Recent Molecular Advances in the Approach to Early Lung Cancer Detection and Intervention

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Lung cancer is a major contributor to overall cancer mortality. Detecting lung cancer while it is still a localized process is a long-cherished goal for improving the outcome of this disease. Recent developments suggest that we are approaching this capability. We next have to think about how to implement a change in our approach to lung cancer management to derive the benefit of better detection capability. This is an area in which our growing understanding of lung cancer biology is providing clues on improving the inhibition of cancer progression. — Environ Health Perspect 105(Suppl 4):935–939 (1997)

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Lung cancer is the most frequently fatal malignancy in much of the developed world and is the leading cause of cancer death for both genders in the United States (1). Significant progress in reducing lung cancer has not occurred despite decades of sustained intensive clinical research efforts. This legacy has forced a reconsideration of fundamental strategies by which to approach this cancer (2). Primary prevention efforts to reduce the number of children beginning to smoke and measures to encourage current smokers to stop smoking are the most efficient approaches to deal with lung cancer. Unfortunately, current and former smokers all have an increased risk of developing lung cancer compared to those who have never smoked. So for the over 90 million current and former smokers in the United States alone, more than primary prevention efforts are required. For the last 20 years there has been only a minimal research effort to develop better approaches to early lung cancer detection.

Recent developments with molecular diagnostic techniques to elucidate the preclinical phase of this disease provide a basis for optimism in significantly improving the early detection of lung cancer. Systematic evaluation of the process of developing an early detection approach is useful in highlighting the critical issues that must be resolved in the evolution of population-based cancer management.

General Considerations

Lung cancer is the result of chronic carcinogen exposure to a range of normal respiratory epithelial cell populations. The many histologies of lung cancer reflect this complicated biology (3). Currently, the precise sequence of genetic events leading to the development of an invasive cancer is speculative. We know that a variety of carcinogens can interact with any of the normal cell populations found in the bronchial epithelium. Depending on the carcinogen exposure and the host’s ability to handle xenobiotic injury, the natural history of the cancer will vary. Effective strategies for early lung cancer detection must reconcile these realities. Another aspect of this challenge is that the probability of developing a lung cancer in the general population is typically less than 0.001%. Defining populations with higher risks of developing lung cancer improves the prospects for successful application of the diagnostic and simplifies clinical trials to validate the effectiveness of early detection tools. Using what we know today about defining high-risk populations, we can only improve the a priori chance of developing cancer by an order of magnitude. The downside of using higher risk populations for marker validation is that such populations are different from the actual screening situation that would be encountered in the general population. Concentrating on high risk populations also implies accepting that some cancers occurring in low-risk populations may not be detected. As with any other public health effort, the strategy for evaluating for lung cancer must be validated carefully. A priority for screening approaches is to ensure that sensitivity of the process is high while the cost of screening is reasonable. Currently, these are complicated calculations. Beyond the issue of direct assay costs, the direct and indirect cost of the clinical consequences associated with false negative and false positive assay determinations is significant. In developing population screening approaches for cancer detection, it is critical to educate the general public about the goals and nature of this type of research. During the early stages in the development of early cancer detection technology there will be problems. Examples of this are currently evident with prostate specific antigen screening for prostate cancer, where the risk/benefit aspects of this early detection effort are controversial (4). Society’s ultimate benefit from comprehensive early cancer detection results will only be achieved through systematic refinement of this and related technologies over many years.

How to Screen Relevant Epithelial Samples for Early Lung Cancer

Inherent to an early lung cancer detection approach is the requirement to identify the disease before the development of metastatic disease. Because metastatic disease is not curable, detection at this stage would only lead to at best a lead-time bias and not a real improvement in cancer-related survival. To detect the process in the early stage, it is logical to look at the process initiation site—the respiratory epithelium. This site can be directly sampled with fiberoptic bronchoscopy or with a sputum specimen. Finding evidence of intrabronchial cancer with bronchial-derived specimens does not exclude the presence of extrathoracic metastatic involvement. In theory, the respiratory compartment would have
evidence of cancer activity for many years before the development of metastatic disease. To avoid detection of advanced-stage lung cancer, the search for the early stage of lung cancer must start years before the study population approaches the peak age of incidence for the disease. Because chronic smoking can cause irreversible genetic damage to epithelial cells, the risk of manifesting a cancer is lifelong. The appropriate frequency of serial evaluation in high-risk populations must be formally defined with clinical studies.

