derivative of E64c is used instead of E64c. E64d penetrates into the cell membranes where endogenous esterases convert it to E64c that covalently binds to the SH group of active center in enzymes. Thus, anti-E64c antibody is a useful probe for *in vivo* analysis of cysteine proteases.

We have succeeded in making an antibody to E64c. First, we tried to establish an antibody against E64c-bound calpain. A peptide corresponding to the active center of calpain was synthesized by using the multiple-antigen peptide system. E64c was chemically introduced into the SH group of active center cysteine under reducing conditions. Rabbits were immunized with the E64c-conjugated calpain-derived peptide without further conjugation with a carrier protein. Unexpectedly, an antibody thus prepared reacted not only with E64c-inactivated calpain but also with E64c-bound other cysteine proteases such as papain and cathepsins. Low antigenicity of peptide region in the immunogen may result in such broad specificity of the antibody. Our antibody is expected to be used for identification of E64c-targeted novel proteases. When cells were treated with E64d, cell growth was suppressed and several proteins were labeled by E64c that is visualized with this antibody on immunoblotting. Structural analysis of these proteins may lead identification of novel cysteine proteases.

Homogenates of *C. elegans* were treated with E64c in the presence or absence of calcium ion, and subjected to electrophoresis/immunoblotting using an anti-E64c antibody. A 55-kDa polypeptide (p55) was labelled with E64c in a calcium ion-dependent manner. In *C. elegans* several calpain-related gene products were identified at the mRNA level, but their physiological function remains to be elucidated.

2. Post-translational modification of proteins during apoptotic cell death

Apoptotic cell death involves various biochemical reactions. Among them, post-translational modification of proteins is intensively investigated in this laboratory. First, intracellular proteolytic enzymes are activated prior to and during apoptosis. Caspases are now established as pivotal apoptosis-executing enzymes that cleave various substrates. Endogenous or viral proteins and synthetic substances inhibitory for caspases suppress the apoptotic cascade and rescue cells from cell death. On the other hand, proteasomes drive the cell cycle by degrading cyclins etc., and also play important parts in apoptosis, since proteasome inhibitors induce apoptotic cell death in growing cells but suppress apoptosis of some cells that is in quiescent state. Furthermore, in some specific cells such as polymorphonuclear leukocytes, other proteases might be involved in cell death.

a. Limited proteolysis of actin in apoptotic neutrophils

Junko Ohmoto and Shinobu Imajoh-Ohmi

Neutrophil actin is proteolyzed to a 40-kDa fragment during preparation/isolation from peripheral blood. The truncated actin lacks amino-terminal region of native protein and presumably cannot copolymerize to F-actin. The 40-kDa actin-derived fragment is apparently related to spontaneous apoptosis of neutrophils. To investigate the role of actin proteolysis, especially cause-and-effect relationship to neutrophil apoptosis, we have made a cleavage-site-directed antibody (#1090pAb) for the 40-kDa form of actin using synthetic peptides as haptens. The antibody reacted with the 40-kDa polypeptide but not with unproteolyzed native actin which remain abundant in the cell. Using this antibody, we have found that (1) the truncated actin is generated during isolation of neutrophils from peripheral blood, (2) neutrophils without the truncated actin can be prepared in the presence of diisopropyl fluorophosphate, and (3) leukocyte elastase is possibly responsible for this limited proteolysis.

Herein we analyzed cellular localization of the truncated actin using #1090pAb. Confocal laser microscopic observation indicated that the plasma membrane of neutrophils were strongly stained with #1090pAb, but that intracellular regions near the membrane were sometimes stained weakly. We examined here whether or not the amino-teriminal region of the 40-kDa actin is on the cell surface of neutrophils using at the same time established antibodies for components of superoxide-generating system composed of transmembranous cytochrome and cytosolic activator proteins. Furthermore, flow-cytometric analysis revealed that #1090pAb stained the cell-surface antigen under the conditions that antibodies for cytosolic proteins did not. Our findings suggest that the truncated actin is, at least in its amino-terminal part, on the surface of neutrophils. However, another antibody against the amino-terminal region of native actin did not stain neutrophils from outside suggesting that the cleavage site is inaccessible to exogenous proteinases.

