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Review

Endoplasmic reticulum stress associated responses in cancer

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ABSTRACT

The endoplasmic reticulum (ER) is responsible for many housekeeping functions within the cell and is an important site for pathways that regulates its state of homeostasis. When cellular states perturb ER functions, a phenomenon termed “ER stress” activates a number of pathways to counteract the associated damages; these pathways are together called the unfolded protein response (UPR). The UPR has a dualistic function; it exists to alleviate damage associated with ER stress, however, if this is not possible, then it signals for cell death through apoptosis. Cancer cells are shown to be very resilient under extreme environmental stress and an increasing number of studies have indicated that this may be largely due to an altered state of the UPR. The role of ER stress and the UPR in cancer is still not clear, however many components are involved and may prove to be promising targets in future anti-cancer therapy. This article is part of a Special Issue entitled: Calcium Signaling in Health and Disease. Guest Editors: Geert Bultynck, Jacques Haiech, Claus W. Heizmann, Joachim Krebs, and Marc Moreau.

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1. Introduction

The optimum state of a cell is necessary for survival and is achieved through a consistent and sustained level of homeostasis. The challenge, however, lies in the fact that the cellular environment is constantly undergoing change, disrupting the internal equilibrium of the cell. To restore this balance cells have evolved numerous ways to adapt to environmental stress and, in cases where the damage is too great, ways to remove the diseased cells, preventing toxicity.

Abbreviations: ASK1, apoptotic signal-regulating kinase 1; ATF4, activating transcription factor 4; ATF6, activating transcription factor 6; Bcl-2, B-cell lymphoma 2; BH3, Bcl-2 homology domain 3; BIK, Bcl-2 interacting killer; BiP, binding immunoglobulin protein; Blimp-1, B lymphocyte-induced maturation protein-1; CHOP, C/EBP homologous protein; EGF-SubA, subtilase cytotoxin catalytic subunit fused with epidermal growth factor; eIF2 α , eukaryotic initiation factor 2 α ; ER, endoplasmic reticulum; ERAD, endoplasmic reticulum associated degradation; ERdj4, endoplasmic reticulum DnaJ homologue 4; ERO1 α , ER oxidoreductin 1 α ; ERp57, endoplasmic reticulum protein of 57kDa; GADD34, growth arrest and DNA damage-inducible protein; GMBP1, gastric cancer multidrug resistance cell-specific binding peptide; GRp78, 78kDa glucose-regulated protein; GRP94, 94kDa glucose-regulated protein; IRE1 α , inositol-requiring enzyme-1 α ; JNK, c-JUN amino-terminal kinase; LAMP3, lysosomal-associated membrane protein 3; LC3B, light chain 3B; MAM, mitochondria associated membrane; MAPK, mitogen-activated protein kinase; mTOR, mammalian target of rapamycin; PERK, protein kinase RNA-like endoplasmic reticulum kinase; PP1, protein phosphatase 1; Rheb, Ras homolog enriched in brain; RIDD, regulated IRE1 α dependant decay; siRNA, small interfering RNA; TNF, tumor necrosis factor; TRAF2, tumor necrosis factor receptor associated factor 2; UPR, unfolded protein response; VCP, valosin containing protein; VEGF, vascular endothelial growth factor; XBP1, X-box binding protein 1

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The endoplasmic reticulum (ER) is a major organelle housing many cellular functions and is therefore an important site for maintaining homeostasis. When pathways within the ER are disturbed, such as ones that regulate protein folding, post-translational modifications, lipid and steroid synthesis, gene expression, cellular metabolism and calcium signaling, ER functions become overwhelmed and the accumulation of misfolded proteins within the ER lumen ultimately leads to ER stress [1]. As a result, the ER launches various coping mechanisms to alleviate the damage, allowing the cell to adapt to the environmental stress. On the other hand, if the recovery of cellular adaptation is not achieved prolonged ER stress triggers apoptosis [2]. Emerging evidence has come to suggest a third pathway, one which allows cells to survive extreme environmental insults and evade cell death through up-regulation of ER adaptive measures [3]. The result is manifested at the organismal level as a disease or disorder and in particular, cancer, which has unique modifications that allow it to capitalize the third phenomenon, allowing survival and growth.

