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CCR Translations

Clinical Cancer Research

# Antigen Presentation Keeps Trending in Immunotherapy Resistance

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Through a gain-of-function kinome screen, *MEX3B* was identified as a mediator of resistance to T-cell immunotherapy not previously identified using CRISPR-based screens. MEX3B is a posttranscriptional regulator of HLA-A, validating the critical role of tumor-intrinsic antigen presentation in T-cell immunotherapy and indicating a new putative molecular target. *Clin Cancer Res; 24(14); 3239–41. ©2018 AACR.*

*See related article by Huang et al., p. 3366*

In this issue of *Clinical Cancer Research*, Huang and colleagues used a kinome library screen and identified *MEX3B* as an important player in melanoma resistance to T-cell immunotherapy (1). Low expression of *MEX3B* was strongly associated with response in a cohort of patients with melanoma treated with anti–PD-1 checkpoint blockade. In functional studies using patient-derived melanoma cell lines and autologous tumor-infiltrating lymphocytes (TIL), MEX3B overexpression inhibited recognition and killing of melanoma by autologous TILs. Notably, this effect of MEX3B was dependent on endogenous expression of HLA-A and could be reversed by overexpression of exogenous HLA-A. In elegant studies using a dual luciferase reporter assay, the authors demonstrate that MEX3B disrupts HLA-A by binding the 3′ untranslated region (UTR) of its mRNA (Fig. 1). Indeed, MEX3B expression was inversely correlated with HLA-A expression in both the anti–PD-1–treated cohort and in the skin cutaneous melanoma The Cancer Genome Atlas (TCGA) cohort.

The authors' work follows in a line of recent studies using molecular screening methods to identify tumor-intrinsic mechanisms of resistance to T-cell immunotherapy. Their approach was a gain-of-function screen to test the impact of a set of 384 genes from a kinome library on the sensitivity of melanoma to direct T-cell killing in culture. In a similar approach, Patel and colleagues used an *in vitro* assay to identify tumor-intrinsic genes important in regulating T-cell antitumor efficacy (2). A CRISPR screen was performed on human melanoma cells in a 12-hour coculture with tumor-specific T cells. Of the genes most enriched in tumor cells surviving T-cell killing, those involved in antigen presentation machinery were most prominent (in particular, HLA-A). The authors also identified APLNR as a critical protein for sensitivity of cancer cells to T-cell killing through its association with JAK1 and interferon (IFN) signaling (Fig. 1). In a similar *in vitro* CRISPR screen approach, Pan and colleagues cocultured B16-F10 murine melanoma with tumor-specific T cells and also identified key genes in antigen presentation (as well as IFN signaling and other pathways) as critical to T-cell–mediated antitumor immunity (3). However, in these CRISPR screens, *MEX3B* was not identified as a resistance mechanism, highlighting the importance of the approach by Huang and colleagues (1).

It is worth noting that mechanisms of resistance to direct T-cell cytotoxicity in short-term *in vitro* coculture may not capture the complexity of resistance mechanisms to immune checkpoint blockade *in vivo*. As such, Manguso and colleagues used an *in vivo* CRISPR screen to identify genes involved in sensitivity or resistance to immune checkpoint blockade (4). Their CRISPR screen was tested in a murine melanoma model treated with an irradiated tumor vaccine with or without anti–PD-1 therapy and identified IFN signaling as a critical pathway in sensitivity to therapy. They also identified *Ptpn2* as a negative regulator of antigen presentation (and therapeutic response) through decreased IFNγ signaling sensitivity (Fig. 1). That antigen presentation is identified as a critical pathway in the Huang and colleagues' kinome library screen (1), the CRISPR screens noted above, as well as the mutations identified in patients who have primary or acquired resistance to immune checkpoint blockade, is highly reassuring and biologically consistent (5). In each model, whether it is an *in vitro* coculture, an *in vivo* model of anti–PD-1 therapy, or even an *in vivo* model of adoptive cell therapy, the end effector of antitumor function is the tumor-specific T cell. Thus, any deficiency in antigen presentation would render the T cells (and the immunotherapy) ineffective.

In conjunction with antigen presentation machinery, tumor IFN signaling has been well defined as a critical component of response to immune checkpoint blockade. Disruptions in both type I and type II IFN signaling, in the form of mutations in *JAK1*, *JAK2*, *APLNR*, and *STAT1*, among others, have been described as mediators of resistance to immune checkpoint blockade. This is at least in part mediated by the indirect role of IFN signaling as an upstream promoter of antigen presentation machinery (Fig. 1). Thus, better understanding of regulators of antigen presentation machinery downstream of IFN signaling could lead to approaches to overcome immunotherapy resistance in IFN signaling–defective tumors. For that reason, *MEX3B* is unique from the targets identified by the