b. Fas, a death receptor, is polymerized to high-molecular weight forms during Fas-mediated apoptosis in Jurkat T cells

Hidehiko Kikuchi, Fotoshi Kuribayashi and Shinobu Imajoh-Ohmi

An apoptotic receptor Fas mediates death signal from Fas ligand. A cell death-inducing monoclonal antibody CH11 mimics Fas ligand and triggers apoptotic signal mediated by Fas molecule. Plasma transglutaminases are found to involved in down-regulation of apoptosis induced by a cytotoxic anti-Fas monoclonal antibody in Jurkat cells. When cells were treated with the antibody in fetal calf serum-containing media, Fas was polymerized to higher-molecular-weight polypeptides as judged by immunoblotting. Under conditions where the transglutaminase activity was eliminated or uppressed, the polymerization of Fas was not observed, and concurrently cell death was hastened. Furthermore, an antibody against blood coagulation factor XIII strongly accelerated the Fas-mediated apoptosis, indicating that plasma transglutaminases catalyze polymerization of Fas and down-regulate apoptotic cell death.

3. Localization of the BRCA2-myosin IIC complex to the midbody during cytokinesis and its role in the completion of cytokinesis

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BRCA2 is mainly localized to the nucleus, where it plays an important role in DNA damage repair. Some of it is also distributed in the midbody of the cell. BRCA2 is phosphorylated by the mitotic polo-like kinase, Plk1, at Ser193. We raised antibodies against phospho-Ser193, which contains the consensus motif for a Plk1 phosphorylation site (Glu / Asp-X-Ser / Thr, where X is any amino acid). The antibodies specifically recognized BRCA2 from the midbody and were specific for phosphorylated BRCA2, since they did not recognize BRCA2 treated with phosphatase (PP2A). This antibody reacted with wild-type BRCA2 in the cell midbody, but it did not react with mutated BRCA2 in which Ser193 was substituted with Ala. Therefore, it is likely that the antibody detects Plk1-dependent phosphorylation of BRCA2 at S193 in the midbody. In addition, cytokinesis is delayed or prevented when BRCA2 is depleted by siRNA. The length of the midbody is significantly extended in HeLa cells treated with BRCA2 siRNA compared with the controls. This phenotype is rescued by the exogenous expression of wild-type BRCA2, but not by the expression of mutated BRCA2 in which Ser193 is substituted by Ala.

However, the role of BRCA2 in the midbody

is unclear. When a cell lysate was fractioned by a glycerol density gradient centrifugation, the majority of endogenous BRCA2 was observed to sediment at approximately 700-800 kDa although the molecular mass of BRCA2 is 380 kDa. In an effort to identify the proteins that physically interacted with BRCA2, some proteins were analyzed using tandem mass spectrometry. After a database search, human nonmuscle myosin heavy chain (NMHC) IIC was identified as the BRCA2-associated protein. Here, we show the interaction of BRCA2 with NMHC IIC by immunoprecipitation and immunostaining analyses. We next conducted an experiment using specific RNA interference to decrease the amount of NMHC IIC or BRCA2 in A549 cells; this resulted in failure in the completion of functional cleavage of the midbody. These findings demonstrate that phosphorylation of BRCA2 by Plk1 is necessary for the recruitment of BRCA2 to the midbody, and that BRCA2 interacts with NMHC IIC to form a complex. This complex may play an essential role in the terminal phase of cytokinesis, and the dysfunction of cytokinesis may lead to breast oncogenesis.

