ENVIRONMENTAL GENOMICS AND CARCINOGENESIS PANELS

[Established in 1972 as the Environmental Mutagenesis and Carcinogenesis Panels; renamed in 2001]

In the 1970s, in parallel with rapid advances in molecular biology, knowledge of the effects of environmental toxins and carcinogens on human health increased dramatically. It was a time of expanding industrialization in Asia, and environmental contamination was becoming a serious concern in many countries, including the United States and Japan. It was also a time of significant change for the U.S.–Japan Program.

In February 1971, the USJCMSP Joint Subcommittee and invited experts met in Honolulu, Hawaii, to discuss the formation of a new panel to address health problems related to the environment. In June of that year, at its seventh meeting, held in Bethesda, Maryland, the Joint Committee adopted specific guidelines for reviewing and evaluating all the USJCMSP panels. At the same meeting, the Joint Committee decided to recommend the formation of a new panel on Environmental Mutagenesis and Carcinogenesis.

Two leading scientists, one in the United States and the other in Japan, urged the creation of the USJCMSP Panel on Environmental Mutagenesis and Carcinogenesis. "In 1972, David Rall was the director of NIEHS," said Dr. James Selkirk in a September 2004 interview. Dr. Selkirk is the Program Officer for the U.S. Environmental Genomics and Carcinogenesis Panel. "At that time, Dr. Sugimura was essentially directing all the carcinogenesis research in Japan." Dr. Takashi Sugimura, who was then Director of the National Cancer Center Research Institute in Tokyo (and is now President Emeritus), became one of the original members of the Japanese Environmental Mutagenesis and Carcinogenesis Panel, and served in that capacity until 1988, when he became Panel Chair. Drs. Sugimura and Rall proposed creating the new panel, says Dr. Selkirk. Research on animal models of carcinogenesis was dominating the field, and many studies involved feeding, painting, or otherwise exposing the animals to various chemicals, to determine which were mutagenic.

Industrial pollution of the environment

The early 1970s was also a time when the *Salmonella* assay was at its height. "This was a bacterial assay based on *Salmonella typhimurium* that was good for detecting potential chemical mutagens," says Dr. Selkirk. Scientists realized that the chemicals that are mutagenic also tend to be carcinogenic, so the *Salmonella* assay also became an assay for carcinogenesis. The connection between mutagenesis and carcinogenesis was the main reason for the panel's original name.

The Panel was organized immediately and established guidelines to study the detection of chemical pollutants that can cause mutation or cancer and to conduct surveillance to determine whether pollutants cause genetic damage in humans. The first chairmen of the new Environmental Mutagenesis and Carcinogenesis Panel were Dr. Frederick J. de Serres, of the U.S. National Institute of Environmental Health Sciences (NIEHS), and Dr. Tadashi Yamamoto, of the Institute of Medical Science, University of Tokyo.

"The first Panel meeting was held in the Ministry of Foreign Affairs in Tokyo in 1972," Takashi Sugimura and Taijiro Matsushima wrote in a "Recollection" published in *Mutation Research*.[1] "The

U.S. delegates were a line-up of eminent scientists including Drs. Elizabeth and James Miller, Bruce Ames, Ernst Freese, Elizabeth Weisburger, Robert Miller, and James V. Neel." Other scientific societies with similar interests were being formed at about the same time: the U.S. Environmental Mutagen Society in 1969, the European Environmental Mutagen Society in 1970, and the Japan Environmental Mutagen Society in 1972. Three "International Conferences on Environmental Mutagens" were held in 1973, 1977, and 1981. Scientists worldwide were recognizing the close relationship between mutagenesis and carcinogenesis and that changes in DNA lay at the root of all cancers.

