Cancer Diagnosis with the Power of Molecular Knowledge

January 27, 2001
International Conference Hall
Aichi Cancer Center
Nagoya, Japan
Aichi Cancer Center
International Symposium VII

Cancer Diagnosis with the Power of Molecular Knowledge

Committee of the Aichi Cancer Center International Symposium
Chairperson: Makoto Ogawa
Ryuzo Ohno
Suketami Tominaga
Joichi Yamada
Katsuhisa Morita
Yoshio Yamamoto
Kazuhiko Ohashi
Toshitada Takahashi
Takashi Takahashi

Organizing Committee of the Seventh Symposium
Chairperson: Toshitada Takahashi
Kazuhiko Ohashi
Shigeo Nakamura
Tetsuya Mitsudomi
Masao Seto
Takashi Takahashi
Yasushi Yatabe
Akira Yamada
Yoko Nakashima

January 27, 2001
Aichi Cancer Center, Nagoya, Japan
# Program of Symposium

9:30-9:40 Opening Remarks: Makoto Ogawa

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<td>Chairperson: Tadaaki Eimoto (Nagoya City University)</td>
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<td>9:40-10:25</td>
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<td>Genetic Reconstruction of Colorectal Tumor Histories: Tumor Histories</td>
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<td>10:25-11:00</td>
<td>Wataru Yasui (Hiroshima University)</td>
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<td>Practical Molecular-pathological Diagnosis of Gastrointestinal Tumors</td>
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<td>11:00-11:25</td>
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<td>Molecular Diagnosis of Malignant Lymphoma Entities in WHO Classification</td>
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<td>11:25-12:10</td>
<td><strong>Impact of Human Genome Project on Molecular Diagnosis</strong></td>
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<td>11:25-12:10</td>
<td>Riccardo Dalla-Favera (Columbia University)</td>
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<td>Gene Expression Profiles of Normal Peripheral Human B-cell Subpopulations and B-cell Malignancies</td>
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<td>12:10-12:45</td>
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<td>Precise Estimation of SNP-allele Frequencies and its Significance in</td>
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<td>Bio-medical sciences</td>
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### Recent Advances in Molecular Diagnosis of Cancer

**Chairperson:** Yasushi Yatabe (Aichi Cancer Center)

**13:40-14:25** Li Mao (M.D. Anderson Cancer Center)  
Molecular Alterations – Roles in Cancer Detection and Risk Assessment

**14:25-15:00** Johji Inazawa (Tokyo Med. and Dent. University)  
Exploring Cancer–related Genes with Novel Amplifications in Various Types of Cancers

**15:00-15:25** Hayao Nakanishi (Aichi Cancer Center)  
Molecular Diagnostic Detection of Free Cancer Cells in the Body Fluids of Gastrointestinal and Bladder Cancer Patients with Real-time PCR

**15:25-15:50** Coffee Break

### Diagnosis and Counseling of Cancer Susceptibility

**Chairperson:** Hiroshi Shiku (Mie University)

**15:50-16:35** Robert Young (South Carolina University)  
Cancer Genetic Counseling: Principles and Practice

**16:35-17:10** Yoshimitsu Fukushima (Shinshu University)  
Proposing Genetic Counseling System in Japan: Experience in the Division of Clinical and Molecular Genetics, Shinshu University Hospital

**17:10-17:35** Nobuyuki Hamajima (Aichi Cancer Center)  
Estimation of Cancer Susceptibility Based on Gene-environment Interaction

**17:35-17:45** Concluding Remarks: Ryuzo Ohno
Welcome Remarks

Makoto Ogawa, M.D.
President, Aichi Cancer Center

On behalf of the organizing committee, I would like to welcome all of you to the Seventh Aichi Cancer Center International Symposium. The first international symposium entitled “From Prevention to Treatment” was held in 1994 when Aichi Cancer Center celebrated its Thirtieth Anniversary. Since then the symposium has been held annually and the organizing committee has selected timely topics on basic research, prevention, diagnosis, treatment and translational research.

This year, the organizing committee has selected the main theme to be “Cancer Diagnosis with the Power of Molecular Knowledge”.

The symposium starts from the session which mainly includes tumor evolution under the term of “molecular clock”, gene abnormalities in gastrointestinal tumors and recent advances of the understanding for lymphomagenesis. The second session will include molecular analysis of hematologic tumors and application of human genome polymorphism for cancer research. The third session will discuss molecular alterations in early lung tumorigenesis, and molecular cytogenetic studies to identify novel cancer-related genes and detection of minimal residual tumors. In the final session, cancer susceptibility and cancer genetic consultation will be discussed.

It is our great pleasure to have this symposium and we really hope that everybody joined will enjoy this symposium.
A recent advance in genetics is the ability to reconstruct evolution or lineages based on sequence comparisons. Histories are recorded in genomes. For example, even though chimpanzee and human genomes are >98% identical, a last common ancestor was nearly five million years ago. Similarly, it should be possible to recreate histories of individual human tumors. Although much is known about the adenoma-cancer sequence, every tumor likely has its own unique progression pathway. When did this tumor start growing? When did metastases occur? These questions are difficult to answer for individual human tumors because most tumors present unexpectedly. It is possible to use somatic mutations in microsatellite (MS) loci to "genotype" mismatch repair deficient (MSI+) tumors. The MS loci essentially "record" past progression and function as "molecular tumor clocks". Preliminary studies of hereditary nonpolyposis colorectal cancers suggest that adenomas are not direct precursors to cancers and that most genetic progression (the accumulation of mutations) occurs in the histologically occult period which precedes a gatekeeper mutation. Molecular clocks have the unique potential to reconstruct progression histories of individual human tumors based on their individual somatic mutations.
Darryl Shibata, M.D.

