Meeting Report

Innovative Approaches to the Prevention, Diagnosis, and Therapy of Cancer

Fourth Joint Conference of the American Association for Cancer Research (AACR) and the Japan Cancer Association (JCA)

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The fourth joint conference of the AACR and Japan Cancer Association entitled “Innovative Approaches to the Prevention, Diagnosis, and Therapy of Cancer,” held February 16–21, 1998, at the Maui Marriott Resort, Maui, Hawaii, was attended by over 350 scientists from Japan and the United States. In addition, representatives from approximately 19 other countries were present. The goals of the joint conferences have been and continue to be informing scientists from both the United States and Japan on various facets of recent cancer research and stimulating potential collaborations between investigators, aimed at the ultimate solution of the cancer problem.

Opening remarks were made by the co-organizers of the conference, Kaoru Abe (National Cancer Center, Tokyo, Japan) and Edward Bresnick (University of Massachusetts Medical Center, Worcester, MA). This introduction was followed by two keynote lectures. In his talk, “Cancer Control through Genetics,” Frederick Li (Dana-Farber Cancer Institute, Boston, MA) addressed the importance of studying populations at high risk for development of cancer, which could provide leads that could be pursued in humans with sporadic incidence of this disease. These types of studies have led to mutational analyses, for example, of the retinoblastoma (Rb), p53, BRCA1 and BRCA2, APC, and the DNA mismatch repair genes. He pointed out the sequential occurrence of second and third cancers in individuals who exhibit Rb and p53 mutations and, therefore, the importance of early diagnosis. The relevance to cancer research of the germ-line p53 mutations in the disease that was named after this investigator, the Li-Fraumeni syndrome, was clearly emphasized. The studies cited by Dr. Li reinforced the key role of early diagnosis through mutational analysis in any cancer prevention program.

The second keynote lecture, by Tadamitsu Kishimoto (Osaka University Medical School, Osaka, Japan), entitled “Cytokines in Cancer: From the Gene to the Clinic,” summarized the recent progress in cytokine research that has led to clinical application in diverse fields. He illustrated the present understanding of cytokine-induced signaling pathways, with particular reference to his discovery of interleukin 6, a protein that evolves a response through a novel regulatory molecule, SSI-1. SSI-1 acts as a negative feedback inhibitor of the Janus-activated kinase-signal transducers and activators of transcription regulatory pathway. Functions of other cytokines, such as cardiotrophin-1, a member of the interleukin 6 family, were discussed. The importance of transgenic and knockout mouse experiments in defining the physiological function of this cytokine was emphasized. This lecture clearly demonstrated the outstanding success of research on cytokines in developing an information base that will contribute to future cancer treatment protocols.

Session 1 of the conference, “Growth Factors, Cytokines, and Signal Transduction,” was cochaired by Kumao Toyoshima (Osaka Medical Center for Cancer and Cardiovascular Diseases, Osaka, Japan) and Harold Moses (Vanderbilt Cancer Center, Nashville, TN). In the first talk in this session, Frank McCormick (University of California, San Francisco, CA) discussed the clever use in cancer therapy of a unique adenoviral vector, which only replicates in cells that contain a mutated p53 and, subsequently, results in their lysis. The efficacy of this vector was initially established in the nude mouse bearing human tumor xenografts. Confirmation of the approach, i.e., effective cancer therapy, was afforded in humans by injection of the vector directly into head and neck cancers. This therapy is currently under investigation in a Phase II clinical trial. Dr. McCormick indicated additional applications of this gene therapy approach in ovarian cancer; prior animal work had shown the localization of the viral vector in peripheral tumors after i.p. administration.

