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Biomarkers of Exposure and Effect

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INTRODUCTION

The majority of cancers have a complex etiology. Usually one or more environmental risk factors, such as lifestyle, diet, chemical pollutants, radiation, and infectious agents, are implicated. Individual response to these factors is influenced by genetic background, co-exposures to environmental agents, and other factors including age, sex, and socio-demographic status. Despite the acknowledged importance of the environment in influencing cancer risk, the precise contribution of individual factors and their interaction, both with each other and with genotype, is difficult to elucidate. This is partially due to the challenges inherent in accurately measuring exposure, not least when the critical period relevant to disease risk may have occurred many years prior to diagnosis (1). It is in response to this need that one of the promises of molecular cancer epidemiology is to provide biomarkers that will refine exposure assessment (2). While the focus of this chapter is on biomarkers, it is important to note that other approaches to refining exposure assessment are also important, including geographic information systems, personal and environmental monitoring, and increasingly sophisticated questionnaires (3,4); it is a combination of tools which is most likely to provide the answers required.

The development of exposure biomarkers, including DNA and protein adducts was well represented among early studies in molecular cancer epidemiology (5,6). However, in the late 1980s and early 1990s, there was a major shift in effort and resources away from exposure biomarkers toward the conduct of gene-disease association studies corresponding to the development of the polymerase chain reaction. This technique permitted analysis of genetic polymorphisms using methodology that was simpler, cheaper, less demanding in terms of sampling, of higher throughput, and quicker than methods required for exposure biomarkers. In addition, genotyping was applicable to the case:control study design, making it suitable for integration into a greater proportion of ongoing epidemiological studies. The accurate assessment of many environmental exposures has remained an outstanding and largely unmet challenge in molecular cancer epidemiology and consequently one that impairs understanding of the complex gene:environment interactions that contribute to the majority of human cancers (7,8).
This chapter will explore not only biomarkers of exposure but also biomarkers of effect. The latter reflect occurrences subsequent to the initial exposure-related events and in general, but not always, may be more persistent than exposure biomarkers. It is difficult to define precisely the scope of this category of biomarker but examples include chromosomal alterations, changes in gene expression, altered protein levels (e.g., growth factors, cytokines), and mutations. Other biomarkers that may be termed biomarkers of altered structure or function, such as precancerous lesions, will not be considered here. These different categories of biomarker have been fully described elsewhere (9).

In one sense, there is only a semantic distinction between the categories of biomarkers of exposure and effect because there are no sharp boundaries in the continuum leading from exposure to disease. A DNA adduct, for example, is not comfortably forced exclusively into one or other category. In addition, many of the required properties of biomarkers in both categories are common, e.g., sensitivity, specificity, validity, and reliability. Nevertheless, the categorization, if held lightly, can be helpful both for descriptive purposes and to inform discussions of disease mechanisms in the context of what, by definition, is the interdisciplinary research embraced by molecular epidemiology (9). Examples of biomarkers of exposure and effect are summarized in Table 1. Throughout the chapter the general principles applying to these types of biomarker are illustrated by examples from the literature.

**THE VALUE OF BIOMARKERS OF EXPOSURE AND EFFECT**

As highlighted above, accurately characterizing an individual’s environmental exposure is critical to establishing disease etiology. Misclassification, both of the exposure of primary interest and of potential confounding factors, introduces uncertainty and limits the power of epidemiological studies. Association between exposures of interest occur to accurate exposure assessment objective measure of “true” biomarkers that have great analytical sensitivity levels occurring in the way biomarkers of smoke (10,11).

Biomarkers of exposure epidemiology than improve biomarkers are also of value between a risk factor and cancer incidence. Alternatively, if a particular biomarker is also linked to higher levels of DNA damage, this would provide mechanistic data are identification and cancer risk assessment.

Another valuable application of the potential to reduce exposure, or monitor either case biomarkers can be established in advance of itself might be the outdoor information pertinent to appropriate doses of chemical.

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Table 2 Applications of Exposure and Effect Biomarkers in Studies of Human Cancer

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of epidemiological studies. This is particularly damaging when the true strength of the association between exposure and disease is modest. In these cases misclassification may blur or completely obscure underlying causal associations. Many environmental exposures of interest occur at low levels but can be ubiquitous, posing further challenges to accurate exposure assessment. In response, biomarkers promise to provide a more objective measure of "true" exposure than previous approaches. The assays used often have great analytical sensitivity and can enable detection and quantification at the low levels occurring in the environment. A good example of the success in this area is the way biomarkers of exposure have been used to study environmental tobacco smoke (10,11).

