A United States–Japan workshop on “Genetic and Environmental Interactions in Cancer Susceptibility in Animal Models” was held at the National Institutes of Health, Bethesda, MD, on March 27–28, 1997 (Fig. 1, group photograph). An introduction to the workshop was provided by Masaaki Terada of the National Cancer Center Research Institute (NCCRI), Tokyo, Japan, who presented a comparison of mortality from common cancers in the two countries. Lung and colon carcinomas are important causes of death in both countries, whereas stomach and liver carcinomas are more important in Japan and breast and prostate carcinomas are more important in the United States. Projections for the years 2001–2010 suggest that the incidence of lung and colon cancers will decline in the United States, Canada, and Europe, while continuing to increase in Japan. Collaboration between our nations can continue to be useful, as it has since 1961 for science generally (United States–Japan Science Collaborative Research Program), since 1965 for medical science (United States–Japan Cooperative Medical Science Program), and since 1974 for cancer research (United States–Japan Cooperative Cancer Research Program). The last of these programs sponsored this workshop, with the goal of examining the use of animal models for the study of genetic and environmental factors that can modify the incidences of cancer and for suggesting new preventive and therapeutic interventions in humans.

An overview of cancer genetics by Alfred Knudson began with a definition of oncogenes as groups into which a population of cancer subjects can be divided according to the etiologic participation of genetic predisposition or environmental agents. A background group, negative for both factors, reflects the contribution of spontaneous somatic mutation to disease development. Two genetic groups, one in which major genes play a critical role, even in the absence of environmental factors and an interactive group that involves both genetic and environmental factors, comprised the subjects of this meeting on cancer susceptibility. The penetrance of major genes can be modulated by environmental or other genetic factors in some instances, and knowledge of the mechanisms might provide new approaches to prevention or treatment. For polymorphic genes that interact with environmental agents, the relative risk of cancer may not be very high, but the attributable risk can be. The study of gene–gene and gene–environment interactions is complicated in humans, so the availability of animal models is particularly welcome and already yielding important findings.

The Eker rat model of hereditary renal carcinoma and other tumors (see below) was discussed by Okio Hino. These rats are heterozygous for mutation of the tuberous sclerosis 2 gene (TSC2 in humans and Tsc2 in rodents); the homozygous mutant state produces fetal lethality. The mutation is caused by insertion of a transposable element into the gene. Introduction of a construct containing the wild-type Tsc2 gene suppresses tumor growth and overcomes fetal lethality in transgenic Eker rat homozygotes. Penetration of the mutation is complete for renal carcinomas and incomplete for hemangiosarcomas of the spleen, leiomyomas of the uterus, and adenomas of the pituitary. Renal carcinogenesis is increased in incidence and the process is greatly accelerated in time by transplacental administration of ethylnitrosourea (ENU). The induced tumors do not show the loss of heterozygosity (LOH) that is found for the majority of spontaneous tumors (1), reflecting the production of point mutations in the second copy of the Tsc2 gene. Of particular interest is the demonstration that tumors induced chemically in rats that do not carry the germline Tsc2 mutation have somatic mutations in the gene, but not in the Vhl (von Hippel-Lindau) gene, which is mutated in most nonhereditary renal carcinomas in humans.

In a study (2) of the induction of excess renal tumors in the Eker rat by dimethylnitrosamine, Cheryl Walker (Science Park–Research Division, M. D. Anderson Cancer Center, Smithville, TX) reported that there was no increase in mesenchymal renal tumors over the number found in rats without the Tsc2 mutation; the mutation was specific for susceptibility to induction of epithelial tumors in the kidney. Rat fetuses homozygous for the Tsc2 mutation exhibit failure of neural tube closure subsequent to overgrowth of neural epithelium. The leiomyomas found in Tsc2 heterozygotes are similar to the corresponding uterine tumors in humans and are, therefore, good models for intervention studies that involve the use of a rapid in vitro screening step followed by in vivo response (3). Tamoxifen treatment and ovariotomy of the host mice are both inhibitory to tumor growth, whereas xenoestrogens are stimulatory. Genistein pro-

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roduces a biphasic response, with stimulation at low doses and inhibition at high doses.

