GENOTYPIC AND PHENOTYPIC BIOMARKER PROFILES FOR INDIVIDUAL RISK ASSESSMENT AND CANCER DETECTION (LESSONS FROM BLADDER CANCER RISK ASSESSMENT IN SYMPTOMATIC PATIENTS AND WORKERS EXPOSED TO BENZIDINE)

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1. ABSTRACT

There is a need for improved methods for detecting individuals at risk for cancer to target subsets of patients for more intensive individual screening and targeted cancer therapy and chemoprevention. One approach for accomplishing this objective is to detect premalignant molecular fingerprints in an organ at risk for cancer or to define biomarkers reflective of treatment selection and response. Bladder cancer is an excellent model for testing this approach; however, comprehending the strategy for biomarker selection and analysis is more complicated than is generally appreciated. The objective of this article is to provide a succinct overview of our experience with the selection of biomarkers for bladder cancer detection, first in symptomatic patients and then in high-risk cohorts of workers at risk for bladder cancer.

Biomarker selection depends on multiple parameters, each of which must be optimized to enhance the utility of a biomarker for clinical application. Many markers that initially show promise fail in the clinical arena for a variety of reasons. Important parameters include when a biomarker is expressed in carcinogenesis (i.e., early vs. late), the sample type, and the method of analysis. These all contribute to the sensitivity, specificity, and ultimate clinical utility of a biomarker. New technologies/ support the notion that all diseases start in the cell, and Seymore West indicated the cell, under appropriate conditions, can function as a microcuvette for biophysical cytochemical analysis. Spectroscopy provides an accurate and sensitive method for quantitative single-cell proteomics. Improved and more stable fluorescence probes will enhance the utility of cellular chemistry, as will a rationale approach for biomarker selection based on the concepts of field cancerization, complemented by improved quantitative analysis of protein markers at the single-cell level. Our laboratory has developed a platform for single-cell proteomic analysis that can be applied to multiple basic science and clinical problems. Single-cell proteomics also facilitates the study of genetic instability and epigenetic signaling (stromal-epithelial interactions) in relation to cancer therapy and diagnosis. Because most cancers arise through multiple signaling pathways and are heterogeneous, the identification of appropriate biomarker profiles provides a number of strategic advantages over a single biomarker. Complex networks of signaling pathways lead to increased cell proliferation, decreased cell adhesion, cellular differentiation, genetic instability, and other functions associated with the malignant phenotype. The purpose of this presentation is to illustrate the fundamental concepts for selection and profile analysis of high-level phenotypic biomarkers developed for bladder cancer risk assessment, screening, and early bladder cancer detection.

2. INTRODUCTION

The purpose of this discussion is 1) to summarize our experience using quantitative fluorescence image analysis (QFIA) to analyze biomarker profiles for bladder cancer risk assessment, and 2) to provide an overview of our experience with the identification, assay and clinical application of biomarkers in symptomatic patients and high-risk populations. An overview of biomarkers as they relate to genitourinary toxicology has been reviewed (1); however, a brief foundation pertaining to the principles of biomarker selection and assay development is supplied (2, 3). Biomarkers by definition relate specifically to any cellular or soluble molecule(s) that may be useful for defining the pathogenesis or detection of a disease. Biomarkers may be related to susceptibility factors, exposure, or the interactive effects of exposure and inherited susceptibility. The end result of the complex set of interactions is the disease itself. Exposures modulating
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(promoting or inhibiting) the disease process may be endogenous or exogenous. For example, chemical exposures, such as to benzidine, may be monitored directly or indirectly as an adduct or other biological change quantitatively reflective of the exposure. Biomarkers may also be quantitated as soluble biomarkers in body fluids, such as urine, or as cell-associated markers. Although cells are not always the most accessible, the driving hypothesis of our research is that biomarkers associated with cells and their microecosystem will be more powerful than soluble biomarkers because of the dilution effects of serum. This is an important consideration, because strong biomarkers are needed for risk assessment, early detection, diagnosis, and screening programs. Molecular markers may be categorized as DNA, RNA, or protein. Since the functional molecules in most instances involve proteins, we theorize that protein biomarkers, or their post-translational modified products, will be stronger because they are more closely linked to regulating the functional genome and the final common pathways of a disease (e.g., translation and functional proteins). Biomarkers of effect are those associated with the disease processes that are the result of inherited genetic susceptibility factors and exposures.