Biomarkers of exposure can provide far more, however, to the field of cancer epidemiology than improvement of the exposure metric (Table 2). For example, such biomarkers are also of value in establishing the biological plausibility of an association between a risk factor and disease. If a particular chemical exposure from ambient air is associated with increased risk, the additional information that exposed individuals have higher levels of DNA damage would add support to the exposure-disease association (11). Alternatively, if a particular genetic polymorphism associated with increased cancer risk is also linked to higher levels of a biomarker on the presumed causal pathway, e.g., DNA adducts, then this would provide support for the original association (12). These types of mechanistic data are increasingly being considered in the processes of hazard identification and cancer risk assessment.

Another valuable application of both biomarkers of exposure and effect is in evaluation of the potential of intervention strategies (13). This may be primary prevention to reduce exposure, or more mechanism-based approaches such as chemoprevention. In either case biomarkers can be used as endpoints, permitting a proof of principle to be established in advance of longer-term interventions where precancerous lesions or cancer itself might be the outcome. The proof-of-principle stage may also provide valuable information pertinent to the design of interventions, for example, in establishing appropriate doses of chemopreventive agents.

Biomarkers of exposure and effect can prove valuable tools in extrapolating data from animals to humans (14). The occurrence of the same biomarker responses across species may, for example, indicate the appropriateness of one model compared to another for carcinogen bioassays. Similarly, biomarker data may help in selection of the most appropriate model systems for initial mechanistic or prevention studies and contribute to the improvement of physiologically based pharmacokinetic models (15).

Biomarkers of exposure and effect may potentially be used to identify individuals at higher risk of development of cancer. This could permit more effective, targeted interventions at the individual level. This application needs careful interpretation though
because these events are indicators of early steps in the carcinogenic process; many other factors will impinge on the subsequent risk of progression to malignancy. It is notable that even in experimental animals few studies have examined this relationship. Among those that have, associations have been reported between biomarkers (both sister chromatid exchanges and adducts) and cancer incidence at the group (i.e., dose) level but not at the level of the individual animal (16,17).

Notwithstanding the above caveat, biomarker levels can be used for biomonitoring or biosurveillance in cross-sectional or longitudinal studies where the prevalence and level of exposure to an environmental carcinogen can be established in a population and act as a signal for the need for interventions. Examples from the occupational field in relation to industrial chemicals are pertinent here (18,19), as are the monitoring of lead or organochlorines and the detection of antibodies to oncogenic viruses.

The types of application of biomarkers of exposure and effect mentioned above hold considerable promise, but there are a number of key questions to be considered, which are generic to their application in epidemiological studies. Among these are what to measure, where to measure, and when to measure? These questions are considered in turn below with examples from biomarkers across the spectrum considered in this chapter.

**WHAT TO MEASURE?**

**Biomarkers of Exposure**

**Internal Dose**

Environmental chemicals or their metabolites in human tissues and body fluids have been used to measure exposure. In these instances, the chemicals are not bound to a critical target in the cell but provide a measure of exposure and absorption and are thus termed markers of internal dose. This type of biomarker can provide an improved metric of exposure compared with levels in ambient air, food, or water. Examples are 1-hydroxypyrene-glucuronide as a measure of polycyclic aromatic hydrocarbon (PAH) exposure (20) and urinary heterocyclic amine metabolites (21). Certain biomarkers of internal dose that are a product of metabolism may also be used to characterize the metabolic phenotype of an individual (22). However, the role of metabolism and other factors such as absorption does mean there may not always be a simple relationship between external exposure and internal dose.

Lipid soluble organochlorines can be detected in serum and plasma as well as adipose tissue (23,24). Other examples of internal dose markers include hormones or nutrients in body fluids, while from the area of infections and cancer (e.g., hepatitis viruses, human papilloma viruses, Helicobacter pylori), the presence of pathogen proteins or antibodies to these proteins indicate that exposure to the infectious agent has occurred. The use of biomarkers in conjunction with questionnaire data to characterize diet should be an area of significant contribution in the future with the measurement of specific nutrients in body fluids, for example (25,26).

**Biologically Effective Dose**

To date a broad range of different DNA adducts have been measured in human biological samples with assays involving 32P-postlabelling, immunoassays, and mass spectrometry among other techniques (5,6). On the basis of the mechanistic role of DNA adducts in chemical carcinogenesis, these have been referred to as biomarkers of biologically effective dose, i.e., a measure of the amount of the carcinogen reaching the critical cellular target. Protein adducts may also be considered, it is important to keep in mind that not only, exogenous distribution, metabolism, etc., but also endogenous factors will impinge on the subsequent risk of progression to malignancy. It is notable that even in experimental animals few studies have examined this relationship. Among those that have, associations have been reported between biomarkers (both sister chromatid exchanges and adducts) and cancer incidence at the group (i.e., dose) level but not at the level of the individual animal (16,17).

