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Abbreviations: ATF6, activating transcription factor 6; CAFs, cancer-associated fibroblasts; CALR, calreticulin; CRYAB, chaperone αB-crystallin; CTLs, cytotoxic T lymphocytes; CX, connexin; DAMPs, damage-associated molecular patterns; DCs, dendritic cells; DLBCL, diffuse large B-cell lymphoma; ECs, endothelial cells; EMT, epithelial-to-mesenchymal transition; ER, endoplasmic reticulum; ERAD, ER-associated degradation; GADD34, growth arrest and DNA damage 34; GCB, germinal center B-cell; GJIC, gap junctional intercellular communication; GRP78, ER chaperone glucose-regulated protein 78; HSPs, heat shock proteins; ICD, immunogenic cell death; IRE1α, inositol requiring enzyme 1 alpha; IRES, internal ribosome entry sites; IRS, integrated stress response; MCP-1, monocyte

chemoattractant protein-1; MDSCs, myeloid-derived suppressor cells; MEF, mouse embryonic fibroblast; MKC, ManKindCorp; MM, multiple myeloma; PERK, protein kinase PKR-like ER kinase; PRRs, pattern recognition receptors; RIDD, regulated IRE1-dependent decay; RIG-I, retinoic acid-inducible gene-I; ROS, reactive oxygen species; TERS, transmissible ER stress; TILs, tumor-infiltrating lymphocytes; TLR2, toll-like receptor 2; TME, tumor microenvironment; TUDCA, tauroursodeoxycholate; uPCA, urokinase plasminogen activator; UPR, unfolded protein response; XBP1, X-box binding protein-1.

Key words: ER stress, unfolded protein response, tumor microenvironment, immunogenic cell death, inflammation.

Abstract

The unfolded protein response (UPR) is a conserved adaptive pathway that helps cells cope with the protein misfolding burden within the endoplasmic reticulum (ER). Imbalance between protein folding demand and capacity in the ER leads to a situation called ER stress that is often observed in highly proliferative and secretory tumor cells. As such, activation of the UPR signaling has emerged as a key adaptive mechanism promoting cancer progression. It is becoming widely acknowledged that, in addition to its intrinsic effect on tumor biology, the UPR can also regulate tumor microenvironment. In this review, we discuss how the UPR coordinates the crosstalk between tumor and stromal cells such as endothelial cells, normal parenchymal cells and immune cells. In addition, we further describe the involvement of ER stress signaling in the response to current treatments as well as its impact on anti-tumor immunity mainly driven by immunogenic cell death. Finally, in this context we discuss the relevance of targeting ER stress/UPR signaling as a potential anti-cancer approach.

1. The UPR is an adaptive mechanism in cancer cells

Excessive endoplasmic reticulum (ER) stress is emerging as a hallmark of solid tumors. Cancer cells due to their high proliferative and secretory demands are at risk of the accumulation of improperly folded proteins in the ER lumen, which perturb the protein homeostasis (referred to as proteostasis) [1]. In addition, tumor cells are constantly exposed to the microenvironmental pressure such as hypoxia, glucose shortage, oxidative stress or low pH, all known to cause ER stress [2]. To cope with

those challenges and restore proteostasis, cells activate the evolutionary conserved adaptive pathway known as unfolded protein response (UPR) by the coordinated action of three ER transmembrane proteins, namely the activating transcription factor 6 (ATF6), the protein kinase PKR-like ER kinase (PERK) and the inositol requiring enzyme 1 alpha (IRE1α, referred to as IRE1 hereafter) [2-4]. The current dogma in mammalian cells indicates that under non-stressed conditions, the ER chaperone glucose-regulated protein 78 (GRP78, also known as BiP) constitutively binds to the luminal domain of the three sensors precluding their activation. However, upon accumulation of unfolded/misfolded proteins in the ER, GRP78 dissociates from those complexes consequently triggering the UPR cascade [4]. It was also proposed that direct association of unfolded proteins to yeast and mammalian IRE1 could induce its activation through conformational change [5, 6]. Upon ER stress, ATF6 is exported from the ER to the Golgi apparatus, where it is activated by the SP1 and SP2-medited proteolytic cleavage, which releases the cytosolic fragment of the protein, ATF6f [7, 8]. The latter is further translocated to the nucleus to regulate the transcription of genes involved in ER-associated degradation (ERAD) and protein folding [9]. To reduce the protein misfolding burden in the ER, activated PERK phosphorylates the eukaryotic translation initiation factor eIF2α at serine 51, hence attenuating global protein synthesis [7, 10]. This mechanism also allows the translational activation of the transcription factor ATF4, which controls the expression of genes impacting on amino acid metabolism, antioxidant response, autophagy, apoptosis and protein folding [2, 7, 11]. Lastly, in response to ER stress, IRE1, which harbors serine/threonine kinase and endoribonuclease (RNase) activities, dimerizes/oligomerizes and auto-transphosphorylates. Active IRE1 catalyzes the unconventional splicing of X-box binding protein-1 (XBP1) mRNA and together with RTCB ligase yields an active transcription factor XBP1s. Consequently, XBP1s modulates the expression of genes involved in glycosylation, ERAD, protein folding and lipid synthesis [4, 12]. IRE1 endoribonuclease activity also targets other mRNAs and micro-RNAs in a process called regulated IRE1-dependent decay (RIDD), which controls cell fate under ER stress conditions [13]. Finally, IRE1 activates the ASK1/JNK1 signaling pathway through the recruitment of TRAF2 to IRE1 [14]. Depending on the time and duration of the ER stress, each arm of the UPR can either trigger the adaptive response to alleviate the ER stress or activate the pro-death signals when ER stress cannot be resolved (terminal UPR; reviewed in [15]). Briefly, the adaptive UPR relies on the activation of among others $p58^{IPK}$, chaperones, foldases and antioxidant enzymes as well as inhibition of the pro-apoptotic CHOP [15]. Pro-death UPR, on the other hand, engages JNK- and NFκB-dependent activation of pro-apoptotic BCL-2 family proteins, induction of CHOP and inhibition of anti-apoptotic miR-106b-25 [15].