As the Environmental Mutagenesis and Carcinogenesis Panel came together, other events affected the USJCMSP. In February 1972, U.S. Delegate Chair Dr. Colin MacLeod died in a London hotel while en route to Dhaka, Bangladesh, to conduct a review for the NIH on the Cholera Research Laboratory. (The USJCMSP second five-year report, released in August 1976, is dedicated to him.[2]) Dr. Ivan L. Bennett, Jr., replaced Dr. MacLeod as U.S. delegate chair. Then, in its second year of existence, scientists associated with the USJCMSP Environmental Mutagenesis and Carcinogenesis Panel made a series of important discoveries that led to a major change in Japanese public health policy.

The discoveries concerned AF-2 [2-(2-furyl)-3-(5-nitro-2-furyl)acrylamide], a nitrofuran derivative, which had been used as a food preservative in Japan since 1965 and had been reported to be noncarcinogenic. However, in 1973, Drs. Tsuneo Kada[3] and Sohei Kondo[4] (Japanese Panel member from 1984–1991) discovered that AF-2 is mutagenic in *E. coli* WP2. The next year, the Environmental Mutagenesis and Carcinogenesis Panel held a meeting entitled "Problems of AF-2, a mutagenic food preservative" in Hakone, Japan. Scientists soon demonstrated the carcinogenicity of AF-2 and in 1974 Japan banned its use. In related studies, scientists found that *S. typhimurium* TA1535 and TA1538, the original bacterial strains used in the Ames method to detect carcinogens, were unable to detect the mutagenicity of AF-2. By including an R-factor plasmid in these two strains of bacteria, Dr. Ames developed two new strains of *Salmonella*, *S. typhimurium* TA98 and TA100,[5] that were more useful for detecting environmental mutagens and carcinogens, including AF-2.

At the same time, researchers in several countries were developing new methods to determine how environmental agents cause those DNA mutations that lead to cancer. "I began to invite people to the Panel meetings who were better versed in new technology," says Dr. Selkirk. "The Japanese Panel did that, too. Soon, it became fairly clear that we were doing more mechanistic studies on disease-causing chemicals. In the 1990s, we began to study which genes were being turned on." It became increasingly clear that certain environmental agents could damage the genome. Dr. Selkirk suggested changing the name of the Panel, and Dr. Sugimura devised its new name.

In July 2001, at its meeting in Princeton, New Jersey, the USJCMSP Joint Committee approved changing the name of the Environmental Mutagenesis and Carcinogenesis Panels to the Environmental Genomics and Carcinogenesis Panels. The change represented continuing scientific progress since the 1972 inception of the Panels, a field that had evolved from descriptive to mechanistic approaches, and that incorporated new technologies, including genomics, to understand how environmental agents alter the genome to cause cancer.

The following is a list of other important science advances in environmental genomics and carcinogenesis research to which USJCMSP scientists have contributed. The list is adapted from information supplied by Dr. Takashi Sugimura (member of the Japanese Environmental Genomics and Carcinogenesis Panel from 1972–1989, and member of the Japanese Delegation from 1989-2001), Dr. James Selkirk (Program Officer for the U.S. Environmental Genomics and Carcinogenesis Panel), and Dr. Keiji Wakabayashi (Chair of the Japanese Environmental Genomics and Carcinogenesis Panel, Japan, from 2000 to the present).

Environmental Genomics and Carcinogenesis Panels

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[Science Advances: 1972–2000]

