

Molecular epidemiology: recent advances and future directions

Frederica P. Perera¹ and I. Bernard Weinstein

Division of Environmental Health Science, Joseph L. Mailman School of Public Health at Columbia University, and Herbert Irving Comprehensive Cancer Center, Columbia University, New York, NY 10032, USA

¹To whom correspondence should be addressed
Email: fpp1@columbia.edu

In 1982 we proposed the concept and a framework for implementing molecular cancer epidemiology. Here, we review progress during the past 17 years in validating and applying this approach to cancer prevention. There have been major advances, notably in the understanding of environment–susceptibility interactions in human cancer. However, a review of major findings to date reveals several urgent research needs to keep pace with the rapid evolution in knowledge of mechanisms in carcinogenesis. Although much valuable progress continues to be made in the study of carcinogens that cause direct DNA damage and are mutagenic, exogenous and endogenous carcinogens can also act by altering gene expression, cell proliferation and differentiation. The mechanisms include aberrant DNA methylation, oxidative damage, effects on metabolism of nitrogen oxide and nitrites, activation of receptors and transcription factors, cyclins and other cell cycle proteins. Sensitive, validated biomarkers are needed to detect these mechanisms in small numbers of cells, tissues or fluids. There is also increasing recognition that individual risk from carcinogen exposure varies as a function of both inherited and acquired factors. Recent advances in genomics, microassay technologies and informatics hold promise for rapid identification of polymorphic variants or changes in expression of genes influencing both response and susceptibility to carcinogens. Another emerging area of molecular epidemiology concerns the role of nutrition and specific dietary factors (including studies on antioxidants, energy metabolism, insulin and various growth factors) and the modulating effect of genetic polymorphisms. Finally, molecular epidemiology has enormous potential in cancer prevention through the early identification of ‘at risk’ populations and the rapid assessment of intervention efficacy. Its success in fully reaching this potential will depend on the application of validated biomarkers, with adherence to sound epidemiologic and ethical principles.

Introduction

In 1982, we proposed the concept and a framework for implementing molecular cancer epidemiology—the merging of molecular biology and epidemiology in the study of cancer causation and, ultimately, in the prevention of this disease (1). Our paper presented a conceptual framework for incorporating biomarkers (biochemical or molecular alterations detectable in

human samples) into epidemiologic studies to assess individual exposure, dose, preclinical effects and susceptibility to carcinogens. We discussed in detail the advantages and limitations of the approach, stressing the need for careful validation of methods. Molecular epidemiology has, in the intervening 17 years, become a major field of research and considerable progress has been made in the validation and application of biomarkers (2–7). Perhaps the greatest contribution of molecular epidemiology has been the insights it has provided into interindividual variation in human cancer risk and the complex interactions between environmental factors and host susceptibility factors, both inherited and acquired, in the multistage process of carcinogenesis (8).

Molecular epidemiology has as its ultimate goal the prevention of cancer, a disease that in the USA claims over 500 000 lives annually, with more than 1 000 000 new cases diagnosed each year (9). Even more clearly than in 1982, various lines of evidence indicate that the great majority of cancer is, in principle, preventable because the factors that determine cancer incidence are largely exogenous (10). This evidence comes mainly from epidemiologic studies and includes: (i) time trends in cancer incidence and mortality; (ii) geographic variations and the effects of migration; (iii) the identification of specific causative factors such as cigarette smoking, occupational and environmental chemicals, radiation, dietary factors and viruses; and (iv) the observation that the majority of human cancers do not show simple patterns of inheritance. Genetic factors are clearly important in terms of influencing individual susceptibility to carcinogens; and in certain rare forms of human cancer, hereditary factors play a decisive role. However, external factors represent the greatest opportunity for primary prevention. This is an optimistic message because it means that the development of cancer is not an inherent consequence of the aging process *per se*, and the human species is not inevitably destined to suffer a high incidence of cancer. This awareness has lent greater urgency to the search for more powerful tools for primary prevention—for early warning systems to identify causal environmental agents and flag risks well before the malignant process is entrenched.

Overview of recent progress in molecular epidemiology

Until recently, the majority of cancer epidemiology studies were limited to assessing possible causative associations between two types of events: exposure to potential causative ‘environmental’ agents (i.e. cigarette smoke, specific chemicals in the workplace, dietary factors, etc.) and disease outcome (i.e. clinically apparent cancer incidence or cancer mortality). The modulation of environmental factors by host susceptibility was rarely evaluated. However, within the past few years, the interaction between environmental factors and host susceptibility factors has become a very active area of research. Increasingly, molecular epidemiologic studies are incorporating panels of biomarkers relevant to exposure, preclinical effects and susceptibility, using samples of blood cells, exfoliated cells,

Abbreviations: AFB₁, aflatoxin B₁; IGF-1, insulin-like growth factor-1; PAH, polycyclic aromatic hydrocarbon.

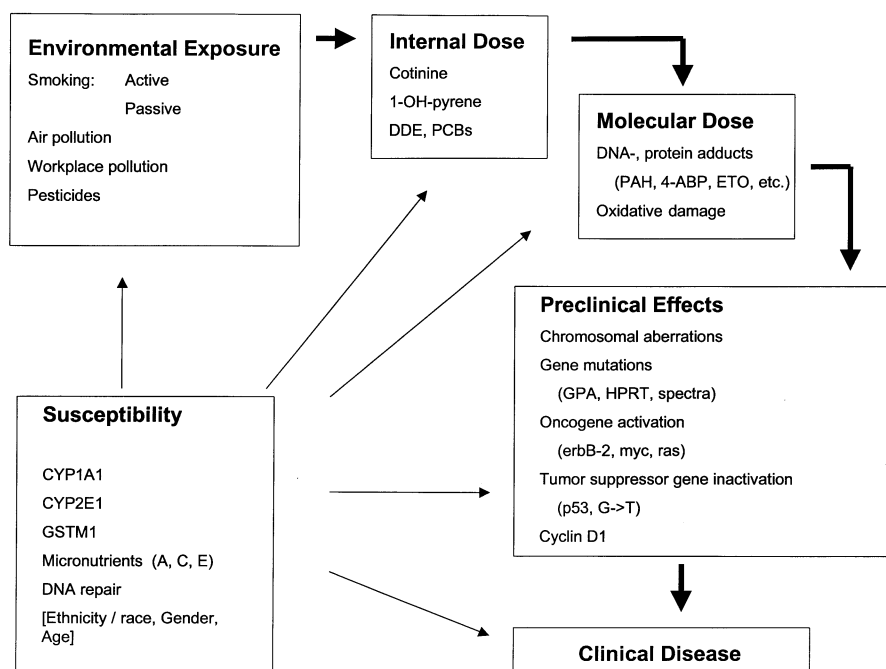


Fig. 1. The continuum of biomarkers with representative examples within each category (based on 1).

tissues and body fluids (7,8). These biomarkers are now being widely used in cross-sectional, retrospective, prospective and nested case-control epidemiologic studies, with the aim of improving our understanding of the causes of specific human cancers.

