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REVIEW

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Activation of the Unfolded Protein Response Pathway in Cytotoxic T Cells: A Comparison Between *in vitro* Stimulation, Infection, and the Tumor Microenvironment

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IRE1α is an extremely conserved intracellular receptor that regulates one branch of the unfolded protein response (UPR†). Homologs of IRE1α are found virtually throughout all eukaryotes. This receptor plays a pivotal role in a cell's reaction to stress, determining whether to take compensatory measures and survive or undergo apoptosis and die. While the role of the unfolded protein response in lower organisms and secretory cells has been comprehensively studied, the precise role of IRE1α in the context of cytotoxic T cells has only begun to be elucidated within the past decade. This review discusses what is known about IRE1α and the unfolded protein response in cytotoxic T cells within the context of development, pathogen response, and cancer cell growth.

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†Abbreviations: α, Anti- (Recognition antigen of an antibody); ATF, Activating Transcription Factor; BiP, Binding Immunoglobulin Protein; Cab45S, 45 kDa Calcium-Binding Protein (also known as SDF4, Stromal Cell Derived Factor 4); CDC37, Cell Division Cycle 37; CHOP, C/EBP (CCAAT-Enhancer-Binding Protein) Homologous Protein; DNAJB9, DnaJ Protein B9, part of the Hsp40 family; EDEM1, ER Degradation-Enhancing Alpha-Mannosidase-Like 1; eIF2α, Eukaryotic Translation Initiation Factor 2A; ER, Endoplasmic Reticulum; ERAD, Endoplasmic-Reticulum-Associated Protein Degradation; ERdj4, Also known as DNAJB9; GADD34, Growth Arrest and DNA Damage-inducible protein; GRP, Glucose-Regulated Protein; HBV, Hepatitis B Virus; HCMV, Human Cytomegalovirus; HCV, Hepatitis C Virus; HSP40, Heat Shock Protein 40; HSP90, Heat Shock Protein 90; HSV-1, Herpes Simplex Virus; I/R, Ischemia/Reperfusion; IRE1α, Inositol-Requiring Enzyme 1α; JNK, c-Jun N-terminal Kinase; M50, Nuclear Egress Protein M50; MCMV, Murine Cytomegalovirus; NS4B, Nonstructural Protein 4 B; OVA, Ovalbumin, Truncated Peptide; PDI, Protein Disulfide-Isomerase; PERK, Protein Kinase RNA-like Endoplasmic Reticulum Kinase; RACK1, Receptor For Activated C Kinase 1; RIDD, Regulated IRE1-Dependent Decay; ROS, Reactive Oxygen Species; RPAP2, RNA Polymerase II Associated Protein 2; rRNA, Ribosomal Ribonucleic Acid; S1P, Sphingosine-1-phosphate; S2P, Site-2 Protease; TCR, T Cell Receptor; TIL, Tumor-Infiltrating Lymphocyte; TRAF2, TNF Receptor Associated Factor 2; UL50, Nuclear Egress Protein 2; UPR, Unfolded Protein Response; US11, Accessory Factor US11; TUDCA, Tauroursodeoxycholic Acid; XBP-1, X-box Binding Protein 1.

Keywords: Unfolded Protein Response, UPR, IRE1α, Cytotoxic T Cells, CD8 T Cells, XBP1

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INTRODUCTION

The unfolded protein response was first recognized with the discovery of two proteins expressed after the infection of chick embryo fibroblasts and rat kidney cells with avian RNA tumor viruses in the 1970s. In both the mammalian and avian cells, viral infection led to increased expression of 73 and 95 kDa proteins [1]. Later studies demonstrated that the proteins are encoded by the host cells, not the viruses, and that the expression of these proteins could be induced by glucose deprivation, calcium disruption, or other conditions that disrupt the endoplasmic reticulum (ER) homeostasis [2]. Since these proteins were induced by glucose deprivation, they were named glucose-regulated proteins (GRP) 78 and 94. In parallel, GRP78 was identified as an immunoglobin heavy chain binding protein, BiP [3]. Today we recognize BiP as a calcium-dependent chaperone and a central regulator of the unfolded protein response (UPR) [4,5].

In humans there are three main activating receptors that constitute the unfolded protein response: IRE1α, PERK, and ATF6. All three receptors reside on the ER with a receptor domain in the ER lumen that can bind to BiP (Figure 1). IRE1α is the most highly conserved receptor of the three, with homologs in mice, yeast, and *Arabidopsis.* In fact, human IRE1α inhibitors show activity in yeast and *Arabidopsis* [6]. IRE1α contains receptor, transmembrane, kinase, and RNase domains. Once BiP dissociates from IRE1α, it is capable of dimerizing and undergoing auto-transphosphorylation. There is some evidence in yeast and human that IRE1α can bind unfolded proteins directly, facilitating this process as well [7,8]. IRE1α itself is the only known target of the IRE1α kinase domain.