The physiological environments of solid tumors differ from that of normal tissues in many ways: it is hypoxic, low in pH and low in nutrients [4]. These environmental factors all contribute to the activation of ER stress and as a result, cancerous cells must possess ways to adapt and prevent the fate of ER stress-induced apoptosis [2,5]. Recent studies have shown the various ways in which cancerous cells utilize altered states of ER stress responses in order to perturb ER associated cell death signaling. The following review will look at some of these studies, ER stress associated responses and their components, as well as targeting these pathways for developing cancer treatments.

2. Endoplasmic reticulum stress

The ER functions to regulate the quality, folding and secretion of newly synthesized proteins. Under normal conditions, correctly folded

proteins are sent through the secretory pathway to their designated cellular location. If proteins are misfolded, they are retained in the ER for further processing by ER protein chaperones, such as calreticulin, calnexin and endoplasmic reticulum protein 57 (ERp57), until they attain the correct conformation for secretion. However, if the correct conformation cannot be achieved, the misfolded protein is sent for ER-associated degradation (ERAD). When environmental factors greatly perturb these processes that maintain ER homeostasis, the ER undergoes stress and activates various ER stress responses termed as the unfolded protein response (UPR).

2.1. Unfolded protein response

The UPR is composed of three different pathways that fall under the control of three respective ER transmembrane proteins: protein kinase RNA-like endoplasmic reticulum kinase (PERK), inositol-requiring enzyme 1 α (IRE1 α) and activating transcription factor 6 (ATF6) [6–8]. Under normal cellular conditions, PERK, IRE1 α and ATF6 form stable complexes with the ER stress sensor binding immunoglobulin protein (BiP) and remain in their inactive state [9]. BiP binds to the ER luminal domains, preventing homodimerization and activity of PERK and IRE1 α [10,11]. The binding of BiP to ATF6 blocks its Golgi-localization signals, retaining ATF6 to the ER membrane, which prevents further processing of ATF6 to its active form [8,12]. In the presence of cellular stress, accumulation of misfolded proteins within the ER binds BiP competitively, causing dissociation of BiP from PERK, IRE1 α and ATF6, thus removing its inhibitory effects [9,10,12]. Release of BiP allows PERK to dimerize and subsequently autophosphorylate, turning on its kinase activity [10]. Activated PERK phosphorylates and inhibits the eukaryotic initiation factor 2 α (eIF2 α), consequently turning off protein synthesis but selectively increasing expression of ATF4 and C/EBP homologous protein (CHOP) [7,10,13]. ATF4 is a transcription factor that regulates pro-survival genes, such as those involved in oxidative stress, amino acid synthesis, protein folding and differentiation [14]. Similarly, upon dissociation from BiP, IRE1 α undergoes dimerization and autophosphorylation, activating its endonuclease activity for mRNA processing [10,15–19]. A particularly important target of IRE1 α is the mRNA encoding X-box binding protein 1 (XBP1). IRE1 α splices a 26 base pair region from the XBP1 mRNA, resulting in an active XBP1 transcription factor which functions to up-regulate gene encoding proteins involved in protein folding, quality control and ERAD [19]. In the case of ATF6, the release of BiP unmasks its Golgi-localization signals, which allows ATF6 to translocate to the Golgi. ATF6 is then sequentially cleaved by the site-1 and site-2 proteases, releasing an N-terminal fragment that acts as a transcription factor to increase transcription of XBP1 and ERAD associated proteins [12,19,20].

2.2. Apoptosis

Under moderate ER stress, the UPR can function as a pro-survival mechanism and return the cell to its state of homeostasis. However, when cellular damage exceeds the capacity of this adaptive response, ER stress is prolonged and continued activation of the UPR signals the cell for apoptosis [21,22]. CHOP is a major pro-apoptotic transcription factor that mediates ER-stress induced apoptosis and is a target for up-regulation by all three arms of the UPR pathway [23]. CHOP induces cell death through regulating expression of various genes. In particular, CHOP suppresses expression of the anti-apoptotic B-cell lymphoma-2 (Bcl-2) protein, which increases production of reactive oxygen species and causes injury to the cell [22,24]. Furthermore, CHOP increases the burden of misfolding in the ER by re-establishing protein folding and it increases the expression of growth arrest and DNA damage-inducible protein (GADD34), a regulatory subunit of protein phosphatase 1 (PP1), which allows dephosphorylation and activation of eIF2 α [25]. CHOP induces ER oxidoreductin 1 α (ERO1 α), hyperoxidizes the ER environment and further commits the cell to apoptosis [25].