**What Markers to Use**

The dominant question regarding early lung cancer detection is what marker or combination of markers should be used to detect all lung cancer. To facilitate this marker selection process, we adapted the functional classification of the lung into three regions. These groups are based on the shared biology of the proximal, mid-, and distal lung (5). For example, the proximal lung shares the biological response to injury of squamous metaplasia. This type of epithelium has a range of defined differentiation markers including morphology, cross-linked envelope formation, and particular cytokeratins. We have previously proposed lists of candidate makers for the pulmonary compartments as well. A number of critical questions exist in optimizing early lung cancer detection, including how to select the most informative of the possible biologic markers and how to determine the number of markers to be used. In our experience, the most relevant gold standard is "what works." We previously published studies on the use of archival sputum specimens derived from an already concluded clinical trial in which eventual lung cancer status was known. In a nonconcurrent, prospective, blinded analysis, we reported that two immunocytochemical markers showed a 91% sensitivity, 88% specificity in determining who would or would not develop lung cancer (6). This analysis was performed on serial sputum specimens obtained annually during the course of a chest X-ray and conventional sputum cytology study. From the known clinical follow-up information, it was established that the 90% accuracy (80% positive predictive value and 94% negative predictive value in the 62 evaluable cases) in predicting lung cancer status was achieved on average 20 months prior to routine clinical detection. Presumably the two monoclonal antibodies are detecting cellular targets that reflect ongoing carcinogenesis.

We recently mapped the expression of the more useful of the two immunocytochemical markers (703D4). We used lung tissues from surgical blocks in which we had determined the morphology in the nonneoplastic epithelial compartments surrounding a resected primary lung cancer (7). This is an exercise in studying field carcinogenesis biology. The pattern of antigen expression in tissues that have had chronic carcinogen exposure may define cellular populations of particular importance in identifying other useful early detection markers. We are keeping the conserved elements of epithelial biology as a central focus for early lung cancer detection approaches.

Using this same strategy and also the same sputum archival specimens from the Johns Hopkins studies (6), investigators from Johns Hopkins reported that determination of a ras mutation in sputa identified individuals who would go on to develop lung cancer (8). Other trials with this strategy are in progress and potentially will provide new clinical/pathologic archival resources to define the true utility of additional markers (9).

**Selecting a Marker for Clinical Early Detection Validation**

A major developmental requirement for a proposed early detection tool is to establish a robust, sensitive, reproducible, efficient assay format (10). We have worked to standardize the handling of bronchial lavage fluids to permit specimen conservation. The goal is to perform a range of assays on small samples of bronchial epithelial cells (11). Early in the process of marker selection, the precision of a proposed biomarker assay must be optimized and standardized. Due to the inherent complexity of detecting all the biology of the different forms of lung cancer, it may be necessary to use a panel of markers. This will provide more information in determining the status of the bronchial epithelium undergoing the carcinogenesis. Rather than relying exclusively on the pattern of expression of a single marker, therefore, the pattern of expression of a battery of markers would be evaluated. Representative component markers could include markers of genetic damage such as particular microsatellite probes, markers of cell proliferation, and expression of growth factors or their receptors. Validation studies of particular biomarker panels with relevant archival material from individuals with known clinical outcomes could establish with defined statistical precision which markers are most informative as early detection probes.