Both Jeffrey Wrana (Hospital for Sick Children, Toronto, Ontario, Canada) and Harold Moses discussed various phases of TGF-β signaling. This cytokine occurs as a superfamily, the members of which must dimerize, in general, to demonstrate biological activity. In addition, Dr. Wrana clearly pointed out the necessity for specific receptors in transducing the signal and for the involvement of Smad proteins as effectors. The latter have been conserved throughout evolution, which is indicative of their importance as signal transducers in normal development. Dr. Moses presented the many biological effects of TGF-β, including chemotaxis, formation of connective tissue, modulation of the immune response, and inhibition of cell proliferation in certain tissues. He also discussed the complexity of interactions between TGF-α and TGF-β in mammary tumorigenesis. Finally, he presented data on the involvement of a number of the Rho family of G proteins in the negative regulation of TGF-β-mediated transcriptional activation, which may be accomplished through an effect upon ubiquitin-facilitated proteolysis.

Masabumi Shibuya (University of Tokyo, Tokyo, Japan) summarized the role of the VEGF/VEGF receptor system in tumor angiogenesis. He also reviewed the role of tumor-derived VEGF in the formation of ascites and in tumor cell growth. He indicated that GFx but not wortmannin inhibited VEGF-induced mitogen-activated protein kinase and DNA synthesis; these were unique aspects of the VEGF receptors in the tyrosine kinase-type receptors. Tadatsugu Taniguchi (University of Tokyo, Tokyo, Japan) presented studies leading to the identification of novel transcription factors, IRF-1 and IRF-2, which bind to the virus-inducible enhancer-like elements of the human IFN-β gene. By use of knockout technology, IRF-1 was shown to be responsible for the Th1-type immune response. He also discussed the role of IRF-1 in the regulation of oncogenesis and demonstrated that this transcription factor acted as a tumor suppressor in concert with p53.

Session 2: “Cell Cycle Control and Transcriptional Activation,” 2

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2 The abbreviations used are: TGF, transforming growth factor; VEGF, vascular endothelial growth factor; CDK, cyclin-dependent kinase; CAD, caspase-activated deoxyribonuclease; ICAD, inhibitor of CAD; MMP, matrix metalloproteinase; LOH, loss of heterozygosity; AGMT, O6-alkylguanine DNA methyltransferase.
was cochaired by Robert Weinberg (Whitehead Institute for Biomedical Research, Cambridge, MA) and Yoji Ikawa (Tokyo Medical and Dental University, Tokyo, Japan). In this session, Kenneth Kinzler (The Johns Hopkins Oncology Center, Baltimore, MD) discussed the genetics of colon cancer, particularly in regard to the roles of the APC and p53 genes. The regulation of transcription by β-catenin has proven to be of major concern in colon cancer. Mutations in the APC gene and in β-catenin expression remove the transcriptional block, thereby playing a prominent role in the early phases of colon carcinogenesis. Preliminary evidence obtained by Dr. Kinzler suggests that inhibiting the coupling between Tcf and β-catenin could serve as a therapeutic goal in this disease. Finally, the master role played by p53 in blocking both the G1 (through p21) and G2 (through 14-3-3) phases of the cell cycle was discussed.

Tetsu Akiyama (Osaka University, Osaka, Japan) continued the discussion on the role of APC. He showed that overexpression of APC blocked the serum-induced cell cycle progression from G0/G1 to the S phase. Mutant APCs, which were observed in familial adenomyoplasia and/or colorectal tumors, were less inhibitory and only partially inhibited the activity of normal APC. The cell cycle blockade induced by APC was alleviated by overexpression of cyclin E/CDK2 or cyclin D1/CDK4. Dr. Akiyama also reported that the APC-β-catenin complex bound to HDLG, the human homologue of Drosophila discs large (dlg) tumor suppressor protein.

Yoichi Taya (National Cancer Center Research Institute, Tokyo, Japan) presented a unique approach to studying Rb and p53. He generated antibodies that recognized about 13 phosphorylation sites of Rb and 11 phosphorylation sites as well as 2 acetylation sites of p53. He showed that CDK4- and CDK2-specific phosphorylation sites can be distinguished in the Rb protein by these antibodies. In p53, phosphorylation of Ser15 occurred when cells were treated with DNA-damaging agents. Moreover, he proposed an interesting hypothesis on the selection of apoptosis and G1 arrest based upon differences in phosphorylation sites on p53.

