

Review Article

The development of molecular epidemiology to elucidate cancer risk and prognosis: a historical perspective

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Abstract: Molecular epidemiology in cancer research grew from the field of chemical carcinogenesis and the use of biomarkers for environmental exposures, with incorporation of principles from early pharmacogenetics. Over the years, molecular epidemiology has become extremely complex, with studies evaluating associations between cancer risk and prognosis and numerous markers of susceptibility, exposure and early effects, as well as epidemiologic factors. In this article, we review the field of molecular epidemiology from a historical perspective, commenting on current status of the field and future directions.

Keywords: Molecular epidemiology, cancer, chemical carcinogenesis, biomarker, polymorphism, cancer prognosis

Today, reference to 'molecular epidemiology' in cancer usually brings to mind studies of genetic variants in relation to disease risk, although the broader application of molecular principles and techniques to epidemiological studies along the cancer continuum is acknowledged. Despite the common perception that molecular epidemiology primarily addresses gene/environment interactions in relation to cancer risk, the field actually grew from the use of biomarkers (mostly non-genomic) to better understand cancer etiology, with at least one foot in toxicology and carcinogenesis. In fact, the earliest work in molecular epidemiology in the field of cancer was related to environmental carcinogenesis. In this commentary, the evolution of the field of molecular epidemiology in cancer research is discussed, the early roots and current applications, as well as future directions.

Roots in chemical carcinogenesis and pharmacogenetics

The tenets of molecular epidemiology derive, to some extent, from the earliest understandings of pharmacogenetics and later application to

environmental toxicology. As noted in a review by Nebert [1], Pythagoras was the first to document glucose-6-phosphate dehydrogenase deficiency in observing the 'dangers of some, but not other, individuals who eat the fava bean', implying a pharmacogenetic response. Although the term 'pharmacogenetics' was not coined until Friedrich Vogel in 1959 [2], the recognition that not all individuals respond in the same manner to similar pharmaceutical or environmental exposures has been quite prevalent throughout time. Primary early clinical examples of pharmacogenetics were the observed associations between variation in inactivation of isoniazid, an anti-tubercular drug, and *N*-acetyltransferase (NAT2) polymorphisms, characterized through phenotypic assays [3]. Similar associations were noted between hypotensive reactions to debrisoquine among a small percentage of patients, later found to be attributed to variable oxidation by cytochrome P4502D6 (CYP2D6) [4]. This paradigm may have been confined to studies of drug metabolism, but the same enzymes involved in drug metabolism are known to activate or detoxify environmental carcinogens, and in 1970, the concept of

'ecogenetics' was presented by a number of scientists, including Mulvihill [5], Motulsky [6], Omenn [7] and Harris [8]. In 1987, Mulvihill and Tulinius published a provocative paper [9] asking "Are cancer epidemiologists ready for molecular geneticists?", indicating the beginnings of the movement for interdisciplinary application of molecular genetics, toxicology and biochemistry to rigorous epidemiological research. In that summary of a meeting of the International Epidemiology Association, the authors had the foresight to suggest that epidemiological studies collect detailed family histories, as well as store sources of DNA, although DNA banking in epidemiological studies did not become common until at least 10 years later.

While the concepts of inter-individual responses to exposures based upon inherited variants were being developed, studies of carcinogenesis were also being applied to humans. Benzo[a]pyrene (BP) is a carcinogenic polycyclic aromatic hydrocarbon, and in 1976, the Harris laboratory published a paper in *Science* [10] in which, using human bronchus tissues from 37 patients, they showed that there was 75-fold inter-individual variation in the metabolic activation and binding of BP diol epoxide to DNA, inferring that inherited differences in drug metabolism could result in subpopulations of individuals who were most susceptible to the effects of chemical carcinogens.