The phosphorylation of IRE1α facilitates the binding of adapter proteins, such as TRAF2 [9], and activates the RNase domain, which mediates regulated IRE1-dependent decay of mRNA (RIDD) and specific splicing of a 26-nt intron from XBP1 mRNA. This leads to the translation of a longer XBP1 isoform, XBP1s [10]. XBP1s is a potent transcription factor that increases pro-survival factors, such as hexosamine pathway enzymes [11], endoplasmic-reticulum-associated degradation (ERAD) proteins [12,13], chaperones [13], and XBP1 itself [14]. Eventually, IRE1α undergoes a higher-order oligomerization and forms foci that appear to have different functional characteristics. Specifically, they seem to promote XBP1 splicing over the generally pro-apoptotic process of RIDD [15]. In humans, there is also an *IRE1β* gene capable of binding TRAF2, splicing XBP1, and RIDD; however, this protein is only expressed in gastrointestinal epithelial cells and aberrant expression of this receptor leads to degradation of 28s rRNA and cell death [16,17].

PERK, like IRE1α, contains receptor, transmembrane, and kinase domains. Also, like IRE1α, PERK undergoes dimerization and auto-transphosphorylation upon dissociation from BiP. Unlike IRE1α, however, PERK is capable of phosphorylating additional factors, namely the translation initiation factor eIF2α. This phosphorylation halts most translation and initiates translation from alternative open reading frames in select transcripts, including ATF4. ATF4 is a transcription factor that initiates accumulation of the pro-apoptotic factors CHOP and GADD34 [18].

ATF6 has divergent properties from IRE1α and PERK, and is the least studied of the three receptors. Once BiP dissociates from ATF6, the receptor translocates to the Golgi apparatus and is cleaved by S1P and S2P to form a 50 kDa soluble transcription factor [19,20]. One target gene of ATF6 includes the IRE1α pathway effector *XBP1*, possibly acting as a priming mechanism for the IRE1α pathway [14]. XBP1s, ATF4, and CHOP further activate an overlapping gene expression program including target genes like *DNAJB11*, *PDIA6*, and *GFAT1*, among others (see overlapping target pathways in Figure 1).

THE REGULATION OF IRE1α

IRE1α activation is predominantly spontaneous upon dimerization/oligomerization, which facilitates the auto-transphosphorylation of the activation loops [21-23]. Classically, BiP is considered sufficient for holding IRE1α as a monomer and inactive [4]; however, several studies have shown that BiP binding to IRE1α (or its homologs) alone is not sufficient. In some instances, direct BiP-IRE1α association modulates the activity of IRE1α rather than completely inactivating the receptor [24-26]. The protein ERdj4 (DNAJB9) binds IRE1α while IRE1α is still dimerized and facilitates ATP hydrolysis, which then increases the affinity of BiP for IRE1α and disrupts IRE1α signaling [27]. Cab45S also stabilizes BiP-IRE1α interactions, decreasing the accumulation of XBP1 protein and phospho-c-JNK [28]. While the binding of the chaperone BiP to IRE1α's ER luminal domain attenuates IRE1α signaling, and binding to the chaperone HSP90 and its co-chaperone CDC37 in IRE1α's cytoplasmic domain also attenuates IRE1α signaling, it appears that the long-term binding of HSP90 to IRE1α stabilizes the protein and maintains the ability of the UPR to activate [29,30]. In addition to interactions with other proteins, an intramolecular IRE1α interaction also prevents IRE1α signaling. Specifically, an intrinsically disordered N-terminal domain called subregion I binds to the core stress-sensing region and prevents it from binding unfolded proteins and IRE1α from forming high-order oligomers [31].

Recently two mechanisms for IRE1α signaling attenuation upon prolonged ER stress have been elucidated. First, phosphorylation of eIF2α by PERK leads to the