Steven Dowdy (Washington University School of Medicine, St. Louis, MO) summarized the regulation of the cell cycle and the migration of cells mediated by protein transduction mechanisms. In particular, he discussed the roles of hypophosphorylated and hyper-phosphorylated forms of Rb in the control of the cell cycle and reviewed evidence for the impact of TGF-β, the CDKs, and the cyclins on this process. In addition, he reviewed the cascade mechanism in which hepatocyte growth factor, c-met, p27, and cdc42 were involved.

Takeharu Nishimoto (Kyushu University, Fukuoka, Japan) discussed the HCF and RCC1 genes, which he and his colleagues had isolated using temperature-sensitive (ts) mutants of the herpes simplex virus in the hamster BHK21 cell line. After viral infection, a complex consisting of HCF, cellular Oct-1, and a virion protein VP16 is formed consisting of HCF, cellular Oct-1, and a virion protein VP16 is formed. In addition, he reviewed the cascade mechanism in which hepatocyte growth factor, c-met, p27, and cdc42 were involved.

Session 3, “Apoptosis,” was cochaired by Yoshiyuki Hashimoto (Sasaki Research Institute, Tokyo, Japan) and Alan Eastman (Norris Cotton Cancer Center, Hanover, NH). The session began with an overview of the current understanding of the pathways of apoptosis. Dr. Eastman emphasized that there are numerous initiators of apoptosis that activate multiple different pathways and that much information on apoptosis may be unique to each specific pathway. These various signals are then integrated through effector pathways, components of which include members of the mitogen-activated protein kinase family; for example, extracellular regulated kinase is commonly antiapoptotic, whereas Jun kinase is frequently proapoptotic.

The consequence of this imbalance in signal transduction is disruption of mitochondrial integrity with release of cytochrome c. Cell lysates from undamaged cells have been used to show that cytochrome c is essential for activation of a cascade of proteases (caspases) that eventually lead to degradation of many proteins, DNA digestion, and the characteristic morphology of apoptosis. Referring to aspects of his own research, he described the cloning and characterization of deoxyribonuclease II as an endonuclease potentially involved in apoptosis and the potential role of protein phosphatase 1 in regulating the release of cytochrome c. Finally, he questioned the requirement of p53 in apoptosis induced by DNA-damaging agents, pointing out that most of our current drugs were originally discovered because of their ability to kill p53-defective cells. He suggested that therapy might be effectively targeted to cell cycle checkpoints that are defective in p53-effective cells.

Shigekazu Nagata (Osaka University Medical School, Osaka, Japan) briefly reviewed the apoptosis pathway activated when a cell is stimulated through the cell surface fas receptor. The activated receptor recruits adapter molecules, which, in turn, recruit and activate Flice/caspase 8. This activates a cascade of proteolytic cleavage events, resulting in destruction of the cell. Most of the presentation focused on identification and characterization of an endonuclease that is activated in response to this protease cascade. This endonuclease has been termed CARD. CAD forms a complex with another protein, ICAD. Activation of the fas receptor leads to cleavage of ICAD, permitting CAD to enter the nucleus and digest DNA. The best evidence for the involvement of CAD in apoptosis comes from experiments in which a cleavage-resistant mutant of ICAD was found to prevent apoptosis induced by several different insults, although, as expected, the cells still died. Many other investigators have identified various endonucleases in apoptosis, but none have presented such convincing evidence for its involvement in apoptosis.

Douglas Hanahan (University of California, San Francisco, CA) discussed the important role of antiapoptosis genes in multistage models of carcinogenesis. Whereas it has appeared logical that suppression of apoptosis should be an early event in the carcinogenic process to keep an initiated cell alive, the results presented demonstrated that suppression of apoptosis is probably a late event. The experiments involved a transgenic mouse model in which the SV40 T antigen is expressed in pancreatic β islet cells. Numerous hyperplastic islets were observed, some of which turned on angiogenesis and became vascularized; a subset of these progressed to small, large, and invasive tumors. The proliferation index increased at each stage in this model, but the apoptotic index also increased, so that there was little net increase in cell number. However, there was a marked suppression of apoptosis in the tumors that correlated with their increase in cell number. It was suggested that the mechanism of resistance to apoptosis in the tumors is attributable to up-regulation of Bcl-X and/or down-regulation of its proapoptotic partner Bak. Changes in insulin-like growth factor 2 and a new candidate tumor suppressor on chromosome 9 may also be implicated in this resistance. Similar results have been obtained in a skin tumor model. The results suggest that continuing apoptosis during tumor development facilitates enhanced proliferation and, therefore, increases the probability of further genomic change and cancer.