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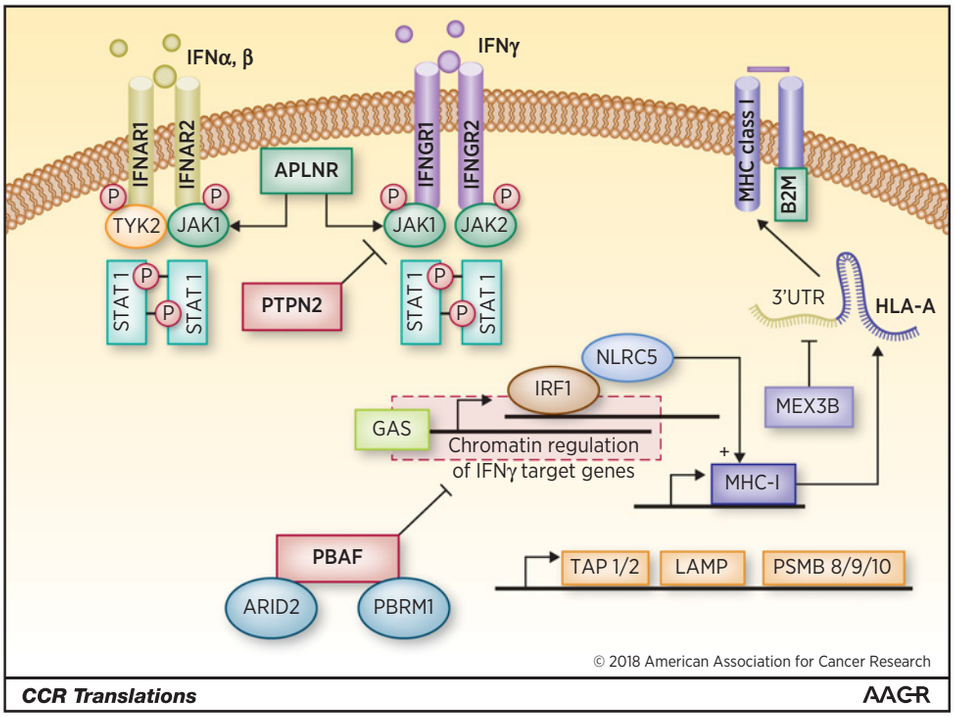


**Figure 1.**

Molecular screens reveal regulators of antigen presentation as key factors in tumor sensitivity to immunotherapy. In the 1990s, it was well-documented that some patients who initially responded to cancer immunotherapies with interleukin-2 or tumor-infiltrating lymphocyte adoptive cell transfer therapy may develop acquired resistance through loss of *B2M*, which leads to absence of surface expression of HLA class I. More recently, we identified defects in *B2M* from patients with acquired resistance to immune checkpoint blockade, along with defects in IFN signaling (*JAK1* and *JAK2*). IFN signaling activates transcription of antigen processing machinery. Two *in vitro* and one *in vivo* CRISPR-based screens have identified *APLNR*, *PTPN2*, and *PBAF* as immunotherapy targets; each is a regulator of IFN signaling, and thereby each indirectly impacts downstream antigen presentation. The CRISPR screens also identified components of antigen processing machinery (e.g., *TAP1*, *TAP2*, and immunoproteasome subunits including *PSMB9*) as determinants of sensitivity to immunotherapy. *NLRC5*, a transcriptional regulator of MHC-I expression, has also been implicated in immunotherapy sensitivity in the CRISPR-based screens. The work reported by Huang and colleagues (1) in this issue of *Clinical Cancer Research* identifies *MEX3B* as a posttranscriptional regulator of HLA-A via binding and disruption of the 3′UTR of the *HLA-A* mRNA.

two CRISPR-based screens described above. Unlike PTPN2 and APLNR, which regulate antigen presentation by impacting IFN sensing, MEX3B regulates antigen presentation at the level of HLA-A mRNA. These and other studies, which provide insight into mechanisms of IFN-independent regulation of antigen presentation, shed light on approaches that may restore antigen presentation in tumors with IFN signaling defects.

More immediately, the relevance of these studies in the clinic relates to patient selection. Across different malignancies, T-cell infiltration, PD-L1 expression, and mutational burden have been used as biomarkers for response to immune checkpoint blockade. However, there are an increasing number of studies describing defined molecular and genetic changes that impact antigen presentation, IFN signaling, and ultimately, response to immune checkpoint blockade. It is plausible that a defined set of these molecular and genetic changes could serve as a biomarker to exclude nonresponding patients and enrich for responding patients.

For defined molecules that negatively impact antigen presentation like MEX3B, development of targeted therapeutic approaches should be considered. The melanoma cell lines used by Huang and colleagues (1) had high basal expression of HLA-A, and thus, disruption of MEX3B was unlikely to have a phenotypic impact. There are subsets of human melanoma with low basal expression of MHC-I molecules, and it would be intriguing to examine whether MEX3B inhibition or deletion could induce MHC-I overexpression. This would be particularly valuable for tumors lacking IFN signaling. Needless to say, Huang and colleagues have identified a regulator of antigen presentation downstream of IFN signaling that is associated with resistance to immune checkpoint blockade and in so doing, piqued interest in this gene as a combinatorial target.

## Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

## Authors' Contributions

**Conception and design:** A. Kalbasi, A. Ribas
**Writing, review, and/or revision of the manuscript:** A. Kalbasi, A. Ribas
**Study supervision:** A. Ribas

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