Department of Human Pathology

Outline and Research Objectives

The Department of Human Pathology was established in 1997, originating from the First Department of Pathology of the Faculty of Medicine. The Department of Human Pathology is specifically dedicated to the research of anatomical and surgical pathology of human diseases.

The Department of Pathology was established in 1887 and has a long history of both anatomical and experimental pathology. Professor Dr. Katsusaburo Yamagiwa (professor: 1905-1923) was the first in the world who succeeded in inducing skin carcinoma on rabbit ears with coal tar in 1908. He was also known to publish a book regarding the carcinogenesis of the stomach. The First and the Second Department Pathology, like as one unit, were engaged in research, diagnosis and teaching. The main research topics of the Department in one hundred years, either anatomical or experimental, varied according to the specialties of its professors and the academic demands of the time in Japan. A brief list of the topics includes clonorchiasis, tsutsugamushi disease, beriberi and salivary gland hormones which were investigated before World War II, and thereafter pathology of atomic bomb injuries, tuberculosis, hepatic disorders, bone marrow pathology and neoplastic diseases of various organs (e.g., stomach, liver, and bone). The experimental aspects of pathology are now succeeded and have been developed by the Department of Molecular Pathology.

The practice of anatomical pathology was a basis of research in the past, but it still constitutes a source of ideas even in the research-oriented pathology. The number of autopsies conducted from 1883 to the present has reached 33,950 and in spite of the current decline in the autopsy rate, one hundred autopsies are still performed each year. Surgical pathology has increasingly become important in the practice of pathology. The laboratory of surgical pathology in the University Hospital, which was established in 1955, became an independent division of the central laboratory of the hospital in 1975. After the integration of the Branch Hospital into the University Hospital in 2001, the numbers of histological and cytological specimens examined each year are expected to be more than 12,000 and 20,000, respectively. The division has been run with the cooperation of the Department of Pathology.

The Department of Human Pathology has succeeded the classical aspects of the Department of Pathology. Its mission is to be the bridge between clinical medicine and basic life sciences. Its research objectives are (1) to discover a new entity of human diseases based on the practice of anatomical and surgical pathology, (2) to clarify disease mechanisms based on morphology, and (3) to function as a "museum" for various forms of human disorders.

Faculties and Students

Professor and Chair	Masashi Fukayama, M.D., D.M.Sc. (1999-)
Associate Professors	Toshiro Niki, M.D., D.M.Sc., Ph.D.
Lecturer	Ja-Mun Chong, M.D., D.M.Sc.
Associate	3
Graduate student	13
Research student	2

Past Research and Major Accomplishments

Our research projects in the past ten years attempt to investigate the role of chronic inflammation in the development and progression of carcinoma. We focused our investigations on the following diseases: (1) Epstein-Barr virus (EBV)-associated neoplasms, such as gastric carcinomas, pyothorax-associated lymphoma (PAL), Hodgkin's disease and AIDS lymphomas, and (2) lung carcinomas in patients with pulmonary fibrosis and lung adenocarcinoma with central scar formation.

1. EBV-associated neoplasms

EBV is the first virus that was identified in a human neoplastic cell in 1963. More than 90% of the world population is infected with EBV before adolescence, and it is thought that certain small populations develop EBV-associated malignancy in an endemic manner, such as Burkitt's lymphoma in equatorial Africa and nasopharyngeal carcinoma in Southern China. However, recent advances in molecular biological techniques have

demonstrated that various neoplasms in the general population are associated with EBV infection.

1-1) EBV-associated gastric carcinoma

EBV-associated gastric carcinoma is now considered to be the most common among EBV-associated neoplasms, and its frequency is 10% or less of gastric carcinomas in Japan. We found that EBV-associated gastric carcinoma is a distinct subgroup of gastric carcinoma, and that it develops against a background of severe atrophic gastritis. In our evaluation of genetic abnormalities, EBV-associated gastric carcinoma was found to be remarkable in its paucity of loss of heterozygosity (LOH) and microsatellite instability.

Since it is difficult to maintain epithelial cells *in vitro* in the latent form of EBV infection, we established one strain of EBV-associated gastric carcinoma transplantable to severe combined immunodeficiency (SCID) mice, which faithfully maintains the original EBV and the expression of EBV-related genes. Applying the DNA-chip analysis to the gastric carcinoma strains with or without EBV-infection in SCID mice, we clarified the expression profile specific to EBV-associated gastric carcinoma, and found that the expression of IL1-β is markedly up-regulated in this type of carcinoma. Of interest is the observation that IL1- β facilitates the growth of a gastric cancer cell line, suggesting that it is an autocrine growth factor in EBV-associated gastric carcinoma.

1-2) EBV-associated lymphomas

PAL develops within the pleural cavity of patients who had a 20-50-year history of pyothorax following tuberculous pleuritis or an artificial pneumothorax for the treatment of pulmonary tuberculosis. Although the reason is not clear at present, most patients of PAL are Japanese. We discovered in 1993 that EBV is causally associated with PAL and that PAL shows the same expression pattern of EBV latency genes as that of AIDS lymphomas.

2. Lung carcinoma

Lung cancer is the major cause of cancer-related deaths among Japanese. Since one of our specialties is the pathology of lung and thymus, two issues are under investigation, that is, the relationship between pulmonary fibrosis and development of lung cancer, and the role of fibrosis in the progression of adenocarcinoma.

2-1) Idiopathic pulmonary fibrosis and lung carcinoma

We evaluated the frequency of lung cancer among the patients with idiopathic pulmonary fibrosis (IPF) by performing autopsy-based case control study. Then, we morphologically evaluated the atypical epithelial proliferation in the honeycombed area. Although squamous metaplasia is much more frequent in the honeycombed area of IPF patients with lung carcinoma, we confirmed the necessity to evaluate the genetic abnormalities in minute foci of atypical epithelial cells in tissue sections.

2-2) Small adenocarcinoma

Among nonsmall cell carcinomas of the lung, the prognosis of adenocarcinoma is relatively poor. One-third of the patients with stage I adenocarcinoma die of the disease within five years after surgery, and the 5-year recurrence rate is as high as 20% for adenocarcinomas of less than 2 cm in diameter. In our study of small adenocarcinoma, inactivation of p16 may play a role in accelerating scar formation and lymph node metastasis, and through these mechanisms p16-inactivation may contribute to a poor prognosis of the patients.

Current Research

1. EBV-associated neoplasms

1-1) Global methylaion in EBV-associated gastric carcinoma

Recently we discovered the phenomenon of global and nonrandom methylation of the promoter region of cancer-associated genes in EBV-associated gastric carcinoma. Such a global methylation was not observed in EBV-negative carcinomas. One of the important roles of DNA methylation is to act as a defense against extrinsic pathogens. The latent infection of viruses in either episomal (EBV), or integrated form (human immunodeficiency virus type 1 or human T-cell leukemia-virus type-1), is restricted by methylation of viral genomes. Thus, we are now investigating a possible mechanism; that is, overridden methylation of cellular genes suppresses exprssion of important genes of EBV-infected epithelial cells, leading to the development of carcinoma.

1-2) EBV-associated lymphomas and human herpes virus 8

Human herpes virus 8 (HHV8) was first discovered in tissues of Kaposi sarcoma in 1994, and is associated with primary effusion lymphoma and multicentric Castleman's disease. However, its epidemiology in lymphoproliferative disorders and its relation to EBV have not been fully clarified. We are now investigating the molecular epidemiology of HHV8-associated diseases in Japan and Asian countries.

2. Lung carcinoma

2-1) Laser capture microscopy-assisted analysis of precursor lesions

Laser capture microscopy (LCM) is now widely applied to analysis of precursor lesions of various carcinomas. We are also applying it to the evaluation of epithelial cells of honeycombed areas in the diseased lungs of IPF patients, and atypical epithelial cells of adenomatous hyperplasia, a precursor of bronchioloalveolar carcinoma of the lung. Nuclei of target cells directly obtained from paraffin-embedded sections are subjected to PCR-based analysis.

Invasion-associated gene expression: Niki et al demonstrated the colocalization of cox-2 and laminin-5 at the invasive front of early-stage lung adenocarcinomas. Current data suggest that p53 abnormalities and EGFR signaling are involved in the aberrant expression of these proteins. Coregulation and interaction of these molecules at the invasive front of cancer may facilitate tumor angiogenesis and invasion in a coordinated manner. Several on-going projects investigate the expression of invasion-associated proteins in lung adenocarcinoma, including S100-related proteins. We are also developing a technique that enables us to apply DNA-chip analysis to tiny tissues of the invasive front using the LCM-assisted sampling.

2-2) Morphological analysis of lung carcinoma and thymic tumors

It is still necessary to review the histology of these neoplasms in order to develop an evaluation system for invasiveness and to identify the specific subgroups of neoplasms.

Future Prospects

There are two opposite directions that pathology is expected to pursue at present and in the future. One is to be included among the fields of life science and cell biology. The other is to remain a basic field for clinical medicine, which in its essence is a good partner and critic of clinical medicine. To keep up with the growing demand for an intimate relationship with clinical medicine, we have decided to combine our department with the Division of Surgical Pathology of the University Hospital. The union is expected to facilitate cross talks between pathology and clinical medicine, which will lead us to our goal, namely, the discovery of a new entity of human diseases and the clarification of their mechanisms based on morphology.

We will also be obligated to maintain the museum function of human pathology. Since we have archives of paraffin blocks, including more than 20,000 autopsy specimens that were accumulated in these past 100 years, a new molecular approach will clarify changes of disease profiles in Japan in the 20th century.

Research Grants

1. 1997-1999: Grant in Aid for Scientific Research (B) from the Ministry of Education, Science, Sports and Culture of Japan. "Pulmonary fibrosis and development of lung carcinoma. Analysis of dysplasic and neoplastic epithelial cells of pulmonary fibrosis."
2. 1999-2002: Grant in Aid for Scientific Research (B) from the Ministry of Education, Science, Sports and Culture of Japan. "Molecular epidemiology of human herpes virus 8-associated diseases in Japan and Asia."
3. 2001: Grant in Aid for Scientific Research on a Priority Area (C) from the Ministry of Education, Science, Sports and Culture of Japan. " Establishment of gene diagnosis system for lung adenocarcinoma."
4. 2002: Grant in Aid for Scientific Research on a Priority Area (C) from the Ministry of Education, Culture, Sports, Science and Technology of Japan. " Establishment of gene diagnosis system for lung adenocarcinoma."
5. 2002-2004: Grant in Aid for Scientific Research (A) from the Ministry of Education, Culture, Sports, Science and Technology of Japan. "Pathogenesis of Epstein-Barr virus-associated neoplasms."

Select Publications

1. Fukayama M, Hayashi Y, Takizawa t, Koike M, Endo S and Okumura H. Glycoprotein hormone alpha-subunit in human stomach. J Histochem Cytochem 34, 995-1001, 1986.
2. Fukayama M, Hayashi Y, Koike M, Hajikano H and Okumura H. Human chorionic gonadotropin in lung and lung tumors. Immunohistochemical study on unbalanced distribution of subunits. Lab Invest 55, 433-443, 1986.
3. Fukayama M, Hayashi Y and Koike M. Human chorionic gonadotropin in gastric carcinoma. An immunohistochemical study suggesting independent regulation of subunits. Virchows Arch (Pathol Anat) 411, 205-212, 1987
4. Fukayama M and Koike M. So-called sclerosing hemangioma of the lung. An immunohistochemical, histochemical and ultrastructural study. Acta Pathol Jpn 38, 627-642, 1988.
5. Fukayama M, Maeda Y, Funata N, Koike M, Saito K, Sakai T and Ikeda T. Pulmonary and pleural thymoma. Diagnostic application of lymphocyte markers to the thymoma of unusual site. Am J Clin Pathol 89, 617-621, 1988.
6. Fukayama M, Funata N, Hayashi Y, Maeda Y, Koike M, Wantanabe J and Okabe T: Brain associated small-cell lung cancer antigen (BASCA) in the lung and lung tumor. Immunohistochemical and immunoelectron microscopic study. J Histochem Cytochem 38, 51-57, 1990.

7. Fukayama M, Hayashi Y, Shiozawa Y, Furukawa E, Funata N and Koike M. Human chorionic gonadotropin α -subunit in endocrine cells of fibrotic and neoplastic lung. Its mode of localization and the size profile of granules. Lab Invest 62, 444-451, 1990.
8. Hayashi Y, Watanabe J, Nakata K, Fukayama M and Ikeda H. A novel diagnostic method of pneumocystis carinii. In situ Hybridization of ribosomal ribonucleic acid with biotinylated oligonucleotide probes. Lab Invest 63, 576-580, 1990.
9. Nakata K, Akagawa KS, Fukayama M, Hayashi Y, Kadokura M and Tokunaga T: Granulocyte-macrophage colony-stimulating factor promotes the proliferation of human alveolar macrophages in vitro. J Immunol 147, 1266-1272, 1991.
10. Fukayama M, Kanno T, Brody S, Kirby M and Crystal RG. Respiratory tract gene transfer. Transplantation of genetically modified T-lymphocytes directly to the respiratory surface. J Biol Chem 266, 18339-1844, 1991
11. Rosenfeld MA, Siegfried W, Yoshimura K, Yoneyama K, Fukayama M, Stier LE, Paakko PK, Gilardi P, Stratford-Perricaudet LD, Perricaudet M, Jallat S, Pavirani A, Lecocq J and Crystal RG. Adenovirus-mediated transfer of a recombinant a1-antitrypsin gene to the lung epithelium in vivo. Sience 252, 431-252, 1991.
12. Rosenfeld MA, Yoshimura K, Trapnell BC, Yoneyama K, Rosenthal ER, Dalemans W, Fukayama M, Bargon J, Stier LE, Stratford-Perricaudet L, Perricaudet M, Guggino WB, Pavirani A, Lecocq J and Crystal RG. In vivo transfer of the human cystic fibrosis transmembrane conductance regulator gene to the airway epithelium. Cell 68, 143-155, 1992.
13. Kikuchi-Yanoshita R, Konishi M, Ito S, Seki M, Tanaka K, Maeda Y, Iino H, Fukayama M, Koike M, Mori T, Sakuraba H, Fukunari H, Iwama T and Miyaki M. Genetic changes of both p53 alleles associated with the conversion from colorectal adenoma to early carcinoma in familial adenomatous polyposis and non-familial adenomatous polyposis patients. Cancer Res 52, 3965-3971, 1992.
14. Fukayama M, Ibuka T, Hayashi Y, Ooba T, Koike M and Mizutani S. Epstein- Barr virus in pyothorax-associated pleural lymphoma. Am J Pathol 143, 1044-1049, 1993.
15. Hayashi Y, Fukayama M, Koike M, Kaseda S, Ikeda T and Yokoyama T. Cell-cycle analysis detecting nuclear antigens: Comparison with BrdU-in vivo labeling and application to lung tumors. Acta Pathol Jpn 43, 313-319, 1993.
16. Fukayama M, Hayashi Y, Iwasaki Y, Chong J, Ooba T, Takizawa T, Koike M, Mizutani S, Miyaki M and Hirai K. Epstein-Barr virus (EBV)-associated gastric carcinoma and EBV-infection of the stomach. Lab Invest 71, 73-81, 1994.
17. Ibuka T, Fukayama M, Hayashi Y, Funata N, Koike M, Ikeda T and Mizutani S. Pyothorax-associated pleural lymphoma: A case evolving from T-cell rich lymphoid infiltration to overt B-cell lymphoma in association with Epstein-Barr virus. Cancer 73, 738-743, 1994.
18. Iino H, Fukayama M, Maeda Y, Koike M, Mori T, Takahashi T, Kikuchi -Yanoshita R, Miyaki M, Mizuno S and Watanabe S. Molecular genetics for clinical management of colorectal carcinoma. 17p, 18q, and 22q loss of heterozygosity and decreased DCC expression are correlated with the metastatic potential. Cancer 73,1324-1331, 1994.
19. Miyaki M, Konishi M, Kikuchi-Yanoshita R, Enomoto M, Igari T, Tanaka K, Muraoka M, Takahashi H, Amada Y, Fukayama M, Maeda M, Iwama T, Mishima Y and Koike M. Characteristics of somatic mutation of the adenomatous polyposis coli gene in colorectal tumors. Cancer Res 54, 3011-3020, 1994.
20. Hishima T, Fukayama M, Fujisawa M, Hayashi Y, Arai K, Funata N, Koike M: CD5 expression in thymic carcinoma. Am J Pathol 145:268-1994.
21. Chong J, Fukayama M, Hayashi Y, Takizawa T, Koike M, Konishi M, Kikuchi-Yanoshita R, Miyaki M: Microsatellite instability in the progression of gastric carcinoma. Cancer Res, 54:4595-4597, 1994.
22. Fukayama M, Hayashi Y, Ooba T, Funata N, Ibuka T, Koike M, Hebisawa A, Kurasawa A, Fukayama M, Nakahiro K and Kudoh S. Pyothorax-associated lymphoma: development of Epstein-Barr virus-associated lymphoma within the inflammatory cavity. Pathol Int 45, 825-831, 1995
23. Egawa N, Fukayama M, Kawaguchi K, Hishima T, Hayashi Y, Funata N, Ibuka T, Koike M, Miyashita H and Tajima T. Relapsing oral and colonic ulcers with monoclonal T-cell infiltration. A low grade mucosal T-lymphoproliferative disease of the digestive tract. Cancer 75, 1728-1733, 1995.
24. Shimada-Hiratuka M, Fukayama M, Hayashi Y, Ushijima T, Suzuki M, Hishima T, Funata N and Koike M, Watanabe T. Primary gastric T-cell lymphoma with and without human T-lymphotropic virus type 1. Cancer 80, 292-303, 1997.
25. Harada M, Suzuki M, Ikeda T, Kaneko T, Harada S and Fukayama M. Clonality in nevocellular nevus and malignant melanoma. An expression-based clonality analysis at the X-linked genes by polymerase chain reaction. J Invest Dermatol 109, 656-660, 1997.
26. Chong J-M, Fukayama M, Hayashi Y, Funata N, Takizawa T, Koike M, Kikuchi-Yanoshita R, Miyaki M and Mizuno S. CD44 variants in gastric carcinoma in association with or without Epstein-Barr virus. Int J Cancer 74:450-454, 1997
27. Chong J, Fukayama M, Hayashi Y, Hishima T, Funata N, Koike M, Matsuya S, Konishi M and Miyaki M. Microsatellite Instability and loss of heterozygosity in gastric lymphoma. Lab Invest 77, 639-645, 1997.
28. Hishima T, Fukayama M, Hayashi Y, Fujii T, Arai K, Shiozawa Y, Funata N and Koike M: Neuroendocrine differentiation in thymic epithelial tumors with special reference to thymic carcinoma and atypical thymoma. Hum Pathol 29, 330-338, 1998.

