

SPOTLIGHT

New insights into IRE1 α activation and function in anti-tumor immunity

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Logue, Gorman, and Samali highlight a study by Guttman and colleagues (2022. *J. Cell Biol.* <https://doi.org/10.1083/jcb.202111068>) that shows exogenous antigen peptides imported into the ER can activate the ER stress sensor IRE1 α , attenuating cross-presentation by dendritic cells.

The accumulation of unfolded proteins in the ER triggers activation of the stress sensor IRE1 α . IRE1 α is the most evolutionarily conserved arm of the mammalian unfolded protein response pathway, working along with PERK and ATF6 in an orchestrated manner to restore proteostasis. IRE1 α is thought to be activated either by dissociation of BiP (Grp78; 1) from its ER luminal domain or, in the case of yeast IRE1 α , by direct binding of unfolded proteins to its ER luminal domain (2). The cytosolic endoribonuclease domain of IRE1 α cleaves ER-located RNAs harboring a CUGCAG sequence within a stem loop structure (3). Its targets are X-box protein 1 (XBPI) mRNA, from which IRE1 α removes a short intron producing XBPI spliced (4), as well as other mRNAs (and miRNAs) whose cleavage triggers their degradation in a process termed regulated IRE1 α -dependent decay (RIDD; 5).

Much of our knowledge of IRE1 α biology has been determined within the context of ER stress induced by addition of exogenous chemicals such as thapsigargin or tunica-mycin. While these approaches have broadened our knowledge of IRE1 α biology and function, our understanding of physiological IRE1 α activation, the mechanism(s) of its activation, as well as downstream consequences, is incomplete.

Selective activation of IRE1 α in the absence of ER stress is not well understood,

but has previously been reported in dendritic cells (DCs; 6–8). These specialized antigen presenting cells internalize, process, and present extracellular antigens to CD8 $+$ T lymphocytes. This pathway, termed cross-presentation, involves entry of the antigen into the cell by pinocytosis and its proteasomal degradation into short peptides followed by their translocation into the ER by the transporter associated with antigen processing 1 (TAPI) protein. In the ER, antigenic peptides are transferred onto major histocompatibility type I (MHC-I), which is then transported to the plasma membrane. Recently, Osorio et al. (7) identified constitutive IRE1 α activation in the absence of ER stress in CD8a $+$ DCs and demonstrated RIDD-dependent defects in antigen cross-presentation by the MHC-I machinery (7), uncovering an important role for IRE1 α signaling in DC biology. However, several important questions remained unanswered, in particular how IRE1 α was selectively activated in this setting and whether this observation could be leveraged for therapeutic benefit. In this issue of *Journal of Cell Biology*, Guttman and colleagues (9) add an important new piece to the puzzle. Using both *in vitro* and *in vivo* approaches, the authors offer important insights into selective activation of IRE1 α in DCs and its functional consequences in antigen cross-presentation

(Fig. 1), finishing with provocative experiments pointing to the therapeutic potential of targeting IRE1 α as an anti-cancer treatment.

The authors first set about demonstrating that peptides derived from exogenous antigens can directly activate IRE1 α in DCs. They initially demonstrated that pulsing mouse bone marrow-derived dendritic cells (BMDCs) with an exogenous antigen, ovalbumin, triggered rapid activation of IRE1 α , and performed careful control experiments to exclude endotoxin-mediated activation of Toll-like receptors as a potential mechanism of IRE1 α activation. They further showed that while heat-denatured and hydrophobic peptide fragments from ovalbumin could bind to a truncated luminal version of IRE1 α , native ovalbumin could not. These experiments support the idea that IRE1 α can bind and become activated by hydrophobic regions of proteins exposed by denaturation or polypeptide degradation. Mutation of the hydrophobic residues in peptide fragments of ovalbumin dramatically reduced their ability to bind IRE1 α , underscoring their importance. In addition to exogenous ovalbumin, cell lysates from various cancer lines (CT26, 4T1, EMT6) also exhibited the ability to activate IRE1 α in BMDCs, indicating this phenomenon is not restricted to ovalbumin.