Associate Professor of Pathology  
Department of Pathology  
University of Southern California  
School of Medicine  
Los Angeles, CA, U.S.A.

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<td>1977</td>
<td>University of California, Los Angeles, B.S. (Biochemistry)</td>
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<td>1979</td>
<td>University of California, San Diego, M.S. (Chemistry)</td>
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<td>1983</td>
<td>University of Southern California (USC), Los Angeles, M.D. (Medicine)</td>
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<td>1984-1989</td>
<td>Post-graduate training (Pathology): University of Southern California, Los Angeles</td>
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<td>1988</td>
<td>American Board of Pathology (anatomic and clinical)</td>
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<td>1989-1995</td>
<td>Assistant Professor of Pathology, USC School of Medicine</td>
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<td>1995-</td>
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Practical Molecular-pathological Diagnosis of Gastrointestinal Tumors

Wataru Yasui, Naohide Oue, Junya Fujimoto, Hiroki Kuniyasu and Hiroshi Yokozaki
First Department of Pathology, Hiroshima University School of Medicine, Hiroshima, Japan

Although the histopathological diagnosis is most valuable in clinical practice, it includes some disadvantages, such as differential diagnosis, diagnosis of the existence of cancer, and limitation on information of biological behavior. By analyzing the genetic and epigenetic alterations in histopathology specimens, we can improve quality of final diagnosis. This is to discuss an overview of multiple genetic and epigenetic alterations during development and progression of the gastrointestinal cancer and to refer an outline and benefit of molecular diagnosis on histopathology specimens that has been routinely implemented in Hiroshima.

In the course of multistep carcinogenesis of the gastrointestinal tract, various genetic alterations of oncogenes, tumor suppressor genes, DNA repair genes, cell cycle regulators and cell adhesion molecules are accumulated. Epigenetic alterations of tumor suppressor genes such as reduced expression by CpG methylation of the promoter region, histone deacetylation, and increased proteosomal degradation are implicated deeply in the gastrointestinal carcinogenesis. Among a variety of genetic and epigenetic alterations, some are found commonly in the esophageal, gastric and colorectal cancers, while others differ depending on the location of cancer and histological type.

Molecular-pathological diagnosis is analyzing genetic and epigenetic alterations, which participate in the development and progression of cancer, in clinical materials and utilizing the molecular findings to clinical diagnosis and treatment. The main purpose of the molecular-pathological diagnosis on gastrointestinal biopsies is to make precise differential diagnosis as well as to foresee the grade of malignancy or patient prognosis. Genetic instability is a good indicator for hereditary non-polyposis colorectal cancer (HNPCC) kindred or patients at high risk for developing multiple primary cancers. The materials are formalin-fixed biopsies, endoscopical muosal resection or surgical specimens that are diagnosed as dysplasia, adenoma, borderline lesion and carcinoma thorough routine histopathological examination. The immunohistochemistry for various biomarkers, such as p53, hMLH1, p27, p21, cyclin D1, cyclin E, TGF-alpha, EGFR and c-erbB2, is carried out first, and some cases are further analyzed by PCR-SSCP, PCR-RFLP and microsatellite assay to obtain genetic information. Final molecular-pathological diagnosis is then made with
molecular findings together with histopathological observation, and reported to the clinicians. More than 10,000 lesions of the gastrointestinal tract had been examined from August 1993 to date, and additional information on differential diagnosis, grade of malignancy, and risk for multiple primary cancers could be obtained in about 20% of the cases examined. Follow-up study demonstrated that the patients diagnosed as high grade malignancy tended to have poorer prognosis and that a half of the cases with microsatellite instability-high (MSI-H) were found to develop multiple primary cancers.

More information on pathogenesis of the gastrointestinal cancer will be available in "post-sequence era". Accumulating findings in transcriptomics using DNA microarray and proteomics will uncover key alterations, which can be novel targets for diagnosis and therapeutics. Molecular pathology must evolve morphological genomics in the field of carcinogenesis.

References

Wataru Yasui, M.D., Ph.D.

Professor and Chairman
First Department of Pathology,
Hiroshima University School of Medicine
Hiroshima, Japan

1982 M.D., Hiroshima University School of Medicine (M.D.)
1982-1986 Ph.D., Hiroshima University Graduate School of Medical Sciences
1986-1987 Assistant, First Department of Pathology, Hiroshima University School of Medicine
1987-1989 Research Associate, Biochemistry Division, Department of Molecular and Experimental Medicine, The Scripps Research Institute, La Jolla
1989-1992 Assistant Professor, First Department of Pathology, Hiroshima University School of Medicine
1992-2000 Associate Professor, First Department of Pathology, Hiroshima University School of Medicine
2000- Professor and Chairman, First Department of Pathology, Hiroshima University School of Medicine
Molecular Diagnosis of Malignant Lymphoma Entities in WHO Classification

Shigeo Nakamura
Department of Pathology and Molecular Diagnostics, Aichi Cancer Center Hospital, Nagoya, Japan

Malignant lymphoma classification is now evolving. It is emphasized that biological approaches, such as immunohistochemistry and molecular biology, play a critical role in the definition of disease entities. This new emphasis is best exemplified by testing for cyclin D1 overexpression in mantle cell lymphomas and anaplastic lymphoma kinase (ALK) in anaplastic large cell lymphomas.

