MOLECULAR EPIDEMIOLOGY

Some Evidences of Molecular Epidemiology in Cancer Research

Kei Nakachi, Kazue Imai, and Kenji Suga

Molecular epidemiology of cancer has been developed along with rapid advances in molecular carcinogenesis, introducing new approaches to etiology and prevention of cancer. First, research activities in Japan are briefly reviewed in this issue focusing on studies of genetic susceptibility, DNA damages, and immunological defense. Then we make an estimation of age periods of initiation for several cancer on the basis of multistaged carcinogenesis. The results are discussed in relation to strategies of cancer prevention in molecular epidemiology, introducing our trials previous and on-going as examples. *J Epidemiol*, 1996 ; 6 : S125-S129.

genetic susceptibility, DNA damage, immunological defense, cancer, Japan

Recent advances in molecular biology introduced new tools into epidemiology, which aims to prevent diseases by studying the association between diseases and environmental causes, i.e., interaction of host with environmental factors in disease incidence or development. Molecular epidemiology discloses the host-environment interaction from the host side in terms of genetic events. Studies made so far have widened the framework of traditional epidemiology in two main respects : genetic host factors and measurement of genotoxic damages on host. The former is based on DNA polymorphisms of several cancer-associated genes, resulting in variation of cancer susceptibility among individuals and, hence, identification of genetically high risk individuals. This approach can provide valuable insights into the problem of "individual difference", which had been treated as a statistical fluctuation in population. The latter has provided biological evidence for known genotoxic environmental factors such as cigarette smoking and occupational exposure to chemicals, and it further enables us to assess genetic damage to host in relation to one's lifestyle.

Recent epidemiologic studies of these lines in Japan are briefly reviewed. Then, strategies of cancer prevention are discussed on the basis of molecular epidemiologic analysis of human carcinogenesis.

MOLECULAR EPIDEMIOLOGIC STUDIES IN JAPAN

A Large-Scale Cohort Study On-Going

Most biological markers are influenced by existence of cancer in host organisms. Accordingly, these markers may not be suitable for use in case-control studies, and cohort studies seem to be the only way to examine directly these markers' association with cancer. However, such studies require large layouts of man-power and funds, as well as epidemiologic surveys and follow-ups. Nevertheless, following the lead of K. Aoki¹⁾, a large-scale cohort study has been conducted by a group of Japanese epidemiologists with collection of serum samples and DNA's isolated from peripheral lymphocytes. This study is a very valuable investment in the future of Japanese epidemiology.

DNA Polymorphisms as a Marker of Genetic Susceptibility to Cancer

In contrast to most biological markers, germline polymorphisms in DNA sequences of genes can be applied to case-control studies especially on inter-individual differences in susceptibility to chemical carcinogenesis, since cancer does not affect germline polymorphisms. The basic concept of gene-environment interaction has been described as "genetic epidemiology"²⁾. Genetic polymorphisms of drug-metabolizing enzymes, which catalyze bioactivation of carcinogens and detoxification of their reactive metabolites, have been studied in association with lung cancer, focusing on the cytochrome P4501A1 (bioactivation of benzo[a]pyrene), P4502E1 (nitrosoamines), P4502D6 (NNK, 4-(methylnitrosamino)-1-(3-

Received and accepted September 21, 1995.

Department for Epidemiology, Saitama Cancer Center Research Institute, Saitama, Japan.

Address for correspondence : Kei Nakachi, Department of Epidemiology, Saitama Cancer Center Research Institute, 818 Komuro Ina-machi Saitama, 362 Japan.

pyridyl)-1-butanone), and a μ -class glutathione *S*-transferase GSTM1 (detoxification of reactive metabolites of benzo[a]pyrene)³⁻¹¹⁾. Most of the studies in this line, carried out in many countries, compared only gene or genotype frequencies among patients and controls. However, epidemiologic studies in Japan estimated genetic risk of lung cancer in relation to cigarette smoking dose^{3-5,9)}. Genetic risk for combined genotyping of P4501A1 and GSTM1 genes was also studied from the aspect of metabolic balance between activation and detoxification of carcinogens^{4,10)}. Inducible phenotype of P4501A1 (aryl hydrocarbon hydroxylase (AHH) activity) was measured in a Japanese population and found to relate to the genetic polymorphisms of P4501A1 gene¹²⁾.

