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J Clin Invest. 2021;131(1):e144344. <https://doi.org/10.1172/JCI144344>.

Commentary

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Immune MAL2-practice: breast cancer immunoevasion via MHC class I degradation

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The success of tumor immunotherapy, while partial, confirms the existence and importance of tumor immunosurveillance. CD8⁺ T cell recognition of tumor-specific peptides bound to MHC class I (MHC-I) molecules is central to this process. In this issue of the *JCI*, Fang, Wang, et al. describe a unique tumor immunoevasion strategy based on endocytosis and degradation of MHC-I complexes mediated by the trafficking factor MAL2. Notably, MAL2 expression was associated with poor prognosis of breast cancer, and its downregulation enhanced CD8⁺ T cell recognition of breast cancer in various experimental models. This work demonstrates that a deeper understanding of tumor interference with MHC-I stability and trafficking has considerable potential for enhancing immunotherapies.

Tumor immunosurveillance and evasion

Tumor immunoevasion is a multifaceted process encompassing expression of immune checkpoints, cytokine production in the tumor microenvironment, and impaired innate and adaptive immune detection of malignant cells. The recent explosion of successful immunotherapies places antigen processing and presentation center stage in T cell-mediated immunosurveillance. Nearly all nucleated cells express MHC class I (MHC-I). By presenting small peptides derived from endogenous proteins, MHC-I enables CD8⁺ T cells to surveil foreign and abnormal translation products, including viral proteins and tumor-associated antigens.

The principal elements of the MHC-I antigen processing pathway have been established in the past 25 years (Figure 1). The proteasome degrades both immature and mature proteins to generate cytosolic peptides (1). The transporter associated with antigen processing (TAP) imports proteasome-generated cytosolic pep-

ptides into the endoplasmic reticulum (ER) where peptides assemble with MHC-I with the assistance of dedicated and general purpose molecular chaperones, followed by trafficking to the cell surface via the standard secretory pathway.

The effectiveness of MHC-I-based immunosurveillance is evident from the ubiquitous evolution of viral proteins that specifically interfere with antigen processing (1, 2). Similarly, many tumors impair antigen processing and peptide presentation to avoid immune eradication (3, 4), a process termed immunoevasion or immunoediting (5). How do tumors sabotage this system? We have likely only scratched the surface of this question, which is critical for distinguishing patients that are most likely to respond to immunotherapy and for devising treatments to convert resistant tumors. The standard approach has predominantly focused on sequencing tumor nucleic acids. This is no surprise, as a needle biopsy provides ample material for determining the transcriptome, exome, or whole genome. Tumor immunoevasion studies have focused mainly on

alterations in the canonical components of the class I assembly complex (6), largely ignoring the fate of correctly generated MHC-I peptide complexes. Yet tumors can also exploit MHC-I trafficking and degradation to evade the immune response.

MAL2 misdirects MHC-I

Enter Fang, Wang, et al., who in this issue of the *JCI* identified genes from publicly available breast cancer data sets that correlate with poor patient survival (7). One top hit, MAL2, was unusual; it failed to regulate breast cancer growth either in vitro or in immunocompromised mice. However, in normal mice, MAL2 expression correlated with tumor size, suggesting a critical role when tumors are under immune pressure. Sure enough, in human triple-negative breast cancers (cancers lacking receptors for estrogen, progesterone, and epidermal growth factor), MAL2 expression in bulk transcriptome and single-cell RNA sequencing data sets correlated with low CD8⁺ T cell cytotoxic signatures, suggesting that MAL2 expression suppresses MHC-I-based immunosurveillance (7).

Fang, Wang, et al. used cell models that expressed peptides from ovalbumin or the human cancer-testis antigen NY-ESO-1 to show that tumor MAL2 inhibits T cell responses during T cell coculture. MHC-I-blocking antibodies abolished the effects of MAL2 expression, indicating that the inhibitory function of MAL2 could be directly attributed to antigen presentation. Thus, a battery of diverse T cell-based assays provide evidence that MAL2 impairs breast cancer antigen presentation, crippling the T cell response (7).