4. S100A4-binding domain peptide of methionine aminopeptidase 2 inhibits angiogenesis and tumor growth

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Angiogenesis is known to be essential for tumor growth and metastasis. We previously reported that the metastasis-associated calcium-binding protein, S100A4, physically interacts with methionine aminopeptidase 2 (MetAP2), a targeting molecule for developing fumagillin-like angiogenic inhibitors. To study a possible physiological significance of this interaction in endothelial cells, an attempt was made to block the binding of both proteins. Here we show that overexpression of the S100A4 binding domain of MetAP2 consisting of 60 amino acid residues spanning from Arg-170 to Ala-229 (named SBD) severely suppressed serum-induced DNA synthesis of endothelial MSS31 cells. Blockage of S100A4-MetAP2 interaction by the SBD was supported by *in vitro* binding assays using the chemically synthesized SBD peptide. The N-terminal 39 amino acid residues of SBD (Arg-170 to Ileu-208, named NBD), was found to contain the essential region for binding with S100A4, based on the results obtained by *in vitro* binding assays and intracellular fluorescence resonance energy transfer analyses. Intriguingly, the NBD peptide, when introduced into MSS31 cells as a complex with atelocollagen, caused a distinct G1 arrest of the cell cycle and a marked suppression of capillary vessel morphogenesis. The directed *in vivo* angiogenesis assay also demonstrated that the angiogenic factor-induced new blood vessel formation was obviously hampered by the NBD peptide complex. A single intratumoral injection of the NBD peptide complex in xenografted nude mice resulted in a significant reduction of vascular development and tumor growth, despite of its inability to affect proliferation of the tumor cells in culture. Taken together, these results clearly indicate that the S100A4-MetAP2 interaction is concerned not only in endothelial cell growth but also in angiogenesis, and blockage of the interaction may offer an effective therapeutic approach for prevention of tumor progression and metastasis.

‹Group II›

1. Dynamic alteration of signaling networks defines drug-resistance properties of breast cancer

Masaaki Oyama, Hiroko Kozuka-Hata, Noriko Yumoto1, Takashi Adachi, Takeshi Nagashima[1], Yoko Kuroki[1], Kazuhiro Ikeda[2], Satoshi Inoue[2], Mariko Hatakeyama[1] and Hiroaki Kitano[3]: [1]Advanced Science Institute, RIKEN, [2]Research Center for Genomic Medicine, Saitama Medical University, [3]Sony Computer Science Laboratories, Inc.

Signal transduction system, in orchestration with subsequent transcriptional regulation, widely regulates complex biological events such as cell proliferation and differentiation. Therefore, a comprehensive and fine description of their dynamic behavior provides a fundamental platform for systematically analyzing the regulatory mechanisms that result in each biological effect. Here we developed a proteomics framework for obtaining time-resolved description of global phosphoproteome dynamics using highly sensitive nanoLC-MS/MS system in combination with the Stable Isotope Labeling by Amino acids in Cell culture (SILAC) technology. Based

on our sophisticated proteomics technology, we analyzed dynamic behaviors of signaling networks mediated by estrogen receptor/ErbB2 pathways, which have long been implicated in drug response of breast cancer. Through shotgun identification and quantification of phosphorylated molecules enriched with anti-phosphotyrosine antibodies/Phos-tag reagents, we obtained a global view of the dynamics regarding breast cancer-related signaling networks upon estrogen (E2) or heregulin (HRG) stimulation. Comparative analysis of wild-type and tamoxifen-resistant MCF-7 cells revealed the altered behaviors of signaling hub dynamics, indicating distinct signaling network properties between these two cell types. Very interestingly, phosphorylation-dependent dynamics of transcription factors was observed in a stimulation or cell-type dependent manner, which suggested their involvement in adapting cellular information systems to acquire drug resistance property against tamoxifen.

