Meeting Report

Genetic Polymorphism and Cancer Susceptibility: Fourteenth Sapporo Cancer Seminar[1]

It has long been recognized since the early studies in Japan by Yamagiwa and Ichikawa with coal tar-induced carcinogenesis that exposure to environmental and dietary chemicals are likely to be responsible for the vast majority of human cancers. A large number of enzymes participate in both activation and inactivation pathways of carcinogen metabolism. These enzymes mainly function in xenobiotic metabolism and are not thought to play critical roles in metabolism of endogenous chemicals such as hormones. A high degree of interindividual variability exists in levels of expression of xenobiotic-metabolizing enzymes, and some of this variability is due to genetic polymorphism. The critical roles of these enzymes in carcinogen metabolism and this genetic variability in their expression lead to the possibility that xenobiotic enzyme polymorphisms are associated with cancer risk. This risk would be manifest with environmental or dietary exposure to carcinogens. A number of genetic polymorphisms in P450s[2] and transferase enzymes have been described, along with phenotyping and genotyping methods for use in their detection. There has also been progress in developing methods to identify DNA and protein adducts that serve as biomarkers for both carcinogen exposure and expression of the activating enzymes. It is, therefore, timely that the subject of the 14th Sapporo Cancer Seminar was "Genetic Polymorphism and Cancer Susceptibility."

Polymorphic Drug Metabolizing Enzymes

F. J. Gonzalez (National Cancer Institute, Bethesda, MD) introduced the P450s and described some of the properties of the four major xenobiotic-metabolizing P450 forms. He noted the large species differences and polymorphisms that exist among the P450s that impact both experimental carcinogenesis and risk assessment. The direct study of human P450s was described as critical to these issues. The CYP2D6 polymorphism was introduced, and the pivotal 1984 study on its association with lung cancer was briefly described. It was emphasized that polymorphisms for the major carcinogen-activating P450s are suspected but have yet to be firmly elucidated at the molecular level. The *CYP2A6* gene is a member of a locus of genes on chromosome 19 that is under study by combining phenotyping with coumarin and direct sequencing of normal and variant/mutant alleles. A low frequency genetic polymorphism is suspected, and it may be associated with cancers, since CYP2A6 can activate a number of carcinogens and it is also expressed in extrahepatic tissues.

Y. Fujii-Kuriyama (Tohoku University, Sendai, Japan) reviewed studies on the mechanism of regulation of CYP1A1, a P450 responsible for metabolic activation of polycyclic aromatic hydrocarbons. The *CYP1A1* gene is controlled by the Ah receptor that, in its active form, consists of a heterodimer with a ligand-binding subunit designated AhR and a protein called Arnt, or AhR nuclear translocator. He described the primary sequences of these two proteins and their conserved domain structures that include the NH₂-terminal helix-

loop-helix domain, the mid-region PAS domain with two short internal repeats, and the glutamine-rich COOH-terminal segment. Studies using gel mobility shifts and specific antibodies were used to determine that the two subunits are part of a complex that binds to the xenobiotic regulatory elements upstream of the *CYP1A1* gene transcription start site. A cDNA expression vector of the AhR was described that was used to determine the amino acids responsible for the differences between the mouse high affinity and low affinity receptors. An A375V mutation and an elongated COOH-terminal sequence due to a nucleotide replacement, T to C at the termination codon of the C57BL receptor, were largely responsible for conversions of high affinity (K_d = 0.27 nM) to low affinity (K_d = 1.86 nM) receptor associated with the C57BL/6N and DBA/2J mouse strains, respectively. Two human AhR receptor cDNAs were described having differences in relative K_d of 1.58 and 0.89 nM that are due to an elongated COOH-terminal sequence. The human receptors also carry Val at the 381 position, which corresponds to position 375 of mouse AhR, another similarity with the mouse AhRs of lower affinity. This difference in receptor may be responsible for a human polymorphism in CYP1A1 induction.