The term 'biochemical' or 'molecular' epidemiology began to emerge in the 1980s, and much of the literature was related to the quickly growing research in carcinogen metabolism and carcinogen-DNA adducts in relation to human cancer, with some of the first papers using the terms published by Perera and Weinstein [11], and by Harris [12]. Numerous carcinogens are metabolically activated, and can bind to DNA, potentially resulting in errors in DNA replication and/or in mutations. With capabilities to detect carcinogen-DNA adducts with a variety of approaches, such as radioimmunoassay, ³²P-postlabeling and mass spectrometry approaches, detection of adducts in humans has provided evidence for exposure to a number of classes of carcinogens, in white blood cells, hemoglobin, and in target tissues. Considered a biomarker of 'biologically effective dose', adducts represent external exposure, with consideration of sum effects of carcinogen absorption and distribution, activation and detoxification,

and DNA repair in some cases [13]. Measurement of adducts has contributed greatly to documenting the role of aflatoxin in relation to hepatocellular cancer [14], as well as occupational exposures to chemical carcinogens, such as polycyclic aromatic hydrocarbons (PAH) in coke oven workers [15], and aromatic amines exposures among dye workers [16]. The identification of carcinogen-DNA adducts in breast tissue and exfoliated mammary ductal epithelial cells in breast milk has also supported a role for chemical carcinogens in human breast cancer [17-19].

In the mid-1980s, Harris drew upon concepts and findings from *in vitro* and *in vivo* studies of chemical carcinogenesis as well as epidemiology and proposed a model of multistage carcinogenesis, considering carcinogen metabolism and subsequent damage to DNA, and incorporating additional exogenous exposures and endogenous factors contributing to conversion and progression to clinical cancer [20]. This model, shown in **Figure 1**, provided a paradigm for molecular epidemiological investigations, not only examining exposures in relation to cancer risk, but also incorporating biomarkers of exposure, dose, and early effects. The development of the technology to determine genetic polymorphisms in key genes involved in carcinogen metabolism and other relevant pathways enabled the addition of genetic susceptibility to this model, particularly the role that variability in carcinogen metabolism could play in modifying associations between exposures and cancer risk, as well as effects on biomarkers along the cancer continuum.

Epidemiology meets environmental carcinogenesis

The publication of Hulka's text 'Biological Markers in Epidemiology' in 1990 [21] and Schulte and Perera's 'Molecular Epidemiology' in 1993 [22] brought much of this work that was previously being conducted primarily by laboratory scientists to the attention of epidemiologists, who began to play a larger role in the development of this field. Before capabilities were developed to determine differences in DNA code that resulted in variable enzymatic activity, phenotypic assays were used to examine inter-individual differences, such as *N*-acetyltransferase phenotypes (rapid, slow, intermediate), involved in metabolism of the bladder carcino-