Mantle cell lymphoma (MCL) is a malignant proliferation of B cells in the mantle zone of lymphoid follicles. MCL is characterized as a monotonous proliferation of small to medium-to-large lymphocytes with scant cytoplasm and slightly irregular nuclei. Immunophenotypic analyses showed co-expression of CD5 and pan B-cell antigens (CD19, CD20, CD22, and CD24), which is characteristic for MCL, as well as for chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL). Clinically, patients with MCL are characterized by advanced stages (III or IV), and frequent involvement of bone marrow/peripheral blood and other extranodal sites. Despite the use of anthracycline-containing combination chemotherapy for aggressive lymphomas, the median survival of patients remains only 3 to 4 years, indicating MCL as one of the mostly incurable lymphomas. Typical MCL contains a cytogenetic abnormality with t(11;14)(q13;q32) translocation, which involves a rearrangement of the BCL-1 locus. The oncogene deregulated by this alteration has subsequently been identified as cyclin D1. Because the breakpoint on chromosome 11q13 covers a wide range between 15kb and more than 400kb distance from the cyclin D1 gene, Southern blotting or polymerase chain reaction (PCR) analysis cannot detect all of t(11;14). Expression of the cyclin D1 protein can now be readily detected by using the 5D4, DCS-6, or P2D11F11 monoclonal antibody. Normal or reactive lymphocytes do not stain for cyclin D1, and the positive staining in the nuclei of lymphoid cells correlates well with the presence of the t(11;14) translocation and the overexpression of cyclin D1 mRNA. Cyclin D1 immunohistochemistry is now essential for the routine diagnosis of patients with MCL, clearly defined a homogeneous group of patients and illuminated their clinical aggressiveness as contrasted with the indolent course of the negative group (5-year survival: 30% versus 86%, P= .0002). This suggested that cyclin D1-positive and negative groups represent different entities and that the former closely fits the characteristics of
classical, typical MCL. The cyclin D1-negative group might be better termed separately form MCL as “cyclin D1-negative MCL-like B-cell lymphoma”.

Anaplastic large cell lymphoma (ALCL) was first described by Stein et al., originally defined by expression of CD30 (Ki-1 antigen) and a peculiar anaplastic morphology that often infiltrates the nodal sinuses and paracortical areas as sheets of lymphoma cells. Although some morphological variants were proposed afterwards, ALCL has been recognized as a distinct disease entity. The nonrandom chromosomal translocation, t(2;5)(p23;q35), is highly associated with ALCL of especially nodal origin, reported in various studies to be present from 15 to 65% of the cases. This translocation has recently been cloned and shown to result in the fusion of the NPM gene located on chromosome 5 to a newly described kinase gene, ALK, located on chromosome 2, which leads to the expression of a novel fusion protein, p80\textsubscript{NPM/ALK}. Although the precise function of the p80 fusion protein is not known at present, it is believed to play an important role in the pathobiology of ALCLs that express it. The identification of this protein in lymphoma cells by immunohistochemistry is now available on paraffin sections, and is believed to be a reliable marker for the p80 fusion protein that results from the t(2;5) translocation, because ALK expression has not been detected in the cells of the hematopoietic system. The polyclonal antibody against p80\textsubscript{NPM/ALK}, which recognizes ALK, and the subsequently established monoclonal antibodies ALK1 and ALKc, have made it possible to further categorize ALCL as an entity separate from Hodgkin’s disease. Using these antibodies, the immunohistochemical detection of ALK also clearly allowed the recognition of ALK-positive ALCL as a distinct subtype with a much younger age distribution, nodal predilection and good prognosis.

Today, we have a wide battery of monoclonal antibodies reactive in paraffin sections. These have transformed our understanding of lymphoid neoplasia and permitted an impartial analysis of questions that have been unresolved when addressed with only H & E sections.
Shigeo Nakamura, M.D.
Chief
Department of Pathology and Molecular Diagnostics
Aichi Cancer Center Hospital,
Nagoya, Japan

1973-1979 Nagoya University School of Medicine, M.D.
1979-1981 Clinical staff, Department of Internal Medicine,
Chukyo Hospital, Nagoya
1981-1985 Department of Pathology, Nagoya University School of Medicine, Ph.D.
(Cell Biology)
1982-1987 Research fellow
National Institute for Physiological Sciences (Cell Biology)
1988-2000 Research Associate,
First Department of Pathology,
Nagoya University School of Medicine
1988-2000 Senior Staff,
Department of Pathology & Clinical Laboratories,
Aichi Cancer Center Hospital
2000- Chief
Department of Pathology and Molecular Diagnostics,
Aichi Cancer Center Hospital
(Surgical Pathology and Hematopathology)
MEMO
Gene Expression Profiles of Normal Peripheral Human B-cell Subpopulations and B-cell Malignancies

Riccardo Dalla-Favera
Institute of Cancer Genetics, Columbia University, New York, NY, U.S.A.

Gene expression profiling provides functional insights into the phenotype of normal cells and allows a comparison with their transformed counterparts from tumor biopsies. Most B-cell malignancies derive from specific functional subsets of mature B cells in lymphoid organs, including naïve B cells, germinal center (GC) centroblasts (CB) and centrocytes (CC), and memory B cells. In order to gain insights into the biology of GC B cells and in their transformed counterparts, we have analyzed the gene expression profiles of normal B cells and major types of B cell malignancy. We have purified all the major B-cell subpopulations [ naïve B cells (IgD\(^+\), CD27\(^-\), CD38\(^{low}\)), CB (CD38\(^{high}\), CD77\(^+\)), CC (CD38\(^{high}\), CD77\(^-\)), and memory B cells (CD27\(^+\), CD38\(^{low}\))] from human tonsils by magnetic cell separation and compared their profiles with purified tumor cell populations from biopsies of diffuse large cell lymphoma, follicular lymphoma, Burkitt lymphoma and chronic lymphocytic leukemia, using a DNA oligonucleotide chip array (Affymetrix U95A, comprising ~12,000 genes). Each of the four B-cell subsets was purified from five individuals. The results were analyzed using the SPLASH (Structural Pattern Localization Analysis by Sequential Histograming) program. The 20 samples correctly clustered in 4 groups representing naïve B cells, CB, CC, and memory B cells. Moreover, the pattern of expression of genes known to be differentially regulated among naïve, GC, and memory B cells validated the identity of the sorted populations. Gene expression profiling of normal B-cell subsets allowed: i) The identification of signatures specific for each B-cell subpopulation; ii) The analysis of the functional status of various signaling pathways in normal and neoplastic cell populations; iii) The identification of the histogenetic derivation of various tumor subtypes.
Riccardo Dalla-Favera, M.D.

