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MEETING REPORT

The 24th International Symposium of Sapporo Cancer Seminar "Pharmacogenomics in Cancer Chemotherapy: Recent Advances in ABC Transporters and Genome Analyses"

Hokkaido University Conference Hall, Sapporo, Japan, June 20 – 22, 2004

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Figure 1 The group picture of participants.

The 24th International Symposium of the Sapporo Cancer Seminar Foundation was held in Sapporo, Japan on June 20 to 22, 2004. A total of 170 research scientists from different countries participated in the symposium (Figure 1). The symposium consisted of four scientific sessions: i.e., oral presentation by invited speakers (Figure 2), poster presentation by participants (Figure 3), luncheon seminar by biotech companies, and corporate exhibition (Figure 4). This symposium was organized by the following organizing committee members: Dr. Tohihisa Ishikawa as the chairman (Tokyo Institute of Technology, Japan), Dr. Yoshikazu Sugimoto (Japanese Foundation of Cancer Research, Japan), Dr. Tetsuya Kamataki (Hokkaido University, Japan), Dr. Piet Borst (Netherlands Cancer Institute, The Netherlands), Dr. Michael M. Gottesman (National Cancer Institute, NIH, USA), and Dr. Victor Ling (British Columbia Cancer Agency, Canada).

SAPPORO CANCER SEMINAR

Before starting the summary of the 24th International Symposium, it might be nice to briefly describe the profile of Sapporo city and the history of Sapporo Cancer Seminar for readers. Sapporo, with a population of more than 1.7 million, is the fifth largest city in Japan, and is the political and business center of Hokkaido, Japan's northernmost island. While there are numerous hot springs throughout the island, Sapporo is internationally known as a winter sports center; the Winter Olympic was held in 1972. The nearest Russian neighbor of Sapporo is Vladiostock, directly to the west across the Japan Sea.

More than twenty years ago, Dr. Hiroshi Kobayashi, Professor Emeritus of Hokkaido University, who had been strongly inspired by the academically liberal atmosphere of the Gordon Research Conferences held

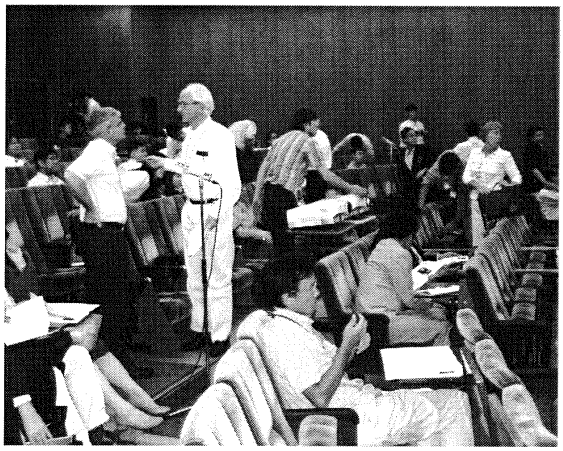

Figure 2 Participants in the conference hall.

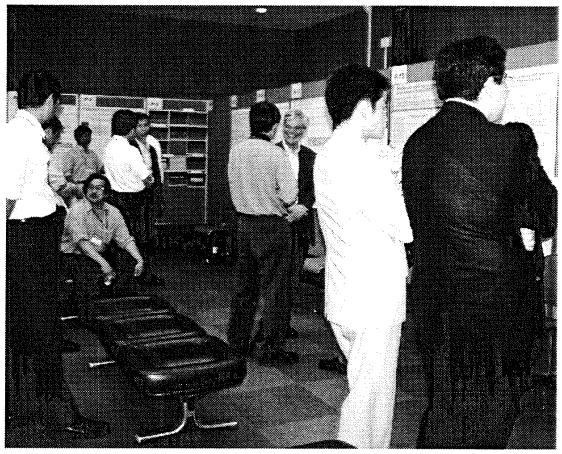

Figure 3 Poster presentation.

in New Hampshire USA, conceived the idea of the Sapporo Cancer Seminar to provide great opportunities for medical researchers to share their knowledge in a relaxed but academic atmosphere. The first Sapporo Cancer Seminar was held in 1981 and thereafter, in 1983, Sapporo Cancer Seminar established itself as a foundation with the warm support of business, pharmaceutical organizations, and the general public. Since 1987 Sapporo Cancer Seminar became a twice-yearly event; seminar is organized in both winter and summer. The winter seminar, which is scheduled to coincide with Sapporo's Snow Festival, an internationally famous event, concentrates upon cancer-related clinical investigations, whereas the summer seminar deals with basic aspects of cancer research.