29. Iwasaki Y, Chong J-M, Hayashi Y, Ikeno R, Arai K, Kitamura M, Koike M, Hirai K and Fukayama M: Establishment and characterization of a human Epstein-Barr virus-associated gastric carcinoma in SCID mice. J Virol 72, 8321-8326, 1998.
30. Sakurai S, Fukayama M, Kaizaki Y, Saito K, Kanazawa K, Kitamura M, Iwasaki Y, Hishima T, Hayashi Y and Koike M: Telomerase Activity in Gastrointestinal Stromal Tumor (GIST). Cancer 83, 2060-2066, 1998.
31. Fukayama M, Chong JM and Kaizaki Y. Epstein-Barr virus and gastric carcinoma. Gastric Cancer 1, 104-114, 1998.
32. Ishida T, Takashima R, Fukayama M, Hamada C, Hippo Y, Fujii T, Moriyama S, Matsuba C, Nakahori Y, Morita H, Yazaki Y, Kodama T, Nishimura S and Aburatani H. New DNA polymorphisms of human MMH/OGG1 gene: prevalence of one polymorphism among lung- adenocarcinoma patients in Japanese. Int J Cancer 80, 18-21, 1999.
33. Sakurai S, Fukasawa T, Chong JM and Fukayama M. Embryonic Form of Smooth Muscle Myosin Heavy Chain (SMemb/MHC-B) in Gastrointestinal Stromal Tumor and Interstitial Cells of Cajal. Am J Pathol 154, 23-28, 1999.
34. Kawabuchi B, Moriyama S, Hironaka M, Fujii T, Koike M, Moriyama H, Nishimura Y, Mizuno S and Fukayama M. p16 inactivation in small sized lung adenocarcinoma: its association of poor prognosis. Int J Cancer 84, 49-53, 1999.
35. Hayashi Y, Fukayama M, Funata N, Hishima T, Oba T and Koike M. Polymerase chain reaction-screening of immunoglobulin heavy chain and T-cell receptor g gene rearrangements. A practical approach tomolecular DNA analysis of non-Hodgkin's lymphoma in a surgical pathology laboratory. Pathol Int 49, 110-117, 1999.
36. Kaizaki Y, Sakurai S, Chong JM and Fukayama M: Atrophic gatritis, Epstein-Barr virus infection, and Epstein-Barr virus-associated gastric carcinoma. Gastric Cancer 2, 101-108, 1999.
37. Koshiishi N, Chong JM, Fukasawa T, Ikeno R, Tanaka A, Kanazawa K, Ogura M, Ebuchi M, Takizawa T, Funata N and Fukayama M. Microsatellite instability and loss of heterozygosity in primary and secondary proliferative lesions of the parathyroid gland. Lab Invest 79, 1051-1058, 1999.
38. Sakurai S, Fukasawa T, Chong JM, Tanaka A and Fukayama M. C-*kit* gene abnormalities in gastrointestinal stromal tumors (tumors of interstitial cell of Cajal). Jpn J Cancer Res 90, 1321-1328, 1999.
39. Hironaka M and Fukayama M. Pulmonary fibrosis and lung carcinoma: A comparative study of metaplastic epithelia in honeycombed areas of usual interstitial pneumonia with or without lung carcinoma. Pathol Int 49, 1060-1066, 1999.
40. Hishima T, Fukayama M, Hayashi Y, Fujii T, Ooba T, Funata N and Koike M. CD70 in thymic carcinoma. Am J Surg Pathol 25, 742-746, 2000.
41. Kurihara K, Shimizu S, Chong JM, Hishima T, Funata N, Kashiwagi H, Nagai H, Miyaki M and Fukayama M. Nuclear localization of immunorective b-catenin is specific to familial adenomatous polyposis in papillary thyroid carcinoma. Jpn J Cancer Res 91, 1100-1102, 2000.
42. Fukasawa T, Chong JM, Sakurai S, Koshiishi N, Ikeno R, Tanaka A, Matsumoto Y, Hayashi Y, Koike M and Fukayama M. Allelic loss of 14q, and 22q, *NF2* mutation, and genetic instability occur independently of c-kit mutation in gastrointestinal stromal tumor. Jpn J Cancer Res 91, 1241-1249, 2000.
43. Fukayama M, Chong JM and Uozaki H. Pathology and molecular pathology of Epstein-Barr virus-associated gastric carcinoma. Curr Top Microbiol Immunol 258, 91-102, 2001.
44. Takeuchi K, Morishita Y, Fukayama M and Mori S. Marked decrease in the Epstein-Barr virus positivity rate in nodular sclerosis subtype Hodgkin's disease in Tokyo: trend between 1955 and 1999. Br J Haematol 113, 429-431, 2001.
45. Sakurai S, Oguni S, Hironaka M, Fukayama M, Morinaga S and Saito K. Mutations in c-kit gene exons 9 and 13 in gastrointestinal stromal tumors among Japanese. Jpn J Cancer Res 92, 494-498, 2001.
46. Hippo Y, Taniguchi H, Tsutsumi S, Machida N, Chong JM, Fukayama M, Kodama T and Aburatani H. Global gene expression analysis of gastric cancer by oligonucleotide microarrays. Cancer Res, 62, 233-240, 2002.
47. Niki T, Kohno T, Iba S, Moriya Y, Takahashi Y, Saito M, Maeshima A, YamadaT, Matsuno Y, Fukayama M, Yokota J and Hirohashi S. Frequent Co-Localization of Cox-2 and Laminin-5 gamma2 Chain at the Invasive Front of Early-Stage Lung Adenocarcinomas. Am J Pathol, 160, 1129-1141, 2002.
48. Chong JM, Sakuma K, Sudo M, Osawa T, Ohara E, Uozaki H, Shibahara J, Kuroiwa K, Tominaga S, Hippo Y, Aburatani H, Funata N and Fukayama M. Interleukin-1beta expression in human gastric carcinoma with Epstein-Barr virus infection. J Virol 76, 6825-6831, 2002.
49. Kakiuchi C, Ishida T, Sato H, Katano H, Ishiko T, Mukai H, Kogi M, Kasuga N, Takeuchi K, Yamane K, Fukayama M and Mori S. Secretion of interleukin-6 and vascular endothelial growth factor by spindle cell sarcoma complicating Castleman's disease (so-called 'vascular neoplasia'). J Pathol 197, 264-271, 2002.
50. Osawa T, Chong J-M, Sudo M, Sakuma K, Uozaki H, Shibahara J, Nagai H, Funata N and Fukayama M. Reduced Expression and Promotor Mehtylation of p16 Gene in Epstein-Barr Virus-associated Gastric Carcinoma. Jpn J Cancer Res in press.

Department of Molecular Pathology

Outline and Research Objectives

Our department has a more than 100-year history from its establishment as the Department of Pathology. The Second Department of Pathology has changed its name to the Department of Molecular Pathology; after the retirement of Dr. Takatoshi Ishikawa, Dr. Miyazono began to work as the Professor of the Department.

Our research is focused on molecular mechanisms of carcinogenesis, using molecular biological techniques. Transforming growth factor-b (TGF-β) is a potent growth inhibitor for many cells, and perturbations of TGF-b signaling result in progression of various cancers. We are interested in the signaling mechanisms of the TGF-β superfamily, including TGF-β and bone morphogenetic proteins (BMPs), and studying how TGF-β signals regulate progression of various cancers, e.g. colorectal cancer.

We are also interested in the pathogenesis of vascular diseases. Signals induced by TGF-β and BMPs play important roles in the development and homeostasis of blood vessels. Abnormalities in TGF-β/BMP signals result in genesis of certain vascular disorders. We have recently started to investigate the mechanisms of growth and differentiation of murine embryonic stem (ES) cells. Using an in vitro system, we are trying to regulate the differentiation of ES cells into smooth muscle cells (SMCs) and endothelial cells (ECs). The results will be useful for the development of new strategies for treatment of cancers and vascular diseases.

Faculty and Students

Professor and Chair	Kohei Miyazono, M.D., D.M.S. (from 2000)
Associate Professors	Keiji Miyazawa, Ph.D.
Associates	2
Postdoctoral Fellow	1
Research Technicians	4
Ph.D. Students	2
Master Course Students	3
Visiting Researcher	1
Visiting Ph.D. Students	7
Secretaries	2

Past Research and Major Accomplishments

Members of the TGF-β superfamily bind to two different types of serine/threonine kinase receptors, and activate intracellular signaling molecules, the Smads. Smads move into the nucleus and regulate transcription of target genes, including c-myc and cyclin-dependent kinase inhibitors. Signaling by Smads is regulated by various molecules, including inhibitory Smads (I-Smads) and transcriptional co-repressors, c-Ski and SnoN (Figure 1).

We isolated cDNAs for TGF-β type I receptor (TbR-I, also called ALK-5) in 1993 [3]. In addition, we have cloned five additional type I receptors, which turned out to be the type I receptors for BMPs and

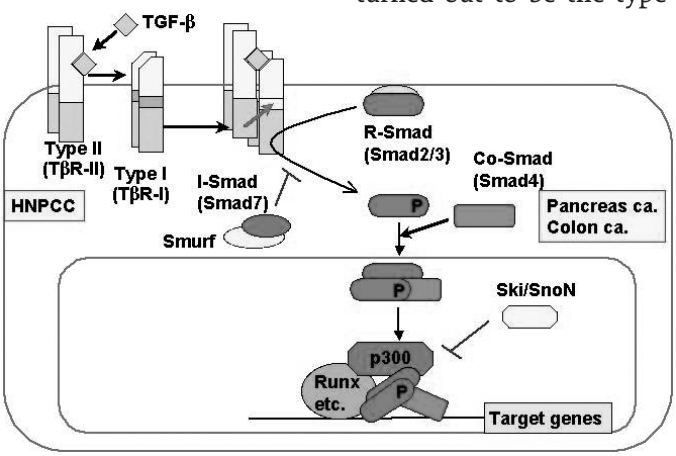

Figure 1. Signaling by TGF-β through serine/threonine kinase receptors and Smads

activins [6], and the type II receptor for BMPs (BMPR-II) [11]. Using these receptors, we demonstrated how the members of the TGF-β superfamily bind to different combinations of type II and type I receptors.

During our studies of intracellular signaling activated by the serine/threonine kinase receptors, we have cloned one of the mammalian I-Smads, Smad6 [17], and reported that Smads can be classified into three subclasses, i.e. R-Smads, Co-Smads, and I-Smads [18]. Moreover, we demonstrated how I-Smads inhibit signaling activities of the TGF-β superfamily cytokines [27, 29, 44]. We have shown that E3 ubiquitin ligases, Smurfs, interact with I-Smads, recruit them from the nucleus to the cell surface receptor complexes, and induce ubiquitination and degradation of the type I receptors [40, 48]. These findings suggested that Smurfs support the inhibitory activity of I-Smads in TGF-β superfamily signaling.

We have also investigated the mechanisms by which Smads regulate transcription of target genes of the TGF-β superfamily cytokines. We have shown that Smads interact with various transcription factors, including the vitamin D3 receptor and Runx proteins (also known as Cbfa/AML/PEBP2α proteins) [25, 30, 37], which play important roles for TGF-β superfamily cytokines in exhibiting various biological effects in different types of cells. In addition, we demonstrated that Smads interact with transcriptional co-activators, p300 and CBP, and transcriptional co-repressors, c-Ski and SnoN [26, 31]. c-Ski was originally identified as an oncogene. Our findings suggest that c-Ski may regulate growth and differentiation of various cells through regulating the actions of Smads.

We are also studying vascular diseases induced by perturbation of the TGF-β/BMP signaling systems. Hereditary hemorrhagic telangiectasias (HHTs) are induced by mutations of the human *endoglin* or *ALK-1* genes. *ALK-1*-null mice exhibit vascular abnormalities reminiscent of human HHT [34]. Primary pulmonary hypertension (PPH) is induced by mutations of BMPR-II; however, we have shown that mutations occur in various portions of the BMPR-II in PPH patients, which may contribute in different fashions to the pathogenesis of this disease [47]. Using adenovirus vectors containing constitutively active forms of TGF-β type I receptor (ALK-5) or ALK-1, we have identified target genes induced by these receptors. These findings will become useful for our future studies designed to elucidate the mechanisms of pathogenesis of vascular diseases induced by abnormalities of TGF-β and BMP signaling.

Current Research

Our current research activities are focused on two major projects. First, we are trying to elucidate the signaling mechanisms of the TGF-β superfamily through Smad proteins, and to modulate TGF-β signaling by various molecules. Many tumor cells are resistant to the growth inhibitory activity of TGF-β. Thus, perturbations of TGF-β superfamily signaling result in progression of various cancers, including hereditary non-polyposis colorectal cancer (HNPCC) and pancreatic cancer (Figure 1). However, the molecular mechanisms of the resistance to effects of TGF-β are still not fully understood. We have recently shown that cis-compound disruption of murine *Smad2* gene accelerates malignant progression of intestinal tumors in *Apc* knockout mice [49]. Moreover, we have shown that TGF-β regulates transcription of c-myc through Smads [45], and we are currently studying the molecules that regulate c-myc transcription together with Smads, including the TCF-4/Lef-1 family of transcription factors.