Validation of Exposure Biomarkers

Validity of a biomarker hinges on whether the levels measured reflect exposure. For example, the levels of aflatoxin metabolites in the diet or the presence of aflatoxin in food samples must be considered. The level of aflatoxin DNA adducts in human liver must reflect aflatoxin exposure. For example, the levels of aflatoxin DNA adducts in human liver must reflect aflatoxin exposure. This is essential to be inherent for the monitoring of aflatoxin exposure in areas where aflatoxin is known to be a significant problem.

Early studies of exposure and effect have demonstrated the presence of aflatoxin DNA adducts in human liver, but it is not clear whether these adducts reflect aflatoxin exposure. This is essential to be inherent for the monitoring of aflatoxin exposure in areas where aflatoxin is known to be a significant problem.

**Specificity of Exposure Biomarkers**

A further consideration for the specificity of exposure biomarkers is the definition of exposure. This is essential to be inherent for the monitoring of aflatoxin exposure in areas where aflatoxin is known to be a significant problem.
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cellular target. Protein adducts, by virtue of sharing bioactivation pathways with DNA
adducts may also be considered measures of biologically effective dose (19,27). However,
it is important to keep in mind that as with certain internal dose biomarkers, adducts
reflect not only exogenous exposure, but also other processes such as absorption,
distribution, metabolism, and DNA repair. As a result the measured adduct level will be a
composite of exposure and these other variables, which will differ among individuals.
Both DNA and protein adducts have been successfully applied in molecular epidemiology
studies for different chemicals and in different study designs such that neither can be said
to be inherently superior to the other. Sample availability has however, at times, been
more of a limitation with DNA than with protein.

Validation of Exposure Biomarkers
Validity of a biomarker has been defined as the (relative) lack of systematic measurement
error when comparing the actual observation with a standard (reference) method, which
represents the “truth” (28). This is true for biomarkers of exposure and effect, which may
both be compared to a “gold standard” of exposure; additionally, biomarkers of effect may
be validated against disease outcome and consequently serve as intermediate endpoints
and predictors of cancer (29). A lack of biomarker validity may simply result from analytical
error in the assay. However, it may result from a more complex set of factors that act to
distort the relationship between the biomarker and the true exposure or disease outcome. In
the case of exposure biomarkers, these factors can include a lack of dose-response
relationship between exposure and biomarker, failure of the biomarker to integrate exposure
over the time period of interest, or interindividual variability in the exposure-biomarker
relationship. Empirical data is therefore required to establish validity of a biomarker.

Early studies of exposure biomarkers in human subjects were indeed able to not only
demonstrate the presence of adducts or carcinogen metabolites, but also to quantitatively
associate the levels with environmental exposures (20,21,30–32). Such studies, therefore,
both established the adequate sensitivity of the analytical methods and contributed to the
above process of validation. However, despite the analytical advances that have permitted
measurement of such biomarkers in human samples over the last 20 years, the number of
cases where the biomarker has been shown to quantitatively reflect exposure remains
relatively few. Unfortunately, exposure biomarkers may be applied to human studies without
being fully validated.

Proceeding without validation is a risk because some biomarkers do not accurately
reflect exposure. For example, urinary aflatoxin Pl, a metabolite of aflatoxin B1, is not a
good indicator of aflatoxin exposure (33), while ochratoxin A in plasma did not correlate
with intake in a detailed duplicate diet study (34). Of course as mentioned above, a perfect
linear relationship between exposure and biomarker will not be expected, both because of
analytical measurement error and because of interindividual differences in, for example,
metabolism. Nevertheless, one may be potentially misled if biomarkers are applied to
epidemiological studies without such validation. A reliably (reproducibly) measured
biomarker in analytical terms does not necessarily translate therefore to a valid biomarker
in the context of epidemiology.

Specificity of Exposure Biomarkers
A further consideration in selecting a biomarker of exposure is that of specificity. A
chemical specific assay of carcinogen-DNA adducts, protein adducts, or metabolites, by
definition, will be specific for the target chemical. However, while some chemicals will
equate to the exposure that is of interest to the epidemiologist, some may not. For example, urinary aflatoxin-DNA adducts are a specific measure for dietary exposure to aflatoxins. However, with benzo(a)pyrene [B(a)P]-DNA adducts, the B(a)P may originate from tobacco smoke, diet, air pollution, or certain occupational exposures. While the marker is chemical specific, giving an accurate reflection of B(a)P exposure, it is not environmental exposure specific. However, it is often the environmental exposure that is the focus of the epidemiological study, not one specific chemical from multiple sources. This fact was illustrated in a study of PAH-DNA adducts in peripheral blood lymphocytes in firefighters (35). While there was no association between adducts and occupational smoke exposure (the main hypothesis), there was a significant correlation with the number of barbecued meals consumed. Here, the dietary source of PAH was more significant than smoke.