2. Global perturbation of phosphotyrosine-dependent signaling networks by Helicobacter pylori infection

Hiroko Kozuka-Hata, Masato Suzuki[4], Kotaro Kiga[4], Shinya Tasaki, Jun-ichiro Inoue, Tadashi Yamamoto[5], Chihiro Sasakawa[4] and Masaaki Oyama: [4]Division of Bacterial Infection, Department of Microbiology and Immunology, IMSUT, [5]Division of Oncology, Department of Cancer Biology, IMSUT

The signal transduction system within a cell regulates complex biological events in response to bacterial infection. The previous analyses of cell signaling in Helicobacter pylori-infected gastric epithelial cells revealed that CagA, a major virulence factor of Helicobacter pylori, is delivered into cells via the type IV secretion system and perturbs phosphotyrosine-dependent protein networks through the interaction with the signaling molecules such as SHP-2, Grb2, Crk/Crk-L, Csk, Met, and ZO-1. Although the biological activity of tyrosine-phosphorylated CagA has intensively been studied, system-wide effects of the virulence factor on cellular signaling have yet to be analyzed. As Helicobacter pylori infection plays pivotal roles in the progression of gastric diseases including carcinogenesis, a comprehensive and fine description of the signaling dynamics would serve as a fundamental platform to theoretically explore for potential drug targets through analyzing the regulatory mechanisms at the system-level. Recent technological advances in mass spectrometry-based proteomics have enabled us to perform large-scale identification and quantification of signaling molecules and even generate their temporal activation profiles on a network-wide scale. Our highly sensitive nanoLC-MS/MS analyses in combination with the Stable Isotope Labeling by Amino acids in Cell culture (SILAC) technology have revealed time-resolved description of the Helicobacter pylori-induced signaling dynamics, which presented a global view of their network activation status including well-known related molecules such as p38 MAPK.

3. Systematic elucidation of the aberrant EGFR signaling by phosphoproteomics-based network description

Shinya Tasaki, Masao Nagasaki[6], Hiroko Kozuka-Hata, Kentaro Semba[7], Noriko Gotoh[8], Seisuke Hattori[9], Jun-ichiro Inoue, Tadashi Yamamoto[5], Satoru Miyano[6], Sumio Sugano[10] and Masaaki Oyama: [6]Laboratory of DNA Information Analysis, Human Genome Center, IMSUT, [7]Department of Life Science and Medical Bio-Science, Waseda University, [8]Division of Systems Biomedical Technology, IMSUT, [9]Department of Biochemistry, School of Pharmaceutical Sciences, Kitasato University, [10]Laboratory of Functional Genomics, Department of Medical Genome Sciences, Graduate School of Frontier Sciences, The University of Tokyo

Mutation of the epidermal growth factor receptor (EGFR) causes a discordant cell signaling, resulting in a number of diseases. However, the mechanism by which such mutation alters downstream signaling is not yet fully understood at the system level. Here, we report a phosphoproteomics-based methodology for characterizing the regulatory mechanism of aberrant EGFR signaling through computational network modeling. Our phosphoproteomic analysis of the mutation at tyrosine 992 (Y992), one of the "hub" autophosphorylation sites of EGFR, revealed network-wide effects of the mutation on the EGF signaling in a time-resolved manner. Computational modeling based on the temporal activation profiles enabled us not only to rediscover already-known protein interactions with Y992 and internalization property of mutated EGFR but to further gain model-driven insights into the effect of cellular content and the regulation of EGFR degradation. Our kinetic model also defined critical reactions to reconstruct the diverse effects of the mutation on the phosphoproteome dynamics. This is the first network-level description of the regulatory mechanism of mutated EGFR signaling, which could provide a systematic strategy toward con-

trolling disease-related cell signaling.

