Fifth Heidelberger Conference on Targets for Cancer Research: Prevention, Differentiation, and Selective Therapy

Dr. Charles Heidelberger (1920–1983) made many important contributions to cancer research. These include: the design and synthesis of 5-FU\(^2\), a drug still widely used in the treatment of solid tumors, the use of radioisotopes to label polycyclic aromatic hydrocarbon carcinogens; and the development of quantitative in vitro assays of carcinogenesis. This meeting, the fifth in a series, organized by former colleagues, postdoctoral fellows, and students of Dr. Heidelberger\(^3\) was held at the East-West Center on the campus of the University of Hawaii at Manoa, Honolulu. Previous meetings were held in Chicago (1984; Ref. 1), Honolulu (1988; Ref. 2), Kyoto (1990; Ref. 3), and Los Angeles (1991; Ref. 4). The intent of the scientific program was to highlight recent advancements in, and applications of, the twin areas of research active in the Heidelberger laboratory: chemical carcinogenesis, with a focus on metabolic activation and development of model systems; and chemotherapy, with a focus on 5-FU and advances in achieving increased antitumor selectivity of this and other chemotherapeutic agents. The symposium was attended by about 60 speakers and participants. We were again fortunate that Mrs. Patricia Heidelberger was able to attend the conference; her presence added a sense of continuity, and her opening remarks refreshed our memories of Charlie.

Session on Molecular Epidemiology

The scientific sessions were commenced by Dr. Gerald Wogan (Massachusetts Institute of Technology, Boston, MA), who reviewed analytical methods being used to detect exposure to environmental carcinogens of both endogenous and exogenous origin by measuring covalent adducts on DNA and blood proteins. These methods are now sufficiently sensitive to detect exposure of humans to tobacco carcinogens, such as polycyclic aromatic hydrocarbons, aromatic amines, and nitrosamines; exposure to dietary aflatoxins, nitrosamines, and heterocyclic amines, exposure to medicinal agents, such as cisplatin, alkylating agents, 8-methoxypsoralen, and UV; occupational exposure to aromatic amines, polycyclic aromatic hydrocarbons, ethylene and styrene oxides; and exposure to endogenous oxidative damage.

Dr. Manfred Rajewsky (University of Essen, Essen, Germany) continued the description of model systems suitable for studies of cell lineage-specific carcinogenesis, in this case, the induction by ethynil-nitrosourea of neural cell tumors in the immature rat. PCR and mutantspecific RFLP allowed early detection of trigeminal Schwann precursor cells carrying a T→A transversion at nucleotide 2012 of the transmembrane region of the neu gene. This mutation is diagnostic for ethynil-nitrosourea-induced schwannomas in the central nervous system.

Received 9/9/94; accepted 11/23/94.

1 This conference was held at the East/West Center, University of Hawaii, Honolulu, Hawaii, February 14–16, 1994. It was sponsored by the Cancer Research Center of Hawaii and supported by the following organizations: U.S. Environmental Protection Agency; Queen’s Medical Center Cancer Institute, Honolulu, HI; Nippon Roche, Kamakura, Japan; Yakult Honsha Company, Ltd., Tokyo, Japan; Enrico Mihich, Roswell Park Cancer Center, Buffalo, NY; and Tom Slaga, University of Texas, Science Park, Smithville, TX.

2 The abbreviations used are: 5-FU, 5-fluorouracil; APL, acute promyelocytic leukemia; BP, benzo(a)pyrene; CHL, chlorhyllin; GIC, gap junctional communication; LV, leukovorin; MCA, methylcholanthrene; DENSPM, N\(^{6}\)-diethylnitrosamine; PMA, phorbol 12-myristate 13-acetate; PML, promyelocytic leukemia; PGS, prostaglandin synthase; PKC, protein kinase C; RAR, retinoic acid receptor; SSAT, spermidine/spermine N\(^{\gamma}\)-acyetyltransferase; TS, thymidylate synthase; IQ, 2-amino-3-methylimidazo[4,5]quinoline; DMBA, 7,12-dimethylbenz[a]anthracene; TNF-\(\gamma\), transforming growth factor \(\gamma\); ATRA, all-trans-retinoic acid.