Other types of assay are less chemical specific than adducts or metabolites and provide a more general measure of exposure. For example, 32P-postlabelling of “bulky” aromatic DNA adducts may reflect a class of chemicals. The single cell gel electrophoresis (“comet”) assay provides an even broader indication of DNA strand breaks, although in combination with DNA repair enzymes may be targeted to more specific types of damage (36).

**Genotoxic and Nongenotoxic Mechanisms**

One notable feature of much of the literature on biomarkers of biologically effective dose is the emphasis on genotoxic pathways of carcinogenesis and the associated measurement of DNA and protein adducts. There is an increasing need however to consider appropriate endpoints for nongenotoxic exposures, given that many carcinogens work through these alternative mechanisms. One example is the mycotoxin, fumonisin B1 (FB1), classed by the IARC as “possibly carcinogenic to humans” (37). FB1 is a structural analogue of ceramide synthase and as such can act as an inhibitor of this enzyme, resulting in alteration of sphingolipid biosynthesis and in the ratio of sphinganine to sphingosine (38). This pathway may also be important in carcinogenesis, and thus the ratio may serve as a biomarker of biologically effective dose (39). The increasing recognition that environmental and dietary factors act through epigenetic mechanisms (40) presents a new and exciting challenge to the field of molecular cancer epidemiology. One of the major areas of future development is likely to be the development of biomarkers of events such as altered gene methylation or histone modification (41). It is possible that the discoveries from “omics” technologies (see later in this chapter) will increasingly lead to identification of relevant nongenotoxic carcinogenic pathways and stimulate the development of associated biomarkers.

**Biomarkers of Effect**

A number of different biomarkers of effect have been applied to human studies. Perhaps the most common are measures in peripheral blood cells of genetic alterations including chromosomal aberrations, micronuclei, and sister chromatid exchanges. In general terms, these biomarkers are nonspecific, reflecting cumulative exposure to a variety of environmental factors. Alternative biomarkers of effect include somatic mutations, either in reporter genes or in the proto-oncogenes and tumor suppressor genes implicated directly in the carcinogenic process. These mutation analyses may consider only the mutation frequency or may also investigate the pattern of mutations to infer something more specific about their environmental origin. In the case of reporter genes, the analyses are generally conducted on tissue samples, but circulating plasma DNA may provide additional biomarkers of exposure.

**Chromosomal Aberrations**

A number of prospective studies have been conducted on circulating plasma DNA, and the association with exposure has been examined in multiple laboratories in central Europe. A key feature of these studies is that elevated chromosomal aberration frequencies had a 1.5- to 3-fold increase compared to controls. This work on both somatic mutations in reporter genes and chromosomal aberrations in peripheral blood lymphocytes generally indicate a suitable endpoint in future studies.

**Somatic Mutations in Reporter Genes**

There have been numerous studies of somatic mutations in reporter genes and in the proto-oncogenes and tumor suppressor genes implicated directly in the carcinogenic process. These mutation analyses may consider only the mutation frequency or may also investigate the pattern of mutations to infer something more specific about their environmental origin. In the case of reporter genes, the analyses are generally conducted on tissue samples, but circulating plasma DNA may provide additional biomarkers of exposure.
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A number of prospective epidemiological studies have reported positive associations between elevated chromosomal aberrations and increased cancer risk (42-47). These positive findings have been generally limited to associations with cancers overall, rather than at specific sites, because of the limited number of cases accrued during follow-up. In the most recent study (47), data on a total of 6430 individuals were collated across nine laboratories in central Europe with follow-up for an average 8.5 years and 200 cancer cases identified. There was a higher relative risk (approximately 1.8) in subjects in the middle and upper tertiles of chromosomal aberrations compared with the lower tertile. In a similar study measuring micronuclei (48), the individuals with medium and high frequencies had a 1.5- to twofold increased relative risk compared with the low-frequency subjects. Sister chromatid exchanges have not been shown to date to be associated with increased risk in prospective studies (49).

This work on both chromosomal aberrations and micronuclei suggests that these biomarkers do indicate a higher risk of cancer at the population level and could be a suitable endpoint in future etiologic or intervention studies. As with the biomarkers of exposure discussed above, the evidence is not yet available to link the biomarker with risk at the individual level.

Somatic Mutations in Reporter Genes and Cancer Genes

There have been a number of assays developed which measure somatic mutation frequency in human population studies. These approaches tend to focus on reporter genes that are not a target for carcinogenesis, but represent the mutational burden of an individual due to environmental exposures. Examples of reporter genes that have been widely applied to human population studies are hypoxanthine-guanine phosphoribosyltransferase (HPRT) (50) and glycophorin A (51), permitting investigation of a number of environmental exposures to physical and chemical carcinogens.