The final two speakers in this session both addressed the mechanism of action of Bcl-2 family members, focusing on their ability to regulate mitochondrial integrity. Yoshihide Tsujimoto (Tokyo Medical and Dental University, Tokyo, Japan) presented results on experiments in isolated mitochondria. He discussed three proposed models as to how Bcl-2 functions: (a) by sequestering procaspases and preventing their activation; (b) by inhibiting release of cytochrome c from mitochondria, which is required for activation of cytosolic caspases; and...
Ca²⁺ and mitochondrial integrity when exposed to various agents such as cadherin-catenin system, which mediates Ca²⁺-dependent homophilic cell-cell adhesion. Mutations occur in the genes encoding E-cadherin and its undercoat proteins, a- and b-catenins; these proteins connect E-cadherin to actin filaments in establishing firm cell-cell adhesions. Mutations in these genes were frequently detected in diffusely infiltrating carcinomas such as scirrhous gastric cancer. Transcriptional activation of E-cadherin caused by CpG methylation in the promoter also plays a significant role in the dedifferentiation of carcinomas. Finally, the cadherin-catenin system is inactivated transiently during metastasis, by the growth factor-stimulated tyrosine phosphorylation of b-catenin through association of the latter with tyrosine kinase growth factor receptors.

Ohkio Hino (Cancer Institute, Tokyo, Japan) presented his findings on multistate carcinogenesis in the Eker rat model of hereditary renal carcinoma. These rats are heterozygous for a mutation of the tuberous sclerosis 2 (Tsc2) gene. He elegantly demonstrated that a second, somatic mutation of wild-type Tsc2 is a rate-limiting step in renal carcinogenesis in the Eker rat and showed the tumor suppressor nature of Tsc2. Histologically, tumors in this model system develop through multiple stages from early preneoplastic lesions, e.g., phenotypically altered renal tubules, to adenomas in virtually all heterozygotes by the age of 1 year, thus facilitating analysis of essential events in carcinogenesis. He has isolated subtracted cDNA clones, including fra-1, that exhibit increased expression in the Eker rat renal carcinoma and is currently evaluating the functions of these genes.

Thomas Kunkel (National Institute of Environmental Health Sciences, Research Triangle Park, NC) reviewed data on DNA replication fidelity, mismatch repair, and genome instability. High-fidelity DNA replication required: (a) accurate selection of appropriate dNTPs by the DNA polymerases; (b) editing of mistakes by exonucleolytic mechanisms; and (c) correction of the few errors that escape the latter by DNA mismatch repair. Failure of any of these processes could lead to genome instability. Dr. Kunkel used results from his own laboratory to demonstrate structure-function analysis with the Klenow fragment of DNA polymerase, HIV-1 reverse transcriptase, and mammalian DNA polymerase β. These structural biological studies established the importance of a number of the subdomains of these polymerases in determining the error rate as manifested through direct miscoding or template-primer slippage. He further explored the correction of replication errors by mismatch repair systems using human cell lines that have mutations in MSH2, MSH3, MSH5, MLH1, or PMS2. The defects in correction processes are alleviated by introducing human chromosomes containing the relevant mismatch repair genes into the mutated cells. Additional studies with cell lines obtained from patients that are predisposed to cancer confirmed the E6 and E7 insertions. The results indicated that viral and other proteins in human cells can disrupt processes that safeguard the genome.