1.1 Activation of the UPR in cancer

The overexpression of UPR sensors has been reported in a large number of human cancers including that of breast, brain, gastrointestinal tract, liver, kidney, pancreas, lung and prostate [8]. In addition, elevated level of the main UPR regulator GRP78 is often found in tumor tissues [16] and is associated with metastasis, poor prognosis and resistance to treatment [17-19]. The UPR involvement in cancer initiation and cell transformation is particularly well-documented in gastrointestinal and blood cancers (reviewed in [10]). For instance, the PERK/eIF2α axis is shown to trigger the loss of stemness in intestinal stem cells, from which most of the colorectal cancers evolve [20, 21]. Similarly, XBP1 deficiency in epithelial cells of the intestine leads to the higher incidence of colorectal cancer and colitis-associated cancer [22]. The IRE1/XBP1 pathway is also necessary for the terminal differentiation of B cells into plasma cells and is frequently upregulated in multiple myeloma (MM) [10]. Moreover, high levels of XBP1s correlate with advanced MM stages and predict poor outcome [23]. The importance of UPR signaling in cancer development is also supported by the number of cancer-associated mutations identified in the three UPR sensor–encoding genes [4]. Interestingly, the somatic mutation profiles of the UPR arms are distinct, with majority of IRE1 and ATF6 mutations reported in gastrointestinal cancers, PERK and ATF6 in urologic and lung cancers, while ATF6 mutations were predominantly found in genital cancers [4]. Finally, elevated ER stress has been also observed in RAS-, BRAF-, MYC-, RET-, and HER2-driven tumorigenesis [24].

1.2 UPR and cancer hallmarks

Mounting evidence suggests that UPR signals support tumor progression by modulating almost all of the cancer hallmarks (as reviewed elsewhere [7, 10, 25, 26]) (**Figure 1**). Genetic ablation of IRE1/XBP1, ATF6 and PERK as well as usage of the specific inhibitors targeting the UPR arms lead to the significant reduction of tumor growth both *in vitro* and *in vivo* [27-29]. PERK/eIF2α signaling is also required for tumor cells to overcome apoptosis under hypoxia, oxidative stress or glucose deprivation [30], which involves various downstream signaling cascades such as AKT activation, induction of glutathione synthesis or mTOR inhibition [31-33]. In contrast, disseminated or circulating cancer cells are often exposed to the inhospitable conditions which prime them to enter a dormancy. Dormant cells are quiescent, arrested in G0/G1 cell cycle phase and show decreased metabolic rate in order to reactivate when more favorable environmental conditions occur [34]. Interestingly, ATF6 that is constitutively activated in quiescent squamous carcinoma cells, promotes cell survival in a RHEB- and mTOR-dependent manner [35]. Moreover, inhibition of ATF6 or RHEB

reverts dormant tumor cell resistance to rapamycin and triggers cell death *in vivo* [35]. Similarly, both GRP78 and PERK/eIF2α are associated with increased survival and drug resistance of dormant cells [36]. Activation of the UPR also promotes cancer progression by impacting on various steps in the metastatic cascade. For instance, PERK activation is required for breast cancer cells to invade and metastasize [37]. Further, by mediating the activation of heme oxygenase 1, PERK protects fibrosarcoma cells from anoikis thereby facilitating lung colonization [38]. PERK also promotes cancer cell migration through ATF4-dependent induction of the metastasis-associated gene LAMP3 [39]. In gastric cancer, PERK, ATF4 and ATF6 induce epithelial-to-mesenchymal transition (EMT) under severe hypoxia, which triggers TGF-β release and the concomitant activation of Smad2/3 and PI3K/AKT signaling [40]. Moreover, IRE1 contributes to the migration and adhesion of glioma cells [41], whereas XBP1 activation promotes lung metastasis in triple negative breast cancer [27]. IRE1 controls glioma cell migration partially through the degradation of SPARC mRNA and consequently inhibition of FAK and RhoA signaling [42]. In addition to its aforementioned cell intrinsic effects on tumor progression, the UPR is now becoming widely explored for its impact on tumor microenvironment, which will be further discussed in the following sections.

2. The UPR in the regulation of tumor microenvironment

Tumor microenvironment (TME) that comprises cancer-associated fibroblasts (CAFs), endothelial cells and immune cells plays a key role in cancer progression. Activated CAFs fuel highly proliferating tumors with glucose, lactate, fatty acids and amino acids, and modulate signaling of adjacent cancer cells by secreting various growth factors and cytokines [43]. They can also support cancer invasion and metastasis by releasing a large number of EMT-inducing soluble factors and by remodeling the extracellular matrix [44]. Endothelial cells, which line tumor blood vessels, are educated by cancer cells to produce pro-angiogenic factors but also to promote migration, metastasis and evade anoikis [45]. Finally, tumor infiltrating leukocytes, that include both effectors of adaptive immunity such as T lymphocytes, dendritic cells (DCs) and B cells, as well as mediators of innate immunity, including macrophages, polymorphonuclear leukocytes and NK cells, are well known to have a dual function in cancer [46]. They can eliminate cancer cells by presenting tumor-associated antigens on the MHC I and MHC II molecules, which consequently activate CD4 helper and CD8 cytotoxic T lymphocytes (CTLs); however, infiltrating leukocytes can also promote tumor growth, metastasis and chronic inflammation leading to the unfavorable patient's outcome [47]. Interestingly, a large body of evidence suggests that the UPR regulates the crosstalk between tumor and non-tumoral cells by impacting on angiogenesis, on inflammation and on the host immune response (**Figure 2**).

2.1 Transmissible UPR

The crosstalk between cancer cells and their environment depends on a variety of chemical and mechanical signals mediated by small molecules, ions, proteins and nucleic acids. This intercellular communication occurs either through physical interactions mediated by gap junctions (Gap Junctional Intercellular Communication; GJIC) or remotely through the secretion of signaling molecules such as growth factors, cytokines and exosomes. GJIC is crucial for the maintenance of tissue homeostasis by controlling growth, differentiation and apoptosis [48, 49]. In contrast, loss of direct cell-cell interaction and the lack of electrical coupling in cells are common features in many tumors. While tumor-promoting chemicals and oncogenes inhibit GJIC, anti-tumor chemicals and anti-oncogene drugs were associated with growth control and loss of tumorigenicity by re-gaining GJIC activity [50-58]. Moreover, the key proteins involved in GJIC, connexins are emerging as tumor suppressors [59]. For instance, the loss of connexin 32 (CX32) leads to a significant increase in liver and lung tumors in mouse models which was reversed by CX32 re-expression [60, 61]. In glioma, CX43 expression was inversely correlated with tumor grade, proliferation and migration capacities, while CX43 downregulation promotes tumor growth [62, 63]. In addition to gene transcription regulation, connexin levels can be regulated by trafficking and degradation mechanisms [64]. Perturbation of protein folding in the ER has been shown to promote CX43 translocation to the cytosol and its subsequent degradation [65-69]. Moreover, CX43 mRNA and protein levels are found to be downregulated during ER stress in numerous human and mouse tumor cell lines, which reduced cell-to-extracellular matrix adhesion and increased migration [50].