F. P. Guengerich (Vanderbilt University, Nashville, TN) pointed out that a single pure chemical can cause cancer in experimental animals and that public concerns about chemicals and cancer must be addressed. He discussed recent studies on a human polymorphism in a θ class GST, designated GSTT1. This polymorphism is distinct from the GSTM1 polymorphism discussed below. His presentation centered on the mechanisms of both activation and detoxication of small chemicals. The dihalomethanes are metabolized to formaldehyde through an S-(1-halomethyl)GSH intermediate, the latter of which may alkylate DNA, resulting in genotoxicity. The rat θ GST was inserted into *Salmonella typhimurium* TA153S and actually shown to be involved in mutagenicity. Dihalomethanes are directly genotoxic, possibly through reaction of a reactive intermediate. A number of other related chemicals were also genotoxic in the bacterial model system. Chemicals such as 4-bromo-1,2-epoxybutane and 1,4-dibromo-2,3-epoxybutane were examined, and a 3-membered ring-GSH-episulfonium ion intermediate was postulated. It was pointed out that the GSH enhancement of genotoxicity of the diepoxides is of human health interest since mice are particularly sensitive to cancer and they are the only species in which the butadiene diepoxide can be detected. Rate constant calculations for the activation pathway indicate that humans are less sensitive. These modeling studies have led to modification of EPA guidelines for exposure to methylene chloride. The molecular mechanism (gene deletion) for the human GSTT1 polymorphism was described. One-third of Caucasians lack this activity, yet it is unrelated to the GSTM1 genetic defect in humans. Questions that require further investigation include tissue site of expression, precise chemical substrates, expression in different ethnic groups, and associations with disease risks.

Y. Yamazoe (Keio University, Tokyo, Japan) described studies on the NAT polymorphism in Syrian golden hamsters. This polymorphism is due to a single base change, C to T in codon 243, producing a stop signal in the hamster *ATII* gene that leads to a prematurely truncated protein. The *ATII* is capable of either inactivation (through NAT) or activation via O-acetylation of arylamine and nitroarene

compounds. He stated that the hamster polymorphism may not be of use in determining the role of *ATII* in arylamine carcinogenesis since another monomorphic transferase ATI has similar substrate specificity. STs are also capable of activating arylamine carcinogens. Three subfamilies, ST1A1, ST1B1 and ST1C1, have been cloned in rats. Their levels of expression vary in the liver, depending on age and sex, and all carry out *p*-nitrophenol sulfonylation. However, ST1C1 is the enzyme most active toward *N*-hydroxy acetylaminofluorene and is the only form capable of *O*-esterification of *N*-hydroxy aminofluorene. The male-specific expression of this form may account for liver tumors in male rats administered *o*-diaminoazobenzene, 2-acetylaminofluorene, and 3′-methyldiaminoazobenzene. Dr. Yamazoe described two human sulfotransferases, ST1A2 and ST1A3, which exhibit 96% amino acid sequence similarity. The latter form is 5-fold more active in esterification of *N*-hydroxy aminofluorene and acetylaminofluorene. Both forms activate the food mutagen *N*-hydroxy PhIP and are much more active than the rat forms, which are rank ordered ST1B1>ST1C1>ST1A1. A genetic polymorphism has been described for a human ST but is only manifest as *in vitro* thermal stability of the platelet enzyme, although activity is found in the liver and intestine.

T. Yokoi (Hokkaido University, Sapporo, Japan) discussed studies using caffeine to phenotype Japanese subjects for the NAT2 polymorphism and variability in caffeine 3-demethylation which is an *in vivo* marker for CYP1A2 expression. Nine % of subjects were deficient in NAT2 activity, and genotyping studies yielded an absolute correlation between phenotype and genotype. The NAT2 *M2* allele was most common in Japanese, followed by the *M3* allele. Fourteen % of Japanese were described as CYP1A2 "poor metabolizers," and studies with families suggested an autosomal recessive mode of inheritance of the poor metabolizer phenotype. Analysis of high and low metabolizers by PCR sequencing did not yield an informative base change associated with either phenotype across all coding exons and including DNA up to −3 kilobase pairs upstream. A single allelic change was found at about −2.9 kilobase pairs, but it was unrelated to metabolic phenotype. Dr. Yokoi noted that liver samples analyzed by his laboratory and others also indicate a wide variation in expression of protein. However, no correlation was found between CYP1A2 proteins and its mRNA. A Chinese hamster cell line was described that was engineered to express the P450 reductase, CYP1A2 and NAT2. This cell was active toward metabolic activation of heterocyclic amines such as IQ, 2-amino-3,4-dimethylimidazo[4,5-f]quinoline (MeIQ), and 2-amino-3,8-dimethylimidazo[4,5-f]quinoxaline (MeIQx). In the absence of NAT2 or with NAT1, the cells were not able to activate these compounds to reactive intermediates. Cells expressing only CYP1A2 were active toward aflatoxin B1.