Activated IRE1 α binds tumor necrosis factor (TNF) receptor associated factor 2 (TRAF2) through its cytosolic domain, recruiting apoptotic signal-regulating kinase 1 (ASK1) into complex formation and communicating ER stress by c-JUN amino-terminal kinase (JNK) activation, a major mediator of apoptosis [26,27]. The association of IRE1 α to TRAF2 also leads to clustering, cleavage and activation of caspase 12, another pro-apoptotic protein that responds only to ER stress [28,29]. There is currently much discrepancy in the activation and role of caspase 12 between human and rodent [30]. Although further investigation is needed to confirm the above pathway in humans and rodents, caspase 12 likely plays a role in ER-stress induced apoptosis through inhibitory effects on NF- κ B, a transcription factor involved in the immune response and apoptosis [31].

Lastly, additional targets of the IRE1 α RNase activity have been found [15,17,32,33]. Apart from XBP1 splicing, activated IRE1 α is also responsible for regulated IRE1 α dependant decay (RIDD) of many mRNAs associated with the ER [15,17]. RIDD plays an important factor in the decision for cellular apoptosis and induction of XBP1 splicing by IRE1 α in the absence of RIDD activity increased cellular survival against tunicamycin induced ER stress and apoptosis [34]. Moreover, IRE1 α may cause cleavage of selective microRNAs responsible for repression of caspase-2 mRNA translation, and this enhances the level of caspase-2 expression resulting in cellular apoptosis [32]. However, the role of ER stress in activation of caspase-2 to initiate apoptosis has been recently challenged [35]. MiR-17, a thioredoxin-interacting protein destabilizing microRNA, is another microRNA target of IRE1 α RNase activity and implicates the role of IRE1 α in inflammation and programmed cell death induced by prolonged UPR activity [33].

3. Cancer

The dualistic response of the UPR initiated by ER stress initially serves as an adaptive measure to protect the cell from irreversible damage. When this damage becomes too great, the UPR then becomes a self-destructive signal to rid the organism of the diseased cell and prevent further toxicity. The metabolic condition of cancer, being highly proliferative under a low vascularized state, creates an unfavorable microenvironment consisting of low pH, low oxygen, and low glucose and other nutrient supply [4]. Low glucose availability affects protein glycosylation and ATP production leading to accumulation of misfolded proteins within the ER [36]. As well in hypoxic conditions, lack of oxygen puts a demand on protein folding, as oxygen is an electron carrier required for disulphide bond formations, contributing to protein misfolding [37]. Under normal conditions, these are all factors that contribute to ER stress mediated cell death, but cancer cells have evolved ways to adapt to this environmental stress and escape the fate of apoptosis.

3.1. GRP78/BiP

The ER protein chaperone BiP, also known as 78-kDa glucose-regulated protein (GRP78), plays a major role in the adaptive response to ER stress and is commonly found to be highly expressed in breast cancer, lung cancer, prostate cancer and other malignancies [38–42]. The elevated expression of BiP plays a major role in the pro-survival and cytoprotective response of cancer cells to major environmental stress through a variety of mechanisms. Overexpression of BiP was found to protect human breast cancer cells from estrogen-starvation induced apoptosis through complex formation and inhibition of BIK, a pro-apoptotic BH3-protein [43]. The ATP binding site of BiP was found to interact with and suppress the activation of caspase-7, preventing apoptotic induction by topoisomerase inhibitors such as etoposide, doxorubicin and camptothecin [44]. Recently, it has been found that clusterin, an ER-stress induced protein chaperone, promotes survival of hepatocellular carcinoma cells under extreme ER stress through interactions with and increased expression of BiP [45]. In contrast, molecules that bind to and inhibit BiP increase cellular susceptibility to ER

stress induced cell death [46]. When BiP expression was reduced with small interfering RNA (siRNA) in HeLa cells, expression of GRP94, CHOP, ERdj4 and P5 increased, suggesting that another mechanism by which BiP prevents cell death is regulation of the UPR [47]. Furthermore, BiP is positively regulated by the mitogen-activated protein kinase (MAPK) pathway. Inhibition of the MAPK pathway in melanoma cells decreased expression of BiP, resulting in increased caspase-4 mediated ER-stress induced apoptosis [48].