Director,
Institute of Cancer Genetics
Joanne and Percy Uris Professor
Professor of Pathology
Professor of Genetics & Development
Columbia University, College of Physicians & Surgeons
New York, NY
U. S. A.

1976 University of Milan School of Medicine, M.D.
1983 - 1987 Assistant Professor, Department of Pathology, New York University School of Medicine
1987 - 1989 Associate Professor, Department of Pathology, New York University School of Medicine
1989 - 1991 Associate Professor, Department of Pathology, Columbia University
1991 - Professor, Department of Pathology, Columbia University, College of Physicians & Surgeons
1992-1998 Director, Division of Experimental Oncology, Department of Pathology, Columbia University
1992- Professor, Department of Genetics & Development, Columbia University
1992- Joanne and Percy Uris Professor, Columbia University
1992-1997 Deputy Director, Cancer Center Columbia University, College of Physicians & Surgeons
1999- Director, Institute of Cancer Genetics, Columbia University
Precise Estimation of SNP-allele Frequencies and its Significance in Bio-medical Sciences

Kenshi Hayashi
Division of Genome Analysis, Institute of Genetic Information, Kyushu University, Fukuoka, Japan

Many genome-widely distributed polymorphic markers are required to pin-point genes responsible for polygenic traits by association studies. The most common polymorphism in the human genome is single-nucleotide polymorphism (SNP). A large scale discovery of SNPs (i.e., finding many SNPs) is relatively easy owing to recently developed various high throughput analysis techniques. However, SNPs are informative (useful in genetic study) only if they are frequently heterozygous. Thus, collection of SNPs with high heterozygosity is important. We introduce here, a technique named PLACE-SSCP, in which PCR products amplified from genomic DNA are fluorescently post-labeled and electrophoretically separated using a DNA sequencer under non-denaturing conditions. SNP alleles are efficiently detected as separated peaks in the electrophoresis at a high specificity. We also show that by pooling DNA samples and analyzing by PLACE-SSCP, SNP alleles are quantitatively detected, and allele frequencies are very precisely estimated. Using this pooling strategy, we found widely different SNP allele frequencies among populations of different ethnicity.
Kenshi Hayashi, Ph.D.

Professor
Division of Genome Analysis
Institute of Genetic Information
Kyushu University
Fukuoka, Japan

Education:
1968  M.S., Department of Biology, Faculty of Science, the University of Tokyo.
1975  Ph.D., the University of Tokyo.

Research and Professional experience:
1969 - 1975  Research Associate at the Faculty of Pharmaceutical Sciences (Prof. Yoshiki Ohba), Kanazawa University, Kanazawa.
1975 - 1983  Research Associate, Biochemistry Division, National Cancer Center Research Institute, Tokyo.
1978 - 1980  International Research Fellow, at Dept. Chemistry (Prof. Norman Davidson), California Institute of Technology, U.S.A.
1983 - 1985  Chief Investigator and Section Head, Biochemistry Division, National Cancer Center Research Institute, Tokyo.
1987 - 1992  Section Head, Oncogene Division, National Cancer Center Research Institute, Tokyo.
1992 - present  Professor, Division of Genome Analysis, Institute of Genetic Information, Kyushu University.
Molecular Alterations
– Roles in Cancer Detection and Risk Assessment

Li Mao
The University of Texas M. D. Anderson Cancer Center, Houston, TX, U.S.A.

The tumorigenic process in most of epithelial cancers is multistep and requires accumulation of multiple genetic and epigenetic alterations. Modern molecular technology has facilitated a rapid and effective identification of these genetic alterations as well as epigenetic alterations in the past decade. I will focus on smoking related cancers, where the concept of “field cancerization” has been widely used to explain an increased risk of multiple tumors in aerodigestive tract and bladder of smokers. The determination of molecular alterations in early carcinogenesis will not only extend our understanding of underlying biology of the carcinogenic process but also provide molecular markers for cancer risk assessment, early detection, and molecular classification. Deletion of chromosomal regions is the most common genetic alteration in early stage carcinogenesis of smoking related cancers. Using microsatellite analysis, deletions in one of two chromosome alleles at particular regions containing tumor suppressor genes can be revealed as loss of heterozygosity or LOH. Microsatellite instability defined as altered number of repeats in microsatellite markers can also be identified in tumor cells or premalignancies. These genetic alterations might reflect the inactivation of tumor suppressor genes and defects in surveillance of genome fidelity. It has been shown that LOH at critical tumor suppressor loci, such as 3p14, 9p21, and 17p13, could be frequently detected in lungs of smokers who have not yet developed lung cancer. More importantly, many of these deletions were found in epithelial areas without histopathologic abnormalities. LOH and microsatellite instability may also be used as markers for diagnosis or monitoring recurrence of cancers such as bladder cancer. In head and neck, we have demonstrated that LOH analysis might provide additional information regarding treatment effects of chemopreventive agents. Its role in cancer risk assessment and molecular classification has also been explored. Several specific tumor suppressor genes and oncogenes have been studied to determine their roles in risk assessment and prediction of outcomes. FHit, located at 3p14.2 is frequently inactivated in multiple tumor types. We analyzed Fhit protein expression status in early stage non-small cell lung cancer (NSCLC) and epithelium of smokers using immunohistochemistry and found that Fhit expression was significantly decreased or undetectable in an about 50% of stage I
NSCLC. Similar results were obtained in a panel of patients with tongue cancers, where loss of Fhit expression strongly associated with poor survival. Of 372 bronchial biopsies from chronic smokers, 86 biopsies (23%) exhibited decreased or lack of Fhit expression. In 37 (43%) of 86 subjects, decreased or lack of Fhit expression was observed in at least one biopsy site. Loss of Fhit expression was significantly higher in bronchial metaplastic lesions (23/49, or 47%) than in histologically normal bronchial epithelium (63/323, or 20%) (P < 0.001). Smokers with a metaplasia index (MI) >15% had a higher frequency of loss of Fhit expression than those with MI ≤15% (P = 0.015). Interestingly, current smokers had a higher rate of loss of Fhit expression than former smokers (P = 0.02). The association between cigarette smoking and Fhit expression suggests a role of \textit{FHIT} in the initiation of smoking related lung tumorigenesis. Epigenetic alterations such as \textit{de novo} DNA hypermethylation play an important role in regulation of gene expression and carcinogenesis. Role of DNA methylation in lung carcinogenesis will be discussed.
Li Mao, M.D.