In terms of somatic mutations in cancer genes, the TP53 tumor suppressor gene has been a major focus of attention with considerable efforts made to relate mutation spectra to environmental exposures (52). In the case of dietary aflatoxins, for example, a specific G to T transversion in codon 249 was geographically correlated with dietary aflatoxin exposure (37). An exciting subsequent development was the discovery of the same mutation in circulating plasma DNA and the demonstration that this was linked to an increased risk of liver cancer (53). Other than aflatoxin, UV sunlight, and cigarette smoke (52), the number of instances to date where this approach has been informative is limited. Nevertheless, interesting data are emerging in other areas, for example, in relation to the potential role of the natural toxin aristolochic acid in the etiology of Balkan endemic nephropathy (54,55). In addition, development of a highly sensitive mutation assay applied to nontumor material in ulcerative colitis cases showed a high frequency of G to A transitions in inflamed tissue; this type of mutation is consistent with free radical induced DNA damage (56). Quantitative mutation assays of this type reflecting specific exposures and applicable to human biopsies may permit assessment of longer-term past exposure or be indicators of increased risk of progression to cancer.
WHERE TO MEASURE?

Biomarkers of exposure and effect have been measured in a number of different biological media, including urine, feces, saliva, plasma, serum, exfoliated cells, white blood cells, biopsies, and other tissue samples. Each of these sources of material may give different qualitative and quantitative information. It is important to bear in mind the information being sought when different biological materials are used.

As described above, a DNA adduct, for example, is believed to be on the pathway from exposure to cancer. The critical dose of that DNA lesion however is defined at the tissue, cell, gene, and even DNA sequence level. Other exposure biomarkers, such as protein adducts or internal dose markers, have the additional caveat of not being on the disease pathway, but hopefully associated with it. These layers of complexity need to be considered when interpreting the information gained from such surrogate molecules or tissues in molecular epidemiology studies.

In practice, biomarkers of exposure and effect can rarely be measured in the target organ. Consequently, measurements are made in more easily available material, typically plasma or serum, white blood cells, or urine; often there is relatively little empirical data to demonstrate the relationship between a biomarker in the target organ and these surrogate materials. Indeed, from animal studies it is known that many carcinogens induce different levels of DNA adducts in different tissues, this being one determinant of the susceptibility of that tissue to carcinogenesis. In a study of four methylating agents, each with a differing primary target organ (colon, esophagus, liver, and lung), DNA adducts were measured in the target organs, the liver, and the peripheral blood cells (PBC) (57). While adducts in PBC and the liver showed a relatively consistent relationship with all four agents, the relationship with the target organ varied markedly. This type of correlation between biomarkers in body fluids and internal organs has rarely been addressed in human studies, although opportunities do exist, for example, where procedures such as bronchoalveolar lavage and endoscopy are performed provide access to target tissues (58–61).

If a biomarker in peripheral blood has been validated in relation to external exposure then the lack of knowledge concerning its relationship with levels in target tissues does not mean that that biomarker is invalid. However, the limitation in understanding this relationship should be borne in mind in the interpretation of the data. There is also a strong case to be made to explore the relationship between target and surrogate tissue biomarker levels where possible; in practice, this is often done in the context of small-scale clinical studies.

WHEN TO MEASURE?

Environmental exposures will most often vary qualitatively and quantitatively during an individual's life because of changes in lifestyle, place of residence, occupation, etc. In addition, a given exposure may have a greater or lesser impact on disease risk at different times of life. Exposures in childhood may be particularly relevant to disease many decades later (1,62). For example, some dietary exposures reveal differing risks depending on the period of life when exposure is assessed (63). It is also well established that age at infection with hepatitis B virus (HBV) is a critical determinant of risk of becoming a chronic HBV carrier, and therefore of developing liver cancer (64). The temporal variation in exposure and the varying significance of that exposure over time poses particular challenges to biomarkers that are, by their inherent nature, transient. It should be remembered how changes in exposure is a intervention strategies, e.g., below), or in ascribing exp

The question of where history of the disease requires variation in exposure, and t serum antibodies to viral ar that studies of infectious d chemical or dietary exposu only 11 years elapsed betv Group 1 human carcinogs measure long-term past ex There are exceptions. persist in the body. For exa and adipose tissue (68). Ne for a chemical exposure v only from a few days throu biomarker investigation m determined empirically. M protein adducts may be in Again studies to derive inl where possible as part of information regarding the analysis of molecular epid

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should be remembered however that on occasion a biomarker responding rapidly to changes in exposure is advantageous. Examples include monitoring the impact of intervention strategies, e.g., antismoking programs (65) or chemoprevention (66) (see also below), or in ascribing exposure to occupation (67).