MAL2 is a 176 residue multispansing transmembrane protein known to participate in transcytosis (8, 9). Pursuing the unique link between antigen presentation and MAL2, Fang, Wang, et al. found that MAL2 abundance indeed controlled cell surface levels of MHC-I. Mass spectrometry analysis of epitope-tagged MAL2-associated proteins identified both MHC-I and

► **Related Article:** <https://doi.org/10.1172/JCI140837>

Conflict of interest: The authors have declared that no conflict of interest exists.

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Reference information: *J Clin Invest.* 2021;131(1):xxxx-yyy. <https://doi.org/10.1172/JCI144344>.

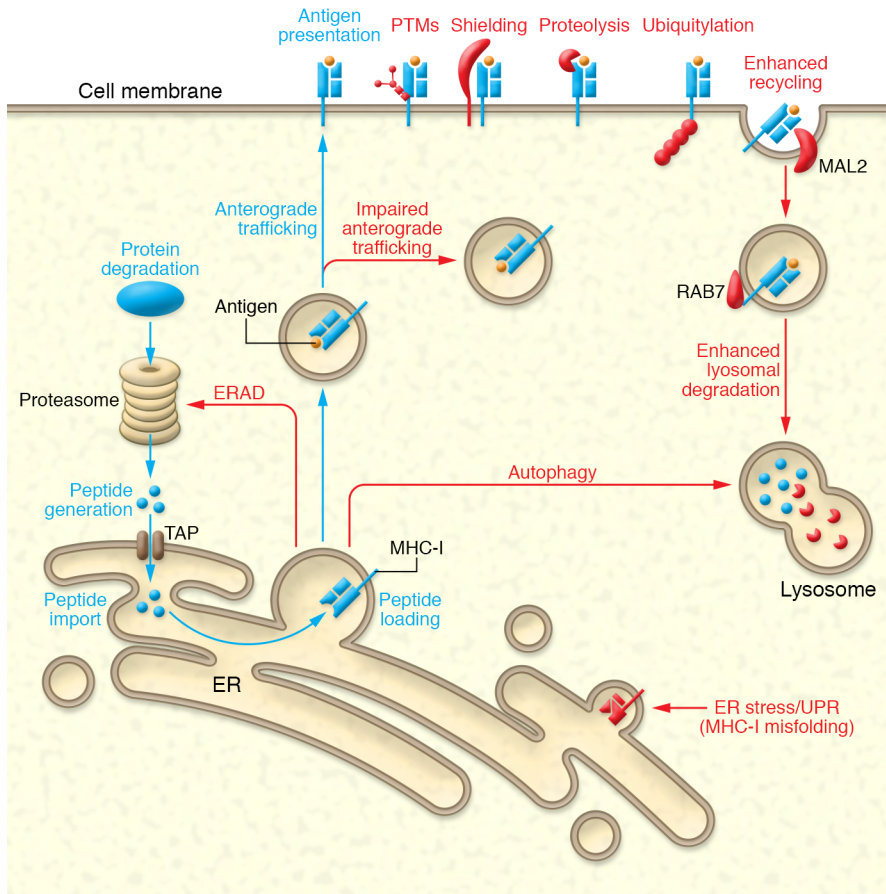


Figure 1. Model for the MHC-I antigen processing pathway and evasion strategies. Oligopeptides destined for MHC-I complexes are typically generated in the cytosol by proteasome-mediated degradation. Peptides are imported into the endoplasmic reticulum (ER) by TAP, the transporter associated with antigen processing. The ER lumen is the site of heterotrimer assembly, where heavy chains, β 2-microglobulin, and a high-affinity peptide associate while bound to TAP with the assistance of dedicated and general purpose chaperones. Assembled MHC-I is delivered to the cell surface via the Golgi complex. Cancer cells often interfere in this process to evade CD8⁺ T cells, although current understanding of immunoevasion has focused on genetic, transcriptomic, and signaling alterations. Highlighted in red are putative and known pathways that can contribute to immunoevasion at the posttranslational level. Fang, Wang, et al. (7) showed that MAL2 promotes endocytosis to reduce tumor antigens on the surface of breast cancer cells. ERAD, ER-associated degradation; PTMs, posttranslational modifications; UPR, unfolded protein response.