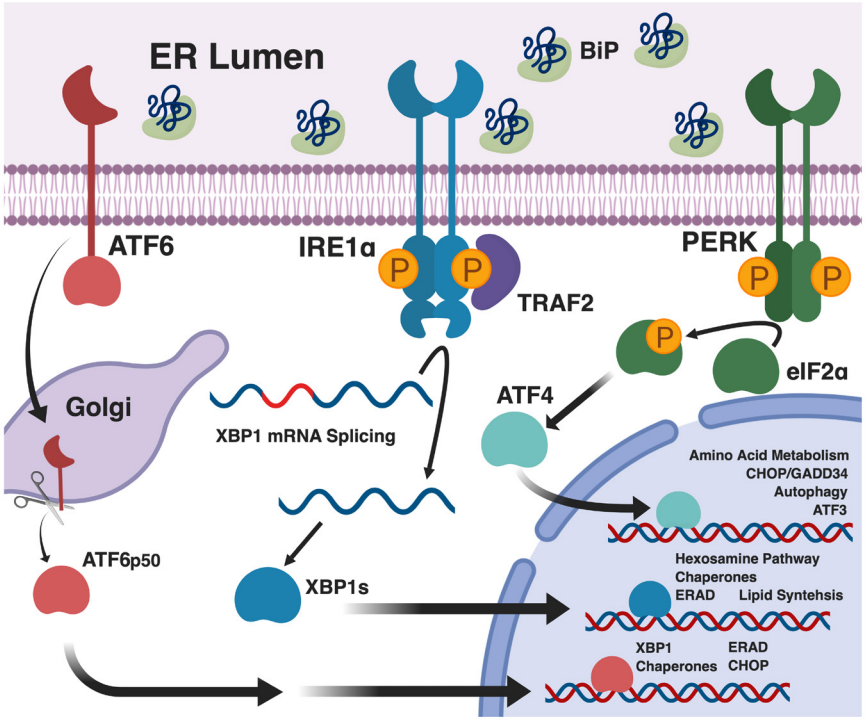

Figure 1. An overview of the 3 unfolded protein response pathways. ATF6 (left) is translocated to the Golgi apparatus after activation and cleaved. The p50 fragment translocates to the nucleus where it functions as a transcription factor. IRE1α (middle) undergoes dimerization and auto-transphosphorylation when activated. Once phosphorylated, IRE1α binds adapter molecules such as TRAF2. Phosphorylation also activates the RNase domain, which is capable of splicing a regulatory intron from XBP1 mRNA. The spliced XBP1 mRNA then encodes a potent transcription factor, XBP1s. IRE1α also decays other mRNAs and undergoes oligomerization (not pictured). PERK (right) undergoes dimerization and auto-transphosphorylation when activated as well. Phosphorylated PERK can phosphorylate eIF2α, leading to alternative translation of ATF4 mRNA. ATF4 is a potent transcription factor. Figure generated with Biorender.

selective translation of the phosphatase RPAP2, which dephosphorylates IRE1α and thereby turns off IRE1α signaling while leaving PERK signaling intact [32]. This first pathway ultimately leads to apoptosis. Second, AKT-mTOR signaling helps re-establish ER-mitochondrial contacts following ER stress, which dephosphorylates IRE1α and attenuates its signaling [33]. This second pathway limits IRE1α without maintaining PERK signaling and thus limits apoptosis and improves cell survival.

Viruses are also potent activators of the unfolded protein response and have evolved to manipulate the IRE1α pathway to increase viral production and decrease apoptosis. Broadly, mammalian viruses actively inhibit the splicing and expression of XBP1, but selectively activate other branches of the IRE1α pathway. For example, the M50 protein from MCMV (murine cytomegalovirus), UL50 protein from HCMV (human cytomegalovirus), and UL41 protein from HSV-1 (Herpes Simplex Virus) all actively suppress the expression of XBP1 [34,35] and XBP1-deficient cells demonstrate decreased apoptosis with HCV (Hepatitis C Virus) infection [36]. HBV (Hepatitis B Virus) similarly appears to have reduced viral production with the expression of the XBP1 target gene *EDEM1* [37]. Conversely, HCMV, HBV, and HCV all appear to promote activation of other branches of the IRE1α pathway (or UPR in general). HCMV uses the US11 protein to promote the degradation (similar to ERAD) of the MHC-I molecules from host cells as a mechanism of immune escape and this is not dependent on XBP1 [38]. Both HBV and HCV target the promoters of IRE1α/UPR genes *BiP* and *Hsp90* to increase chaperone expression using the Large Surface protein or E2 protein for HBV and HCV, respectively [39,40]. The NS4B protein from HCV promotes RIDD to degrade the pro-apoptotic miRNA miR-125a; thereby increasing cell survival and consequently viral production [36]. Broadly, it appears that while viruses utilize the UPR to increase replication, they appear capable of specifically modulating the different components of the UPR in order to optimize viral production.