Biomarkers of effect are also useful for validating in vivo and in vitro models used to study the cancer process. If the quantitative expression of biomarkers of effect in the model is not parallel to that normally expressed in the in vivo system of interest, then the model may not be valid (i.e., alternative signaling pathways may be operative). The validity of the model should be confirmed prior to using it to develop new therapeutic strategies. Thus, quantitative biomarker expression may be used to validate in vitro and in vivo models for elucidating functional pathways and should reflect the microecosystem of the in vivo clinical microecosystem under study. Fundamental reactions, such as nitrous oxide pathways or a specific biochemical pathway may be elucidated in microorganisms (i.e., drosophila) at various stages of evolutionary development, but may serve a different function in different organs or organisms. That is, the biochemical signaling pathway may be associated with a different function and network of signaling pathways. Thus, in this context it must be studied in relation to the appropriate microecosystem. To avoid epiphenomenon, biomarkers may be used to validate models if their expression in the model can be shown to reflect the operative signaling pathway in the host, organ, or organism of interest.

Multiple biomarkers for bladder cancer have been evaluated in preliminary studies, but few have been tested in phase III prospective trials for risk assessment, screening, and bladder cancer detection (3). Because bladder cancer develops in the most simplistic view through [?] at least two separate signaling pathways (e.g., High {p53} and Low {chromosome 9}), it is unlikely that a single biomarker will be effective in identifying all bladder cancers. Hence, a strong rationale exists for defining biomarker profiles for individual risk assessment. The concept of biomarkers profiles is now widely appreciated, as researchers have attempted to analyze complex microarray data in the arena of functional genomics. Here the objective is to define operative signaling pathways relevant to a disease process. Another area where multiple biomarkers are considered in combination is in the thresholds for defining positive chromosomal microsatellites or detecting chromosomal aberrations by fluorescence in situ hybridization assays. Thus, the complexity of analyzing genomic microarrays where there are multiple unknowns is in simplifying the array data by grouping data based on kinetics, gene function, or patterns of biomarker expression (i.e., complexity to simplicity). Considering the algebraic unknowns, this is a formidable task, although highly relevant. An alternative approach is to focus on high-level phenotypic biomarker pathways relevant to the disease. It makes sense to work backward from the simplistic to the more complex. This reduces the number of unknown variables in the signaling pathway of interest by starting with biomarkers tightly linked to carcinogenesis (e.g., apoptosis, cellular differentiation, proliferation, cell adhesion).

A cardinal obstacle to implementing this approach in the past has been assay development and clinical validation of biomarkers studied initially in the research laboratory. The innuendoes and practical obstacles associated with this problem have only been appreciated within the past two decades. In the interim, millions of dollars have been expended without appropriate guidelines for marker evaluation. A recent example is the realization that approximately 20% of patients who receive adjuvant tamoxifen for breast cancer may have been misclassified because of spurious immunohistochemical biomarker analysis (i.e., quantification of ER and Her-2/neu on breast cancer tissues). The problem of marker validation has been a major focus of the Cancer Prevention Branch of the National Cancer Institute, which has established marker networks (EDRN) to provide direction to this effort. However, in some cases scientific bias has diluted the integrity of the effort. Another obstacle to obtaining reproducible results is the method of sample collection and fixation. In addition, logical rules for statistically analyzing biomarker results require further clarification and have been a focal point of several World Health Organization international conferences and a variety of book chapters.

The paradigm evolving from our bladder cancer research serves to illustrate one approach for identifying and validating biomarkers. To accomplish our objective for improved bladder cancer risk assessment and detection, we analyzed biomarkers in the premalignant field of symptomatic bladder cancer patients, analyzed them using risk stratification schemas, and then tested the selected biomarkers in a group of Chinese workers at risk for developing bladder cancer in a six-year longitudinal study.

