Meeting Report

The Role of Biological Markers in Epidemiological Research: Future Directions

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The recent identification of new biological markers (biomarkers) has created unprecedented opportunities for molecular or biochemical epidemiologists in cancer research. Laboratory measurements used with traditional methods of epidemiology have undeniably added a new dimension to studies of causal associations. Although the technological arm of molecular or biochemical epidemiology has grown rapidly, a need still exists for increasing technology transfer from the laboratory to human studies. To date, major research activities have been directed toward biomarker assay development without extending efforts to establish validity in human populations. Of equal concern is the question of the biological relevance of measurements to the processes of carcinogenesis. Similarly, the reported wide ranges of variability in individuals and laboratories as well as the lack of modified statistical methods for data interpretation must be continually addressed. These issues pose a compelling challenge in the continued development and expansion of molecular cancer epidemiology.

To this end, the National Cancer Institute sponsored an advisory workshop on applications of molecular/biochemical epidemiology in cancer research on October 31, 1991, in Kona, Hawaii. With the cooperation of the International Agency for Research on Cancer and the National Center for Toxicological Research, the meeting was held in conjunction with their multidisciplinary conference, "Biomonitoring and Susceptibility Markers in Human Cancer: Applications in Molecular Epidemiology and Risk Assessment." A panel of expert laboratory scientists and cancer epidemiologists was assembled to review the current status of biomarkers in the most active research areas in cancer epidemiology: cancer susceptibility; diet; tobacco; environmental agents; and hormones. A roundtable discussion identified and recommended research needs for epidemiological applications of biomarkers for etiological studies in cancer. Dr. David Schottenfeld, a member of the Board of Scientific Counselors of the Division of Cancer Etiology, served as chairman of the workshop.

Cancer Susceptibility

Dr. C. Harris (National Cancer Institute, Bethesda, MD), in an overview of cancer susceptibility markers, addressed the question of why individuals with similar exposure to risk factors may vary in disease outcome. He postulated that underlying the determinants of variability is genetic predisposition, which by interacting with the processes of carcinogenesis, plays a key role in host responses to carcinogens. Interindividual variability is observed in many in vivo and in vitro assays of biomarkers. Most frequently these are measurements of the enzymatic metabolism of carcinogens: from initial activation of cytochrome activity to fidelity and rates of DNA repair. Metabolic processes may be interacting with confounding exogenous factors. Such differences are then magnified by the normal variations existing among tissues and cells. Animal and epidemiological studies have also demonstrated that variability in tumor promoter responses is attributable to inherited host susceptibility factors. Appropriate biomarkers to indicate this type of susceptibility are not yet available. Dr. Harris added that some determinants of variability may be acting as effect modifiers. For instance, physiological processes which regulate the bioavailability of exogenous carcinogens to target sites are most likely genetically controlled; an example is the degree of mucocilary clearance of tobacco smoke and the influence this may have in the development of tobacco-related cancers of the respiratory system. At the DNA (gene) level, recent advances in molecular genetics have identified candidate genes as discrete determinants of inherited predisposition to cancer development. Conditions associated with these include: the Li-Fraumeni syndrome (p53), retinoblastoma (Rb), familial polyposis coli (APC), debrisoquine phenotype (CYP2D6), and xeroderma pigmentosum (XPC). The scientific capability to identify high-risk individuals has raised public concern, suggesting that bioethical considerations should be incorporated into the planning.
phase of study protocols. A group discussion of debrisoquine metabolic phenotyping expounded the difficulties in interpreting phenotyping assays using surrogate chemicals. The participants emphasized five points: (a) data must relate to the specific substrate of interest; (b) both inducers and inhibitors can influence the results; (c) genotyping, which measures a specific pattern of inheritance and expression, may be useful, although different mutations may exhibit different biological activities; (d) concurrent assessment of exposure history would help delineate changes over time; and (e) future investigations of regulatory genes in addition to the cytochrome P-450 isoenzymes may be elucidating.

At the present time, there are few available cancer susceptibility markers for large-scale epidemiological studies. It was predicted that they would become available within the next ten years due to the rapid advances in molecular biology and genetics. In the interim, epidemiologists need to pursue studies and comparisons of biomarkers in ethnic populations, family units, and other population subgroups to clarify an operational definition of genetic susceptibility. Future data analyses should include exposure stratification by known susceptibility factors to enhance the power of epidemiological studies to resolve exposure-cancer associations.