J. Doehmer (Institute for Toxicology, Munich, Germany) reported on the use of genetically engineered Chinese hamster V79 cells to study carcinogen metabolism. He noted that caution should be exercised when using a particular system of cDNA-expressed P450s and that certain systems may be preferable for specific questions. The rat and human CYP1A1 were individually expressed in separate cell lines and compared for their abilities to activate polycyclic aromatic hydrocarbons, phenanthrene, benz(*a*)anthracene, and benzo(*a*)pyrene. The substrates were directly incubated with culture cells, and metabolites were assayed after three days by HPLC and gas chromatography/mass spectrometry. Both rat and human CYP1A1 had similar ethoxyresorufin *O*-deethylase activities. However, human CYP1A1 was found to preferentially produce the diol epoxide from benzo(*a*)pyrene-7,8-diol as compared to the rat form. The 9,10-oxide was also produced by human CYP1A1; while the rat form preferentially generated the benzo(*a*)pyrene-4,5 and benzo(*a*)pyrene-9,10 epoxides. Human CYP1A1-expressing cells had increased cytotoxic sensitivity to benzo(*a*)pyrene as compared to the rat cells. Human and rat

CYP1A1 also differed in the ratios of various metabolites produced from the noncarcinogen phenanthrene. Dr. Doehmer stated that species differences in metabolism as assessed by V79 cells engineered to express P450s cells should be considered in risk assessment and biomarker studies.

H. Esumi (National Cancer Center Research Institute East, Chiba, Japan) described the nitric oxide synthase enzymes and their possible roles in carcinogenesis. The different forms of NO synthase and their roles in cellular physiology were noted. He also discussed the reactivity of NO in producing mutations at 5-methylcytosine. NO can participate in the generation of endogenous nitrosamines through production of nitrate and nitrite, and this has been implicated in cancers associated with chronic inflammation, where macrophage NO synthesis is high. Endogenous nitrate and nitrite production can exceed the intake of these chemicals in the diet. In this context, infections with the liver fluke *Ospistorchis viverrini* result in the high urinary excretion of nitrates, possibly producing elevated nitrosamines in these patients that have increased risk for cholangiocarcinoma. There was no correlation between N_2O_3 and N_2O_4 with chronic hepatitis. The tissue-specific expression and inducibility of the three known NO synthases were reviewed. An alternatively spliced noninducible NO synthase protein, designated n-NOS-2, was described as lacking 105 amino acids due to skipping of two exons during pre-mRNA splicing. This was found in both mouse and humans, suggesting a physiologically important role of the truncated form. L-Arg, the substrate, is known to bind to the deleted region. The cDNA-expressed n-NOS-2 is catalytically inactive as a conventional NO synthase, but it is active as an oxidoreductase of unknown substrate specificity. It is known that in the absence of L-Arg, NO synthase produces oxyradicals, thus suggesting the possibility that n-NOS-2 is a radical producer. Variation in expression of the i-NOS was found in brain tumor astrocytes but not in surrounding normal tissues. The upstream elements of i-NOS were studied, and possible *cis*-elements were found, including one that interacts with nuclear factor-kB. The gene is regulated by a variety of cytokines. An *Xba*I RFLP was found associated with the i-NOS gene, but it is not known to be of functional significance.

N. Harada (Fujita Health University, Aichi, Japan) reported on aromatase gene expression in breast cancer. Aromatase, CYP19A1, is the rate-limiting enzyme for biosynthesis of estrogen. One-third of all breast cancers are estrogen dependent and, in this case, estrogen produced through a paracrine mechanism (by adipose stromal cells) stimulates tumor growth. The *CYP19* gene is composed of at least six exons 1, and the use of various of these exons is tissue-specific and governed by regulatory elements upstream of the various exons. Exon 1b predominates in fetal and adult liver and skin fibroblast. Exon 1a is used in placenta, a nd exons 1c and 1d are preferentially transcribed in ovary. Exon 1f is used in the brain, and exon 1e expression is found in all tissues. The placental gene is regulated by a variety of agents. It is induced by 12-*O*-tetradecanoylphorbol-13-acetate and suppressed by insulin, glucagon, and insulin-like growth factor-1, depending on the cell type. Adipose stromal cells of the breast stain for aromatase expression. To determine expression of various spliced mRNA, a fluorescence-based PCR assay was developed and automated for quantification of exons 1a-, 1b-, 1c-, 1c′-, and 1d-expressed forms. Breast tissue from noncancer patients had the 1b-type mRNA, while stromal cells from most patients expressed the 1c-, 1c′-, or 1d-type. Cells adjacent to the tumors had the highest expression of these forms, while adipose stromal cells that were distal to the carcinoma usually transcribed exon 1b. Patients with metastatic nodes almost always transcribed 1c. Twenty-three of 49 (47%) patients transcribed exon 1b, while 16 of 16 tissues from controls expressed this exon. Twenty-six of 49 (53%) cancer patients expressed exon 1c. For nodal metas-

