Cancer Genomics and Molecular Diagnosis — The Nineteenth International Symposium of Sapporo Cancer Seminar

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The Nineteenth International Symposium on Cancer, which was held in Sapporo from 7 to 9 July 1999, focused on three main topics: a) the genomic analysis of cancer, b) the molecular basis of cancer and c) the molecular diagnosis of cancer. The Seminar was inaugurated by Dr. Kobayashi, who reminded the audience of the origin of this Seminar series nearly twenty years ago, after his experience at the Gordon Conferences in the US. The topics of the meeting were selected from among the most rapidly advancing areas of genomics. Dr. Nakamura stressed in his opening remarks that with the progress in the Human Genome Project there will be a huge amount of information that should be exploited for better management of cancer patients. Forty-eight scientific papers were presented (23 talks and 25 posters), with 107 participants (96 from Japan and 11 from the US, Europe and Australia).

The first two sessions dealt with the genomic analysis of cancer, with special emphasis on DNA microarrays ("DNA chips") and comparative genomic hybridization (CGH). In the first talk, Sumio Sugano (Tokyo) presented recent results obtained with a new method to clone full-length cDNA, a crucial requirement in the coming age of "computer cloning," because the ETS are not sufficient to provide clues to gene product function. The procedure represents a significant improvement, especially for long messages. The method is based on the protection of the 5′-end of the mRNA by artificial capping, which leads to enrichment of full-length cDNAs, because only the capped molecules are ligated by tobacco acid pyrophosphatase. The method has generated an impressive amount of data on the features of the gene transcription start sites in both TATA box positive and negative genes. Molecular cytogenetics by the CGH approach was the topic of the next three talks by Johji Inazawa (Tokyo), Olli Kallioniemi (Bethesda) and Joe Gray (San Francisco). The complexity of the quantitative chromosomal changes undergone by the cancer cell was shown by Inazawa, with a large CGH dataset for glioma and esophageal carcinoma. Frequent regions with increased copies were found in several chromosomes and the complex structure of the amplicons was revealed. This makes cumbersome the task of pinpointing the relevant gene under positive selection during tumorigenesis. Despite these difficulties, a novel cancer gene candidate called MASL-1 was identified in the 8p23.1 region in histiocytoma cell lines. Olli Kalloniemi gave a progress report on the new tissue microarrays ("tissue chips") approach for the massive parallel analysis of large numbers of tissues. The tissue chips can be used not only for immunochemistry, but also for gene expression by *in situ* hybridization and also for *in situ* FISH, ("fish on chips") although this last technology still requires further improvement. Prostate and breast cancer were chosen as examples of the problems that can be addressed using this powerful strategy. More than 100 genes were overexpressed in prostate cancer and more than 30 regions exhibited detectable increases of copy number in breast cancer. The potentially important issues of tumor heterogeneity and message abundance were raised in the lively discussion that ensued between Carlos Caldas and Yusuke Nakamura. Joe Gray continued the CGH story and described in detail three topics. First, the application in the clinical arena of global genomic alterations, second, the preliminary stages of development of DNA array approaches for CGH, leading to high-resolution analysis for gene discovery, and finally, the issue previously raised of genomic instability of the cancer cell and its concomitant result of tumor heterogeneity. The first topic led to the gloomy conclusion that due to the enormous complexity of the alterations in cancer, combinatorial approaches for correlative clinical studies would require much more powerful computers then are currently available to handle the nearly infinite possibilities. The second issue also showed the complicated nature of the system, with more than three candidate cancer genes already localized in the relatively tiny area of chromosome 20q13.2. The third topic also provided a somewhat perplexing view of the huge degree of heterogeneity in tumors despite the paradoxical karyotype stability of tumor cell lines.

