Establishment of the Basic Concept that Cancer is a Disease of DNA

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There is abundant evidence that cancer has existed from the prehistoric era and is not a disease only associated with modernization of our society. As a good anecdotal example, the presence of a breast cancer can be recognized in a picture draw of his wife, the famous Dutch painter, Rembrandt over 300 years ago. However, it is also true that under a particular occupational conditions, certain cancers may be especially frequent. Thus scrotum cancer in the chimney sweeps of London attracted the attention of Sir William Pott in 1775 and urinary bladder cancer was often found among workers in the aniline-dye industry in the late 19th century (Rehn, 1895). These examples of so-called occupational cancers facilitated the search for the nature of cancer. On the basis of such clues, Prof. Katsusaburo Yamagiwa painted coal tar on the ears of rabbits and first succeeded experimentally in producing skin cancer. Dr. Kennaway in United Kingdom first isolated pure 1,2,5,6-dibenzanthracene as a chemical producing skin cancer (1930). Drs. Takaoki Sasaki and Tomizo Yoshida were the first in the world to produce cancers in the viscera of animals (hepatoma) by feeding o-aminoazotoluol on rats (1932). As we can see, the tradition of carcinogenesis research in Japan goes back a very long way.

It is now a commonplace that cancer cells are converted from normal cells. Dr. Boveri (1914) and Dr. Bauer (1928) who carefully studied the carcinogenic process and abnormalities in chromosomal features and numbers, very early proposed the hypothesis that cancer cells are the outcome of somatic mutations. Mathematical analysis of the age of onset of stomach, colon and lung cancers led Drs. Armitage and Doll (1954) to conclude that the underlying carcinogenic processes involve several events. Similarly adult T-cell leukemia is triggered by infection with HTLV-1 at an early stage of life through breast milk feeding but the onset of disease has a peak at 55-65 years old. Berenblum had in fact already demonstrated the presence of at least two qualitatively distinct steps with their experiments of painting of benzo [a]pyrene followed by croton oil in 1941.

The above background suggests that carcinogenesis might be due to multiple-step alteration of genes. I had the good fortune with my mentor Dr. Waro Nakahara (1957) to prove that the mutagen 4-nitroquinoline 1-oxide (4NQO), could cause mouse skin tumors. In a series of studies, we demonstrated formation of 4NQO-derived adducts in DNA base after *in vivo* injection of the carcinogen (1967), then revealed metabolic conversion of 4NQO to 4-hydroxylaminoquinoline 1-oxide (4HAQO), and production of single strand DNA scission by 4HAQO (1968). An enzyme which converts 4NQO to 4HAQO, was purified from the rat liver (1966). We then demonstrated the carcinogenicity of *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG) in rats by noting fibrosarcoma development with subcutaneous injections (1966) and gastric carcinomas after oral administration as a drinking water solution (1967). Subsequently we found remarkable differences in the susceptibility to induction of stomach cancer by genetic crosses (1983) and now this subject is a major theme in the rat genome project (1996).

The food additive, AF-2, which had been used as a preservative was demonstrated to be mutagenic by Dr. Sohei Kondo and his associates. It was proven to be carcinogenic some years later, and its usage was banned.

The fact that typical carcinogens such as polycyclic aromatic hydrocarbons and azodyes could not initially be demonstrated to be mutagenic in microbes was at first confusing. However, Prof. Bruce Ames overcome this problem when he invented the so-called "Ames Test". The principle consists of incubation of test-substances with a *Salmonella typhimurium* strain requiring L-histidine and a metabolic system obtained from the liver of rats treated with PCBs.

Typical carcinogens thereby undergo metabolism by cytochrome P450 and eventual activation to ultimate forms (electrophilic compounds) which can bind to DNA and proteins (nucleophilic compounds), as unequivocally investigated by Drs. E.C. Miller and J. Miller.

4NQO and AF-2 are metabolically activated by a pathway shown by common microbes and mammalian cells. MNNG itself can react with DNA through decomposition in water. However, most carcinogens require metabolic activation by cyticgrine P450 and therefore can only be demonstrated by Ames type approaches.

We took great advantage of tha Ames method. It was known that tar of cigarette smoke contained many mutagens/carcinogens. We were fortunate in that this research led us to observe that smoke yielded by broiling fish also contained mutagenic agents (1977). As a result we undertook further intensive studies of pyrolysates of amino acids and proteins and also of heated meat (fish, beef etc.) and demonstrated the existence of new heterocyclic amines (HCAs) like pyridoindole, dipyridoimidazole and imidazoquinoline (IQ) as well as imidazoquinoxaline (IQx) derivatives. It is now clear that these are ubiquitously found in meat cooked under very ordinary conditions. They are thus contaminants of daily food and the presence of HCA-DNA base adducts has already been proven in man. Human urine contains HCAs and their metabolites. HCAs are carcinogens in rodents (1981-1991). Dr. Felton found one of HCAs, being phenylimidazopyridine (PhIP). We demonstrated that PhIP can in fact induce many types of cancers, in the breast of female and colon and prostate of male rats and in the lymphatics of mice. All those cancers are currently on the increase in humans.

Dr. Jägerstad discovered that the precursors for IQ and IQx derivatives are creatin(in)e, sugars and amino acids in meat. HCAs are metabolically activated by CYP1A2 to their hydroxyamino derivatives. The hydroxyamino derivatives are further activated by esterification with acetic acid and sulfuric acid to ultimate forms, producing DNA adducts, especially with guanine.

Human beings are not only exposed to HCAs but also to other genotoxic agents such as polycyclic aromatic hydrocarbons and active oxygen species which can damage DNA. Chronic inflammation yields active oxygen and nitric oxide. Inflammation and tissue damage stimulates cell division and increases the chance of errors occurring with DNA replication. This may be one reason for the observed link between viruses and bacteria infection and human carcinogenesis.

The presence of mutations in cancer cells has been demonstrated for oncogenic virus studies on the one side, and transformation experiments with NIH 3T3 cells on the other. Furthermore, the identification of families led to the discovery of cancer related genes, such as *RB*, *APC* and *BRCA1*.

We were lucky to early on demonstrate the presence of multiple genetic alterations in pancreas (1986) and then lung small cell cancers (1987) In the same vein, clonal growth of hepatoma cells with accumulation of genetic alterations was demonstrated for hepatitis B virus infected hepatocarcinogenesis. In animal experiments, colon cancer induced by PhIP often showed truncation of *APC* gene as with cases of human colon neoplasia. In addition, microsatellite mutations were frequently observed in both experimental and clinical studies.

An awarenes of "Cancer is a disease of DNA" facilitates development of new weapons for early cancer diagnosis, deciding on the most appropriate therapy for individual cancer patients, gene therapy, better consultation of cancer-family members, cancer prevention, and inhibition of multiple tumor in patients at high risk.