Although molecular epidemiology has the advantage of being directly relevant to human risk, unlike animal or other experimental models that require extrapolation to humans, it is also subject to many of the general limitations of epidemiologic studies, such as vulnerability to confounding factors. In addition, many of the biomarkers used in molecular epidemiologic studies require further validation; as discussed in detail elsewhere (5,11). Furthermore, without adequate precautions the use of these biomarkers carries the potential for serious social harm (reviewed in refs 12 and 13). At the present time, many biomarkers can be useful in assessing exposure, early deleterious molecular and biologic effects and potential risk for a group or population; most are not, however, sufficiently characterized or validated for routine use in screening, diagnosis or quantitative estimation of individual risk of developing cancer.

As we proposed in 1982 (1), biomarkers have generally been classified into four major categories: (i) internal dose; (ii) biologically effective dose; (iii) preclinical biologic effects; and (iv) susceptibility, with some overlap between them (14) (Figure 1). Of the four categories, internal dosimeters have been most widely used, particularly in the workplace, to determine exposure to carcinogens and other toxicants. Because of their precision, reliability, and relevance to individual risk, biomarkers of internal dose have been used to great advantage in conjunction with more traditional approaches: monitoring of ambient or workplace concentrations of the selected agent(s), and analysis of workers' records or questionnaires, including information on smoking, food consumption and other environmental exposures.

Highly sensitive analytical procedures and immunoassays now make it possible to measure very low concentrations of

a potential chemical carcinogen or its metabolites in a variety of cells, tissues or body fluids. Biomarkers of internal dose take into account individual differences in absorption, metabolism, bioaccumulation or excretion of the compound in question and indicate the actual level of the compound within the body and in specific tissues or compartments. However, their half-lives range from a few hours to decades, so their interpretation and applicability to different study designs vary accordingly (15). Examples include cotinine in serum or urine, resulting from cigarette smoke exposure; urinary levels of 1-hydroxypyrene from exposure to polycyclic aromatic hydrocarbons (PAHs); aflatoxin B₁ (AFB₁) levels in urine, reflecting dietary sources; *N*-nitroso compounds or their metabolites in urine from dietary or endogenous sources; DDT, polychlorinated biphenyls (PCBs), dioxins and furans in serum or adipose tissue biopsies, due to workplace and environmental contamination; infectious agents in target tissues such as human papillomavirus (HPV); and mutagenicity of urine, a generic dosimeter of genotoxic agents present in the body as a consequence of exposure to cigarette smoke or other environmental sources.

Although markers of internal dose are a valuable supplement to conventional methods of assessing exposure, they do not indicate the extent to which a given compound has interacted with critical cellular targets. Therefore, assays have been developed to measure the 'biologically effective dose' of a compound, i.e. the amount that has reacted with critical cellular macromolecules, usually DNA, or a surrogate, such as specific proteins in the blood. The rationale for measuring carcinogen-DNA adducts derives from the fact that a number of chemical carcinogens or their metabolites exert their biological effects by binding covalently to cellular DNA, thereby inducing mutations in critical cellular genes. Measures of specific carcinogen-DNA adducts reflect not only individual differences in absorption and distribution but also differences in metabolism (activation versus detoxification) of the chemical, as well as the extent of repair of carcinogen-DNA adducts that may have taken place subsequent to carcinogen exposure

(2,16–18). For many studies in humans, DNA from the target tissue is not readily accessible and thus surrogate cells or tissues are often used (e.g. peripheral blood cells, buccal cells or placenta). The relationships between the types and levels of adducts in the more readily available surrogate samples and those in the target tissue have not been well characterized in humans, but have been established for certain carcinogens in experimental animals (19). Another limitation is that, because of DNA repair and tissue renewal, levels of carcinogen–DNA adducts generally reflect exposure during the previous several months, rather than exposure in the distant past (20).

Since 1982, when the *in vivo* occurrence of carcinogen–DNA adducts was first reported in a human population (21), many different types of carcinogen–DNA adducts have been detected in human cells and tissues (17,18,22–24). [There was one prior report of methylated purine in two samples of human liver as a result of intentional poisoning (25).] Carcinogen–protein adducts, especially adducts formed with hemoglobin or albumin, have also been widely measured as surrogates for DNA damage (26,27). The use of longer-lived adducts with histone and collagen molecules is now being explored (26). The carcinogens whose derivatives have been found to covalently bind to DNA or protein include such diverse agents as ethylene oxide, 4-aminobiphenyl, PAH, AFB₁, nitrosamines and cisplatin (17,18,22,26–28). Not surprisingly, in many different populations, the adduct data show a general dose–response relationship for the genotoxic carcinogens studied, with no apparent threshold. Adducts have gained relevance as a potential risk marker from the recent finding that patients with lung cancer had markedly higher PAH–DNA adduct levels in their peripheral white blood cells than individuals without cancer, after taking into account differences in the amount of smoking and other potential confounders (17,29). Other molecular epidemiologic studies have prospectively linked DNA damage from AFB₁ to liver cancer risk in Chinese and Taiwanese populations (30–32). In virtually every study, the levels of DNA damage or protein adducts have been found to vary considerably between persons with apparently similar exposure (20,33,34). As has been shown with PAH–DNA adducts, although the observed variability reflects a combination of true biologic variability, unaccounted for differences in exposure, within-person variability, and laboratory variation, a significant fraction is apparently contributed by biologic factors (35). This aspect is discussed later under the topic of biomarkers of susceptibility and gene–environment interactions.

Although chemical-specific damage to DNA by exogenous agents remains a major mechanism in carcinogenesis, there is increasing recognition of the importance of other types of damage to DNA by both endogenous and exogenous factors, as a driving force in carcinogenesis. The types of non-specific DNA damage include deaminations, alkylations, base losses and oxidations. The magnitude of these effects can be influenced by inflammatory processes and co-exposure to dietary and environmental agents. Therefore, biomarkers that assess the extent of these types of DNA damage will play an increasingly important role in molecular epidemiologic studies. An example is the development of antibodies that recognize 8-hydroxyguanosine, one of the major DNA adducts produced by oxygen radicals (36).

The next category of biomarkers, which reflects subsequent events in the multi-step sequence of carcinogenesis, includes markers of early biologic effects resulting from exposure. Like

the preceding categories of markers, these effects can be measured directly in target tissues or in a surrogate source of cells, such as peripheral white blood cells. Most of the available biomarkers assess various types of genotoxicity, including chromosomal aberrations, small deletions [loss of heterozygosity (LOH)], and point mutations.

