Journal of Cellular Biochemistry, Supplement 16G:33-38 (1992)

Intermediate Biomarkers in Upper Aerodigestive Tract and Lung Chemoprevention Trials

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Abstract Chemoprevention trials in lung and upper aerodigestive tract (UADT) cancer are guided by the field cancerization hypothesis. Inhaled carcinogens place the entire epithelial lining at risk for the development of cancer. The hypothesis is supported by the occurrence of premalignant lesions, such as leukoplakia or squamous metaplasia, and multiple primary tumors within the field. The concept of carcinogenesis as a multistep process suggests the possibility of blocking or reversing the progression to invasive cancer with systemic treatment. A series of ongoing clinical trials will determine the efficacy of retinoid chemoprevention and will attempt to develop intermediate biomarkers. Biomarkers which reliably reflect progression towards cancer could be used to dramatically improve the efficiency of chemoprevention trials and also would aid in screening potential chemoprevention agents. Genomic biomarkers include non-specific estimates of ongoing DNA injury, such as micronuclei, as well as development of aneuploidy and alterations in oncogenes. A class of biomarkers of increasing importance assess proliferation and growth regulation, and include proliferating cell nuclear antigen (PCNA), TGF-β, EGFR and retinoid receptors. Other markers, such as the blood group antigens, reflect differentiation and may be associated with the development of premalignant lesions. Preliminary data from several of these markers has suggested an association with carcinogenic exposures and premalignant lesions, but none of these markers either alone or in panels have yet been validated as a reliable surrogate for the development of invasive cancer.

Key words: chemoprevention, field cancerization, intermediate biomarker, premalignant lesions, upper aerodigestive tract cancer

Understanding that carcinogenesis is a multistep process raises the possibility that individuals may be identified who are at increased risk for the development of cancer. Intervention studies have often targeted high risk patient populations. Intense interest has developed in the possibility that carcinogenesis may be blocked or reversed by the systemic administration of drugs, prior to the development of an invasive cancer [1]. There are many ongoing chemoprevention studies attempting to determine if natural agents such as retinol or beta-carotene, or synthetic drugs such as the retinoid 13-*cis*-retinoic acid may be used to prevent the development of the cancer [2]. Completion of these trials has been hampered by the necessity of relying on cancer incidence as the study endpoint [3].The time from administration of the chemopreventive agent until the development of a clinically apparent tumor may be several years. In addition, while information is gained for the population concerning the effects of the chemopreventive agent, less is known about the impact of the drug on individual patients. Even in a high risk population, only a minority of patients would ultimately develop cancer if left untreated.

A critical aspect in the development of future chemoprevention efforts therefore, is the development and validation of reliable intermediate markers of carcinogenesis [4]. If such markers could be developed, they would be extremely useful in identifying the patients most likely to benefit from chemoprevention. In addition, it would be possible to assess the impact of the chemopreventive agent in a wider group of

Supported in part by grants (CA-48369, CA-18019) from the National Cancer Institute

patients than the individuals who ultimately develop a cancer. Biomarkers would improve the feasibility of performing chemoprevention trials by serving as intermediate endpoints for these studies. At the present time, none of these markers have as yet been validated as a reliable surrogate for the development of cancer. There are however, a number of candidate biomarkers which are currently being evaluated.

FIELD CANCERIZATION

The development of chemoprevention trials in the lung and upper aerodigestive tract (UADT) have been guided by the field cancerization hypothesis. Slaughter et al. first coined this term to describe the diffuse mucosal changes observed in specimens resected from patients with squamous cell cancer of the head and neck [5]. Examination of 783 specimens demonstrated the presence of multi-focal tumors in 11.2%. In every case, epithelial changes including hyperplasia, hyperkeratinization, dyskaryosis and marked atypism were observed. The field cancerization hypothesis suggests that inhaled carcinogens, such as tobacco smoke, damage the entire epithelial lining of the UADT and lung. A condemned mucosa develops in which both changes resulting from carcinogen exposure and an increased risk of cancer may be observed.

The concept of field cancerization is supported by the observation of premalignant lesions in the carcinogen-exposed field [6]. A premalignant lesion which occurs in the oral cavity is leukoplakia. Leukoplakia has been defined as the presence of "a white patch or plaque that cannot be characterized clinically or pathologically as any other disease"[7]. Despite this nonspecific definition, careful follow-up of leukoplakia patients has demonstrated the development of oral squamous cell cancers in 17.5% of 257 patients followed for a mean of 7.2 years [8]. Among the patients with both leukoplakia and dysplasia, 36.4% developed oral cancer during the follow-up period.