Also of interest is the fact that a genetic polymorphism of low K_m aldehyde dehydrogenase (ALDH2) was studied among Japanese population, who showed an increased frequency of the enzyme deficiency compared with Caucasians, revealing that this polymorphism is closely linked to alcohol-associated symptoms and drinking behavior¹³⁾. This work will provide a useful tool for studies of alcohol-associated cancer in terms of a genetic factor regulating alcohol drinking behavior or ethanol metabolism.

DNA Damages Due to Chemical Carcinogens and Oxygen Radicals

The measurement of various DNA adducts has been developed, and it is expected to be a marker for exposure levels to carcinogens or for oxidative damage to DNA, although most epidemiological studies are restricted by types of biological materials available, i.e., peripheral blood. A representative marker for oxidative DNA damages, 8-hydroxydeoxyguanosine (8-OHdG) level, was measured by a strict assay protocol among Japanese individuals to examine its association with lifestyle : no such association was found¹⁴⁾. However, an increased level was observed in Fanconi's anemia patients, who are known to have high risk of cancer¹⁵⁾. Highly sensitive detection of DNA alkylation products has been developed for use in studies of molecular epidemiology, and detectable levels of the products were found in peripheral lymphocytes of general individuals¹⁶⁾. Application of DNA adduct assay to epidemiology is in general limited to cross-sectional studies of assessing environmental risk factors in small numbers of study subjects in the general population, since the adduct levels in dividing cells seem to reflect only recent genotoxic events or exposure to carcinogens ; hence, the measurement on cancer patients provides little meaning for case-control studies. Apart from DNA adduct, increased frequency of sister chromatid exchanges (SCE) was found to relate to poor health practices¹⁷⁾, suggesting that SCE can be a marker to assess generalized health.

Immunological Markers and Lifestyle

Immunological defense against cancer, which may come into action in the late stage of promotion or subsequent stages, has

been intensively studied in Japan, although, strictly speaking, it may not belong to the category of molecular epidemiology. Namely, association of natural killer cell activities with living habits has been reported in cross-sectional studies, revealing that some good health practices, already known to reduce cancer risks, also enhance the natural immune against cancer^{18,19)}. Their results suggest that immunological host defense may play an important role in prevention of cancer development, not only in immunodeficient patients but in general population. Recent advances in molecular immunology including cloning of natural killer cell stimulating factor (NKSF) may provide a molecular basis for these studies soon. Although follow-up studies are needed to confirm association of biological markers with cancer incidence, these studies have demonstrated that certain types of immune may underlie so-called good health practices, resulting in reduction of cancer risk.

Present Limitation of Molecular Epidemiology

Molecular epidemiology in Japan and other countries may need to be expanded in the following respects. Most molecular epidemiologic studies have paid attention only to genetic affairs concerned with exposure to carcinogens or genetic predisposition to initiation. This limitation was partially due to a rule of epidemiology ; namely, studies have to include general individuals as controls (to compare with cancer patients) or as parent population from which cancer incidences take place. Hence, the biological markers or materials were restricted to those measurable for, or available in, general population. In the light of this, is it possible to develop methodology, and study the events followed by "exposure", for cancer prevention? It is important to expand our preventive measures even to those who have already experienced the first genetic alteration, since the first genetic event in carcinogenic process may not be the only important factor for cancer prevention. To discuss this problem further, we next make an estimation of carcinogenic process as a function of age and try to characterize human carcinogenesis from the standpoint of prevention.