The powerful PCR method and variations thereof have greatly facilitated the analysis of somatic mutations in molecular epidemiologic studies (37). It is now possible to detect such mutations in tissues of exposed individuals at a very early stage in the carcinogenic process; that is, even before cells carrying mutated genes have undergone clonal expansion. Characteristic patterns of mutations in tumors have provided valuable, albeit circumstantial, molecular evidence of the importance of specific environmental carcinogens. For example, because mutations of the *p53* tumor suppressor gene and the *ras* oncogene are common events in human cancer, they can be useful reporters of carcinogenic events involved in the development of cancer. In a number of cases, the patterns of *p53* and *ras* mutations in human tumors have been consistent with the types of DNA adducts implicated in their causation and with the types of mutations induced experimentally by the specific environmental carcinogens under investigation (38,39). Thus, the mutation profile or spectrum found in certain genes may, in some instances, serve as a fingerprint of the causative agent. In recent studies, PCR-based methods have demonstrated that histologically ‘normal’ areas of the bronchial epithelium of cigarette smokers can display specific chromosomal areas of LOH that are often found in lung cancers (40). LOH can be considered a biomarker of biologic effect that may be useful in identifying individuals who do not yet have cancer but are at high risk.

Other types of biomarkers may be indicative of the dose and effects of agents that enhance the carcinogenic process without forming covalent adducts with cellular DNA or proteins, and without directly inducing gene mutations. These agents appear to act by disrupting gene expression, cell growth, apoptosis and/or differentiation. They include certain tumor promoters (12-*O*-tetradecanoylphorbol-13-acetate and phenobarbital), xenobiotics (such as 2,3,7,8-tetrachlorodibenzo-*p*-dioxin, PCBs and various pesticides) and natural and synthetic estrogens and androgens. Assays for the biologic effects of these agents are only now being developed, and include occupancy of specific high affinity receptors that mediate the action of these compounds, levels of specific mediators (growth factors, growth factor receptors, second messengers like cAMP and diacylglycerol, protein kinases and the phosphorylation of specific proteins), and the expression of genes related to tumor promotion, cell proliferation, apoptosis and differentiation. Examples of markers related to cell proliferation are proliferating cell nuclear antigen (a nuclear antigen associated with cell division) and cyclins (proteins associated with cell-cycle regulation) (41). In the latter category, cyclin D1 may prove to be a very useful biomarker since it is induced by various mitogens and is frequently overexpressed in a wide variety of human cancers (42). Recently, overexpression of cyclin D1 in plasma has been associated with increased risk of breast cancer (D.L.Tang, A.Rundle, Q.W.Chen, J.Z.Zhou and P.Brandt-Rauf, manuscript submitted).

The fourth category of biomarkers includes those that relate to inherited or acquired variations in host susceptibility. This category is a burgeoning area of research and, among all the categories of biomarkers, generates the greatest concern about

ethical issues (8,43–45). The basic principles of multistage carcinogenesis and of the molecular biology of cancer predict that a number of factors, in addition to exposure to specific causative agents, influence the likelihood that tumors will develop in a given individual. These individual susceptibility factors can be acquired or inherited. Examples include inter-individual differences in the metabolism (activation and detoxification) of carcinogenic chemicals, DNA repair, and the functions of protooncogenes or tumor suppressor genes, as well as interindividual differences in nutritional, hormonal and immunologic factors.

With respect to genetic factors, rare, highly penetrant dominant mutations in genes, such as p53 in the Li–Fraumeni syndrome and Rb in familial bilateral retinoblastoma confer high individual risk, but account for a small percent of all cancer (12,46–48). In contrast, relatively common genetic traits that regulate metabolism and detoxification of carcinogens can have a major impact on the population attributable risk of cancer, even though their individual risk is low. For example, cytochrome P450 ‘phase I’ enzymes can produce highly reactive DNA-damaging intermediates during the normal process of converting chemical carcinogens to excretable forms. CYP1A1, a cytochrome P450 enzyme that metabolizes PAH, is normally inducible. Thus, its level can vary 20-fold in the human liver and 50-fold in the human lung (49,50). Inducibility also varies between individuals, and high CYP1A1 inducibility has been correlated in a number of studies with risk of lung cancer (51,52). Polymorphic variations in the sequences of CYP1A1, CYP1A2, CYP1B1 and other P450s exist in the human population and specific forms have been associated with increased cancer risk in various populations (43).

Interindividual variation in ‘Phase II’ detoxifying enzymes, such as GSTM1, can also contribute to individual susceptibility. About 40% of the population have a deletion at this locus which has been linked to increased risk of bladder and lung cancers (53–56). Another common genetic factor related to increased cancer risk is the inheritance of a homozygous recessive mutation in the *N*-acetyltransferase gene resulting in the inability to efficiently detoxify aromatic amines via *N*-acetylation. The mutation is carried by ~50% of the population. It has been demonstrated that slow acetylators are at increased risk of bladder cancer, especially those occupationally exposed to aromatic amine bladder carcinogens (57). Furthermore, in a study of blond and black tobacco smokers, slow acetylators had higher levels of 4-aminobiphenyl-hemoglobin (4-AB-Hb) adducts in their red blood cells than fast acetylators who smoked the same type and quantity of cigarettes (58,59). In contrast to its protective effect in bladder cancer, the fast acetylator phenotype is associated with an increased risk of colon cancer (60,61), demonstrating the complexity of gene–environment interactions with respect to cancer susceptibility. Moreover, combinations of metabolic polymorphisms are increasingly being linked to increased cancer risk (8).

Acquired or inherited variations in the efficiency or fidelity of DNA repair can also influence individual susceptibility to cancer. This principle is illustrated by the rare autosomal recessive disease xeroderma pigmentosum, in which defects in the excision of pyrimidine dimers and other bulky DNA lesions lead to a marked increase in susceptibility to skin cancers induced by sunlight (62). Indeed, studies of human lymphocytes have identified a ~5-fold interindividual variation in unscheduled DNA synthesis induced by UV exposure; and DNA repair deficiency (measured phenotypically) has been

linked to increased cancer risk (63,64). In one study, compared with healthy controls, patients with lung cancer were five times more likely to have reduced ability to repair damage induced by the PAH metabolite, benzo[*a*]pyrene diol epoxide (65). Recent studies suggest that polymorphisms in the *XPD* nucleotide excision repair gene influence the risk of basal cell carcinomas in patients with psoriasis (66). In addition, studies of Taiwanese suggest that polymorphisms in another nucleotide excision repair gene, *XRCC1*, result in deficient DNA repair (67).