3 Members of the organizing committee were: John Bertram, University of Hawaii, Honolulu, HI; Eli Huberman, Argonne National Laboratories, Argonne, IL; Toshio Kuroki, University of Tokyo, Tokyo, Japan; Enrico Mihich, Roswell Park Cancer Center, Buffalo, NY; and Tom Slaga, University of Texas, Science Park, Smithville, TX.

 between carcinogen adducts and the formation of mutations in human tumors.

Dr. Robert Cooney (University of Hawaii, Honolulu, HI) discussed evidence for the endogenous production of genotoxic damage by nitric oxide. Using the 10T\(1/2\) cell assay for neoplastic transformation, developed in Dr. Heidelberger’s group in the early 1970s, he demonstrated that inhibitors of nitric oxide synthase can prevent the formation of transformed foci induced by the chemical carcinogen methylcholanthrene. Conversely, treatment with \(\gamma\)-interferon or bacterial lipopolysaccharide, which can induce nitric oxide synthesis, transforms cells in the absence of methylcholanthrene. \(\gamma\)-Tocopherol can interfere with nitric oxide-mediated cellular damage, a finding which may explain the epidemiological association of reduced lung cancer incidence in individuals with high serum levels of \(\gamma\)-tocopherol. Dr. Cooney finally discussed the potential role of endogenous nitric oxide synthesis produced during chronic infection and/or inflammation as contributing to the increased cancer rates seen in such conditions.

Session on Carcinogenesis

Dr. Stephen Nesnow (U.S. Environmental Protection Agency) presented data on the genotoxicity of two environmental polycyclic aromatic hydrocarbons, namely, cyclopenta[cde]pyrene and benz[a]anthracene. Both compounds are highly genotoxic inducing mutations, morphological transformation in 10T\(1/2\) cells, and tumors in mouse skin. In A-J mice, both were potent inducers of lung adenoma and were five to 20 times more active than BP. Both carcinogens produced high frequencies (50–65%) of the rare codon 12 mutation, GGT to CTG, on K-ras. 3\(\beta\)-post labeling analysis of treated mouse lung revealed that the most likely source of such mutations was the high frequency production of cyclopenta-ring oxide-2'-deoxyguanosine adducts.

Dr. Henry Pitot (University of Wisconsin, Madison, WI) discussed the development of a multistage rat hepatic carcinogenesis model in which sequential stages of initiation, promotion, and progression can be distinguished, characterized, and quantitated by the use of appropriate protein markers. A placent al isozyme of glutathione-S-transferase was used to identify single glutathione-S-transferase-positive hepatocytes, believed to represent initiated cells, following initiation by a single dose of diethylamino. Whereas approximately 10\(^6\) GSTP-positive hepatocytes are initially formed, only about 10\(^4\) altered hepatic foci result after 4 months feeding with phenobarbital. Cells in such foci are karyotypically normal and will regress following removal of phenobarbital. Dr. Pitot’s group is currently evaluating the expression of transforming growth factor \(\alpha\) as a marker of those hepatocytes capable of progression to neoplasia and is developing this model system to estimate the risk from exposure to environmental chemical carcinogens in humans.
Cells bearing this neu mutation were found to exhibit sustained proliferative activity and expression of gp185 neu. In contrast, terminal differentiation occurred in the wild-type neighbors of these mutant cells. Mutant cells are being traced through the stage of formation of trigeminal schwannomas in order to define genetic and functional alterations associated with postinitiation steps of carcinogenesis in this cell lineage. Recently, a candidate gene has been identified which codes for a cell surface glycoprotein and which is associated with lineage-specific differentiation and ethynitrosourea-induced oncogenesis in rat brain.

