Lung Epithelial Proliferation: a Biomarker for Chemoprevention Trials?

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Despite major advances in our understanding of the molecular pathogenesis of lung cancer, progress in reducing the death rate from lung cancer has been slow. Lung cancer remains the leading cause of cancer death in the United States, resulting in more deaths each year than the next three major causes of cancer death (cancers of the breast, prostate, and colorectum) summed together. In 2001, it is estimated that there will be 169 500 new cases and 157 400 deaths attributable to lung cancer *(1)*. Clearly, control of this tremendous public health burden will require multiple paths of investigation, ranging from primary prevention and early detection to targeted treatment of advanced malignancy.

Chemoprevention, the use of agents to treat the early phases of carcinogenesis and thereby prevent the development of invasive cancer, offers a promising means of controlling lung cancer by intervening during the earlier, potentially treatable phases of this lengthy disease process. The success of tamoxifen in reducing breast cancer risk and celecoxib in reducing adenoma burden in familial adenomatous polyposis (as recognized by Food and Drug Administration approval of these agents for these indications) provides a strong rationale for testing chemopreventive strategies in other epithelial malignancies *(2,3)*. Moreover, high-dose 13-*cis*-retinoic acid has shown promise in the treatment of oral premalignant lesions and in the prevention of second primary cancers of the head and neck *(4,5)*. However, chemoprevention of lung cancer has, thus far, proven to be more challenging. Several large phase III randomized studies examining β-carotene, vitamin E, vitamin A, *N*-acetylcysteine, and 13-*cis*-retinoic acid have shown no efficacy for these agents alone or in various combinations in preventing primary or second primary lung cancers in a variety of high-risk cohorts *(6–9)*. In fact, a worse outcome in current smokers was noted in several of these trials *(6,7,9)*. How should we proceed?

Successful cancer prevention clinical trials are predicated on the following: 1) identification of efficacious agents used at pharmacologically appropriate doses, 2) selection of appropriate high-risk patient cohorts, and 3) definition of primary study endpoints that are predictive of reduced cancer incidence. Clearly, delineation of the molecular pathways integral to the process of lung carcinogenesis is key to developing effective targeted agents, and effective agents are the minimum requirement for successful clinical trials. In the same fashion, identifying the proper patient cohort with assessable preinvasive disease or with a high enough risk so that an efficacious intervention could actually be discerned in a clinical study is essential. This underscores the inherent difficulty in patient accrual to cancer prevention trials, because individuals at high risk for cancer who do not yet bear a cancer diagnosis are particularly difficult to identify and are difficult to convince to participate in treatment studies. Finally, and equally important in the context of phase II preliminary efficacy clinical trials, the identification of intermediate endpoints that are sufficiently predictive for cancer incidence and mortality to serve as primary study endpoints in early phases of drug development represents a third minimum requirement for successful trials. Without an appropriate study endpoint, effective agents can be missed and ineffective agents may receive more attention than is deserved.

The study by Lee et al. *(10)* in this issue of the Journal examines the proliferative marker Ki-67 as a potential biomarker for lung cancer risk and chemopreventive response. The investigators report that Ki-67 staining is markedly elevated in current smokers but decreases substantially after smoking cessation, even though it remains measurable in former smokers. The Ki-67-labeling index correlates with histologic abnormalities, such as squamous metaplasia. However, there is considerable heterogeneity of expression, even in the absence of metaplasia, suggesting the attractive, but as yet unproven, hypothesis that individuals with increased bronchial epithelial cell proliferation may be at increased risk compared with those with low Ki-67 expression. Needless to say, long-term follow-up will be needed to determine if that is indeed the case.

Whether Ki-67 would be a good marker for chemoprevention studies is a separate issue from its potential ability to predict lung cancer risk. There are several characteristics of a marker that would make it appropriate for use in chemoprevention trials *(11,12)*. A marker should be integrally involved in the process of carcinogenesis, such that modulation of expression correlates highly with disease course. The expression of a marker should differ in normal versus premalignant or at-risk epithelium, and it should be easily and reproducibly measurable from biologic specimens likely to be obtained during clinical trials. The expression of a marker should be able to be modulated by efficacious chemopreventive interventions, but it should not fluctuate spontaneously or have a high spontaneous remission rate. Putative markers must subsequently undergo validation in prospective clinical trials. How well does Ki-67 meet these criteria?