Rapid progress has been made in identifying the numerous genes that play a role in DNA repair pathways in mammalian cells, including direct damage reversal (via the enzyme *O*⁶-methylguanine-DNA methyltransferase), base excision repair, nucleotide excision repair, mismatch repair and double-strand break repair (68). It will be of interest to determine to what extent heritable polymorphic variations in some of these enzymes influence individual susceptibility to specific types of human cancer.

Using combinations of biomarkers, molecular epidemiology has reinforced prior evidence that risk from carcinogenic exposures can vary significantly with ethnicity, age or stage of development, gender, pre-existing health impairment and nutritional factors (for review see ref. 8). Biologically based interindividual variation in only a few susceptibility factors can lead to a significant increase in population risk over that expected based on an assumption of uniform susceptibility, possibly by an order of magnitude or more (69). Based on biomarker data, Hattis *et al.* have calculated that the variability in metabolic activity, detoxification and DNA repair among 95% of the US population could be as high as 85–500-fold (69) with correspondingly high variability in cancer risk.

Future directions and research needs in the field of molecular epidemiology

There has been dramatic progress in the application of biomarkers to human studies of cancer causation. The populations studied have included: cigarette smokers, workers in specific industries, residents exposed to air pollution, persons with specific dietary patterns and cancer patients given chemotherapy. Although many of the early studies were ‘transitional’ in nature, and therefore small in scale and of limited design, they established a battery of methods that are adequately sensitive for human studies and have the potential to provide valuable information on group and individual risks. Progress has been made in the development and validation of biomarkers that are directly relevant to the carcinogenic process and that can be used in large-scale epidemiologic studies. Study designs have become increasingly complex, with greater attention to the need to incorporate appropriate controls and account for potential confounders. Multiple markers, each reflecting a different stage or mechanism in carcinogenesis, are frequently being assessed in the same biologic samples as well as in surrogate and target tissues, to clarify the relationships between them. A number of longitudinal or nested case-control studies have been undertaken to establish the predictive value of biomarkers.

However, as knowledge of mechanisms in carcinogenesis has evolved, the available armamentarium of biomarkers is no longer sufficient. As discussed above, the majority of the available biomarkers used in molecular epidemiologic studies relate to agents that cause DNA damage and are mutagenic.

At the same time, we know that a large number of chemicals (hormones, various tumor promoters, pesticides, retinoids, etc.) can enhance or inhibit the carcinogenic process through indirect genotoxic or epigenetic mechanisms. They appear to act by altering gene expression, cell proliferation, differentiation and/or apoptosis (programmed cell death). Therefore, a current challenge is to develop more biomarkers for this category of agents and to incorporate them into molecular epidemiology studies.

For example, there is increasing evidence that in addition to mutations due to damage to DNA, the carcinogenic process is often associated with aberrant hypermethylation of CpG islands in the promoter regions of various genes, leading to their transcriptional inactivation. These genes encode various proteins including the cell-cycle inhibitory protein p16^{ink4}, the DNA mismatch repair protein hMLH1, and the detoxification enzyme glutathione *S*-transferase π (70). Since these changes can enhance the process of tumor progression, they might provide informative biomarkers in cell samples obtained from individuals at risk of developing cancer.

Additional assays are needed to study oxygen radicals and oxidant stress, the metabolism of nitrogen oxide and nitrites, alterations in DNA methylation, the activities of specific protein kinases, activation of various cytoplasmic and nuclear receptors and transcription factors, cyclins and other cell-cycle control proteins, and aberrant cell proliferation, differentiation, apoptosis and angiogenesis. During the multistage process of carcinogenesis, mutations and/or epigenetic disorders in the expression of numerous genes can occur, and this can lead to marked changes in the overall circuitry of signal transduction and cell cycle control (for review see ref. 71). Recent advances in genomics, microarray technology and informatics have made it possible to analyze small numbers of cells for their profiles of expression of many different genes. As these techniques become more available they can be applied to exfoliated cells and small biopsies of normal tissues, precursor lesions and tumors.

As mentioned earlier, polymorphic variation in several types of genes may influence cancer susceptibility at the population level. In addition to the metabolism of xenobiotics, these polymorphisms can affect the metabolism of various dietary factors, the endogenous synthesis, metabolism and action of hormones, DNA repair, immune and inflammatory processes, oxidant stress, signal transduction and cell-cycle control. Therefore, recently launched nationwide efforts to assemble and characterize a large panel of such polymorphic genes through the Human Genome and Environmental Genome Project (72,73) should provide powerful new biomarkers to the field of molecular epidemiology. Automated DNA-chip technology will greatly facilitate the large scale application of this new technology.

An extremely important area for future application of the concepts and methods of molecular epidemiology is the role of nutrition and diet in cancer. Although epidemiologic studies suggest that nutritional factors play a causative role in >30% of human cancers, the precise roles of specific dietary factors are uncertain and often controversial. Current issues include: (i) the identification of specific cancer-preventive chemicals in fruits, vegetables and various phytochemicals, including antioxidants; (ii) the influence of genetic factors on individual cancer risk from dietary factors; (iii) the carcinogenic role of heterocyclic amines that are generated by cooking meat at high temperatures; and (iv) the contribution of specific dietary

fats versus caloric excess *per se* to increased cancer risk. New types of biomarkers need to be developed to analyze these questions in specific human populations.

For example, it is well known that calorie restriction can markedly inhibit tumor formation in mice. A variety of mechanisms have been invoked. The level of insulin-like growth factor-1 (IGF-1) may play a critical role since the serum level of this protein is reduced in calorically restricted mice and continuous infusion of IGF-1 into these mice enhances tumor formation (74). A variety of other experimental studies implicate IGF-1 and signalling through its receptor (IGF-1r) in carcinogenesis. Furthermore, several types of human tumors, including breast, colon and lung carcinomas, overexpress either IGF-1 or IGF-1r (for review see ref. 75). High serum levels of IGF1 have been implicated in human cancer of the prostate (76) and breast (77). It is known that diets high in fat and high glycemic index carbohydrates can lead to a state of insulin resistance, with hyperglycemia, impaired glucose tolerance and hyperinsulinemia. One hypothesis is that this process can enhance colon carcinogenesis because of the mitogenic effects of insulin and related factors (for review see ref. 78). Therefore, assays for serum levels of IGF-1, insulin and other polypeptide growth factors may provide informative biomarkers in molecular epidemiologic studies on nutrition and cancer. A related issue is whether biomarkers can be developed to better assess the putative role of exercise as a protective factor for breast and other cancers.