Exosomes are small endosome-derived extracellular vesicles of 30–100 nm size secreted by a wide range of mammalian cell types, which act as mediators of cell-cell communication [70-72]. They contain a conserved set of proteins, and although they are deprived of any cellular organelles, they can still transmit variety of bioactive molecules [73], depending on the cell and tissue of origin [74, 75]. Exosomes secreted by cancer cells support disease spread in both autocrine and paracrine manners by impacting on major tumor-associated pathways including cancer stemness, angiogenesis and metastasis [76]. Exosomes can also play an important role in drug resistance mechanisms such as the expulsion of intracellular drugs and their metabolites, and neutralization of antibody-based therapies [77-82]. Due to their unique stability, selective cargo and resemblance to the cells of origin, exosomes have great potential to serve as a reservoir of cancer biomarkers for disease detection, clinical diagnosis and selection of therapy [83-85]. Small amounts of exosomes collected from non-invasive liquid biopsies including saliva, urine and blood [86-90] can provide multiple dynamic informations from different tumors [91]. For example, exosomes released from human

brain tumors were shown to contain miRNAs, different heat-shock proteins and other tumor promoting immunomodulatory agents that drive macrophages polarization towards pro-tumoral M2 phenotype *in vitro* [92-97]. Intriguingly, ER stress and the UPR activation enhance the exosomes' secretion. As such, tunicamycin-induced ER stress increases the multi-vesicular body formation in cervical cancer cells and promotes exosomes secretion in IRE1- and PERK-dependent manner, which is abrogated by PERK and IRE1 inhibition [98].

The UPR can also enhance the tumor overall fitness by being transmitted from cancer cells to the cells of TME. This transmissible ER stress (TERS) has multiple effects on the recipient cells *in vitro* and *in vivo*. For instance, TERS alters the function and cross-priming of bone marrow derived dendritic cells (DCs) by transcriptional upregulation of different tumorigenic and immunosuppressive molecules, as well as inflammatory cytokines [99]. This phenomenon is independent of Toll-Like Receptor 2 (TLR2) or IL-6R but relies on the TLR4, which senses and potentiates TERS and conditions macrophages to mirror tumor cells [100]. Moreover, ER stress signaling pathways are triggered in mice receiving ER stress-conditioned medium that facilitates pro-tumorigenic characteristics [100, 101]. In prostate cancer cells, TERS promotes survival and drug resistance to the proteasome inhibition-mediated toxicity by transmitting a unique UPR signal to the juxtaposed cancer cells. It induces Wnt signaling in an IRE1-dependent manner, while the induced enhancement of cell survival is mediated by PERK activation [102]. TERS secreted from CVB3-infected myocardiocytes also promote the pathogenesis of viral myocarditis by augmenting the pro-inflammatory responses of cardiac infiltrating macrophages [103].

In conclusion, activation of the UPR has a broad range of targets that affects and regulates protein secretion including those involved in the intercellular communications. This important role in protein trafficking and the fact that the UPR itself can be transmitted position the UPR to influence cell-cell communication pathways and to coordinate physiological processes between cells and tissues.

2.2 UPR and angiogenesis

Angiogenesis, which is a process of remodeling existing blood vessels, involving sprouting, migration, and proliferation of endothelial cells (ECs), is mediated by several factors including PDGF, FGF, IL-8, and VEGF [104]. A growing evidence suggests that UPR plays a key role in angiogenesis induction. It has been reported that XBP1 and ATF4 can both directly bind and transactivate VEGFA promoter in response to ER stress, an event that is even more prominent that hypoxia-driven VEGFA activation [105]. Moreover, VEGFA upregulation after oxygen or glucose deprivation is blunted in tumor cells

expressing a dominant negative IRE1 as well as in IRE1 deficient mouse embryonic fibroblast（MEFs) [106]. Inhibition of IRE1 signaling decreases glioma vascular density and vessel perfusion *in vivo*, which are rescued by the expression of a transgene of IL-6 [41]. Interestingly, in diabetic bone marrow-derived progenitor cells, loss of IRE1 results in decreased angiopoietin 1 expression and disrupts angiogenesis, due to inefficient RIDD of miR-466 and miR-200 families [107]. In line with this observation, the PERK/ATF4 pathway regulates the angiogenic switch in human tumors, by increasing the expression of many proangiogenic modulators, including VEGF, FGF-2 and IL-6, with the concomitant decrease in the expression of the angiogenic inhibitors THBS1, CXCL14 and CXCL10 mRNA [108]. *In vivo*, PERK knockout in κ-Ras transformed MEFs leads to angiogenesis inhibition and reduced tumor mass compared to the wild-type counterparts [109]. More recently it has been demonstrated that in response to acute hypoxic stress, PERK triggers the cap-independent internal ribosome entry sites (IRES)-mediated translation of VEGF and FGF-2 [110]. In addition, ER stress triggered by tunicamycin, thapsigargin or glucose deprivation also increases the expression of the pro-angiogenic factors FGF-2, IL-1α IL-6, IL-8, angiopoietin-2 and TGFβ2 [105]. Finally, at the post-translational level, ER stress induces the ER chaperone ORP150 which facilitates VEGF processing and secretion [111].