The question of when to measure a biomarker of exposure in relation to the natural history of the disease requires some notion of the critical exposure period, the temporal variation in exposure, and the inherent stability of the biomarker concerned. With viruses, serum antibodies to viral antigens often persist and indicate past exposure. This has meant that studies of infectious disease can progress more rapidly than perhaps is the case for chemical or dietary exposures. For example, with Helicobacter pylori and gastric cancer, only 11 years elapsed between identification of the organism and its classification as a Group 1 human carcinogen by the IARC (5). For chemical exposures the ability to measure long-term past exposure is more difficult to achieve.

There are exceptions, where chemical exposures can be assessed by biomarkers that persist in the body. For example, certain pesticide residues have long half-lives in plasma and adipose tissue (68). Nevertheless, in the majority of instances the biomarker half-life for a chemical exposure will be relatively short and consequently provide information only from a few days through to a few months prior to sampling. As with other areas of biomarker investigation mentioned above, there are few occasions where this has been determined empirically. More often it is implied from other evidence, e.g., the half-life of protein adducts may be inferred from the biological turnover of the protein concerned. Again studies to derive information on the persistence of biomarkers should be obtained where possible as part of the validation process. In all cases, the actual or assumed information regarding the biomarker should be taken into account in the design and analysis of molecular epidemiology studies.

One attempt to reconstruct past exposures has been to seek longer-lived protein adducts by examining binding of chemicals to proteins that persist through cell division, such as histones (69). Alternatively, as discussed above, the analysis of mutation spectra may be informative concerning past exposure (53,56,70–72).

As mentioned earlier, the most common design to assess cancer etiology has been the case:control study. While genotyping is well suited to this design, biomarkers of exposure and possibly effect applied to biological samples at the time of or soon after cancer diagnosis may not reflect what has occurred in the past. In addition, the presence of disease could potentially influence the biomarker. This may be because the patient changes their lifestyle postdiagnosis or because the presence of disease alters physiology such that the biomarker is affected, resulting in reverse causation. Despite the above limitations, DNA and protein adducts, for example, have been applied in case:control studies with positive findings (73–75).

An alternative is to establish prospective cohorts. A nested case:control study within the cohort or a case:cohort design (76) can then be used to limit the resources needed for biomarker analysis. As the aim is to have all individuals healthy at entry to the study, when biological samples are collected, the problems of reverse causation may be avoided. The design also provides an opportunity for one or more measures of exposure prior to disease onset. In practice, however, the periods of follow-up still tend to encompass a relatively short fraction of the carcinogenic process (a few years rather than decades), thus presenting some residual risk of reverse causation and often involve only a single time point (most often recruitment) for biological sampling.

Despite the caveats mentioned above, it is the prospective cohort design that is better adapted to most currently available biomarkers of both exposure and effect. Huge
investment is being made in large prospective cohort studies that include collection and banking of biological material (77–79). These studies are predicated on the exploitation of the associated biobanks using appropriate biomarkers to elucidate the genetic and environmental basis of common chronic disorders. The need for biomarkers of exposure to complement those of genetic factors in order to fully benefit from these investments has been stressed on (7,8). Given the fact that many of these biomarkers inherently are reflective of only recent past exposure also implies value in repeat samples being collected from the same individual wherever possible.

It is within the prospective cohort design that the most successful examples of biomarkers and disease outcome are found. For example, DNA and protein adducts as well as urinary metabolites have provided key evidence in establishing an exposure-disease association between aflatoxins and liver cancer (33,80). Other studies have associated “bulky” DNA adducts with lung cancer in smokers (81) and never- or ex-smokers (82). Similarly, the chromosomal aberration and micronuclei studies mentioned earlier, although not strictly from a cohort design, were related prospectively to increased cancer risk (47,48).

**BIOLOGICAL PLAUSIBILITY**

One of the criteria for establishing a causal association between an exposure and disease is biological plausibility. In this context, biomarkers may contribute by illuminating some of the carcinogenic steps linked to a particular risk factor. This is therefore an additional area, and possibly an undervalued one, where biomarkers can make significant contributions to cancer epidemiology. Some examples are described below.

One of the much-investigated risk factors for colorectal cancer is high meat intake, but the causal factor(s) remain unknown. One hypothesis is that red meat stimulates endogenous intestinal N-nitrosation, resulting in formation of N-nitroso compounds (NOC). Lewin and colleagues (61) studied volunteers in a metabolic suite consuming defined diets high in red meat compared with individuals consuming a vegetarian diet. They demonstrated that both the formation of NOC in feces and NOC-related DNA adducts (O6-carboxymethylguanine) in exfoliated cells in feces were higher in people consuming the red meat diet. At the individual level there was a correlation between the two biomarkers. Such studies are often demanding to perform. For example, in this instance, a metabolic suite was required with provision of controlled diets and rapid processing of fecal samples. Consequently, studies are normally limited to relatively small numbers of subjects. Nevertheless, this study serves as a good example, whereby carefully designed biomarker experiments in human volunteers can be used to investigate mechanisms of carcinogenesis and provide evidence for the biological plausibility of putative etiologic associations.