David Bowtell (Melbourne) opened the second session by giving an overview of the different stages and technical aspects of the global approach to gene expression analysis with DNA chip technology, which was equated to a productive fishing expedition. An example of such a task was presented by Kenshi Komatsu (Hiroshima), who described the isolation by positional cloning of a new tumor suppres-

sor gene responsible for the Nijmegen breakage syndrome (NBS), a cancer-predisposing condition similar to ataxia-telangiectasia (AT). The *NBS* gene appears to be involved in DNA repair, interacting with other members of the family of genes implicated in genome integrity, such as *Rad50* and *mre11*, and possibly with *ATM*, although it does not possess a kinase domain, in contrast with DNA protein kinase. Misao Ohki (Tokyo) presented his studies on the role of the *MG8* (*ETO*) gene family of histone deacetylase complexes in leukemia pathogenesis. The application of a DNA microarray approach yielded many differences in gene expression (up- and down-regulation) in a single elegant experiment. Yoshinori Murakami (Tokyo) described a genomics approach for the localization of a tumor suppressor gene in non-small cell lung cancer on chromosome 11q23. The work provided an excellent example of the difficulties of positional cloning of cancer genes in the absence of germline mutations that narrow down the region containing the gene in question. After tenacious work, the gene(s) has been localized to a 700 kb region, but the first attempts to identify the culprit gene have been unsuccessful and no repressor activity was detected in gene transfer assays of PAC clones. The similarity to the *DCC* gene was discussed and pointed out to be somewhat misleading as the gene's tumor suppressor function is currently doubtful. Mark Skolnick (Salt Lake City) gave a lively talk on the genetic analyses of common cancers, using as examples the breast and ovarian hereditary cancer syndromes and the *BRCA1* and *BRCA2* genes. To a great extent the approach involved a new combination of molecular biology techniques, automation and high-throughput genomic analysis, and informatics. For instance, he described sequencing software that facilitates mutation detection. The current screening for germline mutations in these two genes provides a paradigm for this type of research because their huge size and the technical problem of distinguishing true mutations from polymorphisms. The biology of the system was also dealt with, and interesting functional and structural data linking these proteins with Rad51, and with cell cycle checkpoints and apoptosis was reported. Other familial cancers such as prostate and colon cancer were also discussed, and the opinion was expressed that the frequency of involvement of mismatch repair genes in familial colon cancer has been exaggerated.

In the third session, Yoichi Taya (Tokyo) initiated the talks on the molecular basis of cancer, summarizing his studies on the role of p53 in apoptosis and growth arrest, and the regulation of these activities by phosphorylation and acetylation. The data suggest that the apoptosis and growth arrest domains are different and independent, since deletion mutagenesis of a domain localized to residues 43–63 abolished the former but not the latter. The DNA damage-apoptotic chain appears to differentiate UV damage with involvement of the ATR gene product, while gamma ray damage induces apoptosis through a pathway involving ATM. Continuing with the p53 subject, Takashi Tokino (Sapporo), reported a simple and elegant genome scanning strategy for identifying targets for p53. The strategy involved a combination of approaches using a 20 bp consensus sequence to p53 binding and screening by hybridization, followed by selection in yeast of the cloned sequences by means of a functional assay. From the initial 57 sequences showing p53 binding, 8 represented novel genes regulated by wild-type p53. One of these, called BAI1, which was expressed in brain and repressed in 8/9 glioblastomas, was further characterized to inhibit neovascularization in the rat cornea by basic FGF. Another p53-induced gene exhibited a restricted expression in the thymus and was not induced by mutant p53. The protein was localized to the mitochondria. Overexpression of this protein (TP53AIP1) suppressed cell growth and induced apoptosis. The results suggest that this is a new target for p53 involved in the apoptosis pathway.