We have shown that I-Smads and c-Ski/SnoN negatively regulate TGF-β signaling. Based on their three-dimensional structures, we are trying to elucidate how these inhibitory molecules interact with the TGF-β receptors or Smads. We are also trying to identify novel molecules that regulate TGF-β/BMP signaling by yeast two-hybrid systems and DNA microarray analyses. Experiments using *Xenopus* embryos which

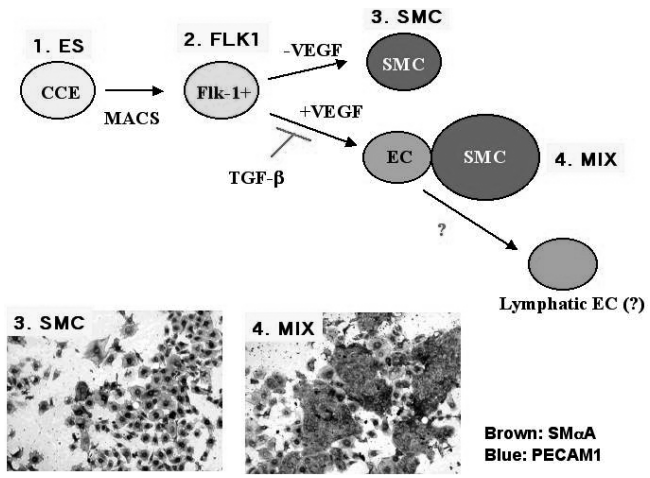

Figure 2. Differentiation of ES cells into ECs and SMCs in vitro.

we have recently started in our laboratory will be useful for examining the biological activities regulated by these molecules. Although perturbations of TGF-β signaling result in malignant progression of tumors, tumor cells by themselves produce large amounts of TGF-β, which induce progression of tumors through induction of neovascularization, inhibition of immune function, and accumulation of extracellular matrices. We have recently found that adenoviruses containing Smad7 and c-Ski prevented metastasis of breast tumors in nude mice. Thus, molecules that negatively regulate TGF-β signaling may be useful for regulation of progression and/or metastasis of certain cancers.

Our second project is to study the growth and differentiation of murine ES cells. Formation of new blood vessels involves the proliferation and differentiation of endothelial progenitor cells (EPCs) circulating in peripheral blood. We are studying whether TGF-β superfamily signals play roles during the differentiation of EPCs into endothelial cells (ECs) and smooth muscle cells (SMCs) using an *in vitro* ES cell system (Figure 2). Our preliminary findings suggest that the balance of two major signaling pathways of the TGF-β superfamily cytokines, i.e. TGF-β-like signals and BMP-like signals, plays important roles in the differentiation of EPCs into ECs and SMCs. We also found that TGF-β regulates differentiation and formation of tight junction of ECs. Moreover, we have obtained some evidence that certain fractions of ECs express specific markers for lymphatic ECs, suggesting that lymphangiogenesis may be induced from EPCs.

Future Prospects

Our future goals will include further elucidation of signaling mechanisms of the TGF-β superfamily cytokines. Inhibition of TGF-β signaling by I-Smads or transcriptional co-repressors appears to be a powerful method of preventing tissue fibrosis and cancer metastasis. Development of molecules that regulate TGF-β signaling pathways may thus be useful for treatment of various clinical disorders. We will also extend our research to generate cells of different lineages from murine ES cells. Our knowledge of TGF-β and other cytokine signaling pathways will be useful in these projects. Generation of ECs and SMCs from ES cells will be valuable for treatment of various vascular diseases. Moreover, generation of lymphatic ECs will be useful for identification of genes specifically expressed in lymphatic tissues. These studies will thus be beneficial not only for application of these cells in the field of regenerative medicine, but also for development of new strategies for diagnosis and treatment of metastatic tumors.

Research Grants

1. Research for the Future Program: Study on the Signal Transduction by Serine-Threonine Kinase Receptors (1996-2000) Kohei Miyazono (284,485,000 JPY) Supported by the Japan Society for the Promotion of Science.
2. Grant-in-Aid for Scientific Research on Priority Areas (Advanced Research on Cancer): Signaling Mechanisms of TGF-β and Their Abnormalities in Human Cancer (2000-2004) Kohei Miyazono (224,270,000 JPY in 2000-2002) Supported by the Ministry of Education, Culture, Sports, Science, and Technology of Japan.
3. Grant-in-Aid for Scientific Research (A): Study on the Biological Activities of Serine-Threonine Kinase Receptors (2002-2005) Kohei Miyazono (40,900,000 JPY) Supported by the Japan Society for the Promotion of Science.
4 Grant-in-Aid for Scientific Research (B)(2): Signal Transduction by Smad Proteins and Their Biological Activities (1999-2001) Kohei Miyazono (14,900,000 JPY) Supported by the Ministry of Education, Culture, Sports, Science, and Technology of Japan and the Japan Society for the Promotion of Science.
5. Grant-in-Aid for Scientific Research (B) (2): Signal Transduction and Biological Activities of the Members of the TGF-β Superfamily (1997-1998) Kohei Miyazono (15,100,000 JPY) Supported by the Ministry of Education, Culture, Sports, Science, and Technology of Japan.

Select Publications (* indicates 10 select Publications, for which copies are attached)

1. Miyazono, K., Olofsson, A., Colosetti, P., and Heldin, C.-H. A role of the latent TGF-b1-binding protein in the assembly and secretion of TGF-b1. *EMBO J.* 10, 1091-1101, 1991.
2. ten Dijke, P., Ichijo, H., Franzén, P., Schulz, P., Saras, J., Toyoshima, H., Heldin, C.-H., and Miyazono, K. Activin receptor-like kinases: A novel subclass of cell surface receptors with predicted serine/threonine kinase activity. *Oncogene* 8, 2879-2887, 1993.

3.* Franzén, P., ten Dijke, P., Ichijo, H., Yamashita, H., Schulz, P., Heldin, C.-H., and Miyazono, K. Cloning of a TGFb type I receptor that forms a heteromeric complex with the TGFb type II receptor. *Cell* 75, 781-792, 1993.

4. Yamashita, H., Ichijo, H., Grimsby, S., Morén, A., ten Dijke, P., and Miyazono, K. Endoglin forms a heteromeric complex with the signaling receptors for transforming growth factor-b. *J. Biol. Chem.* 269, 1995-2001, 1994.
5. Taipale, J., Miyazono, K., Heldin, C.-H., and Keski-Oja, J. Latent transforming growth factor-b1 associates to fibroblast extracellular matrix via latent TGF-β binding protein. *J. Cell Biol.* 124, 171-181, 1994.

6.* ten Dijke, P., Yamashita, H., Ichijo, H., Franzén, P., Laiho, M., Miyazono, K., and Heldin, C.-H.

Characterization of type I receptors for transfoming growth factor-b and activin. *Science* 264, 101-104, 1994.

7. ten Dijke, P., Yamashita, H., Sampath, T.K., Reddi, A.H., Estevez, M., Riddle, D.L., Ichijo, H., Heldin, C.-H., and Miyazono, K. Identification of type I receptors for osteogenic protein-1 and bone morphogenetic protein-4. *J. Biol. Chem.* 269, 16985-16988, 1994.
8. Okadome, T., Yamashita, H., Franzén, P., Morén, A., Heldin, C.-H., and Miyazono, K. Distinct roles of the intracellular domains of transforming growth factor-b type I and type II receptors in signal transduction. *J. Biol. Chem.* 269, 30753-30756, 1994.
9. Yamashita, H., Okadome, T., Franzén, P., ten Dijke, P., Heldin, C.-H., and Miyazono, K. A rat pituitary tumor cell line (GH3) expresses type I and type II receptors and other cell surface binding protein(s) for transforming growth factor-b. *J. Biol. Chem.* 270, 770-774, 1995.
10. * Yamashita, H., ten Dijke, P., Huylebroeck, D., Sampath, T.K., Andries, M., Smith, J.C., Heldin, C.-H., and Miyazono, K. Osteogenic protein-1 binds to activin type II receptors and induces certain activin-like effects. *J. Cell Biol.* 130, 217-226, 1995.
11. Rosenzweig, B.L., Imamura, T., Okadome, T., Cox, G.N., Yamashita, H., ten Dijke, P., Heldin, C.-H., and Miyazono, K. Cloning and characterization of a human type II receptor for bone morphogenetic proteins. *Proc. Natl. Acad. Sci. USA* 92, 7632-7636, 1995.
12. Nishitoh, H., Ichijo, H., Kimura, M., Matsumoto, T., Makishima, F., Yamaguchi, A., Yamashita, H., Enomoto, S., and Miyazono, K. Identification of type I and type II serine/threonine kinase receptors for growth/differentiation factor-5. *J. Biol. Chem.* 271, 21345-21352, 1996.
13. Souchelnytskyi, S., ten Dijke, P., Miyazono, K., and Heldin, C.-H. Phosphorylation of Ser165 in TGF-β type I receptor modulates TGF-b1-induced cellular responses. *EMBO J.* 15, 6231-6240, 1996.
14. * Ichijo, H., Nishida, E., Irie, K., ten Dijke, P., Saitoh, M., Moriguchi, T., Takagi, M., Matsumoto, K., Miyazono, K., and Gotoh, Y. Induction of apoptosis by ASK1, a mammalian MAPKKK that activates SAPK/JNK and p38 signaling pathways. *Science* 275, 90-94, 1997.
15. Nakajima, Y., Miyazono, K., Kato, M., Takase, M., Yamagishi, T., and Nakamura, H.Extracellular fibrillar structure of latent TGFb binding protein-1: Role in TGFb-dependent endothelial-mesenchymal transformation during endocardial cushion tissue formation in mouse embryonic heart. *J. Cell Biol.* 136, 193-204, 1997.
16. Nakao, A., Imamura, T., Souchelnytskyi, S., Kawabata, M., Ishisaki, A., Oeda, E., Tamaki, K., Hanai, J.-i., Heldin, C.-H., Miyazono, K., and ten Dijke, P. TGF-β receptor-mediated signalling through Smad2, Smad3 and Smad4. *EMBO J.* 16, 5353-5362, 1997.
17. * Imamura, T., Takase, M., Nishihara, A., Oeda, E., Hanai, J.-i., Kawabata, M. and Miyazono, K. Smad6 inhibits signalling by the TGF-β superfamily. *Nature* 389, 622-626, 1997.
18. * Heldin, C.-H., Miyazono, K., and ten Dijke, P. TGF-β signalling from cell membrane to nucleus through Smad proteins. *Nature* 390, 465-471, 1997.
19. Saitoh, M., Nishitoh, H., Fujii, M., Takeda, K., Tobiume, K., Sawada, Y., Kawabata, M., Miyazono, K., and Ichijo, H. Mammalian thioreodxin is a direct inhibitor of apoptosis signal-regulating kinase (ASK) 1. *EMBO J.* 17, 2596-2606, 1998.
20. Takeda, K., Ichijo, H., Fujii, M., Mochida, Y., Saitoh, M., Nishitoh, H., Sampath, T.K., and Miyazono, K. Identification of a novel bone morphogenetic protein-responsive gene that may function as a noncoding RNA. *J. Biol. Chem.* 273, 17079-17085, 1998.
21. Inoue, H., Imamura, T., Ishidou, Y., Takase, M., Udagawa, Y., Tsuneizumi, K., Tabata, T., Miyazono, K., and Kawabata, M. Interplay of signal mediators of Decapentaplegic (Dpp): Molecular characterization of Mothers aganst dpp, Medea, and Daughters against Dpp. *Mol. Biol. Cell* 9, 2145-2156, 1998.
22. * Kawabata, M., Inoue, H., Hanyu, A., Imamura T., and Miyazono, K. Smad proteins exist as monomers in vivo and undergo homo- and hetero-oligomerization upon activation by serine/threonine kinase receptors. *EMBO J.* 17, 4056-4065, 1998.
23. Nishitoh, H., Saitoh, M., Mochida, Y., Takeda, K., Nakano, H., Rothe, M., Miyazono, K., and Ichijo, H. ASK1 is essential for JNK/SAPK activation by TRAF2. *Mol. Cell* 2, 389-395, 1998.
24. Yagi, K., Goto, D, Hamamoto, T., Takenoshita, S., Kato, M. and Miyazono, K. Alternatively-spliced variant of Smad2 lacking exon 3: Comparison with wild-type Smad2 and Smad3. *J. Biol. Chem.* 274, 703-709, 1999.
25. Yanagisawa, J., Yanagi, Y., Masuhiro, Y., Suzawa, M., Toriyabe, T., Kashiwagi, K., Watanabe, M., Kawabata, M., Miyazono, K., and Kato, S. Convergence of transforming growth factor-b and vitamin D signaling pathways on SMAD transcriptional coactivators. *Science* 283, 1317-1321, 1999.
26. Nakashima, K., Yanagisawa, M., Arakawa, H., Kimura, N., Hisatsune, T., Kawabata, M., Miyazono, K., and Taga, T. Synergistic signaling in fetal brain by STAT3-Smad1 complex bridged by p300. *Science* 284, 479-482, 1999.
27. Nakao, A., Fujii, M., Matsumura, R., Kumano, K., Saito, Y., Miyazono, K., and Iwamoto, I. Transient gene transfer and expression of Smad7 prevents bleomycin-induced lung fibrosis in mice. *J. Clin. Invest.* 104, 5-11, 1999.
28. Nishihara, A., Hanai, J.-i., Imamura, T., Miyazono, K., and Kawabata, M. E1A inhibits transforming growth factor-b signaling through binding to Smad proteins. *J. Biol. Chem.* 274, 28716-28723, 1999.
29. * Fujii, M., Takeda, K., Imamura, T., Aoki, H., Sampath, T.K., Enomoto, S., Kawabata, M., Kato, M., Ichijo, H., Miyazono, K. Roles of bone morphogenetic

protein type I receptors and Smad proteins in osteoblastic and chondroblastic differentiation. *Mol. Biol. Cell* 10, 3801-3813, 1999.

30. Hanai, J.-i., Chen, L.F., Kanno, T., Ohtani-Fujita, N., Kim, W.Y., Guo, W.-H., Imamura, T., Ishidou, Y., Fukuchi, M., Shi, M.-J., Stavnezer, J., Kawabata, M., Miyazono, K., and Ito, Y. Interaction and functional cooperation of PEBP2/CBF with Smads: Synergistic induction of the immunoglobulin germline Ca promoter. *J. Biol. Chem.* 274, 31577-31582, 1999.
31. Akiyoshi, S., Inoue, H., Hanai, J.-i., Kusanagi, K., Nemoto, N., Miyazono, K., and Kawabata, M. c-Ski acts as a transcriptional co-repressor in TGF-β signaling through interaction with Smads. *J. Biol. Chem.* 274, 35269-35278, 1999.
32. Kusanagi, K., Inoue, H., Ishidou, Y., Mishima, H.K., Kawabata, M., and Miyazono, K. Characterization of a bone morphogenetic protein-responsive Smad binding element. *Mol. Biol. Cell* 11, 555-565, 2000.
33. Ishida, W., Hamamoto, K., Kusanagi, K., Yagi, K., Kawabata, M., Takehara, K., Sampath, T.K., Kato, M., and Miyazono, K. Smad6 is a Smad1/5-induced Smad inhibitor: Characterization of bone morphogenetic protein-responsive element in the mouse *Smad6* promoter. *J. Biol. Chem.* 275, 6075-6079, 2000.
34. Oh, S.P., Seki, T., Goss, K.A., Imamura, T., Yi, Y., Donahoe, P.K., Li, L., Miyazono, K., ten Dijke, P., Kim, S., and Li, E.) Activin receptor-like kinase 1 modulates transforming growth factor-b1 signaling in the regulation of angiogenesis *Proc. Natl. Acad. Sci. USA* 97, 2626-2631, 200.
35. Miyazono, K. Positive and negative regulation of TGF-β signaling. *J. Cell Sci.* 113, 1101-1109, 2000.
36. Beppu,, H., Kawabata, M., Hamamoto, T., Chytil, A., Minowa, O., Noda, T., and Miyazono, K. BMP type II receptor is required for gastrulation and early development of mouse embryos. *Dev. Biol.* 221, 249-258, 2000.
37. Zhang, Y.-W., Yasui, N., Huang, G., Fujii, M., Hanai, J.-i., Nogami, H., Ochi, T., Miyazono, K., and Ito, Y. A RUNX2/PEBP2αA/CBFA1 mutation displaying impaired transactivation and Smad interaction in cleidocranial dysplasia. *Proc. Natl. Acad. Sci. USA* 97, 10549-10554, 2000.
38. Miura, S., Takeshita, T., Asao, H., Kimura, Y., Murata, K., Sasaki, Y., Hanai, J.-i., Beppu, H., Tsukazaki, T., Wrana, J.L., Miyazono, K., and Sugamura, K. Hgs (Hrs), a FYVE domain protein, is involved in Smad signaling through cooperation with SARA. *Mol. Cell. Biol.* 20, 9346-9355, 2000.
39. Yoshida, Y., Tanaka, S., Umemori, H., Minowa, M., Usui, M., Ikematsu, N., Hosoda, E., Imamura, T., Kuno, J., Yamashita, T., Miyazono, K., Noda, M., Noda, T., and Yamamoto, T. Negative regulation of BMP/Smad signaling by Tob in osteoblasts. *Cell* 103, 1085-1097, 2000.
40.* Ebisawa, T., Fukuchi, M., Murakami, G., Chiba, T., Tanaka, K., Imamura, T., and Miyazono, K. Smurf1 interacts with transforming growth factor-b type I receptor through Smad7 and induces receptor degradation. *J. Biol. Chem.* 276, 12477-12480, 2001.
41. Fukuchi, M., Imamura, T., Chiba, T., Ebisawa, T., Kawabata, M., Tanaka, K., and Miyazono, K.Ligand-dependent degradation of Smad3 by a ubiquitin ligase complex of ROC1 and associated proteins. *Mol. Biol. Cell* 12, 1431-1443, 2001.
42. Furuhashi, M.,Yagi, K.,Yamamoto, H., Furukawa, Y., Shimada, S., Nakamura, Y., Kikuchi, A., Miyazono, K., and Kato, M. Axin facilitates Smad3 activation in the transforming growth factor-b signaling pathway. *Mol. Cell. Biol.* 21, 5132-5141, 2001.
43. Kusanagi, K., Kawabata, M., Mishima, H.K., and Miyazono, K. a-Helix 2 in the amino-terminal Mad homology 1 domain is responsible for specific DNA-binding of Smad3. *J. Biol. Chem.* 276, 28155-28163, 2001.
44.* Hanyu, A., Ishidou, Y., Ebisawa, T., Shimanuki, T., Imamura, T., and Miyazono, K. The N-domain of Smad7 is essential for specific inhibition of transforming growth factor-b signaling. *J. Cell Biol.* 155, 1017-1028, 2001.
45. Yagi, K., Furuhashi, M., Aoki, H., Goto, D., Kuwano, H., Sugamura, K., Miyazono, K., and Kato, M. c-myc is a downstream target of Smad pathway. *J. Biol. Chem.* 277, 854-861, 2002.
46. Ring, C., Ogata, S., Meek, L., Song, J., Ohta, T., Miyazono, K., and Cho, K.W.Y. The role of a Williams-Beuren syndrome-associated helix-loop-helix domain-containing transcription factor in activin/nodal signaling. *Genes Dev.* 16, 820-835, 2002.
47. Nishihara, N., Watabe, T., Imamura, T., and Miyazono, K. Functional heterogeneity of BMPR-II mutants found in patients with primary pulmonary hypertension. *Mol. Biol. Cell* 22, 5212-5221, 2002.
48. Suzuki, C., Murakami, G., Fukuchi, M., Shimanuki, T., Shikauchi, Y., Imamura, T., and Miyazono, K. (2002) Smurf1 regulates the inhibitory activity of Smad7 by targeting Smad7 to the plasma membrane. *J. Biol. Chem.* in press.
49. Hamamoto, T., Beppu, H., Okada, H., Kawabata, M., Kitamura, T., Miyazono, K., and Kato, M. (2002) Compound disruption of *Smad2* accelerates malignant progression of intestinal tumors in *Apc* knockout mice. *Cancer Res.* in press.
50. Miyazono, K., and Miyazawa, K. (2002) Id: A target of BMP signaling. *Science's STKE* in press.