PHARMACOGENOMICS IN CANCER CHEMOTHERAPY

In the 21st century, emerging genomic technologies (i.e., bioinformatics, functional genomics, and pharmacogenomics) are shifting the paradigm of drug discovery research and improving the strategy of medical care for patients. In order to realize personalized medicine, it is critically important to understand molecular mechanisms underlying inter-individual differences in drug response, namely, pharmacological effect vs. side effect. The occurrence of the variations among persons in the drug response may involve many different causes, for example, genetic variations and/or expression levels of drug target molecules including membrane receptors, nuclear receptors, signal transduction components, enzymes, etc. as well as those of drug metabolizing enzymes and drug transporters. Observations of inter-individual variations in different drug responses have led to the development of pharmacogenetics and pharmacogenomics.

Cancer is one of the gene-associated diseases, involving multiple factors in its cause and development. Despite enormous efforts spent in the development of cancer chemotherapies, often these therapies are effective only in a relatively small proportion of cancer patients. Acquired and intrinsic drug resistance in cancer is the major obstacle to long-term, sustained patient response to chemotherapy. It has been long recognized that the effectiveness of anticancer drugs can vary significantly among individual patients. It is obvious that the susceptibility of cancer cells to particular anticancer drugs cannot be predicted by a single factor but is determined by many factors that influence overall sensitivity. Cancer cells appear to have the capacity to generate variants resistant to anticancer drugs, as part of biological responses to external challenges. Tumors, and even individual cancer cells, can exhibit multiple mechanisms of resistance simultaneously. Therefore, it is critically important to understand molecular mechanisms underlying the multidrug resistance in cancer cells.

TOPICS PRESENTED AT THE 24TH INTERNATIONAL SYMPOSIUM

ABC transpoters and other transporters involved in drug resistance

Multidrug resistance in human cancer is the major obstacle to long-term, sustained patient response to chemotherapy. Several ATP-binding cassette (ABC) transporters cause multidrug resistance in cancer cells by actively extruding the clinically administered

chemotherapeutic drugs. By far, the best-known major drug transporters, i.e., ABCB1 (P-glycoprotein or MDR1), ABCC1 (MRP1), ABCC2 (MRP2, cMOAT), and ABCG2 (BCRP), have been characterized in detail with respect to their structure and function. At the symposium, we have expanded our knowledge with respect to pharmacogenomics and gene regulation of ABC transporters.

Dr. Michael M. Gottesman (National Cancer Institute, NIH, USA) presented two approaches to evaluate the role of the ABC transporters in drug resistance: (a) quantitative RT-PCR analysis of all 48 ABC transporters in 60 NCI established cancer cell lines whose resistance to approximately 100,000 different drugs is known and (b) a microarray approach using a chip enriched in cDNAs and oligos for the ABC transporters and other detoxifying genes (ABC Toxi-chip). These approaches have led to the identification of several more ABC transporters associated with drug resistance both in cancer cell lines unselected in anticancer drugs, and in highly selected cell lines. In addition to identifying these transporters, this approach also yields information about substrates for these ABC transporters. Furthermore, Dr. Gottesman demonstrated that the RT-PCR and microarray chip technology was easily applicable to clinical samples and could be used to discover whether ABC transporter expression is associated with anticancer drug resistance in human cancers.

Dr. Morimasa Wada (Kyushu Univ., Japa) presented his recent study on SNPs in ABCC2 and ABCB1 genes as well as their clinical impact in drug response and carcinogenesis. He has analyzed the nucleotide sequence polymorphisms in the 5' upstream regulatory region of the gene spanning 4kb from the transcriptional start site of *ABCB1* and *ABCC2* in addition to coding region and adjacent intron sequence, and tried to identify any associations between the polymorphisms and the expression. In normal colorectal mucosa, diplotypes at the regulatory region showed more significant association with the expression level of ABCB1 mRNA than each SNP did. In an in vitro reporter assay, transcription activity of the minor-type construct carrying haplotypes 2 and 3 was significantly lower than that of the major-type construct carrying haplotype 1. Dr. Wada further identified two DNA binding proteins those bound to the nucleotide sequence carrying major type alleles but not or significantly weaker to that carrying minor alleles. His data suggested the significance of the SNPs invariable expression in colon inter-individually. The association between the regulatory SNPs / haplotypes of *ABCB1* and *ABCC2* genes and response to anti-cancer drugs or incidence of colorectal carcinogenesis, were also presented.

Dr. Balázs Sarkadi (National Medical Center, Hungary) addressed the critical role of amino acid 482 in ABCG2 (BCRP/MXR/ABCP). He has characterized several naturally occurring polymorphic, as well as drug-selection generated mutant variants of this protein. ABCG2 mutants, containing Arg (wild-type), Gly, or Thr at amino acid position 482, showed different substrate recognition and drug-transport patterns. Based on these results we have generated several other amino acid 482 mutants, with variable substrate recognition and transport patterns, in the hope of obtaining relevant structure-function information on this protein. It was demonstrated that ABCG2 had a high-affinity interaction with several tyrosine kinase inhibitors, currently under development or already in clinical use in cancer therapy. This interaction is considered to significantly modify the anticancer activity, as well as absorption, distribution and toxicity of these compounds. In addition, Dr. Sarkadi presented a special application of the ABCG2 protein in gene therapy, as a tailor-made selectable marker for the protection of genetically modified stem cells.

