SPOTLIGHT

New insights into IRE1α 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 IRE1α, attenuating cross-presentation by dendritic cells.

The accumulation of unfolded proteins in the ER triggers activation of the stress sensor IRE1α. IRE1α 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. IRE1α is thought to be activated either by dissociation of BIP (Grp78; 1) from its ER lumenal domain or, in the case of yeast IRE1α, by direct binding of unfolded proteins to its ER lumenal domain (2). The cytosolic endoribonuclease domain of IRE1α cleaves ER-located RNAs harboring a CUG/CAG sequence within a stem loop structure (3). Its targets are X-box protein 1 (XBP1) mRNA, from which IRE1α removes a short intron producing XBP1 spliced (4), as well as other mRNAs (and miRNAs) whose cleavage triggers their degradation in a process termed regulated IRE1α-dependent decay (RIDD; 5).

Much of our knowledge of IRE1α biology has been determined within the context of ER stress induced by addition of exogenous chemicals such as thapsigargin or tunicamycin. While these approaches have broadened our knowledge of IRE1α biology and function, our understanding of physiological IRE1α activation, the mechanism(s) of its activation, as well as downstream consequences, is incomplete.

Selective activation of IRE1α 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 (TAP1) 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 IRE1α activation in the absence of ER stress in CD8α+ DCs and demonstrated RIDD-dependent defects in antigen cross-presentation by the MHC-I machinery (7), uncovering an important role for IRE1α signaling in DC biology. However, several important questions remained unanswered, in particular how IRE1α 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 IRE1α in DCs and its functional consequences in antigen cross-presentation (Fig. 1), finishing with provocative experiments pointing to the therapeutic potential of targeting IRE1α as an anti-cancer treatment.

The authors first set about demonstrating that peptides derived from exogenous antigens can directly activate IRE1α in DCs. They initially demonstrated that pulsing mouse bone marrow–derived dendritic cells (BMDCs) with an exogenous antigen, ovalbumin, triggered rapid activation of IRE1α, and performed careful control experiments to exclude endotoxin-mediated activation of Toll-like receptors as a potential mechanism of IRE1α activation. They further showed that while heat-denatured and hydrophobic peptide fragments from ovalbumin could bind to a truncated luminal version of IRE1α, native ovalbumin could not. These experiments support the idea that IRE1α 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 IRE1α, underscoring their importance. In addition to exogenous ovalbumin, cell lysates from various cancer lines (CT26, 4T1, EMT6) also exhibited the ability to activate IRE1α in BMDCs, indicating this phenomenon is not restricted to ovalbumin.

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Guttman et al. (9) next explored the consequences of IRE1α activation for antigen cross-presentation by DCs. They verified that IRE1α 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 IRE1α, 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 IRE1α inhibition. Together, these results suggest a model where antigen import into the ER triggers IRE1α 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 IRE1α inhibition to enhance antigen cross-presentation raises an intriguing and pressing question—could IRE1α 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 IRE1α signaling in tumor cells themselves. In the case of 4T1, the growth inhibition observed with G9668 was greater than that observed in IRE1α knockout 4T1 tumors, suggesting an additive effect due to IRE1α blockade within the wider tumor microenvironment. Excised tumor xenografts indicated systemic IRE1α 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.

Figure 1. Effect of IRE1α 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 IRE1α. Left: The endoribonuclease activity of IRE1α 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 IRE1α 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.
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 IRE1α biology from the fundamental mechanisms controlling its activation to the therapeutic potential of IRE1α inhibitors in cancer treatment. As the authors speculate, antigen peptide-mediated activation of IRE1α 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 IRE1α functional in all DC subtypes? Indeed, is it restricted to DC cells? Can additional peptide modalities trigger selective IRE1α 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 IRE1α activation and its role in anti-tumor immunity.

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