tasis, all patients (11 of 11) produced the exon 1c variant. Altered regulation in cancer patients is responsible for the exon switch, but the mechanism is not known. Studies were also described using CAT reporter constructs in which a shift from exon 1a to 1b was noted in J3 cells that was dependent on time after transfection. Dibutyryl cAMP caused a shift to the 1c exon start site, and 10% FCS resulted in exon 1b expression. These studies suggest that a hormonal mechanism may be responsible for the alternative use of CYP19A1 gene promoters.

Genetic Epidemiology and Carcinogen Metabolizing Enzymes

H. Bartsch (German Cancer Research Center, Heidelberg, Germany) pointed out that cigarette smoking is associated with all histological types of lung cancer with the most common types being squamous cell and small cell carcinomas. It has been well established that tobacco carcinogens require metabolic activation by P450s. He discussed studies that determined the effect of tobacco smoking on xenobiotic-metabolizing enzymes. Surgical lung tissue from lung cancer patients who were recent smokers exhibited an elevated level of CYP1A1 and its enzymic activity towards activation of benzo-(a)pyrene as compared to lung tissues of nonsmokers. In contrast to CYP1A1 induction, certain phase II enzymes (glutathione transferase) are repressed in lungs of smokers. A highly significant correlation was found between the extent of CYP1A1 activity and levels of benzo-(a)pyrene-diol-epoxide DNA adducts. There was little correlation between this activity and bulky adducts characteristic of aromatic amines. These data set lend support to the notion that CYP1A1 inducibility controls polycyclic aromatic hydrocarbon DNA adduct formation in tobacco smokers and suggest that inducibility is associated with lung cancer risk. Case-control studies have suggested an association between extent of CYP1A1 induction and lung cancer and a polymorphism associated with the CYP1A1 gene indicates a higher risk with a specific genotype (see the summary of the talk of Dr. Kawajiri). These studies also showed a risk associated with GSTM1 null genotype and lung cancer risk. In this connection, Dr. Bartsch presented data showing that smokers with the null genotype excreted four times more urinary mutagens than individuals with an active gene. However, no difference was found between urine mutagenicity between slow and rapid NAT2 genotypes. These studies give a biochemical basis for GSTM1 association with smoking-related cancers. Although there was no association between NAT2 genotype and lung cancer, slow acetylators are at increased risk for bladder cancer but probably more so at low doses of aromatic amines. This is reflected in differentially shaped dose-response curves between aromatic amine dose and aromatic amine-hemoglobin adducts in slow and fast acetylators.

N. Caporaso (National Cancer Institute, Bethesda, MD) reviewed the known conditions in which a single gene is involved in cancer susceptibility such as retinoblastomas, adenomatous polyposis coli, and basal cell nervous syndrome. These disorders are due to single genes that are necessary and sufficient to cause cancer. This is contrasted with susceptibility genes which act only in the presence of a specific environmental agent to alter the probability of cancer in the host. Examples of such agents that exhibit wide interindividual variation in the population due to hereditary factors include: alcohol, which causes esophageal, liver, and oropharynx cancer; arylamines, that cause bladder cancer; and tobacco, which causes lung, bladder, and other neoplasms. In the case of lung cancer, susceptibility multigenic factors are suggested based on case control and family studies. He noted that traditional linkage analysis approaches are difficult since cancer families are rarely large enough due to the high incidence of death from both cancer and smoking-related nonneoplastic disease.

Environmental-gene interactions that are secondary for single gene disorders are critical for susceptibility genes. An example of a gene-environment interaction was presented using glucose 6-phosphate dehydrogenase deficiency and consumption of fava beans, resulting in hemolytic anemia. The requirement for both the exposure and genetic component for disease is indicated with the quote "carry the gene and eat the bean." Drug toxicity due to genetic polymorphism was given as a paradigm for gene-environment interaction. Dr. Caporaso cited the need to develop genotyping assays for use in molecular epidemiology studies instead of reliance on phenotyping since the latter cannot identify heterozygotes, can be effected by disease and diet, and relies on administration of a probe drug. He stated that the gene frequency of the polymorphism, sensitivity, and specificity will all contribute to the efficiency of gene-disease association studies. The CYP2D6 polymorphism was reviewed with regard to an association to lung cancer. The various alleles for CYP2D6 have been identified and include null alleles, slow-metabolizing alleles, and a rare, ultrarapid allele due to amplification of the CYP2D6 gene. Taken together, all studies published to date show a significant trend toward the PM having mutant CPY2D6 genes being protected against non-adenocarcinoma-type lung cancer. Dr. Caporaso said that the mechanism for this association is still unclear, and a better appreciation of the mechanism will be important in understanding heterogeneous study findings. New studies have focused on the neurological aspects of the CYP2D6 polymorphism. PMs appear to be more anxious, less assertive, and less socialized. Perhaps there is a link to the σ receptor in the brain related to the dopamine neurotransmitters or the metabolism of endogenous opiates. Dr. Caporaso noted the importance of ethnic differences in frequencies of polymorphisms and the need for inter-ethnic studies such as the Japanese-American collaboration in the 1950s that elucidated population differences in acetylation phenotype. Finally, he emphasized that cancer epidemiology studies that do not address exposure are of little use.