Recent studies suggest that individuals who have a methyl-deficient diet are at higher risk of colon cancer if they carry a defective polymorphic form of the enzyme methylene tetrahydrofolate reductase (79). Moreover, a decreased level of PAH-DNA adducts in the peripheral white blood cells of cigarette smokers has been reported in persons with high plasma levels of α -tocopherol, but only in those individuals who lack the GSTM1 detoxifying gene, which is detected in 30–50% of the Caucasian population (80,81). Studies are needed also on polymorphic variations in genes involved in energy metabolism or in the metabolism of lipids.

Specific viruses or bacteria may be important cofactors in the causation of certain types of human cancer, acting in concert with environmental chemical agents, dietary factors or hereditary susceptibility factors. Examples include hepatitis B and C viruses in the causation of liver cancer, Epstein-Barr virus (EBV) in Burkitt's lymphoma and nasopharyngeal carcinoma, HPV in cervical cancer, human Herpes virus in Kaposi's sarcoma and *Helicobacter pylori* in gastric cancer (82). Therefore, it is important to include biomarkers for various microorganisms in molecular epidemiologic studies on specific types of human cancer.

A recent trend that brings together cancer researchers interested in cancer epidemiology, chemoprevention and therapy is the increasing recognition that biomarkers developed in the field of molecular epidemiology may also be useful as early or intermediate endpoints in studies on cancer prevention by identifying 'at risk' populations and then assessing the efficacy of various types of intervention. For example, in interventions to prevent first or second malignancies, biomarkers can help identify populations or individuals at high risk of cancer resulting from specific environment-gene interactions. Measures could then be taken to reduce exposure (i.e. through clean-up of the workplace, modification of the diet, tighter air quality regulations, etc.), to carry out early cancer detection screening, or to initiate chemoprevention when

appropriate. In some situations a combination of these measures could be used. Appropriate biomarkers could then be used to provide feedback during these interventions (83–85).

The field of molecular epidemiology is especially relevant to the very promising and rapidly expanding field of cancer chemoprevention, i.e. the use of specific, synthetic or naturally occurring compounds to inhibit the carcinogenic process before the development of malignant tumors (86). Both approaches emphasize the multistage nature of carcinogenesis. Specific agents used in cancer chemoprevention appear to act by inhibiting carcinogenic damage to DNA, mutagenesis, tumor promotion and/or tumor progression. In addition to identifying appropriate candidates for cancer chemoprevention studies, many of the biomarkers used in molecular epidemiology are useful as early or intermediate endpoints, to assess the efficacy of chemopreventive agents in humans. These biomarkers include assays for DNA adducts, mutations in specific genes, abnormalities in cell proliferation and alterations in gene expression. Thus, new bridges are being built between the fields of basic cancer research, epidemiology, prevention and therapy (71).

Finally, with respect to prevention, the use of biomarkers to quantify interindividual variability in response to exposure has significant implications for carcinogenic risk assessment and associated regulatory actions (8). The assumption underlying current risk assessment models, that all humans respond homogeneously to a specific carcinogen or mixture of carcinogens, is belied by the large interindividual variation observed within human populations exposed to similar levels of diverse carcinogens. These observations suggest the need for more stringent regulation of exposures to certain ambient or workplace pollutants or environmental tobacco smoke to protect the more sensitive subpopulations. Further molecular epidemiologic research on the relationship between exposure and susceptibility should also provide a better estimate of the percentage of cancer cases resulting from specific environmental exposures. Such estimates of attributable risk can be used to guide public policy toward reduction or elimination of the most significant causative factors and toward interventions targeted at specific subpopulations or individuals.

In conclusion, we believe that the potential benefits of biomarkers and molecular epidemiology in cancer prevention justify a major commitment to the further development and use of this approach and to addressing the ethical concerns involved in its application to cancer prevention. We are hopeful that this new discipline will continue to provide new insights into the causation of specific human cancers and will help us arrive at more effective strategies for cancer prevention.

Acknowledgements

F.P.P. is a Professor in the Division of Environmental Health Sciences, Joseph L. Mailman School of Public Health at Columbia University, and Director of the Columbia Center for Children's Environmental Health. I.B.W. is the Frode Jensen Professor of Medicine and a Professor in the Joseph L. Mailman School of Public Health at Columbia University and the Department of Genetics and Development of Columbia University. F.P.P. was supported by the National Institutes of Health, grant no. 5R01 CA53772; Cancer Center Core grant no. 5 P30 CA13696-23; The National Institute of Environmental Health Science, grant no. NCI 5R01 CA69094, 1R01 ES06722; US Army, grant no. DAMD17-94-J-4251; Department of Energy, grant no. DE-FG02-93 ER61719; NIH/EPA, 1P50ES09600-01; NIH-1P30, ES09089-01; NIEHS grant no. 1 R01 ES08977-01; and awards from the Gladys and Roland Harriman Foundation, The Bauman Family Foundation, The Robert Wood Johnson Foundation, W.Alton Jones Foundation, New York Community Trust and the Irving A. Hansen Memorial Foundation. I.B.W. was supported by the

National Institutes of Health, grant nos NCI R01CA63467, NCI R37CA26056, 5P30 CA 13696; US Army, grant no. DAMD17-94-J-4104 and awards from the National Foundation for Cancer Research, the TJ Martell Foundation and the Alma Toorock Memorial for Cancer Research.