Motoharu Seiki (University of Tokyo, Tokyo, Japan) discussed the role of MMPs in multistage carcinogenesis. These enzymes are required for the localized degradation of the extracellular matrix on the cell surface, an important process in tumor cell invasion. MT1-MMP, a new class of MMPs discovered by this group, is expressed in both cancer cells and surrounding fibroblasts; this enzyme is required for the activation of progelatinase A. Tissue inhibitor of metalloproteinase-2 is required as an adaptor for the binding of MT1-MMP to progelatinase A, which is subsequently accompanied by the efficient processing of neighboring MT1-MMP. The latter enzyme represents a key molecule in the assembly of type IV collagenase (gelatinase A) at the invasive tumor cell surface.

Setsuo Hirohashi (National Cancer Center Research Institute, Tokyo, Japan) presented results on multistage carcinogenesis, showing the inactivation of the cadherin-catenin system, which mediates Ca²⁺-dependent homophilic cell-cell adhesion. Mutations occur in the genes encoding E-cadherin and its undercoat proteins, a- and b-catenins; these proteins connect E-cadherin to actin filaments in establishing firm cell-cell adhesions. Mutations in these genes were frequently detected in diffusely infiltrating carcinomas such as scirrhous gastric cancer. Transcriptional activation of E-cadherin caused by CpG methylation in the promoter also plays a significant role in the dedifferentiation of carcinomas. Finally, the cadherin-catenin system is inactivated transiently during metastasis, by the growth factor-stimulated tyrosine phosphorylation of b-catenin through association of the latter with tyrosine kinase growth factor receptors.

Dr. Thompson also presented a model of multistage carcinogenesis in which Bcl-X was targeted to skin by expression from a keratin promoter. The first notable observation was that these cells did not die when they were subjected to UV light (no “sunburn cells”), but there was no increased incidence of tumors. Bcl-X could not substitute for Bcl-2 when they were subjected to UV light (no “sunburn cells”), but there was no increased incidence of tumors. Bcl-X could not substitute for Bcl-2. Bcl-2 also prevented hyperpolarization of the mitochondrial inner membrane, rather than loss of ΔΨ. This hyperpolarization leads to mitochondrial swelling, rupture of the outer mitochondrial membrane, and release of cytochrome c, so that it can then activate caspases. Furthermore, decrease in ΔΨ is not required for release of cytochrome c; rather, it occurs as a consequence of caspase activity. The importance of cytochrome c release was demonstrated in experiments in which microinjection of cytochrome c into the cytosol was found to be a potent activator of the caspase cascade. Bcl-X was suggested to maintain a decreased ΔΨ and prevent the swelling and rupture of the outer mitochondrial membrane. These results are consistent with the crystal structure and physiology studies showing Bcl-X may function as an ion channel.

Session 4, “Multistage Carcinogenesis,” was cochaired by Thea Tlsty (University of California, San Francisco, CA) and Hiroshi Kobayashi (Sapporo Cancer Seminar, Sapporo, Japan). In her talk, Dr. Tlsty pointed out the importance of assessing genomic integrity and the definition of cellular activities to determine whether the cell cycle is to progress or halt. Mutations in any of the many cellular pathways may perturb the system through a disruption in chromosomal integrity and loss of genomic stability. The protein products of the p53 and Rb tumor suppressor genes are key components in controlling genomic stability and, therefore, in determining neoplasia. Their importance was demonstrated through an assessment of the ability of cells that had been transformed to express E6 and E7 of the human papillomavirus type 16 to amplify the endogenous CAD gene after exposure to chemotherapeutic agent. Additional studies with cells obtained from patients that are predisposed to cancer confirmed the E6 and E7 investigations. The results indicated that viral and other proteins in human cells can disrupt processes that safeguard the genome.

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In Session 5, “New Frontiers in Cancer Diagnosis,” which was cochaired by Fumimaro Takaku (Jichi Medical School, Tochigi, Japan) and David Sidransky (The Johns Hopkins University School of Medicine, Baltimore, MD), a variety of new technologies for the early diagnosis of cancer and for assessing progression of the disease were explored. Dr. Sidransky talked about the clonal genetic alterations that are detected in the multistages of human cancer and their utility in early diagnosis. During the course of progression from the normal...
through hyperplasia, dysplasia, cancer in situ, and frank cancer, LOH as well as specific chromosomal changes occur, thus providing a fertile ground for exploration of early detection. Dr. Sidransky explored various opportunities in this regard, including the use of shed cells in bodily fluids (urine, plasma, and saliva) or in stool. He provided results on the determining recurrence of bladder cancer by looking at LOH at chromosome 9 in shed cells excreted in urine. He stressed the importance of developing technology for high throughput and with increased sensitivity.