3. QUANTITATIVE FLUORESCENCE IMAGE ANALYSIS OF BIOMARKERS IN SINGLE CELLS

Biophysical cytochemistry of single cells was heralded by Ploem, Casperson and West over 30 years ago. Only recently has the power of utilizing a cell as a microcuvette for quantitative biomarker analysis been
appreciated. Although the concept of sub-classifying leukemias and lymphomas based on a qualitative biomarker alteration has played a cardinal role for patient management, precise quantification of biomarkers carries their value to another level. This approach is based on the concept that all diseases start in cells, and most diseases initially reflect subtle dysregulations of normal cell functions, including changes in premalignant lesions associated with bladder cancer. Precise quantification of biomarkers is highly relevant. In bladder cancer, because of the low prevalence of the disease and the need for biomarker profiles, quantification of biomarkers in single cells (less than 10% variance) with rare event detection (e.g., 2/10,000) is now possible due to recent advances in computerized microscope systems, increased computer power, and fluorescence probes with improved signal stability. This capability may now be used to enhance the clinical use of cellular biomarkers for single-cell genomic and proteomic analysis. However, retrospective studies on archival tissue samples are now possible because of new analytical approaches and the increased sensitivity of marker reagents. For example, the new Tyramide reagents facilitate quantitation of biomarkers in formalin-fixed tissue sections because of the increased amplification of the biomarker above background fluorescence associated with formalin fixation (e.g., variance 25%). The use of these reagents, coupled with precise instrument calibration, enhances the use of biomarkers for immediate analysis of archival clinical samples. Florence microscopes with reliable scanning stages, high-resolution image storage, and stable light sources or laser signals are now cost-effective platforms that further contribute to improved quantitative accuracy.

5. HIGH-LEVEL PHENOTYPIC BIOMARKERS

Cancer cells display a number of fundamental physiologic changes. These include increased cellular proliferation, dedifferentiation, decreased cell adhesion, increased cell motility, and the potential for invasion and metastasis, a feature frequently associated with genetic instability and tumor heterogeneity. Quantitative or qualitative alterations in macromolecules associated with one or more of these fundamental processes are likely to be strong biomarkers. Initially, biomarkers with a high specificity and sensitivity are identified in the primary neoplasm (1). To identify a strong marker, the marker is evaluated in 12-14 cancer cases and in an equal number of controls. If the marker is expressed in greater than 75% of the cancers and none of the controls, it can be considered a strong biomarker. Next, the assay method is evaluated and the reproducibility established under defined conditions. Once the assay is well established, a two-phase study is then carried out in 50 bladder cancer cases and 40 to 50 symptomatic and asymptomatic controls (3). Longitudinal monitoring of individuals with a previous history of cancer provides insight into whether the biomarker is expressed early or late during carcinogenesis (4). Next, the biomarker’s expression may be mapped in the cancer, the areas adjacent to the cancer, and the normal-appearing cells in an organ harboring the cancer. Rao et al followed Koss’s lead and mapped the biomarker expression in the malignant area, adjacent area, and the normal-appearing cells in the organ at risk in 30 patients with bladder cancer. The segregation of these biomarkers was then evaluated in a cluster analysis. In this case, G-actin and EGFR, DNA ploidy Her-2 neu, and cytology were grouped together (5). A variety of other approaches are available for selecting biomarkers, including the risk assessment strategy. In our experience, biomarkers expressed in the normal-appearing cells in the cancer field will generally be expressed early in carcinogenesis. These normal-appearing cells may be altered early in carcinogenesis or may be regulated by a secondary epigenetic signal from the primary neoplasm (6). Another approach is to use a risk stratification schema to determine if a marker is expressed early or late in carcinogenesis. If the percentage of individuals positive for a biomarker increases as the clinical risk increases, then the marker may be useful. This depends on the percentage of individuals with disease who are positive, compared to the true negatives. In general, biomarkers expressed in the more normal cells will be expressed early and will be more useful for risk assessment; however, due to their poorer specificity, they will be most useful for targeting chemoprevention. Later markers will have a higher specificity, and thus are more useful for cancer detection.
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Utilizing the combination of methods discussed above (4), we have studied a number of markers, including DNA ploidy, a tumor-associated antigen (p300), cytосkeletal markers G & F-actin, and a another very promising tumor-associated antigen (DD23) (5). A prime example in the case of bladder cancer is an alteration in the cytосkeletal proteins G-actin and F-actin or their ratio (7). Cellular changes in the G-actin/F-actin ratio distinguish between early premalignant changes and those associated with inflammatory conditions, characteristically a difficult dilemma for the pathologist. Subsequent studies have shown that alteration in the cytосkeletal is a general phenomena associated with carcinogenesis in number of epithelial malignancies (ref bladder, prostate, breast). While depolymerization of cytoplasmic actin heralds collapse of the cytосkeleton (dedifferentiation) genetically regulated by ras and rho, the change in the nuclear actin ratio heralds malignant transformation (8).