K. Kawajiri (Saitama Cancer Center Research Institute, Saitama, Japan) reported that polycyclic aromatic hydrocarbons can be activated by CYP1A1 or inactivated by GSTM1, a form of GST. An allele of CYP1A1 was found that is associated with an MspI RFLP in the 3' flanking region of the gene and an Ile-Val amino acid change in exon 7. The Val form is a rare allele and, when homozygous, is associated with an increased risk for lung cancer, particularly the squamous cell and undifferentiated types that are both associated with tobacco smoking. The more weakly smoking-related adenocarcinoma shows frequency of Val/Val genotype similar to matched control. When the GSTM1 polymorphism (in which 40% of individuals are homozygous for two gene deletions) are examined, a combined genotype of Val/Val and GSTM1 −/− results in marked elevated risk of up to 7 for squamous cell carcinoma. When cigarette dose was factored in, the risk was found to be low for the nonsusceptible Ile/Ile, GSTM1-positive genotype but increased with an increased dose of cigarettes. At low dose, the susceptible genotype was at high risk. Mutations in the tumor suppressor gene p53 were examined as associated with CYP1A1 genotypes. In this case, the MspI genotypes were analyzed. The low frequency genotype C was found to be related to increased p53 mutations as compared to genotypes A and B. The molecular mechanisms for the CYP1A1 association are still unknown. Dr. Kawajiri mentioned possibilities of differences in catalytic activities between the two allelic gene products, differences in extent of induction, and the association of the rare Val allele with cancer susceptibility genes. Studies were discussed on analyzing the induction of CYP1A1 by the Ah receptor. As noted earlier, this receptor consists of a ligand-binding domain called Ahr and the Arnt protein. The levels of the mRNAs encoding two proteins were found to be highly correlated. To search for a polymorphism in the AhR, SSCP was performed

across all 11 exons of the gene in 25 individuals. A single silent base change was found in exon 2, and a G to A base change causing an Arg to Lys amino acid change was detected in exon 10 at codon 554. The allelic frequencies were almost 1:1, but no significant association was found with lung cancer risk.

R. A. Branch (University of Pittsburgh Medical Center, Pittsburgh, PA) discussed the known polymorphisms in P450s and noted the high variability within the extensive metabolizers. The mephenytoin polymorphism is due to mutations in the CYP2C19 gene. The CYP2D6 and CYP2C19 polymorphisms can be diagnosed by phenotyping and genotyping. Variability in CYP3A4 can be detected by phenotyping using dapsone, but to date no genetic polymorphism has been described. Some decreases in activity with age was found, but this was attributed to an increase in variance as a function of age. To simultaneously determine levels of CYP2D6, CYP2C19, and CYP3A4, a three-drug cocktail was used to determine metabolic phenotypes, and the association with bladder cancer was examined. This cancer has been associated with occupational exposure to arylamines, smoking, and alcohol. No CYP2D6 PMs were found in aggressive bladder cancer, whereas the nonaggressive type had a PM frequency similar to matched controls. CYP2D6 mRNA was detected in bladder tissue, and levels correlated with phenotype. Dapsone hydroxylation recovery ratios were also lower in individuals with aggressive tumors. These patients also had an elevation in GSTM1 −/− genotype and a history of excess alcohol intake. The S-mephenytoin polymorphism was not associated with increased risk, and the NAT2 polymorphism was not significant alone. High alcohol intake, increased dapsone recovery ratios, and GSTM1 −/− were found in 25% of aggressive tumor-type patients as compared to only 3% of matched controls. Thus, multi-risk factors can contribute to each other to increase the risk of cancer. Knowing these factors will possibly allow modifications in risk associations, diagnosis, prognosis and therapy.

