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Candidate Biomarkers for Application as Intermediate End Points of Lung Carcinogenesis

James L. Mulshine, R. Ilona Linnoila, Anthony M. Treston, Frank M. Scott, Kathryn Quinn, Ingalill Avis, Gail L. Shaw, Sandra M. Jensen, Powell Brown, Michael J. Birrer and Frank Cuttitta

Biomarkers and Prevention Research Branch, Division of Cancer Prevention and Control, National Cancer Institute, Bethesda, Maryland 20889

Abstract The need for validated intermediate end point markers to facilitate lung cancer chemointervention research is compelling. Three major classes of lung markers are relevant for this application. Since lung cancer includes four distinct histologies, markers that map degrees of histologic differentiation are important. Many of the markers for squamous differentiation overlap with the candidates for application in the study of head and neck cancer. Production of tissue-specific cell products especially for surfactant or CEA is of interest, because the gene structure is known and many differentiation-related polymorphisms exist. This strategy would be useful for adenomatous type tissue. A second type of marker is the broad group of differentiation markers. The carbohydrate or blood group-like antigens comprise a representative example. Carbohydrate structures are expressed in a specific sequence during fetal processes, and this sequence appears to reverse with the development of a cancer. Retrodifferentiation of specific differentiation markers is the basis of a major effort to effect earlier lung cancer detection using sputum immunocytochemistry. The final class includes markers which affect either positive or negative aspects of growth. Candidates in this area include growth factors or their receptors, or genes that regulate growth. If the intermediate end point marker reflects tumor biology and that biology is in the causal path of tumor progression, serial observation of that parameter should indicate the success of the intervention. In all three of these examples, the clinical material to be analyzed could be sputum specimens, bronchial biopsies or resected lung tissue. Systematic analysis of these markers in context of intervention trials is required to validate their utility. Long term clinical follow-up will demonstrate the degree of concordance between biomarkers and more traditional clinical trial end points and will establish if such tools can play a role in catalyzing the rate of prevention research.

Key words: carcinogenesis, chemoprevention, intermediate end point, biomarkers, differentiation, growth factors, lung cancer

Lung cancer occurs most commonly in individuals who were chronic smokers. The interval between initial cigarette exposure and the development of lung cancer is typically on the order of 10–20 years. During this time, the focus of lung carcinogenesis is the bronchial epithelium, which is the interface between the substance of the lung and the inhaled cigarette smoke. Although the precise details are unclear, it is generally accepted that cigarette smoke exposure leads to genetic injury. Eventually, this exposure results in transformed clones of bronchial epithelial cells that may evolve the competence for tissue invasion and metastatic growth. Typically with chronic smokers, the entire upper aerodigestive epithelium is bathed by carcinogen. The appearance of independent primary cancers arising in the head and neck region, esophagus and lung are considered to be a manifestation of a field of cancerization [1]. Throughout that upper aerodigestive epithelium, the tissues affected by tobacco exposure may be focally involved in various stages of carcinogenesis. Dr. Michael Sporn has identified this field of carcinogenesis, and not overt cancer, as the appropriate focus for oncology research [2]. He points out that waiting for cancer to clinically manifest usually results in a lethal outcome for the patient despite the prompt application of the best medical care. Yet strategies to treat carcinogenesis to effect better cancer control have not been defined. A significant aspect of this problem is to define what are

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the parameters that define a field of cancerization, to identify which subjects would require an intervention and whether markers that reflect those same parameters could be serially monitored to determine the success of intervention therapy.

Based on the recent work of the M.D. Anderson group using retinoids as an intervention approach for patients with previously treated head and neck cancer, the enthusiasm for intervention approaches to cancer control has intensified [3]. The rationale for the success of retinoids as intervention tools is based on their ability to drive certain transformed cells to differentiate. A class of intervention approaches could be derived by attempting to systematically neutralize the biochemical processes thought to underlie the processes central to carcinogenesis.

Tumor promotion has been identified as the most reasonable target for the development of new intervention approaches [4]. An example for this approach would be to consider tumor growth factors as a class of promotion factors. A model for this approach is provided by the pulmonary autocrine growth factor, gastrin releasing peptide (GRP) [5]. This molecule is a critical factor in fetal lung development, is elevated in the airways of smokers and is frequently overexpressed in small cell lung cancer cells. Therefore, GRP in lung cancer may play a role similar to estrogen in breast cancer progression. We have used a monoclonal antibody to neutralize the effects of GRP in a clinical trial with advanced small cell lung cancer patients [6]. The absence of clinical toxicity, coupled with preliminary data suggesting the success of the antibody in neutralizing the GRP effect, encourage the consideration of the anti-GRP monoclonal antibody as a potential lung cancer intervention agent. Many other molecules produced by lung cancer cells can also mediate autocrine growth effects including epidermal growth factor (EGF), insulin-like growth factor I (IGF-I) and a transferrin-like molecule [7]. The interactions of each of these molecules alone and in combinations may be of critical importance in promoting lung carcinogenesis [5,8].