Genetic instability and tumor heterogeneity is another high-level phenotypic change and is the hallmark of most solid neoplasms, including bladder cancer (9). DNA ploidy may be detected by a number of different techniques, including aneuploidy, which is defined as abnormal DNA peaks assayed by flow cytometry or image analysis. However, image analysis has the advantage of high sensitivity and specificity among all possible cutoff points or more biomarkers should be selected to optimize the sensitivity and specificity between early premalignant changes and those associated with inflammatory conditions, characteristically a difficult dilemma for the pathologist. Subsequent studies have shown that alteration in the cytосkeletal is a general phenomena associated with carcinogenesis in number of epithelial malignancies (ref bladder, prostate, breast). While depolymerization of cytoplasmic actin heralds collapse of the cytосkeleton (dedifferentiation) genetically regulated by ras and rho, the change in the nuclear actin ratio heralds malignant transformation (8).

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6. A STATISTICAL FRAMEWORK FOR ANALYZING BIOMARKER PROFILES

The objective of defining a biomarker profile not only involves biomarker selection, but also the optimization of the sensitivity and specificity of the profile. Because of the low incidence of bladder cancer, the false positive rate of the profile must be low to avoid costly evaluation of patients without disease. However, this will come at the cost of low sensitivity. In the extreme case, a biomarker cutoff point that eliminates all normal controls found beyond the point will have 100% specificity (the percentage of patients whose biomarker was tested as negative in all non-cancer patients), but usually will also have a very bad sensitivity (the percentage of patients whose biomarker was tested as positive in all cancer patients). Conversely, a biomarker cutoff point that eliminates all cancer cases found below the point will have a 100% sensitivity, but usually will have a very bad specificity. However, optimizing the cutoff points for markers that are independent of each other in a profile may yield a higher sensitivity and specificity than a single marker.

