Cancer Research

Progress in Understanding Complexity and Determinants of Immune-Related Prognostic Subsets in Primary Melanoma

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Gene signatures are increasingly being used to infer the immune composition of the tumor microenvironment. This strategy holds the promise for earlier detection, identification of patients at higher risk of progression, and for understanding therapeutic response and resistance to immunotherapy. This gene signature approach is now being integrated with information from genomic changes, gene networks, and master immunoregulatory genes, and this development can lead to identify the main determinants shaping the tumor immune landscape. A study in this issue of *Cancer Research* indicates a way forward to discover prognostic immune-related subsets in primary melanoma and to understand major determinants impairing protective immunity in early stage of disease.

See related article by Poźniak et al., p. 2684

Clinical management of cutaneous melanoma faces two seemingly unrelated, but equally urgent needs. The first is the identification of effective early-disease prognostic factors to predict the risk of clinical progression. The second is an understanding of the mechanisms controlling intrinsic and acquired resistance to immunotherapy. These two goals are related by a shared biological process (defective/impaired immune response), which contributes to clinical progression in primary disease and to resistance to immunotherapy in metastatic disease. Achieving these goals of identifying patients in early stage of disease with favorable prognosis and to predict patients more likely to respond to immunotherapy would inform clinical decisions regarding treatment options and foster the development of new or combinatorial immunotherapy regimens.

Since Clark and colleagues published their seminal study on the prognostic relevance of tumor-infiltrating lymphocytes (TIL) in stage I melanoma (1), increasing evidence has strengthened the concept that an active immune microenvironment contributes to the prevention of metastatic spread and to improvement in prognosis of both early and advanced disease. The presence of a brisk TIL infiltrate in primary melanoma has been reported to be associated with a lower probability of positive sentinel lymph node when compared with melanomas without TIL infiltration (2). In addition, the immune-related gene expression in metastatic lymph nodes has been shown to be positively correlated with survival (3), and a melanoma genomic classification study by the ATLAS collaboration, which focused primarily on metastatic samples (80%), found that only a transcriptomic subset characterized by enrichment for immune gene expression was associated with improved patients' survival, whereas no correlation between genomic features and outcome was found (4).

According to the eight American Joint Committee on Cancer (AJCC) staging system (5), prognosis for patients with clinically localized primary melanoma is based on the histopathologic features of Breslow thickness and ulceration; mitotic rate is no longer a determinant of prognosis or thinner lesions. This histopathologic classification captures only a fraction of the biological complexity and heterogeneity of the disease. Despite decades of investigation and the identification of a wide array of prognostic parameters through IHC, genomic analysis, and gene signatures, integration of molecular, biological, and immunologic characteristics into the current histopathologic classification has not been successful, possibly due to the lack of confirmatory evidence following the initial identification of these emerging prognostic factors.

In this issue of *Cancer Research*, Poźniak and colleagues make a significant step toward the identification of prognostic immune subgroups in primary melanoma and in understanding the major determinants that may negatively impact the development of immune response in early-stage disease (6). The authors took advantage of transcriptomic data from a subset of patients in the Leeds Melanoma Cohort (LMC) that included 703 primary melanomas with a median follow-up of 7.5 years and is fully annotated for characteristics including site of disease, BRAF/NRAS mutations, Breslow thickness, ulceration, mitotic index, AJCC stage, and TILs. To identify the immune-related prognostic subsets, the authors expanded on an approach pioneered by Galon and colleagues based on a compendium of 1,980 carefully selected immune genes defining 31 distinct immune cell types (7). Crucially, the authors subjected this compendium to a stringent, three-level filtering process. To this end, the authors removed from the initial compendium all the genes expressed also in normal and neoplastic cells of the melanocyte lineage. Then they took into consideration the genes defining each immune cell subset. Within each immune cell-specific list, genes that did not show

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the expected positive correlations were also deleted from the compendium. This step reduced the compendium to 27 immune cell type–specific scores based on 376 genes.