VEGF itself was shown to induce ER stress in ECs and consequently activate all three UPR branches, IRE1, ATF6 and PERK, through a PLCγ/mTORC1 pathway [112]. This VEGF-driven UPR activation is necessary for ECs survival and angiogenesis, and is mediated by AKT phosphorylation and decrease in CHOP mRNA level [112]. The UPR can be also triggered in ECs by low pH and GRP78 has been reported to play a key role in such activation [113]. Strikingly, targeting the GRP78 in acid-stressed ECs abrogates sunitinib chemoresistance, partially through the induction of caspase 7 cleavage [113]. In renal cell carcinoma, GRP78 knockdown suppresses tumor progression and enhances the anti-tumor effects of anti-angiogenic therapy *in vivo* [114]. The UPR activation can be also triggered in ECs by imposing stress from adjacent cancer cells. As such, breast cancer cell-stimulated ECs upregulate the chaperone αB-crystallin (CRYAB) that acts downstream of IRE1 and ATF6 inducing VEGF expression and secretion [115]. Furthermore, by protecting endogenous VEGF from proteolytic degradation, CRYAB supports ECs proliferation and survival [115]. Taken together, activation of the UPR in cancer cells promotes angiogenesis by directly upregulating the expression of pro-angiogenic factors or transmitting the pro-angiogenic signals to the surrounding ECs.

2.3 *UPR activation in immune cells*

The UPR is known to control immune cell development, function and survival both in pathological and physiological conditions. For example, highly secretory cancer cells, like B cells in multiple myeloma, produce high levels of immunoglobulins and as a consequence suffer chronic ER stress [116]. In addition, XBP1s is among the key regulators required for the activation of B cell terminal differentiation [117]. This coincides with the fact that the upregulation of the leptin-receptor upon fasting blocks acute lymphoblastic leukemia development by activating cell differentiation, which depends on the increase of the mRNA and protein levels of key transcription factors like XBP1, BLIMP1, and IRF4 [118]. In the same line, in the germinal center B-cell like (GCB)- diffuse large B-cell lymphoma (DLBCL), characterized by gain-of-function mutations of EZH2, IRE1 expression levels are reduced by the binding of high amounts of H3K27me3-repressive marks to its promoter, impairing the induction of an effective ER stress response. In result, GCB-DLBCLs do not induce XBP1 splicing, contributing to accelerate tumor growth [119]. Several other studies have also suggested the importance of XBP1 during the terminal differentiation and expansion of antigen-specific CD8 T cells [120].

The IRE1/XBP1 pathway also affects conventional DCs in a tissue-specific manner. Indeed, intestinal and splenic conventional DCs survive the loss of XBP1, although with defects on their ability to cross-present dead cell-associated antigens [121, 122]. This survival adaptive mechanism involves the induction of the eIF2α/ATF4/4E-BP1 pathway to avoid excessive protein loading and the IRE1/RIDD pathway to lower mRNA abundance and protein folding in the ER. Conversely, XBP1 loss affects the survival of lung and other peripheral-tissue-resident conventional DCs in a CHOP-independent manner [122]. In the context of cancer, tumor associated DCs induce XBP1 expression in response to increased ROS, thus modulating intracellular lipid homeostasis. This increase in XBP1s promotes ovarian tumor growth by impairing T cell activation [123]. In macrophages, TLR signaling inhibits the translation of ATF4 mRNA, thereby affecting the expression levels of its pro-apoptotic target CHOP. As such, macrophages can survive during the activation of the immune response [124]. However, CHOP deficiency in myeloid-derived suppressor cells (MDSCs) shows a decreased capacity to affect T cell responses, enhancing T cell function and inducing an anti-tumor response [125]. Additionally, in the MUP-uPA mouse, in which hepatocytes express high levels of urokinase plasminogen activator (uPA), and therefore undergo transient ER stress; a high fat diet induces hepatocellular carcinoma, through an ER stress-mediated mechanism that includes TNFα production by infiltrating

inflammatory macrophages. Finally, macrophage infiltration and TNF expression are both inhibited by treatment with the bile acid thought to act as a chemical chaperone, tauroursodeoxycholate (TUDCA) [126].

2.4 *UPR and tumor-promoting inflammation*

The UPR controls the production of inflammatory cytokines at the transcriptional and post-transcriptional level, thus having a direct impact on tumor progression. For example, XBP1s can bind to the promoter regions of IL-6 and TNFα in response to ER stress, inducing their expression in macrophages [124]. Similarly, CHOP directly regulates IL-23p19 expression in DCs [127]. IRE1, via the activation of GSK3β, induces gene expression of pro-inflammatory IL-1β, independently of its action on XBP1s signaling [128]. In addition, mTOR stimulation, also through IRE1, activates JNK and triggers IL-8 secretion in response to glutamine deprivation [129]. Interestingly, cytokines can in turn induce ER stress and regulate the UPR *per se*, thereby creating a feedback loop that results in the amplification of the inflammatory response. For example, in response to TNFα treatment, IKKβ phosphorylates and stabilizes XBP1s [130]. IL-10 blocks TNF-dependent translocation of cleaved ATF6 (ATF6f) to the nucleus via p38 MAPK signaling [131]. Pro-inflammatory IL-1β, IL-6 and TNFα induce the UPR and activate genes involved in the acute phase response (C-reactive protein and serum amyloid P-component) through the increased cleavage of the membrane anchored transcription factor CREBH [132]. Conversely to the great amount of studies that describe the inflammatory-dependent activation of the UPR, it has been recently described that obesity-related chronic inflammation can induce the S-nitrosylation of IRE1, thus shutting down its endoribonucelase activity without affecting its kinase domain. As a result, this modification of IRE1 contributes to metabolic and inflammatory stress, compromising the adaptive UPR response through the decrease of XBP1s followed by an increase in JNK levels [133].

Interestingly, aberrant lipid composition of the ER membrane (also known as lipid bilayer stress) can activate IRE1. It has been suggested that IRE1, due to its localization and mechanistic properties, can sense and signal lipid changes in the ER membrane independently of misfolded protein accumulation in the ER lumen. This occurs by inducing IRE1 oligomerization and signaling, hence recognizing IRE1 as a molecular link between protein and lipid homeostasis [134, 135]. IRE1 activation also allows the crosstalk between the ER and the mitochondria through the ROS-dependent activation of the NLRP3 inflammasome. This results in the activation of caspase-2, the cleavage of the pro-apoptotic factor BID and the release of mitochondrial contents [136]. These

studies bring new membrane-based perspectives to the role of lipids in the ER stress-related pathophysiological conditions.