References

- Perera,F.P. and Weinstein,I.B. (1982) Molecular epidemiology and carcinogen–DNA adduct detection: new approaches to studies of human cancer causation. *J. Chron. Dis.*, **35**, 581–600.
- Perera,F.P. (1987) Molecular cancer epidemiology: a new tool in cancer prevention. *J. Natl Cancer Inst.*, **78**, 887–898.
- Hulka,B.S. (1991) Epidemiological studies using biological markers: issues for epidemiologists. *Cancer Epidemiol. Biomarkers Prev.*, **1**, 13–19.
- Harris,C. (1991) Chemical and physical carcinogenesis: advances and perspectives for the 1990s. *Cancer Res.*, **51** (suppl.), 5023S–5044S.
- Schulte,P.A. and Perera,F.P. (eds) (1993) *Molecular Epidemiology: Principles and Practices*. Academic Press, New York, NY.
- IARC (1994) Intereaction of cancer susceptibility genes and environmental carcinogens. *Cancer Res.*, **54**, 4243–4247.
- Ambrosone,C. and Kadlubar,F. (1997) Toward an integrated approach to molecular epidemiology. *Am. J. Epidemiol.*, **146**, 912–918.
- Perera,F.P. (1997) Environment and cancer: who are susceptible? *Science*, **278**, 1068–1073.
- ACS (1996) *Cancer Facts and Figures, 1996*. American Cancer Society, Atlanta, GA.
- Weinstein,I.B., Santella,R.M. and Perera,F.P. (1993) The molecular biology and molecular epidemiology of cancer. In P. Greenwald,P. (ed.) *The Science and Practice of Cancer Prevention and Control*. Marcel-Dekker, New York, NY.
- Hulka,B.S., Griffith,J.D. and Wilcosky,T.C. (1990) *Biologic Markers in Epidemiology*. Oxford University Press, New York, NY.
- Omenn,G.S. (1991) Future research directions in cancer ecogenetics. *Mutat. Res.*, **247**, 283–291.
- Schulte,P.A. and Sweeney,M.H. (1995) Ethical considerations, confidentiality issues, rights of human subjects and uses of monitoring data in research and regulation. *Environ. Health Perspect.*, **103** (Suppl. 3), 69–74.
- Anonymous (1987) Biological markers in environmental health research. Committee on Biological Markers of the National Research Council. *Environ. Health Perspect.*, **74**, 3–9.
- Coggon,D. and Friesen,M. (1997) Markers of internal dose: chemical agents. In Toniolo,P., Boffetta,P., Shuker,D., Rothman,N., Hulka,B. and Pearce,N. (eds) *Application of Biomarkers in Cancer Epidemiology*. IARC Scientific Publications No. 142, IARC, Lyon, pp. 95–102.
- Rothman,N., Shield,P.G., Poirier,M.C., Harrington,A.M., Ford,D.P. and Strickland,P. (1995) The impact of glutathione S-transferase M1 and cytochrome P450 1A1 genotype on white-blood cell polycyclic aromatic hydrocarbon–DNA adduct levels in humans. *Mol. Carcinog.*, **14**, 63–68.
- Bartsch,J. and Hietanen,E. (1996) The role of individual susceptibility in cancer burden related to environmental exposure. *Environ. Health Perspect.*, **104** (Suppl. 3), 569–577.
- Hemminki,K. (1997) DNA adducts and mutations in occupational and environmental biomonitoring. *Environ. Health Perspect.*, **105** (Suppl. 4), 832–827.
- Anderson,M.W. and Stowers,S.J. (1984) Ubiquitous binding of benzo(a)pyrene metabolites to DNA and protein in tissues of the mouse and rabbit. *Chem. Biol. Interact.*, **51**, 151.
- Mooney,L.A., Santella,R.M., Covey,L., Jeffrey,A.M., Bigbee,W., Randall,M.C., Cooper,T.B., Ottman,R., Tsai,W.-Y., Wazneh,L. *et al.* (1995) Decline in DNA damage and other biomarkers in peripheral blood following smoking cessation. *Cancer Epidemiol. Biomarkers Prev.*, **4**, 627–634.
- Perera,F.P., Poirier,M.C., Yuspa,S.H., Nakayama,J., Jaretski,A., Curnen,M.M., Knowles,D.M. and Weinstein,I.B. (1982) A pilot project in molecular cancer epidemiology: determination of benzo[a]pyrene–DNA adducts in animal and human tissues by immunoassays. *Carcinogenesis*, **3**, 1405–1410.
- Santella,R.M. (1990) Immunologic methods for the detection of carcinogen adducts in humans. *Basic Life Sci.*, **53**, 33–44.
- Poirier,M.C. (1991) Development of immunoassays for the detection of carcinogen–DNA adducts. In Groopman,J.D. and Skipper,P.L. (eds) *Molecular Dosimetry and Human Cancer: Analytical, Epidemiological and Social Considerations*. CRC Press Inc., Boca Raton, FL, pp. 211–230.
- Phillips,D. (1997) Detection of DNA modifications by the ³²P-post-labelling assay. *Mutat. Res.*, **378**, 1–12.