Other still undefined growth factors may also be involved in the process of cancer promotion. These facts suggest a formidable task for cancer intervention research. It is apparent that a large number of trials would need to be performed, and the most relevant outcome in these types of trials would be to identify chemointervention approaches that mediate a reduction in the number of subjects eventually succumbing to lung cancer death. Such trials are complicated because of the size and study duration required to achieve statistically valid determinations. Faced with the logistical challenges presented by intervention research, investigators have proposed the use of intermediate end point markers as surrogates to the definitive end point of mortality [8]. Historically, a number of markers such as micronuclei number or proliferation index have been proposed for this application. In the specific circumstance of lung cancer where a considerable understanding of its pathogenesis exists, deliberate evaluation of specific types of markers to define which will function most robustly as an intermediate end point marker appears possible.

DEFINING THE LUNG CANCER FIELD

Prior to focusing on a unitary panel of "lung intermediate end point markers," the existence of multiple tissue types in the lung must be reconciled. In the normal lung, the central airways share the epidermal epithelium, which comprises the proximal aerodigestive epithelium. The central airways also contain elements of neuroendocrine differentiation. The neuroendocrine elements are often found at sites of central airway bifurcation. The cell population changes to a more adenomatous type with transition to characteristic papillary cell types in the terminal alveoli. Although there are clear gradients, the dominant histologies of squamous, small cell, large cell and adenocarcinoma reflect the transformed correlates of the normal bronchial architecture [10].

In considering the use of biomarkers which represent all aspects of the biology of lung carcinogenesis, a complex range of options needs to be considered. Our group has proposed to organize consideration of potential biomarkers in the context of tissue specific differentiation as outlined in Table 1. For

TABLE I. Markers of Lung Cancer Differentiation

Neuroendocrine	Squamous
Chromogranin A	Cytokeratin
Leu 7	Involucrin
Neuron-Specific Enolase	Epidermal Growth Factor Receptor
Dopa Decarboxylase	Transglutaminase

Adenomatous
Clara 10-kilodalton Protein
Surfactant Associated Peptide
CEA
ras Oncogene

instance, during squamous carcinogenesis, the histology of the epithelium may demonstrate areas of squamous metaplasia. In these areas, focal elevations of transglutaminase activity and EGF receptor expression may be observed. The goal of biomarker research would be to define levels of transglutaminase or EGF (possibly sustained over a defined interval, i.e., 2 months) which would be indicative of cancer field dynamics. During intervention trials, serial analysis of critical parameters such as EGF receptor status (or EGF peptide) could indicate the success of a particular intervention in neutralizing the field carcinogenesis [5,7].

We have previously discussed the role of GRP as an element of neuroendocrine differentiation which is frequently expressed in small cell lung cancer cells. Neuroendocrine elements are thought to be central to fetal organogenesis as well as wound repair. Neuroendocrine cells produce a number of vasoactive products which can recruit appropriate effector cells into the area to enable regeneration of normal cellular architecture. As already discussed, the potential for autocrine effects of neuroendocrine products suggests a fundamental role in cancer field dynamics. If neuroendocrine hyperplasia is found to be an integral part of cancer field promotion, neuroendocrine elements may emerge as the most important class of surrogate markers.

Despite some overlap, the adenomatous differentiation breaks down into two biologically distinct groups. The adenomatous epithelium of the small caliber airway is distinct from the differentiated cell population of the terminal alveoli. The terminal alveoli includes Type II pneumocytes which produce specialized products including surfactant [11]. Another important cell population of the terminal alveoli is the Clara cell which is involved in xenobiotic metabolism. These cells produce the distinct 10-kilodalton protein (CLR-10) which can be used to define that type of differentiation. These cells are felt to be the progenitor populations for the development of a particular subset of adenocarcinomas called bronchioalveolar carcinomas.

The final major histology is large cell cancer. This histology is a diagnosis by exclusion since it lacks any of the differentiated properties of the other three histologies.

In using biomarkers either for early detection or for intermediate end point application, a precise understanding of the sequence of expression of the differentiated products is critical to discern the state of the epithelium relative to the process of carcinogenesis. From consideration of carbohydrate antigen expression on cancer cells, Hakomori contends that tumor cells have similar patterns of antigen display to fetal cells [12]. Based on Hakomori's work, it is postulated that cancer is a process of retroversion of fetal development. The early tumor cells would differ only slightly from a normal phenotype but the advanced cancer cells would be expected to display a considerably more primitive, fetal antigenic pattern.

In considering such biologically relevant markers for application as surrogate markers for intervention trials, more research is required to establish which class of differentiation antigen is most useful as an intermediate end point marker [13]. Ultimately, lung specific markers may complement the use of generic intermediate end point markers (such as a proliferation marker) to give a very accurate measure as to the state of carcinogenesis proceeding on the bronchial epithelium. If this application of biomarkers can be eventually validated, the pace of cancer intervention research can be greatly accelerated.

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