Associate Professor of Medicine
Director, Molecular Biology Laboratory,
Department of Thoracic/Head and Neck Medical Oncology,
University of Texas M. D. Anderson Cancer Center
1515 Holcombe Blvd.,
Houston, TX 77030
U. S. A.

1982 Nanjing Medical University, M.D.
1982-1987 Surgical Resident, The First Affiliated Hospital of Nanjing Medical University
1987-1992 Instructor and Attending Surgeon, Department of Surgery, First Affiliated Hospital of Nanjing Medical University
1992-1995 Research Fellow, Johns Hopkins University School of Medicine
1995-1999 Assistant Professor, Department of Thoracic/Head and Neck Medical Oncology, University of Texas M.D. Anderson Cancer Center
1998- Director, Molecular Biology Laboratory
1999- Associate Professor, Department of Thoracic/Head and Neck Medical Oncology, University of Texas M.D. Anderson Cancer Center
Exploring Cancer-related Genes within Novel Amplifications in Various Types of Cancer

Johji Inazawa
Department of Molecular Cytogenetics, Medical Research Institute, Tokyo Medical & Dental University, Tokyo, Japan

Amplification of DNA in certain chromosomal regions frequently activates genes that are associated with tumorigenesis in human tissues. Therefore, exploring novel amplifications and characterizing genes within these amplicons can provide important insights into the pathogenesis of cancer. Comparative genomic hybridization (CGH) is a powerful molecular cytogenetic tool that allows comprehensive analysis at the entire chromosome level and has now become the most popular genome scanning technique. Using CGH we have been investigating DNA copy number aberrations including amplification in various types of tumors and detected novel amplifications at specific regions of certain chromosomes. Of them, we focused on 1q32, 3q26-27, 5p12-13, 8p23.1, 9p23-24, 11q22-23, 13q34, 14q12-21, 15q26, 18p11.3, and 20q, we performed molecular cytogenetic characterization of these amplicons, with the goal for the identification of novel oncogenes and/or cancer-related genes. Until now we have isolated novel genes including MASL1 and GASC1 from the common amplicons at 8p23.1 and 9p23-24, respectively. Expression of the MASL1 (MFH-amplified sequences with leucine-rich tandem repeats 1) was enhanced significantly in malignant fibrous histiocytoma (MFH) tumors bearing the 8p amplicon. The primary structure of its deduced product revealed an ATP/GTP-binding site, three leucine-zipper domains, and a leucine-rich tandem repeat, all of which are important structural or functional elements for interactions among proteins related to the cell cycle. GASC1 (gene amplified in squamous cell carcinoma 1) was isolated from the common amplicons among esophageal squamous cell carcinoma (ESC) cell lines. This gene encodes protein containing PHD and PX domains. The PHD finger is a zinc-finger-like motif widely found in nuclear proteins involved in chromatin-mediated transcriptional regulators. Amplification and consequent over-expression of GASC1 in ESC cell lines suggest that this gene may involved in the progression of a subset of ESC as well as other several types of tumors.
Johji Inazawa, M.D., Ph.D.

Professor
Department of Molecular Cytogenetics,
Division of Genetics,
Medical Research Institute,
Tokyo Medical and Dental University
Tokyo, Japan

1982 Kyoto Prefectural University of Medicine, M.D.
1991 Kyoto Prefectural University of Medicine, Ph.D.
1987-1996 Assistant Professor, Kyoto Prefectural University of Medicine
1996-1998 Assistant Professor, Human Genome Center, Institute of Medical Science,
The University of Tokyo
1998- Professor, Department of Molecular Cytogenetics, Division of Genetics,
Medical Research Institute, Tokyo Medical and Dental University
Molecular Diagnostic Detection of Free Cancer Cells in the Body Fluids of Gastrointestinal and Bladder Cancer Patients with Real-time PCR

Hayao Nakanishi
Division of Oncological Pathology, Aichi Cancer Center Research Institute, Nagoya, Japan

Peritoneal dissemination is the most frequent type of recurrence after curative resection in patients with gastric and ovarian cancers in Japan and Western countries. Free cancer cells derived from serosal invasion might be an indicator of early peritoneal seeding with subsequent formation of metastatic colonies. Their detection, therefore, is likely to be a useful tool for prediction of outcome in such cases. Cytological examination of peritoneal washes has been gold standard for assessment of peritoneal recurrence in gastric cancer patients. Nevertheless, some of the patients with negative cytology results die of peritoneal metastasis after curative surgery. Recently, detection of free cancer cells in the peritoneal cavity by reverse transcriptase-polymerase chain reaction (PCR) using CEA as a target gene has been found to be a more sensitive predictor of peritoneal dissemination than conventional cytology (CY) in gastric cancer patients. Difficulties with this method are the lack of quantitative assessment of free cancer cells and the time required for completion. In this paper, a rapid and quantitative detection method using a real-time fluorescence PCR system (LightCycler) which is newly established to overcome these problems is reported.