- Demonstrated the mutagenicity and carcinogenicity of AF-2 [2-(2-furyl)-3-(5-nitro-2-furyl) acrylamide], which had been used as a food preservative in Japan since 1965. The discoveries led to banning the use of AF-2 in Japan. [See discussion above.]
- Identified mutagenic and carcinogenic heterocyclic amines (HCAs) in cooked food, which provided a strong stimulus to search for new environmental carcinogens in daily foods. Using the Ames mutagenicity assays, Dr. Takashi Sugimura and his colleagues discovered the presence of potent chemical mutagens in cooked meat and fish, as well as charred material of amino acids and proteins.[6] Scientists identified the mutagenic HCAs including imidazoquinoline (IQ) and phenylimidazopyridine (PhIP) compounds in cooked food.[7] Dr. Sugimura and his colleagues showed the mutagenic HCAs were carcinogenic in mice and rats, inducing tumors in the liver, colon, prostate and mammary gland, etc.,[8] while others demonstrated the carcinogenicity of IQ in monkeys. These and other studies elucidated: the mechanism by which HCAs form as fish and meat are heated; metabolic activation pathways; structures of HCA-DNA adducts; and mechanisms of mutagenicity and carcinogenicity of HCAs. An assay based on the quantitative measurement of HCAs in cooked foods and human urine samples was developed to estimate the magnitude of exposure of humans to HCAs. Moreover, epidemiologic surveys indicated positive links between the consumption of well-done meats and an elevated risk of colon and breast cancers. Thus, HCAs are a group of environmental carcinogens that are produced in cooking under routine conditions, which makes human exposure to them almost unavoidable.
- Demonstrated that the water in three Asian rivers contains chemicals that can cause DNA mutations. Water pollution is becoming a serious problem for public health in many countries. In collaboration with Dr. Wannee R. Kusamran of the National Cancer Institute, Thailand, Japanese Panel Chair Dr. Keiji Wakabayashi and his colleagues tested the mutagenicity of water samples from the Chao Phraya river and some connected canals in Bangkok, Thailand, and from the Sumida and Ara rivers in Tokyo, Japan, using blue rayon to adsorb the mutagens. All of these samples showed a mutagenicity of 87-1213 revertants per 0.05 g blue rayon extract towards *S. typhimurium* YG1024 in the presence of S9 mix. These results demonstrated that the water in all three rivers contained some frameshift mutagens.
- Identified genes responsible for multistage carcinogenesis. Genomic instability, accompanied by the amplification or deletion of certain subsets of genes, plays a key role in various types of cancer. The coexistence of amplification of mutated K-*RAS* and c-*MYC* genes was first observed in human pancreatic cancer. Former Chair of the Japanese Panel on Environmental Genomics and Carcinogenesis Dr. Masaaki Terada and his colleagues demonstrated that K-*SAM* and *cyclin D1* gene amplification are among these changes.[9] In other studies, Dr. Misao Ohki and his colleagues analyzed a specific chromosome translocation frequently found in a subset of human acute myeloid leukemia, and showed that the production of a chimeric transcription factor encoded by the *AML1* (*RUNX1*)-*MTG8* fusion gene may contribute to the development of acute myeloid leukemia.[10]
- Developed new animal models (including DNA-repair deficient fruit flies and, later, transgenic mice), and short-term screening tests (including *in vitro* and *in vivo* assays) to help identify potential mutagens and carcinogens. This has resulted in improved methods for characterizing carcinogens, mutagens, and tumor promoters in the environment as well as in dietary constituents. Novel animal models are required to evaluate more precisely the mutagenic and carcinogenic potential of environmental agents. Dr. Takehiko Nohmi and his colleagues developed a new *in vivo* mutagenicity test system using *gpt* delta transgenic mice.[11] Recently, many other transgenic mouse lines harboring human oncogenes such as c-*MYC* and TGFα have been established, and some of them were reported to be highly susceptible to tumor induction by certain carcinogens and therefore of possible use for rapid screening purposes. Dr. Motoya Katsuki and his colleagues developed transgenic mice that harbor a human prototype c-Ha-ras gene, and demonstrated the rapid induction of more malignant tumors by various genotoxic carcinogens.[12]
- Developed an animal model that demonstrates the relationship between *Helicobacter pylori* (*Hp*) infection and gastric carcinogenesis. Dr. Masae Tatematsu and his colleagues reported that *Hp* infection increases the incidence of N-methyl-N-nitrosourea (MNU)-induced adenocarcinoma of the glandular stomach in Mongolian gerbils.[13] Moreover, *Hp* eradication was shown to be useful as a preventive approach against stomach cancer in this animal model.
- Identified and characterized naturally occurring carcinogens such as polycyclic hydrocarbons and aflatoxin, and applied molecular biology techniques to assess the biological relevance of exposure to a variety of mutagens and carcinogens. This has greatly increased the sensitivity of human epidemiology studies.
- Identified a susceptibility gene for renal cancer in an animal model. Dr. Okio Hino and his colleagues identified the *Tsc2* gene as a tumor-suppressor gene that, when mutated, causes a heritable form of renal cancer in the Eker renal carcinoma (RC) rat.[14] Both spontaneous and ENU-induced RCs in the Eker

