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Sequencing the Events That Mediate Progression of Premalignant Lung Lesions

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Analysis of a large group of patients with multifocal premalignant disease by Krysan and colleagues in this issue of *Cancer Research* provides an informative view of the processes that may underlie progression of these lesions to invasive adenocarcinoma of the lung. The identification of the type and distribution of mutational changes reveals that common processes may be occurring within individuals but that these are generally unique between patients at risk for developing lung cancer. Furthermore, predicted neoantigens are identified and associated with characteristics of immune infiltrates supporting the role of alterations in adaptive immune surveillance in progression of these premalignant lesions. These findings provide critical insights that will help establish a foundation of knowledge for developing personalized prevention strategies with the potential to significantly impact overall mortality in lung cancer.

See related article by Krysan et al., p. 5022

The two major subtypes of non–small cell lung carcinoma, adenocarcinoma and squamous cell carcinoma, are associated with established premalignant lesions that demonstrate well-defined histopathologic characteristics. Bronchial dysplasias are precursors of invasive squamous cell carcinoma (SCC). Atypical adenomatous hyperplasia (AAH), adenocarcinoma *in situ* (AIS), and minimally invasive adenocarcinoma represent a spectrum of lesions that define the progression from premalignant to invasive disease in lung adenocarcinoma. AAH is the earliest premalignant change that can be identified histologically or radiographically and is characterized by the growth of atypical cells along alveolar septae (lepidic growth pattern) without invasion of underlying stromal tissue. While bronchial dysplasia can be detected bronchoscopically and sampled over time, the location and nature of AAH precludes such longitudinal tissue sampling and histopathologic characterization. Radiographically, AAH appears as ground glass opacities (GGO), which on progression will evolve to part-solid or fully solid invasive nodules. This radiographic appearance is not specific as other nonneoplastic processes can also cause GGOs, but the detection of these adenocarcinoma precursor lesions is becoming common with the institution of low-dose CT lung cancer screening. Importantly, these premalignant lesions represent potential targets for the prevention of lung cancer.

Like bronchial dysplasias, the large majority of adenocarcinoma precursor lesions regress spontaneously (1, 2), although lack of entirely specific radiographic features makes determination of the exact rate uncertain. To recognize high-risk lesions and develop effective preventive strategies, we must describe the mechanisms that underlie progression to invasive adenocarcinoma. The report by Krysan and colleagues significantly advances our understanding of the processes underlying the development and progression of adenocarcinoma-associated premalignant lung lesions (3). The impact that development of effective preventive therapies could have in the realm of lung cancer, although hard to predict, could be very substantial. In the past two decades, advances in early detection and molecular-targeted therapies for the treatment of established lung cancer have led to increases in 5-year survival that translate to several thousands of additional lives saved every year in the United States alone. However, when considering the impact prevention has had in other cancer types such as cervix and colon where estimated decreases in incidence and mortality range from 20% to 60% (4, 5), decreases in lung cancer–related mortality through prevention could represent an additional order of magnitude compared with that achieved with recent advances in care for invasive lung cancer. A key component of this study by Krysan and colleagues that provides strong insight is the focus on cases with multiple premalignant lesions within a single-resection specimen. Using whole-exome sequencing (WES), the authors demonstrated genomic alterations that uncover progression-related alterations and also, along with supportive *in situ* analyses, used this sequence data to analyze immunogenic epitopes that help define the role of the immune contexture in the progression of these lesions. While preventive measures in cervix and colon have largely involved procedural removal of premalignant lesions, the general size of the lung field affected by premalignant change will likely mandate a chemopreventive approach. As our understanding of the molecular underpinnings of carcinogenesis and our ability to target these processes increase, the potential to develop chemopreventive approaches will be based on findings from studies that elucidate these processes.

Results from the genomic analysis of independent premalignant AAHs versus AIS and invasive adenocarcinomas were used to classify mutational events as those found only in premalignant lesions, those found in both AAH and AIS/adenocarcinoma groups [termed progression-associated mutations (PAM)] and those associated only with the