Using this device with hybridization probes as fluorophores, we could detect CEA mRNA in peritoneal washes during surgery (within 3 hours) without any post-PCR procedure. This method could reproducibly quantitate 10-10^5 CEA expressing colon carcinoma cells per 10^7 peripheral blood leukocytes, a sensitivity comparable to that of conventional RT-PCR with a wide linear measuring range. Analysis of peritoneal washes from 190 gastric cancer patients with this assay revealed relative values of CEA transcripts in peritoneal washes to correlate well with the depth of tumor invasion (p<0.01). Sensitivity and specificity of real-time RT-PCR using a CEA mRNA cut-off value of 0.1, as determined by ROC curve analysis, were 84 % and 83%, respectively. Survival curve of the 30 patients who were CY(-)PCR(+) was intermediate between the 34 patients who were CY(+)PCR(+) and the 126 with PCR(-) result. Peritoneal recurrence was frequent among PCR(+) patients but rare in the PCR (-) patients. Thus, the CEA mRNA value was a significant independent prognostic factor along with the presence of nodal metastasis and peritoneal metastasis at surgery, whereas CY(+) was not. The results suggest that quantitative real-time RT-PCR of the peritoneal
washes can replace and overcome cytology examination as a tool to assess the degree of risk for peritoneal recurrence in gastric cancer patients.

The diagnostic utility of cytokeratin 20 (CK 20) mRNA quantitation as a urinary marker of urothelial transitional cell carcinoma (TCC) was also evaluated by quantitative real-time RT-PCR analysis on the LightCycler instrument. Spontaneously voided urine was obtained from 27 urothelial TCC patients, 19 patients with other urologic diseases (non-TCC group) and 27 healthy volunteers (Control group). Mean CK 20 mRNA values in TCC (12,937) were significantly higher than those of non-TCC (171) and healthy control (4.55) (p<0.0005 and p<0.0001, respectively). Urinary CK 20 mRNA values were significantly correlated with tumour grade and urinary cytological class, but not with depth of tumour invasion. Sensitivity and specificity of real-time RT-PCR using a cut-off value of 15 were 74% and 83%, respectively, against 30% and 100% with conventional cytology, respectively. Therefore, real-time CK 20 RT-PCR is a more sensitive method to detect free cancer cells in the urine and may be useful to monitor recurrence of urothelial TCC noninvasively.

We conclude that real-time RT-PCR with hybridization probes is a sensitive, quantitative, specific and rapid method to detect free cancer cells in peritoneal washes and urine. This clinically applicable system can also be employed to detect micrometastasis in the lymph node and blood, and has the potential to become routine technique to evaluate the risk of various patterns of recurrence in patients with gastrointestinal and urothelial cancer.
Hayao Nakanishi, M.D.

Section Head
Division of Oncological Pathology,
Aichi Cancer Center Research Institute,
Nagoya, Japan

1979 M.D., Ehime University School of Medicine
1983 Ph.D., Ehime University School of Medicine
1992-1993 Guest Researcher, National Institute on Aging, Baltimore
1994-2000 Section Head, Laboratory of Pathology,
Aichi Cancer Center Research Institute
2000- Section Head, Division of Oncological Pathology,
Aichi Cancer Center Research Institute
Cancer Genetic Counseling: Principles and Practice

S. Robert Young
Department of Obstetrics and Gynecology, University of South Carolina School of Medicine, Columbia, SC, U.S.A..

Genetic counseling is the communication process which deals with the evaluation of genetic risk, conveying of complex genetic information and helping the patient understand the ramifications of some very far-reaching situations. Individuals trained in genetics and counseling can assist the practicing physician in providing this delicate, time-consuming information to patients. Genetic counselors have been working with pediatricians and obstetricians in providing this service for over 30 years. With the tremendous advances in cancer genetics the new field of Cancer Genetic Counseling has emerged.

The Cancer Center at the University of South Carolina now has a Section of Cancer Genetics with Board-certified Genetic Counselors specializing in Cancer Genetics. Oncologists and surgeons refer patients with high risk of inherited cancer susceptibility for evaluation, counseling and possible testing. When testing is carried out, patients return to the Center to discuss the results. Letters describing the sessions and laboratory studies are sent to the referring physician.

A negative cancer genetic counseling/testing session can provide the patient a sense of relief at being no more at risk than the general population for the particular malignancy. A positive cancer genetic counseling session can lead the patient into selecting life-style changes, heightened surveillance, and chemopreventive strategies. More recently, findings of BRCA1/2 carrier status in a breast-ovarian cancer patient can influence surgical decisions. Other genetic markers may prove to be important in other malignancies, as well.

In just a few short years we have learned the importance of genetics in oncology. It is now generally recognized that all cancer has a genetic etiology and progression. The cancer geneticist, the genetic counselor and genetic testing are taking their places with the team of primary care physicians, oncologists, surgeons, and other cancer specialists in providing up-to-date comprehensive oncology services.
Samuel Robert Young, Ph.D., F.A.C.M.G.

Professor,
Director, Cancer and Research Genetics,
Department of Obstetrics and Gynecology
University of South Carolina School of Medicine
Columbia, SC 29203
U. S. A.