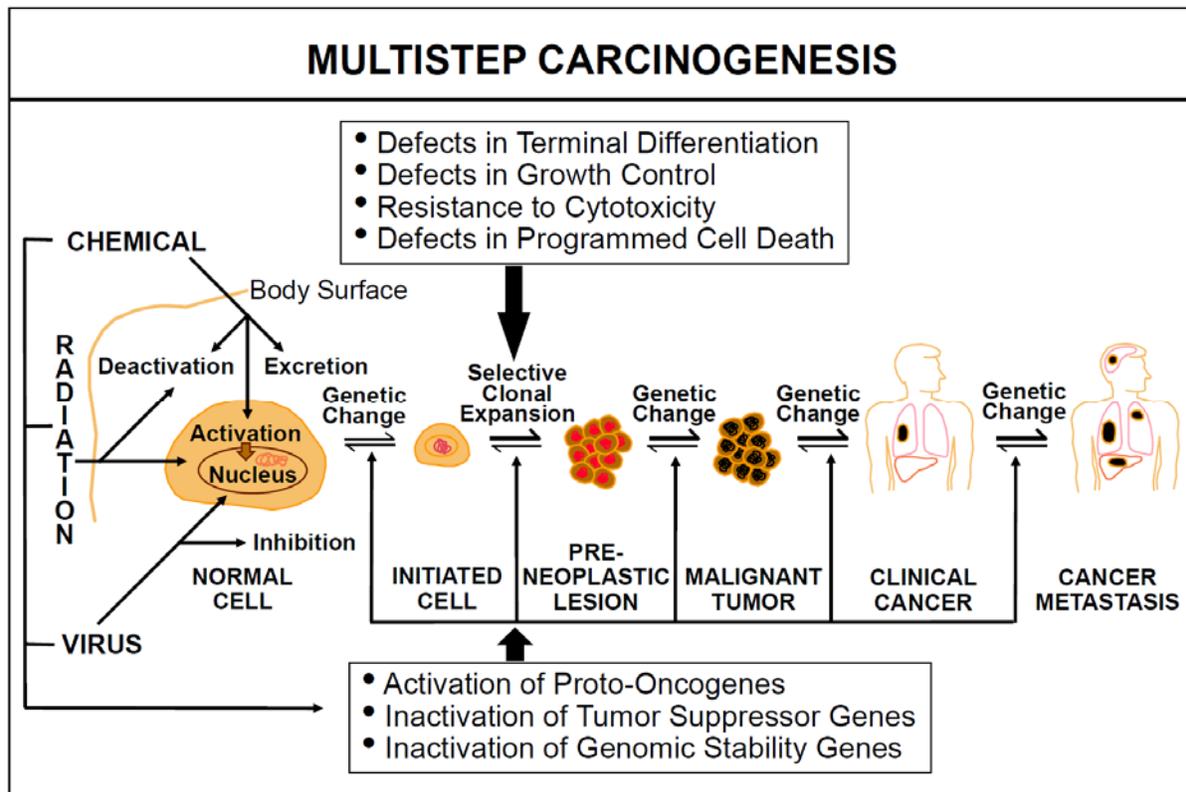


Figure 1. Schematic representation of the multistage process of carcinogenesis, building upon studies of experimental carcinogenesis, and forming the basis for molecular epidemiology.

gens aromatic amines, in relation to risk [23, 24]. However, the discovery of polymerase chain reaction (PCR) to amplify targeted segments of DNA and restriction fragment length polymorphism (RFLP) assay to identify DNA fragments of differential sizes from the same gene locus enabled the incorporation of genotyping assays into epidemiological studies to examine differential susceptibility to cancer. PCR-RFLP was first applied to studies of polymorphisms in oncogenes, such as *ras* and *myc* in relation to cancer risk, but the application of genetic polymorphisms to epidemiological studies primarily focused on associations between cancer risk and polymorphisms in key enzymes that activate (phase I) and detoxify (phase II) chemical carcinogens, particularly in relation to exposures. Some of the earliest work in this area was conducted by Kawajiri and Nakachi in relation to cytochrome P4501A1 genetic polymorphisms and lung cancer risk. Kawajiri first showed that the *CYP1A1* Msp1 polymorphism was more common in lung cancer patients than in controls [25], and in a classic model for molecular epidemiological studies, they also

showed that individuals with polymorphisms in *CYP1A1* and *GSTM1*, phase I and phase II enzymes, respectively, were at increased risk of lung cancer at a low dose of smoking [26]. Greater cancer risk at lower doses of exposure among those with susceptible genotypes was also supported by research showing that levels of 4-aminobiphenyl adducts in bladder cells and in hemoglobin were highest among those with slower *N*-acetyltransferase genotypes at low or null nicotine-cotinine levels, whereas there were no differences at higher levels of tobacco-smoke exposure [27].

For several years, molecular epidemiology studies were directed primarily towards cancers with known causal relationships with chemical carcinogens, and research focused on examining genetic variants in carcinogen metabolism and DNA repair pathways in relation to those cancers, such as lung cancer and bladder cancer [28]. When RFLP was the primary method for assessing genetic polymorphisms, research was limited by the presence of restriction sites that would differentiate genetic variants in candidate