The study of BiP in cancer has increased significantly and has proven to be a highly relevant therapeutic target. Many anticancer treatments function through activation of ER stress and as a result, induce BiP activity. BiP activation has been shown to contribute to resistance of cancer to these anti-cancer therapies. As previously mentioned, BiP expression plays a role in chemoresistance to topoisomerase inhibitors and subsequently, examining BiP expression in breast cancer prior to treatment can predict the prognosis of chemotherapy [44,49]. On the other hand, BiP expression is a positive indicator of therapeutic benefit from adjuvant chemotherapy in patients with colorectal cancer and this discrepancy may be due to differences in treatment or tissue [50]. In addition to acting as a biomarker for predicting treatment outcomes, BiP can be targeted for inhibition during traditional anti-cancer therapy to reduce its cytoprotective effects. Subtilase cytotoxin catalytic subunit fused with epidermal growth factor (EGF-SubA) is a BiP inhibitor and when used to treat cancer cells during photodynamic therapy, can prevent BiP mediated resistance [51]. Similarly, gastric cancer multidrug resistance cell-specific binding peptide (GMBP1) binds the surface of gastric cancer cells and prevents multidrug resistance through interacting with and decreasing expression of BiP [52].

3.2. IRE1 α and XBP1

The role of IRE1 α and XBP1 signaling has been investigated in solid tumors, breast cancer, hepatocellular carcinoma and multiple myeloma [2,53,54]. XBP1 is a major transcriptional factor involved in the adaptive response to ER stress and is required for solid tumor growth and survival under hypoxic stress [55]. An increase in XBP1 expression and splicing was observed in hepatocellular carcinoma and breast cancer, which may result from the regulatory function of XBP1 towards BiP [56,57].

Numerous reports have shown the importance of the IRE1 α and XBP1 signaling pathway in multiple myeloma [54]. Notably, XBP1 is overexpressed in plasma cells from myeloma and is an important transcription factor known to be required for plasma cell differentiation [58–61]. The overexpression of XBP1 may be a critical component that induces multiple myeloma [61]. Blimp-1, another transcription factor involved in plasma cell differentiation functions upstream of and up-regulates XBP1 which in turn induces the secretory pathway, enlarges the size of the ER and increases protein synthesis [60]. Furthermore, IRE1 α induces cellular proliferation through XBP1 splicing, increasing expression of genes that encode for ER-Golgi, plasma membrane, secretory proteins and in particular, cyclin A1 a cell cycle regulatory protein [62]. In addition, the IRE1 α -XBP1 pathway appears to be regulated by a family of Bcl-2 proteins. BAX and BAK were necessary to activate IRE1 α through a direct interaction with the cytosolic domain [63]. Bim and PUMA were also identified as IRE1 α interacting Bcl-2 family proteins that modulate XBP1 splicing activity [64]. These studies suggest a direct association between the UPR and the apoptotic pathway and provide a new regime in cancer therapy.

Studies indicate that the IRE1 α -XBP1 pathway is a major therapeutic target in treatment of cancer, more prominently in multiple myeloma [65]. Inhibition of XBP1 splicing by MKC-3946 reduced growth of multiple myeloma cells and enhanced the pro-apoptotic effects of drugs such as Bortezomib and 17-AAG [66]. Specific inhibition of IRE1 α endonuclease activity by STF-083010 sensitized multiple myeloma cells to ER stress and reduced survival [67]. A small molecule, 4u8C, that binds and blocks the active site of IRE1 α RNase activity inhibited XBP1

splicing and RIDD leading to disrupted ER mediated expansion and secretion [68]. Another inhibitor, toyocamycin, was found to specifically target and prevent splicing of XBP1 by IRE1 α , inhibiting XBP1 expression and induction through ER stress [69]. Analogs of 4-phenylbutyric acid that inhibit IRE1 α and XBP1 splicing have been developed and show strong therapeutic promise in their ability to block IRE1 α signaling pathways [70].