Dr. Allan Conney (Rutgers University) introduced the theme of protection from carcinogenic damage by dietary constituents. He presented evidence that administration of green or black tea in the drinking water of SKH-1 mice, previously initiated with DMBA, could inhibit subsequent UVB-induced skin carcinogenesis. In other animal models, administration of green tea was also shown to inhibit diethylnitrosamine-induced forestomach and lung tumors and 4-(methyl nitrosamine)-1-(3-pyridyl)-1-butanone-induced lung tumors. He presented the surprising observation that, in some studies, green tea not only inhibited the frequency of tumors but also reduced the size of tumors that did appear. It is presently unclear whether these separate effects are due to the same or different components of green tea.

Dr. Roderick Dashwood (University of Hawaii, Honolulu, HI) continued the discussion of the protective effects of natural compounds against carcinogenesis. His studies involve the use of CHL, a water-soluble salt of chlorophyll, that inhibits the mutagenic activity of heterocyclic amines such as IQ. He presented in vitro studies suggesting that inhibition of mutagenicity involves direct interaction between CHL and IQ-type compounds, thereby limiting the bioavailability of the carcinogen. When tested in vivo, coadministration of IQ and CHL to F344 rats produced dose-related inhibition of IQ-DNA binding in three target organs for carcinogenesis, i.e., the small intestine, large intestine, and liver. Further, CHL was shown to alter the distribution pattern of the carcinogen in the tissues and excreta, leading to increased elimination of intact parent compound in the feces and decreased binding of IQ to DNA. IQ was most effective when ingested simultaneously with the carcinogen. This protocol also protected against the development of foci of aberrant crypts in the colonic mucosa thought to represent preneoplastic lesions. However, postinitiation exposure to 0.1% CHL in the drinking water for 5 weeks caused a 2–3-fold increase in the number of aberrant crypts. These findings suggest the need for further study of the relative risks versus benefits of CHL treatment, particularly in light of the fact that CHL is used clinically and is sold as a health supplement.

Dr. Kiyoi Tanaka (Osaka University, Osaka, Japan) presented studies on protection from carcinogenesis by the DNA repair functions of the group A xenoderma pigmentosum gene (XPA). This protein, alone or in combination with other proteins, is involved in the recognition step of nucleotide excision repair since it bound preferentially to DNA damaged by UV, cisplatin, or osmium tetroxide. Fibroblasts removed from XPA-deficient mice produced by targeted knock-out of the XPA gene were, as expected, highly sensitive to UV killing and were deficient in nucleotide excision repair. However, a wider role for this protein was implied by experiments demonstrating that treatment of deficient mice with topical DMBA caused rapid skin ulceration and a high frequency induction of papillomas without the necessity of a tumor promoter. This experiment provides direct evidence that XPA-mediated excision repair can protect mice against DNA damage elicited by a chemical carcinogen.

Dr. Hirota Fujiki (Saitama Cancer Center Research Institute, Saitama, Japan) directed our attention towards novel tumor promoters, the okadaic acid class of compounds, which inhibit protein phosphatases 1 and 2A. Interestingly, cell messengers such as TNF-α and interleukin 1 also induce changes in protein phosphorylation patterns and in expression of early response genes similar to those produced by the okadaic acid class of promoter. Moreover, TNF-α stimulated the transformation of methylicholanthrene-initiated BALB/3T3 cells and is released from okadaic acid-treated 3T3 cells. These data may help explain the relationship between cell proliferation, inflammation, and tumor promotion. Sarcophytol A, a marine natural product, and (-)-epigallocatechin gallate found in tea, inhibited tumor promotion induced by okadaic acid in mouse skin, and both compounds were found to inhibit TNF-α release from okadaic acid-treated 3T3 cells. This suggests that a novel mechanism of cancer chemoprevention could be achieved through inhibition of TNF-α release or function.

Dr. Cheryl Walker (University of Texas, M. D. Anderson Cancer Center, Houston, TX) further implicated TNF-α in carcinogenesis in her studies of the Eker rat as a model for induction of renal cell carcinoma. In the Eker rat, renal carcinomas arise in high frequency and contain molecular alterations similar to those that occur in human disease. Overexpression of TGF-α was discovered to be a very early event in the histogenesis of renal cell carcinoma. Dysplastic tubules, the earliest preneoplastic lesions recognizable histologically, as well as carcinomas and adenomas, were found to express abundant TGF-α. Moreover, tumor cell lines, but not cultured proximal tubule cells, secrete TGF-α into cell culture medium. These data indicate that altered expression of TGF-α occurs early during tumor development, may influence disease progression, and may be a useful marker for early stage disease.

