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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.

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.

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

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; eIF2α, eukaryotic initiation factor 2α; ER, endoplasmic reticulum; ERAD, endoplasmic reticulum associated degradation; ERdj4, endoplasmic reticulum DnaJ homologue 4; ERO1a, ER oxidoreductin 1α; 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; IRE1α, inositol-requiring enzyme-1α; 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 IRE1α 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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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 1α (IRE1α) and activating transcription factor 6 (ATF6) [6–8]. Under normal cellular conditions, PERK, IRE1α 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 IRE1α [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, IRE1α 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 2α (eIF2α), 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, IRE1α undergoes dimerization and autophosphorylation, activating its endonuclease activity for mRNA processing [10,15–19]. A particularly important target of IRE1α is the mRNA encoding X-box binding protein 1 (XBP1). IRE1α 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 eIF2α [25]. CHOP induces ER oxidoreductin 1α (ERO1α), hyperoxidizes the ER environment and further commits the cell to apoptosis [25].

Activated IRE1α 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 IRE1α 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-κB, a transcription factor involved in the immune response and apoptosis [31].

Lastly, additional targets of the IRE1α RNase activity have been found [15,17,32,33]. Apart from XBP1 splicing, activated IRE1α is also responsible for regulated IRE1α 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 IRE1α in the absence of RIDD activity increased cellular survival against tunicamycin induced ER stress and apoptosis [34]. Moreover, IRE1α 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 IRE1α RNase activity and implicates the role of IRE1α 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