To explain this concept, we present the mathematical and graphic logic of this approach. For one biomarker, a cutoff point will define the positive interval of the biomarker. Any patient whose biomarker value is greater than the cutoff point will be considered positive, while a patient whose biomarker value is less than or equal to the cutoff point will be considered negative. Several biomarkers’ profiles (cutoff points) may be combined in order to obtain better specificity and sensitivity than a single profile would have. Usually, the positive of two or more biomarkers was defined by a critical (positive) region. A patient is classified as positive (at risk of cancer) if the values of his/her measured biomarkers belong to the region, or as negative (at no risk of cancer) if not. However, a simple combination of cutoff points for two or more biomarkers may not reserve the original specificity or sensitivity of the single profile, and will vary upon different combinations. For example, taking only all biomarkers positive as positive (Figure 1) will produce the highest specificity among all single profiles, but will significantly reduce sensitivity, while to take either biomarkers positive as positive (Figure 2) will produce the highest sensitivity among all single profiles, but will significantly reduce specificity. Therefore, either a cutoff point for one biomarker or a combined critical (positive) region for two or more biomarkers should be selected to optimize the sensitivity and specificity among all possible cutoff points or all possible regions. Let $X_1$, $X_2$, and $X_3$ be three biomarkers, and $a$ and $b$ be the cutoff point of $X_1$ and $X_2$, (a patient has a positive $X_1$, if his/her $X_1>b$, a negative $X_1$, otherwise; a patient has a positive $X_2$, if his/her $X_2>a$, a negative $X_2$, otherwise), respectively. Figure 1 shows a critical region that takes both $X_1$ and $X_2$ positive as positive (that is, a patient is treated as positive only if he/she has
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both positive $X_1$ and $X_2$, in other words, if his/her $X_1>a$ and $X_2>b$, and Figure 2 shows a critical region that takes either $X_1$ or $X_2$ positive as positive (that is, a patient is treated as positive if he/she has either positive $X_1$ or $X_2$, in other words, if his/her $X_1>a$ or $X_2>b$). In general, a critical (positive) region $C$ for two biomarkers can be defined as $C = \{(X_i, X_j) : f(X_i, X_j) \in K\}$, where $f$ is a function of $X_i$ and $X_j$ and $K$ is a set of numbers. A patient is classified as positive if his/her $(X_i, X_j) \in C$, and is classified as negative otherwise. For example, a critical region for two biomarkers may be a two-dimensional region bounded by a line (Figure 3) (where $f = 2X_1 + 5X_2$ is a linear function of $X_1$ and $X_2$, $K = \{\text{any number} > 10\}$; and a patient is classified as positive if his/her $(X_1, X_2)$ has $2X_1 + 5X_2 > 10$ and negative otherwise), or by several lines (Figure 4), or by a curve (Figure 5). Similarly, a general form for a critical (positive) region $C$ for three biomarkers can be defined as $C = \{(X_1, X_2, X_3) : f(X_1, X_2, X_3) \in K\}$. For example, a critical region may be a three-dimensional region bounded by a plane (any point $(X_1, X_2, X_3)$ above the plane was considered positive) (Figure 6), or by a surface (any point $(X_1, X_2, X_3)$ above the surface was considered positive) (Figure 7). The methods of discriminate analysis and logistic regression analysis are usually used to find the cutoff point or bounds that maximize, in terms of both specificity and sensitivity, the identification ability of the given biomarker(s). That is, they are used to find the cutoff point of a biomarker or the boundary of two or more biomarkers that is the best among all possible cutoff points or bounds in separating cancer cases (to the positive region) from non-cancer controls (to the negative region). Readers who are interested in details are referred to respective statistical books and papers, e.g. Hand (10) and Wang (11). Usually, the cutoff points/critical regions for two or more biomarkers used for screening an asymptomatic population are different from those used for screening a symptomatic population that has a higher prevalence of the cancer. The cutoff points/critical regions for two or more biomarkers for cancer risk assessments are also different from those for cancer detection.

7. HIGH-RISK POPULATIONS AND BLADDER CANCER RISK ASSESSMENT

Occupationally exposed workers at risk for bladder cancer provide a living model of carcinogenesis. The latency period for bladder cancer is 15 to 20 years. This long latency provides an adequate time interval for detecting the molecular fingerprints driving carcinogenesis in the premalignant field prior to the onset of genetic instability. Rao et al demonstrated that there are premalignant molecular changes in normal-appearing cells in a bladder harboring cancer. Cells bearing molecular fingerprints are exfoliated in the urine, thus providing an opportunity to continuously monitor the molecular changes associated with carcinogenesis. The President Hubert Humphrey case confirms that in selected cases of bladder cancer, molecular changes in P53 were present in cytologic specimens prior to the primary neoplasm. However, until recently high-risk occupational groups or smokers have not been followed longitudinally to monitor biomarker expression. This approach could be adopted to confirm the concept that biomarkers of effect are a result of the endogenous and exogenous exposures and cancer genes, rather than the result of a pre-established neoplasm.
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Over the past two decades, we have studied several different cohorts at high risk, each of which has provided insight into our comprehension of biomarkers and the power of biomarker profiles for individual risk assessment, early detection, and diagnosis.