Session on Growth and Differentiation

Dr. Tosio Kuroki (University of Tokyo, Tokyo, Japan) emphasized the importance of studies of epithelial cells from which 90% of human cancers arise. Dr. Kuroki described the isolation and characterization of the η isoform of protein kinase C (nPKCη) from a cDNA library of mouse skin. In situ hybridization and immunohistochemical staining revealed that nPKCη is predominately localized in differentiated or differentiating epithelial cells of the skin and the gastrointestinal and respiratory tracts. While the activity of other isoforms of protein kinase C is regulated by interactions with polar head-groups of memhrane phospholipids such as phosphatidyserine and diacylglycerol, the η isoform was found to be preferentially activated by cholesterol sulfate, a cholesterol metabolite with a sulfonic head-group. Since cholesterol sulfate is formed during squamous differentiation, the activation of nPKCη by cholesterol sulfate may mediate squamous differentiation and possibly other signal transduction pathways in epithelia.

Dr. I. Bernard Weinstein (Columbia-Presbyterian Cancer Center, New York, NY) explored the role of cyclin genes as potential targets involved in multistage carcinogenesis. These studies were initiated because of the central role that cyclins and cyclin-related genes play in cell cycle progression. Studies were focused on cyclin D1 because of its location on chromosome region 11q13, a region often amplified in human esophageal tumors. Examination of DNA from 4 human esophageal carcinoma cell lines and 50 primary esophageal carcinomas from China, Italy, and France revealed a 3–10-fold amplification of the cyclin D1 gene and a marked increase in cyclin D1 protein in approximately 30% of these tumors. Because of evidence that cyclin D1 can oppose growth inhibition mediated by the pRb tumor suppressor gene, expression of pRb was measured in these tumors and tumor cell lines. Normal pRb levels were expressed in those tumors exhibiting cyclin D1 gene amplification, whereas in those tumors that did not express pRb, no amplification of cyclin D1 was detected. This
suggests a model of tumor development where the inhibitory effects of pRB on cell cycle progression may be abrogated, either by loss of expression of RB or by increased expression of cyclin D1. To examine the influence of cyclin D1 in a more controlled system, it was stably transfected into R6 rat fibroblast cells. Although transfected cells were found to have the same doubling time as vector control cells, they grew to higher saturation densities, formed colonies in soft agar, and were tumorigenic in nude mice. Furthermore, increased expression of cyclin A, c-myc, and c-jun were detected, providing the first evidence that cyclin D1 can alter the expression of several genes involved in growth control. These findings in human tumors indicate that perturbation in this repertoire of cell cycle genes can play a critical role in multistage carcinogenesis.

Dr. Harvey Hershman (University of California, Los Angeles, CA) described the cloning of a mitogen-inducible gene encoding a novel prostaglandin synthase/cyclooxygenase (TIS10/PGS2). This enzyme is induced by a variety of mitogens, by the v-src oncogene in fibroblasts, and by multiple exogenous factors. In all cases, induction is blocked by glucocorticoid hormones. Many cells express prostaglandin synthase 1 (PGS1) constitutively but require induction by appropriate ligands for TIS10/PGS2 expression. In the case of mitogen-induced prostaglandin production in fibroblasts and endotoxin-induced prostaglandin production in macrophages, induction of TIS10/ PGS2 is required for conversion of endogenous arachidonic acid to prostaglandins, despite the presence of PGS1 in both cell types. This suggests that this gene may be central to many inflammatory processes.

Dr. Eli Huberman (Argonne National Laboratory, Argonne, IL) described research into the control of cell replication and differentiation by examining the role of PKC isoenzymes in mediating PMA-induced differentiation in human HL-60 promyelocytic leukemia cells sensitive or resistant to PMA. Resistant cells had reduced expression of genes encoding PKC β- and δ-like isozymes. Transfection of resistant cells with plasmids containing PKC β1 or β2 cDNA resulted in cells that regained their response to PMA. Thus, PKC β appears to be an essential element in PMA-induced signal transduction that leads to macrophage differentiation in HL60 cells and perhaps in other related cell types.