Dr. Yoshikazu Sugimoto (Cancer Chemotherapy Center, JFCR, Japan) identified two *ABCG2* SNPs, 376C>T (Q126Stop) and 421C>A (Q141K), that greatly diminish BCRP expression. No active ABCG2 will be made from the 376C>T allele. Cells transfected with BCRP(421C>A) cDNA expressed low amount of BCRP protein and showed low-level drug resistance. In addition, he recently identified two new naturally occurring base changes (infrequent SNPs) in the *ABCB1* gene that were also associated with low ABCB1expression in the transfectants. Polymorphisms of the *ABCG2* and *MDR1* genes that cause low transporter expression would be associated with hypersensitivity of normal cells to substrate anticancer agents. Since such transporters play crucial roles in the absorption and excretion of certain drugs, SNPs of these genes should be considered in the clinical development of new anticancer agents and multidrug resistance-reversing agents.

Dr. Roohangiz Safaei (Univ. California san Diego, USA) provided new aspects of copper transporters in the regulation of cellular pharmacology and intracellular trafficking of the platinum-containing drugs. By means of digital imaging and confocal microscopy, Dr. Safaei could demonstrate that the Pt-containing drug cisplatin co-localizes with Cu transporters ATP7A and ATP7B and induces the subcellular trafficking of these proteins. Furthermore, negative association was observed between the expression levels of ATP7A and ATP7B and the outcome of Pt therapy in many tumor types. Therefore, these copper transporters may be involved in cellular resistance to cisplatin and other platinum-containing anticancer agents.

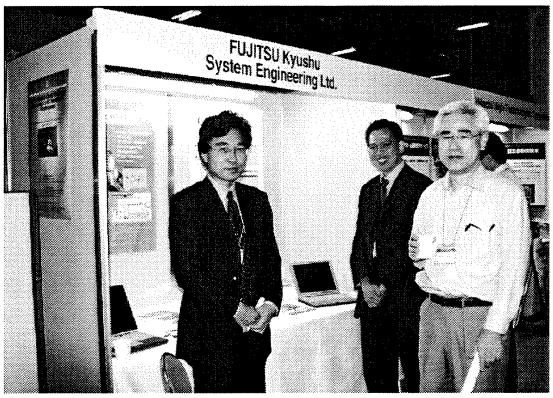

Figure 4 Corporate exhibition.

Clinical Aspects

Clinical evidence conveys important information as to the development of new drugs and therapeutic strategies. It is now known that inherited differences among individuals may also affect drug efficacy and toxicity. Such differences include genetic polymorphisms in drug targets and drug-metabolizing enzymes, as well as in drug transporters. This symposium addressed current clinical issues regarding the individual difference in drug efficacy and side effects to gain insight into the underlying molecular and genetic mechanisms.

Dr. Maja de Jonge (Erasmus Medical Center, the Netherlands) examined potential links between genetic polymorphisms and individual differences in drug exposure. Irinotecan was administered to 65 cancer patients as a 90-min infusion (dose, 200–350 mg/m^2), and pharmacokinetic data were obtained during the first cycle. All patients were genotyped for variants in genes encoding ABCB1, ABCC1 and ABCC2, ABCG2, carboxylesterases (CES1, CES2), cytochrome P450 isozymes (CYP3A4, CYP3A5), UDP glucuronosyltransferase (UGT1A1), and a DNA-repair enzyme (XRCC1), which was included as a nonmechanistic control. Eighteen genetic variants were found in nine genes of putative importance for irinotecan disposition. The homozygous T allele of the ABCB1 1236C>T polymorphism was associated with significantly increased exposure to irinotecan (P=0.038) and its active metabolite SN-38 (P=0.031). Pharmacokinetic parameters were not related to any of the other multiple variant genotypes, possibly because of the low allele frequency. The extent of SN-38 glucuronidation was slightly impaired in homozygous variants of UGT1A1*28, although differences were not statistically significant (P=0.22). However, in a subsequent prospective trial, a significant relationship was found between the homozygous UGT1A1*28 allele and an increased plasma exposition to SN-38. No significant changes in irinotecan pharmacokinetics were observed in relation to the ABCG2 421C>A genotype