3.3. PERK, eIF2 α and ATF4

The activity of the PERK/eIF2 α pathway contributes greatly to the growth and survival of cancer under hypoxic stress [2,71]. Hypoxia has been shown to greatly down-regulate protein synthesis through an increase in PERK inhibition and phosphorylation of eIF2 α at Ser51. When the PERK/eIF2 α pathway is abolished, whether through PERK knockout or transfection with mutant alleles of PERK or eIF2 α , the cells experienced a decrease in protein synthesis inhibition and tolerance to hypoxic conditions [72,73]. Moreover, a microarray analysis of PERK^{+/+} and PERK^{-/-} mouse embryonic fibroblasts under aerobic and hypoxic conditions revealed mechanistically that PERK is responsible for activation of many angiogenic genes as well as an adhesion protein, vascular endothelial growth factor (VEGF) and type 1 collagen inducible protein [74].

While PERK inhibits general protein synthesis through eIF2 α phosphorylation, there is still active translation of ATF4, a transcription factor that regulates pro-survival genes, such as those involved in oxidative stress, amino acid synthesis, protein folding and differentiation [2,75,76]. ATF4 is overexpressed in many human solid tumors and is involved in promoting proliferation and survival of nutrient deprivation through regulating expression of asparagine synthetase [77]. Another mechanism by which ATF4 expression contributes to cancer formation is through negatively regulating genes involved in cellular senescence, while ATF4 repression inducing cellular senescence and preventing oncogenic transformation [78]. Furthermore, ATF4 mediates survival during severe hypoxia through up-regulation of LC3B [79]. LC3B is a component of the autophagosomal membrane, and up-regulation induces autophagy, a process by which cells increase lysosomal degradation of unnecessary cellular components, thereby conserving energy for survival under nutrient deprivation [79]. As well, the lysosomal-associated membrane protein 3 (LAMP3), which promotes hypoxia-driven metastasis, is highly expressed in primary human cervix tumors and is induced through the PERK/ATF4 pathway [80]. Lastly, induction of tumorigenesis by forced expression of myc, a transcription factor that controls cell growth and protein synthesis, requires activation of the UPR [81]. Myc-transformed cells create intrinsic stress, such as accumulation of unfolded proteins, which under normal conditions, lead to cell death. Activation of UPR is therefore necessary to deal with myc-induced stress, by activation of autophagy through the PERK/eIF2 α /ATF4 pathway [81,82].

Hypoxia is a very common stress factor in cancer and as a result, the PERK/eIF2 α /ATF4 pathway is a highly promising therapeutic target. When compared with inhibition of IRE1 α signaling, the PERK inhibitor, GSK-compound 39 was more effective in reducing adaptation and survival of cells to hypoxia-induced ER stress [83]. A potent and selective inhibitor of PERK activity, GSK2606414, was found to reduce tumor growth in vitro and in vivo as demonstrated in cells and human tumor xenographs in mice [84]. Similarly, GSK2656153, an ATP-competitive inhibitor of PERK, also exhibits anti-tumor effects, inhibiting growth of human tumor xenographs in mice in a dosage dependant manner [85]. Moreover, the role of the PERK/eIF2 α /ATF4 pathway in cancer progression is mediated by various mechanisms which can all be targeted for therapeutic development. For example, sinulariolide, an effective anti-tumor compound used to kill melanoma and bladder cancer cells, works through activation of the PERK/eIF2 α /ATF4/CHOP apoptotic pathway [86]. The nonsteroidal anti-inflammatory drug tofenamic acid suppresses progression of colorectal cancer cells through promotion of ER

stress and activation of the PERK/eIF2 α /ATF4 pathway [87]. In addition, when the PERK/ATF4/LAMP3 pathway was inhibited, the DNA-damage response was disrupted and breast cancer cells were sensitized to radiotherapy [88]. More recently, the active, Ser 245 phosphorylated ATF4 was found to be overexpressed in non-small cell lung cancer cells and could be further investigated as a potential biological marker for cancer [89].

3.4. ATF6

In comparison with the PERK and IRE1 α pathway, there have been fewer studies on ATF6 in association with cancer. Nevertheless, it has been shown that ATF6 does play a role in human cancers, that its expression is necessary for malignancy and that this is mainly dependant on interaction with the other arms of the UPR [54]. In a recent study, a missense single nucleotide polymorphism (SNP) was identified in the ATF6 gene, correlating with a decrease in ATF6 mRNA and ATF6 target gene expression, contributing to an increase in hepatocellular carcinoma susceptibility [90]. When ATF6 is knocked down in multiple myeloma cells, PERK phosphorylation of eIF2 α increases, which suppressed apoptosis and induced autophagy [54,91]. Furthermore, ATF6 is responsible for up-regulation of XBP1 expression [19] and the activity of both pathways increases BiP expression, which is required for hepatocarcinogenesis [57].