rat demonstrated loss of heterozygosity (LOH) or intragenic somatic mutations in the remaining allele of the *Tsc2* gene. Moreover, substantial effects of genetic background on renal carcinogenesis were also demonstrated, indicating a substantial contribution of a modifier gene(s) on the development of cancers, even in a dominantly predisposing cancer-related gene.

- Evaluated comparative experimental and epidemiologic data on the carcinogenicity of environmental toxicants from experiments gathered from many countries
- Used cDNA (complementary DNA) chips for monitoring several thousand human genes to determine the effects of toxic chemicals in the United States and Japan
- Monitored mutagens and carcinogens in human populations, including chlorinated dioxins and environmental chemicals that may have estrogen-like activity, and found that some of these compounds acted in a similar manner to natural estrogens
- Characterized and identified genes that increase susceptibility to certain forms of cancer; particularly *BRCA1*, the first-discovered breast cancer susceptibility gene, and *KAII*, a prostate tumor-suppressor gene

[Science Advances: 2000 – 2004]

- Increased understanding of the molecular mechanism underlying HCA-induced carcinogenesis. Ten HCAs have been found to be carcinogenic to rats or mice, and five of them give rise to colon tumors in rats. Genetic alterations in the rodent *Apc* or β-*catenin* gene are frequently observed in HCA-induced colon cancers, as in the case of human colon carcinogenesis. Furthermore, genetic backgrounds of animals were demonstrated to have a significant effect on the susceptibility to the development of colon cancers. After the discovery of a candidate locus on rat chromosome 16 for genetic susceptibility to PhIP-induced colon carcinogenesis, Dr. Hitoshi Nakagama and his colleagues mapped loci for the resistant traits on chromosomes 6 and 9.[15] Further investigation to identify responsible genes is ongoing.
- Increased understanding of the relationship between individual genetic differences and lung cancer risk in smokers. It is becoming increasingly clear that individual differences in specific genes can have profound effects on the ability to metabolically activate or detoxify chemical carcinogens, including those in cigarette smoke. Cytochrome P450 2A6 (CYP2A6) is one of the forms of CYP expressed in the human respiratory tract and is responsible for activating tobacco-specific nitrosamines. Dr. Tetsuya Kamataki and his colleagues investigated the effects of the CYP2A6*4, an entire CYP2A6 gene deletion-type polymorphism, on lung cancer risk in Japanese male smokers.[16] The homozygous deletion (*4/*4) genotype was not found in patients with squamous cell carcinoma (0 of 105) or small cell carcinoma (0 of 44), indicating that subjects with the *4/*4 genotype have low risk for lung cancers, particularly those caused by tobacco smoke. Consistent with these data, pretreatment with 8-methoxypsoralen, a potent human CYP2A6 inhibitor, strongly inhibited lung tumorigenesis induced by tobacco-specific nitrosamine in mice.[17]
- Continued to develop new animal models of cancer, including human c-Ha-ras proto-oncogene transgenic rats, which were developed by Dr. Hiroyuki Tsuda and his colleagues. These rats are highly susceptible to mammary tumor induction, which suggests a possible application for screening environmental carcinogens.[18]

Footnotes — Environmental Genomics and Carcinogenesis Panels

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