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Immunosurveillance and regression in the context of squamous pulmonary premalignancy

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Summary.

In this issue of Cancer Discovery, Pennycuick *et al.* comprehensively evaluate the immune contexture of progressive and regressive lesions in squamous pulmonary premalignancy (1). The authors dissect the molecular features of these lesions and the potential pathways of immune escape operative in progression to invasive cancer.

Epithelial cells in the field of lung injury can give rise to distinct premalignant lesions bearing unique genetic aberrations. Over time, a subset of these lesions may escape immune surveillance and progress to invasive cancer, whereas others regress without therapeutic intervention. Recognized as a hallmark of cancer, immune escape has been established as a critical determinant of tumor progression. The majority of earlier studies focused on the epithelial component of premalignant lesions (2) and more recently have begun to encompass inflammation, immunity and the microenvironment as determinants of progression (3). The centrality of the immune response, including effector recognition of premalignant neoantigens, is now the focus as a potential key determinant of premalignant lesion fate. With the advent of lung cancer screening, more patients are presenting with imaging studies consistent with focal or multifocal pulmonary premalignancy with unknown malignant potential. The premalignancy mutational, neoantigen and gene expression landscapes that predict progression have not yet been fully elucidated. The concepts of immunosurveillance and immunoediting suggest that the host immune response is capable of both recognizing and preventing the outgrowth of invasive malignancy at the earliest points of cancer development. Are immunosurveillance and immunoediting operative in pulmonary premalignancy? While immune recognition is evident in the earliest stages of disease, immunosuppressive networks may dominate as pulmonary premalignancy progresses to invasive cancer. This gives rise to the suggestion that scrutiny of the immune contexture in developing premalignancy could enable cancer interception, a strategy that seeks to block the progression of premalignancy to invasive cancer.

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Disclosure of Potential Conflicts of Interest

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A study of premalignant atypical adenomatous hyperplasia (AAH) lesions in early stage lung adenocarcinoma (LUAD) found that the percentages of progression-associated neoantigens (those found in both premalignant AAH lesions and the associated cancer) were significantly associated with the levels of $CD8^+$ T cell infiltration in AAH. Infiltration of $CD4^+$ T cell and PD-L1 expression correlated with the neoantigen load of AAH lesions. These findings suggest specific immune recognition of neoantigens at the earliest points of LUAD development and the potential for lung cancer interception (4). Teixeira *et al.* performed a comprehensive genomic, transcriptomic and epigenomic evaluation of high-grade pre-invasive carcinoma *in situ* (CIS) pulmonary lesions. The authors defined the molecular signatures associated with progression as well as marked differences between the premalignant lesions that will progress to cancer and those that will regress (5). Chromosomal instability was among the key pathways found to be associated with progression but the role of immune surveillance remained unclear.

Premalignant lesions (PMLs) reside within a complex multicellular ecosystem comprised of premalignant epithelium, immune and other cell types. Alterations in premalignant cell gene expression may also induce changes in the surrounding cells. Beane *et al.* assessed gene expression in squamous premalignant lesions obtained via autofluorescence bronchoscopy from subjects at high risk for developing lung cancer (6). The authors defined a gene expression signature characteristic for squamous premalignancy. The analysis of three public datasets revealed that many of the biological pathways altered in premalignancy were also altered in invasive LUSC. In a subsequent longitudinal study, the authors evaluated bronchial biopsies from subjects at high risk for lung cancer by transcriptomic profiling (7). The biopsies were classified into four transcriptionally distinct groups, including one designated “Proliferative” that was found to be associated with bronchial dysplasia. Patients with Proliferative premalignancy could also be identified via gene expression measured from cells in non-involved large airway epithelium. Persistent and progressive Proliferative PMLs were characterized by decreased expression of genes involved in interferon signaling and antigen processing and presentation pathways. These lesions were depleted of $CD68^+$/$CD163^+$ macrophages and $CD8^+$ T cells and demonstrated lower expression of human leukocyte antigen (HLA) class I genes. These findings suggest the potential for identification of PMLs that will be more likely to progress to invasive lung cancer.