Department of Microbiology

Outline and Research Objectives

Microbial disease has been recognized as the major threat to human health throughout the history. Despite the development of preventive and therapeutic interventions against some pathogenic microbes, infectious disease is still one of the most significant medical problems. On the other hand, microbial organisms have served as a useful model as well for elucidating the human molecular mechanisms of a variety of biological events, providing useful insights into life science. Efforts have also been initiated by a number of research group to utilize animal viruses as a tool for human gene therapy. In order to familialize students with these issues, importance of microbiology in medical education is increasing more rapidly than ever. To fulfill this requirement, our department, as the only basic microbiology unit in the Faculty and Graduate School, currently assumes a responsibility for teaching bacteriology and virology to medical undergraduates, and for educating graduate students in a field of virology on animal RNA viruses.

Our major research interest is to elucidate molecular mechanisms for species-, tissue-, and cell-specific pathogenicity of poliovirus and hepatitis C virus. For this purpose, we attempt to identify viral and host factors required for the viral replication and dissemination in animal model. Tissue and cell distribution of host factors in host species or animal model may give an insight into molecular pathogenesis of those RNA viruses. Thus, the main objectives in our department are as follows;

I. Replication, dissemination, and neurovirulence of poliovirus

Poliovirus (PV), the causative agent of poliomyelitis, is a human enterovirus that belongs to the Picornaviridae. The genome of PV is a single-stranded RNA of positive polarity and consists of approximately 7500 nucleotides (nt). To this RNA, a small protein VPg is attached at the 5'end, and poly(A) at the 3' end. PV mRNA does not have VPg and starts with simple pU. Thus PV mRNA is an exceptional eukaryotic mRNA. An internal ribosome entry site (IRES) was identified in the 5' untranslated region (UTR) of the RNA.

Natural PV infection in humans is thought to begin with oral ingestion. After ingestion, the virus multiplies in the alimentary mucosa; the tonsils and Peyer's patches are possibly also invaded early in the course of infection. Following multiplication in these loci, the virus moves into the deep cervical and mesenteric lymph nodes and then into the blood. Neutralizing antibodies to PV in the blood prevent the development of poliomyelitis, which suggests that viremia is necessary for the spread of virus to the central nervous system (CNS). The circulating virus then invades the CNS and replicates in neurons, particularly the motor neurons. There are two possible routes through which PV can enter the CNS. One is virus permeation through the blood-brain barrier (BBB) and the other is virus transmission via peripheral nerves. Paralytic poliomyelitis occurs as a result of neuronal destruction. Specifically, we are working on the following projects.

1. Roles of human PV receptor (hPVR) in PV infection.
2. Molecular mechanisms for BBB permeation of PV.
3. Molecular mechanisms for retrograde axonal transport of PV.
4. Replication of PV in neurons.

II. Replication and pathogenesis of hepatitis C virus

Hepatitis C virus (HCV), the main causative agent of non-A, non-B hepatitis, is a member of Flaviviridae. Chronic infection of HCV frequently leads to liver cirrhosis and hepatocellular carcinoma (HCC). The genome of HCV is a single-stranded RNA of positive polarity and consists of approximately 9500 nt. An IRES was found in the 5' portion of the HCV RNA like PV RNA, although it is not known whether the 5' end is capped or not. As for the 3'

UTR, the HCV RNA does not have a poly(A) tail but a poly(U/C) tract of 80-130 nt followed by a highly conserved 98 nt sequence among HCV isolates, termed the X region. Thus, HCV RNA has an unique structure as an eukaryotic mRNA. Specifically, we are working on the following projects.

1. Molecular mechanisms for development of HCC by HCV infection.
2. Replication mechanisms of HCV RNA replicon.

III. IRES-dependent virus tropism

Virulent strains of PV can replicate well both in the CNS and the alimentary mucosa, whereas attenuated strains of PV cannot replicate well in the CNS although these strains have a strong replicating capacity in the alimentary mucosa. Therefore, virulent and attenuated PV strains have different tissue tropism each other. Relatively strong determinant for the attenuation phenotype was identified in the 5' UTR within the IRES. It is possible that IRES activity differ in tissues, and that viral replication capacity reflects the IRES activity.

IRES was first described for picornavirus RNAs, and later for many cellular mRNAs as well as other viral RNAs. Nucleotide sequences that serve as IRES, so far discovered, have a variety of lengths and predicted secondary structures, although all of them have a similar function in translation initiation. Existence of a variety of IRES structures suggests that different sets of trans-acting cellular factors are required for activity of individual IRESs. Cumulative evidence suggests that cellular factors required for IRES activities are quantitatively and/or qualitatively different in individual IRESs. Specifically, we are working on the following projects.

1. Construction of recombinant viruses that have mutated IRESs.
2. Identification of host factors required for activity of various IRESs.
3. Establishment of method for measuring IRES activity in vivo.

Faculties and Students

Professor and Chair Akio Nomoto, Ph.D. (since 1999)
Associate Professor Tetsuro Matano, M.D., Ph.D.
Associate 2
Postdoctoral Fellow 3
Graduate Students 12
Secretary 2

Past Research and Major Accomplishments

1. Structural analysis of the 5' termini of all the PV specific RNAs and proposal of VPg-primer theory of PV RNA synthesis.
2. Determination of the primary structure of the genomes of all three attenuated PV serotypes
3. Construction of infectious cDNA clone of the attenuated PV strain (Sabin 1 strain) and first gene manipulation of animal RNA virus using the infectious clone.
4. Proposal of new copy choice model of RNA genome for its deletion and recombination events.
5. Molecular genetic analysis of the attenuation phenotype of type 1 PV.
6. Construction of new attenuated PV strains based on knowledge of stability of attenuation mutations.
7. Discovery of hPVR.
8. Establishment of hPVR/transgenic (Tg) mice susceptible to PV.
9. Analysis of interaction between PV and hPVR.
10. Formation of a concept "IRES-dependent virus tropism".
11. Study on dissemination pathway of PV using Tg mouse model.
12. Subcellular localization of hPVR in polarized cells.
13. Response of neural cells to poliovirus infection.
14. Discovery of HCV IRES

Current Research

1. There are two isoforms of functional human PV receptors, hPVR α and hPVR δ. These two isoforms differ only in a part of amino acid sequence within the cytoplasmic domain. Basolateral sorting signal was identified in the cytoplasmic domain of hPVR α but not hPVR δ. Indeed, hPVR α localizes to the basolateral membran and hPVR δ apical membrane. It is possible that PV infection occurs by using hPVR δ in the human alimentary tract.
2. Using a hPVR/Tg mouse model, we have shown that PV permeates the BBB at a fairly high rate, independently of the presence of hPVR or virus strain-specific effects. Thus, some host cell molecules other than hPVR must be involved in the BBB permeability of PV. We are currently investi-

gating which host molecules are involved in this dissemination process.

3. Our recent results strongly suggest the following; Intramuscularly inoculated PV is incorporated by hPVR-mediated endocytosis at synapses without conformational change. The endosomes containing PV is associated with dynein motor molecule, and retrogradely transported to neuron cell body along microtubules. After the retrograde transport through the axon, lytic replication of PV occurs in the cell body. The virulent and attenuated PV strains do not show significant difference in efficiency of this dissemination route in a hPVR/Tg mouse model. We are attempting to prove the above bypothesis.
4. PV-infected neural cells do not show cytopathic effect when the infected cell cultures are added with anti-PV or anti-PVR antibodies 2 hours after the infection. Similar phenomenon cannot be observed in PV-infected HeLa cells. We precisely analyzed this phenomenon, and found that PV IRES activity was inhibited in the infected neural cells, and that PV-specific 2A protease was transported to the nucleus, and therefore translation initiation factor eIF4G was restored in the infected cells. We are currently attempting to elucidate why PV IRES activity is inhibited in the infected neural cells.
5. Development of cancers always accompanies mutations in chromosomes. Although HCV genome does not have any specific tumor gene, it is possible that some HCV gene products induce instability of human chromosome structure. We found that nuclear transport system was disturbed by HCV core protein. This may result in instability of human chromosome structure in the HCV-infected hepatocytes. We are now investigating interaction between HCV core protein and nuclear transport receptors.
6. Chimeric PV whose IRES was replaced by HCV IRES was constructed. This recombinant virus can replicate well in the liver of hPVR/Tg mice but not in the brain of the mice. This indicates that HCV IRES is active in the liver but not in the brain. The results support our notion " IRES-dependent virus tropism".

Future Prospects

PV is now one of the most well characterized virus and recent studies of its molecular biology have made great progress. Additionally, the development of a transgenic mouse model for poliomyelitis has not only facilitated investigations of PV dissemination but has transformed PV research into an important area of neuroscience. The elucidation of the molecular basis of PV neurovirulence is only at its starting point, and some basic questions proposed in section of " Current Research" must be answered if we are to understand fully the molecular mechanisms responsible. This research could eventually lead to the development of new strategies to control poliomyelitis and other viral diseases.

It is an important question in life science why viruses without tumor gene are able to cause cancers. Extensive effort have been made to elucidate mechanisms for development of HCC by HCV infection. However, little is known, as yet, about the mechanisms responsible. Since development of effective vaccine to control HCV infection seems to be difficult, and altogether may be impossible, only sure way to control HCV is to elucidate molecular mechanisms responsible for onset of HCV disease, resulting in the development of new strategies to control the viral disease.

Our approach to study pathogenicity of RNA viruses may also provide useful insights into general life sciences.

Research Grants

1995-1999 Grant-in-aid for Specially Promoted Research (total ¥273,000,000)
2000-2004 Grant-in-aid for Specially Promoted Research (~2002 ¥182,000,000)
2001-2005 Research Grant from the Organization for Pharmaceutical Safety and Research (~2002 ¥65,000,000)

Select Publications

1. Akio Nomoto, Yuan Fon Lee & Eckard Wimmer. The 5' end of poliovirus mRNA is not capped with m7G(5')ppp(5')Np.
   Proc. Natl. Acad. Sci. USA 73: 375-380, 1976
2. Yuan Fon Lee, Akio Nomoto, Barbara M. Detjen & Eckard Wimmer. A protein covalently linked to poliovirus genome RNA.
   Proc. Natl. Acad. Sci. USA 74(1): 59-63, 1977
3. Akio Nomoto, Barbara M. Detjen, Rudy Pozzatti & Eckard Wimmer. The location of the polio genome protein in viral RNAs and its implication for RNA synthesis.
   Nature 268(5617): 208-213, 1977
4. Akio Nomoto, Naomi Kitamura, Fred Golini & Eckard Wimmer. The 5'-terminal structures of poliovirus RNA and poliovirus mRNA differ only in the genome-linked protein VPg.
   Proc. Natl. Acad. Sci. USA 72(12): 5345-5349, 1977
5. Akio Nomoto, Ann Jacobson, Yuan Fon Lee, John Dunn & Eckard Wimmer. Defective interfering particles of poliovirus: Mapping of the deletion and evi-