S. Ikawa (Tohoku University, Sendai, Japan) emphasized that experimental animals are difficult to use in order to study and predict human cancer risk. Studies in human populations will be required in order to identify human polymorphisms that might be associated with susceptibilities to certain cancers. An RFLP due to a DraI site in intron 6 of CYP2E1 was examined in Japanese lung cancer patients. Heterozygotes were found to be overrepresented in the cancer cases compared to matched controls, with a higher percentage of heterozygotes identified in the low-pack-year smokers. Analysis of CYP2E1 mRNA revealed high levels in heterozygotes. The CYP1A1 gene in humans is also under investigation for cancer risk. The AhR, which controls CYP1A1 inducibility, is being cloned from cosmid libraries to determine the existence of a polymorphism similar to that described in the mouse. Analysis of the association of the CYP1A1 allelic variants with lung cancer revealed results at variance to those described above by the Saitama Cancer Center group. In this case, the Leu/Leu genotype was more frequent in lung cancer patients as compared to controls, emphasizing the need for further confirmatory studies in Japan.

Biomonitoring of Carcinogen Metabolites and DNA Adducts

F. Kadlubar (National Center for Toxicological Research, Jefferson, AR) presented a list of potential carcinogenic agents to which humans are exposed, their known sources (diet, smoking, or environmental) and associations with certain cancers, and methods and type of carcinogen-adduct detected, e.g., 4-aminobiphenyl (in cigarette smoke) and 4,4'-methylenebischloroaniline (an occupational hazard) for urinary bladder cancer, the food mutagen PhIP and colon cancer, and even H_2O_2 and O_2^- that are liberated under oxidative stress and their possible roles in liver and lung cancer. Most importantly, DNA

adducts produced by these agents can be measured and quantified in human tissues. DNA adduct levels derived from complex mixtures have now been shown to be correlated with tumor incidence in several mouse cancer models. Human larynx cancer is smoking related, and risk is increased 4-fold by heavy alcohol consumption. Candidate carcinogens include the polycyclic aromatic hydrocarbons and arylamine carcinogens. Two types of adducts derived from different types of carcinogens were found by ^{32}P-postlabeling in the larynx of smokers. The butanol-enhanced adducts are due to aromatic amine exposure, and the P1-sensitive adducts are due to polycyclic aromatic hydrocarbon adducts. Both adducts were found in the larynx, and levels correlated with levels of CYP1A1, CYP3A4, and one or more CYP2C P450s. Epidemiological risk factors for the pancreas are poorly understood, although it is positively associated with cigarette smoking and meat consumption and inversely associated with fruit and vegetable consumption. No P450s are detected in these tissues, but P450 reductase, nitroreductase, and N-acetyl transferase 1 are found. Thus, the DNA adducts could be due to arylamine hydroxylation by CYP1A2 in liver, followed by O-acetylation in pancreas, that can result in activation through intramolecular rearrangement. Four uses of adducts in human studies were outlined: (a) as a biomarker of exposure; (b) to identify a specific activation pathway; (c) to study the relationship to mutagenic activity; and (d) to predict tumor incidence with steady-state adducts. Colon cancer risk is increased in the rapid CYP1A2 phenotypes in combination with the active N-acetyltransferase 2 alleles. Risk is increased even further by red meat preference. The food mutagen PhIP was suggested as the etiological agent since this compound produces adducts in human colon.

J. Groopman (The Johns Hopkins University, Baltimore, MD) reviewed statistics in cancer incidence at various sites in developed and underdeveloped countries. New liver cancer cases were 46,000 and 168,000 in developed and underdeveloped countries, respectively. This cancer is particularly insidious with the morbidity equal to the mortality. The two etiological agents responsible for the vast majority of cancers in the underdeveloped world are hepatitis B virus and AFB. In Gambia and China, there are 300,000 deaths/year, and in certain hepatitis B virus endemic areas, infants are infected by the age of two. AFB exposure also begins early in life. In the United States, AFB exposure is 25–75 ng/day, while in Southern China, it can be as high as 75–250 μg/day. Cancer begins to be seen at age 30, and there is a linear relationship between incidence and age. AFB is activated by epoxidation at the 8,9-position by a P450. Bulky carcinogen adducts can be found in urine along with the glutathione conjugate of AFB. The AFB adducts DNA ten times more than those of benzo(a)pyrene and 4-aminobiphenyl in exposed individuals. Dr. Groopman discussed the development of molecular biomarkers for AFB and the use of animal models to validate the markers. The AFB adducts found in rat urine are linearly related to target tissue adducts, suggesting a constant rate of removal. A large prospective study on association of AFB exposure and DNA adducts and liver cancer is under way in Shanghai. Epidemiology studies to date have demonstrated a 3.4-fold risk for AFB exposure and a 7.3-fold risk for hepatitis B virus surface antigen but a 59-fold risk for combined AFB and hepatitis B virus surface antigen. Olitpraz, an antischistomiasis drug that induces a GST in rats responsible for AFB epoxide conjugation, protects rats from AFB carcinogenesis. This drug also induced a human α class GST and is a candidate for a chemopreventive agent for hepatocarcinogenesis.