1962 Millikin University, B.A.
1966 Northern Illinois University, M.S. (Biology)
1968 University of Michigan, M.S. (Human Genetics)
1972 University of Michigan, Ph.D. (Human Genetics)
1970-1972 Genetic Counselor/Cytogeneticist,
         Heredity Clinic, University of Michigan
1972-1977 Chief, Genetics Laboratory,
         William S. Hall Psychiatric Institute
         South Carolina Department of Mental Health
1977- Professor of Obstetrics and Gynecology,
         University of South Carolina School of Medicine
1978-1991 Director, Division of Clinical Genetics
1991-1994 Director, Division of Genetics
1994- Director, Cancer and Research Genetics,
         Department of Obstetrics and Gynecology,
         University of South Carolina School of Medicine
Proposing Genetic Counseling System in Japan: Experience in the Division of Clinical and Molecular Genetics, Shinshu University Hospital

Yoshimitsu Fukushima
Division of Clinical and Molecular Genetics, Shinshu University Hospital, Matsumoto, Japan

Genetic testing has been widely available and useful in several kinds of familial cancer. Although genetic counseling is fundamental before and after the genetic testing, there is no official system for genetic counseling in Japan. In the United States, the genetic counseling system is established especially in the field of birth defects and is extending to the field of cancer genetics.

In this symposium, I would like to introduce our activity at the Division of Clinical and Molecular Genetics, Shinshu University Hospital and also would like to propose the team approach of genetic counseling, which would become available in the Japanese medical system. Shinshu University Hospital established a division of clinical and molecular genetics as one of its central service departments in 1996 and officially approved by Monbusho in 2000. Our division is composed of several MDs from the departments of neurology, endocrinology, pediatrics, oncology, laboratory medicine and medical genetics, a clinical psychologist, and a genetic nurse. All of the MD staffs are certified members of the Japanese Board of Medical Genetics, Clinical Geneticists or as its trainees.

Persons who seek genetic counseling or genetic testing, visit our clinic usually twice. At the first visit, a staff member collects complete information including family history, correct diagnosis and examination results, and he clarifies the counselee's request. We have a staff meeting once a week to discuss each case for providing the next suitable counseling and the ethical, legal and social issues (ELSI). At the second visit, genetic counseling is provided by an MD specialist and a clinical psychologist or a genetic nurse. Information of genetics or genetic testing is explained by an MD specialist, and psychological support is offered by a clinical psychologist or genetic nurse.

Three hundred and eleven counsellees visited the clinic from May 1996 to March 2000: 170 for birth defects, 37 for obstetric problems, 47 for familial cancers, 29 for neurological disorders and 28 for other reasons. Regarding to familial cancer, we performed genetic testing in 44 cases, including familial adenomatous polyposis, multiple endocrine neoplasia type 1 and 2, familial breast cancer, von Hippel-Lindau disease and Li-Fraumeni syndrome.
Discussion by many kinds of specialists at the staff meeting is very valuable to solve the ELSI in each case. Our team approach of genetic counseling by combination of an MD specialist and a clinical psychologist or a genetic nurse is very practical in Japan. This is the first officially approved clinical genetics department in the National University Hospitals in Japan. We hope our system is familiarized to other hospitals and genetic services in Japan are improved.
Yoshimitsu Fukushima, M.D., Ph.D.

Professor,
Department of Hygiene and Medical Genetics,
Shinshu University School of Medicine
Director,
Division of Clinical and Molecular Genetics,
Shinshu University Hospital
Matsumoto, Japan

1977 M.D., Hokkaido University School of Medicine
1977-1981 Resident, Department of Pediatrics, Hokkaido University Hospital
1981-1985 Medical Staff, Division of Medical Genetics, Kanagawa Children's Medical Center
1985-1986 Medical Chief, Division of Medical Genetics, Saitama Children's Medical Center
1986-1988 Visiting Research Associate, Department of Human Genetics, Roswell-Park Memorial Institute, NY
1988-1995 Medical Chief, Division of Medical Genetics, Saitama Children's Medical Center
1995-present Professor, Department of Hygiene and Medical Genetics, Shinshu University School of Medicine
2000-present Director, Division of Clinical and Molecular Genetics, Shinshu University Hospital
Estimation of Cancer Susceptibility Based on Gene-environment Interaction

Nobuyuki Hamajima and Kazuo Tajima
Division of Epidemiology and Prevention, Aichi Cancer Center Research Institute, Nagoya, Japan

As PCR technology for genotyping develops, the findings on the associations between disease susceptibility and genetic polymorphisms have been accumulated at a rapid pace. Especially, in the field of cancer epidemiology, high risk combinations of genotypes and environmental exposures have been reported for several cancers, indicating that the gene-environment interactions play an important role in carcinogenesis.

In Aichi Cancer Center, HERPACC-II (the Advanced Hospital-based Epidemiologic Research Program at Aichi Cancer Center for gene-environment interactions) launched in 2000, in which all first-visit patients are invited to the Program consisting of lifestyle questionnaire and blood sampling. The expected participants are 2,000 cancer patients and 8,000 non-cancer patients within 2 years. Although the research products have not yet obtained from HERPACC-II, a pilot study for 1,300 participants (950 cancer patients and 350 non-cancer patients) sampled during 1997 to 1999 has been providing several important findings concerning the gene-environment interactions.

*Helicobacter pylori* infection is a well-known cause of stomach cancer. The persistent infection is possibly determined by both genetic traits and lifestyle factors. We found that 1) polymorphisms of inflammation-associated enzymes determined the infection rate, 2) the rate was modified by salty food, fruits intake, and smoking, and 3) there were gene-gene interactions. A polymorphism of L-myc was reported to increase the risk of oral cancer, colorectal cancer, lung cancer, and so forth. We observed that the polymorphism modified the effect of smoking on cancers of the esophagus and lung. Those findings generate useful information for cancer prevention in the near future.