The UPR crosstalks with numerous other signaling pathways to regulate tumor-host interactions. The three branches of the UPR have been shown to induce the pro-inflammatory NFκB pathway. Firstly, IRE1 interacts with TRAF2, recruiting IKK and inducing the phosphorylation and degradation of IκB, which allows NFκB to translocate to the nucleus. Secondly, IκB has a shorter half-life than NFκB and for this reason, changes in protein translation under ER stress stimuli that activate the PERK pathway, induce the NFκB pathway by decreasing the IκB:NFκB ratio. Finally, ATF6 can induce NFκB through the phosphorylation of AKT [120].

2.5 UPR and anti-tumor immune response

There is a growing evidence that the UPR can also control the anti-tumor immune response by acting as an innate immune machinery. The UPR can regulate the release of damage-associated molecular patterns (DAMPs), which can act as "eat me" signals, "find me" signals or chemoattractants. In summary, DAMPs are intracellular molecules that are hidden from the immune system's recognition under normal conditions. However, upon cellular stress or death, cells can induce an immunogenic response by the pre-apoptotic expression of DAMPs on the cell surface (e.g. calreticulin (CALR) and heat shock proteins (HSPs)) or by releasing or secreting them (e.g. ATP and HMGB1) [120, 137]. This type of cell death is known as immunogenic cell death (ICD). Interestingly, ICD has to be preceded by the ER stress in order to induce CALR and HSPs surface exposure. In the case of CALR exposure, it occurs through the activation of the PERK/eIF2α pathway [138], however the exact respective contribution of PERK activation and eIF2α phosphorylation need to be further explored. On the other hand, ATP release depends on pre-mortem autophagy, and the secretion of HMGB1 on secondary necrosis [137].

These DAMPs are recognized by specific receptors: CALR binds to CD91, ATP binds to purinergic receptors (P2Y2 or P2X7) and HMGB1 binds to TLR4, respectively [137]. These receptors are found on DCs and promote engulfment of dying cells, attraction of DCs into the tumor bed, production of IL-1β and tumor antigen presentation. CALR is a highly conserved calcium-binding ER lectin that has important functions in the immune response. For example, CALR chaperones MHC class I molecules, thus regulating antigen presentation hence affecting recognition by CD8 T cells [139]. It is also associated with the increased expression of CD86, CD80 and MHC II in the cell surface of DCs, leading to an efficient anti-cancer CD8 T cell response [120]. Furthermore, CALR exposure at the cell

surface plays an important role in the immunosurveillance mechanism induced by cells that have increased ploidy [140]. Even though ER stress induces CALR exposure to the cell surface during ICD, the mechanism by which this phenomenon happens remains elusive.

HMGB1 secretion during cell death can activate the UPR in DCs by increasing GRP78 expression and XBP1 splicing [120]. XBP1 silencing leads to the downregulation of CD86 and CD80 cell surface activation markers and MHC class II expression. These events result in the decrease of T cell proliferation and differentiation affecting the activation of T cells in *ex vivo* co-cultures [120]. In more recent studies, increased expression of HMGB1, HMGN1, XBP1 and peIF2α is correlated with a high amount of tumor infiltrating lymphocytes in triple negative breast cancer patients [141]. Besides DAMPs, there are also "don't eat me" signals that will help cancer cells avoid the immune system's recognition. ER stress regulated proteins also control these signals. For example, GRP78 inhibition in BALB/c and athymic tumor-bearing mice increases monocyte chemoattractant protein-1 (MCP-1) serum levels and regulates CD47, a glycoprotein of the immunoglobulin superfamily critical in self-recognition. Normal tissue increases the CD47 "don't eat me" signal in response to GRP78 inhibition, while the tumoral tissue decreases its expression. In this way, GRP78 inhibition stimulates macrophage infiltration and reduction of estrogen receptor–positive breast cancers [142].

The similarities between the antigen-specific immune response triggered by ICD and those induced by pathogen infection have led scientists to look into these pathways in order to try to apply this knowledge in cancer research. This is the case of TLRs, which are pattern recognition receptors (PRRs) that recognize pathogen-associated molecular patterns. Activation of TLRs and the IRE1/XBP1s pathway are interconnected and result in the induction of the innate immune surveillance in response to pathogen infection. In macrophages, TLR activation induces a ROS-dependent specific activation of the IRE1α/XBP1s pathway, but not of the other arms of the UPR. Then, XBP1s induces IL-6 and IFN-β cytokine production [124]. This kind of response is not restricted to TLRs, as there is a clear link between the UPR and retinoic acid-inducible gene-I (RIG-I)-like receptors (RLRs). RLRs are RNA helicases that sense pathogenic RNA and initiate antiviral immunity. Recent studies have linked IRE1 with the RIG-I pathway upon pathogen infection [143] and pathological conditions [144]. Upon the activation of IRE1 RNase activity, the cleavage of endogenous RNA through RIDD may produce fragments that resemble those of pathogens as they lack 5'-caps or 3'-polyA-tails that mark mRNA as "self". These fragments in turn activate RIG-I that induces an innate immune response.

In the context of cancer, endogenous RNAs that are not shielded by RNA binding proteins have already been shown to act as DAMPs for PRRs. In primary human breast cancers, activated stromal cells present unshielded RNA in exosomes in order to propagate anti-viral signaling to the TME. This unshielded RNA in stromal exosomes results in an inflammatory response when transferred to immune cells and in tumor growth and invasion when transferred to breast cancer cells [145]. In immunocompetent mice, RIG-I activation induces the secretion of extracellular vesicles by melanoma cells that act as immune activating agents favoring the anti-cancer immune response [146]. Administration of a BCL-2 siRNA activates RIG-I efficiently and leads to tumor growth inhibition through an anti-tumor immune response. This anti-tumor response involves myeloid and plasmacytoid DCs activation, NK cells, CD4 and CD8 T cells and is associated with the secretion of type I cytokines (IFN-α, IL-12p40 and IFN-γ) [147]. Furthermore, RIG-I has been proposed as a tumor suppressor in hepatocellular carcinoma (HCC) as RIG-I deficiency promotes HCC carcinogenesis [148]. Other studies in highly immunodeficient mice suggest that RIG-I can inhibit tumor growth by inducing apoptosis through the regulation of BH3-only proteins [149]. Additionally, pancreatic cancer cells treated with RIG-I–like helicase ligands die through ICD. This ICD occurs through the translocation of CALR to the cell surface followed by the release of HMGB1 that activates DCs and cytotoxic CD8 T cells [150]. One could speculate that stimuli activating the IRE1/RIDD pathway in tumor cells could potentially activate RIG-I, inducing ICD and an anti-cancer immune response. Altogether, these studies highlight the importance of studying the regulation of the UPR in the context of cancer in order to understand immunogenicity and to improve the anti-tumor immune responses and therapies.