4. Time-resolved description of tyrosine-phosphoproteome signaling dynamics reveals Src family-mediated global regulatory networks

Hiroko Kozuka-Hata, Masaaki Oyama, Shinya Tasaki, Kentaro Semba[7], Seisuke Hattori[9], Sumio Sugano[10], Jun-ichiro Inoue and Tadashi Yamamoto[5]

It is well-known that signal transduction system within a cell leads to the determination of diverse cell fates, such as proliferation, differentiation, or apoptosis. As phosphotyrosine-dependent networks play a key role in transmitting signals, time-resolved description of their dynamics provides a fundamental platform for analyzing the regulatory mechanisms at the system level. Here we established a mass spectrometry-based framework for analyzing tyrosine-phosphoproteome dynamics through temporal network perturbation and applied our methodology to the signaling networks that worked in human A431 cells as a model system. The dynamic behavior upon EGF stimulation revealed the property of multi-phase network activation, comprising spike signal transmission within 1 min followed by prolonged activation of multiple Src-related molecules. Temporal perturbation of Src-family kinases with the corresponding inhibitor PP2 in the prolonged activation phase enabled us to clearly distinguish between sensitive and robust pathways to this treatment, providing a system-level view of Src function in EGF signaling of A431 cells. Our methodology enables us to refine literature-based network structure into cell type-specific architecture. We expect that mathematical analyses on cell type-specific network models will lead us to efficient identification of potential drug targets in each disease condition and also enable us to theoretically estimate the effect of their corresponding drugs on a network-wide scale prior to clinical application.

5. Large-scale identification of small proteins by 2DnanoLC-MS/MS system defines increased complexity of the human short ORFeome

Masaaki Oyama, Hiroko Kozuka-Hata, Sumio Sugano[10], Tadashi Yamamoto[5] and Jun-ichiro Inoue

In parallel with the human genome projects, human full-length cDNA data has also been intensively accumulated. Large-scale analysis of their 5′-UTRs revealed that about half of these had a short ORF upstream of the coding region. Experimental verification as to whether such upstream ORFs are translated is essential to reconsider the generality of the classical scanning mechanism for initiation of translation and define the real outline of the human proteome. Our previous proteomics analysis of small proteins expressed in human K562 cells provided the first direct evidence of translation of upstream ORFs in human full-length cDNAs (Oyama et al., Genome Res, 14: 2048-2052, 2004). In order to grasp an expanded landscape of the human short ORFeome, we have performed an in-depth proteomics analysis of human K562 and HEK293 cells using a two-dimensional nanoLC-MS/MS system. The results led to the identification of eight protein-coding regions besides 197 small proteins with a theoretical mass less than 20 kDa that were already annotated coding sequences in the curated mRNA database. In addition to the upstream ORFs in the presumed 5′-untranslated regions of mRNAs, bioinformatics analysis based on accumulated 5′-end cDNA sequence data provided evidence of novel short coding regions that were likely to be translated from the upstream non-AUG start site or from the new short transcript variants generated by utilization of downstream alternative promoters. Protein expression analysis of the *GRINL1A* gene revealed that translation from the most upstream start site occurred on the minor alternative splicing transcript, whereas this initiation site was not utilized on the major mRNA, resulting in translation of the downstream ORF from the second initiation codon. These findings reveal a novel post-transcriptional system that can augment the human proteome via the alternative use of diverse translation start sites coupled with transcriptional regulation through alternative promoters or splicing, leading to increased complexity of short protein-coding regions defined by the human transcriptome (Oyama et al., Mol Cell Proteomics, 6: 1000-1006, 2007).

‹Group III›

The main activity of this group is to offer supports for the research projects those need electron microscopic analysis. The services available in this group are the conventional thin section transmission electron microscopy, immuno-electron microscopy, negative staining techniques and scanning electron microscopy. By using these individual technique or combination of some of these, we can offer direct visual evidence that can not be acquired by other methods. In addition to the supportive work, we have been examining to develop new methods

to reveal the electron microscopic localization of GFP and other fluorescent markers without using immuno-electrom microscopy. Another project to establish new techniques to get serial thin sections and to observe with both transmission and scanning electron microscope are in progress.