Another area where biomarkers have been applied to investigate the plausibility of etiologic hypotheses is that of genetic susceptibility. For example, associations have been predicted between genetic polymorphisms in carcinogen metabolizing or DNA repair genes and higher DNA adduct levels. In one study in healthy individuals, a dose-response relationship between “bulky” DNA adducts in lymphocytes and polymorphisms in three different DNA repair enzymes was reported (83). This type of study strengthens the rationale for examining the relationship between polymorphisms and cancer risk precisely because they provide evidence that the former affect a significant step on the mechanistic pathway to cancer.

**INTERVENTION STUDY**

Biomarkers can be incorporated into an intervention study design. The objective may be to examine an intervention pathway, perhaps using a case-control study design. The objective may be to test endpoints in intervention studies to further assess the mechanistic-orientation of a full-scale intervention study and application of biomarkers below.

Epidemiological evidence of cancer at a number of sites has been equivocal (85). In some cases, vitamin supplementation (86) has been shown to have beneficial effects on cancer risk. For example, vitamin C supplementation in a cohort of Norwegian men was associated with a reduced risk of cancer (87). The mechanism by which these effects are achieved is not yet fully understood, but it is likely that vitamin C may act as a chain-breaking antioxidant, preventing DNA damage and cell proliferation.

Another example is the use of aflatoxin biomarkers in the groundnut crop in Ghana. Aflatoxin contamination has been a significant problem in the food supply, and the use of aflatoxin biomarkers has been an effective intervention to reduce aflatoxin exposure. The aflatoxin biomarkers used in this study were urinary metabolites and confirmatory adducts in tissues. The results demonstrated a significant reduction in aflatoxin exposure in the intervention group compared to the control group.

These examples illustrate the potential for the use of biomarkers in intervention studies to assess the impact of an intervention on cancer risk. Further research is needed to fully understand the mechanisms by which these interventions work and to determine the best methods for the application of biomarkers in cancer prevention and control.
Biomarkers of Exposure and Effect

INTERVENTION STUDIES

Biomarkers can be incorporated into intervention studies for different purposes. First, the objective may be to examine the possibility to modulate a particular biochemical pathway, perhaps using a micronutrient or pharmaceutical agent. This type of intervention study tends to be conducted on a small scale with intense analysis of one or more biomarkers. The outcome is a better understanding in vivo of mechanisms of carcinogenesis and a stronger scientific rationale for public health interventions. Second, the objective may be to use biomarkers of exposure or effect as surrogates for disease endpoints in intervention studies. This type of study will tend to be on a larger scale than the more mechanism-orientated studies and may serve as a prelude to the conduct of a full-scale intervention study with disease as the outcome. Some illustrations of the application of biomarkers in these types of intervention study are discussed briefly below.

Epidemiological evidence links fruit and vegetable consumption to decreased risk of cancer at a number of sites (84). One hypothesis is that antioxidants may be at least partially responsible by protecting cells from oxidative DNA damage. However, when large-scale intervention studies with antioxidants have been conducted the results have been equivocal (85). In each case, the precise mechanism by which the vitamin supplementation may be effective and the dose required were poorly understood. A number of small-scale intervention studies have been conducted to examine whether vitamin supplementation can decrease DNA damage in vivo, in order to provide some additional supportive data to the underlying mechanistic hypotheses. For example, Collins and colleagues (86) reported that kiwi fruit consumption reduced both endogenous oxidative DNA damage and damage induced by an ex vivo challenge in peripheral lymphocytes. In addition, DNA repair capacity was enhanced by fruit consumption. These observations illustrate that an additional mechanism, notably induction of DNA repair capacity, may be of relevance. In another intervention study, in smokers, increased flavonoid consumption resulted in an increase in urine antimutagenicity (87) but no consistent effects on DNA adducts in exfoliated bladder cells (88). This type of data can add valuable information to the scientific rationale behind intervention studies using micronutrients.

Another example where biomarkers have been used in intervention studies is in the case of aflatoxins. In a community-based postharvest primary prevention trial, targeted at the groundnut crop in Guinea, West Africa (89), aflatoxin-albumin adduct levels were more than 50% lower in subjects in the intervention villages. A panel of aflatoxin biomarkers, including urinary DNA adducts and metabolites, have also been applied successfully in an elegant series of chemoprevention studies in China using oltipraz and chlorophyllin (66,90-92). These studies were able to demonstrate the modulation of aflatoxin metabolism in exposed individuals and a decrease in the level of aflatoxin-N7-guanine adducts.