Dr. Laurent Degos (Hospital Saint Louis, Paris, France) directed our attention towards the clinical success of differentiation therapy with ATRA in APL. In a multicenter randomized trial in APL, a 12-month event-free survival of about 80% has been achieved with ATRA plus chemotherapy versus only 50% when compared with chemotherapy alone. Molecular studies have shown that APL is associated with a translocation event fusing RAR-α gene with a PML gene to yield a fusion product. In normal cells, the PML gene product has been shown to be localized into the outer shell of nuclear bodies which are disrupted in leukemic cells. Treatment with ATRA causes the normalization of the location of the PML gene product. A role for the PML/RAR fusion product itself in malignancy was indicated by gene transfer experiments. Transfection of the fusion product into HL60 cells resulted in an impaired ability to respond to ATRA. Treatment of APL with ATRA appears to be the first model of differentiation therapy in human malignancy and the first specific treatment for a genetic defect.

Session on Tumor Suppressor Genes and Cell Cycle Control

Dr. Akira Hori (National Cancer Institute, Tokyo, Japan) presented data on the analysis of APC mutations, the gene responsible for familial adenomatous polyposis and found mutated in 70% of colorectal tumors. To analyze the potential contribution of APC mutations in the genesis of other tumors, mutations at this locus were investigated in tumors of brain, lung, esophagus, kidney, liver, pancreas, and stomach. Frequent mutations were detected in pancreatic and gastric cancers and in gastric flat adenomas, implying that APC mutations are important in several tumor types and may play an early role in both colorectal and gastric carcinogenesis. Preliminary data was presented on the potential role of mismatch repair systems in patients with multiple primary cancers. By measuring replication errors in several microsatellite markers in human tumors, approximately 75% of patients were shown to possess the replication error-positive phenotype. This phenotype thus appears to play an important role in the development of multiple primary cancers and may prove to be a useful tool to detect high-risk individuals.

Dr. Eric Stanbridge (University of California, Irvine, CA) presented research into the potential functional interactions between oncogenes and tumor suppressor genes using colon carcinoma as the paradigm. While multiple genetic defects clearly occur during neoplastic progression, including the activation of ras oncogenes and loss-of-function of several tumor suppressor genes, including APC, DCC and p53, the correction of any single tumor suppressor gene defect (via monoclonal chromosome transfer or cDNA transfection) resulted in tumor suppression. Additional evidence for the dominant role of the tumor suppressor genes was presented: (a) tumor suppression in somatic cell hybrids occurs despite the continued expression of the endogenous activated oncogene present in the cancer cell genome; (b) introduction of multiple activated oncogenes (e.g., ras and myc) into normal human diploid fibroblasts via cDNA transfection has no reproducible immortalizing or transforming effect; (c) transfection of activated ras oncogenes into immortalized human cells results in only rare neoplastic transfectants; and (d) deletion of an endogenous activated N-ras oncogene in human fibrosarcoma cells results in the formation of flat revertants but retention of their tumor-forming ability. Therefore, the activation of ras oncogenes is neither sufficient for neoplastic conversion nor is their expression in cancer cells necessary for continued maintenance of the malignant phenotype. He speculated that ras expression leads to genomic instability, which may cause the additional genetic alterations critical for neoplastic progression.