dence that the deletions in the genomes of DI (1), (2) and (3) are located in the same region.
J. Mol. Biol. 128(2): 179-196, 1979
6. Akio Nomoto, Toshiko Omata, Haruka Toyoda, Shusuke Kuge, Hitoshi Horie, Yutaka Kataoka, Yuko Genba, Yasuko Nakano & Nobumasa Imura. Complete nucleotide sequence of the attenuated poliovirus Sabin 1 strain Genome.
Proc. Natl. Acad. Sci. USA 79(19): 5793-5797, 1982
7. Haruka Toyoda, Michinori Kohara, Yutaka Kataoka, Toshiyuki Suganuma, Toshiko Omata, Nobumasa Imura & Akio Nomoto. Complete nucleotide sequences of all three poliovirus serotype genomes: Implication for genetic relationship, gene function and antigenic determinants.
J. Mol. Biol. 174(4): 561-585, 1984
8. Toshiko Omata, Michinori Kohara, Yuko Sakai, Atsuko Kameda, Nobumasa Imura & Akio Nomoto. Cloned infectious complementary DNA of poliovirus Sabin 1 genome: biochemical and biological properties of the recovered virus.
Gene 32 (Part I): 1-10, 1984
9. Michinori Kohara, Toshiko Omata, Atsuko Kameda, Bert L. Semler, Heihachi Itoh, Eckard Wimmer & Akio Nomoto. In vitro phenotypic markers of a poliovirus recombinant constructed from infectious cDNA clones of the neurovirulent Mahoney and attenuated Sabin 1 strains.
J. Virol. 53(3): 786-792, 1985
10. Toshiko Omata, Michinori Kohara, Shusuke Kuge, Toshihiko Komatsu, Shinobu Abe, Bert L. Semler, Atsuko Kameda, Heihachi Itoh, Mineo Arita, Eckard Wimmer & Akio Nomoto. Genetic analysis of the attenuation phenotype of poliovirus type 1.
J. Virol. 58(2): 348-358, 1986
11. Michinori Kohara, Shinobu Abe, Shusuke Kuge, Bert L. Semler, Toshihiko Komatsu, Mineo Arita, Heihachi Itoh & Akio Nomoto. An infectious cDNA clone of the poliovirus Sabin strain could be used as a stable repository and inoculum for the oral polio live vaccine.
Virology 151(1): 21-30, 1986
12. Shusuke Kuge, Izumu Saito & Akio Nomoto. Primary structure of poliovirus defective interfering particle genomes and possible generation mechanisms of the particle.
J. Mol. Biol. 192: 473-487, 1986
13. Shusuke Kuge & Akio Nomoto. Construction of viable deletion and insertion mutants of the Sabin strain of type 1 poliovirus: Function of the 5' noncoding sequence in viral replication.
J. Virol. 61(5): 1478-1487, 1987
14. Akio Nomoto, Narushi Iizuka, Michinori Kohara & Mineo Arita. Strategy for construction of live picornavirus vaccines.
Vaccine 6(2): 134-137, 1988
15. Michinori Kohara, Shinobu Abe, Toshihiko Komatsu, Katsuhiko Tago, Mineo Arita & Akio Nomoto. A recombinant virus between the Sabin 1 and Sabin 3 vaccine strains of poliovirus as a possible candidate for a new type 3 poliovirus live vaccine strain.
J. Virol. 62(8): 2828-2835, 1988
16. Shusuke Kuge, Noriyuki Kawamura & Akio Nomoto. Genetic variation occurring on the genome of an in vitro insertion mutant of poliovirus type 1.
J. Virol. 63(3): 1069-1075, 1989
17. Noriyuki Kawamura, Michinori Kohara, Shinobu Abe, Toshihiko Komatsu, Katsuhiko Tago, Mineo Arita & Akio Nomoto. Determinants in the 5' noncoding region of poliovirus Sabin 1 RNA that influence the attenuation phenotype.
J. Virol. 63(3): 1302-1309, 1989
18. Shusuke Kuge, Noriyuki Kawamura & Akio Nomoto. Strong inclination toward transition mutation in nucleotide substitutions by poliovirus replicase.
J. Mol. Biol. 207(1): 175-182, 1989
19. Narushi Iizuka, Michinori Kohara, Kimiko Hagino-Yamagishi, Shinobu Abe, Toshihiko Komatsu, Katsuhiko Tago, Mineo Arita & Akio Nomoto. Construction of less neurovirulent polioviruses by introducing deletions into the 5' noncoding sequence of the genome.
J. Virol. 63(12): 5354-5363, 1989
20. Kimiko Hagino-Yamagishi & Akio Nomoto. In vitro construction of poliovirus defective-interfering particles.
J. Virol. 63(12): 5386-5392, 1989
21. Satoshi Koike, Hitoshi Horie, Iku Ise, Akira Okitsu, Michihiro Yoshida, Narushi Iizuka, Kenji Takeuchi Tsutomu Takegami & Akio Nomoto. The poliovirus receptor protein is produced both as membrane-bound and secreted forms.
EMBO J. 9(10): 3217-3224, 1990
22. Satoshi Koike, Choji Taya, Takeshi Kurata, Shinobu Abe, Iku Ise, Hiromichi Yonekawa & Akio Nomoto. Transgenic mice susceptible to poliovirus.
Proc. Natl. Acad. Sci. USA 88(3): 951-955, 1991
23. Kyoko Tsukiyama-Kohara, Michinori Kohara, Kenjiro Yamaguchi, Noboru Maki, Ayumi Toyoshima, Keizaburo Miki, Satoshi Tanaka, Nobu Hattori & Akio Nomoto. A second group of hepatitis C virus.
Virus Genes 5(3): 243-254, 1991
24. Satoshi Koike, Iku Ise & Akio Nomoto. Functional domains of the poliovirus receptor.
Proc. Natl. Acad. Sci. USA 88(5): 4104-4108, 1991
25. Narushi Iizuka, Hiromichi Yonekawa & Akio Nomoto. Nucleotide sequences important for translation initiation of enterovirus RNA.
J. Virol. 65(9): 4867-4873, 1991
26. Kyoko Tsukiyama-Kohara, Narushi Iizuka, Michinori Kohara & Akio Nomoto. Internal ribosome entry site within hepatitis C virus RNA.
J. Virol. 66(3): 1476-1483, 1992
27. Satoshi Koike, Iku Ise, Yasuko Sato, Kotoko Mitsui, Hitoshi Horie, Hideaki Umeyama & Akio Nomoto. Early events of poliovirus infection.
Seminars in Virology, Vol.3, 1992, Cellular Receptors for Virus Infection (A. Nomoto, ed.) pp109-115, Saunders Scientific Publications/ Academic Press

28. Satoshi Koike, Iku Ise, Yasuko Sato, Hiromichi Yonekawa, Osamu Gotoh & Akio Nomoto. A second gene for African green monkey poliovirus receptor that has no putative N-glycosylation site in the functional N-terminal immunoglobulin-like domain.
J. Virol. 66(12): 7059-7066, 1992
29. Akio Nomoto. Recombinant polioviruses as candidates for oral poliovaccines.
Microbiol. Immunol. 37(3): 169-174, 1993
30. Akio Nomoto, Satoshi Koike & Junken Aoki. Tissue tropism and species specificity of poliovirus infection.
Trends in Microbiology, Vol. 2, No.2, 47-51, 1994
31. Hitoshi Horie, Satoshi Koike, Takeshi Kurata, Yasuko Sato-Yoshida, Iku Ise, Yoshihiro Ota, Shinobu Abe, Kyoji Hioki, Hideki Kato, Choji Taya, Tatsuji Nomura, Sou Hashizume, Hiromichi Yonekawa & Akio Nomoto. Transgenic mice carrying the human poliovirus receptor: new animal model for study of poliovirus neurovirulence.
J. Virol. 68 (2): 681-688, 1994
32. Junken Aoki, Satoshi Koike, Iku Ise, Yasuko Sato-Yoshida & Akio Nomoto. Amino acid residues on human poliovirus receptor involved in interaction with poliovirus.
J. Biol. Chem. 269 (11) : 8431-8438, 1994
33. Shinobu Abe, Yoshihiro Ota, Satoshi Koike, Takeshi Kurata, Hitoshi Horie, Tatsuji Nomura, So Hashizume & Akio Nomoto. Neurovirulence test for oral live poliovaccines using poliovirus-sensitive transgenic mice.
Virology 206: 1075-1083, 1995
34. Kazuko Shiroki, Toshihiko Ishii, Takahiro Aoki, Yoshihiro Ohta, Wei-Xing Yang, Toshihiko Komatsu, Yasushi Ami, Mineo Arita, Shinobu Abe, So Hashizume & Akio Nomoto. Host range phenotype induced by mutations in the internal ribosomal entry site of poliovirus RNA.
J. Virol. 71 (1): 1-8, 1997
35. Wei-Xing Yang, Tetsuya Terasaki, Kazuko Shiroki, Seii Ohka, Junken Aoki, Shuichi Tanabe, Tatsuji Nomura, Eiji Terada, Yuichi Sugiyama & Akio Nomoto. Efficient delivery of circulating poliovirus to the central nervous system independently of poliovirus receptor.
Virology 229 (2): 421-428, 1997
36. Nobuhiko Kamoshita, Kyoko Tsukiyama-Kohara, Michinori Kohara & Akio Nomoto. Genetic analysis of internal ribosomal entry site on hepatitis C virus RNA: implication for involvement of the highly-ordered structure and cell type specific trans-acting factors.
Virology 223 (1): 9-18, 1997
37. Junken Aoki, Satoshi Koike, Hiroaki Asou, Iku Ise, Hiroshi Suwa, Toshiyuki Tanaka, Masayuki Miyasaka & Akio Nomoto. Mouse homolog of poliovirus receptor-related gene 2 product, mPRR2, mediates cell aggregation.
Exp. Cell Res. 235: 374-384, 1997
38. Minetaro Arita, Satoshi Koike, Junken Aoki, Hitoshi Horie & Akio Nomoto. Interaction of poliovirus with its purified receptor and conformational alteration in the virion.
J. Virol. 72 (5): 3578-3586, 1998
39. Seii Ohka, Wei-Xing Yang, Eiji Terada, Kuniko Iwasaki & Akio Nomoto. Retrograde transport of intact poliovirus through the axon via the fast transport system.
Virology 250 (1): 67-75, 1998
40. Minetaro Arita, Hitoshi Horie, Mineo Arita & Akio Nomoto. Interaction of poliovirus with its receptor affords a high level of infectivity to the virion in poliovirus infections mediated by the Fc receptor.
J. Virol. 73(2): 1066-1074, 1999
41. Kazuko Shiroki, Takeshi Isoyama, Shusuke Kuge, Toshihiko Ishii, Shinobu Ohmi, Syoji Hata, Koichi Suzuki, Yoshinari Takasaki & Akio Nomoto. Intracellular redistribution of truncated La protein produced by poliovirus 3Cpro-mediated cleavage.
J. Virol.73(3): 2193-2200, 1999
42. Qingmei Jia, Seii Ohka, Kuniko Iwasaki & Akio Nomoto. Isolation and molecular characterization of a poliovirus type 1 mutant that replicates in the spinal cords of mice.
J. Virol. 73(7): 6041-6047, 1999
43. Takeshi Isoyama, Nobuhiko Kamoshita, Kotaro Yasui, Atsushi Iwai, Kazuko Shiroki, Haruka Toyoda, Akio Yamada & Akio Nomoto. Lower concentration of La protein required for internal ribosome entry on hepatitis C virus than on poliovirus RNA.
J. Gen. Virol. 80(9): 2319-2327, 1999
44. Kyoko Murakami, Michikazu Abe, Tsutomu Kageyama, Nobuhiko Kamoshita & Akio Nomoto. Down regulation of translation driven by hepatitis C virus internal ribosomal entry site by the 3' untranslated region of RNA.
Arch. Virol. 146(4): 729-741, 2001
45. Qingmei Jia, James M. Hogle, Tsutomu Hashikawa & Akio Nomoto. Molecular genetic analysis of revertants from a poliovirus mutant that is specifically adapted to the spinal cord of mice.
J. Virol., 75(23): 11766-11772, 2001
46. Seii Ohka, Hitoshi Horie, Koujiro Tohyama & Akio Nomoto. Basolateral sorting of human poliovirus receptor a involves an interaction with the m1B subunit of the clathrin adaptor complex in polarized epithelial cells.
Biochem. Biophys. Res. Comm., 287: 941-948, 2001
Seii Ohka & Akio Nomoto. Recent insights into poliovirus pathogenesis.
Trends in Microbiology, 9(10): 501-506, 2001
48. Qingmei Jia, Fengyi Liang, Seii Ohka, Akio Nomoto & Tsutomu Hashikawa. Expression of brain-derived neurotrophic factor in the central nervous system of mice using a poliovirus-based vector.
J. NeuroVirology, 8(1): 14-23, 2002
49. Akio Nomoto & Isao Arita. Eradication of Poliomyelitis
Nature Immunology, 3(3): 205-208, 2002

50. Takeshi Isoyama, Shusuke Kuge & Akio Nomoto. The core protein of hepatitis C virus is imported into the nucleus by transport receptor Kap123p but inhibits Kap121p-dependent nuclear import of yeast-AP1-like factor in yeast cells.
J. Biol. Chem., 277(42): 39634-39641, 2002
Publications indicated by bold letters are attached.

Department of Infection Control and Prevention

Outline and Research Objectives

The Department of Infection Control and Prevention was founded in 1994 in order to minimize hospital infections. This department is, therefore, very new, but is the oldest one in the field of infection control in Japan. It started at first as the Division of Hospital Infection and Control Services on January 23, 1991. This division developed into the Division of Infection Control and Prevention on September 1, 1993 and the present department on June 24, 1994.

Research and practice of hospital infection control in Japan has been very primitives, and at least 15-20 years behind those in USA and European countries. The main objectives of our department are; 1) to control and prevent hospital infections, 2) to develop and propagate new effective methods to control and prevent hospital infections and 3) to be a model and lead of infection control in Japan. Therefore, surveillance of hospital infections, early detection of outbreaks, analyses of the causes of outbreaks and intervention are the most important parts of our activities. Education of medical and comedical staffs and students is also a very important role of our department.

Faculties and Students

Professor and Chair Satoshi Kimura, MD, PhD (1996～)
Lecturer (Hospital) Kyoji Moriya, MD, PhD (2002～)
Associate (Hospital) 2
Postdoctoral Fellow 1
Research student.....2
Secretary 2

Past Research and Major Accomplishments

1. Establishment of hospital infection surveillance system in the University of Tokyo Hospital (comprehensive and targeted).
2. Reduction of surgical site infections by replacing razor with clipper for preoperational removal of body hair at surgical sites.
3. Introduction of EPINet system into Japan and conduction of nationwide surveillance of needlestick injuries in health care workers in Japan.
4. Reduction of needlestick injuries among health care workers by employing safety devices.
5. Epidemiological analyses of Methicillin-resistant *Staphylococcus aureus* (MRSA) and other drug resistant microbes by using PFGE and/or PCR in the University of Tokyo Hospital.
6. Control of MRSA outbreak in NICU by unselected use of mupirocin ointment.
7. Control of *Ralstonia pickettii* colonization of patients in an obstetric ward.
8. Evaluation of disinfectant and antiseptics.
9. Nationwide surveillance of opportunistic infections in HIV infection.
10. Development of PCR systems for diagnosis of opportunistic infections in HIV/AIDS.
11. Quantitative analyses of cytomegalovirus (CMV) in the blood using real-time PCR for early diagnosis, monitoring and prospecting of CMV infections.
12. Evaluation of clinical efficacy of antiretroviral agents and highly active antiretroviral therapy (HAART).
13. Pharmacodynanic analyses of anti-HIV agents.
14. Evaluation of clinical significance of drug-resistance mutations of HIV against protease inhibitors.
15. Analyses of hospital visits among HIV-infected persons and AIDS cases in Japan.
16. Anti-HIV activities of oligodeoxyribonucleotides, ribozymes, fluoroquinolones etc..
17. Elucidation of molecular mechanism of viral hepatocarcinogenesis (HBV and HCV).
18. Elucidation of molecular mechanism of chemotaxis.
19. Elucidation of mode of action of defensin and cloning of a nevel mouse β-defensin.
20. Establishment of certification system of Infection Control Doctors (ICD) in Japan by organizing Japanese Council of ICD consisting of 16 scientific societies in the field of infectious diseases.

Current Research

We have been mainly studying on following subjects:

1. Surveillance, analyses of hospital infections, and planning to reduce hospital infections and occupational infections, such as needle stick injuries.

2. Epidemiological analyses of methicillin-resistant *Staphylococcus aureus* and other drug-resistant microbes using PFGE, AP-PCR, etc.
3. Evaluation of disinfectants and antiseptics.
4. Development of sensitive and rapid methods to detect pathogens.
5. Evaluation of multiple-drug therapies for HIV infection.
6. Quantification of DNA viruses in the blood using real-time PCR.
7. Preparation of guideline for appropriate use of antimicrobials.
8. Molecular mechanism o viral hepatocarcinogenesis.

Future Prospect

Surveillance is most important for the analysis of causes of outbreaks, and for the development of new preventive measures. So far, we have established surveillance system in the University of Tokyo Hospital. ICD system established by Prof. S. Kimura in 1999 greatly stimulated the trend to start surveillance in many other hospitals all over Japan. Thus, level of infection control and prevention in Japan will be rapidly elevated. In this process, staffs in our department will gain initiative.

By reducing hospital infections, duration of hospital stay of patients is to be shortened, medical expense be reduced, prognosis of patients be better, and quality of life be improved.