The discovery of the Min (multiple intestinal neoplasia) mouse has stimulated considerable interest in the investigation of tumor growth modification caused by mutations in the responsible Apc (adenomatous polyposis coli) gene. Makoto Taketo (University of Tokyo, Japan) has employed a knockout mouse with a deletion mutation in codon 716 (Apc\(^{3716}\)) to study the polyps, which are homologous to those found in humans with familial adenomatous polyposis. In heterozygous mice, small tumors begin to form in crypts and spread under the cover of normal villous epithelial cells. When this normal cell cover in the mouse polyps is removed and the underlying tumors are analyzed, even very small lesions show loss of the remaining wild-type allele. Since polyps can be precursors of malignant tumors, any means of reducing them in this animal model would have implications for humans. Nonsteroidal anti-inflammatory agents reduce prostaglandin production and polyps, so reduction of the cyclooxygenases (COX1 and COX2) responsible for the conversion of arachidonic acid to prostaglandins might reduce polyp formation. The inducible COX-2 gene Ptgs2 (i.e., prostaglandin–endoperoxide synthase 2) is induced in many colon carcinomas and even in small adenomatous polyps. An Apc plus Ptgs2 double-knockout mouse shows a dramatic reduction in the number and size of the polyps, as does the Apc knockout mouse treated with a COX-2 inhibitor [e.g., MF–tricyclic, which is a very effective suppressor of polyp formation (4)].

The discoverer of the Min mouse, William Dove (University of Wisconsin, Madison), also mapped a resistance modifier of Min, called Mom1, which was identified in the AKR strain of mice. Mice either heterozygous or homozygous for the resistant form of Mom1 show large decreases in both the size and the number of tumors (5). Mom1 does not seem to be acting as a tumor suppressor gene, inasmuch as it does not show LOH in the tumors that form in heterozygous Mom1 mice, i.e., there is maintenance of heterozygosity. A candidate gene for Mom1 is a secretory phospholipase of intestinal Paneth cells. The phenotype of Min is unresponsive to germline (i.e., genetic) or immune status, but adenoma formation is reduced by nonsteroidal anti-inflammatory agents. Administration of the carcinogen ENU at 10 days of age increases the number of adenomas considerably, whereas its administration at 30 days of age causes numerous mammary tumors as well. The Min mouse made nullizygous for p53 (i.e., loss of p53 function) shows a marginal increase in adenomas but a great increase in desmoid tumors, as occurs in humans with Gardner’s syndrome. Nullizygosity for COX-2 greatly reduces tumor number, and heterozygosity for loss of DNA methylase causes a reduction in the number of tumors as well.

Differences among rat strains to colon carcinogenesis induced by the carcinogen 2-amino-1-methyl-6-phenylimidazo-[4,5-b]pyridine (PhIP), by which humans are exposed in cooked meat, was discussed by Minako Nagao (NCCRI) (6). She found that, using an assay for aberrant crypt foci (ACF), BUF rats were sensitive, F344 rats were somewhat so, and ACI rats were resistant to PhIP colon carcinogenesis. Frameshift mutations were found in the Apc gene. Since the rat strains she used yielded almost equivalent levels of PhIP–DNA adducts in the colonic mucosa and since there were no differences in ACF sizes (number of aberrant crypts/focus) among these strains, the gene(s) regulating initial event(s) after adduct formation are expected to be identified. She is now mapping the chromosomal loci responsible for cancer susceptibility by use of representational difference analysis. William Dove pointed out that the number of responsible genes might be estimated from the variance in ACF numbers observed in backcross animals.

Allan Balmain (Onyx Pharmaceuticals, Richmond, CA) has been analyzing multistep skin carcinogenesis in mice. Somatic mutations in the H-Ras gene are virtually universal in the tumors and apparently play a role in initiation, promotion, and progression, as judged by transgenic use of activated H-Ras. Tumor progression can be influenced according to the cell type that is targeted. If keratin-containing cells are targeted, only papillomas result; with the targeting of basal cells, the papillomas progress to carcinomas. Effects are also noted with knockout mice. Thus, cyclin D1+/− heterozygous mice develop fewer chemically induced tumors, and −/− mice develop many fewer. Only a slight difference is found with p53+/− heterozygous mice; the p53−/−...
homozygous mouse shows no effect on tumor number, but the tumors are more likely to progress. In a study of mice with p53+/− heterozygosity and activated Ras, progression to carcinoma of the salivary glands was accelerated. Considerable strain differences in tumor incidence and progression among mice have been observed in an ongoing study that has already identified several chromosomal regions as putative sites of modifying genes (7).