To predict probability of adverse effects on paclitaxel treatment, Dr. Yoshio Miki (Tokyo Med. Dent. Univ./ Genome Center, JFCR, Japan) conducted genotyping analysis on breast cancer patients enrolled onto neo-adjuvant weekly paclitaxel therapy. Patients exhibiting almost the same stage of breast cancer were administrated single agent of paclitaxel (80mg/m^2). Adverse effects were evaluated every 7 days and graded according to NCI-CTC grading. To elucidate SNPs associated with granulocytopenia, genotypes of 2,727 SNPs over 298 genes on 54 participants were determined. Associations were examined by comparing genotypes of patients with and without granulocytopenia. The association analyses revealed SNPs on two loci were associated with granulocytopenia (P= 0.0020, OR = 10.7, 95% C.I.: 2.09 55.6 and P=0.0062, OR=5.11, 95% C.I.: 1.41 18.5). In addition, the probability of granulocytopenia on paclitaxel treatment was calculated more precisely by a combination of genotype on two loci. Based on the frequency in Japanese population, it is strongly suggetsed that those studies for prediction of granulocytopenia by paclitaxel treatment would benefit more than 50% of Japanese patients.

Dr. Takashi Tsuruo (Univ. Tokyo, Japan) has demonstrated the development of a new MDR reversal drug, MS-209. At present, new multidrug resistance-reversal agents have been developed and are currently in clinical trials. MS-209, a quinoline derivative, showed a good profile in phase 1 clinical study, and showed promising effects in phase 2 study in combination with CAF-therapy against drug resistant breast cancer. At the symposium, Dr. Tsuruo announced that the drug has recently entered the phase 3 clinical study.

Corporate exhibition and luncheon seminar

New technologies are emerging for screening of new drug candidates. Biotechnology and information technology companies presented their new technologies and products in corporate exhibition (Figure 4) and luncheon seminar sessions.

To quantitatively evaluate the effect of genetic polymorphisms on the substrate specificity of ABC transporters, such as ABCB1 (P-gp/MDR1) and ABCG2 (BCRP/ABCP), BioTec Co., Ltd. (Tokyo, Japan) and GS PlatZ Co, Ltd. (Tokyo, Japan), in collaboration with Ishikawa laboratory (Tokyo Inst. Tech., Japan), have developed high-speed screening systems (i.e., ATPase activity and vesicle transport measurements), as well as a new structure-activity relationship (SAR) analysis

Figure 5 Dinner at Sapporo Beer Garden.

method. By using the Markush TOPFRAG program, chemical fragmentation codes were generated for test compounds and the multiple linear regression analysis was carried out to gain a structure-activity relationship (SAR) between the substrate specificity and the chemical fragmentation codes thus generated. Thereby several sets of chemical fragmentation codes were identified. The new SAR analysis using chemical fragmentation codes is considered as a powerful tool to quantify the impact of amino acid substitutions on the function of ABC transporters.

On the other hand, surface activity is known to be an important characteristic of drugs, influencing their formulation as well as correlating to their solubility and ADME/Tox properties. The measurement of surface activity has, however, been exceedingly slow and consuming large amounts of material. To resolve this bottleneck, Dr. Paavo K.J. Kinnunen (Kibron Inc., Finland) has developed a new platform, Delta-8 multichannel microtensiometer, allowing to determine the surface activity of compounds using the standard 96-well microplate format and requiring only 5-30 micrograms of compound. Readout for the plate takes less than two minutes and yields the surface activity profile for 6-8 compounds, with CMC/solubility, air/water partitioning coefficient, and cross sectional area. Comparison of the measured data with those in literature demonstrates correlation to passive permeation through the blood brain barrier as well as susceptibility to transfer by ABCB1 (P-gp/MDR1). Surface activity profile further correlates to long-term toxicity, phospholipidosis in particular.

SOCIAL PROGRAM: BAR-B-Q DINNER AT SAPPORO BEER GARDEN

In the evening of Jun 21, all participants enjoyed wonderful food and conversation at Sapporo Beer Garden (Figure 5). The old brewery in Sapporo reproduces the liveliness of a beer factory in the good old days. In giant "Kessel" (iron pan) constructed in 1912 for the fermentation of beer sits in the middle of the high-ceiling atrium, where symposium participants enjoyed the Bar-B-Q dinner. In the dinner menu, choicest Hokkaido's seafood was also provided to all participants in the unique northern style, namely, "dynamic and adventurous". This significantly stimulated the friendly atmosphere.

Conclusion

The 24th International Symposium of the Sapporo Cancer Seminar Foundation was very successful. With the help of all experts of the Organizing Committee and the Sapporo Cancer Seminar Foundation, we could assemble an outstanding scientific program in the most topical fields of cancer therapeutics and their clinical applications. On behalf of the Organizing Committee, the author cordially thanks all of the participants for their contribution to the international symposium. The abstract and symposium photos are available at the web site: http://www.humanABC.bio.titech.ac.jp.