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The peripheral immune response and lung cancer prognosis

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Abbreviations: NSCLC, non-small cell lung cancer; PBMC, peripheral blood mononuclear cells; miRNA, microRNA; HR, hazard ratio

Attempts to refine and improve outcome predictions using tumor gene expression have been recently reported. We show that peripheral blood mononuclear cell (PBMC)-associated gene signatures can predict outcome in non-small cell lung carcinoma patients independent of demographic data or TNM staging, and that this information may persist after tumor resection.

Tumors and the immune system have been suggested to co-evolve during cancer progression, based on studies of tumor-associated immune cells. Gene expression profiles from peripheral blood mononuclear cells (PBMCs), as identified in our laboratory and by others, show that the peripheral immune system responds in specific ways to different cancers.[1] Our previous studies could identify, in Stage I non-small cell lung carcinoma (NSCLC) patients, a cancer-associated PBMC gene profile that diminishes or reverts to a non-cancer profile after tumor resection.[2] By direct comparison of gene expression in matched pre- and post-surgery samples, we identified differences in mRNA and miRNA expression that correlated with tumor resection.[3]

Gene expression profiles in tumor cells reported to be prognostic for lung cancer recurrence and patient survival have recently been reviewed.[4] To determine whether patient survival is a function of immune responses, we studied whether gene expression data from NSCLC PBMCs also contain prognostic information. To address this possibility, we analyzed survival data in 108 NSCLC patients[2] as a function of their PBMC gene expression profiles.[5] Using only 54 of the patients, we identified a panel of 26 genes that could be used to predict survival. This result was confirmed on an independent validation set constituted by the remaining half of the patients, with a statistical significance of $p = 0.009$.[5] While the survival of the patient cohort showed the expected correlation with age and stage, the gene prognostic score (GPS) based on the expression levels of the 26 genes that we identified represented an independent indicator of survival. A predictor based on tumor stage and gene expression correctly reassigned 5 of the 54 patients from the testing set that were misassigned using only stage: two from the low- to the high-risk group and three from the high- to low-risk group.

To understand the basis for prognosis based on immune gene expression, we performed a functional classification of 1704 genes significantly associated with survival and found a significant representation in two main functional categories. Higher expression of genes associated with protein synthesis (ribosomal structure and function) was associated with better survival, while higher expression of genes associated with cell cycle or metaphase was associated with worse survival. This possibly contradictory result suggested an involvement of multiple cell types.

Analysis of cell type-specific gene expression identified only the T-cell and myeloid lineages (**Fig. 1**) to be significantly associated with survival. Among 23 survival-related genes specific to T cells, 8 were associated with better survival and included T-cell transcriptional activators and cytotoxic-T-lymphocyte (CTL) response genes, which are important for tumor destruction. The remaining 15 genes, indicative of poor survival, were associated with cell cycle, the functional category also linked to poor survival. These might have represented $CD4^+$ Th2 responses, which are known to inhibit tumor immunity. Six of these 15 genes, namely, CCNA2, CCNB1, CCNB2, CDK1, CDCA5 and UBE2C are also significantly associated with lung adenocarcinomas.[6,7] It is highly unlikely that we would be detecting circulating tumor cells in these early-stage cancer patients. Rather, as tumors contain information from infiltrating T-cells (the 72 tumor gene prognostic signature of Ropeman[8] indeed also includes immune cell-specific genes), and this expression may be similar to that detected in peripheral T cells.

The 21 myeloid-specific prognostic genes were exclusively negatively associated with survival, i.e., high expression correlated with poor survival. The association with poor outcome is consistent with observations showing that increased