CARCINOGENIC PROCESS IN HUMAN POPULATION

Studies on molecular carcinogenesis have engendered the concept of multistaged carcinogenesis where normal cells become cancerous according to sequential stages but with cumulative effects, i.e., initiation, promotion, conversion, and progression. In chemical carcinogenesis, the first step in initiation is exposure to various carcinogens in one's environment, and most chemical carcinogens require metabolic activation to become reactive electrophilic forms, which react with DNA resulting in formation of DNA adducts. This reaction seems to occur within only a few hours of exposure. The chemical damage to DNA causes genetic alterations during DNA replication, and these alterations become fixed once they evade DNA repair processes. Some of these genetic damages pro-

Figure 1. Age periods of initiation estimated for several cancers on the basis of multistaged carcinogenesis theory. In numerical calculation for this estimate, age-dependences of relative risk for cancers (peak positions and gradients of relative risks to age) were used as parameters in the formula derived by Whittemore, varying the numbers of stages from 2 to 7 taken as another parameter.

duce the initiated cells where the activation of oncogenes or inactivation of tumor suppressor genes takes place. The change of normal cells to initiated cells may take only a few days. The longest stage in human carcinogenesis is promotion, where the initiated cells are clonally expanded over periods of ten years or more. In the following stages of conversion and progression, the cells in promotion stage undergo additional genetic alterations and acquire malignant phenotypes of invasion and metastasis. Importance of each stage differs according to the purpose of a study, i.e., host status (exposure level or susceptibility to carcinogens) in initiation is useful for the identification of high risk individuals or significant carcinogens in environment; promotion is the most important stage for prevention of cancer for the reason described below.

Initiation occurs repeatedly at various cells in the human body throughout a human life span, and initiation that results in cancer within an average lifetime seems to take place rather early in life. We estimated age periods of initiation for several cancers in Figure 1, by a numerical analysis on cancer mortality data among Japanese populations with different lifestyles, using the formulation reported by Whittemore²⁰. Considering that progression takes one or two years, the age period between initiation and cancer incidence in Figure 1 is mostly of promotion stage. Initiation of lung and stomach cancer takes place in the twenties or thirties, while that of colon and liver occurs even earlier: in some cases, the latter possibly occurs at birth, suggesting contribution of hereditary factors (as in breast cancer) and viral infections in fetal or infant life. It is also interesting to note that initiation (or the initial step) in hormone-associated cancers of the breast and uterus somehow overlap with age period of most frequent incidence, suggesting that their

carcinogenesis is quite different from that of the other cancers.

This scheme of carcinogenic stages and age may be applied not only to cancer patients but more or less to most of general population, since the cumulative incidence rates of cancer show rapid increase of probability of contracting cancer with age: among Japanese men, 38% by age 79, 57% by age 89, and 71% by age 99; among Japanese women, 21% by age 79, 33% by age 89, and 44% by age 99. To summarize, large portions of men and women are thought to have expanding initiated-cells in their organisms throughout a considerably long period of their lives. This tells us why regulation of tumor promotion is the most important factor for cancer prevention.

DISCUSSION

A recent criticism on epidemiology, "Epidemiology Faces Its Limits"²¹, has pointed out and summarized the problems in methodology and interpretation of findings confronting epidemiologists. Furthermore, advances in molecular carcinogenesis have widened the gap between epidemiology and basic research, which asks epidemiologists to present epidemiologic findings on carcinogenic mechanisms in a more interpretable way and to assess exposure to risk factors in terms of biological measurement. To overcome these problems, we epidemiologists may need to expand and improve our methodology and ideas, while retaining the values of traditional epidemiology. Introduction of molecular biological concept and techniques into classical epidemiology is one way for us to achieve this purpose.