In 1982, under the auspices of NIOSH and the Workers’ Institute of Occupational Safety and Health, the first prospective government notification program was initiated to inform a group of workers known to be at high risk for bladder cancer. Workers in Augusta, Georgia, who had been exposed to beta-napthylamine and were at risk for bladder cancer, were notified and screened longitudinally. The DNA ploidy biomarker expressed as the 5C DNA ER, in combination with Papanicolaou cytology and hematuria testing, detected eight bladder cancers in this cohort. The study confirmed that DNA ploidy correlated with occupational exposure and represented a significant risk factor for the development of bladder cancer (10). There was also a correlation between smoking and DNA 5CER (clastogenic effect). Following the Augusta model, a second group of beta-napthylamine-exposed workers in Pennsylvania have been followed longitudinally for more than fourteen years. This community-based program, facilitated by the University of Pittsburgh, detected two cases of bladder cancer and several workers at risk, adding further credence to the biomarker approach and demonstrated the practicality of biomarker programs. The third high-risk group to be studied, a unique cohort of benzidine-exposed workers in China, represented a once-in-a-lifetime opportunity to monitor the premalignant changes in individuals at risk for bladder cancer. Participants for this study were based on a cohort study organized by the Chinese Academy of Preventive Medicine, a World Health Organization Center in Beijing. The program was sponsored jointly by NIOSH and the National Cancer Institute, and was initiated in 1982. The cohort study revealed an exceedingly high bladder cancer rate of in the exposed group (RR32.3) compared to the controls. Because this group had not yet reached its peak incidence rate in the late 1980s, we proposed to study this cohort to confirm our approach for selecting biomarkers based on mapping the biomarker expression in the cancer field. The six-year longitudinal study, which was recently published, confirmed that exposed workers can be stratified into different levels of risk based on a predefined biomarker profile. These groups then can be screened accordingly, and effectively monitored for early cancer detection.

The five-city Chinese study prospectively confirmed that biomarker changes occur in normal-appearing cells in the premalignant field years in advance of the primary neoplasm. Initially, exposed workers (n=1778) and age- and sex-matched controls (n=373) were divided into high-, moderate- and low-risk groups based on their biomarker profiles. The biomarkers selected for this study were those available in 1991, based on studies in symptomatic patients. They included DNA ploidy expressed as DNA 5C ER; a tumor-associated antigen, p300; and a cytoskeletal protein, G-actin. Thresholds for the biomarkers were established prior to the onset of the
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study based on ROC plots of the markers in symptomatic patients and asymptomatic controls. QFIA of the biomarkers detected as few as 2/10,000 cells positive for the p300 antigen. The optical resolution for this antigen detected by fluorescence is more sensitive than conventional immunohistochemistry. The markers were optimized by measuring cellular DNA, ploidy expressed as DNA 5C ER, detected by machine sensible parameters using QFIA. Image analysis detects rare event cells, resulting in a higher specificity and enhancing the clinical utility of the biomarker profile. However, if one marker was strongly positive and the other markers were negative, the test profile was considered positive for high risk. A test was also defined as positive if one the conventional markers (Papanicolaou or hematuria) were positive. Establishing rules for biomarker profile based on the mathematical considerations discussed above provides a paradigm for marker combinations.

Based on the biomarker profile, two markers positive or one marker strongly positive constituted the high-risk group. These subjects were screened every six months, and cystoscopy was recommended. Biomarkers were analyzed for risk assessment at six-month intervals or at the time of tumor detection. The results confirm the utility of the M344 antibody to the p300 antigen and DNA ploidy (5C DNA ER) for detecting bladder cancer. Individuals positive by M344 and DNA ploidy had a relative risk of 81, compared to workers with a negative test based on a COX logistic regression model. The overall sensitivity of the biomarkers was 87%, with a specificity of 73%. DNA ploidy was the most specific and sensitive marker for risk assessment and detection. Longitudinal screening detected 90% of the bladder cancers early in the course of the disease (only four of 30 cases were invasive cancer: T2/T3). Importantly, individuals in the moderate risk group were positive a mean of 33 months prior to cancer detection, while the high-risk group was positive a mean of 19 months compared to PAP cytology (9 months) and hematuria testing (3 months). Hematuria, the cardinal sign of bladder cancer, is usually positive late in the pathogenesis of the disease, because only then do the neovascularity and bleeding associated with tumor-releasing angiogenic factors occur.