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AIS/adenocarcinoma group [malignant-specific mutations (MSM); ref. 3]. This useful classification allowed for the identification within each patient of AAHs with close relationship (homogeneity) to the invasive lesions. This suggests commonalities in the pathways for progression in multiple AAHs within the lungs of a given patient. Notably, however, when PAMs were compared between patients, there was very little homogeneity, indicating that mechanisms of progression are unique from individual to individual. In respect to the PAMs, an intriguing finding was the demonstration within subjects that individual AAHs often demonstrated numerous identical mutations to those of the anatomically independent AIS/adenocarcinoma. Most patients showed independent AAHs with 5%–10% of mutations being PAMs shared with AIS/adenocarcinoma, and approximately a third showed 20% or more shared PAMs. It seems certain that this extensive list of shared mutations cannot represent random events, and the finding has implications regarding field effects that may promote carcinogenesis in the lung. Interestingly, this same phenomenon of shared mutations in anatomically independent premalignant lesions has also been reported in bronchial dysplasia (6, 7). Two considerations regarding the mechanism underlying these shared PAMs would be either migration of premalignant cells through the lung, with reseeding at distant sites, or an environment that promotes similar molecular mechanisms of carcinogenesis within the lungs of an individual. Adaptive oncogenesis is a proposed process that might lead to the latter consideration. This process involves selection of certain mutant clones when specific conditions in the microenvironment provide an advantage for the expansion of that premalignant clone, a process that has been demonstrated to occur in models of hematologic malignancies (8). Another interesting finding was the demonstration that most mutational driver oncogenic events were identified only in the MSM group of mutations, suggesting that these events occur late in the progression to invasive adenocarcinoma. Interestingly, this finding has been previously reported although involving classic driver mutations of *BRAF* in premalignant lesions that progressed to *EGFR* mutation–positive invasive lung adenocarcinomas (9). Thus, it appears that other mutational events and distinct pathways may be important for initiation of premalignant change and may provide a cellular population in which progression-associated events occur.

A novel aspect of the analysis performed by Krysan and colleagues is the use of the WES data to evaluate potential immunogenic epitopes associated with the identified somatic mutations. Recent advances in our understanding of the mechanisms controlling immune surveillance of invasive cancers has led to major breakthroughs in cancer treatment, and the demonstration of characteristic immunogenic epitopes, as well as evidence of related expression of factors controlling immune checkpoint activity, gives strong support to the contention that immune surveillance is a key component controlling progression of premalignant lesions also. Utilizing patient HLA genotypes and *in silico* models of antigens corresponding to detected somatic mutations, the authors demonstrate the presence of significant numbers of AAH premalignancy-associated neoantigens (PrN). Not surprisingly, the number of PrNs strongly correlated with the number of mutations in a premalignant lesion. Importantly, they also correlated with increased numbers of progression-associated neoantigens (PAN) that are also found in the AIS/adenocarcinoma lesions within the same patient. Using IHC staining of sections from these same AAH lesions, the load of PrNs was correlated with both CD4-positive T-lymphocyte infiltration and PDL1 expression in AAHs, and PAN loads were correlated with CD8-positive T-lymphocyte infiltration of AAHs. Although not subclassified in this analysis, these findings correspond with RNA expression data in previous studies of both AAH and bronchial dysplasia that indicate altered pro- versus antitumorigenic adaptive T-lymphocyte–mediated immune responses and macrophage-associated antigen presentation that have been demonstrated to distinguish progressive from regressive premalignant lesions (9, 10). While the authors note that the identified neoantigens are generally not derived from classic lung adenocarcinoma driver oncogenes, they describe an intriguing common association of PANs between patients with *MUC4*, in which neoantigens associated with mutations in this gene were found in approximately 75% of patients. Overall, greater than 90% of patients showed PANs that persist during progression of AAHs and could represent effective targets for immune-based preventive therapy.

Changes in adaptive immune responses in premalignant lesions of the lung are now being recognized as common and, furthermore, appear to involve similar reprogramming mechanisms in both precursor lesions of lung adenocarcinoma and SCC. Furthermore, descriptions of genomic alterations that are associated with progression of premalignant lesions to invasive carcinoma will reveal molecular mechanisms of progression that can be targeted in chemopreventive therapy. The data presented by Krysan and colleagues reveals new characteristics and helps confirm some previously described unique aspects of premalignant disease. Premalignant lesions may facilitate the acquisition of driver oncogenic events, but they frequently appear to develop without these alterations. Thus, unique pathways will likely need to be targeted to prevent malignant progression. In addition, while common mechanisms of progression appear to occur at multiple foci within single patients, mechanisms of progression between patients appear most often to be unique in nature. This suggests that personalized approaches to preventive therapy will be required. However, it also may indicate that identification of specific progression mechanisms in a given patient may allow highly efficacious preventive therapy by targeting one or a few particular pathways. The continued collection of information on the biology of premalignant progression in lung cancer is critical to developing preventive measures that could have a dramatic impact on the overall mortality associated with lung cancer.

Disclosure of Potential Conflicts of Interest

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