The authors would like to introduce our past and current studies as follows, in hopes that our difficulties in molecular

epidemiology may provide a humble example. We started our molecular epidemiology in 1988 with the aim to identify individuals at genetically high risk for lung cancer. We have tried several approaches to study variation in cancer susceptibility among individuals in terms of difference in dose-risk relationship from an epidemiologic standpoint. Genetic susceptibility is one of the important problems in advanced countries where a wide variety of carcinogens exist in our environment at relatively low concentrations, and lung cancer provides a good model for host-environment interaction to study this subject, especially when susceptible individuals contract the carcinoma with lesser cigarette dose. However, exposure to a number of carcinogens in our life means that avoidance of initiation is very difficult as we discussed in the previous section, and hence we have to address the problem of what truly effective prevention of cancer consists in, apart from the identification of high risk individuals. Our cohort study, which started in 1986 and combined molecular, serological, and immunological approaches with epidemiological survey, gave us a breakthrough in this point, showing that green tea is the most promising candidate for prevention of cancer as well as prevention of cardiovascular disease²². Specifically, delayed cancer onset was associated with increased consumption of green tea, and a significant slowdown in the increase of cancer incidence with age was observed among those who consume a large amount of green tea. Since initiation is irreversible and difficult to avoid, our aim in cancer prevention is to delay cancer onset indefinitely by inhibiting growth of initiated cells and subsequent events. Green tea provides us a good model for the effective prevention of this line. Molecular biological concepts and methodology need to be introduced in the next step of study: intervention trials using green tea extract.

We recently started a breast cancer study, investigating mRNA expression levels and mutations of several genes in tumor, hormone concentrations in breast tissue and peripheral blood, and epidemiologic characteristics of patients. These measurements will be associated with invasive and metastatic feature of tumor in individual patients, aiming to find host factors, in development of breast cancer, that may be described as interaction of tumor with its neighboring tissues. Slowing down tumor growth or inhibiting malignant transformation will be studied for their possible association with lifestyle. This is useful for cancer prevention of the above concept.

To summarize, epidemiology has to develop along with the rapid advances in molecular carcinogenesis. The term "molecular epidemiology" may be meaningless, if it merely indicates the introduction of molecular biological tools. We have to follow advancing ideas of cancer research and apply them to prevention of human carcinogenesis, because human cancer involves not only biological events but also cultural, behavioral, and psychological factors. Epidemiology is just now in a position to bring together all of these factors in the science of prevention.

ACKNOWLEDGEMENT

This study was supported in part by Grants-in-Aid for Cancer Research from the Ministry of Education, Science and Culture, from the Ministry of Health and Welfare, Japan, and a grant from the Smoking Research Foundation of Japan.

REFERENCES

1. Annual report of the research by "grant-in-aid for scientific research on priority areas-cancer-" of Ministry of Education, Science and Culture. 1994 : pp42-45 (in Japanese).
2. Khoury MJ, Adama MJ, and Flanders WD. An epidemiologic approach to ecogenetics. *Am J Hum Genet*, 1988 ; 42 : 89-95.
3. Nakachi K, Imai K, Hayashi S-I, Watanabe J, and Kawajiri K. Genetic susceptibility to squamous cell carcinoma of the lung in relation to cigarette smoking dose. *Cancer Res*, 1991 ; 51 : 5177-5180.
4. Nakachi K, Imai K, Hayashi S-I, and Kawajiri K. Polymorphisms of the *CYP1A1* and glutathione S-transferase genes associated with susceptibility to lung cancer in relation to cigarette dose in a Japanese population. *Cancer Res*, 1993 ; 53 : 2994-2999.
5. Nakachi K, Hayashi S-I, Kawajiri K, and Imai K. Association of cigarette smoking and *CYP1A1* polymorphisms with adenocarcinoma of the lung by grades of differentiation. *Carcinogenesis*, 1995 ; 16 : 2209-2213.
6. Hamada GS, Sugimura H, Suzuki I, Nagura K, Kiyokawa E, Iwase T, Tanaka M, Takahashi T, Watanabe S, Kino I, and Tsugane S. The heme-binding region polymorphism of cytochrome P450IA1 (*Cyp1A1*), rather than the RsaI polymorphism of IIE1 (*Cyp1IE1*), is associated with lung cancer in Rio de Janeiro. *Cancer Epidemiol Biomarkers & Prev*, 1995 ; 4 : 63-67.
7. Sugimura H, Hamada GS, Suzuki I, Iwase T, Kiyokawa E, Kino I, and Tsugane S. *CYP1A1* and *CYP2E1* polymorphism and lung cancer, case-control study in Rio de Janeiro, Brazil. *Pharmacogenetics*, 1995 ; 5 : S145-S148.
8. Uematsu F, Kikuchi H, Motomiya M, Abe T, Sagami I, Ohmachi T, Wakui A, Kanamaru R, Watanabe M. Association between restriction fragment length and polymorphism of the human cytochrome *P450IIE1* gene and susceptibility to lung cancer. *Jpn J Cancer Res*, 1991 ; 82 : 254-256.
9. Kihara M, Kihara M, and Noda K. Lung cancer risk of *GSTM1* null genotype is dependent on the extent of tobacco smoke exposure. *Carcinogenesis*, 1994 ; 15 : 415-418.
10. Kihara M, Kihara M, and Noda K. Risk of smoking for squamous and small cell carcinoma of the lung modulated by combinations of *CYP1A1* and *GSTM1* gene polymorphisms in a Japanese population. *Carcinogenesis*,