2.6 Impact of UPR activation in tumor-surrounding parenchyma

Tumors develop in particular tissular environments that are composed by a multiple of non-tumoral cell types. Next to ECs and immune cells (presented above), other stromal/parenchymal cells as such stellate cells, epithelial cells, fibroblasts or astrocytes and neurons could also be affected by UPR downstream signals provided by tumor cells. Little if any of such interactions is up-to-now reported in the context of neoplasia and only few indirect evidences are described so far. For instance, pancreatic stellate cells are key stromal cells in pancreatic cancer for secreting extracellular matrix proteins and inflammatory mediators. Under metabolic stress, the PERK/CHOP branch of the UPR is activated in pancreatic stellate cells, thus protecting them from apoptosis [151]. Furthermore, under these conditions, stellate cell fibrogenic activity is reduced and the profile of secreted cytokines is modulated (i.e. reduction of IL-6 expression and increase of the immune modulator IL-4), thereby

contributing to the modulation of TME. Several UPR-induced genes have clear impact on the stromal cells surrounding tumors. For instance, Serpin B2 expressed by the cancer-associated fibroblasts limits metastasis in pancreatic ductal adenocarcinoma due to its collagen remodelling capacity [152]. ATF3, a downstream effector of the PERK/ATF4 pathway, is a key regulator of tumor-associated stromal cell reprogramming leading to increase in their proliferation ability, which in turn supports tumor growth [153]. The impact of the UPR on stromal cells is well documented in other pathologic situations such as neurogenerative diseases [154]. Indeed, instead of improving protein quality control and protein folding, prolonged ER stress leads to neuronal cell apoptosis, synaptic dysfunction and axonal degeneration. One could speculate that brain tumor transmissible UPR would affect neighboring brain resident cells such as oligodendrocytes, astrocytes and neurons leading to neuronal dysfunctions and tumor cell bedding. Further studies are required to investigate such stroma/tumor cell communications through UPR activation.

3. Targeting UPR as anti-cancer approach

As exemplify above, the UPR has a broad impact on tumor-associated processes such as sustained growth, resistance to apoptosis, metastasis, inflammation or escape from immune recognition, which creates a rationale for targeting ER stress pathways as a potential anti-cancer approach. This can be achieved either by exploiting the pro-death UPR signaling to effectively kill cancer cells or to impede UPR-mediated adaptive responses which help tumor cells propagate in harsh TME conditions and resist the treatment. As such, ER stress- triggered apoptosis has been observed in various cancer models both *in vitro* and *in vivo*. For instance, in glioblastoma, a large number of small molecules including FDA-approved drugs nelfinavir, quinine and celecoxib, has been reported to induce cell death by perturbing ER proteostasis, which is mainly mediated by the upregulation of GRP78 and/or CHOP mRNA or protein levels [29]. Further, many natural and chemical agents are shown to promote cell death by generating reactive oxygen species (ROS) and consequently triggering ER stress in lung, breast, liver or colon cancer [155]. As discussed above, the UPR has a very important role in ICD induction and constitutes a promising target for the development of novel anti-cancer strategies. Remarkably, patients can only benefit of checkpoint blockade immunotherapies if tumors are infiltrated by tumor-infiltrating lymphocytes (TILs) previous to the treatment. Importantly, tumors without TILs can be sensitized to checkpoint blockade immunotherapies when combined with ICD-inducing drugs [123]. In this sense, the co-administration of chemotherapies that do not induce ICD with immunogenic chemotherapies capable of inducing the UPR should be considered. Many of the ICD inducers are intensively used in

the clinical practice and are divided into the type I and type II ICD inducers [156, 157]. Type I inducers such as bortezomib, anthracyclines and oxaliplatin, trigger apoptosis via non-ER targets (e.g. through the DNA damage or proteasomal inhibition) with the parallel 'off-target' impact on the ER stress signaling [157]. On the other hand, type II ICD inducers (involving hypericin-photodynamic therapy and oncolytic viruses) drive apoptosis through the selective activation of ROS-mediated ER stress [157]. Interestingly, it has been recently demonstrated that cells resistant to ER stress and chemotherapy acquire a multidrug resistant phenotype through the activation of the PERK/NRF2/MRP1 signaling axis. Targeting this axis restores chemosensitivity in resistant cancer cells and diminishes tumor growth *in vivo* [158].

In the past decade, various inhibitors targeting each of the UPR arm have been developed and have been shown to yield a promising anti-tumor response (**Figure 3**). As such, four compounds are reported to modulate ATF6 signaling: 16F16 (a PDI inhibitor necessary for ATF6 activation), caepins and two non-toxic ATF6 activators – compounds 147 and 263 [29]. Interestingly, impairing ATF6 signaling with 16F16 restores imatinib sensitivity in imatinib-resistant leukemia K562 cells [159]. More recently, it has been showed that melatonin blocks the ATF6 signaling in HCC leading to a decrease in COX-2 expression and consequently promoting cell apoptosis under tunicamycin-induced ER stress [160]. The IRE1 modulators developed so far include both RNase inhibitors such as 4μ8C, ManKindCorp (MKC) analogs, 3-methoxy-6-bromosalicylaldehyde and STF-083010, as well as agents targeting kinase domain – KIRAs and ATP kinase inhibitor compound 3 [29]. Those inhibitors are shown to kill cancer cells or sensitize them to common chemo- or radiotherapies. For instance, STF-083010 significantly inhibits the growth of human multiple myeloma xenografts [161]. Moreover, it restores tamoxifen sensitivity in resistant breast cancer cells, while when administered synergistically with tamoxifen it suppresses breast tumor progression *in vivo* [162]. Similarly, MKC-3946 decreases multiple myeloma growth and shows therapeutic activity in the combination with the proteasome inhibitor bortezomib [163]. Finally, the KIRA6 inhibitor and the optimized KIRA, KIRA8 which is a mono-selective IRE1 inhibitor with a single digit nanomolar potency, block IRE1 *in vivo* and promote cell survival under ER stress in several mouse models [118, 164]. Regarding PERK inhibitors, GSK2656157, ISRIB, salubrinal, guanabenz and sephin 1/IFB-088 are shown to modify PERK phosphorylation or its downstream signaling by targeting the eIF2α complexes [29]. GSK2606414 and the related drug GSK2656157 impact on cancer progression by decreasing tumor growth and reducing tumor-associated angiogenesis, respectively [165, 166]. Moreover, in an orthotopic model of pancreatic ductal adenocarcinoma, ISRIB enhances the gemcitabine chemosensitivity by suppressing the integrated stress response (ISR) and its downstream anti-apoptotic pathways [167]. In line, salubrinal, an inhibitor of growth arrest and DNA damage 34