Dr. Peter Jones (University of Southern California, Los Angeles, CA) discussed evidence linking the methylation of cytosine residues to mutation of the p53 tumor suppressor gene, which contains many CpG sites serving as methyl acceptor sites. These can function as mutational hot spots in the germ line as well as hot spots for mutation in certain human cancers, particularly colon cancer, through a process of spontaneous deamination of 5-methylcytosine to thymine. Interestingly, the rate of deamination as determined in solution is several orders of magnitude greater than that needed to explain the rate of mutation at these sites in intact cells. This emphasizes the importance of DNA repair systems in protecting cells from deamination. Preliminary evidence was presented suggesting that the DNA methyltransferase might bind strongly to mispairs at methylation sites, thus inhibiting or transforming effect: (c) transfection of activated ras oncogenes into immortalized human cells results in only rare neoplastic transfectants; and (d) deletion of an endogenous activated N-ras oncogene in human fibrosarcoma cells results in the formation of flat revertants but retention of their tumor-forming ability. Therefore, the activation of ras oncogenes is neither sufficient for neoplastic conversion nor is their expression in cancer cells necessary for continued maintenance of the malignant phenotype. He speculated that ras expression leads to genomic instability, which may cause the additional genetic alterations critical for neoplastic progression.

Dr. John Bertram (University of Hawaii, Honolulu, HI) discussed the mechanism of action of retinoids and carotenoids as cancer preventive agents in carcinogen-treated 10T½ cells. Inhibition of neoplastic transformation was statistically correlated with increased GJC. Up-regulated GJC was due to increased steady-state levels of mRNA and protein of the gap junctional gene connexin 43. Several experiments indicated that the activity of carotenoids was not due to conversion to active retinoids. The ability of retinoic acid to up-regulate connexin 43 expression is not limited to 10T½ cells; treatment of human skin 14 days prior to surgery or human keratinocytes in
organotypic culture also caused a major up-regulation of connexin43 expression in suprabasal keratinocytes. In several systems, increased GJC is associated with enhanced growth control of normal and malignant cells, suggesting a model in which retinoids or carotenoids, by stimulating GJC between normal and carcinogen-initiated cells, cause growth inhibition of initiated cells, thereby preventing their transformation.

Dr. Magnus Pfahl (La Jolla Cancer Research Foundation, La Jolla, CA) continued the discussion of the chemopreventive properties of retinoids. While retinoids have great promise as novel drugs for the prevention and treatment of cancer, a number of undesirable side effects limit their application to the clinic. Fortunately, recent advances in the identification of nuclear retinoic acid receptors, the RARs and the RXRs, now allow a rational approach to the development of new retinoids with selective biological activities. Specific retinoids that selectively activate RAR or RXR subtypes have recently been defined; these may have fewer side effects than the presently available retinoids which indiscriminately activate retinoid receptors. In addition to their role as direct gene regulators, retinoid receptors can also inhibit the transcription factor AP-1 and its components, c-Jun and c-Fos. Since these transcription factors appear to regulate the cell cycle, the antiproliferative activity of retinoids may be mediated through this pathway. Novel retinoids have been designed which inhibit the c-Jun and c-Fos transcription factors, inhibit the growth of cancer cell lines, and yet do not induce differentiation and do not function as transcriptional inducers. Thus, the deciphering of retinoid signaling mechanisms at the molecular level may make feasible the design of retinoids with optimal clinical efficacy and reduced side effects.

Dr. Tom Slaga (University of Texas, M.D. Anderson Cancer Center) described the development of specific inhibitors of the initiation and the promotion phase of carcinogenesis. Irreversible inhibitors of polycyclic aromatic hydrocarbon metabolism were developed by measuring the inhibition of BP metabolism in liver microsomes. Two compounds, 1-ethylypyrene and 1-vinlypyrene, were further evaluated in the mouse skin model of carcinogenesis. 1-Ethylpyrene was found to be a dose-dependent inhibitor of binding of both DMBA and BP to DNA and to inhibit skin tumor initiation. Ethylpyrene was much more effective in inhibiting DMBA initiation than initiation induced by BP, while vinylpyrene was less active than ethylpyrene against initiation by both carcinogens. Neither compound was effective against initiation induced by direct-acting carcinogens such as N-methyl-N'-nitro-N-nitrosoguanidine. In studies designed to identify agents active as antipromoters the compound AD-19, a potent free radical scavenging agent, was found to suppress PMA-induced hyperplasia and to prevent the formation of carcinomas during the promotion phase of two-stage skin carcinogenesis. A rational and effective approach to inhibit cancer induction by environmental agents may be the administration of combinations of agents that inhibit both initiation and promotion.