F. Oesch (University of Mainz, Mainz, Germany) reported on a new method for detection of DNA damage which allows determination of individual exposure and susceptibility to genotoxins in humans. An alkaline filter elution protocol was developed that determines levels of alkali-labile DNA damage, DNA strand breaks, and DNA cross-links

in peripheral mononuclear blood cells of humans *in vivo*. Evidence was presented showing the use of this technique in biomonitoring the genotoxic effects of occupational and habitual exposure to toxins and for monitoring of chemotherapy. Metal workers exposed to dialkylamines, such as *N*-nitrosodiethanolamine, were examined, and the extent of DNA damage was proportional to the air content of the chemical in the workplace up to maximum of 1000 ng/m^3. Firefighters acutely exposed to *o*-nitroanisole were found to have statistically significant elevated strand breaks. Roofers exposed to bitumens were also elevated in strand breaks, whereas road pavers showed no difference from matched controls. Exposure to ethylene oxide was also detected by the alkaline elution method. This exposure was associated with work-related complaints, and two groups were identified as more and less sensitive. No statistically significant correlation was found between the two groups and the known polymorphisms in GSTM1 or GSTT1. These enzymes might be expected to conjugate ethylene oxide. Microsomal and soluble epoxide hydrolase were also examined and, based on use of inhibitors, were found to both contribute to ethylene oxide hydration. Polymorphisms in these enzymes have not been demonstrated. Dr. Oesch also reported on the effect of chemotherapy on DNA damage. Nurses who took precautions when preparing cyclophosphamide were found to have no detectable damage above controls. Those who did not exercise care had significant damage. In patients receiving combination chemotherapy, individuals who showed favorable response, indicated by a low level of tumor marker, had a 100 to 500% increase in DNA strand breaks in their lymphocytes. Patients in which the tumor marker was unchanged showed only a very small or no DNA damage. Thus, evidence was found for a correlation of DNA damage caused by chemotherapy and the response to the chemotherapy, with the potential of early prediction of the suitability or nonsuitability of chemotherapeutic protocols for individual patients.

Experimental Carcinogenesis in Animals

Y. Hashimoto (Tohoku University, Sendai, Japan) spoke on the susceptibility of mice and rats to carcinogenic heterocyclic amines. These chemicals, including Trp-P-1, induce CYP1A2 in CDF1 mice. In this strain, liver cancer induced by Trp-P-1 is higher in females than males. Mutagenic activation of this compound is also higher when female liver microsomes are used in the Ames test. Rats showed no sex difference in induction or Trp-P-1 hepatocarcinogenicity. The levels of P450s CYP1A1, CYP1A2, and GST-P were monitored in a rat liver cancer protocol in which animals were initiated with diethylnitrosamines, fed 2-acetylaminofluorene for 4 weeks (during which time a partial hepatectomy was performed), and then fed a basal diet for 7 weeks. CYP1A1 was induced in all areas of the liver, whereas CYP1A2 was not elevated in the preneoplastic islets. An inverse relationship was found between the induction of GST-P and CYP1A2 expression. Dr. Hashimoto postulated that high levels of adducts in preneoplastic lesions were due to low DNA repair enzymes. The effect of metal ions on P450 induction was investigated. Lead was found to specifically reduce CYP1A2 induction by 3-methoxy-4-aminoazobenzene and 3-methylcholanthrene. In contrast, GST-P was induced by lead nitrate. Nickel, copper, and cadmium had no effect. The reduction in CYP1A2 and induction of GST-P resembles the situation found in hyperplastic nodules.