genes, and not all polymorphisms investigated were known to have functional effects. In these early years of molecular epidemiological research and the study of the effects of genetic variants on cancer risk, there were numerous small studies conducted, with often inconsistent results. It was uncommon for laboratory scientists with expertise and knowledge in carcinogenesis and carcinogen metabolism, and capabilities to perform PCR-RFLP to identify genotypes, to have access to large study populations with DNA available. On the other hand, the majority of epidemiologists were unfamiliar with the concepts driving chemical carcinogenesis, and investigation of susceptibility to environmental carcinogens was foreign territory. To address these gaps in cross-training and education, and to solidify molecular epidemiology as a credible field in cancer research, Kadlubar and Ambrosone proposed that it is essential that epidemiologists who use biomarkers in their studies obtain training and basic skills in biochemistry and molecular biology, and that laboratory scientists and clinicians applying concepts to human populations should be grounded in training in epidemiological methods and study design [29]. Toward this end, they and others organized a Molecular Epidemiology Working Group, which first met in 1997, with the following proposed mission statement: "The Molecular Epidemiology Working Group is a professional organization dedicated to an interdisciplinary approach to the study of chronic disease etiology. The Molecular Epidemiology Working Group promotes the incorporation of molecular and biochemical concepts and techniques into well-designed epidemiological studies by providing a forum for discussion and development of sound approaches to the conduct and interpretation of molecular epidemiological studies, sponsoring of educational activities, and fostering of partnerships among scientists in different disciplines. The Molecular Epidemiology Working Group is an organization for epidemiologists, molecular biologists, toxicologists, nutritionists, statisticians, clinicians, and all other scientists who are interested in working together and merging their efforts toward an integrated approach to gain further insights into disease etiology and to promote public health." [30]. This Molecular Epidemiology Group (MEG) eventually became part of the American Association for Cancer Research (AACR), and with support from AACR, has played an active role in highlighting molecular epidemi-

ological research at the annual AACR meetings and through AACR Special Conferences developed by MEG to address topical issues in molecular epidemiology. These activities have been extremely effective for educating the molecular epidemiology community on growing areas in the field. MEG/AACR also partners with Molecular Epidemiology groups in Europe, and has participated in joint meetings.

With a growing cadre of cancer researchers who are cross-trained in fundamentals of epidemiology, biology, carcinogenesis and statistics to lead rigorous, well-designed molecular epidemiological studies, and with major technological advances enabling high-throughput genotyping, there has been explosive growth in investigations of gene/environment interactions in cancer risk. Many of these studies have served to greatly elucidate carcinogenic pathways, particularly those undertaken in populations with documented occupational and/or environmental exposures. Using candidate gene approaches, investigations have expanded to examine hormone metabolism pathways in cancers that are hormonally-related, such as breast, endometrium and prostate, and key genes in enzymatic pathways related to exposures that may increase or decrease risk, such as nutritional factors, pharmaceuticals, and other lifestyle factors. Accumulating data from a number of these research areas are contributing to elucidation of etiologic pathways for a number of cancers, but because of the breadth of substrates for numerous enzymatic pathways, and the complex exposures to factors that increase as well as decrease risk of cancer, it is unlikely that there will soon be application for individual risk assessment, except for susceptibility to carcinogens with clear rate-limiting metabolic pathways. The value of these studies of gene/environment interactions may be in their allowing refinement of risk estimates associated with specific exposures, by focusing primarily on those who are most susceptible and 'at-risk'. In heterogeneous populations, there may be weak or null associations between cancer risk and particular exposures, but with examination of only those who are most susceptible, associations may become evident. For example, although the evidence was weak for an association between cigarette smoking and breast cancer risk, Ambrosone, Freudenheim and Shields showed that, when stratified by genotypes for *N*-acetyltransferase, women with

genotypes that resulted in slower detoxification of aromatic amines, present in tobacco smoke, were at increased risk with smoking [31]. Risk was reduced among those with rapid *NAT2* genotypes. In a recent pooled and meta-analysis of 13 studies, this significant statistical interaction between smoking, *NAT2* and breast cancer risk was confirmed [32]. Here, the concept of genetic susceptibility was used to clarify risk relationships between smoking and breast cancer, and identify potentially susceptible subsets.

SNPs on chips

With the capabilities to conduct high-throughput genotyping, there has been a movement in the last several years to examine effects of single nucleotide polymorphisms (SNPs), singly or in pathways, in relation to cancer risk. Gene selection may be hypothesis-driven, and chips or arrays constructed with SNPs in candidate genes selected, or an agnostic approach may be taken, as in genome-wide association studies. To facilitate these types of analyses, a number of consortia have been formed to pool data and samples or genotyping results. Many of these studies have resulted in null findings, and conclusions drawn that the candidate genes evaluated do not play a role in cancer risk.