Research Grants

1. 1998：Grant-in-Aid from the Ministry of Health and Welfare of Japan（H-9-AIDS-002）"Studies on HIV Infection" ¥216,400,000
2. Grant-in-Aid from the Ministry of Health and Welfare of Japan "Studies on Epidemiology of HIV" ¥500,000
3. Grant-in-Aid for International Medical Cooperation"Studies on International Medical Cooperation against HIV Pandemic" ¥22,000,000
4. Grant-in-Aid from the Organization for Pharmaceutical Safety and Research"Epidemiological and Clinical Studies on Complications of HIV Infections" ¥90,000,000
5. 1999：Grant-in-Aid from the Ministry of Health and Welfare of Japan（H-9-AIDS-002）"Studies on HIV Infection" ¥216,426,000
6. Grant-in-Aid from the Ministry of Health and Welfare of Japan "Studies on Epidemiology of HIV" ¥500,000
7. Grant-in-Aid from the Organization for Pharmaceutical Safety and Research " Epidemiological and Clinical Studies on Complications of HIV Infections" ¥90,000,000
8. 2000：Grant-in-Aid from the Ministry of Health, Labor and Welfare of Japan（H-12-AIDS-004）"Studies on the Management of Complication in HIV/AIDS" ¥70,000,000
9. Grant-in-Aid from the Ministry of Health, Labor and Welfare of Japan "Socio-epidemiological Studies on the Trends of HIV/AIDS in Japan and Its Intervention" ¥500,000
10. 2001：Grant-in-Aid from the Ministry of Health, Labor and Welfare of Japan（H-12-AIDS-004）"Studies on the Management of Complication in HIV/AIDS" ¥1,005,000,000
11. Grant-in-Aid from the Ministry of Health, Labor and Welfare of Japan "Socio-epidemiological Studies on the Trends of HIV/AIDS in Japan and Its Intervention" ¥500,000
12. 2002：Grant-in-Aid from the Ministry of Health, Labor and Welfare of Japan（H-12-AIDS-004）"Studies on the Management of Complication in HIV/AIDS" ¥63,000,000
13. Grant-in-Aid from the Ministry of Foreign Affairs of Japan, US-Japan Cooperative Medical Science Program AIDS Panel ¥6,500,000
14. Grant-in-Aid from the Ministry of Health, Labor and Welfare of Japan "Socio-epidemiological Studies on the Trends of HIV/AIDS in Japan and Its Intervention" ¥500,000

Select Publications

1. S. Kimura, J. Matsuda, S. Ikematsu, K. Miyazono, A. Ito, T. Nakahata, M. Minamitani, K. Shimada, Y. Shiokawa, and F. Takaku; Efficacy of recombinant human granulocyte colony-stimulating factor on neutropenia in patients with AIDS. AIDS 4: 1251-1255, 1990
2. S. Oka, K. Urayama, Y. Hirabayashi, K. Ohnishi, H. Goto, K. Mitamura, S. Kimura, and K. Shimada; Quantitative estimation of human immunodeficiency virus type-1 provirus in CD4+ T lymphocytes using the polymerase chain reaction. Mol. and Cellular Probes 5: 137-142, 1991
3. T. Toyoshima, S. Kimura, S. Muramatsu, H. Takahagi, and K. Shimada; A sensitive nonisotopic method for the determination of intracellular azidothymidine 5'-mono-, 5'-di-, and 5'-tri-phosphate. Analyt. Biochem. 196: 302-307, 1991
4. K. Kitada, S. Oka, S. Kimura, K. Shimada, T. Serikawa, J. Yamada, H. Tsunoo, K. Egawa, and Y. Nakamura; Detection of *Pneumocystis carinii* sequence by polymerase chain reaction: animal models and clinical application to noninvasive specimens. J. Clin. Microbiol. 29: 1985-1990, 1991
5. S. Oka, K. Urayama, Y. Hirabayashi, S. Kimura, K. Mitamura, and K. Shimada; Human immunodeficiency virus DNA copies as a virologic marker in a clinical trial with β-interferon. J. Acquir. Immun. Def. Synd. 5: 707-711, 1992

6. S. Kimura; Current state of AIDS treatment - Can AIDS be controlled? - . Asian Med. J. 35: 251-259, 1992
7. S. Kimura, S. Oka, T. Toyoshima, Y. Hirabayashi, Y. Kikuchi, K. Mitamura, and K. Shimada; A randomized trial of reduced doses of azidothymidine in Japanese patients with human immunodeficiency virus type 1 infection. Intern. Med. 31: 871-876, 1992
8. S. Oka, M. Goto, Y. Kaji, S. Kimura, K. Matsuda, Y. Asai, M. Sanada, S. Nakagawa, M. Inoue, and K. Shimada; Synergistic activity of imipenem/cilastatin combined with cefotiam against methicillin-resistant *Stapylococcus aureus*. J. Antimicrob. Chemother. 31: 533-541, 1993
9. K. Yamada, S. Kimura, M. Negishi, J. Takamatsu, M. Inagaki, M. Aihara, Y. Nishida, K. Mori, K. Fukutake, J. Mimaya, N. Takata, and K. Shimada. Phase I/II trial of didanosine (2', 3'-dideoxyinosine) in hemophiliac patients with AIDS or AIDS-related complex. Clin. Diagnostic Virol. 1: 245-256, 1993
10. S. Oka, K. Kitada, T. Kohjin, Y. Nakamura, S. Kimura, and K. Shimada; Direct monitoring as well as sensitive diagnosis of *Pneumocystis carinii* pneumonia by polymerase chain reaction on sputum samples. Mol. Cellular Probes 7; 419-424, 1993
11. J. Hoy, E. J. Wright, and S. Kimura; Early HIV infection. AIDS 8 (suppl 2): s29-s35, 1994
12. T. Shioda, S. Oka, S. Ida, K. Nokihara, H. Toriyoshi, S. Mori, Y. Takebe, S. Kimura, K. Shimada, and Y. Nagai; A naturally occurring single basic amino acid substitution in the V3 region of HIV-1 Env protein alters the viral cellular host range and antigenic structure. J. Virol. 68: 7689-7696, 1994
13. H. Furukawa, K. Momota, T. Agatsuma, I. Yamamoto, S. Kimura, and K. Shimada; Mechanism of inhibition of HIV-1 infection *in vitro* by guanine-rich oligonucleotides modified at the 5' terminal by dimethoxytrityl residue. Nucleic Acids Res. 22: 5621-5627, 1994
14. S. Watanabe, M. Shinkai, S. Hitomi, H. Kozuka, S. Kimura, K. Shimada, R. Hondo, and N. Yamaguchi; A polymorphic region of the human cytomegalovirus genome encoding putative glycoproteins. Arch. Virol. 137: 117-121, 1994
15. A. Yasuoka, S. Oka, K. Komuro, H. Shimizu, K. Kitada, S. Shibahara, T. Takeuchi, S. Kondo, K. Shimada, and S. Kimura; Successful treatment with Benanomicin A (ME1451) of *Pneumocystis carinii* pneumonia in mice. Antimicrobial Agents Chemother. 39: 720-724, 1995
16. A. Suzaki, M. Kimurua, S. Kimura, K. Shimada, M. Miyaji, and L. Kaufman; An outbreak of histoplasmosis contacted by Japanese visitors to a bat-inhabited cave near Manaus, Brazil. J. Mycol. Med. 5: 40-43, 1995
17. M. Goto, S. Oka, N. Tachikawa, K. Kitada, M. Wada, C. Abe, K. Shimada, and S. Kimura; *KatG* sequence deletion is not the major cause of isoniazid resistance in Japanese and Yemei *Mycobacterium tuberculosis* isolates. Molecular and Cellular Probes 9: 433-439, 1995
18. A. Yasuoka, N. Tachikawa, K. Kaoru, S. Kimura, and S. Oka; (1-3) β -D-glucan as a quantitative serological marker for *Pneumocystis carinii* pneumonia. Clin. Diag. Lab. Immunol. 3: 197-199, 1996
19. H. Hotoda, K. Momota, H. Furukawa, T. Nakamura, and M. Kaneko S. Kimura and K. Shimada; Biologically active oligodeoxyribonucleotides- IV: Anti-HIV activity of TGGGAG having hydrophobic substituent at 5'-end via phosphodiester linkage. Nucleosides & Nucleotides, 15: 531-538, 1996
20. H. Shiga, T. Shioda, H. Tomiyama, Y. Takamiya, S. Oka, S. Kimura, Y. Yamaguchi, T. Gojoubori, H-G. Rammensee, K. Miwa, and M. Takiguchi; Identification of multiple HIV-1 cytotoxic T-cell epitopes presented by human leukocyte antigen B35 molecules. AIDS. 10: 1075-1083, 1996
21. S. Oka, Y. Nagata, Y. Fujino, A. Yasuoka, N. Tachikawa, K. Shimada, S. Kimura, A. Iwamoto, M. Mochizuki; CD8+ T lymphocytes in cytomegalovirus retinitis in a patient with AIDS. Brit. J. Ophtalmol. 80: 1108-1109, 1996
22. S. Kimura; Neurologic manifestations in human immunodeficiency virus infection/ acquired immunodeficiency syndrome (Editorial): Intrenal Medicine 36: 160-161,1997
23. S. Ida, H. Gatanaga, T. Shioda, Y. Nagai, N. Kobayashi, K. Shimada, S. Kimura, A. Iwamoto, and S. Oka; HIV type 1 V3 variation dynamics *in vivo*: Long-term persistence of non-syncytium-inducing genotypes and transient presence of syncytium-inducing genotypes during the course of progressive AIDS. AIDS Research and Human Retroviruses 13: 1597-1609, 1997
24. K. Momota, H. Furukawa, S. Kimura, H. Hotoda, S. Oka, and K. Shimada; Antiviral and biological properties of dimethoxytrityl-linked guanine-rich oligodeoxynucleotides that inhibit replication by primary clinical human immunodeficiency virus type-1 isolates. Antiviral Chem. Chemother. : 8: 497-505, 1997
25. H. Yotsuyanagi, K. Yasuda, S. Iino, K. Moriya, Y. Shintani, H. Fujie, T. Tsutsumi, S. Kimura, and K. Koike; Persistent viremia after recovery from self-limited acute hepatitis B. Hepatology 27: 1377-1382, 1998
26. K. Moriya, H. Fujie, Y. Shimtani, H. Yotsuyanagi, T. Tsutsumi, K. Ishibashi, Y. Matsuura, S. Kimura, T. Miyamura and K. Koike; The core protein of hepatitis C virus induces hepatocellular carcinoma in transgenic mice. Nature Medicine, 4 (9): 1065-1067, 1998.
27. S. Hitomi, S. Baba, H. Yano, Y. Morisawa and S. Kimura; Antimicrobial effects of electrolytic products of sodium chloride - Comparative evaluation with sodium hypochlorite solution and efficacy in handwashing. J. Jpn. Assoc. Infect. Dis. 72 (11): 1176-1181, 1998
28. K. Koike, K. Moriya, H. Yotsuyanagi, Y. Shintani, H. Fujie, T. Tsutsumi, and S. Kimura; Compensatory

apoptosis in preneoplastic liver of a transgenic mouse model for viral hepatocarcinogenesis. Cancer Lett 134 (2): 181-186, 1998
29. M. Koizumi, Y. Ozawa, R. Yagi, T. Nishigaki, M. Kaneko, S. Oka, S. Kimura, A. Iwamoto, Y. Komatsu, and E. Otsuka; Design and anti-HIV-1 activity of hammerhead and hairpin ribozymes containing a stable loop. Nucleosides & Nucleotides 17 (1-3) : 207-218, 1998
30. H. Kashiwase, K. Momota, T. Ohmine, T. Komai, T. Kimura, T. Katube, T. Nishigaki, S. Kimura, K. Shimada and H. Furukawa; A new fluoroquinolone derivative exhibits inhibitory activity against human immunodeficiency virus type 1 replication. Chemotherapy 45: 48-55, 1999
31. N. Tachikawa, M. Goto, Y. Hoshino, H. Gatanaga, A. Yasuoka, T. Wakabayashi, Y. Katano, S. Kimura, S. Oka, and A. Iwamoto; PCR detects *Toxoplasma gondii*, Epstein-Barr virus, and JC virus DNAs in the cerebrospinal fluid in AIDS patients with focal CNS complications. Intern. Med. 38: 556-562, 1999
32. H. Fujie, H. Yotsuyanagi, K. Moriya, Y. Shintani, T. Tsutsumi, T. Takayama, M. Makuuchi, Y. Matsuura, T. Miyamura, S. Kimura, and K. Koike; Steatosis and intrahepatic hepatitis C virus in chronic hepatitis. J. Med. Virol. 59: 141-145, 1999
33. Y. Shintani, H. Yotsuyanagi, K. Moriya, H. Fujie, T. Tsutsumi, Y. Kanegae, S. Kimura, I. Saito, and K. Koike; Induction of apoptosis upon the switch-on of the hepatitis B virus X gene mediated by Cre/loxP recombinase system. J. Gen. Virol. 180: 3257-3266, 1999
34. E. Noiri, T. Yokomizo, A. Nakao, T. Izumi, T. Fujita, S. Kimura, and T. Shimizu; An *in vivo* approach showing the chemotactic activity of leukotriene B4 in acute renal ischemic-reperfusion injury. Proc. Natl. Acad. Sci. 97 (2): 823-828, 2000
35. S. Hashimoto, T. Matsumoto, M. Nagai, Y. Matsuyama, Y. Nakamura, T. Umeda, M. Kamakura, S. Ichikawa, S. Kimura, K. Fukutomi, and M. Kihara; Delays and continuation of hospital visits among HIV-infected persons and AIDS cases in Japan. J. Epidemiol. 10 (1): 65-70, 2000
36. Y. Shintani, H. Yotsuyanagi, K. Moriya, H. Fujie, T. Tsutsumi, T. Takayama, M. Makuuchi, S. Kimura, and K. Koike; The significance of hepatitis B virus DNA detected in hepatocellular carcinoma of patients with hepatitis C. Cancer 88 (11): 2479-2486, 2000
37. U. Machida, M. Kami, T. Fukui, Y. Kazuyama, M. Kinoshita, Y. Tanaka, Y. Kanda, S. Ogawa, H. Honda, S. Chiba, K. Mitani, Y. Muto, K. Osumi, S. Kimura, and H. Hirai; Real-time automated PCR for early diagnosis and monitoring of cytomegalovirus infection after bone marrow transplantation. J. Clin. Microbiol. 38 (7): 2536-2542, 2000
38. A. Yoneyama, H. Yano, S. Hitomi, K. Okuzumi, R. Suzuki, and S. Kimura; *Ralstonia pickettii* colonization of patients in an obstetric ward caused by a contaminated irrigation system. J. Hosp. Infection 46: 77-80, 2000
39. S. Hitomi, M. Kubota, N. Mori, S. Baba, H. Yano, K. Okuzumi, and S. Kimura; Control of a methicillin-resistant *Staphylococcus aureus* outbreak in a neonatal intensive care unit by unselective use of nasal mupirocin ointment. J. Hosp. Infection 46: 123-129, 2000
40. H. Yotsuyanagi, Y. Shintani, K. Moriya, H. Fujie, T. Tsutsumi, T. Kato, K. Nishioka, T. Takayama, M. Makuuchi, S. Iino, S. Kimura, and K. Koike; Virologic analysis of non-B, non-C hepatocellular carcinoma in Japan: frequent involvement of hepatitis B virus. J. Infect. Dis. 181: 1920-8, 2000
41. E. Noiri, A. Nakao, A. Oya, T. Fujita, and S. Kimura; Hepatitis C virus in blood and dialysate in hemodialysis. Am. J. Kidney Dis. 37 (1): 38-42, 2001
42. H. Fujie, K. Moriya, Y. Shintani, H. Yotsuyanagi, S. Iino, S. Kimura, and K. Koike; Hepatitis B virus genotypes and hepatocellular carcinoma in Japan. Gastroenterology 120: 1564-1565, 2001
43. K. Tsuchiya, S. Matsuoka, A. Hachiya, A. Yasuoka, N. Tachikawa, Y. Kikuchi, I. Genka, K. Teruya, S. Kimura and S. Oka; Accumulation of lopinavir resistance-associated mutations over 3 years follow-up of patients on highly active antiretroviral therapy: implication in salvage therapy. AIDS 15 (9): 1183-1184, 2001
44. H. Fujie, K. Moriya, Y. Shintani, T. Tsutsumi, T. Takayama, M. Makuuchi, S. Kimura and K. Koike; Frequent β-catenin aberration in human hepatocellular carcinoma. Hepatology Research 20: 39-51, 2001
45. K. Moriya, K. Nakagawa, T. Santa, Y. Shintani, H. Fujie, H. Miyoshi, T. Tsutsumi, T. Miyazawa, K. Ishibashi, T. Horie, K. Imai. T. Todoroki, S. Kimura and K. Koike; Oxidative stress in the absence of inflammation in a mouse model for hepatitis C virus-associated hepatocarcinogenesis. Cancer Res. 61: 4365-4370, 2001
46. Y. Yamaguchi, S. Fukuhara, T. Nagase, T. Tomita, S. Hitomi, S. Kimura, H. Kurihara and Y. Ouchi; A novel mouse β-defensin, mBD-6, predominantly expressed in skeletal muscle. J. Biol. Chem. 276 (34): 31510-31514, 2001
47. A. Yoshida, S. Hitomi, T. Fukui, H. Endo, Y. Morisawa, Y. Kazuyama, K. Osumi, S. Oka and S. Kimura; Diagnosis and monitoring of human cytomegalovirus diseases in patients with human immunodeficiency virus infection by use of a real-time PCR assay. Clinical Infectious Diseases 33: 1756-1761, 2001
48. M. Tanaka, C. Yasuoka, I. Genka, N. Tachikawa, Y. Kikuchi, K. Teruya, A. Yasuoka, Y. Hirabayashi, S. Kimura and S. Oka; Sustained cytomegalovirus-specific CD4+ T cell response associated with prevention of recurrence of cytomegalovirus retinitis without secondary prophylaxis after highly active antiretroviral therapy in patients with AIDS. AIDS Res. Human Retroviruses 17 (18): 1749-1756, 2001

49. S. Ida, N. Tachikawa, A. Nakajima, M. Daikoku, M. Yano, Y. Kikuchi, A. Yasuoka, S. Kimura and S. Oka; Influence of human immunodeficiency virus type 1 infection on acute hepatitis A virus infection. Clinical Infectious Diseases 34, 379-85, 2002
50. K. Koike, K. Moriya and S. Kimura; Role of hepatitis C virus in the development of hepatocellular carcinoma: Transgenic approach to viral hepatocarcinogenesis. J. Gastroenterol. Hepatol. 17: 394-400, 2002

Department of Immunology

Outline and Research Objectives

Research projects, currently being conducted in our Department, have stemmed from the original identification of two cytokine genes between the late 1970s and early 1980s, namely, the genes encoding human fibroblast interferon (renamed thereafter as IFN-β) and interleukin-2 (IL-2), and their molecular characterization. In fact, the molecular distinction and consolidation of an enormous number of cytokine molecules, which are usually produced simultaneously at low levels in many cell types, have made it possible to study each cytokine as a single molecule and to elucidate their intracellular signaling mechanisms as well as their gene regulation mechanisms.