In another effort to identify new tumor-modifying genes, Hiroshi Hiai (Kyoto University, Tokyo, Japan) has investigated urethane-induced pulmonary tumors in recombinant inbred (SMXA) mouse strains (8). One strain carried four known susceptibility genes, including K-Ras2, but was highly resistant to tumor development because of the presence of a dominant pulmonary adenoma resistance (Par) gene(s). Previous work has identified one gene (Par1) on chromosome 11 and another (Par2) on chromosome 18. This work locates one gene on chromosome 11 (probably Par1) and a new one (Par3) on chromosome 12. A candidate gene for the latter may be nPkceta, which is expressed exclusively in skin and lung and down-regulated (i.e., as expressed at lower levels) in the pulmonary tumors. This protein kinase C gene appears to act synergistically with Par1.

Genetic investigations of a very different kind are illustrated by the comparative species approach of Sayabrata Nandi (University of California, Berkeley), who began by observing that the incidence of breast cancer is relatively high in humans and in four other well-studied mammalian species (domestic cats and dogs and laboratory mice and rats), all of which may be exposed to many of the same environmental risk factors. Induced tumors in mice and rats are drastically different with respect to the presence of estrogen receptors: Approximately 90% of rat tumors are estrogen receptor (ER) positive, whereas 80%–100% of mouse tumors are ER negative and apparently do not arise from ER-positive tumors. Nandi hypothesizes that this difference may relate to differences in fetal hormonal environments, with a stronger influence of fetal androgens in the rat.

The responsible gene for gastric cancer susceptibility is being studied by Toshikazu Ushijima (NCCRI). The ACI rat is known to be highly susceptible to N-nitro-N-nitrosoguanidine (MNNG)-induced stomach carcinogenesis (9), and the excessive amount of cell proliferation in reaction to mucosal damage is suggested as the mechanism. To map the gene responsible for the high susceptibility to stomach cancer and the gene(s) regulating cell proliferation after mucosal damage, Toyota et al. (10) have developed a time- and money-saving gene mapping system for the rat. The system is comprised of more than 200 representational difference analysis (RDA) markers, 52 interspersed repetitive sequence (IRS)–RDA markers, and 12 CAAT sequence–RDA markers. Most of the RDA markers and all of the IRS–RDA and CAAT–RDA markers can be used to genotype hundreds of rats, simply by conducting hybridization analyses using filters onto which genomic DNA (containing the relevant amplicons) has been dotblotted. The gene(s) regulating cell proliferation are now being mapped using these markers. On the other hand, Toyota et al. pooled DNA from rats with stomach cancers after a carcinogenicity test involving use of backcross rats. By performing RDA using the DNA pool and DNA from an inbred-resistant strain, BUF, they identified several clones, possibly linked to cancer development. The clones are now being verified and should reveal the relationship between the excessive cell proliferation and cancer development as well as the genetic loci for the two traits.

Peroxisome proliferators (PPs) and their mechanism of hepatocarcinogenesis in rats and mice were described by Frank Gonzalez (National Cancer Institute, Bethesda, MD). The effects of these agents depend on a member of the superfamily of steroid receptors, PP-activated receptor (PPARα), whose activity can be assessed with trans-activated reporter genes that contain a response element. The receptor’s function depends on dimerization with retinoic acid receptor and 9-cis-retinoic acid. Mice made PPARα null exhibit no obvious phenotype until later age, when lipid microdroplets accumulate in the liver and kidney. The failure of these mice to respond in the usual way to clofibrate (i.e., antihyperlipidemic) peroxisome proliferators demonstrates the critical requirement of PPARα; there were no observed increases in hydrogen peroxide production, in DNA adducts, in markers of DNA synthesis, or in apoptosis (11). Human cells are resistant to peroxisome proliferation, so the effect upon them of transgenically active PPARα is being tested.

Direct evidence that DNA repair can protect mice from chemically induced cancer was presented by Takatoshi Ishikawa (University of Tokyo), who developed the ada mouse by transfecting germ cells with the Escherichia coli O9-methylguanine DNA methyltransferase gene (MT), under the control of the metallothionein I promoter. Mice normally express much less MT activity than humans, but the administration of zinc to the ada mouse causes high MT expression and resistance to diethylnitrosamine- and dimethylnitrosamine-induced liver tumors (12). In another experimental system, involving induction of skin ulcers and papillomas in mice following a single administration of 7,12-dimethylbenz[a]anthracene and tissue plasminogen activator, (TPA), knockout mice for the xeroderma pigmentosum A (XPA) gene showed enhanced ulceration and 100% incidence of tumors after 16 weeks of treatment. Wild-type mice and mice heterozygous for XPA had only a few papillomas. In yet another set of experiments, pregnant p53 heterozygous knockout mice given ENU (25 mg/kg) produced p53 null offspring with a 70% incidence of brain tumors (glioblastomas and medulloblastomas) (13). The few tumors that developed in heterozygous offspring had lost the wild-type allele. This finding directly demonstrates the importance of p53 in early brain tumorigenesis.