Diet

During the past decades questionnaires estimating dietary intake have been invaluable in generating diet-cancer hypotheses. Yet, much remains unknown about the relationship between diet, interaction of diet and nondietary factors, and cancer. Biochemical indicators have become useful as surrogate measurements for actual dietary intake of certain nutrients; more recently, they have become validation instruments for estimated dietary assessments. Their potential value in etiological studies depends upon improved analytical methods for better characterization and identification of variance components in measurements. Validated, accurate biomarkers are needed to quantitate the intake of several important nutrients and to reflect long-term dietary exposures. Ongoing clinical trials in chemoprevention could utilize biomarkers as indicators of short-term dietary effects or as intermediate end points. Dr. J. Felton (Lawrence Livermore National Laboratory, Livermore, CA) presented an overview of technological advances that are highly sensitive and could be considered for future application in studies of dietary effects. In referring to current dietary biomarkers of cancer risk, he noted their capability to detect low-dose exposures to potent compounds such as the heterocyclic aromatic amines. Their presence reflects internal doses which result from combined metabolic and repair processes. Laboratory methods that could improve the resolution of these measurements are: (a) DNA-protein interactions measured by 32P postlabeling, immunochromography, mass spectrometry, or fluorescent techniques; (b) detection of metabolites or DNA adducts in urine; (c) metabolic and/or DNA repair phenotyping; (d) gene-specific assays of hypoxanthine phosphoribosyl transferase or glycophorin-A; and (e) cyto genetic methodology including sister chromatid exchange, micronuclei counts, and chromosomal painting of specific DNA adducts.

Workshop participants commented on the need to improve the specificity of present techniques such as 32P postlabeling and immunochemistry. They concluded that a reasonable approach would be to combine batteries of assays, adding those biomarkers with the highest specificity, for the most meaningful results. Suggested topics for future research included: time-integrated characterization of specific dietary components; more targeted assessments of dietary effects in decreasing cancer risk; and validated markers of (a) tissue damage (e.g., peroxidation products, oxidants); (b) early premorbid stages in tumor development; (c) intermediate end points; (d) effects of dietary intervention; and (e) refinements of nonlaboratory (questionnaire) measures of dietary intake.

Tobacco

Research questions, dealing with the complexity of tobacco smoke, have extended beyond the known consequences associated with smoking habits. Dr. S. Hecht (American Health Foundation, Valhalla, NY) commented that tobacco users comprise an excellent study population in which to apply biomarker technology due to the known associations between tobacco and cancers of various sites and the existing capacity to quantify exposure to a mixture of tobacco carcinogens. Thus, much of ongoing scientific activity is focused on groups such as snuff dippers, betel quid chewers, and aerodigestive cancer patients with second primary malignancies. Current laboratory efforts are concentrated in two major areas: mechanistic studies and identification of markers of individual susceptibility. Experimental measurements have been facilitated by technological advances (e.g., gas chromatography, mass spectrometry, synchronous fluoroscent spectrometry, 32P postlabeling, immunoassays, and polymerase chain reaction with amplification). Assays of components of tobacco smoke, their metabolites, and markers of smoke exposure are now available for application in human population studies (e.g., aromatic amines, N-nitrosamines, polycyclic aromatic hydrocarbons, DNA and hemoglobin adducts, cytochrome P-450 family phenotyping and genotyping, mutational spectra analyses, and nicotine metabolites). Projected research directions were identified as including large epidemiological studies using multiple assays within the nested case-control design and additional mechanistic studies of biomarkers targeted for use in epidemiological research. Further work on risk modification by diet and pharmaceuticals could provide useful information for models of chemoprevention. Two additional concerns were added by the panel. First, there is a need for biomarker research on the effects and interactions of receptor systems in target tissues of smokers. Second, laboratory implementation for population testing will require reorientation of objectives, personnel, and mechanisms of funding support; developmental activities must be replaced by the repeated application of standardized assays of validated biomarkers selected for known utility in epidemiological research.

Environmental Agents

Dr. F. Perera (Columbia University, New York, NY) noted the marked progress during the past decade in biomarker development for research in environmental carcinogenesis. The initial enthusiasm created by technological advances has become tempered by the recognition of methodological issues and clarification of the role of
biomarkers in etiological research. The indisputable strength of existing biomarkers lies in their sensitivity to low biologically effective doses of carcinogens and their feasibility as indicators of molecular host response. Conversely, their limitations are related to the processes of measurement. Biological plausibility, relating to the stages of neoplastic transformation and the outcome of interest, is a primary requisite. Consequently, Dr. Perera stressed the importance of recording the chronology of measured events which fit within the continuum between exposure and disease. Combinations or batteries of assays should be used for detecting mechanistic interactions between external dose, internal dose, metabolism, and other aspects of host susceptibility. This approach was exemplified by a description of the integrated laboratories in the Molecular Epidemiology Program, Columbia School of Public Health. Individual laboratories function as autonomous units for assay analyses, sharing the same sources of specimens within the Program for multiple testing. On the national level, methodological concerns such as reproducibility, intra- and interlaboratory variability, intra- and interindividual variability, source and exposure specificity, and use of surrogate tissues are being addressed by independent and collaborating laboratories and/or investigators. It was added that, whereas the laboratory component is important, epidemiological study designs should account for background exposures and confounding variables. Combining measurements of dose or response (carcinogen-DNA, carcinogen-protein adducts) with markers of susceptibility (cytochrome P-450 isoenzymes, p53, DNA repair) was proposed to minimize interindividual variability. Dr. Perera predicted that methods for cell fractionation of peripheral blood and tissues will facilitate future cellular and tissue comparisons of biomarker detection.