Dr. Reuben Lotan (University of Texas, M.D. Anderson Cancer Center) described studies into the mechanism of action of retinoic acid as a preventive agent in upper aerodigestive tract cancer. To determine whether expression of RAR or RXR mRNAs are related to the development of squamous cell carcinomas, he examined by in situ hybridization their presence in specimens from normal volunteers and from head and neck cancer patients. Whereas no differences in expression of RXR isofoms were detected, and only minor changes in expression of RAR-α and RAR-γ were seen in tumor versus normal samples, expression of RAR-β was detected in only 56% of dysplastic lesions and in 35% of squamous cell carcinomas. This decrease in RAR-β expression appeared to be an early change, since only 40% of oral leukoplakia specimens expressed RAR-β versus 100% expression in mucosal specimens from normal volunteers. Furthermore, regression of oral leukoplakia induced by 13-cis-retinoic acid was associated with a significant increase in the expression of RAR-β transcripts. Similar decreases in RAR-β transcripts were also found in preneoplastic and neoplastic lung tissue than in normal lung. These studies indicate that loss of RAR-β expression is an early event in the development of many head and neck and lung cancers and that induction of this gene by retinoic acid may be a useful intermediate marker of response in chemoprevention studies.

Dr. Frank Meyskens (University of California, Irvine, CA) reviewed the rationale behind clinical cancer chemoprevention trials, the record of past successes, and new directions for the future. In a departure from the chemoprevention of solid tumors with retinoids, Dr. Meyskens presented recent data indicating that retinol palmitate together with busulfan may prevent the progression of chronic myeloid leukemia to the blast-phase crisis. In this study, the progression-free survival interval was prolonged, and the overall survival was increased in comparison to patients taking busulfan alone. The chronic stage of this disease was proposed as analogous to the preneoplastic, in situ stages of carcinomas, suggesting that the long-term potential for cancer chemoprevention may not be limited to solid tumors.

Session on Chemotherapy

Dr. Peter Danenberg (University of Southern California, Los Angeles, CA) described studies aimed at predicting which tumors will respond to 5-FU, originally synthesized by Dr. Heidelberger. Because TS is believed to be the target enzyme for 5-FU, the hypothesis was tested that the tumor specific expression of this enzyme is a major determinant of clinical response. Using a newly developed PCR-based method, TS expression was found to vary by as much as 100-fold among gastric and colorectal tumors; expression was found to significantly correlate (P = 0.003) with the lack of response to 5-FU/leucovorin therapy. No instance was found of a tumor with high TS expression which responded to therapy, although some tumors with low TS expression also did not respond. Quantification of TS expression may allow the identification of nonresponsive patients and represents a step towards the specific tailoring of therapy to individuals.

Dr. Youcef Rustum (Roswell Park Cancer Center, Buffalo, NY) described approaches to the metabolic modulation of 5-FU metabolism with a view to increasing clinical effectiveness by circumventing the rapid emergence of drug resistance. He described how the successful translation of data on modulation of 5-FU metabolism by leucovorin (LV) and N-(phosphoacetyl)-L-aspartate from model systems to the clinic has led to advances in therapy. Optimization of the dose and the schedule of 5-FU and LV demonstrated that prolonged infusion of high dose 5-FU and LV resulted in a 3–4-fold increase in clinical response rates in previously untreated patients with advanced colorectal cancer. In model systems, use of 5-FU prodrugs such as Ftorafor, modulation of levels the newly generated 5-FU by LV, and inhibition of its degradation by uracil and/or 5-ethynyluracil have demonstrated additional improvement in the therapeutic selectivity of 5-FU. Thus, the concept of metabolic modulation of fluoropyrimidines has demonstrated significant clinical potential and should provide new approaches towards achieving greater selectivity of fluoropyrimidines and other agents.