T. Watabe (Tokyo College of Pharmacy, Tokyo, Japan) discussed experiments demonstrating the role of hydroxysteroid sulfotransferases in metabolic activation of carcinogenic polycyclic arylmethanols, 7-hydroxymethyl-12-methylbenz[*a*]anthracene and 5-hydroxymethylchrysene. These chemicals undergo *O*-sulfation to yield higher reactive sulfate esters that are potent mutagens in the Ames test

and produce DNA adducts both *in vitro* and *in vivo*. Adenosine 3′-phosphate 5′-phosphosulfate and cytosol are required for increased mutagenesis. Sulfate esters of 7-hydroxymethyl-12-methylbenz[*a*]anthracene are more potent than epoxides in the Ames test. The direct mutagen DHBA 7-sulfate, which preferentially binds G residues, is deactivated by addition of GSH. Dr. Watabe also discussed purification of rat sulfotransferases designated Sta, STb, and STc. A sex difference was found in expression of these enzymes in the rat. In humans, no sex differences have been reported, albeit a polymorphism was reported in which 25% of the population are high dehydroepiandrosterone conjugators. Sulfate esters, *e.g.*, 5-methylchyrsenes, can be rapidly inactivated by the θ class of GSTs in adult rats. The human counterpart is subject to a polymorphism in which 38% of Caucasians lack activity. This suggests a possible link between deactivation of sulfate esters and a human genetic polymorphism.

Genetic Polymorphism and Susceptibility to Cancer: Where Are We Going?

T. Sugimura (National Cancer Center Research Institute, Tokyo, Japan) discussed studies on 4-nitroquinoline-1-oxide, a carcinogen that does not require metabolic activation by P450. The NO$_2$ moiety is reduced to HNOH, which leads to adducts. UV-sensitive mutants of yeast are sensitive to 4-nitroquinoline-1-oxide. Xeroderma pigmentosum patients are also sensitive to this agent. The carcinogen *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine also does not require metabolic activation and modifies guanine and lysine. The buffalo rat stain is resistant to *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine-induced stomach cancer. This resistance is unrelated to metabolism and is inherited as an autosomal dominant gene trait. No differences in adduct levels are found between sensitive and resistant rats, but the sensitive strains have a higher labeling index, with bromodeoxyuridine suggesting a genetic difference in cell proliferation. Dr. Sugimura also discussed breast cancer in Japan. Between 1960 and 1990, the incidence of breast cancer has doubled to over 20 per 100,000 with an onset age of 30. In the United States in Caucasians, early onset bilateral cancer represents about 5% of all cancers and is due to the *BRCA1*, a 17q21 autosomal dominant gene. This gene is also responsible for ovary cancers. In Japanese cancer patients, there is no evidence for allele loss on 17q21 and no germ line p53 mutations. The question of cancer cure was also discussed. Ten % of all cancer patients that are considered cured develop secondary cancers. In all cases, these are second primary cancers, not recurrences. Dr. Sugimura ended the symposium with a discussion of the "iceberg" theory of cancer research. Each cancer research scientist is studying some specific aspect of the problem, such as carcinogen metabolism, oncogenes, tumor suppressor genes, metastasis, etc., and each piece of information represents the tip of a single iceberg. Cancer scientists must not lose site of the reality that understanding cancer requires knowledge gained from many individual large icebergs.

Summary and Recommendations for Future Studies

The consensus from the presentations at this meeting is that genetic polymorphisms do indeed play a role in cancer susceptibility. The cancers affected are probably those that are considered "spontaneous" cancers, where there is little evidence for familial inheritance. Since the polymorphic xenobiotic-metabolizing enzymes activate and/or inactivate procarcinogens, they must act in concert with chemicals to exert their effect. This is most evident by the dose-association studies with smoking and lung cancer and the dietary factors of colon cancer. More well-designed case-control epidemiology studies need to be performed in different populations with adequate case number to yield

statistical significance. Prospective studies should also be initiated. More sensitive and specific biomonitoring studies should be carried out to test for exposure with an aim to determine the existence of perhaps other polymorphic susceptibility genes. Positive associations of cancer with a polymorphism in an enzyme should also be investigated to determine the mechanism of the association. This can be accomplished by use of the appropriate animal model systems or other *in vitro* systems.

Once the association of one or more genetic polymorphisms with a particular cancer is firmly established, this information can be useful in the public health arena to advise on cancer risk. For example, on the basis of genotype, an individual can be be told that he or she will be predisposed to cancer as compared to the general population when engaged in a particular activity that might be hazardous, like smoking or even a low level workplace exposure to chemicals like aromatic amines. There is also the possibility of using chemopreventive measures to lower risk when a person has a known genotype. Thus, it is a hope of the organizers that the information gained at the Sapporo Cancer Seminar will stimulate more studies, particularly interinstitutional collaborative efforts to launch meaningful lines of experimentation.

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