1. Thin section electron microscopy and immuno-electron microscopy

Thin section electron microscopy is the most widely used technique to observe the inner structure of cells and tissues. In this method, samples are fixed and embedded in epoxy resin, thin sections with about 70nm thickness are cut and observed in the electron microscope. In case of immuno-electron microscopy, thin sections are obtained by similar procedure, and the antigen epitopes exposed on the surface of the sections are marked by sequentially reacted with appropriate primary antibodies and colloidal gold labeled secondary antibodies. This year, thin section electron microscopy combined with immuno-electron microscopy were used in many collaborative works.

a. Ultrastructural analysis of entry and assembly of Herpes Simplex Virus.

We have been performing several studies with research groups in Dr. Kawaguchi[1]'s laboratory: [1]Department of Infectious Disease Control, International Research Center for Infectious Diseases, regarding the infection/replication processes of herpes simplex virus (HSV). This year, two studies were published through this colaboration. In the work regarding the infection process, a viral entry pathway dependent on the paired immunoglobulin-like type 2 receptor (PILR) α was analyzed with electron microscopy. In contrast to the typical way of entry mediated by endocytosis, the entry of the herpes virus into the PILRα transduced chinese hamster ovary cells was revealed to be mediated by virus-cell fusion at the cell surface. This and other results indicated that expression of PILRα produced an alternative HSV-1 entry pathway (ref. Arii[1] *et al*). In another study, electron microscopy was used to figure the replication process of the HSV mutants. We showed that the reguration of Us3 kinase activity by autophosphorylation play a critical role in viral replication (ref. Sagou[1] *et al*).

b. Ultrastructural analysis of tear duct-associated lymphoid tissue (TALT).

In this study, we analyzed the ultrastructure of the TALT epithelium by both transmission electron microscopy and scanning electron microscopy and showed the existence of M cells with typical short microvilli and a lymphocyte including pocket. This and other data showed that TALT shares immunological features with mucosa-asociated lymphoid tissues (MALTs) but has distinct tissue genesis mechanism and plays a key role in ocular immunity (ref. Nagatake[2] *et al*) [2]Division of Mucosal Immunology, Department of Microbiology and Immunology.

Some other collaborative research works using thin section electron microscopy and/or immuno-electron microscopy were done with Dr. Nagatsuma[3] *et al*, in [3]Department of Pathology, The Jikei University School of Medicine (ref. Nagatsuma *et al*), Dr. Masaike[4] *et al*, in [4]Integrated Research Institute, Tokyo Institute of Technology (ref. Masaike *et al*) and Dr. Chida[5] *et al*, in [5]Department of Pathology, Research Institute, Internal Medical Center of Japan. Also, other works are now in progress with Dr. Noda[6] *et al* in [6]Division of Virology, Department of Microbiology and Immunology and with Dr. Terao[7] *et al* in [7]Laboratory Animal Center.

2. Negative staining techniques

Negative staining techniques are simple and quick method to observe the morphology of the macro molecules. This year, the negative staining method were used in collaborative work with Dr. Noda[6] *et al*. In this study, negative staining techniques were used to analyze the roles of influenza viral proteins in nucleocapsid formation.

3. Scanning electron microscopy

Scanning electron microscopy is a technique used to examine the surface structure of the cells, tissues or other non-biological materials. The collaborative works using scanning electron microscopy were done with Dr. Uchida[8] *et al*, [8]Department of Histology, School of life Dentistry, The Nippon Dental University, to analyze the factors those affect the bone regeneration processes (ref Uchida *et al*). Other works are in progress with Dr. Sanada[9] *et al*, [9]Department of Gerontological Nursing, Division of Health Science and Nursing, Graduate School of Medicine, to analyze the effects of diabetes or bacterial infection during wound repair.

Publications

‹Group I›

Niwa T, Saito H, Imajoh-Ohmi S, Kaminishi M, Seto Y, Miki Y, and Nakanishi A. BRCA2 interacts with the cytoskeletal linker protein plectin to form a complex controlling centrosome localization. Cancer Sci, 100: 2115-2125, 2009.