THE ROLE OF IRE1α IN LEUKOCYTES

IRE1α and XBP1 knockouts are embryonic lethal due to a malformation of placental tissue [41], so most studies to-date have used conditional cell lineage deletion. One consistent theme emerges – IRE1α and XBP1 are essential in highly secretory leukocytes. The most canonical example is that XBP1 is essential to the end-stage differentiation of B cells into plasma cells. Without XBP1, plasma cells do not form and immunoglobin production is inhibited [42]. Inhibition of XBP1 splicing in plasma cells is lethal and has even been proposed as a potential therapeutic strategy [43]. In addition, IRE1α/XBP1 signaling is essential for effector functions in many other highly secretory leukocytes such as pro-inflammatory macrophages, CD8+ dendritic cells, NK cells, eosinophils, and Th2 (CD4+) T cells. This is evident by a reduction in cytokine production, effector molecule release, and antigen processing/presentation upon loss of IRE1α or XBP1 [44-50].

THE ROLE OF IRE1α IN HYPOXIA

Analysis of ischemia-reperfusion injury may offer some perspective of T cell behavior in tumors, as both scenarios have to navigate a hypoxic environment. Ischemia-reperfusion injury (I/R) is when a temporary occlusion of an artery prevents circulation to a part of the body and is followed by a resurgence of blood flow to the affected area. Often, this is studied in the context of the heart as a myocardial infarction, but also occurs in the brain, liver, and intestines. The process of I/R mimics known activators of the UPR – the initial hypoxic environment with ischemia causes perturbations in Ca^{2+} flux and the subsequent reperfusion leads to the production of reactive oxygen species (ROS) in the heart [51]. Despite the fact that these alterations activate all three branches of the UPR [52], the PERK pathway appears to increase cellular damage while the IRE1α pathway is cytoprotective. CHOP deficient mice (a PERK pathway protein) experience less tissue damage, less DNA damage, and reduced inflammatory gene expression after I/R compared to wild-type mice despite increases in XBP1 after injury [53].

Comparatively, XBP1 is central to the cytoprotective roles of the IRE1α pathway and splicing of XBP1 can be detected in as little as 5 minutes after the initiation of ischemia [11]. XBP1 as a transcription factor then stimulates the hexosamine pathway and expression of BiP to help decrease injury during and after I/R [11,54]. Outside of XBP1, the IRE1α pathway also increases Protein Disulfide-Isomerase (PDI) expression to increase survival and stimulates the RACK1 pathway to prevent apoptosis by increasing the phosphorylation of BCL-2 [54,55]. Broadly, pro-apoptotic, necrotic, and inflammatory aspects of the PERK pathway during I/R appear to overshadow the cytoprotective aspects of IRE1α since globally decreasing ER stress with 4-phenylbutyric acid or TUDCA reduces apoptosis, necrosis, and inflammation [52,56]. Thus, under the hypoxic stress of I/R inhibiting the PERK pathway or stimulating the IRE1α pathway reduces cellular damage. Similar changes may also help protect T cells in the hypoxic tumor microenvironment. Interestingly, culturing cytotoxic T cells under 1% oxygen increased their production of granzyme-B and their ability to kill target cancer cells [57]. Although a transcriptomic analysis of these hypoxic T cells revealed higher levels of Glut-1 and glycolysis-related genes, full mRNA profiling was not reported. It is interesting to speculate whether changes in the UPR might correspond to those observed in I/R injury.

THE UNFOLDED PROTEIN RESPONSE IN T CELLS DURING ACTIVATION AND INFECTION

Multiple studies have shown that the UPR is activated almost immediately after TCR stimulation in CD8+ T cells. Cao *et al*. demonstrated that PERK and IRE1α phosphorylation occurs within 48 hours of αCD3/αCD28 stimulation and is accompanied by the expression of downstream effector molecules [58]. The phosphorylation of these receptors is one of the first steps in the activation of the UPR pathways, but is more difficult to measure than mRNA splicing. Also, Cao *et al*. did not measure extremely early timepoints after stimulation. Kamimura and Bevan showed that IRE1α splicing of XBP1 mRNA is detectable in as little as 2 hours after αCD3/αCD28 stimulation *in vitro* [59]. The splicing of XBP1 and phosphorylation of IRE1α and PERK are not dependent on transcription or translation, so these are the earliest detectable changes. Further down the IRE1α pathway, the XBP1 target gene BiP (GRP78) is up-regulated at 6 hours after CD3 stimulation or alternative activation with PMA/ionomycin and is critical to preventing activation-induced apoptosis [60]. This 6-hour timepoint was the earliest timepoint measured by Takano *et al*. *In vivo*, the activation of the CD8+ T cells is thought to be slower and this would explain why XBP1 splicing was observed at day 5 after infection with LCMV or Ova-*Listeria* [59], or how increases in BiP occurred in T cells 2 days following stimulation in a H2-K^b-recognizing transgenic mouse model [61].