RAB7, an endosome recycling factor (10). After confirming the RAB7-MHC-I interaction by coimmunoprecipitation, Fang, Wang, et al. showed that MAL2 overexpression enhanced the MHC-I-RAB7 interaction, including on cell-derived endosomes (7). Notably, MAL2 overexpression enhanced MHC-I degradation, which was blocked by filipin, an endocytosis inhibitor (7). These findings are consistent with MAL2-RAB7-mediated endosomal/lysosomal degradation of MHC-I.

Fang, Wang, and colleagues next examined MAL2 function in vivo using a mouse breast cancer model to generate single-cell RNA sequencing data for tens of thousands of tumor-infiltrating CD3⁺ T cells. The sequencing data matched their analyses of published human data sets, showing that tumor MAL2 expression impaired the T cell cytotoxicity signature and furthermore strongly reduced the number of infiltrating effector T cells (7). Extending these experiments, the authors showed that MAL2 overexpression blocked autologous T cells from targeting primary human breast cancer organoids.

Finally, immunohistochemical staining of hundreds of primary human biopsies for MAL2, CD8, and cytolytic granules (GZMB) demonstrated that tumors with high MAL2 protein levels displayed relatively weak CD8⁺ T cell activity (7).

Altogether, Fang, Wang, et al. provide robust evidence for an inhibitory effect of MAL2 on T cell recruitment and cytotoxicity in breast cancers, based on posttranslational downregulation of MHC-I (7).

Clinical implications

Immunoevasion is a critical step in the development of clinical tumors. Malignant cells frequently hijack existing cell biological processes, e.g., coopting epigenetic regulation of MHC-I machinery (11) to reduce levels of antigen processing and presentation components. Subversion of MHC-I trafficking by MAL2 fits this modus operandi by utilizing preexisting protein trafficking pathways. It will be of great interest to determine the extent to which other tumors exploit MAL2 misdirection of MHC. Consistently, MAL2 is overexpressed in colorectal cancer (12),

renal cell carcinoma (13), and ovarian carcinoma (14). It is important to note that NK-based immunosurveillance of MHC-I-deficient cells constrains tumor escape and necessitates that tumor cells deploy additional changes to walk the razor’s edge between NK and CD8⁺ T cell immune pressure.

The findings described by Fang, Wang, et al. (7) raise the unexplored possibility of a MAL2-based therapy, either in the form of inhibitors or even anti-MAL2 monoclonal antibodies, given that MAL2 was predominantly expressed on the surface of breast cancer cells. This MAL2 therapy could be particularly important in combination with antibodies targeting PD-L1/PD-1 checkpoints.

Although posttranslational manipulation of antigen presentation has been historically less studied than genetic and transcriptomic analyses, the work of Fang, Wang, et al. serves as a shining example of how trafficking and protein-centric mechanisms can be relevant to immune-based control over tumors. Appropriately, recently published studies expand on

these modes of regulation in a number of cancers: (a) In triple-negative breast cancer, expression of the long noncoding RNA *LINK-A* enhances ubiquitin-mediated degradation of peptide loading complex components, hampering MHC-I folding and peptide loading (15). (b) In pancreatic ductal adenocarcinoma, MHC-I is strongly downregulated via autophagy and the cargo receptor NBR1 (16). Inhibition of autophagy restored MHC-I surface levels and improved checkpoint blockade therapies in mouse models. (c) In diffuse large B cell lymphoma, unbiased genome-wide CRISPR screens identified dozens of previously unknown antigen presentation regulators, spanning epigenetics, transcription, peptide loading, and protein trafficking (17). Numerous proteins implicated in endocytosis and endosomal/lysosomal delivery were identified as negative regulators, likely delineating lymphoma-specific recycling and degradation pathways. (d) Desialylating cell surface MHC-I heavy chain is associated with decreased degradation (18), which may have important implications for (frequently hypersialylated) cancer cells. Oncogenes themselves may even play direct roles in MHC-I internalization and immune escape (19).

The cancer-specific examples of regulation discussed above highlight some of the myriad mechanisms that tumor cells exploit to evade T cells — additional putative avenues for posttranslational control of antigen presentation are depicted in Figure 1. Clearly, there are avenues galore for aspiring cancer cell biologists to

explore tumor antigen processing and ultimately generate new drugs and treatments to enhance cancer immunotherapy.

Acknowledgments

DD and JWY are supported by the NIH Intramural Research Program.

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