Premalignant lesions also develop in the lung in response to carcinogen exposure. Heavy tobacco smokers frequently develop squamous metaplasia of the bronchial epithelium [9]. Squamous metaplasia has also been described in association with the invasive lung cancers [10]. Dysplasia is not necessary for the diagnosis of leukoplakia or squamous metaplasia, but when present may identify a patient population at increased risk for the development of cancer.

The notion of field cancerization is also supported by the frequent occurrence of multiple primary tumors. There is a significant risk for patients who have had an initial squamous cell cancer of the head and neck to develop a second primary tumor (SPT) [11]. SPTs develop primarily within the exposed field, occurring most commonly in the head and neck, lung or esophagus [12]. The lifetime risk of a second primary tumor, following squamous cell cancer of the head and neck, is between 20% and 40% [13]. SPT's are also observed following resection of primary lung cancer, both small cell and non-small cell types [14,15]. The exact rate of SPTs following resection of early stage non-small cell lung cancers is less certain but appears to be between 10% and 20% [16].

Patients with premalignant lesions or a history of a previous primary tumor are ideal candidates for chemoprevention trials. In conjunction with these clinical trials, efforts are being made to determine the biology of field cancerization, in part through biomarker studies.

CANDIDATE BIOMARKERS

In the lung and UADT chemoprevention trials, several candidate biomarkers are under evaluation (Table I). Potential intermediate endpoints have been categorized as genomic markers, proliferation and growth regulation markers, or differentiation markers.

GENOMIC MARKERS

The most extensively studied biomarker in lung and UADT cancer is micronuclei frequency. The ability to quantitatively assess micronuclei counts in exfoliated cells has led researchers to evaluate this marker in a number of chemoprevention studies [17-19]. Micronuclei have been most commonly measured in oral leukoplakia studies, but more recently have also been included in studies of squamous metaplasia of the bronchial epithelium.

Micronuclei are extranuclear fragments of DNA formed as the result of clastogenic exposure during division [20]. Micronuclei represent a nonspecific but quantifiable assessment of the

TABLE I. Biomarkers in Current Lung and UADT Chemoprevention Trials.

Genomic Markers
Micronuclei
DNA content
Genetic Alterations (oncogene expression)
Proliferation and Growth Regulation Markers
Proliferating Cell Nuclear Antigen (PCNA)
TGF - B
Epidermal Growth Factor Receptor (EGFR)
Retinoic Acid Receptors (RARs)
Differential Markers
Squamous Differentiation: transglutaminase, involucrin and keratins
Blood Group Antigen

extent of ongoing DNA injury. Micronuclei counts are expected to change as the extent of carcinogenic exposure varies. Stich and Rosen have described the changes in micronuclei counts associated with exposure to tobacco and among beetle nut chewers [21]. They have clearly shown elevation of micronuclei counts among individuals with these exposures and the tendency for the highest micronuclei counts to be observed in areas where the tobacco quids are held [22]. Lippman et al. demonstrated similar findings in carinal mucosa, with active smokers shown to have higher micronuclei counts than individuals who never smoked [23].

In patients with leukoplakia, both the lesions and micronuclei counts have been reported to decrease with the administration of chemoprevention agents. Beta-carotene, Vitamin A and 13-*cis*-retinoic acid have all been associated with a reduction in micronuclei counts among oral leukoplakia patients. Reduction in micronuclei counts has not, however, completely reflected the clinical pattern of these lesions. An ongoing study of squamous metaplasia among heavy cigarette smokers will assess the response of micronuclei counts to administration of 13-*cis*-retinoic acid. Interim analysis of this study of lung squamous metaplasia has suggested wide intra-individual variation in micronuclei frequency from the six simultaneously obtained biopsies.

Attempts to validate micronuclei counts as a predictor of the development of invasive cancer have not yet been successful. A large chemoprevention trial performed in China demonstrated a reduction in esophageal micronuclei counts with the administration of retinol, riboflavin and zinc, but no histologic improvement was observed [24].

Another genomic marker is based on the measurement of DNA content. This measurement may be performed using flow cytometry. It has been hypothesized that abnormalities in DNA content may precede the development of invasive cancer. More specific probes of DNA content are also under development. The techniques of premature chromosome condensation and *in situ* hybridization using chromosome specific probes are employed to determine if aneuploidy is present [25,26]. These techniques may be quite helpful in determining if abnormalities in specific chromosome counts are associated with field cancerization.