Beyond these few studies focused on the UPR in CD8+ T cells, are several global genomic studies on cytotoxic T cell activation. Best *et al*. gathered broad transcriptomic data from CD8+ T cells activated using an *in vivo* Ova-*Listeria* model of infection. An investi-

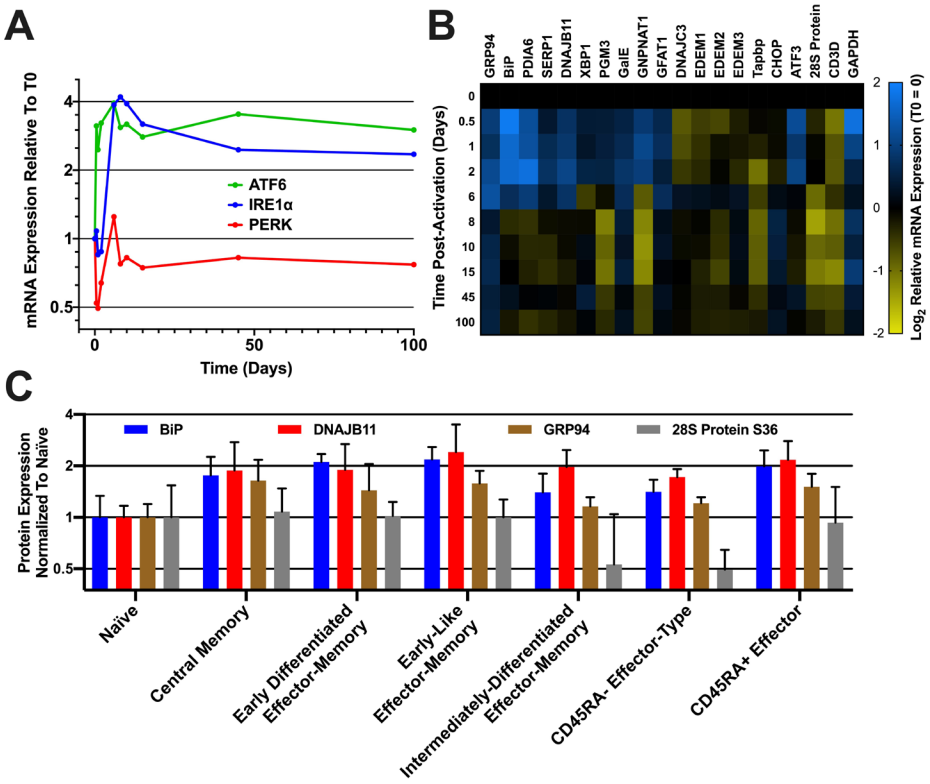

Figure 2. Expression of UPR regulated genes in T cells. A. Transcription of unfolded protein response receptors after introduction of an OVA-expressing *Listeria monocytogenes* model antigen *in vivo* to C57BL/6 mice with T cells that have a T cell receptor that recognizes OVA. T0 is RNA obtained from mouse CD8+ T cells at time 0 before infection. **B.** Transcription of IRE1α/XBP1-target mRNAs, PERK pathway mRNAs, and reference mRNAs after *in vivo* activation of CD8+ murine T cells. Genes 1-14 (GRP94 through EDEM3) are XBP1 target genes; PGM3, GalE, GNAPNAT1, and GFAT1 are also hexosamine pathway genes. Tapbp is a target of IRE1α decay and decreases with IRE1α activation. CHOP and ATF3 are PERK pathway genes. 28S Protein, CD3D, and GAPDH are included for reference. **C.** Protein expression of XBP1 target genes and ribosomal 28S protein S36 in sorted peripheral human CD8+ T cells. Transcriptomic data is from Best *et al*. 2013 and then microarray data is available from the accession number: GEO: microarray data, GSE15907. Mass spectrometry data is from Aalderen *et al*. 2017 with the raw data available from the accession number: ProteomeXchange Consortium: PXD004637.

gation of chaperones and other IRE1α/XBP1 target gene mRNAs, reveals that these genes are acutely up-regulated in as little as 12 hours after stimulation (Figure 2A, B). Following this acute increase in IRE1α pathway gene mRNA expression, there is a chronic increase in IRE1α mRNA. Comparatively, PERK mRNA is unchanged or mildly decreased following activation [62].