Given the fact that these cytokines are intimately involved in the regulation of immunity and oncogenesis, the above initial studies have led us to further extend the characterization of these cytokine systems in the context of the regulation of immune responses and oncogenesis. More recently, we have also initiated a project linking the immune system and bone remodeling in the context of cytokine signaling cross talk. Thus, our current research objectives are aimed at clarifying the following.

1. The mechanisms of signaling and transcription networks operating in the IFN-α/β system in innate immune responses.
2. The function and regulation of the transcription factor family, i.e., the interferon regulatory factors (IRFs), in innate and adaptive immune responses.
3. The mechanisms of regulation of antigen-presenting cells (APCs), typically dendrite cells (DCs), by IFNs and other cytokines and toll-like receptors (TLRs).
4. The regulation of ontogenesis by IFN-α/β and the tumor suppressor p53, particularly their mutual cooperation and function on target genes.
5. The regulation of bone remodeling by RANKL of the TNF family member and its signaling cross talks with IFNs and other cytokines.

The above projects are being conducted in a coordinated manner with much emphasis on interaction with researchers and students inside and outside of the Department, and collaborations, including international ones, are highly encouraged. The important objectives of our Department include the fostering of independent scientists with international experience, and search for new concepts critical for future developments in immunology and cancer biology.

Faculties and Students

Professor and Chair	Tadatsugu Taniguchi, Ph.D. (1995~)
Lecturer	Akinori Takaoka, M.D., Ph.D. (2000~)
Associate	2
Postdoctoral Fellow	4
Graduate student	12
Research student	1
Technical Assistant	2
Secretary	2

Past Research and Major Accomplishments

T. Taniguchi, Professor and Chairman of this Department, obtained his Ph. D. at the University of Zurich in 1978 (Title of doctoral dissertation: RNA-RNA interactions in the process of 70S initiation complex formation between E. coli ribosomes and bacteriophage Qb RNA), and returned to work at The Cancer Institute, Japanese Foundation for Cancer Research in Tokyo, wherein the molecular characterization of soluble mediators of the immune response, now collectively termed cytokines, was initiated. In fact, we initially identified and characterized the genes encoding IFN-β and IL-2, and established the production of cytokines by recombinant DNA technology (Refs 1-6). The subsequent characterizations of the promoters of these genes, performed at the institute, were further extended after we moved to the Institute for Molecular and Cellular Biology of Osaka University, and we identified distinct regulatory *cis*-elements within the promoter of *IFN-β* gene, which function as a virus-inducible enhancer (Refs. 7, 12). The work on the *IFN-β* enhancer led us to the

discovery of a novel family of transcription factors, the IRF family, which now has nine members (Refs. 15, 18, 48). In fact, *IFN-α/β* genes as well as a number of IFN-inducible genes were shown to be involved in regulating cell proliferation and to be potential critical target genes of IRFs. We also identified the regulatory sequences of the *IL-2* gene, which have since been extensively studied in the context of T cell activation and anergy by many other groups (Ref. 9). We also continued working on the mechanisms of IL-2 signaling by characterizing and analyzing the structure and function of the IL-2 receptor complex. The achievements, made during and after transition to the current Department, are briefly summarized below on each project.

(1) Gene regulation in IFN-α/β system; Operation of "revving up" mechanism for robust antiviral responses.

The work on the *IFN-β* enhancer led us to discover the IRF family of transcription factors. A number of IFN-inducible genes were shown to be involved in regulating cell proliferation and to be potential critical target genes of IRFs (Ref. 48). In fact, to evaluate the diverse biological effect of IRFs in vivo, we initially established the mice deficient in *IRF-1* or *IRF-2* gene in collaboration with T. Mak and his colleagues, University of Toronto (Ref. 29). We first found that IRF-1 is a critical regulator for the antiviral response by IFNs, but that it is not essential for virus-induced *IFN-α/β* gene expression (Refs. 29, 31). More recently, we and others have focused on other two related IRF members, IRF-3 and IRF-7. These two factors reside in the cytoplasm of uninfected cells and undergo translocation to the nucleus after viral infection. We adduced experimental evidence that these two IRFs indeed activate the *IFN* promoters, and the exact role of these factors became clear when we generated mice deficient in these genes (Refs. 45, 48, 49). It was found that cells have an acquired auto-amplification mechanism for efficient *IFN-α/β* gene expression. Briefly, viral infections first result in the phosphorylation of the constitutively expressed IRF-3, and the phosphorylated IRF-3 primarily activates the *IFN-β* promoter. Once IFN-β is produced, it signals the cell to activate ISGF3, a heterotrimer complex consisting of STAT1, STAT2 and IRF-9; ISGF3 in turn induces *IRF-7* gene expression. The de novo produced IRF-7 then undergoes virus-induced phosphorylation, similar to IRF-3, and activates the *IFN-α/β* promoters. Thus, massive IFN-α/β production can be achieved through the positive-feedback loop (Refs. 48, 49).

We also found that the constitutive, IRF-3/IRF-7-independent production of IFN-α/β in uninfected cells is critical for setting the IRF-7 expression levels that determine whether or not the above-mentioned positive-feedback mechanism will operate effectively upon viral infection. We discussed the significance of spontaneous *IFN-α/β* gene expression, in the context of what we proposed as the "revving up" model. In fact, a weak IFN-α/β signaling also provides a foundation for cells to respond efficiently to other cytokines, such as IFN-γ and IL-6 (Ref. 49). In the "revving up" by the IFN-α/β signal, signaling molecules remain constantly activated, albeit weakly, and the expression level of target genes such as IRF-7 (and probably many others) is maintained, thereby providing a foundation for more efficient signaling, either in that pathway or in different pathways. Thus, the consumption of cellular resources is not futile but a regulated "trade-off" to provide the cell with a greater dynamic range (signal-to-noise) in its response to stimuli (Ref. 49). It is likely that mechanisms similar to what we found in the context of the "revving up" model may be operational in other immune responses, since the immune system generally requires such mechanisms so as to render the operation against invading pathogens effective.

(2) Function of IRF-1 and IRF-2: Regulation of immunity and oncogenesis.

In continuation of our analysis of the *IRF-1*-deficient mice, we found that IRF-1 is absolutely required for Th1 type of $CD4^+$ T cell responses and NK cell differentiation. In fact, IRF-1 is essential for the induction of IL-12 and inducible nitric oxide synthase, which are critical for the induction and effector phase of Th1 response, respectively, and it is also essential for IL-15 gene expression in stroma cells to induce NK cell development (Refs. 37, 38, 48). In addition, IRF-1 is found to be a tumor susceptibility gene. IRF-1 is required in the induction of apoptosis of oncogene-expressing fibroblasts and DNA-damaged, proliferating mature T cells (Refs. 35, 36, 42). In DNA-damaged fibroblasts, IRF-1 cooperates with p53 to induce cell cycle arrest by activating the $p21^{WAF1/CIP1}$ gene. We have shown that the loss of *IRF-1* alleles *per se* has no effect on spontaneous tumor development in the mouse, but that it dramatically exacerbates previous tumor predispositions caused by the *c-Ha-ras* transgene or nullizygosity for *p53.* In addition, notable alterations in the tumor spectrum of *IRF-1, p53* double-deficient mice indicated that IRF-1 is not hypostatic to p53. We also adduced evidence that the loss-of-function mutation in the *IRF-1* gene may be involved in cancer development in humans (Ref. 42, 48). In continuation of this project, we attempted to isolate genes functioning downstream of IRF-1/p53, and identified a novel gene, *Noxa.* The *Noxa* gene encodes a BH3-only protein of the Bcl-2 family, and its expression is actually dependent on p53; it also induces apoptosis when it is overexpressed (Ref. 43).

Although IRF-2 was originally identified as a negative regulator of IRF-1, it has a unique function in negatively regulating IFN-α/β signaling. IRF-2 is a very stable nuclear factor that attenuates transcription by the IFN-activated ISGF3. Interestingly, IRF-2-deficient mice spontaneously develop an inflammatory skin disease, resembling psoriasis. $CD8^+$ T cells appear to be involved in the pathogenesis of this condition, and $CD8^+$ T cells from these mice are hyper-responsive to antigen stimulation *in vitro*, which is accompanied by a markedly upregulated expression of genes induced by IFN-α/β (Refs.46, 48). Thus, IRF-2 is necessary for balancing the beneficial and harmful effects of the spontaneous IFN-α/β signaling, as described above.

(3) Characterization and functional studies on the IL-2 receptor complex.

The use of recombinant IL-2 made it possible to study this "T cell growth factor" in the context of regulation of intracellular signaling mechanisms. T. Waldmann and colleagues first discovered the IL-2 receptor, as an IL-2Rα chain, and it turned out that the functional receptor consists of subunits (Refs. 28, 34). We then isolated the second chain, IL-2Rβ chain, and showed for the first time that this cytokine receptor, lacking any catalytic domains, can transmit the signals to the cell interior by recruiting non-receptor tyrosine kinases (Refs. 8, 10, 17, 19, 34). In addition, it was also shown that multiple signaling molecules are recruited to the receptor, which play distinct functions. It is now known that IL-2R consists of IL-2Rα, β (termed cβ) and γ (termed cγ) chains.

We also took an *in vivo* approach to study the distinct domains of the IL-2Rβ cytoplasmic region by introducing each mutant cDNA that lacked one of these domains into *IL-2Rβ*-deficient mice. We showed that the lack of the membrane-distal H-region, which mediates activation of STAT5/STAT3 transcription factors, selectively affects the development of natural killer (NK) cells and T cells bearing γδ T cell receptors. In contrast, the A-region, which is located next to the H-region and mediates the activation of Src-family protein tyrosine kinases, contributes to the downregulation of the T cell proliferation function. The IL-2Rβc null mutant mice develop severe autoimmune symptoms, but these are all suppressed following the expression of either of the mutant cDNAs (Ref. 40).

(4) Osteoimmunology: Signaling cross talks between IFNs and RANKL in bone remodeling.

The regulation of osteoclast differentiation is an aspect central to the understanding of the pathogenesis and the treatment of bone diseases such as autoimmune arthritis and osteoporosis. In fact, excessive signaling by RANKL, a TNF family member essential for osteoclastogenesis, may contribute to such pathological conditions. We found unique signaling cross talks between RANKL and IFNs. We provided evidence that activated T cells maintain bone homeostasis by counterbalancing the action of RANKL through IFN-γ production. IFN-γ induces the rapid degradation of the RANK adapter protein, TRAF6, resulting in the strong inhibition of the RANKL-induced activation of NF-κB and JNK (Ref. 47). This work was introduced as a new area of research called osteoimmunology [Arron, J. R. et al. *Nature*, 408, 535, 2000]. More recently, we also found that RANKL induces the *IFN-β* gene, but not *IFN-α* genes, in osteoclast precursor cells, and that IFN-β strongly inhibits osteoclast differentiation by interfering with the RANKL-induced c-Fos expression (Ref. 50). The series of in vivo experiments revealed that these two IFN-mediated regulatory mechanisms, distinct with one another, are both important to keep balancing the homeostatic bone resorption. Collectively, these studies revealed novel aspects of the two types of IFNs, beyond their original roles in immune response, and may offer a molecular basis for the treatment of bone diseases.

Current Research

As described above, we have gained new insight into the mechanisms of signaling and transcription networks operating in the IFN-α/β system in immune responses. The current research projects in our laboratory are summarized as follows:

1. Function and regulation of IRFs.

Still elusive is the mechanism(s) by which IRF-3 and IRF-7 are activated by viruses. We have been trying to identify a virus-activated kinase(s), which is responsible for this activation by taking a proteomics approach. We have identified several molecules that are associated with IRF-3, and these molecules are currently subjected to functional analyses to determine their involvement in IRF-3 regulation. We have generated *IRF-7*-deficent mice and are now confirming the role of IRF-7 in *IFN-α/β* gene induction in distinct cell populations, particularly, in bone marrow-derived cells, such as DCs. Our preliminary data indicate that germinal center formation is impaired in the mutant mice, and we are going to analyze the underlying mechanisms. The generation of *IRF-3/IRF-7* double deficient mice is also under way. As for IRF-1, we have been working on the role of IRF-1 in $CD4^+$ T cell differentiation, particularly the identification of the IRF-1 target genes.

2. Mechanisms of regulation of antigen-presenting cells (APCs), typically dendritic cells (DCs).

We found that the IFN-α/β system is critically

involved in DC maturation, which is induced by TLRs. We are currently studying the mechanism by which TLR expression is regulated by the IFN-α/β system. We are also working on the differentiation and maturation of DCs, which are found to be abnormal in *IRF-2*-deficient mice.

3. Regulation of oncogenesis by IFN-α/β and the tumor suppressor p53.

A critical function of p53 in tumor suppression is the induction of apoptosis in cells exposed to noxious stresses, such as DNA damage. We have previously shown that Noxa belongs to the BH3-only subfamily of the Bcl-2 family proteins and that Noxa overexpression causes apoptosis of some cells. More recently, we generated mice deficient in the *Noxa* gene or in both *Noxa* and *Bax* genes. In addition, we are continuously studying on the cooperation between IFN signaling and p53 pathways in relation to oncogenesis using mutant mice that are defective in some of these pathways.

4. Regulation of osteoclast differentiation.

During the differentiation process of hematopoietic stem cells to osteoclasts with bone-resorbing activity, transcription factors such as PU.1, c-Fos, NF-κB, and MITF play a critical and specific role. To date, however, little is known about how RANKL, but not other cytokines that activate similar pathways, specifically induces the terminal differentiation of osteoclasts through a specific transcriptional program. Previous observations suggest that RANKL signaling activates an as yet unknown pathway(s) that specifically invokes the transcriptional program leading the cells to undergo terminal differentiation. To gain insight into the mechanism of the RANKL-specific induction of the osteoclast differentiation program, we took a genome-wide screening approach to identify genes specifically induced by RANKL, but not by IL-1, and came up with the NFATc1 transcription factor. We are currently analyzing the complex signaling and transcription networks in the RANKL-c-Fos/TRAF6-NFATc1 pathways to gain more insight into the differentiation programs.

Future Prospects

From the inception of the molecular analyses of the cytokine systems in the late 1970s, research projects in our laboratory have progressed and differentiated in many ways. This was natural and important for our laboratory, particularly in fostering the next-generation scientists who are independent and have international experience. In fact, more than ten former colleagues have been promoted to full professor in renowned universities. The discovery and functional analyses of the IRF family of transcription factors provided new insight into the molecular mechanisms underlying the efficient induction of IFN-α/β genes, as well as the regulation of immune responses and oncogenesis. As for future prospects, it is important to elucidate the mechanisms of activation of these transcription factors in virus-infected cells. Considering the increasing interest in the IFN-α/β system induction by nonviral pathogens in antigen presenting cells, it will also be important to elucidate the role of IFNs and IRFs in linking the innate and adaptive immune systems. We are beginning to see a new link between IFN systems and p53, and this will hopefully reveal a new and interesting area of research in cancer biology. We also continue elucidating the RANKL-mediated mechanisms of bone remodeling. We hope that our studies will provide novel therapeutic strategies for controlling infectious and neoplastic diseases, as well as bone diseases. In fact, it is also our long-term goal to gain insight into the mechanisms of autoimmunity and tumor immunity, so as to provide a means for the treatment of these diseases.