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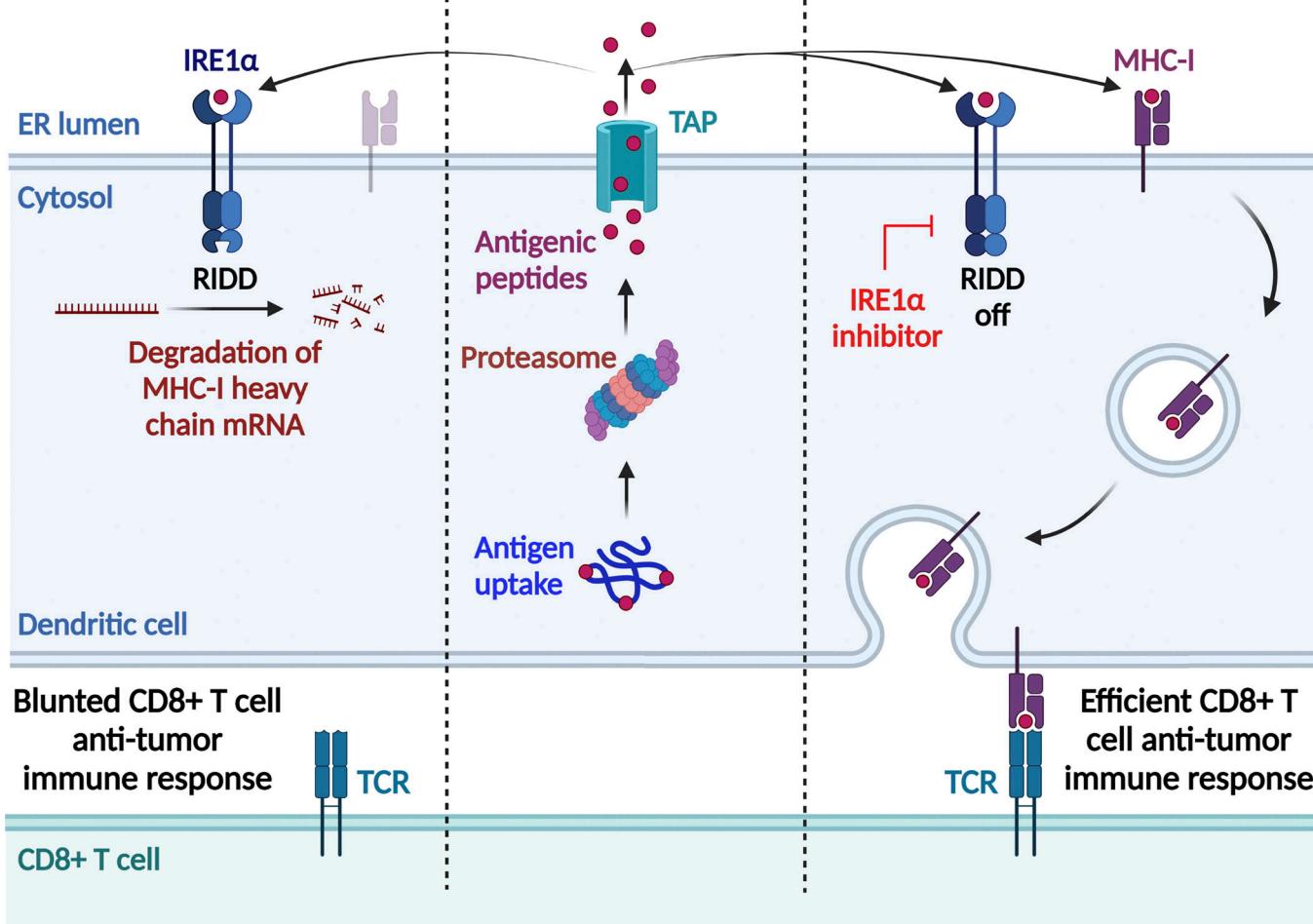
IRE1 α activeIRE1 α inhibited