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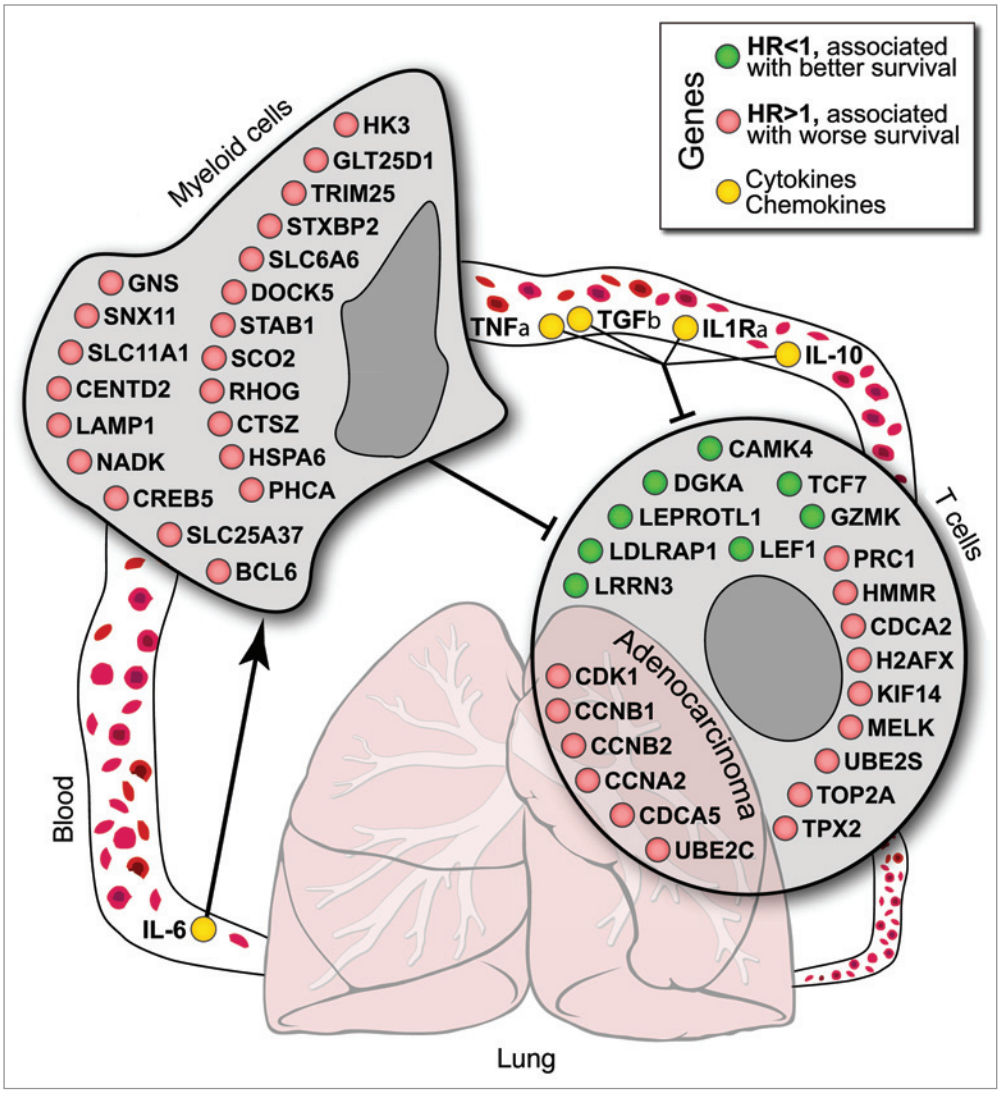

Figure 1. Genes specific to T-cell and myeloid lineages significantly associated with survival. Genes significantly associated with survival were found to be enriched for genes specific to myeloid cells (21 genes, all with hazard ratio, HR, > 1) and T cells (15 genes with HR > 1, 8 genes with HR < 1), indicating an association of those lineages with the survival signature. While our samples were derived from the peripheral blood, 6 T-cell specific genes have also been found by other studies to be differentially expressed in lung adenocarcinoma tumor samples, reflecting the infiltration of T cells into lung lesions. These are shown in the T-cell overlap with the lung. Cytokines and chemokines detected in the serum of lung cancer patients that can promote or interfere with immune responses are also indicated.

numbers of immature myeloid cells in late stage cancers can suppress antitumor immune responses and potently inhibit $CD4^+$ and $CD8^+$ T cells.[9,10] It is known that NSCLC influences the levels of cytokines and other circulating effectors including transforming growth factor β (TGFβ), tumor necrosis factor α (TNFα), IL1Rα and interleukin (IL-10), all of which are associated with immunosuppression, as well as of IL-6, which is associated with myeloid differentiation. As demonstrated by the fact that most of the patients in this study were affected by early stage disease, the association of myeloid suppression with poor outcome could be detected in PBMCs even in early stage cancers, hence constituting a target for early intervention.

PBMC gene expression measured before tumor resection is an independent predictor of patient survival. Because cancer-associated PBMC gene profiles revert after surgery,[5] we asked whether outcome-related information persists in post-resection gene expression. Out of 1704 genes associated with outcome, we identified 383 with altered expression after lung resection, of which 236 were expressed to higher levels in the presence of the tumor and were associated with a better prognosis and 92 had higher post-surgery expression and were linked to poor survival. The first group includes genes such as CXCR4 and genes coding for ribosomal proteins and translation elongation factors, whose upregulation in some classes of T cells could be important for antitumor effects (and by extension outcome). The second group contains genes associated with oxygenation and hemoglobin, suggestive of an increased presence of circulating erythroblasts in response to tumor-induced poor oxygenation.[6] One of these genes, SLC11A1, is indirectly regulated by hypoxia.

Prognosis based on tumor gene expression can only be assessed at the time of surgical resection, for operable cases. On the contrary, prognostic and diagnostic scores based on gene expression as detected in circulating lymphocytes continue to be measureable after tumor removal. By continuously monitoring these immune signatures, it may be possible to detect changes that anticipate recurrence before other clinical manifestations are detected.

PBMC gene signatures that can predict tumor recurrence and patient survival have important implications for treatment after surgery, and may also provide insights that can direct the development of new therapeutic approaches. Moreover, the analysis of tumor-specific changes in circulating immune cells has the potential to reveal immune differentiation, which can be either counteracted or enhanced to promote productive antitumor immune responses.

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