In the second session on the molecular basis of cancer, Fumitoshi Ishino (Yokohama) discussed the imprinting of Peg3, a gene encoding a large zinc finger protein only expressed from the paternal allele at high levels in the adult brain. The gene was growth-inhibitory in soft agar assays and tumor-suppressive in nude mouse assays after overexpression in glioma cells, suggesting a tumor suppressor role in brain carcinogenesis. Tetsuo Noda (Sendai) gave a talk providing an excellent example of a reverse genetics approach to the arduous task of detecting the molecular events occurring at the initiation of carcinogenesis. The mouse model system utilizes the *Apc* tumor suppressor gene and a Cre-lox expression system that targets the inactivation of the endogenous gene after infection with an adenovirus vector. While the phenotype was seen very quickly (after two weeks of infection), the results suggested that inactivation of the remaining wild-type *Apc* allele was not sufficient for neoplastic transformation, since only a few adenomas were observed. The viral targeting system also made it possible to examine the specificity of APC inactivation. No tumors developed after infection of the pancreas, but tumors developed in the biliary tract. Manuel Perucho (La Jolla) reviewed the application as an unbiased molecular karyotyping method of arbitrarily primed PCR (AP-PCR) DNA fingerprinting, which was instrumental in the discovery of the microsatellite mutator phenotype pathway for colon cancer. The peculiar features of these tumors were also described and a model was presented to explain the abundance of heterozygous mutations in functional sequences (such as frameshift mutations in *BAX* and *TGFβRII*) while accumulating ubiquitous biallelic mutations in other longer non-functional sequences, such as the poly(A) tails of *Alu* repeats. The model proposes that due to the exacerbated mutator phenotype of these tumor cells, the accumulation

of multiple monoallelic mutations in different genes is as probable as biallelic mutation in a single gene, and that the loss of half the amount of gene products may be sufficient to express the tumor phenotype. The accumulative haplo-insufficiency model is not restricted to genes involved in cell growth or survival, but also applies to genes involved in genome integrity. A scanning approach to detect alterations in DNA methylation was also described and the widespread occurrence of homeotic epigenetic alterations in solid tumors was reported.

Yusuke Nakamura (Tokyo) selected two examples of the cancer gene cloning efforts of his group in the first talk of the third and last session on the molecular basis of cancer. A gene at 3p21.3 was isolated by a large-scale genomic sequencing effort directed at this chromosomal region. The gene, named *DLC1* (deleted in lung cancer 1), exhibits suppressor activity *in vitro* and alterations in gene expression were common in several malignancies, suggesting that the gene may be involved in carcinogenesis of the lung, esophagus and kidney. A second candidate cancer gene was isolated by a differential display approach from murine colon carcinoma cell lines with and without metastatic potential. The human counterpart of the *ream* gene (reduced expression associated with metastasis) was localized to 8p21.3-p22, a region commonly lost in human colon cancer. Repression and somatic *ream* gene mutations were found, suggestive evidence that at least one of the long-sought suppressor genes at the short arm of chromosome 8 has finally been isolated. The talk by Gregory Higgins (Durham) focused on the progress of the serial analysis of gene expression (SAGE) efforts at several collaborative institutions and the establishment of a public electronic database. In the pilot experiment, gene expression in human glioblastomas was compared to that in normal human brain. Among nearly 500 differentially expressed transcripts, genes were classified as angiogenesis-related, transcription factors, and cell cycle genes. Akira Horii (Sendai) reported a thorough genomics analysis of human pancreatic cancer. The data give a vastly complex picture of the many alterations found in this aggressive neoplasm, with high incidences of mutated *K-ras* oncogene, and p16, p53 and *DPC4/SMAD4* suppressor genes. Other chromosomal regions were also frequently lost (1p, 6q, and 12q) or gained (8q and 20q) as detected by CGH analysis, indicating the presence of cancer genes subjected to both negative and positive selective pressure during tumorigenesis. Moreover, enhanced microsatellite instability is present in some pancreatic carcinomas, although its incidence may have been overestimated. None of the few cancers positive for microsatellite instability had mutations in the target genes for the microsatellite mutator phenotype, such as *TGFβRII* or *BAX*. The potential clinical application of these studies was highlighted by the early detection of some of these genetic alterations in