Although 'sub-group' analyses may be disparaged as data dredging [33], it is clear that the effects of some gene variants may only be observed among those with relevant exposures [34, 35]. There are numerous examples of this from the literature with a body of fairly consistent findings, including associations between *N*-acetyltransferase, smoking and breast cancer risk [32] as well as *NAT2*, meat consumption and risk of colon cancer [36]. Polymorphisms in manganese superoxide dismutase (*SOD2*) were not associated with breast cancer risk in a meta-analysis of 9,710 cases and 11,041 controls [37], but were associated with increased risk among women who were low consumers of dietary anti-oxidants [38, 39] and with low plasma anti-oxidants in the Nurse's Health Study [40]. Similar associations have been noted for prostate cancer, with no main effect observed for MnSOD polymorphisms in meta-analysis [41], but increased risk among men with lower plasma carotenoids in the Health Professionals Study [42], low plasma anti-oxidants in the Physicians' Health Study [43] low vitamin E in the CARET study [44], and low selenoprotein P in a

large (n=4,871) population based study in Sweden [45]. Furthermore, there may be no effects of polymorphisms in alcohol dehydrogenase on cancer risk, unless evaluating among consumers of alcohol [46]. Thus, although the identification of common variants that may increase risk of cancer in and of themselves may be informative for further investigation of etiologic pathways, it is likely that for most cancers, which are extremely complex and likely arise from multiple genetic and environmental factors, identification of these SNPs that result in small increases in risk may have a negligible impact on public health.

Genetics and cancer treatment outcomes

The application of pharmacogenetic concepts to cancer treatment outcomes has resulted in clinically relevant findings. For example, a small proportion of children with leukemia experience life-threatening myelosuppression when treated with mercaptopurine. Because thiopurine methyltransferase (*TPMT*) polymorphism, encoding no activity, results in excess of active drug metabolites, children are now genotyped prior to treatment, and dose reduced approximately 10-fold in those who are homozygous for the polymorphism [47]. Similarly, polymorphisms in di-phosphoglucuronosyltransferase 1A1 (*UGT1A1*) are associated with toxicities associated with irinotecan [48]. For both of these chemotherapy agents, drug labeling references variability by genotypes in relation to suggested doses.

There has been less success in progress in pharmacogenetic studies for cancers that are treated with multiple drug agents, or with chemotherapeutics with complex metabolic pathways, although there have been some consistent data for more global mechanisms, such as oxidative stress and DNA repair pathways. A shortcoming of many studies of cancer prognosis is that they are often relatively small studies conducted in clinical populations. Molecular epidemiology studies are often conducted by pooling data from several study groups, resulting in tens of thousands of study participants. However, the majority of pharmacogenetic studies in the past were either conducted in heterogeneous populations, or in trials that were relatively small, thus reducing statistical power to detect effects of genetic loci.

One area that holds exceptional promise is the

application of GWAS to cancer treatment outcomes. In studies of cancer etiology, most of the alleles identified for risk of cancer infer risks that are slight, as would be expected when examining gene variants that are common in populations, and it is likely that many high prevalence (> 1%) polymorphisms will not, in and of themselves, increase risk of cancer, but will only become penetrant in the presence of exposures that are relevant for disease etiology. However, for studies of treatment-related toxicities, the exposure is overwhelming, known, and common to all receiving treatment, yet not all patients experience the same side effects. Thus, it is likely that a GWAS will have the capabilities to detect genetic variants that play an extremely important role in susceptibility to severe drug toxicities, identifying alleles that, in the context of overwhelming exposure (chemotherapy), have much larger effect sizes than those observed in etiologic studies where exposures are heterogeneous and not taken into account, although large sample sizes are required. In the future, GWAS with larger coverage of the genome will likely provide even more useful information. The impact of newer technology and expansion to other areas in molecular epidemiology is reflected in a new text on molecular epidemiology published in 2008 by Rebbeck, Ambrosone and Shields [49].