(GADD34), potentiates the cytotoxic effect of doxorubicin in doxorubicin-resistant breast cancer cells *in vitro* [168]. Thus, molecules that generate irremediable ER stress in tumor cells or specifically target the UPR branches represent interesting therapeutic options alone or in combination with other commonly used drugs.

4. Concluding remarks

The UPR signaling has a broad impact on cancer biology. It not only provides tumor cells with the selective advantages to survive and propagate in harsh environmental conditions, but also educates the surrounding non-tumoral cells to even further promote cancer progression. As discussed herein, signals emerging from the ER impact on the tumor secretome, which in turn supports new vessels formation, inflammation or immune suppression. Interestingly, a growing evidence suggests the UPR involvement in the regulation of anti-tumor host response. Nevertheless, we still need to uncover what triggers these opposite outputs. Apparently, the difference lays in a combination of the type of UPR-inducing stimulus and which pathways are engaged in response to it. It is tempting to think that it all comes down to a fine-tuning of the different UPR proteins downstream of the master sensors. For example, in the case of IRE1, which has an interesting dual role in both cell death and immunosurveillance, several pathways ramify downstream of its activation. Is the outcome the same if we activate more the XBP1s downstream pathway than the RIDD pathway and *vice versa*? Further studies are needed in order to complete the puzzle that is the UPR in the TME and its control of cell death and the immune system response. For these reasons, modulating ER stress in tumor cells and the TME represents an additional level of therapeutic intervention.

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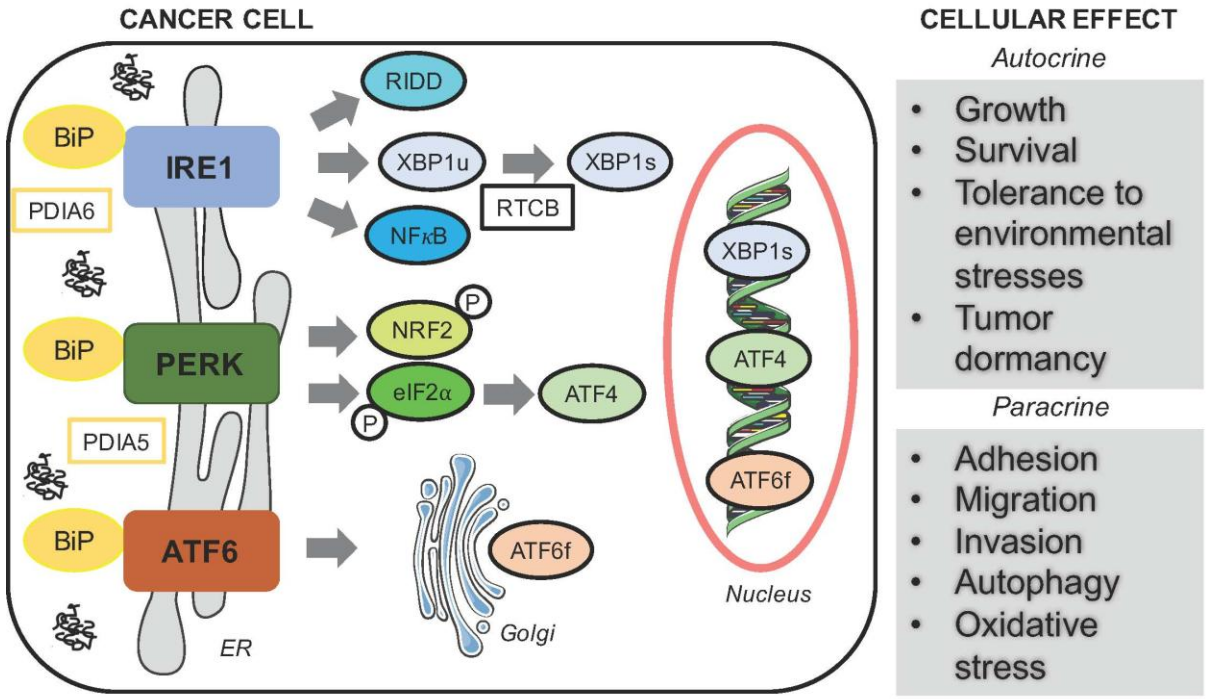

Figure 1: Cell intrinsic effects of the UPR on cancer progression. Activation of the UPR arms – IRE1, PERK and ATF6 activate the downstream signaling cascade driven by transcription factors XBP1s, ATF4 and ATF6f, respectively. In addition, IRE1 controls degradation of target mRNAs through RIDD and activates NFκB signaling, whereas PERK activation leads to the phosphorylation of NRF2. The integration of these outputs trigger a large number of biological effects supporting tumor progression in auto- and paracrine manner.