25. Herron, D. and Shank, R. (1980) Methylated purines in human liver DNA after probable dimethylnitrosamine poisoning. *Cancer Res.*, **40**, 3116–3117.
26. Skipper, P.L. and Tannenbaum, S.R. (1990) Protein adducts in the molecular dosimetry of chemical carcinogens. *Carcinogenesis*, **11**, 507–518.
27. Wild, C. and Pisani, P. (1997) Carcinogen–DNA and carcinogen–protein adducts in molecular epidemiology. In Toniolo, P., Boffetta, P., Shuker, D., Rothman, N., Hulka, B. and Pearce, N. (eds) *Application of Biomarkers in Cancer Epidemiology*. IARC Scientific Publications No. 142, IARC, Lyon, pp. 143–156.
28. Poirier, M. and Weston, A. (1991) DNA adduct determination in humans. *Prog. Clin. Biol. Res.*, **372**, 205–218.
29. Tang, D.L., Chiamprasert, S., Santella, R.M. and Perera, F.P. (1995) Molecular epidemiology of lung cancer: carcinogen–DNA adducts, GSTM1 and risk. *Proc. Am. Assoc. Cancer Res.*, **36**, 284.
30. Ross, R.K., Yuan, J.M., Yu, M.C., Wogan, G.N., Qian, G.S., Tu, J.P., Groopman, J.D., Gao, Y.T. and Henderson, B.E. (1992) Urinary aflatoxin biomarkers and risk of hepatocellular carcinoma. *Lancet*, **339**, 943–946.
31. Groopman, J.D., Wogan, G.N., Roebuck, B.D. and Kensler, T.W. (1994) Molecular biomarkers for aflatoxins and their application to human cancer prevention. *Cancer Res.*, **54**, 1907s–1911s.
32. Yu, M., Lien, J., Chiu, Y., Santella, R., Liaw, Y. and Chen, C. (1997) Effect of aflatoxin metabolism and DNA adduct formation on hepatocellular carcinoma among chronic hepatitis B carriers in Taiwan. *J. Hepatol.*, **27**, 320–330.
33. Bryant, M.S., Skipper, P.L., Tannenbaum, S.R. and Niure, M. (1987) Hemoglobin adducts of 4-aminobiphenyl in smokers and nonsmokers. *Cancer Res.*, **47**, 612–618.
34. Perera, F.P. (1994) *Biomarkers and Molecular Epidemiology of Cancer. Proceedings of the 9th International Symposium in Epidemiology in Occupational Health, 1992*. National Institute for Occupational Safety and Health, Cincinnati, OH, pp. 54–66.
35. Dickey, C., Santella, R., Hattis, D., Tang, D., Hsu, Y., Cooper, T., Young, T. and Perera, F. (1997) Variability in PAH–DNA adduct measurements in peripheral mononuclear cells: implications for quantitative cancer risk assessment. *Risk Anal.*, **17**, 649–655.
36. Yarborough, A., Zhang, Y., Hsu, T. and Santella, R. (1996) Immunoperoxidase detection of 8-hydroxydeoxyguanosine in aflatoxin B₁-treated rat liver and human oral mucosal cells. *Cancer Res.*, **56**, 683–688.
37. Erlich, H.A., Gelfand, D. and Sninsky, J.J. (1991) Recent advances in the polymerase chain reaction. *Science*, **252**, 1642–1651.
38. Hollstein, M., Sidransky, D., Vogelstein, B. and Harris, C.C. (1991) p53 mutations in human cancers. *Science*, **253**, 49–52.
39. Denissenko, M.P. (1996) Preferential formation of benzo[a]pyrene adducts at lung cancer mutational hotspots in p53. *Science*, **274**, 430–432.
40. Gazdar, A. and Minna, J. (1999) Molecular detection of early lung cancer. *J. Natl Cancer Inst.*, **91**, 299–301.
41. Weinstein, I.B. (1995) The contributions of molecular biology to cancer epidemiology. *Ann. N Y Acad. Sci.*, **768**, 30–40.
42. Zhou, P., Jiang, W., Weghorst, C. and Weinstein, I. (1996) Overexpression of cyclin D1 enhances gene amplification. *Cancer Res.*, **56**, 36–39.
43. Caporaso, N. and Goldstein, A. (1997) Issues involving biomarkers in the study of the genetics of human cancer. In Toniolo, P., Boffetta, P., Shuker, D., Rothman, N., Hulka, B. and Pearce, N. (eds) *Application of Biomarkers in Cancer Epidemiology*. IARC Scientific Publications No. 142, IARC, Lyon, 237–250.
44. Garte, S., Zocchetti, C. and Taioli, E. (1997) Gene–environment interactions in the application of biomarkers of cancer susceptibility in epidemiology. In Toniolo, P., Boffetta, P., Shuker, D., Rothman, N., Hulka, B. and Pearce, N. (eds) *Application of Biomarkers in Cancer Epidemiology*. IARC Scientific Publications No. 142, IARC, Lyon, p. 251.
45. Schulte, P., Hunter, D. and Rothman, N. (1997) Ethical and social issues in the use of biomarkers in epidemiological studies. In Toniolo, P., Boffetta, P., Shuker, D., Rothman, N., Hulka, B. and Pearce, N. (eds) *Application of Biomarkers in Cancer Epidemiology*. IARC Scientific Publications No. 142, IARC, Lyon, p. 313.
46. Knudson, A.G. (1994) Hereditary cancer, oncogenes and antioncogenes. *Cancer Res.*, **45**, 1437–1443.
47. Venitt, S. (1994) Mechanisms of carcinogenesis and individual susceptibility to cancer. *Clin. Chem.*, **40**, 1421–1425.
48. Cavenee, W.K. and White, R.L. (1995) The genetic basis of cancer. *Sci. Am.*, **272**, 72–79.
49. Petruzzelli, S., Camus, A.M., Carozzi, L., Ghelarducci, L., Rindi, M., Menocconi, G., Angeletti, C.A., Ahotupa, M., Hietanen, E., Aitio, A. et al. (1988) Long-lasting effects of tobacco smoking on pulmonary drug-metabolizing enzymes: a case-control study on lung cancer patients. *Cancer Res.*, **48**, 4695–4700.
50. Schweikl, H., Taylor, J.A., Kitarewan, S., Linko, P., Nagorney, D. and Goldstein, J.A. (1993) Expression of CYP1A1 and CYP1A2 genes in human liver. *Pharmacogenetics*, **3**, 239–249.
51. Kellerman, G., Shaw, C.R. and Kellermann, M.L. (1973) Aryl hydrocarbon hydroxylase inducibility and bronchogenic carcinoma. *N. Engl. J. Med.*, **289**, 934–937.
52. Kouri, R.E., McKinney, C.E., Slomiany, D.J., Snodgrass, D.R., Wray, N.P. and McLemore, T.L. (1982) Positive correlation between high aryl hydroxylase activity and primary lung cancer as analyzed in cryopreserved lymphocytes. *Cancer Res.*, **45**, 5030–5037.
53. Seidegard, J., Pero, R.W., Markowitz, M.M., Roush, G., Miller, D.G. and Beattie, E.J. (1990) Isoenzyme(s) of glutathione transferase (class mu) as a marker for the susceptibility to lung cancer: a follow-up study. *Carcinogenesis*, **11**, 33–36.
54. Bell, D.A., Taylor, J.A., Paulson, D.F., Robertson, C.N., Mohler, J.L. and Lucier, G.W. (1993) Genetic risk and carcinogen exposure: a common inherited defect of the carcinogen–metabolism gene glutathione S-transferase M1 (GSTM1) that increases susceptibility to bladder cancer. *J. Natl Cancer Inst.*, **85**, 1159–1164.
55. Vineis, P. and Caporaso, N. (1995) Tobacco and cancer: epidemiology and the laboratory. *Environ. Health Perspect.*, **103**, 156–160.
56. McWilliams, J.E., Sanderson, B.J.S., Harris, E.L., Richert-Boe, K.E. and Henner, W.D. (1995) Glutathione S-transferase M1 (GSTM1) deficiency and lung cancer risk. *Cancer Epidemiol. Biomarkers Prev.*, **4**, 589–594.
57. Cartwright, R.A., Glashan, R.W., Rogers, H.J., Barham-Hall, D., Ahmad, R.A., Higgins, E. and Kahn, M.A. (1982) Role of N-acetyltransferase phenotypes in bladder carcinogenesis: a pharmacokinetic epidemiological approach to bladder cancer. *Lancet*, **2**, 842–846.
58. Vineis, P., Caporaso, N., Tannenbaum, S.R., Skipper, P.L., Glogowski, J., Bartsch, H., Coda, M., Talaska, G. and Kadlubar, F. (1990) Acetylation phenotype, carcinogen–hemoglobin adducts and cigarette smoking. *Cancer Res.*, **50**, 3002–3004.
59. Bartsch, H., Caporaso, N., Coda, M., Kadlubar, F., Malaveille, C., Skipper, P., Talaska, G. and Tannenbaum, S.R. (1990) Carcinogen hemoglobin adducts, urinary mutagenicity and metabolic phenotype in active and passive cigarette smokers. *J. Natl Cancer Inst.*, **82**, 1826–1831.
60. Ilett, K.F., David, B.M., Dethon, P., Castleden, W.M. and Kwa, R. (1987) Acetylation phenotype in colorectal carcinoma. *Cancer Res.*, **47**, 1466–1469.
61. Wohlleb, J.C., Hunter, C.F., Blass, B., Kadlubar, F.F., Chu, D.Z.J. and Lang, N.P. (1990) Aromatic amine acetyltransferase as a marker for colorectal cancer: environmental and demographic associations. *Int. J. Cancer*, **46**, 22.
62. Lindahl, T., Wood, R.D. and Karran, P. (1991) Molecular deficiencies in cancer-prone syndromes associated with hypersensitivity to DNA damaging agents. In Brugge, J., Curren, T., Harlow, E. and McCormick, F. (eds) *Origins of Human Cancer: A Comprehensive Review*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, NY, pp. 163–170.
63. Pero, R.W., Johnson, D., Markowitz, M., Doyle, G., Lund-Pero, M., Halper, M. and Miller, D.G. (1989) DNA repair synthesis in mononuclear leukocytes of individuals with and without a familial history of cancer. *Carcinogenesis*, **10**, 693–697.
64. Rudiger, H.W., Schwartz, U., Serrand, E., Stief, M., Krause, T., Nowak, D., Doerjter, G. and Lehnert, G. (1989) Reduced O⁶-methylguanine repair in fibroblast cultures from patients with lung cancer. *Cancer Res.*, **49**, 5623–5626.
65. Wei, Q., Gu, J., Cheng, L., Bondy, M.L., Jiang, H., Hong, W.K. and Spitz, M.R. (1996) Benzo(a)pyrene diol epoxide-induced chromosomal aberrations and risk of lung cancer. *Cancer Res.*, **56**, 3975–3979.
66. Dybdahl, M., Vogel, U., Frentz, G., Wallin, H. and Nexø, B. (1999) Polymorphisms in the DNA repair gene XPD: correlations with risk and age at onset of basal cell carcinoma. *Cancer Epidemiol. Biomarkers Prev.*, **8**, 77–81.
67. Lunn, R., Langlois, R., Hsieh, L., Thompson, C. and Bell, D. (1999) Polymorphisms: effects on aflatoxin B₁-DNA adducts and glycophorin A variant frequency. *Cancer Res.*, **59**, 2557–2561.
68. Hannawalt, P. (1995) DNA repair comes of age. *Mutat. Res.*, **336**, 101–113.
69. Hattis, D., Erdreich, L. and DiMauro, T. (1986) *Human Variability in Parameters that are Potentially Related to Susceptibility to Carcinogenesis—I. Preliminary Observations*. Center for Technology, Policy and Industrial Development, MIT, Cambridge, MA.
70. Baylin, S., Herman, J., Graff, J., Vertino, P. and Issa, J. (1998) Alterations in DNA methylation: a fundamental aspect of neoplasia. *Cancer Res.*, **72**, 141–196.
71. Weinstein, I. and Zhou, P. (1997) *Cell Cycle Control Gene Defects and Human Cancer. Encyclopedia of Cancer*. Vol. 1. Academic Press, New York, NY, pp. 256–267.