pancreatic juice by microallelotyping and FISH analysis, although the reliability of FISH in this kind of material was the subject of some discussion and caution was recommended in the interpretation of the data. Lauri Aaltonen (Helsinki) gave an interesting overview on the molecular screening of hereditary intestinal cancer syndromes, with particular emphasis on juvenile polyposis and the Peutz-Jeghers hamartomatous polyp and HNPCC familial syndromes. The PJS *LKB-11* gene initially identified at 19p by CGH has been the subject of an extensive mutational analysis, with only a few tumors found positive. Not much is known about the protein function other than its suppressor activity in cells lacking the gene and its Ser-Thr kinase and autophosphorylation activity, which is decreased by non-truncating mutations. Not much progress was reported with the juvenile polyposis syndrome, and the question of which is the culprit gene is still open. An update on the screening of Finnish families for HNPCC germline mutations and counseling issues gave an optimistic outlook since there have been no casualties reported since the screening was implemented a few years ago. Screening of the population at large with a first test for enhanced microsatellite instability (MSI) was shown to be an efficient strategy for the diagnosis of HNPCC. Among over 500 unselected patients with colon cancer, 12% were positive for enhanced MSI and about one-third of them carried a DNA mismatch repair germline mutation, thus qualifying for HNPCC. The study illustrates the power of a simple molecular test for enhanced MSI for cancer diagnosis and prognosis, as well as for diagnosis of cancer susceptibility. The topic of molecular diagnosis of cancer was continued by Mitsuru Emi (Kawasaki) for breast and thyroid cancers. The impressive data showed over 18 chromosomal regions with frequent loss of heterozygosity (LOH) in breast carcinoma, several of which exhibited clear prognostic significance. Several survival curves depending on the presence or absence of LOH at these chromosomal regions (i.e., 1p34) and their combinations (i.e., 1p34 and 17p13.3 or 13q12 and 17p13.3) were strong prognostic indicators, illustrating the power of this molecular genomics approach in clinical oncology. The data on thyroid cancer was no less impressive, despite the histopathological heterogeneity of the tumors and their less well studied molecular genetics.

Takashi Takahashi (Nagoya) gave an overview on the genetic alterations in lung cancer. Mutations in the *Smad2* and *Smad4* genes were found in about 5–10% of cancers, especially in adenocarcinomas. Since the proportion of LOH at 18q is clearly higher (about 40%), the discrepancy was discussed and several possibilities were suggested, in addition to the obvious possible existence of an additional suppressor gene in the region. Chikashi Ishioka (Sendai) reported an elegant assay in yeast to detect the functionality of missense mutations in DNA mismatch repair muta-

tor genes. The assay is based on a dominant mutator effect of overexpression of hMLH1 in yeast, and revealed the functionality of many of the about 30% missense mutations found in HNPCC. Shinzaburo Noguchi (Osaka) gave a couple of excellent examples on the molecular diagnosis of breast cancer. A real-time automated PCR system was worked out for the detection of bone marrow micrometastases using the cytokeratin 19 gene. The results showed the clinical value of such a sophisticated molecular approach, since the results were shown to be useful prognostic indicators. Another example was reported for the diagnosis of breast cancer by FISH of chromosomes 1, 11 and 17 in fine needle aspirates. The findings were the subject of intense discussion to decide the cut-off values of such an approach. The meeting was closed by a lively talk by Carlos Caldas (Cambridge) on a topic of great importance in Japan, hereditary gastric cancer. Confirmation of a cancer predisposition by identification of truncation mutations in the E-cadherin gene, and the description of a family with gastric cancer exhibiting microsatellite instability, even though no mutations were detected in the known mismatch repair genes, were among the highlights of his talk.

The 19th Sapporo Cancer Seminar ended with the general feeling among participants that the meeting had been highly successful and stimulating, with lively discussions after most presentations reflecting a great interest in the topics despite the demanding schedule of the Seminar.