Yusuke Nakamura (University of Tokyo, Tokyo, Japan) reported the identification of novel p53-targeted genes by using mRNA differential display and a new method of direct cloning of functional p53-binding sites. Twenty p53-inducible genes were isolated and the biological functions of three, GML, P2XM, and BAI1, have been characterized to date. GML appears to be involved in p53-dependent apoptosis, P2XM has a role in proliferation and differentiation of skeletal muscle, and BAI1 may function in inhibiting angiogenesis. Direct cloning of binding proteins appears to be a useful technique in identifying target genes.

Johji Inazawa (University of Tokyo, Tokyo, Japan) discussed molecular cytogenetic techniques for examining gene alterations in human neoplasms. One such technique, fluorescence in situ hybridization is adaptable to all forms of cytology specimens, including smears, fine-needle aspirations, and touch preparations. This method proved useful in the diagnosis of breast cancer and the prediction of clinical prognosis in meningioma. Two advanced fluorescence in situ hybridization techniques, comparative genomic hybridization and spectral karyotyping/multiplex, were also discussed: novel gene amplifications of chromosome 8p23 were demonstrated by his laboratory in patients with advanced gastric cancers.

Scott Lowe (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY) stressed the importance of apoptosis to cancer therapy and reinforced the effect of p53 status on the response to therapeutic agents. The basic model under study in his laboratory was the normal cell (mouse embryo fibroblasts) into which various key genes had been inserted. The fibroblasts were usually obtained from normal or knockout mice. Studies with Adriamycin and a variety of other agents using these systems supported the need for normal p53 in reducing cell survival. In addition to p53, Dr. Lowe presented data demonstrating minimal toxicity in normal cells that possess Rb function and considerable killing in cancer cells with an altered Rb function. He concluded that a need existed for the development of therapeutic strategies that would specifically enhance apoptosis in cancer cells.

Session 6, “Biomarkers and Cancer Prevention,” was cochaired by Peter Greenwald (National Cancer Institute, Bethesda, MD) and Nobouki Ito (Nagoya City University Medical School, Nagoya, Japan). Dr. Greenwald indicated the goal of cancer prevention to be the inhibition or reversal of the process of carcinogenesis, thus preventing or delaying the occurrence of cancer. The presentations by Gary Kelloff (National Cancer Institute, Bethesda, MD) and David Alberts (University of Arizona Cancer Center, Phoenix, AZ) were complementary in pointing the importance of appropriate biological model systems in the development of an effective cancer chemoprevention program. In addition, Dr. Alberts provided examples of the effective use of these agents in humans.

All speakers indicated that surrogate end point biomarkers are required to overcome the requirement for many hundreds or thousands of study participants in clinical prevention trials, who need to be followed for many years. Surrogate end point biomarkers are defined as biological events that take place between some exposure to a carcinogen (endogenous or exogenous) and the later development of a cancer. A candidate biomarker must be measured reliably in the laboratory and should be a valid predictor of cancer incidence (which has yet to be proven for nearly all available biomarkers). The biomarker may have biological relevance to the carcinogenic pathway or may demonstrate the effect of a particular chemopreventive agent. Intraepithelial neoplasia is a primary example of a histological biomarker suitable for cancer prevention trials. Intraepithelial neoplasia includes colorectal adenomas (useful in the examples provided by Dr. Alberts), prostatic intraepithelial neoplasia, breast ductal carcinoma in situ, oral dysplastic leukoplasia, and others. More recently, a number of genotypic and molecular biomarkers has been developed which are currently being validated. All speakers concluded with the necessity for developing an effective cancer prevention program, components of which will be the use of chemopreventive agents and the availability of fully validated surrogate end points.

