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.

Nutrition, Cancer, and The Degenerative Diseases of Aging

Bruce N. Ames

Aging appears to be in good part due to the oxidants produced as by-products of normal metabolism by mitochondria. The degenerative diseases of aging such as cancer, cardiovascular disease, cataracts, and brain dysfunction, are increasingly found to have, in good part, an oxidative origin. The main source of dietary antioxidants is fruits and vegetables. The quarter of the American population that eats the fewest fruits and vegetables (5 portions a day is advised) has about double the cancer rate for most types of cancer of the quarter that eats the most. Deficiency of antioxidants causes the same damage to DNA as radiation.

Many micronutrients protect against DNA damage. For example, folate deficiency is one of the most common vitamin deficiencies, occurring in nearly half of low income (mainly African-American) elderly, and adolescents. Folate deficiency is associated with increased chromosome breaks cancer, heart disease, neural tube defects in the fetus, and cognitive defects in adults. Folate deficiency causes extensive incorporation of uracil into human DNA (4 million/cell), leading to chromosomal breaks. Elevated DNA uracil levels and chromosome breakage are reversed by folate administration. This mechanism is the likely cause of the increased cancer risk and cognitive defects in humans and emphasizes the importance of fruit and vegetable intake for a healthy life.

Men with low Vitamin C intake have low Vitamin C in their seminal fluid and much more oxidative damage to the DNA in their sperm. Male smokers are particularly at risk as they have depleted antioxidant pools (cigarette smoke is extremely high in oxidants). A smoker must eat 2 to 3 times as much Vitamin C as a non-smoker to maintain an equal plasma level, yet smokers tend to eat worse diets than non-smokers. Indeed, male smokers may have a considerably higher risk of having children with birth defects and childhood cancer.

Two major causes of cancer are smoking (1/3 of cancer & 1/4 of heart disease) and dietary imbalances (excess fat and calories; inadequate intake of fruits, vegetables, fiber, and micronutrients). Another major contributor to cancer is chronic infections leading to chronic inflammation (hepatitis B and C viruses, Helicobacter pylori infection, schistosomiasis, etc.). Chronic inflammation is a major cause of cancer in the world because it releases powerful oxidants which both stimulate cell division and are mutagens. Gamma-tocopherol, the main source of Vitamin E in the diet, is a mutagen trap and defends against NOx and other mutagens released during inflammation or smoking, and thus complements alpha-tocopherol, the antioxidant sold as a supplement. Past occupational exposures might cause about 2% of current human cancer, a major part being asbestos exposure in smokers, and industiral or synthetic chemical pollution causes less than 1%, in my view. The age-adjusted cancer death rate in the U.S. for all cancers combined (excluding those attributable to smoking) has declined 15% in the U.S. since 1950, while life expectancy increases every year.

Two factors are critical in the formation of mutations: lesions in DNA, formed when DNA is damaged, and cell division, which converts DNA lesions to mutations. Agents increasing either lesions or cell division increase mutations and as a consequence increase cancer incidence. Hormones stimulating cell division increase cancer incidence (e.g. levels of estrogen in breast cancer and testosterone in prostate cancer); hormones may be a risk factor in about 20% of human cancer.

Animal cancer tests, which are done at the maximum tolerated dose (MTD), are being misinterpreted to mean that low doses of the chemicals tested and found positive are thereby relevant to human cancer. Animal cancer tests are mainly done on synthetic chemicals and industrial pollutants, yet half of all natural chemicals that

have been tested at the MTD are rodent carcinogens. It is argued that the explanation for the high frequency of positive results in animal cancer tests is that high dose animal cancer tests are mainly measuring increases in cell division due to cell killing and compensatory cell division; this is a high dose effect that does not occur at low doses. In any case, 99.9% or more of the chemicals we eat are natural. For example 99.99% of the pesticides we eat are natural chemicals that are present in plants to ward off insects and other predators. More than half of those natural pesticides tested in high dose animal tests are rodent carcinogens. There are about 10,000 of so different natural pestivides in our diet, and they are usually present at enormously higher levels than synthetic pesticides. Cooking food also generates thousands of chemicals. There are over 1000 chemicals reported in a cup of coffee. Only 26 have been tested in animal cancer tests and more than half are rodent carcinogens; there are still a thousand chamicals left to test. The amount of potentially carcinogenic pesticide residues consumed in a year is less than the known amount of rodent carcinogens in a cup of coffee.

The reason we can eat the tremendous variety of natural chemical rodent carcinogens in our food is that animals are extremely well defended against all chemicals by many general defense systems. These enzymes, e.g. DNA repair and glutathione transferases which defend against reactive compounds such as mutagens, are all inducible (more of them are made when they are in use). They are equally effective against natural and synthetic reactive chemicals. Thus, animals are extremely well defended against low doses of chemicals. One does not expect, nor does one find, a general difference between synthetic and natural chemicals in their carcinogenicity, and though less well studied, the same would be expected for mutagenicity, teratogenicity, and acute toxicity.