The authors found that the consensus clustering of the 27 immune cell scores defined three distinct melanoma subsets coded as "high immune," "intermediate immune," or "low immune." These subsets were validated in The Cancer Genome Atlas (TCGA) cohort. In both datasets, the three immune subsets were associated with significantly different survival, with the "high immune" subset showing a lower hazard of melanoma death compared with "low" and "intermediate" subsets. Interestingly, there was a relatively small overlap of 70 shared genes between gene sets identified by Poźniak and colleagues and TCGA prognostic immune signature. Furthermore, their 3-class signature showed only moderate concordance with TCGA 3-class signature ("immune," "keratin," and "MITF low"), as only the "high immune" subgroup overlapped well with TCGA "immune" class. These not fully concordant results suggest that different data analysis and signature selection strategies may impact the resulting prognostic subsets.

The "high immune" subset identified in the LMC cohort was enriched for thinner tumors and for tumors with more TILs and lower mitotic rate, suggesting that strong activation of immunity, with prognostic significance, may be more frequent in thinner, less advanced lesions before additional immune escape mechanisms develop, and in tumors that have not yet achieved a strong proliferative activity (low mitotic number).

The authors then focused on the identification of determinants and master regulators that may be associated with the "high immune" and "low immune" classes. In agreement with the current understanding of the main immune circuits of adaptive immunity in tumor tissue, the most highly enriched pathways in the "high immune" subset were IFNα/β signaling, antigen processing/presentation, IFNγ, and NF-κB signaling. Interestingly, their analysis uncovered a deleterious effect of smoking on survival in the "high immune subset," a finding that was associated with the higher expression of GPR15 gene. GPR15 is a known biomarker of tobacco smoke exposure encoding a protein involved in T-cell trafficking and was expressed at higher levels in tumors from "ever smokers" in the "high immune" subset.

The analysis of the "low immune" subset led to the discovery of a set of molecular features that together may impair the development of antitumor immunity. By network analysis of genes enriched in the "low immune" subset, the oncogene MYC showed the highest centrality. *MYC* was found to be amplified in a higher fraction of tumors in this subgroup compared with the "intermediate" and "high immune" subsets. The "low immune" subset also contained more frequent deletions in NF-κB and IFNγ pathway genes. Subsequent investigation of the transcriptomic data revealed a negative correlation between *MYC* and genes involved in antigen processing and presentation. This evidence was corroborated by IHC analysis of tissues where MYC expression and HLA-B showed a negative correlation. In addition, tumors in the "low immune" subset contained more frequent overexpression of the CTNNB1 gene (encoding β-catenin), a finding in agreement with the role of β-catenin in promoting a "non–T-cell inflamed" profile (i.e., T-cell exclusion) in tumor lesions, as reported by Gajewski and colleagues (8). Collectively, these data suggest that multiple factors including master regulators (β-catenin and MYC expression) and genetic factors (*MYC* amplification and NF-κB/IFNγ pathway gene deletions) may together suppress/impair the development of protective immunity in a subset of early-stage melanomas.

Taken together, what is the clinical relevance of these findings? First, three previously unrecognized immune-related prognostic primary melanoma subgroups were clearly identified (and validated in the TCGA melanoma dataset). This observation was possible because of synergy in the design of the study: the availability of transcriptomic data from a large and fully annotated primary melanoma cohort and the careful strategy adopted for selecting the genes to be enclosed in the immune cell–specific scores. Clearly, additional validation studies in independent primary melanoma cohorts are needed to corroborate these results and to assess the potential added value resulting from integration of the immune prognostic subsets with the conventional histopathologic parameters. Second, the study provided strong evidence for genetic and molecular determinants that may together shape the "low immune" subgroup. This latter evidence intersects the similar studies being carried out to understand resistance to immunotherapy. One possibility is that mechanisms that shape the "low immune" subset may be active even in the context of immunotherapy resistance and vice versa. For example, one open question is the role of MYC in melanoma resistance to immunotherapy, possibly by regulating the antigen presentation genes or other mechanisms. Conversely, it is crucial to understand whether the recently described signatures of intrinsic resistance to anti-PD-1 (9), or the cancer cell program promoting T-cell exclusion and resistance to immunotherapy (10) play a role in shaping the "low immune" primary melanoma subgroup. In conclusion, the study by Poźniak and colleagues describes the progress toward the goal of improving risk stratification and treatment decisions in primary melanoma.

Disclosure of Potential Conflicts of Interest

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