**Technological Developments Relevant to Population-based Screening**

In the current cost-sensitive environment, an efficient and economical assay is required. Over the next decade, progress in computerization and microchip-based technology may revise current concepts of population-based screening. These experimental assay formats for gene probes may enable the throughput of large assay numbers. This technology will enable validating pilot trials to determine which assays provide the kind of clinically useful information that would justify population-based application. These assays will be multiplexed so that a number of probes will be analyzed simultaneously, providing sufficient biological information to sort through the complicated biological issues already discussed. Recent developments with microchip-based assays suggest that this technology will be a more economical assay platform on which to accomplish molecular analysis of many clinical specimens. Refinements in several technologies have contributed to this possibility. The use of microjet technology accomplishes the precise, reproducible deposition of target nucleotide probes on a test matrix. Nucleotide probes that have been formulated to orient optimally for hybridization are sprayed on a silicon surface. Using microelectronic circuitry, surface charges are modified to allow reagent exposure, probe hybridization, and disruption of low affinity binding interactions (12,13). A variety of biotechnology companies have pioneered different approaches to this technology (14).

Another enabling aspect of this technology is an efficient detection system. Collaborators from the Genosensor Consortium developed an integrated charge-coupled detector (12). This consortium included members from industry and academe with support from the Advanced Technology Program (Gaithersburg, MD) and the National Center for Human Genome Research (Bethesda, MD). A charge-coupled device has been developed that detects photons or radioactive decay products at pixel locations where the sample has bound to complementary probes. The number of electrons is calibrated to be proportional to the number of molecular
binding events in that area so that quantitative information can be obtained. Because of silicon's sensitivity to electromagnetic fields, this matrix material is a favorable medium. The abundance of silicon means that the cost of using this material as a matrix is slight, which is an essential design consideration for population-based screening applications.

The enthusiasm for this molecular diagnostic platform is growing but this new family of molecular diagnostics must be carefully validated. First, the specificity and reproducibility of this approach must be established in model systems. Next, the level of sensitivity must be optimized to ensure signal detection in clinically relevant specimens. The current generation of biosensor assays may not include polymerase chain reaction amplification steps. Whether informative markers can be monitored within the existing range of detection with direct hybridization remains to be established. The necessity of a signal amplification step would significantly increase the complexity and the cost of these procedures. Therefore, all the specimen-handling procedures must be optimized to prevent clinical specimen loss. At least initially in assay development, the focus must be on abundantly expressed target molecules. Innovative strategies to recover epithelial cells from clinical specimens are being considered to increase the information obtained by these techniques.

Ultimately, the information provided by these automated, robotically driven assays will include many molecular probes analyzed on serial clinical specimens obtained during large clinical trials. Because this technology evaluates multiple genetic markers at once, systematic clinical investigation will be required to discern the most informative markers for inclusion in a detection panel. The potential information load generated with this type of technology will soon be overwhelming. Developing rigorous epidemiological and biometric approaches for optimal management and retrieval of information contained in such data sets will be instrumental in revealing directions for continuing improvement in this technology.