To date these intervention studies have employed biomarkers of chemical metabolites or adducts, rather than biomarkers of effect such as chromosomal aberrations or mutations. This fact has mainly reflected the aims of the study, to establish proof of principle, and the associated requirement for biomarkers that respond rapidly to the intervention. In the future, it can be envisaged that longer-term interventions may use biomarkers of effect to monitor early outcomes. These may be considered analogous to the use of serum HBV surface antigen as a biomarker to demonstrate that HBV vaccination has been effective in preventing chronic HBV carriage (93). An argument
could be made to incorporate biomarkers of effect in longer-term intervention trials, as early indicators for any evidence of adverse effects.

FUTURE BIOMARKERS OF EXPOSURE AND EFFECT

It is pertinent to ask whether the increasing application of transcriptomics, proteomics, and metabonomics in cancer research can contribute to the development of biomarkers of exposure and effect (15). Their value will primarily depend on whether specific exposures are reflected by altered levels of mRNA, proteins, or metabolites. Will there be signatures or fingerprints of environmental exposures across a broad spectrum of mechanisms of action, both genotoxic and nongenotoxic? Similarly, there is a need to examine whether the particular biological effects consequent to exposure will be represented by characteristic patterns of gene expression, proteins, or metabolites. If so, these new technologies may provide a step-change in the development of biomarkers of both exposure and effect.

There are some early indications that this is a fruitful area of research, albeit one that to date is relatively unexplored. For example, naturally occurring or industrial compounds with estrogen activity do alter the expression of similar genes in vitro (94), while ionizing irradiation also altered expression of specific genes in human lymphocytes (95,96). There have also been a few gene expression studies in relation to exposure in a population setting focusing on smoking (97), benzene (98), arsenic (99), metal fumes (100), and environmental air pollution (101). These preliminary data show that different exposures do elicit different changes in gene expression and encourage the further exploration of the sensitivity, specificity, and stability of these changes.

Examination of the 3000 or so major metabolites (102) that constitute the metabonome also offers opportunities to address exposure assessment. In an experimental mouse study involving infection with Schistosoma mansoni, differences in urinary metabolite fingerprints were obtained, which indicated effects on certain metabolic pathways (103). In terms of human studies, the change from nonsoy to soy-containing diet was shown to be associated with some changes in plasma metabonome (104). The potential application of “omics” technologies to characterize dietary exposures and to understand the biological effects of diet at the cellular level therefore receives some support from these early investigations (104,105).

This new generation of technologies requires further extensive research before deciding whether it can provide a marked advance in biomarkers of exposure and effect. Notably, it remains to be seen if, in principle, mRNA, protein, or metabolite expression can be specific and sensitive enough to define exposures at low levels in human populations. It will be important to understand whether complex mixtures or families of chemicals act through the same pathways and can be represented by common targets on common mechanistic pathways. The dynamic nature of each of these systems may militate against long-term exposure assessment, unless some of the changes prove stable over time. The technology will also need to be tailored in terms of such properties as sensitivity, sample requirement, throughput, and cost to be applicable to population studies. Purification procedures in the case of metabonomics and proteomics will be essential to measure rare, and possibly more informative, proteins or metabolites among the background of quantitatively more dominant species. Although not explicitly discussed here, the need for sophisticated statistical analysis emerges as crucial to any eventual application. As with the earlier generation of exposure biomarkers, a carefully planned strategy, starting with model systems and small-scale human studies, is likely to be most successful (33).
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SUMMARY

The new generation of mega-cohort studies (78,106–108) provides the framework for investigations of genetic variation, environment, lifestyle, and chronic disease. These studies represent substantial investment, with U.K. Biobank, for example, recruiting 500,000 adults at a cost of around £60 million in the initial phase. A considerable part of this cost is driven by the collection and banking of biological material. This investment is at least partially justified on the assumption that biochemical and molecular measures on this material will help resolve important etiologic questions. It is self-evident that unraveling complex environmental and genetic etiologies to plan effective public health interventions demands that both environmental exposures and genetic variation are reliably measured. Advances in statistical methods and in bioinformatics in relation to large data sets are also of critical importance. The further development, validation, and application of biomarkers of exposure and effect in this context are manifestly a critical part of the future of cancer epidemiology in the 21st century.

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INTRODUCTION

The goal of epidemiologic subject exposure and disease prevention: to identify risk-exposures that might be eradicated.

One strength of epidemiology is that, in general, findings are generalizable to other populations. One limitation of epidemiology is that experimentation poses a threat to experimentation to increase the effect of prostate cancer among low-risk prostate cancer in average modifiers tamoxifen and raloxifene.

Another limitation is inconsistency. Measuring the impact of intervention in the association between exposure and outcome not be controlled for, can have effects on both exposures such as genetic predisposition the treatment and even more profound effect is studying the effects of exposure-disease relations. Some of these sources can be experimentally manipulated, some analysis of human e