Research Grants

1. A grant for Advanced Research on Cancer from the Ministry of Education, Culture, Sports, Science, and Technology of Japan (1998-to date)
2. The research for the Future Program (96L0037) from Japan Society for the Promotion of Sciences (1995-1999).
3. Human Frontier Science Program (1996-1998); In collaboration with Drs. D. Littman (USA), B. Malissen and O. Acuto (France)

Selected Papers (50)

1. Taniguchi, T., Sakai, M., Fujii-Kuriyama, Y., Muramatsu, M., Kobayashi, S.and Sudo,T. ; Construction and identification of a bacterial plasmid containing the human fibroblast interferon gene sequence. *Proc. Jpn. Acad.*, 55B, 464-469, 1979.
2. Taniguchi, T., Ohno, S., Fujii-Kuriyama, Y. and Muramatsu, M. ; The nucleotide sequence of human fibloblast interferon cDNA. *Gene*, 10, 11-15, 1980.
3. Taniguchi, T., Mantei, N., Schwarzstain, M., Nagata, S., Muramatsu, M. and Weissmann, C. ; Human leukocyte and fibroblast interferons are structurally related. *Nature*, 285, 547-549, 1980.
4. Ohno, S. and Taniguchi, T. ; Structure of a chromosomal gene for human interferon-β. *Proc. Natl. Acad. Sci. USA*, 78, 5305-5309, 1981.
5. Taniguchi, T., Matsui, H., Fujita, T., Takaoka, C., Kashima, N., Yoshimoto, R. and Hamuro, J. ; Structure and expression of a cloned cDNA for human interleukin-2. *Nature*, 302, 305-310, 1983.

6. Kashima, N., Nishi-Takaoka, C., Fujita, T., Taki, S., Yamada, G., Hamuro, J. and Taniguchi,T. ; Unique structure of murine interleukin-2 as deduced from cloned cDNAs. *Nature*, 313, 402-404, 1985.
7. Fujita, T., Ohno, S., Yasumitsu, H. and Taniguchi, T. ; Delimitation and properties of DNA sequences required for the regulated expression of human interferon-β gene. *Cell*, 41, 489-496, 1985.
8. Hatakeyama, M., Minamoto, S., Uchiyama, T., Hardy, R. R., Yamada, G. and Taniguchi, T. ; Reconstitution of functional receptor for human interleukin-2 in mouse cells. *Nature*, 318, 467-470, 1985.
9. Fujita, T., Shibuya, H., Ohashi, T. Yamanishi, K. and Taniguchi, T. ; Regulation of human interleukin-2 gene. : Functional DNA sequences in the 5' flanking region for the gene expression in activated T lymphocytes. *Cell*, 46, 401-407, 1986.
10. Taniguchi, T., Fujita ,T., Hatakeyama, M., Mori, H., Matsui, H., Sato, T., Hamuro, J., Minamoto, S., Yamada, G. and Shibuya, H. ; Interleukin-2 and its receptor: Structure and functional expression of the genes. *Cold Spring Harbor Symp.*, LI, 577-586, 1986.
11. Maruyama, M., Shibuya, H., Harada, H., Hatakeyama, M., Seiki, M., Fujita, T., Inoue, J-I., Yoshida, M. and Taniguchi, T. ; Evidence for aberrant activation of the interleukin-2 autocrine loop by HTLV-1-encoded p40x and T3/Ti complex triggering. *Cell*, 48, 343-350, 1987.
12. Fujita, T., Shibuya, H., Hotta, H., Yamanishi, K. and Taniguchi, T. ; Interferon-β gene regulation: Tandemly repeated sequences of a synthetic 6bp oligomer function as a virus inducible enhancer. *Cell*, 49, 357-367, 1987.
13. Yamada, G., Kitamura, Y., Sonoda ,H., Harada, H., Taki, S., Mulligan, R.C., Osawa, H., Diamantstein, T., Yokoyama, S. and Taniguchi, T. ; Retroviral expression of the human IL-2 gene in a murine T cell line results in cell growth autonomy and tumorigenicity. *EMBO J.*, 6, 2705-2709, 1987.
14. Taniguchi, T. ; Regulation of cytokine gene expression. *Annu. Rev. Immunol.*, 6, 439-464, 1988.
15. Miyamoto, M.,Fujita, T., Kimura, Y., Maruyama, M., Harada, H., Sudo, Y., Miyata, T. and Taniguchi, T. ; Regulated expression of a gene encoding a nuclear factor, IRF-1, that specifically binds to IFN-β gene regulatory elements. *Cell*, 54, 903-913, 1988.
16. Fujita, T., Kimura, Y., Miyamoto, M., Barsoumian, E. L. and Taniguchi, T. ; Induction of endogenous IFN-α and IFN-β gene by a regulatory transcription factor, IRF-1. *Nature*, 337, 270-272, 1989.
17. Hatakeyama, M., Tsudo,M., Minamoto, S., Kono, T., Doi, T., Miyata, T., Miyasaka, M. and Taniguchi, T. ; Interleukin-2 receptor β chain gene: Generation of three receptor forms by cloned human α and β chain cDNA's. *Science*, 244, 551-556, 1989.
18. Harada, H., Fujita, T., Miyamoto, M., Kimura, Y., Maruyama, M., Furia, A., Miyata, T. and Taniguchi, T. ; Structurally similar but functionally distinct factors, IRF-1 and IRF-2, bind to the same regulatory elements of IFN and IFN-inducible genes. *Cell*, 58, 729-739, 1989.
19. Hatakeyama, M., Mori, H., Doi, T. and Taniguchi, T. ; A restricted cytoplasmic region of IL-2 receptor β chain is essential for growth signal transduction but not for ligand binding and internalization. *Cell*, 59, 837-845, 1989.
20. Taniguchi, T., Hatakeyama, M., Minamoto, S., Kono, T., Doi, T., Tsudo, M., Miyasaka, M. and Miyata, T. ; Interleukin-2 receptor β chain: Molecular cloning and functional expression of the human cDNA. *Cold Spring Harbor Symposia on Quantitative Biology*, Vol. LIV, 689-694, 1989.
21. Harada, H., Willison, K., Sakakibara, J., Miyamoto, M., Fujita, T. and Taniguchi, T. ; Absence of the type I IFN system in EC cells: Transcriptional activator (IRF-1) and repressor (IRF-2) genes are developmentally regulated. *Cell*, 63, 303-312, 1990.
22. Hatakeyama, M., Kono, T., Kobayashi, N., Kawahara, A., Levin, S. D., Perlmutter, R. M., and Taniguchi, T. ; Interaction of the IL-2 receptor with the src-family kinase p56lck: Identification of novel intermolecular association. *Science*, 252, 1523-1528, 1991.
23. Shibuya, H., Yoneyama, M., Ninomiya-Tsuji, J., Matsumoto, K., and Taniguchi, T. ; IL-2 and EGF receptors stimulate the hematopoietic cell cycle via different signaling pathways: Demonstration of a novel role for c-myc. *Cell*, 70, 57-67, 1992.
24. Minami, Y., Kono, T., Yamada, K., Kobayashi, N., Kawahara, A., Perlmutter, R. M., and Taniguchi, T. ; Association of p56lck with IL-2 receptor β chain is critical for the IL-2 induced activation of p56lck. *EMBO J.*, 12. 759-768, 1993.
25. Harada H., Kitagawa, M., Tanaka,N., Yamamoto, H., Harada, K., Ishihara, M., and Taniguchi, T. ; Anti-oncogenic and oncogenic potentials of interferon regulatory factors-1 and-2. *Science*, 259, 971-974, 1993.
26. Willman, C. L., Sever C. E., Pallavicini, M. G., Harada, H., Tanaka, N., Slovak, M. L., Yamamoto, H., Harada, K., Meeker, T. C., List, A. F., and Taniguchi, T. ; Deletion of *IRF 1*, mapping to chromosome 5q31.1, in human leukemia and preleukemic myelodysplasia. *Science*, 259, 968-971, 1993.
27. Minami, Y., Kono, T., Miyazaki, T., and Taniguchi, T. ; The IL-2 receptor complex: Its structure, function, and target genes. *Annu. Rev. Immunol.*, 11, 245-267, 1993.
28. Taniguchi, T., and Minami, Y. ; The IL-2/IL-2 receptor system: A current overview. *Cell*, 73, 5-8, 1993.
29. Matsuyama, T., Kimura, T., Kitagawa, M., Pfeffer, K., Kawakami, T., Watanabe, N., K dig, M. T., Amakawa, R., Kishihara, K., Wakaeham, A., Potter, J., Furlonger, L. C., Narendran, A., Suzuki, H., Ohashi, P. S., Paige, J. C., Taniguchi, T. and Mak, T. W.; Targeted disruption of IRF-1 or IRF-2 results in abnormal type I IFN gene induction and aberrant lymphocyte development. *Cell*, 75, 83-97, 1993.
30. Tanaka, N., Ishihara, M., Kitagawa, M., Harada, H., Kimura, T., Matsuyama, T.,Lamphier, M. S., Aizawa, S., Mak, T. W. and Taniguchi, T.; Cellular commit-

ment to oncogene-induced transformation or apoptosis is dependent on the transcription factor IRF 1. *Cell*, 77, 829-839, 1994.

31. Kimura, T., Nakayama, K., Penninger, J., Kitagawa, M., Harada, H., Matsuyama, T., Tanaka, N., Kamijo, R., Vilcek, J., Mak, T. W., Taniguchi, T. ; Involvement of the IRF-1 transcription factor in antiviral responses to interferons. *Science*, 264, 1921-1924, 1994.
32. Miyazaki, T., Kawahara, A., Fujii, H., Nakagawa, Y., Minami, Y., Liu, Z-J., Oishi, I., Silvennoinen, O., Witthuhn, B.A., Ihle, J.N., and Taniguchi, T.; Functional activation of Jak 1 and Jak3 by selective association with IL-2 receptor subunits. *Science*, 266, 1045-1047, 1994.
33. Minami, Y., Nakagawa, Y., Kawahara, A., Miyazaki, T., Sada, K., Yamamura, H. and Taniguchi, T. ; Protein tyrosine kinase Syk is associated with and activated by the IL-2 receptor, possible link with the c-myc induction pathway. *Immunity*, 2, 89-100, 1995.
34. Taniguchi, T. ; Cytokine signaling through nonreceptor protein tyrosine kinases. *Science*, 268, 251-255, 1995.
35. Tamura, T., Ishihara, M., Lamphire, M.S., Tanaka, N., Oishi, I., Aizawa, S., Matsuyama, T., Mak, T. W., Taki, S. and Taniguchi, T. ; An IRF-1-dependent pathway of DNA damage-induced apoptosis in mitogen-activated T lymphosytes. *Nature*, 376, 596-599, 1995.
36. Tanaka, N., Ishihara, M., Lamphier, M., Nozawa, H., Matsuyama, T., Mak, T.W., Aizawa, S., Tokino, T., Oren, M. and Taniguchi, T. ; Cooperation of the tumor suppressors IRF-1 and p53 in response to DNA damage. *Nature*, 382, 816-818, 1996.
37. Taki, S., Sato, T., Ogasawara, K., Fukuda, T., Sato, M., Hida, S., Suzuki, G.,Mitsuyama, M., Shin, E.-H., Kojima, S., Taniguchi, T. and Asano, Y. ; Multistage regulation of Th1-type immune responses by the transcription factor IRF-1. *Immunity*, 6, 673-679, 1997.
38. Ogasawara, K., Hida, S., Azimi, N., Tagaya, Y., Sato, T., Yokochi-Fukuda, T.,Waldmann, T.A., Taniguchi, T. and Taki, S. ; Requirement of IRF-1 for the microenvironment supporting natural killer cell development. *Nature*, 391, 700-703, 1998.
39. Miyazaki, T., Takaoka, A., Noguerira, L., Dikic, I., Fujii, H., Tsujino, S., Mitani, Y., Maeda, M., Schlessinger, J. and Taniguchi, T. ; Pyk2 is a downstream mediator of the IL-2 receptor-coupled Jak-signaling pathway. *Genes. Dev.* 12, 770-775, 1998.
40. Fujii, H., Ogasawara, K., Otsuka, H., Suzuki, M, Yamamura, K., Yokochi, T, Miyazaki, T., Suzuki, H., Mak, T. W., Taki, S. and Taniguchi, T.; Functional dissection of the cytoplasmic subregions of the IL-2 receptor β c chain in primary lymphocyte populations. *EMBO J.*, 17, 6551-6557, 1998.
41. Takaoka, A., Tanaka, N., Mitani, Y., Miyazaki, T., Fujii, H., Sato, M., Kovarik, P,. Decker, T., Schlessinger, J. and Taniguchi, T.; Protein tyrosine kinase Pyk2 mediates the Jak-dependent activation of MAPK and Stat1 in the IFN-γ , but not IFN-α , signaling. *EMBO. J.*, 18, 2480-2488, 1999.
42. Nozawa, H., Oda, E., Nakao, K., Ishihara, M., Ueda, S., Yokochi, T., Ogasawara, K., Nakatsuru, Y., Shimizu, S., Ohira, Y., Hioki, K., Aizawa, S., Ishikawa, T., Katsuki, M., Muto, T., Taniguchi, T., and Tanaka, N.; Loss of transcription factor IRF-1 affects tumor susceptibility in mice carrying the Ha-*ras* transgene or nullizygosity for *p53*. *Genes. Dev.* 12, 1240-1245, 1999.
43. Oda, E., Ohki, R., Murasawa, H., Nemoto, J., Shibue, T., Yamashita,T., Tokino, T., Taniguchi, T. and Tanaka, N.; Noxa, a BH3-only member of the Bcl-2 family, and candidate mediator of p53-induced apoptosis. *Science*, 288, 1053-1058, 2000.
44. Takaoka, A., Mitani, Y., Suemori, H., Sato, S., Yokochi, T., Noguchi, S., Tanaka, N., and Taniguchi, T.; Crosstalk between interferon-γ and-α / β signaling components at caveolar membrane domain. *Science*, 288, 2357-2360, 2000.
45. Sato, M., Suemori, H., Hata, N., Asagiri, M., Ogasawara, K., Nakao, K., Nakaya, T., Katsuki, M., Noguchi, S., Tanaka, N., and Taniguchi, T.;Distinct and essential roles of transcription factors IRF-3 and IRF-7 in response to viruses for IFN-α / β gene induction. *Immunity*, 13, 539-548, 2000.
46. Hida, S., Ogasawara, K., Sato, K., Abe, M., Takayanagi, H., Yokochi, T., Sato, T., Hirose, S., Shirai, T., Taki, S. and Tanicuhi, T.;CD8+ T cell-mediated skin disease in mice lacking IRF-2, the transcriptional attenuator of interferon-α / β signaling. *Immunity*,13, 643-655, 2000.
47. Takayanagi, H., Ogasawara, K., Hida, S., Chiba, T., Murata, S., Sato, K., Takaoka, A., Yokochi, T., Oda, H., Tanaka, K., Nakamura, k. and Taniguchi, T.; T-cell-mediated regulation of osteoclastogenesis by signalling cross-talk between RANKL and IFN-γ . *Nature*, 408, 600-605, 2000.
48. Taniguchi, T., Ogasawara, K., Takaoka, A, and Tanaka, N.; IRF family of transcription factors as regulators of host defense. *Ann. Rev. Immunol.* 19, 623-655, 2001.
49 Taniguchi, T. and Takaoka, A. ; A weak signal for strong responses: Interferon - α / β Revisited. *Nature Rev. Mol. Cell Biology*, 2, 378-386, 2001.
50. Takayanagi, H., Kim, S., Matsuo, K., Suzuki, H., Suzuki, T., Sato, K., Yokochi, T., Oda, H., Nakamura, K., Ida, N., Wagner, E. F., and Taniguchi, T.; RANKL maintains bone homeostasis through c-Fos-dependent induction of interferon-β. *Nature*, 416, 744-749, 2002.