Nakamura M, Morisawa H, Imajoh-Ohmi S, Takamura C, Fukuda H, and Toda T. Proteomic analysis of protein complexes in human SH-SY5Y neuroblastoma cells by using blue-native gel electrophoresis: an increase in lamin A/C associated with heat shock protein 90 in response to 6-hydroxydopamine-induced oxidative stress. Exp Gerontol, 44: 375-382, 2009.

‹Group II›

Oyama M*, Kozuka-Hata H*, Tasaki S, Semba K, Hattori S, Sugano S, Inoue J, and Yamamoto T. (*; equal contribution) Temporal perturbation of tyrosine-phosphoproteome dynamics reveals the system-wide regulatory networks. Mol Cell Proteomics, 8: 226-231, 2009.

Oyama M. Comprehensive description of signal transduction networks by quantitative proteomics and systems biology. Curr Bioinform, 4: 149-153, 2009.

Fukui R, Saitoh S, Matsumoto F, Kozuka-Hata H, Oyama M, Tabeta K, Beutler B, and Miyake K. Unc93B1 biases Toll-like receptor responses to nucleic acid in dendritic cells towards DNA- but against RNA-sensing. J Exp Med, 206: 1339-1350, 2009.

Tsujita K, Itoh T, Kondo A, Oyama M, Kozuka-Hata H, Irino Y, Hasegawa J, and Takenawa T. Proteome of acidic phospholipid-binding proteins: Spatial and temporal regulation of coronin 1A by phosphoinositides. J Biol Chem, 2009 Dec 22. [Epub ahead of print]

Oyama M, Tasaki S, and Kozuka-Hata H. Tyrosine-Phosphoproteome Dynamics In Systems Biology for Signaling Networks (ed. Choi, S), Springer, in press.

尾山大明，秦裕子「リン酸化時系列情報から見えるシグナルダイナミクス」実験医学vol 27，No. 16，2576−2581，2009.

‹Group III›

Nagatsuma K, Hayashi Y, Hano H, Sagara H, Murakami K, Saito M, Masaki T, Lu T, Tanaka M, Enzan H, Aizawa Y, Tajiri H and Matsuura T. Lecithin: retinol acyltransferase protein is distributed in both hepatic stellate cells and endothelial cells of normal rodent and human liver. Liver Int, 29: 47-54, 2009.

Uchida M, Agata H, Sagara H, Shinohara Y, Kagami H and Asahina I. Mixing conditions for cell scaffolds affect the bone formation induced by bone engineering with human bone marrow stromal cells, beta-tricalcium phosphate granules, and rhBMP-2. J Biomed Mater Res A, 91: 84-91, 2009.

Arii J, Uema M, Morimoto T, Sagara H, Akashi H, Ono E, Arase H and Kawaguchi Y. Entry of herpes simplex virus 1 and other alphaherpesviruses via the paired immunoglobulin-like type 2 receptor α. J Virol, 83: 4520-4527, 2009.

Sagou K, Imai T, Sagara H, Uema M and Kawaguchi Y. Regulation of the catalytic activity of herpes simplex virus 1 protein kinase Us3 by autophosphorylation and its role in pathogenesis. J Virol, 83: 5773-5783, 2009.

Nagatake T, Fukuyama S, Kim DY, Goda K, Igarashi O, Sato S, Nochi T, Sagara H, Yokota Y, Jetten AM, Kaisho T, Akira S, Mimuro H, Sasakawa C, Fukui Y, Fujihashi K, Akiyama T, Inoue J, Penninger JM, Kunisawa J and Kiyono H. Id2-, RORgammat-, and LTbetaR-independent initiation of lymphoid organogenesis in ocular immunity. J Exp Med, 206: 2351-2364, 2009.

Masaike Y, Takagi T, Hirota M, Yamada J, Ishihara S, Yung TM, Inoue T, Sawa C, Sagara H, Sakamoto S, Kabe Y, Takahashi Y, Yamaguchi Y and Handa H. Identification of dynamin-2-mediated endocytosis as a new target of osteoporosis drugs, bisphosphonates. Mol Pharmacol, in press.