Cytotoxic T cell activation shares many characteristics with known activators of the UPR, so it is logical that they would be activated together. First, CD3ζ signaling leads to calcium efflux from the ER into the cytoplasm through the IP3 receptor. This process is critical to T cell activation, and similar to the potent UPR activators thapsigargin and calcium ionophore A23187 [63]. While the calcium ionophore ionomycin is not potent enough to activate the UPR, it is used in combination with PMA to activate T cells [61,64]. In fact, PKC activation following T cell stimulation was necessary for UPR activation, as measured by increased expression of BiP, other chaperones, and other ER proteins [61]. Second, T cell activation is followed by a massive up-regulation in protein production that eventually leads to a doubling of T cell volume from ~5μm in diameter to ~10μm [65]. Similarly, most secretory cells (plasma cells, goblet cells, acinar cells, and Paneth cells) activate IRE1α in order to properly fold and maintain turnover of the massive amounts of protein they produce [66]. Third, activated T cells increase the production of reactive oxygen species (ROS) as effector molecules (*e.g.* NO, O_2^-, or $OH^\bullet$), as secondary messenger molecules for activation, or as a byproduct of increased metabolism, which are all then countered chiefly by increased production of the reducing agent glutathione [67,68]. Similarly, both oxidizing and reducing agents (*e.g.* ROS and DTT, respectively) are potent stimulators of the UPR because they cause proteins to denature, partially through the disruption of

disulfide bonds [69,70]. Fourth, activated CD8+ T cells greatly increase their glutamine and glucose metabolism, which through the hexosamine pathway increase the production of the amino sugar UDP-GlucNAc, an essential metabolite for glycoprotein and glycolipid synthesis [71]. In other cell types, XBP1 is a potent stimulator of the hexosamine pathway by directly up-regulating the expression of hexosamine pathway proteins, including the rate-limiting enzyme GFAT1, thereby relieving stressed cells by increasing GlucNAc and protein glycosylation [11]. In contrast, tunicamycin inhibits N-linked glycosylation and is a potent UPR activator [63]. Moreover, while TRAF2 is an important adapter molecule for IRE1α signaling, it is also the central mediator of 4-1BB signaling (a T cell costimulatory receptor) [9,72]. Taken together, there are multiple reasons why the IRE1α pathway would be stimulated during CD8+ T cell activation and be cytoprotective.

During T cell differentiation, XBP1 splicing positively correlates with CD8 expression, indicating activation of IRE1α. Once thymic T cells mature into double positive (CD4+, CD8+) lymphocytes, XBP1 splicing is up-regulated, but the activity of IRE1α is partially lost once T cells migrate out of the thymus. Splenic CD8+ T cells maintain this increase in IRE1α activity, whereas splenic CD4+ T cells as a whole lose IRE1α activation in C57B/6 mice (a strain that contains more Th1 CD4+ T cells than Th2 CD4+ T cells [73]), indicating a potential preference for IRE1α in one lineage over the other [74]. Based on a study in CD8+ T cells, this increase in IRE1α activity without attenuation by PERK may be due to an increase in IRE1α protein expression, similar to the increase in IRE1α mRNA expression that occurs in CD8+ T cells with stimulation (Figure 2A). In particular, IRE1α is important for production of IL-4, IL-5, and IL-13 from Th2 T cells, but does not contribute to IFN-γ or IL-17 in Th1 or Th17 T cells, respectively [49,50].

Best *et al*. also noted distinct transcriptional signatures were associated with different terminal differentiation states in CD8+ T cell lineages. Within these transcriptional signatures, BiP and other chaperones were classified as part of the initial effector response, IRE1α with late effector-memory T cells, PERK with early effector and late memory T cells, and ATF6 was left un-clustered during the un-biased analysis [62]. ATF6 might act as a priming mechanism for IRE1α because ATF6 increases XBP1 RNA expression [14]. The association of IRE1α with differentiation past effector-memory cells is collaborated by the fact that *Xbp1*$^{-/-}$ CD8+ T cells demonstrate reduced end-stage differentiation into effector CD8+ T cells *in vivo* [59]. As further evidence of the role for IRE1α and chaperones in CD8+ T cell differentiation, BiP and other XBP1 target chaperones are up-regulated through CD8+ T cell differentiation based on proteomic data from Aalderen *et al*. (Figure 2C) [75] and BiP-deficient mice demonstrate reduced CD8+ T cell effector functions [76].

Altogether it appears that the IRE1α pathway is involved in the natural process of CD8+ T cell activation and differentiation. While there is limited functional data on the role of IRE1α in T cell activation and differentiation, there is a strong body of correlational expression evidence suggesting a role for IRE1α with the natural CD8+ T cell effector response.