The signaling pathway of ATF6 and its relation to the other two UPR pathways may be complex and worth investigating as a target for anti-cancer treatment. In a study that looked at the dependence of pancreatic β -cells on active ATF6-p50 expression under non-stressed conditions, knockdown of ATF6-p50 induced apoptosis through phosphorylation of JNK, p38 and c-Jun [92]. SiRNA induced knockdown of C₁₆-ceramide was found to activate ATF6, increasing CHOP expression and leading to an increase in ER stress mediated apoptosis in human head and

neck squamous cell carcinomas [93]. ATF6 α expression was shown to enhance tumor cell survival through increased expression and activity of a cell surviving Rheb-mTOR signaling pathway [94]. While most studies show that ATF6 promotes survival in cancer, in myoblast cells, over-expression of ATF6 caused apoptosis. This occurs through an increased expression of pro-apoptotic WW domain binding protein 1 and suppression of the anti-apoptotic protein, myeloid cell leukemia sequence 1 [95]. The contradiction in ATF6 function in cancer may be due to the difference in ATF6 isoforms.

3.5. Sigma-1 receptor

The sigma-1 receptor is an ER protein chaperone up-regulated in many human cancer cell lines [96]. Importantly, the sigma-1 receptor interacts with other ER protein chaperones like BiP and IRE1 α at mitochondria associated membranes (MAMs), a specific region of the ER where the membrane may connect directly to the mitochondria [97]. The gathering of sigma-1 receptors at MAMs allows for the receptors to efficiently modulate cell survival signals from the ER to the mitochondria. When ER calcium is depleted, BiP dissociates from the sigma-1 receptor, enabling its function to stabilize inositol 1,4,5-triphosphate receptors at MAMs and facilitate calcium signaling to the mitochondria [97]. Additionally, under ER stress conditions, the sigma-1 receptor was also found to interact with and stabilize IRE1 α at MAMs and enhance cellular survival through prolonged activation of the IRE1 α -XBP1 pathway [98].

Increased expression of the sigma-1 receptor promotes the adaptive response against ER stress, while a decreased expression leads to apoptosis [97]. Inhibitors of the sigma-1 receptor may become promising therapeutic targets in cancer. Antagonists that inhibit sigma-1 receptor signaling caused a reduction in tumor growth by enhancing apoptosis