Developing the molecular epidemiology of cancer prognosis

Although there has been extensive work conducted to determine risk factors for cancer etiology, much less is known regarding the molecular epidemiology of cancer prognosis, particularly for establishment of guidelines for prevention of cancer-related morbidities and mortality. In particular, little is known regarding the potential effects of dietary factors, supplements, physical activity, alcohol consumption and complementary and alternative medicines on treatment outcomes. Because of the growing populations of cancer survivors, research in this area is sorely needed to provide information to enhance patient well-being and decrease mortality among cancer survivors. As reviewed by Demark-Wahnefried [50], there have been recommendations made for cancer survivors regarding weight management, diet, exercise and smoking cessation, and some interventions have been conducted. The Women's Healthy Eating and Living trial tested the potential effects of a diet

high in vegetables and low in fat on breast cancer outcomes among women who were up to four years post-diagnosis, finding no survival benefit for the high vegetable, low-fat diet [51]. However, the women who participated in the study were already eating quite healthy diets to begin with. In contrast to the findings in the WHEL study, the Women's Intervention Nutrition Study (WINS), which was a randomized trial to test the effect of reduced fat intake, did find that there was a significantly lower relapse rate among women in the intervention group than among the controls [52], particularly for women with estrogen receptor-negative tumors. There have also been limited studies with physical activity interventions among cancer survivors, with findings of enhanced quality of life and improved function, as reviewed by Demark-Wahnefried [50]. However, there is still a paucity of data on lifestyle/behavior changes that could impact cancer progression and/or survival. In addition to interventions in patient populations, there are also growing data from observational studies, including follow-up of cases from case-control studies; prospective studies of cancer patients with data collected at diagnosis and throughout treatment, and some Cooperative Group studies that include collection of questionnaire data on lifestyle habits in the context of clinical trials [53]. Through these approaches, the effects, if any, of not only behavioral factors, but also biologic and genetic factors, can be incorporated, and studies of gene/environment interactions in cancer treatment outcomes assessed.

Tumor heterogeneity

There has been growing recognition that not all site-specific tumors are the same, and for some cancers, such as lymphoma and breast cancer, treatments are given depending upon tumor characteristics; genetic alterations for lymphomas, and gene expression arrays (or immunohistochemistry markers) for breast cancer. These advances in clinical medicine hold great utility for molecular epidemiology. For example, through the use of gene expression patterns, breast tumors have been classified into five distinct subtypes, with luminal A (ER+) cancers having the best prognosis, and basal like tumors, the worst [54, 55]. Because basal like breast cancers are the most aggressive, public health would be well-served by focusing our attention on identification of genetic and envi-

ronmental factors associated with increased risk of this subtype. With the Tumor Genome Atlas Project, it is likely that, within time, there will be distinct classifications of tumors that can be evaluated for causal factors. While associations between risk factors and an all-inclusive tumor site might dilute the strength of the relationship, by clearly refining the tumor phenotype through genetic characteristics, stronger associations are likely to be observed to better understand causal risk factors in distinct cancer subtypes.

Conclusion

In the last 30 years, there has been enormous growth in cancer research, aided by the rapid advances in technological capabilities. With the growth in understanding of carcinogenesis and the human genome, as well as the capacity to conduct high-throughput analyses for a number of biomarkers, there has also been an evolution in the field of molecular epidemiology. Although the interest in identification of associations between common gene variants (through GWAS, etc), may have peaked, it is likely that over the next few years, the literature will be filled with results from these studies, as well as fine mapping of identified loci and their functional validation. It is hoped that the next step will be to examine SNPs from GWAS results among subgroups of the populations, based upon exposures known to be associated with risk. There has also been growing interest in areas other than nuclear genetic polymorphisms, including copy number variation, mitochondrial DNA, variation in microRNAs and other factors that regulate gene expression, such as gene methylation. With discoveries in basic science driving the field of cancer research, epidemiologists, now more than ever, will need to be well versed in these areas, and part of multidisciplinary research teams to effectively make meaningful contributions to the prevention and cure of cancer.

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