Utilizing autofluorescence bronchoscopy biopsies from patients with nine morphological stages of LUSC, Mascaux *et al.* delineated the co-evolution of cancer and immune responses (8). This study highlighted the gene expression changes that lead to the alteration of the microenvironment, shifting from the initial anti-tumor immune response in low grade pre-invasive lesions, gradual immune escape in high-grade lesions, followed by immune exhaustion in invasive LUSC.

Extending their prior investigations (5), Pennycuick and colleagues complemented the genomic, transcriptomic and epigenetic characterization of the aggressive and regressive CIS lesions with comprehensive assessment of the immune contexture of the lesion microenvironment (1). The authors found that the regressive lesions had significantly higher levels of infiltrating $CD8^+$ T cells compared to the lesions that progressed to invasive cancer. However, the proportion of $CD8^+$ T cells expressing granzyme B was similar in progressive

and regressive lesions, suggesting that the lack of recruitment, but not impairment of the cytolytic capacity, was critical for immune evasion. The number of infiltrating $CD4^+$ T cells, $FOXP3^+$ regulatory T cells, B cells and macrophages did not significantly differ between the progressive and regressive lesions. Hierarchical clustering of various immune cells demonstrated that immune "cold" lesions predominantly progressed to cancer, supporting the concept that highly immunogenic lesions are eliminated early while lesions with low immunogenicity tend to progress. These findings suggest that immune surveillance appears to play a critical role in pre-invasive lesion regression. Progressive lesions had significantly higher mutational burden and levels of putative neoantigens compared to lesions that regressed. Previous studies have shown a survival advantage for lung cancer patients whose tumors had higher mutational burdens following immune checkpoint blockade therapy. The findings in the current research suggest that in pre-invasive pulmonary squamous lesions the abundance of neoantigens can lead to immune exhaustion and subsequent escape from immune surveillance. In accord with previously reported findings (4), there was no overlap in putative neoantigens between patients, suggesting that future therapeutic approaches targeting neoantigens will require tailoring for individual patients. Pennycuick *et al.* observed genetic and epigenetic alterations of antigen presentation-related genes in progressive lesions, highlighting yet another mechanism of immune escape. The involvement of promoter hypermethylation warrants further investigation to determine if demethylating agents could play a role in limiting premalignant lesion progression.

The authors found an increase of pro-inflammatory cytokines, some of which are clearly associated with augmentation of anti-tumor immunity, including *IL2, TNF, and IL12A,* in lesions that regressed. Comparative gene expression analysis of immunomodulatory genes identified a significant and consistent downregulation of the T cell activation marker, *TNFRSF9*, in progressive lesions. CCL27 and CXCL8 were found to be upregulated chemokines in progressive premalignant lesions. While the overexpression of CXCL8 has been shown to be pro-tumorigenic in the context of several malignancies, the role of CCL27 is less clear. In contrast to the findings of Pennycuick *et al.*, others have found that a decrement of CCL27 may be responsible for tumor evasion of T cell-mediated antitumor immune responses (9). Members of this chemokine family, including CCL21, have documented anti-tumor properties and are being evaluated for lung cancer therapy in the context of *in situ* vaccines (10) in ongoing clinical trials (NCT03546361).

The findings of Pennycuick *et al.* contribute to the mounting evidence suggesting that pulmonary premalignancy may be held in check by specific cell-mediated antitumor immune responses. It appears that the immunosuppressive microenvironment, that has been well-documented in advanced stage disease, is operative at the earliest points in the development of lung cancer and may serve to promote the progression to invasive disease (Figure 1).

Many questions and challenges remain. In the context of tobacco-induced lung cancer, a field of injury induces epithelium at risk throughout the respiratory tree (11). Invasive disease may develop in areas distinct from lesions under investigation. Does a change in the microenvironment in one premalignant lesion portend invasive disease in other areas at risk? What are the key features of immunosuppression or limitations in antigen presentation that

should prompt interception interventions? Which types of immune regulatory interception should be applied? Could local therapies augment systemic responses that protect other areas of epithelium at risk? Additional studies, such as this one by Pennycuick *et al.*, will be required to build the pulmonary pre-cancer atlas that will facilitate the application of immune-mediated interception for pulmonary premalignancy.