72. Anonymous (1997) Environmental genome project advances. *Environ. Health Perspect.*, **105**, 1298.
73. Collins,F., Patrinos,A., Jordan,E., Chakravarti,A., Gesteland,R. and Walters,L. (1998) New goals for the U.S. Human Genome Project: 1998–2003. *Science*, **282**, 682–689.
74. Dunn,S., Kari,F., French,J., Leininger,J., Travlos,G., Wilson,R. and Barrett,J. (1997) Dietary restriction reduces insulin-like growth factor I levels, which modulate apoptosis, cell proliferation and tumor progression in p53-deficient mice. *Cancer Res.*, **57**, 4667–4672.
75. Wilker,E., Bol,D., Kiguchi,K., Rupp,T., Beltran,L. and DiGiovanni,J. (1999) Enhancement of susceptibility to diverse skin tumor promoters by activation of the insulin-like growth factor 1 receptor in the epidermis of transgenic mice. *Mol. Carcinog.*, **25**, 122–131.
76. Chan,J., Stampfer,M., Giovannucci,E., Gann,P., Ma,J., Wilkinson,P., Hennekens,C. and Pollak,M. (1998) Plasma insulin-like growth factor-I and prostate cancer risk: a prospective study. *Science*, **279**, 563–566.
77. Hankinson,S., Willett,W., Colditz,G., Hunter,D., Michaud,D., Deroo,B., Rosner,B., Speizer,F. and Pollak,M. (1998) Circulating concentrations of insulin-like growth factor-I and risk of breast cancer. *Lancet*, **351**, 1393–1396.
78. Bruce,W., Archer,M., Corpet,D., Medline,A., Minkin,S., Stamp,D., Yin,Y. and Zhang,X. (1993) Diet, aberrant crypt foci and colorectal cancer. *Mutat. Res.*, **290**, 111–118.
79. Chen,J.G.E., Kelsey,K., Rimm,E.B., Stampfer,M.J., Colditz,G.A., Spiegelman,D., Willett,W.C. and Hunter,D.J. (1996) A methylenetetrahydrofolate reductase polymorphism and the risk of colorectal cancer. *Cancer Res.*, **56**, 4862–4864.
80. Grinberg-Funes,R., Singh,V., Perera,F., Bell,D., Young,T., Dickey,C., Wang,L. and Santella,R. (1994) Polycyclic aromatic hydrocarbon–DNA adducts in smokers and their relationship to micronutrient levels and the glutathione-S-transferase M1 genotype. *Carcinogenesis*, **15**, 2449–2454.
81. Mooney,L.A., Bell,D.A., Santella,R.M., Van Bennesum,A.M., Ottman,R., Paik,M., Blaner,W.S., Lucier,G.W., Covey,L., Young,T.L., Cooper,T.B., Glassman,A.H. and Perera,F.P. (1997) Contribution of genetic and nutritional factors to DNA damage in heavy smokers. *Carcinogenesis*, **18**, 503–509.
82. Mueller,N. (1995) Overview: viral agents and cancer. *Environ. Health Perspect.*, **103** (Suppl. 8), 259–261.
83. Gritz,E.R. (1992) Paving the road from basic research to policy: cigarette smoking as a prototype issue for cancer control science. *Cancer Epidemiol. Biomarkers Prev.*, **1**, 427–434.
84. Perera,F.P. and Mooney,L.A. (1993) The role of molecular epidemiology in cancer prevention. In DeVita,V.T.Jr, Hellman,S. and Rosenberg,S.A. (eds) *Cancer Prevention*. J.B. Lippincott Co., Philadelphia, PA, pp. 1–15.
85. Khoury,M.J. and the Genetics Working Group (1996) From genes to public health: the applications of genetic technology in disease prevention. *Am. J. Pub. Health*, **86**, 1717–1722.
86. Sporn,M.B. and Suh,N. (2000) Chemoprevention of cancer. *Carcinogenesis*, **21**, 525–530.

Received July 7, 1999; accepted August 3, 1999