- 1995 ; 16 : 2331-2336.
11. Gough AC, Miles JS, Spurr NK, Moss JE, Gaedigk A, Eichelbaum M, and Wolf CR. Identification of the primary gene defect at the cytochrome P₄₅₀ *CYP2D* locus. *Nature*, 1990 ; 347 : 773-776.
 12. Kiyohara C, Hirohata T, and Inutsuka S. The relationship between aryl hydrocarbon hydroxylase and polymorphisms of the *CYP1A1* gene. *Jpn J Cancer Res*, 1996 ; 87 : 18-24.
 13. Takeshita T, Morimoto K, Mao X-Q, Hashimoto T, and Furuyama J-I. Phenotypic differences in low K_m aldehyde dehydrogenase in Japanese workers. *Lancet*, 1993 ; 341 : 837-838.
 14. Takeuchi T, Nakajima M, Ohta Y, Mure K, Takeshita T, and Morimoto K. Evaluation of 8-hydroxydeoxyguanosine, a typical oxidative DNA damage, in human leukocytes. *Carcinogenesis*, 1994 ; 15 : 1519-1523.
 15. Takeuchi T and Morimoto K. Increased formation of 8-hydroxydeoxyguanosine, an oxidative DNA damage, in lymphoblasts from Fanconi's anemia patients due to possible catalase deficiency. *Carcinogenesis*, 1993 ; 14 : 1115-1120.
 16. Kang H, Konishi C, Eberle G, Rajewsky MF, Kuroki T, and Huh N. Highly sensitive, specific detection of O⁶-methylguanine, O⁴-methylthymine, and O⁴-Ethylthymine by the combination of high-performance liquid chromatography prefraction, ³²P postlabeling, and immunoprecipitation. *Cancer Res*, 1992 ; 52 : 5307-5312.
 17. Morimoto K, Takeshita T, Takeuchi T, Maruyama S, Ezoe S, Mure K, and Inoue C. Chromosome alternations in peripheral lymphocytes as indices of lifestyle and genotoxicity. *Int Arch Occup Environ Health*, 1993 ; 65 : S37-S41.
 18. Nakachi K and Imai K. Environmental and physiological influences on human natural killer cell activity in relation to good health practices. *Jpn J Cancer Res*, 1992 ; 83 : 798-805.
 19. Kusaka Y, Kondou H, and Morimoto K. Healthy lifestyles are associated with higher natural killer cell activity. *Preventive Medicine*, 1992 ; 21 : 602-615.
 20. Whittemore AS. The age distribution of human cancer for carcinogenic exposure of varying intensity. *Am J Epidemiol*, 1977 ; 106 : 418-432.
 21. Taubes G. Epidemiology faces its limits. *Science*, 1995 ; 269 : 164-169.
 22. Imai K and Nakachi K. Cross sectional study of effects of drinking green tea on cardiovascular and liver diseases. *BMJ*, 1995 ; 310 : 693-696.