Epistatic Oncogenic Interactions Determine Cancer Susceptibility to Immunotherapy

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Summary: Cancer genetic alterations and epigenetics control the malignant phenotype of tumor cells and the stroma. Synergistic oncogenic alterations may cooperatively dictate immunogenicity, level of infiltration by immune system cells, and response to immunotherapy in an epistatic fashion. The work of Skoulidis and colleagues shows that concomitant RAS and STK11/LKB1 mutations in non–small cell lung adenocarcinomas result in primary resistance to PD-1–based immunotherapy and poor T-cell infiltration. Cancer Discov; 8(7); 794–6. ©2018 AACR.

See related article by Skoulidis et al., p. 822 (4).

In classic genetics, epistatic mutations are those that render different phenotypic effects when acting individually or in combination. With regard to malignant transformation, cooperativity in oncogenesis was first found for RAS and MYC in classic fibroblast transformation assays by Weinberg and colleagues (1). Indeed, most cancers are the result of multiple oncogenic driver effects due both to the activation or overexpression of oncogenes as well as to the mutations or epigenetic silencing of cancer suppressor genes (2).

Recently, as a result of the PD-1/PD-L1 immunotherapy revolution, much attention is being given to the potential oncogenic pathways at determining the immunogenicity of cancer lesions and the response to immunotherapy (3). To date, most of these correlates of immune responsiveness had been made with single genetic events rather than with cooperative interactions in collusion against immunotherapy, as a result of more than one oncogenic genetic alteration.

In this issue, Skoulidis and colleagues (4) provide the evidence that, in RAS-driven lung adenocarcinoma, coexistence of a mutation of the STK11/SKB1 cancer suppressor gene determines primary resistance to checkpoint inhibitor immunotherapy with anti–PD-1 and anti–PD-L1 mAbs when retrospectively analyzing objective response and overall survival from various clinical trials. Furthermore, in two mouse lung adenocarcinoma cell lines driven by mutated RAS, knocking down STK11/SKB1 confers resistance to PD-1 immunotherapy and leads to poor T-cell infiltration.

In this regard, previous evidence had been reported on the potential cooperativity of STK11/LKB1 and RAS mutations to reduce immunogenicity (5). In that previous report, inducible transgenic mice developing double-mutant RAS and STK11/LKB1 lung adenocarcinomas were found to be less infiltrated by CD4⁺ and CD8⁺ T lymphocytes. Those lymphocytes showed evidence for a more immune-exhausted phenotype in the tumor microenvironment. Furthermore, such experimental tumors attracted immunosuppressive neutrophils or granulocytic myeloid-derived suppressor cells (5). IL6 together with IL23 and CXCL7 were found to be potential mediators in shaping the immunosuppressive tumor microenvironment. Of note, double-mutant RAS and STK11/LKB1 lung adenocarcinoma mouse cell lines in the report by Skoulidis and colleagues (4) are not more intensely infiltrated by polymorphonuclear leukocytes, but both studies coincide in showing a paucity of infiltration by T lymphocytes. The discrepancy regarding myeloid cells is probably related to inherent differences in the experimental systems. Other authors studying gene expression profiles had independently reached the conclusion that in human lung adenocarcinomas, concomitant RAS and STK11/LKB1 mutations resulted in a reduced T cell–related transcriptional signature (6).

Given the importance of the resulting immune phenotype, the next relevant question is how both oncogenic events mechanistically interact to cause T-cell exclusion. Unbiased genetic screenings and differential gene expression profiling studies are ongoing. This question is extremely important because silencing or induction of druggable inflammatory or anti-inflammatory mediators is likely to be involved, therefore potentially offering routes to intervene in the resistance against anti–PD-1/PD-L1 agents in double RAS and STK11/LKB1 mutant cases. In this sense, it is tempting to speculate that in RAS and STK11/LKB1 double-mutated adenocarcinoma cases, IL6 inhibition by drugs already approved (tocilizumab and siluteiximab) might be synergistic with PD-1/PD-L1 blockade (7).

Cooperativity at driving an immunosuppressive phenotype has also been reported for RAS mutation and MYC overexpression that not only accelerate tumorigenesis in vivo but also shape the immune tumor microenvironment (8). This was observed in oncogene transgenic mice in which MYC expression can be pharmacologically turned on and off. In this
RAS- and MYC-driven setting, overproduction of CCL9 and IL23 seems to be the critical factor attracting macrophages and excluding T cells and, importantly, natural killer (NK) cells.

The concept that oncogenic genetic alterations could dictate tumor stroma features with absence of T cells (immune desert cancers) was pioneered by Tom Gajewski’s group (9). Seminal observations were made indicating that in metastatic skin melanoma, alterations of the WNT/β-catenin pathway correlated with poor T-cell infiltrates. Experimental evidence was gathered in transgenic models of melanoma elicited by activation of mutated BRAF and loss of PTEN in conjunction with a transgenic experimental model antigen to ensure CD8+ T-cell recognition. Melanomas were locally induced by tattooing tamoxifen to induce transformation and, as a consequence, lack of infiltrates by tumor antigen-presenting dendritic cells that are thought to be responsible in turn for T-cell infiltration.