Ensuing remarks by workshop participants emphasized the need for a consensus on terminology. In particular, the concept of validation elicited varying definitions from laboratory scientists and epidemiologists. If the determination of validity is based on whether a factor is absent from or present in (e.g., as determined by a measurement such as a biomarker) a population screened for the absence or presence of an outcome (e.g., cancer), the definition relates to the extent to which an instrument (or procedure) measures what it purports to measure. Molecular epidemiologists find it functionally useful to dichotomize the meaning of validity when speaking of measurements; i.e., two components must be satisfied: (a) the analytical or laboratory-based component which includes low-dose sensitivity, accuracy, precision, reproducibility, persistence, and biological relevance; and (b) the epidemiological or population-based component which includes inter- and intra-individual variability, sensitivity (to exposure or disease), specificity, predictive value, and prediction of disease (a desirable characteristic that may not be feasible). It was agreed that the most commonly held tenet is the correct association of a measurement with a disease, that this supersedes any other validity criterion, and that the type of "validity" should be stated or defined whenever the term is used. Additional comments stressed the need for quality control of appropriate reference materials and internal standards used in laboratory analyses.

Hormones
Dr. B. Hulka (University of North Carolina, Chapel Hill, NC) described one conceptual approach to molecular epidemiology by citing a study assessing the association of estrogen receptors, representing an intermediate biomarker, and breast cancer. The research question arose from the observed promotional and carcinogenic effects of estrogen in animal models, studies of reproductive events in women demonstrating the influential role of endogenous estrogen, and epidemiological data that show weak or no increase in breast cancer risk due to exogenous estrogens (oral contraceptives, estrogen replacement therapy). When the content of estrogen receptors was measured in breast tumor tissue, results differed among oral contraceptive users depending upon recency of use and hormonal composition of the contraceptive. No effect was significantly apparent in women who had received estrogen replacement therapy. Both a normal and a malignant breast epithelial cell were used as models to link this data to postulated cellular events. Dr. Hulka emphasized that observed data should be fitted into a scheme that will facilitate understanding of mechanisms of malignant processes. Such a basic model could be applied in prostate cancer research as well.

Promising hormonal biomarkers include: various hormones (estrogen, progesterone, prolactin, and other peptides); estrone metabolites; growth factors and their receptors; and genetic determinants (oncogenes, tumor suppressor genes, and susceptibility genes such as rare alleles of H-ras). Appropriate sources of samples need to be investigated further and may include serum, WBC, breast tissue, nipple aspirate, or urine. General group discussion among workshop participants identified the need for more basic and applied research activity in hormonal carcinogenesis. One topic of special interest was the possibility of significant hormonal interactions with genetic constituents and whether these could be associated with carcinogenesis. Laboratory scientists added that recent advances in the understanding of mechanisms of carcinogenesis and tumor progression will require concerted efforts in biotechnology transfer. Moreover, an understanding of how estrogen turns on cell division could lead to effective strategies for identifying high-risk individuals. Epidemiologists noted that the wide inter- and intralaboratory variations in present measurements of serum hormones preclude large definitive studies. They added that correlation studies of hormone and receptor levels in serum, tissues, and cells, especially in ethnic populations, would be helpful in achieving the goal of validating these measurements.

Future Directions
From the workshop presentations and discussions, the participants summarized research needs for the further development and application of biomarkers. Recommendations encompassed four general areas: methodology, resources, collaboration and training, and the development of new biomarkers. General consensus emphasized the great need and importance of validating existing and promising biomarkers in tissues, individuals, and populations. A concerted effort should be focused on assessing inter- and intra-individual variability as well as inter- and intralaboratory variability. Selection of laboratory measurements should be based on relevance to the end
Meeting Report: The Role of Biological Markers

point (e.g., predisposing disease state, early stage of cancer) and to the research hypothesis. Epidemiological study designs which incorporate biomarkers must take into account the methodological issues unique to their application. The role of specimen repositories and laboratory networks is often discussed, although organization (networking) and funding mechanisms have not yet been addressed in detail. Implementation will require visionary planning and effective alliances. Future experimental approaches will necessitate cooperation, collaboration, and communication between multidisciplinary research teams, developing and maintaining integrated research efforts throughout all phases of research and analyses. To facilitate this process, regular interdisciplinary conferences could encourage interactions, improve the understanding of technical and logistic problems, and clarify data interpretation. Of future impact would be the establishment of training programs to produce scientists with expertise in multiple disciplines (laboratory sciences, epidemiology, population studies).

The rapid advances in molecular biology and genetics have stimulated efforts to develop a variety of new biochemical, molecular, genetic, and immunologic biomarkers. The selection of biomarkers for further development can be guided by their potential utility for preventive interventions, elucidation of mechanisms of carcinogenesis, or identification of genetic determinants that act independently or interact with environmental factors.

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