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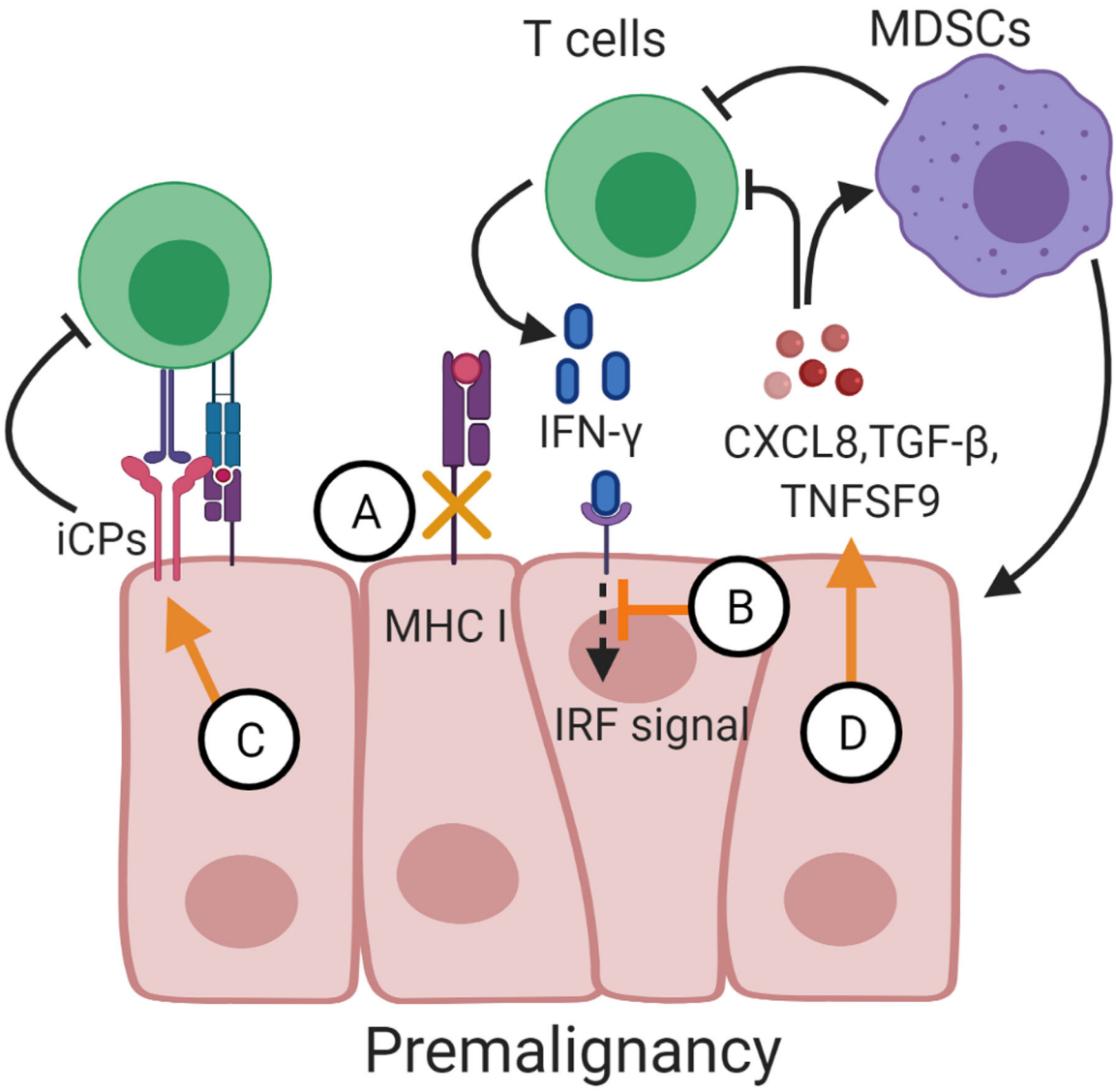

Figure 1:

Progression of squamous premalignancy may be associated with suppression of immune surveillance through: (**A**) impairment of antigen processing and presentation capacity, (**B**) impairment of IFN-g signaling, (**C**) increased expression of immune check-points (iCPs), and (**D**) the release of cytokines inhibiting T cell activation while promoting recruitment of myeloid-derived suppressor cells (MDSCs).