In light of this new idea of epistatic anti-immune cooperativity of oncogenic events, WNT/β-catenin pathway mutations are to be reexplored to see whether this immune-desert phenotype requires contribution by other partnering genetic events, including PTEN deficiency or defective expression, as induced in the transgenic mouse models used for the proof of concept (9).

Indeed, PTEN loss is a genetic alteration linked to poor response to immunotherapy, as discovered by Peng and colleagues (10). Loss of PTEN leads to much fewer T cells dwelling in the tumor microenvironment. Interestingly, in melanomas with loss of this tumor suppressor gene, there is an elevation of CCL2 and VEGF transcripts that might reshape the immune tumor microenvironment, fostering myeloid suppressor cells and conditioning tumor endothelial cells. Very informatively, in melanoma lesions with PTEN heterogeneity, the areas of PTEN loss are T-cell underpopulated, as opposed to PTEN-proficient areas. However, the mechanism of immune resistance could be multifaceted because PTEN loss also renders melanoma cells more resistant to CTL killing. These findings have been conducive to research testing PI3-kinase inhibitors in combination with PD-1/PD-L1 blockade in cases of PTEN loss (3).

The common theme seems to be that single or multiple genetic alterations control a key limiting factor for tumor immunotherapy, that is, trafficking and infiltration by NK and T cells (11). The molecular mediators responsible downstream for preventing T-cell infiltration may vary and are likely to mainly involve instructions to endothelial cells, thus becoming refractory to giving way to T cells, while permitting the entrance of immunosuppressive and protumoral leukocyte populations. In these scenarios, chemokines are perceived as key functional components.

A key event seems to be the entrance of a small number of NK cells and dendritic cells of the DC1 (BATF3-dependent) subset (12). Presence of these latter cellular actors in the tumor niche nicely correlates with the levels of T-cell infiltration across multiple malignancies (13), and their presence in the organism is key for PD-1/PD-L1 inhibitors to be effective (14).

Genetic alterations conditioning treatment responses to cancer immunotherapy are not necessarily oncogenic and may arise as an adaptive response to the selective pressure of tumor resistance of tumor cells to IFNγ and IFNα/β.

Genetic and epigenetic control of primary resistance to PD-1/PD-L1 therapy can result from multiple etiologies. Single-gene effects or epistatic interactions of oncogenes can, in addition to causing cell transformation, interfere with anticancer immune responses. Cancer cell epigenetic changes might play an as yet understudied important role. There are also multiple lines of evidence that tumor cell-extrinsic factors dictate PD-1/PD-L1 responsiveness. In a multifactorial fashion, epistatic genetic interactions are postulated to orchestrate mechanistic phenotypes in the tumor microenvironment that frequently prevent the success of PD-1-based immunotherapies.

**Figure 1.** Genetic and epigenetic control of primary resistance to PD-1/PD-L1 therapy can result from multiple etiologies. Single-gene effects or epistatic interactions of oncogenes can, in addition to causing cell transformation, interfere with anticancer immune responses. Cancer cell epigenetic changes might play an as yet understudied important role. There are also multiple lines of evidence that tumor cell-extrinsic factors dictate PD-1/PD-L1 responsiveness. In a multifactorial fashion, epistatic genetic interactions are postulated to orchestrate mechanistic phenotypes in the tumor microenvironment that frequently prevent the success of PD-1-based immunotherapies.
immunity even at early stages of carcinogenesis (immune editing). For instance, loss of strong neoantigens, loss of antigen presentation machinery, or loss of response to type I and II IFNs are known to occur (15). In line with this, much research is ongoing, defining the genetic requirements for the success of immunotherapy using loss-of-function genetic screenings in mouse models, some of which find proteins previously unknown to be related to oncogenesis or immune functions. Likewise, it is possible that cooperative interactions resulting in local immunosuppression could involve an oncogenic driver in conjunction with an epigenetic or genetic change in a gene previously unrelated to cancer biology. It must be considered that the oncogenic alterations leading to immune resistance could take the form of loss-of-function mutations, gain-of-function mutations, overexpression, or epigenetic silencing (Fig. 1). Comprehensive correlative studies with T-cell infiltrates and responsiveness to immunotherapy might lead to the discovery of more epistatic interactions.

Two important consequences derive from the notion that epistatic oncogenic alterations may shape the tumor microenvironment to mediate primary or even acquired resistance to immunotherapy: (i) because some of these pathways might be druggable, this allows the design of combination strategies with immunotherapy; (ii) gene alterations considered in pairs or trios may become useful predictive biomarkers as is already the case with the STK11/LKB1 mutation in KRAS-driven lung adenocarcinoma (4). The notion that immunotherapy refractoriness may be lurking in multiple genetic events, potentially acting cooperatively, complicates the scene but offers opportunities for synthetic synergistic immunotherapies and biomarker identification.

Disclosure of Potential Conflicts of Interest

I. Melero has received commercial research grants from BMS, Roche, and Alligator; has received honoraria from the speakers bureau of MSD; and is an external advisor for BMS, Bioncotech, Merck Serono, Roche, AstraZeneca, F-Star, Tusk, Alligator, and Cytomx. No potential conflicts of interest were disclosed by the other authors.

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