THE UNFOLDED PROTEIN RESPONSE IN THE T CELL RESPONSE TO TUMORS

Given the emerging role of the UPR in T cells responding to infections, it is reasonable to hypothesize that the UPR might also contribute to T cells responding to cancer cells. Activation of the UPR within cancer cells by dietary restriction leads to cytokine production, recruitment of immune cells and a CD8+ T cell mediated response [77]. However, to our knowledge, there are only two papers specifically investigating the role of the UPR in tumor-infiltrating lymphocytes (TILs) themselves. The first, published in late 2018, examines the role of IRE1α-XBP1 in (IFNγ+, Th1-like) CD4+ TILs [78] and the second, published in early 2019, investigates the role of PERK-CHOP in CD8+ TILs [58]. Together these papers evaluate two distinct subsets of T cells with two distinct roles in the tumor microenvironment (TME). As CD8+ T cells naturally activate IRE1α during differentiation, while CD4+ T cells do not [74], these papers also exhibit a difference in UPR activation in the tumor microenvironment compared to normal differentiation.

In the first paper, Song *et al*. noted that XBP1 splicing (and by extension IRE1α activation) is induced in the TME in T cells. Interestingly, the induction of XBP1 splicing in the TME compared to peripheral blood is seen most dramatically in the CD4+ T cells, whereas CD8+ T cells have statistically equivalent XBP1 mRNA splicing in peripheral blood and the TME and equivalent to the XBP1 mRNA splicing found in peripheral blood CD4+ T cells [78]. The expression of XBP1s was deleterious to glutamine metabolism, which is necessary for T cells to function under the hypoglycemic stress of the TME. Proteasomal inhibition appeared to mitigate some of the effects of XBP1 splicing, possibly indicating that XBP1 increased ERAD-mediated decay of glutamine transporters, thereby decreasing glutamine metabolism. Song *et al*. also noted a statistically significant correlation between IRE1α/XBP1 activation and CHOP mRNA expression, and a significant inverse correlation between TIL penetration and CHOP expression [78]. Overall, it appears that the IRE1α pathway is activated in CD4+ T cells in the TME, but possibly not CD8+ T cells.

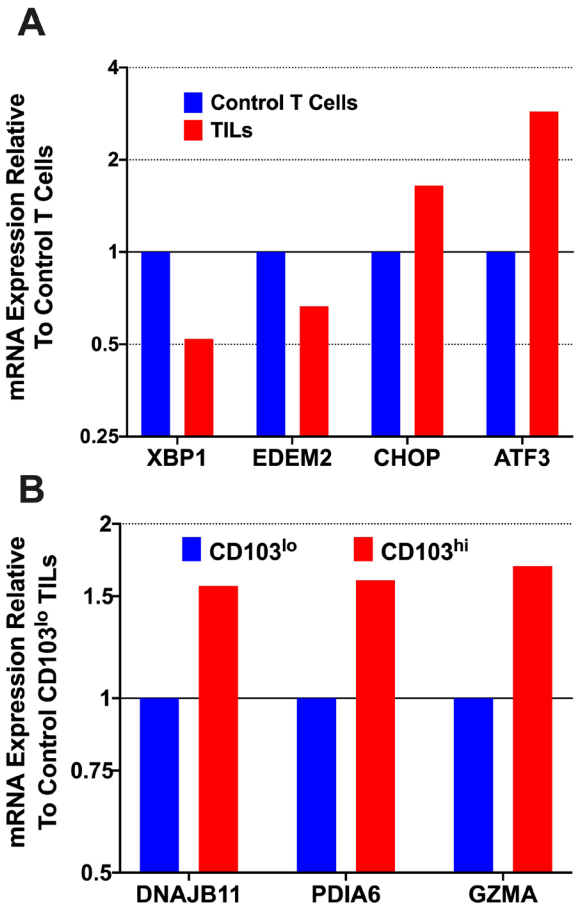

Figure 3. Expression of UPR related genes in TILs. A. Transcription of IRE1α pathway and PERK pathway in tumor infiltrating CD8+ T cells compared to control CD8+ T cells from adjacent, tumor-free tissue (n=36). XBP1 and EDEM2 mRNA is increased when the IRE1α pathway is activated and CHOP and ATF3 mRNA is increased when the PERK pathway is activated. **B.** Comparative mRNA expression of IRE1α pathway genes (*DNAJB11* and *PDIA6*) and Granzyme A (GZMA) from CD8+ tumor-infiltrating T cells with enhanced ($CD103^{hi}$; n=7) or decreased ($CD103^{lo}$; n=5) cytotoxicity. For **A** and **B**, all differences between samples are significant (adjusted $p < 0.05$). Transcriptomic data is from Ganesan *et al*. 2017 and available from the accession number: GEO: GSE90730.