Figure 1. Effect of IRE1 α on antigen cross-presentation and anti-tumor immune response. Antigenic peptides are transported into the ER lumen by the TAP transporter protein, where they can directly activate IRE1 α . Left: The endoribonuclease activity of IRE1 α stimulates RIDD, leading to degradation of the mRNA for MHC-I heavy chain. This causes a reduction in antigen presentation on the cell surface and reduced CD8 $+$ T cell anti-tumor immunity. Right: Inhibition of IRE1 α prevents RIDD, and thus increases MHC-I antigen presentation on the cell surface as well as CD8 $+$ T cell-mediated anti-tumor immunity. Created with BioRender.com.

Guttman et al. (9) next explored the consequences of IRE1 α activation for antigen cross-presentation by DCs. They verified that IRE1 α activation by exogenous ovalbumin in BMDCs required pinocytosis-dependent internalization of antigen and TAP1-mediated translocation to the ER. Using G9668, a highly selectively small molecule inhibitor of IRE1 α , they further demonstrated that BMDCs pulsed with ovalbumin in the presence of G9668 exhibited a much higher capacity to activate antigen-specific CD8 $+$ T cells than those cells treated with vehicle alone. To determine the mechanism, the authors assessed the abundance of mRNA transcripts encoding core components of the cross-presentation machinery. They identified mRNA encoding H-2K, a mouse MHC-I heavy chain, as a

potential RIDD substrate. Complementary *in vitro* and *in vivo* experiments verified RIDD mediated degradation of H-2K transcripts, which could be reversed by IRE1 α inhibition. Together, these results suggest a model where antigen import into the ER triggers IRE1 α RIDD activity in BMDCs, driving the degradation of core components of the MHC-I machinery and attenuating antigen cross-presentation (Fig. 1).

The ability of IRE1 α inhibition to enhance antigen cross-presentation raises an intriguing and pressing question—could IRE1 α inhibitors be leveraged as novel cancer therapeutics to increase anti-tumor immune responses? Guttman and colleagues (9) showed that treatment with G9668 significantly reduced growth of three syngeneic tumors, an effect that was not

dependent on IRE1 α signaling in tumor cells themselves. In the case of 4T1, the growth inhibition observed with G9668 was greater than that observed in IRE1 α knockout 4T1 tumors, suggesting an additive effect due to IRE1 α blockade within the wider tumor microenvironment. Excised tumor xeno-grafts indicated systemic IRE1 α inhibition increased MHC-I heavy chain transcript and surface expression. This increase was accompanied by increased infiltration and activation of CD8 $+$ T cells in G9668 treated mice compared to those administered vehicle alone. While immunotherapies such as programmed death ligand 1 (PD-L1) inhibitors are effective as a stand-alone treatment for some tumors, combination therapies are being explored as a way to increase their effectiveness in other cancers

such as triple-negative breast cancer (TNBC; 10). The authors tested if combination with G9668 could enhance the effectiveness of PD-L1 inhibition in orthotopic EMT6 TNBC tumor xenografts. While both G9668 and anti-mouse PD-L1 monoclonal antibody 6E11 displayed effectiveness as single agents, this was significantly enhanced by combination.

The insights provided by Guttman and colleagues (9) in this study span IRE1 α biology from the fundamental mechanisms controlling its activation to the therapeutic potential of IRE1 α inhibitors in cancer treatment. As the authors speculate, antigen peptide-mediated activation of IRE1 α in DCs could have evolved to act as a safety mechanism to reduce cross-presentation and dampen immune activation within settings such as sterile injury. However, the results presented raise several additional questions. Is peptide-mediated activation of IRE1 α functional in all DC

subtypes? Indeed, is it restricted to DC cells? Can additional peptide modalities trigger selective IRE1 α activation in other cell types? While further studies are required to answer these questions this work by Guttman et al. offers new and important insights into IRE1 α activation and its role in anti-tumor immunity.

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