More than 20 projects are now on going for 60 genetic polymorphisms and 7 sites of cancers. A new method, PCT-CTPP (PCR with Confronting Two-Pair Primers) was invented in the course of establishing an effective genotyping system in our laboratory. The HERPACC-II will produce many findings on gene-environment interactions for Japanese.
Nobuyuki Hamajima, M.D., Dr.Med.Sci., M.P.H.
Section head, 
Division of Epidemiology and Prevention 
Aichi Cancer Center Research Institute, Nagoya, Japan

1980 Nagoya University School of Medicine (M.D.)
1984 Nagoya University School of Medicine (Dr.Med.Sci)
1984-1986 Research Associate, Department of Preventive Medicine, Nagoya University 
School of Medicine
1986-1987 University of Washington, School of Public Health and Community Medicine, 
Seattle, U.S.A. (M.P.H.)
1987-1991 Assistant Professor, Department of Preventive Medicine, Nagoya University 
School of Medicine
1991-1993 Associate Professor, Department of Public Health, Gifu University School of 
Medicine
1992 Visiting Scientist, Department of Public Health, University of Sydney, 
Australia
1993-2000 Section Head, Division of Epidemiology
2000- Section Head, Division of Epidemiology and Prevention
MEMO
List of Speakers and Chairpersons

Riccardo Dalla-Favera, M.D.  Joanne and Percy Uris Professor  Director, Institute of Cancer Genetics  Columbia University  1150 St. Nicholas Avenue  New York, NY 10032  U. S. A.  Phone:  212-304-7381  Fax:  212-304-5537  E-mail:  ct289@columbia.edu

Tadaaki Eimoto, M.D., Ph.D.  Professor  Second Department of Pathology  Nagoya City University School of Medicine  1 Kawasumi, Mizuho-ku, Nagoya 467-8601  Japan  Phone:  052-853-8159  Fax:  052-851-4166  E-mail:  teimoto@med.nagoya-cu.ac.jp

Yoshimitsu Fukushima, M.D., Ph.D.  Professor  Department of Hygiene and Medical Genetics  Shinshu University School of Medicine  3-1-1 Asahi, Matsumoto, Nagano 390-8621  Japan  Phone:  0263-37-2617  Fax:  0263-37-2619  E-mail:  yfukush@schmd.shinshu-u.ac.jp

Nobuyuki Hamajima, M.D., Dr.Med.Sci., M.P.H.  Section Head  Division of Epidemiology and Prevention  Aichi Cancer Center Research Institute-Chikusa-ku, Nagoya 464-8681  Japan  Phone:  052-764-2988  Fax:  052-763-5233  E-mail:  nhamajim@aichi-cc.pref.aichi.jp
Kenshi Hayashi, M.D., Ph.D.  Professor
Division of Genome Analysis, Institute of Genetic Information, Kyushu University
3-1-1 Maidashi, Fukuoka 812-8582 Japan
Phone: 092-642-6170
Fax: 092-632-2376
E-mail: khayashi@gen.kyushu-u.ac.jp

Johji Inazawa, M.D., Ph.D.  Professor
Department of Molecular Cytogenetics-Medical Research Institute-
Tokyo Medical & Dental University
1-5-45 Yushima, Bunkyo-ku, Tokyo 113-8510 Japan
Phone: 03-5803-5821
Fax: 03-5803-0244
E-mail: Johinaz.cgen@mri.tmd.ac.jp

Li Mao, M.D.  Associate Professor
Department of Thoracic/Head and Neck Medical Oncology
University of Texas M.D. Anderson Cancer Center
Houston, TX 77030 U. S. A.
Phone: 713-792-6363
Fax: 713-796-8655
E-mail: lmao@notes.mdacc.tmc.edu

Shigeo Nakamura, M.D., Ph.D.  Chief
Department of Pathology and Molecular Diagnostics
Aichi Cancer Center Hospital
1-1 Kanokoden, Chikusa-ku, Nagoya 464-8681 Japan
Phone: 052-762-6111
Fax: 052-763-5233
E-mail: snakamura@aichi-cc.pref.aichi.jp
Hiroshi Shiku, M.D., Ph.D.  Dean
Professor
Second Department of Internal Medicine
Mie University School of Medicine
2-174 Edobashi, Tsu, Mie 514-8507
Japan
Phone:  0592- 31-5016
Fax:  0592- 31-5200
E-mail:  shiku@clin.medic.mie-u.ac.jp

Wataru Yasui, M.D., Ph.D.  Professor
First Department of Pathology-
Hiroshima University School of Medicine-
1-2-3 Kasumi, Minami-ku, Hiroshima 734-8551
Japan
Phone:  082- 257-5145
Fax:  082-257-5149
E-mail:  wyasui@mcai.med.hiroshima-u.ac.jp

Yasushi Yatabe, M.D.  Section Head
Department of Pathology and Molecular Diagnostics
Aichi Cancer Center Hospital
Chikusa-ku, Nagoya 464-8681
Japan
Phone:  052-762-6111, Ext. 3432
Fax:  052-763-5233
E-mail:  yyatabe@aichi-cc.pref.aichi.jp

Robert Young, Ph.D.  Professor,
Director, Cancer and Research Genetics-
Department of Obstetrics and Gynecology
University of South Carolina School of Medicine-
Columbia, SC 29203
U. S. A.
Phone:  803-779-4928, Ext. 230
Fax:  803-434-4699
E-mail:  byoung@richmed.medpark.sc.edu