8. INHERITED SUSCEPTIBILITY MARKERS AND EXPOSURE IN RELATION TO BIOMARKERS OF EFFECT

This study and other studies raise a provocative question regarding the power of genotypic polymorphisms for individual risk assessment in combination with exposure data: How can we best incorporate the momentous advances of the human genome project to assist us in individual risk assessment? Provided we can identify all the genetic polymorphisms relevant to a malignancy, we could analyze the power of susceptibility biomarkers in relation to exposures to determine individual risk. Biomarkers of effect may also be used in combination with susceptibility markers to evaluate the significance of exposures, instead of using clinical cancer as an endpoint. This approach may well reduce the sample sizes required to study the relevance of nutritional and environmental factors. Bladder cancer serves as a useful example to illustrate how to use biomarkers of effect, exposure, and susceptibility in combination. [Scandinavian studies support the view that there will be multiple genetic
polymorphism involved in bladder cancer, or there is variable gene penetrance, or multiple epigenetic factors because only approximately 1% of bladder cancers are strongly linked to inherited genetic factors. Thus, the other 99% require genetic polymorphisms and smoking (50%) or other exposures (i.e., occupational (25%), etc.) Several limited studies in lung cancer, a disease where smoking exposure can be approximated, suggest that it is possible to obtain OR's approaching six or seven for individual risk assessment. However, this approach also does not consider epigenetic effects and variable gene penetrance. Dr. Peter Greenwald, Chief of the Cancer Prevention Branch at the NCI, some years ago brought into focus the cancer incidence in twins as a potential model for estimating the power of genetics alone for individual risk assessment. An analysis of the bladder cancer in identical twins has not been reported, but other recent Scandinavian studies confirm a cancer rates ranging between 27-47% in twins. These twin studies and population family studies serve to formulate the limitations of genetic polymorphisms SNP analysis for genetic risk assessment. Based on this logic, combining biomarkers of susceptibility with biomarkers of exposure and/or effect predictably would be more powerful for individual risk assessment.

9. GENETIC POLYMORPHISMS FOR INDIVIDUAL RISK ASSESSMENT

To test the relevance of the genetic polymorphism to bladder cancer, in addition to evaluating biomarkers of effect in the premalignant field, a subset of workers with bladder cancer were compared to controls for genetic polymorphisms known to be associated with bladder cancer risk. Genetic polymorphisms included in the profile were phenotypic and genotypic markers for slow and fast acetylation NAT2 and the NAT1 genotype, as well as the GSTM1 genotype. Previous studies confirm workers exposed to a mixture of aromatic amines reveal that slow acetylation is generally associated with an increased risk for bladder cancer (OR~1.5-3.0). On the other hand, workers exposed only to benzidine experience a protective effect from the slow acetylation phenotype because of the metabolic inactivation of benzidine. Results of this study confirmed that rapid acetylation is a protective factor for benzidine (OR= 0.3, 95%CI 0.1-1.0). Comparing the results of this study, with a meta-analysis risk estimate of case-control studies of NAT2 acetylation and bladder cancer in Asian populations without occupational arylamine exposures, supports the existence of a gene environment interaction. (OR=0.3, 95%CI 0.10-1.0). (ref)

10. PERSPECTIVES

Studying the biomarker expression in the premalignant field and the cancer compared to normal tissues provides a useful paradigm for identifying biomarkers relevant to carcinogenesis. Biomarkers of effect are the result of the genetic polymorphisms and endogenous and exogenous exposures that are frequently difficult to quantify. Biomarkers of effect, when accurately quantitated in single cells, can be incorporated into a useful biomarker profile for individual risk assessment, cancer screening, and detection. Combining biomarkers of effect with defined genetic polymorphisms may further enhance individual risk assessment. Future challenges to incorporating this information into new models for individual risk assessment include variable genetic penetrance, epigenetic factors, and the inability to precisely quantitate endogenous and exogenous exposures. In the interim, the powerful approach of using high-level phenotypic biomarkers of effect remains a tool for predicting individuals at risk for cancer years in advance of clinically manifest disease. These individuals can be stratified for more intensive surveillance. This may serve to target individuals as candidates for chemoprevention trials, and may be useful for monitoring and designing new cancer prevention strategies.

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12. REFERENCES


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