The Ki-67 protein was originally defined by a monoclonal antibody that was generated by immunizing mice with nuclei from the Hodgkin's lymphoma cell line L428 *(13)*. This protein, whose functional role is yet to be defined, is present in the nuclei of human cells in all phases of the active cell division cycle but not in the G_0 resting state. Unlike the proliferating cell nuclear antigen (PCNA), another commonly used marker of proliferation, Ki-67 does not appear to be involved in DNA repair *(14)*. For this reason, the Ki-67-labeling index (the proportion of cells staining positively for Ki-67) is widely accepted to be a good marker for the proliferative compartment *(15)*. In understanding what this means, however, it is important to keep in mind that Ki-67 staining relates only to the number of proliferative cells and not to the rate of proliferation. Cells in early G_1, before the restriction point and commitment to mitosis, as well as cells that become growth arrested in G_2 or M (i.e., by chemotherapeutic

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agents), also stain positively for Ki-67, even though they may not finish transiting through the cell cycle. Thus, Ki-67 expression provides a measure of the proliferating and potentially proliferating cell burden but is not an accurate measure of successful cell division. This has implications for the use of Ki-67 to follow therapeutic interventions that may lead to cell cycle arrest at points other than G_0.

Despite the existence of abundant literature relating to Ki-67 (4245 references, PubMed, June 2001), it is unclear what the Ki-67-labeling index would be in never-smoking individuals in comparison to the current and former smokers studied by Lee et al. *(10)*. This information is particularly important for judging the potential usefulness of this marker in chemoprevention trials of former smokers, because, as stated already, a useful marker should differ in expression between normal and at-risk individuals. Animal studies *(16,17)* have suggested that proliferation is low in normal lung epithelia, with approximately 0.5% of airway cells actively dividing at any given time. Organ culture of biopsy specimens from human large airways had a Ki-67-labeling index of 1.7% at the time of explantation, which rose transiently to 30%, and subsequently stabilized around 5% *(18)*. How well these organ culture conditions reflect what is going on *in vivo* is not at all clear, but they do emphasize that proliferation is a dynamic process that responds quickly to stress conditions. Barsky et al. *(19)*, in examining Ki-67 in nonsmokers, tobacco smokers, and habitual smokers of marijuana or cocaine, found that 29% of their control nonsmoker group (28 individuals) had 5% or more Ki-67-positive cells in their bronchial biopsy specimens. However, some of those individuals were clearly ex-tobacco smokers, albeit with low total exposure *(19)*. Conversely, it must be kept in mind that cell proliferation is a response to injury that also occurs in many noncancer disease states. Animal studies *(20)* show that multiple irritants, such as high oxygen content, ozone, sulfur dioxide, mechanical irritation, and infection, all increase lung epithelial proliferation. Thus, although it appears that the proliferative rate differs between high-risk individuals (current smokers and possibly some former smokers) and low-risk individuals (never smokers), increased proliferative rate is not specific for lung carcinogenesis.

How about the ability of Ki-67 to be modulated by chemopreventive interventions? Although the study by Lee et al. *(10)* did not address this directly, the same group of investigators *(21)* recently published data showing that modulation of the proliferative index PCNA during a chemoprevention trial was associated with reversion of histologic squamous metaplasia, which, in turn, was associated with quitting smoking. This association reaffirms the tight correlation between smoking status and proliferative index, but we need to await positive chemoprevention trials to determine if there is additional value provided by proliferative indices in assessing response.

Where does this leave Ki-67? The current study *(10)* begins to explore the biologic differences between current and former smokers, which have yet to be taken into account in tailoring chemopreventive interventions appropriately. Tobacco smoke is known to increase epithelial proliferation in animals *(20)*, and this has now been shown to hold true in humans in the current study. Cell proliferation is a universal process that is dysregulated during tumorigenesis, and further understanding of how this dysregulation occurs may well provide better targets and more specific markers for chemoprevention clinical trials. The proliferative indices, such as Ki-67, represent only one class of many potential markers for use as intermediate endpoints, even though none have been validated to date. In the meantime, further studies of Ki-67, as well as of other markers, will be necessary to best define their usefulness.

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