Shoji Fukushima (Osaka City University Medical School, Osaka, Japan) concentrated on the organosulfur compounds and described results using a liver medium term test and a multiorgan carcinogenesis bioassay. Chronic treatment with many oil-soluble agents were found to promote hepatocarcinogenesis, causing increases in both the numbers and areas of preneoplastic foci, whereas water-soluble components inhibited these processes. Cysteine and S-methylcysteine inhibited the formation of preneoplastic lesions in both liver and colon. These studies pointed out the necessity of in vivo studies in the assessment of chemopreventive efficacy.

Makoto Taketo (University of Tokyo, Tokyo, Japan) discussed his research on genes that cause the formation of intestinal tumors in mice and, in particular, the appearance of mutations of the APC gene in colorectal, stomach, and esophageal cancers. The histogenesis of polypos in the APC-knockout mice was described and the loss of the wild-type allele as confirmed, thus suggesting LOH as the responsible feature. In addition, he discussed the essential role of cyclooxygenase-2 in the development of polypos and indicated the role of inhibitors of this enzyme in a chemoprevention strategy. Finally, he introduced a novel mechanism whereby inactivation of biallelic homeobox-containing Cdx2 leads to colon tumorigenesis.

In the last talk in this session, Minako Nagao (National Cancer Center Research Institute, Tokyo, Japan) presented findings on the genetic differences in susceptibility to digestive tract carcinogens, stressing the importance of this difference in the 90–95% of nonfamilial cancer. She reported the establishment of a method for the analysis of chromosomal loci using representational analysis to isolate polymorphic markers. She used back-cross experiments in rats to clarify the gene(s) involved in gastric and colon cancer and was able to map stomach cancer-resistant loci to chromosomes 3 and 4. In addition, she determined the high susceptibility of the F344 rat to PhIP-induced aberrant crypt foci formation to be linked to chromosome 16. Her presentation reinforced the necessity for considering strain variation in investigating organ-specific carcinogenesis.

Session 7, “New Approaches for Targeting Drug Resistance,” was cochaired by Haruo Sagano (Cancer Institute, Tokyo, Japan) and Joe Bertino (Memorial Sloan-Kettering Cancer Center, New York, NY). A number of interesting approaches to cancer chemotherapy were presented by the speakers in this session. Allen Oliff (Merck Research Laboratories, West Point, PA) talked about the use of farnesyl transferase inhibitors in the therapy of cancer. Considerable basic knowledge has evolved on the mechanisms of signal transduction during the past 5 years. The enzymatic steps in the transduction pathway offer some avenues of opportunity in drug development. For example, ras is responsible for initiating a G protein cascade that culminates in a mitotic signal to the nucleus. The ras protein must be activated under normal conditions, and it operates in a negative feedback mechanism. In many cancers, however, the ras protein requires insertion into mem-

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brane, a process that is accomplished through farnesylation. Dr. Oliff therefore targeted this step for the design of therapeutic agents. He spoke of the efficacy of a number of these substances using H-ras-infected rat-1 cells, xenografts in nude mice, and, ultimately, in human subjects Phase 1 clinical trials. In addition, he presented results with several new models for cancer, the ras transgene in mice (induces breast cancer), the p53-null mouse, and the K-ras transgenic mouse. The farnesyl transferase inhibitors thus provide a new approach to the therapy of cancer.

Takashi Tsuruo (University of Tokyo, Tokyo, Japan) discussed mechanisms of drug-induced apoptosis. Caspase 3 (CPP-32/apopain) is activated during the onset of apoptosis and actin appears to be one of the specific substrates for this cell death enzyme, both in vitro and in vivo. The upstream components of the signal pathway were examined, and JUK 1 was shown to trigger the apoptotic response in U937 cells by activating Z-Asp-sensitive caspases. Dr. Tsuruo has purified a apoptosis-inducing factor from snake venom that was identified as a novel protein, apoxin. Hydrogen peroxide, which is produced by and methylating agents. One of the actions of these alkylating agents is activated during the onset of apoptosis and actin appears to be one of the specific substrates for this cell death enzyme, both in vitro and in vivo. The upstream components of the signal pathway were examined, and JUK 1 was shown to trigger the apoptotic response in U937 cells by activating Z-Asp-sensitive caspases. Dr. Tsuruo has purified a apoptosis-inducing factor from snake venom that was identified as a novel protein, apoxin. Hydrogen peroxide, which is produced by apoxins through 1-amino acid oxidation, is involved in the apoptotic pathway. An understanding of the precise molecular bases for induction of and resistance to apoptosis would provide a very useful strategy in future approaches in cancer chemotherapy.