The most specific genomic markers assess changes in gene products associated with carcinogenesis. The products of oncogenes c-erb-B1, ras, and myc have all been reported to be elevated in head and neck tumor specimens [27,28]. Similarly, abnormalities in the oncogenes or in the expression in the products have been observed in lung cancer lines. Abnormalities affecting ras, myc, and p-53 have all been reported for lung cancer [29,30].

PROLIFERATION AND GROWTH REGULATION MARKERS

Abnormalities in proliferation and growth regulation are thought to be central to carcinogenesis. The proliferating cell nuclear antigen (PCNA) is one potential marker of this process [31]. Specimens obtained during resection of non-small cell lung cancers have demonstrated a greater proportion of PCNA-positive cells in areas containing metaplasia, dysplasia or carcinoma *in situ* than in normal epithelium. PCNA expression is also increased within the cancer, especially for squamous cell histology [32].

PCNA expression is also being assessed in an ongoing retinoid chemoprevention study with specimens obtained from six standardized endobronchial biopsy sites. Preliminary data suggests that with the progression from normal epithelium to hyperplastic and ultimately

dysplastic sites, PCNA expression is both increased and abnormal in staining pattern [33].

Other markers of proliferation include the thymidine labeling index and the percent S phase cells obtained from flow cytometry. Other potential markers of proliferation and growth regulation under evaluation include, TGF-β, ornithine decarboxylase and polyamines, EGFR, and retinoid receptors.

Results of clinical trials in the UADT indicate that 13-*cis*-retinoic acid is able to reverse premalignant lesions and may prevent the development of SPTs [34-35]. Consequently, efforts have been made to determine the mechanism by which retinoids act as chemopreventive agents. Retinoid acid receptors (RARs) including RAR-α, RAR-β, RAR-γ, RXR, may be important in the modulation of premalignant cells by retinoids [36]. Preliminary data has suggested varying levels of these nuclear receptors according to the site of origin of the normal epithelium. RARs may also vary in squamous cell cancers. Efforts to characterize the expression of retinoic acid receptors in oral premalignancy and squamous cell carcinoma of the aerodigestive tract will continue as part of ongoing 13-*cis*-retinoic acid chemoprevention studies.

DIFFERENTIATION MARKERS

Abnormal expression of cellular differentiation markers may also be observed in the process of carcinogenesis. Efforts are under way to determine if expression of these markers may be used to identify sites at risk for the development of cancer. Head and neck cancers are predominately of squamous cell histology, so in this model, markers of squamous differentiation have been examined. These markers include: transglutaminase, involucrin and keratins [37]. The relationship of expression of these markers to the development of cancer has not been established.

Another marker of differentiation which has attracted considerable interest is the expression of the ABH blood group antigens. Interest in ABH antigen expression developed when studies of the epidermal growth factor receptor suggested that an antibody which cross-reacted with blood group antigen A identified patients with a favorable outcome in non-small cell lung cancer [38]. In a retrospective study, Lee et al. demonstrated that for patients with blood group type A or AB who retained expression of blood group antigen A in the tumor, a marked improvement in survival was observed [39]. An intermediate prognosis was observed in patients who have blood types B or O, and the worst prognosis was seen in patients with blood types A or AB when blood group A antigen expression was not observed in the tumor. Evidence that this antigen expression is a prognostic factor in non-small cell lung cancer prompted interest in the antigen as an intermediate marker of carcinogenesis.

Investigators have observed altered blood group antigen exposure associated with leukoplakia. Among these studies, Lee et al. demonstrated that treatment of leukoplakia with 13-*cis*-retinoic acid resulted in increased expression of blood group antigen A in some patients [40]. Loss of blood group antigen A has also been reported in bronchial epithelium specimens obtained from heavy smokers as part of an ongoing chemoprevention study.

SUMMARY

Chemoprevention trials represent an exciting opportunity to develop effective cancer control strategies. Logistic difficulties in performing these trials relating to sample size and duration of the studies have hampered advances in the field. The establishment of reliable intermediate markers which may act as surrogates for the development of invasive cancer would greatly improve the efficiency of these studies. A wide range of potential intermediate markers are currently under evaluation. At present, however, the field is in its infancy and markers have not yet been developed which may be used as the sole endpoints for chemoprevention trials. The development of intermediate markers represents a tremendous opportunity for collaboration between clinicians and basic scientists in order to move recent advances in cellular and molecular biology into the clinic.

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