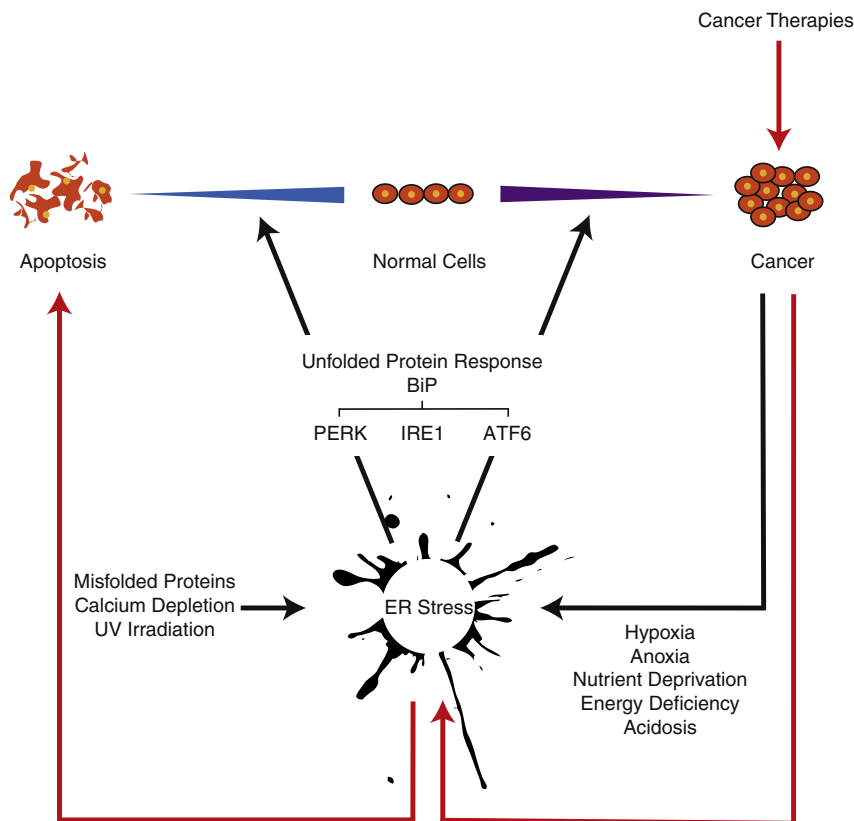


Fig. 1. ER stress and cancer. Under mild ER stress, the UPR is activated to cope with the disturbance in homeostasis, however when ER stress is prolonged apoptosis is activated to kill the cell. Studies have shown that an altered state of the UPR acts as a driver of malignancy but in other cases cancers alter the state of the UPR to adapt to its environmental stress (i.e. hypoxia, nutrient deprivation and low pH). The UPR can therefore become a target for anti-cancer therapy, with a focus on how to activate the apoptotic pathway.

through a caspase-dependent manner [99]. Furthermore, inhibition or knockdown of sigma-1 receptors reduced tumor cell mass through translational repression, revealing a role for sigma-1 receptors in protein synthesis and providing a new avenue for anti-cancer therapy [100].

3.6. ERAD

Maintaining homeostasis between protein synthesis and degradation is another important factor of normal cellular metabolism. ERAD is an integral part of the ER stress response responsible for the degradation of excess or misfolded intracellular proteins. By itself, the disruption of ERAD or the balance between protein synthesis and degradation can lead to accumulation of misfolded proteins, cytotoxicity, ER stress and eventual apoptosis. Therefore the effect of ERAD on ER stress and ER stress mediated apoptosis has been utilized in the development of potent anti-cancer drug treatments [65,101]. Proteasome inhibitors such as MG-132 and Bortezomib can elicit a strong stress response followed by apoptosis and specifically, Bortezomib has shown potential in clinical trials against non-Hodgkin lymphoma, acute myeloid leukemia and multiple myeloma [102–104]. In combination with Bortezomib, treatment with ER stress inducer celebrex or ERAD inhibitor Eeyarestatin I enhances the antitumor activity of Bortezomib and reveal the additive toxicity of ERAD inhibition with ER stress [105,106].

The AAA ATPase molecular chaperone valosin containing protein (VCP) or p97 has been shown to be up-regulated in many different types of cancer and may become a potential target in anticancer therapy [107]. VCP is involved in a wide variety of biological functions such as protein folding, cell cycle control and apoptosis but as a part of ERAD, it controls ubiquitin mediated degradation of misfolded proteins [107]. Development of VCP inhibitors has come under way and many are potential candidates in treatment of cancer. Eeyarestatin I, as mentioned above, is an ERAD inhibitor and functions through interaction with VCP, blocking its protein degradation activity [108]. VCP inhibition with Eeyarestatin I suppressed tumor growth and induced apoptosis in non-small cell lung carcinoma in vitro and in xenograft murine models [109]. DBeQ is a potent small molecule inhibitor of VCP that targets its ATPase activity and is effective in suppressing tumor growth and activating caspase-3 and caspase-7 in executing apoptosis [110]. Lastly, a novel group of allosteric VCP inhibitors, alkylsulfanyl-1,2,4-triazoles, were identified and may be further developed as a basis for future cancer therapy drugs [107].

4. Conclusion

Cancer cells deviate from normal cells in many aspects: they are highly proliferative, anaerobic and they often outgrow their blood supply. These physiological differences result in a tumor microenvironment that is hypoxic, low in nutrients and low in pH, factors that can cause resistance to anti-cancer therapy [4]. This microenvironment is unfavorable to cancer cells because it causes ER stress, a phenomenon that if prolonged, can lead to cell death through apoptosis (Fig. 1). Before triggering apoptosis, ER stress activates a number of adaptive responses, termed the UPR, to bring the cell back to homeostasis. Many human cancers have alterations in the UPR, and can therefore adapt to chronic stress and avoid cell death. Whether changes in the UPR are the driving force of or a consequence of malignancy is still unknown but components of the UPR may become a useful therapeutic target for anti-cancer therapies (Fig. 1).

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