In the second paper, Cao *et al*. investigated the role of the PERK pathway in CD8+ T cells in the TME. They found that PERK was activated in the TME for CD8+ T cells and that canonical ATF4-induced CHOP expression directly repressed the anti-tumor activity of T cells through a direct repression of T-bet, a master regulator of CD8+ T cell effector functions [58]. This is in agreement with a study by Baitsch *et al*. that found increased levels of ATF4 as well as several genes associated with T cell exhaustion in CD8+ T cells in lymph nodes infiltrated by melanoma compared to peripheral blood CD8+ T cells [79]. It is possible that this aberrant PERK activation results in an increased negative feedback on IRE1α activation through RPAP2, possibly explaining why IRE1α activation was not detected in CD8+ TILs from Song *et al*. [78].

In contrast to Cao *et al*.'s study demonstrating a negative role for ATF4 in the tumor response of CD8+ TILs, in peripheral blood CD4+ T cells ATF4 is necessary to drive a metabolic adaptive response to amino acid starvation and oxidative stress [80]. The difference between this beneficial effect of ATF4 on metabolic reprogramming in CD4+ T cells and its negative effect on anti-tumor activity in CD8+ T cells could be due to differences between the tumor microenvironment and stressed peripheral blood T cells, intrinsic differences between CD4+ and CD8+ T cells, such as alternate transcription factors in CD4+ T cells like Eomes modifying the response, or differences in the functional assays that were tested, where a positive metabolic response of increased amino acid production and glycolysis does not necessarily result in better cytotoxicity.

Further insight on the role and status of IRE1α in CD8+ TILs may be derived from larger transcriptomic

studies. Ganesan *et al*. performed whole-population RNA sequencing on CD8+ TILs from patients with treatment-naïve non-small cell lung cancer and CD8+ T cells from adjacent tumor-free tissues as controls [81]. Comparing CD8+ TILs and control T cells reveals a significant up-regulation of PERK pathway genes and a down-regulation of IRE1α/XBP1-pathway genes (Figure 3A). This provides further evidence that there is an impairment in the UPR activation in T cells in the TME compared to activated T cells in an infection. Ganesan *et al*. also found that tumors enriched for tissue-resident memory T cell genes (*e.g.* CD103) were strongly correlated with a favorable patient prognosis and increased T cell effector functions. Among the genes more significantly expressed in the CD8+ TILs of those tumors enriched for $CD103^{hi}$ expression were *DNAJB11* and *PDIA6*, two IRE1α/XBP1 pathway genes (Figure 3B) [81-83]. This could possibly indicate that IRE1α provides a fitness advantage to CD8+ T cells in the tumor microenvironment. However, to confirm this, further studies on IRE1α itself, its multiple regulators, and the interdynamics of the three UPR pathways (IRE1α, PERK, and ATF6) are necessary.

CONCLUSIONS AND OUTLOOK

Cell death is inevitable without a proper reaction to stress, and sometimes a cell decides that death through apoptosis is better than risking the life of the entire organism. The unfolded protein response handles that stress and decides between life and death on a molecular level. In multiple different cell lineages, the IRE1α pathway is activated constitutively or as a cytoprotective mechanism to cell stress. The IRE1α/XBP1 pathway plays a cytoprotective role in cells with external stress (*e.g.*, ischemia/reperfusion injury) or internal stress (*e.g.*, having a large secretory burden). This is because the IRE1α/XBP1 reduces ER stress and enhances the protein-folding capacity of these cell types, whereas the PERK/CHOP pathway typically is pro-apoptotic and counters the IRE1α/XBP1 pathway. The third UPR pathway, ATF6, is the least studied of the three pathways and can be seen as a primer for the IRE1α pathway by increasing XBP1 mRNA expression before XBP1 is active and forms a positive feedback loop.

Cytotoxic T cell activation causes radical cellular changes and causes activation of the UPR. It is logical that CD8+ T cells would use the IRE1α pathway as a cytoprotective mechanism to maintain the secretory capacity of these cells and reduce the amount of ER stress since this is shared behavior observed in other cell lineages. However, few studies have directly investigated the roles of the IRE1α pathway in cytotoxic T cell differentiation and effector functions. Those that have, support the hypothesis that IRE1α promotes CD8+ T cell health, and the rest show a large amount of correlational evidence between IRE1α activity and CD8+ T effector functions during activation and infection. However, it appears that the IRE1α pathway is not activated in CD8+ T cells in the tumor microenvironment and instead the PERK pathway is predominantly active at the loss of CD8+ T cell effector function. This may be one potential explanation why T cells are unable to successfully eliminate cancer cells in a tumor. Further studies into the roles of IRE1α and the UPR pathways in CD8+ T cell survival and effector functions will help guide future cancer therapies and increase our understanding of T cell physiology.

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