**Rational Interventions for Early Lung Cancer**

Assuming that the feasibility of population-based screening continues to improve, the critical need then becomes to complement this positive diagnostic development. Effective epithelial-directed interventions that arrest the early phase of lung cancer must be defined. Fortunately, the study of cancer chemoprevention is rapidly developing (15). Recent studies of chemoprevention using β-carotene have pointed out the complexity of this approach. β-Carotene is a precursor of vitamin A and it was assumed that the oral supplementation of this compound would lead to a replacement of deficient vitamin A stores and potentially reduce cancer frequency (16–18). These β-carotene chemoprevention trials have not shown any benefit and raise the question of whether they may cause harm. An unexpected frequency of lung cancer occurred in two of the three intervention trials for the group of subjects receiving β-carotene. This experience suggests the importance of understanding the underlying biology of the intervention. The biosynthesis of vitamin A can be impaired under certain circumstances, as with heavy alcohol ingestion (19). Whether random events or an unanticipated factor, such as an imbalance in the accrual of subjects with high alcohol consumption, contributed to the unexpected finding may never be absolutely determined. However, the preclinical information about retinoid chemoprevention is much more extensive than was the rationale for the use of β-carotene. In contrast to the β-carotene experience, retinoids have been shown to significantly reduce the frequency of second primary cancers of the upper aerodigestive tract, including lung cancer (15,20). The frequency of side effects with 13-cis-retinoic acid as a chemoprevention agent—including headache, mouth sores, itchiness, and hyperlipidemia—has reduced enthusiasm for this experimental approach. We recently reported that 13-cis-retinoic acid binds to albumin and is significantly less bioactive in that state (21). In another recently reported study, French investigators found that intracellular levels of all-trans retinoic acid best correlate with patients in complete remission of their acute promyelocytic leukemia (22). This was supported in part by the finding that the leukemic cells from patients clinically resistant to trans retinoic acid were still sensitive to low dose all-trans retinoic acid in vitro. From this experience, they also conclude that bioavailability of retinoic acid to target cell populations is a major barrier to therapeutic benefit. To optimize the availability of retinoic acid to the transformed bronchial epithelial cells, an aerosolized drug delivery approach may be required. This direct epithelial delivery would not only increase the bioavailability of the retinoic acid to the target bronchial epithelial cells but also would decrease the potential for systemic toxicity. We propose to evaluate this approach clinically.

Another intervention approach that arises from consideration of the tumor biology of lung cancer involves arachidonic acid metabolism. This pathway may be involved in the signal transduction of lung cancer growth factors. We have identified the enzyme 5-lipoxygenase as playing a key role in cell signaling because this pathway appears to be activated by two important lung cancer-associated autocrine growth factors (23). Insulin-like growth factor-I (IGF-I) and gastrin releasing peptide both cause prompt elevation in the production of a specific 5-lipoxygenase dependent cell product, 5-HETE. We found low concentrations of 5-HETE stimulate the growth of lung cancer cell lines in serum-free media. Using several inhibitors that block the lipoxygenase pathway through different mechanisms, a consistent growth reduction is observed for most lung cancer cell lines. In both in vitro and in vivo conditions, the exposure of the lipoxygenase inhibitors to the lung cancer cell lines results in an increase of apoptotic cells.

We propose the following model to explain these findings: The role of autocrine growth factors in lung biology may be to function as promotion factors. We postulated that chemoprevention strategies based on neutralizing the effects of autocrine growth factors could be valuable in arresting tumor promotion (24). For this reason, we are evaluating the use of lipoxygenase inhibitors as an intervention strategy to control early cancer progression. This approach is summarized in Figure 1. If we think about carcinogenesis as a dysregulation of normal cellular homeostasis,
we see that the malignant cell has devised a way to circumvent growth regulation. Such a shunt may be sustained through the action of constitutive activation of an IGF-I receptor circuit, as suggested by Baserga (25). The action of the lipoxigenase inhibitor eliminates the shunt and permits the reestablishment of normal cellular regulation, leading to normal differentiated functions. The most important normal cellular pathway that is reexpressed after lipoxigenase inhibition may be apoptotic cell regulation. The most important role of a tumor-promotion factor such as the activators of the IGF receptor may be to protect the clonally expanding early cancer from apoptotic clearance. New information is rapidly expanding our appreciation of the importance of the control of cell division relative to the process of cancer (26). Understanding cell cycle regulation has implications for both early detection and intervention research. A new paradigm for translational approaches to lung cancer intervention is summarized in Figure 2. If we can identify, at an early stage in carcinogenesis, critical tumor promotion factors that thwart normal cell-cycle regulation, neutralizing the stimulatory effect of those factors would potentially allow the regulatory effect of apoptosis to be reestablished. Candidate agents to be evaluated for this type of approach include inhibitors of cyclooxygenase protein kinase C and other critical signal-transduction molecules. This strategy forms a blueprint for the future. Coordinated research involving molecular detection of early epithelial phase carcinogenesis with the use of defined molecular antagonists that upregulate apoptosis is emerging as a promising strategy to improve lung cancer mortality.

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