Motivated by a desire to avoid the usual 2-year mouse bioassay for the carcinogenic activity of chemicals, John French (National Institute of Environmental Health Sciences, Triangle Park, NC) has developed a 6-month assay with a viral H-Ras-transfected mouse. An associated advantage is the low background incidence of tumors at 6 months. The carcinogen is applied with TPA to the skin of homozygous-transfected mice. Dose and dose-rate dependence are observed for tumors produced by both mutagenic and nonmutagenic carcinogens. In nontransfected mice, only epidermal hyperplasia is observed. Tumors are also produced in mice that are heterozygous for mutation of p53. Bladder tumors result also, but the wild-type p53 allele is not lost in cells of these tumors; however, a high rate of such loss is observed after animals are exposed to benzene.

The c-kit gene, which produces a receptor tyrosine kinase,
and its ligand, stem cell factor, have been investigated by Yuki-hiko Kitamura (Osaka University Medical School, Japan). The mouse W locus and the rat Ws locus encode c-KIT protein and the mouse Sl locus encodes stem cell factor. Homozygous loss mutations at either of these loci result in deficiencies in melanocytes, erythrocytes, germ cells, mast cells, and interstitial cells of Cajals (ICC), as well as in foestrochome papillomas and antral ulcers. The last are produced as a result of bile reflux that occurs subsequent to incontinence of the pyloric us caused by lack of ICC’s (14). Gain of function mutations in the c-kit gene lead to mast cell tumor formation (15). A mutation in the tyrosine kinase domain of the protein is found in these tumors in mice, rats, and humans. Mutation or deletion at the juxtaminembrane domain may cause tumors of the gastrointestinal stroma of humans; these tumors are thought to be of ICC origin. 

Gene–gene interactions among Nf1, Nf2, and p53 in mice have been the subject of investigation by Andrea McClatchey (Center for Cancer Research, Massachusetts Institute of Technology, Cambridge). Although these genes are on different chromosomal arms in humans (17q, 22q, and 17p, respectively), they are all on chromosome 11 in the mouse. There is an interesting cis/trans effect in heterozygous double mutants. Thus, transmutants (p53–Nf2+/p53+Nf2−) show small tumor (osteosarcoma and rhabdomyosarcoma) effects, whereas cis mutant (p53−Nf2−/p53+Nf2+) mice show large effects. In both instances, the tumors show high LOH rates for wild-type alleles. In the mouse, loss or somatic recombination in a single chromosomal arm could lead to loss at both loci, whereas in humans, this would require two separate events. Parallel results are observed for Nf1/Nf2 mice, although the tumor spectrum is different. Mice that are chimeric for wild-type and double Nf1/Nf2 knockout cells develop many neurofibromas.

Ryo Kominami (Niigata University Medical School, Japan) presented his results on the induction of thymic lymphomas by gamma irradiation in wild-type mice or mice constitutionally mutant at one of four loci: p53, N- or K-Ras, Tsr1 (thymic lymphoma suppressor region on chromosome 4), and Lyr (lymphoma resistance, also on chromosome 4). Loss of either Tsr1 or Lyr in combination with heterozygosity for p53 mutation resulted in a high incidence of lymphomas, with a high rate of loss of the remaining wild-type p53 allele observed in the tumors. New regions of chromosomes 12 and 16 show a high rate of LOH in some tumors, suggesting the importance of other tumor suppressor genes for the process. However, the loss is not sensitive to p53 status for the chromosome 12 site, but it is for the chromosome 16 locus.

The meeting provided numerous examples of the interaction of tumor susceptibility genes with other genes and with environmental agents. Such studies have been aided enormously by the introduction of directed mutagenesis in mice. Because of the availability of a large number of inbred strains of both mice and rats, it is possible to study the modification of the penetrance of major genes by other genes as well as by environmental agents. The cloning of new major genes in humans can lead to the production of mutants in mice, a search there for modifying genes, and a return to humans to determine whether such modifiers operate there as well. In addition, polymorphic genes that interact with environmental agents can be studied in rodents more efficiently than in humans. Thus, mice can be produced that are of specific genotypes for two or more loci and then examined for responses to environmental agents. The knowledge gained from rodent studies should provide clues to preventive or therapeutic interventions in humans.

References


Note

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