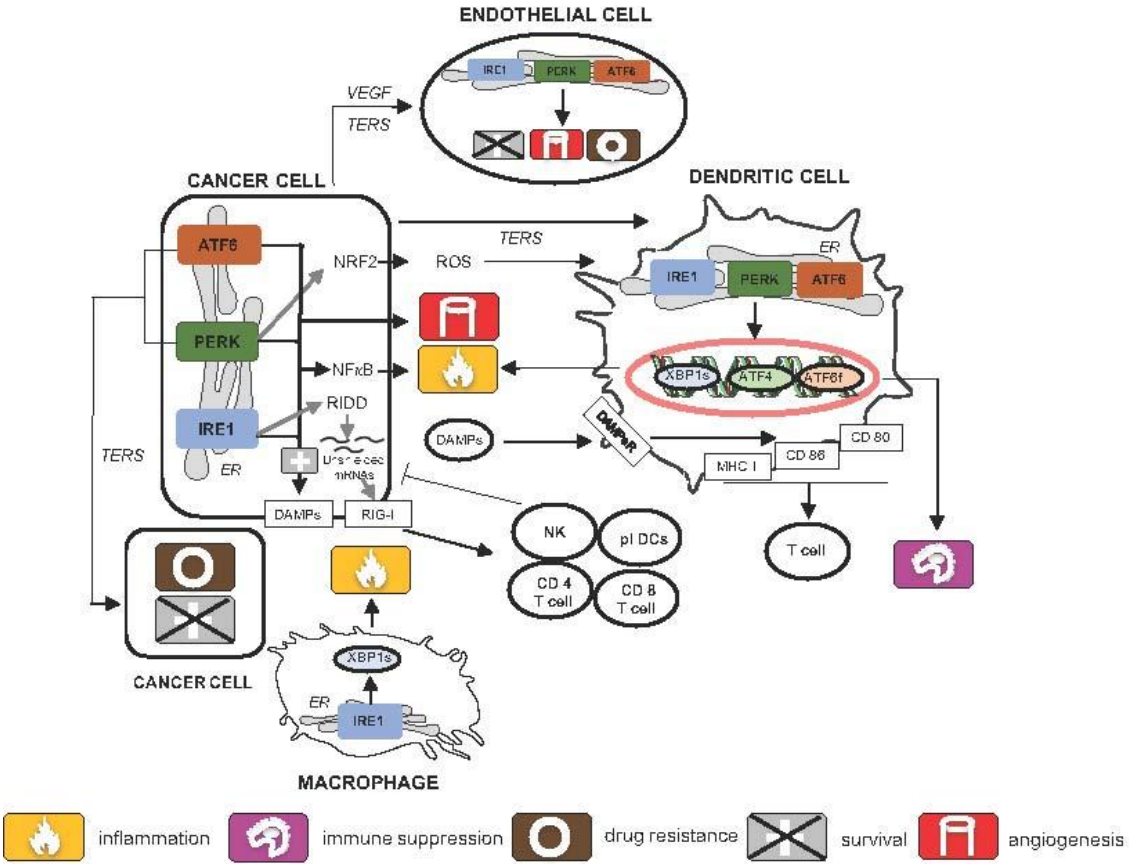

Figure 2: UPR-mediated crosstalk between tumor and non-tumoral cells. The UPR activation in cancer cells shapes tumor microenvironment by impacting on neighboring cancer, stromal and immune cells. This reciprocal communication is mediated by secretion of various soluble factors including ROS, pro-angiogenetic and pro-inflammatory molecules or by transmitting the ER stress from one cell to another (known as TERS). In that manner, TERS-imprinted juxtaposed cancer cells are resistant to apoptosis and chemotherapy. On the other hand, the non-tumoral cells, such as endothelial cells, macrophages and dendritic cells support cancer progression by inducing angiogenesis, inflammation and escape from immune surveillance. However, UPR in dying cancer cells can also control the induction and/or secretion of DAMPs, which trigger the anti-cancer immune response through the activation of dendritic cells and consequently T cells. DAMPs, damage-associated molecular patterns; DCs, dendritic cells; NK, natural killers; pl DCs, plasmacytoid dendritic cells; ROS, reactive oxygen species; TERS, transmissible ER stress.

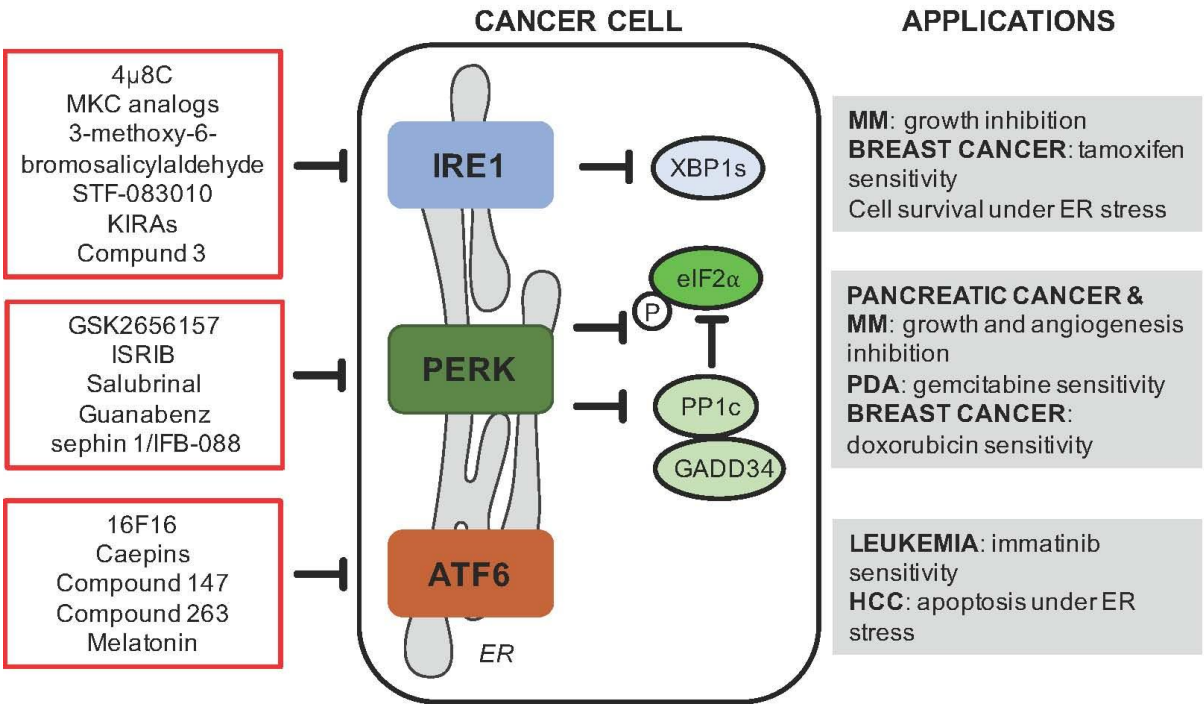

Figure 3. Anti-cancer effects of the UPR-targeting drugs. Many molecules specifically targeting each of the UPR branches show potential anti-cancer activities either by inhibiting tumor growth or restoring chemosensitivity in drug-resistant cells.