Anthony Pegg (Hershey Medical Center, Hershey, PA) provided rationale for introducing a “resistance” gene into bone marrow to avoid the toxic manifestations of cancer therapy with chloroethylating and methylating agents. One of the actions of these alkylating agents is the production of O6-alkylated guanine in DNA that miscodes. This DNA-guanine analogue is repaired by AGMT, a protein that transfers the alkyl group from the guanine derivative to a cysteine moiety, restoring the integrity of the DNA. Unfortunately, bone marrow cells have little expression of this protein and, therefore, are uniquely sensitive to the cytotoxic action of these alkylating agents. Therefore, a rationale for overcoming the cytotoxicity to bone marrow by gene transfer of the AGMT gene into these cells was designed. Results from transfected cells and from mice that bear the AGMT gene support the rationale. In addition, Dr. Pegg told of structural biological studies with AGMT and various substrate/inhibitors in which agents were developed that could effectively block this repair protein and, thereby, enhance therapy by chloroethylating and methylating (e.g., temozolamide) agents in tumors that contain AGMT, e.g., in certain brain tumors. One such agent is O6-benzylguanine. Phase II clinical trials are underway with this approach. Structural biopsy has also suggested the development of resistance to these guanine analogues. Consequently, Dr. Pegg and his colleagues are actively developing O6-benzylguanine derivatives that are more tumor specific and that could inhibit the resistant form of AGMT.

Nagahiro Saijo (National Cancer Center Hospital, Tokyo, Japan) discussed the role of transporters in cancer chemotherapy. Transfection of the γ-glutamylcysteine synthetase (γ-GCS) gene into SBC-3 cells increased the intracellular content of glutathione and GSX pump activity, resulting in a decreased sensitivity to cis-platin and reduced platinum accumulation. GSX pump activity was not decreased by treatment with BSO. The gene was isolated from cisplatin-resistant cells, PC-14/cisplatin and the protein product was identified as SMRP, which consists of 946 amino acids and is a member of the ABC superfamily. It was noted that KM-966, an antibody against the ganglioside GM-2, which is specifically expressed in Adriamycin-resistant cells, can overcome drug resistance. The antibody results in strong antitumor activity in vitro and in vivo. Its use in the treatment of resistant tumor cells is currently being tested in clinical trials.

Joe Bertino discussed the general approaches by which tumors become resistant to cancer therapy. He reinforced Dr. Pegg’s rationale of attempting to use gene therapy to modify the phenotype of bone marrow cells from sensitive to cytotoxicity by cancer therapeutic agents to resistant. Dr. Bertino and colleagues have been using new gene therapy vectors to optimize the gene transfer into CD34+ bone marrow cells. As an example of this approach, he has used vectors which contain the dihydrofolate reductase gene, which accords resistance to antifolates such as methotrexate. His results in mice that have received bone marrow cells into which this gene had been transferred suggest the effectiveness of the rationale. In addition, his laboratory has used detailed information on the interactions between substrates (and inhibitors) and the dihydrofolate reductase protein to design agents that would still inhibit the mutated enzyme (which now is unable to bind methotrexate). Dr. Bertino also relayed information of the generation of mutated dTMP synthases that still retain excellent enzymatic activity despite losing the ability to interact with 5-fluorodeoxyuridine-monophosphate. These approaches offer considerable opportunity in genetically altering bone marrow cells so that they become more resistant to excellent cancer therapeutic agents that, unfortunately, have myelosuppression as a dose-limiting toxicity.

The fourth joint conference, which was viewed by the participants as successful, was closed by Kaoru Abe and Ed Bresnick.
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Kaoru Abe and Edward Bresnick


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