Dr. Hideo Ishitsuka (Nippon Roche, Japan) presented other approaches to increasing the selectivity of action of 5-FU by the use of doxifluridine (Furtulon), a 5-FU analogue. Doxifluridine is converted to the active drug, 5-FU, by the enzyme, pyrimidine nucleoside phosphorylase. The advantages of this produg over 5-FU appear to be due to up-regulation of the activating nucleoside phosphorylases by inflammatory cytokines and growth factors often present in a tumor
environment. In a mouse model system, nucleoside phosphorylase levels were demonstrated to be lower in normal tissues than in tumor tissues, thus allowing selective production of 5-FU in the tumor. In a mouse artificial metastasis model using Lewis lung carcinoma cells, doxifluridine but not 5-FU was shown to inhibit lung metastases at doses lower than those required to inhibit s.c. growth of the same tumor. In an attempt to overcome the dose-limiting side effect of diarrhea produced by oral doxifluridine, novel prodrugs [N-carbonyl substituted-5'-deoxy-5-fluorocytidine derivatives] have been designed to pass unchanged through the gastrointestinal tract and subsequently to be metabolized to doxifluridine and 5-FU by sequential activation in the liver and tumor tissues. Preliminary data shows that these new doxifluridine analogues have improved safety profiles when tested in monkeys.

Dr. Carl Porter (Roswell Park Cancer Institute, Buffalo, NY) discussed other approaches to achieving drug selectivity and described the development of novel polyamine analogues, which mimic natural polyamines by down-regulating the two key polyamine biosynthetic enzymes, and potently up-regulated (500-fold) a polyamine acetylating enzyme, SSAT. This event greatly augments polyamine depletion by facilitating polyamine excretion and catabolism. Thus, natural polyamines are rapidly depleted and replaced with dysfunctional analogues incapable of supporting cell growth. One analogue, DENSPM, is active in the low micromolar range against human lung cancer and melanoma cell lines, including multidrug-resistant sublines. Activity was correlated with induction of SSAT. In human tumor xenograft systems, DENSPM has achieved long-term tumor growth suppression and cures of human melanoma, lung adenocarcinoma, and ovarian carcinoma. Here, DENSPM induces SSAT and depletes polyamines to a much greater extent in tumor tissue than in normal tissue. It is currently undergoing Phase I clinical trials against solid tumors. This polyamine analogue is representative of a new class of anticancer agents and is unique in its apparent mode of action.

Dr. Tetsuo Taguchi (Osaka University, Osaka, Japan) presented new clinical studies of a novel camptothecin derivative, CPT-11, which is believed to act via inhibition of DNA topoisomerase I. Phase II clinical studies conducted since 1989 indicate that CPT-11 has a wide range of activity against solid and hematological malignancies. Response rates (complete plus partial remissions) of between 25 and 40% were achieved in the following cancers: non-small cell lung, small cell lung, cervical, ovarian, colorectal, gastric, breast, squamous cell carcinoma of skin, and non-Hodgkin's lymphoma. Adverse reactions, described as tolerable and reversible, were leukopenia, diarrhea, nausea, and vomiting. Thus, CPT-11 is another example of a novel class of chemotherapeutic agents which appears to possess very promising selective chemotherapeutic potential.

Dr. Andrew Seidman, (Memorial Sloan Kettering Cancer Center, New York, NY) gave the final lecture of the conference and presented recent results with Taxol in a new group of 26 breast cancer patients with stage IV disease. The median age of patients was 52 years, 82% had more than two sites of metastasis and 58% had received prior adjuvant chemotherapy. Taxol, at a dose of 250 mg/m², was given via 24-h infusion every 21 days with dose escalation or reduction based on toxicity. Recombinant tumor granulocyte colony-stimulating factor was given between days 3 and 10 to ameliorate anticipated myelosuppression. Sixty-two % of patients responded (13 partial and 3 complete remissions), and responses were observed at all anatomic sites. Based on these encouraging results and recent breakthroughs in the synthesis of quantities adequate for additional clinical trials, Taxol is currently being evaluated as part of a postoperative adjuvant regimen in patients with early-stage disease.

References
Fifth Heidelberger Conference on Targets for Cancer Research: Prevention, Differentiation, and Selective Therapy

John S. Bertram


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/55/3/705.citation

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.