The Unfolded Protein Response in Inflammatory Bowel Disease

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The endoplasmic reticulum (ER) is a multifunctional organelle playing a vital role in maintaining cell homeostasis, and disruptions to its functions can have detrimental effects on cells. Dysregulated ER stress and the unfolded protein response (UPR) have been linked to various human diseases. For example, ER stress and the activation of the UPR signaling pathways in intestinal epithelial cells can either exacerbate or alleviate the severity of inflammatory bowel disease (IBD), contingent on the degree and conditions of activation.

Keywords: endoplasmic reticulum stress ; unfolded protein response ; EPICERTIN ; intestinal homeostasis

1. Endoplasmic Reticulum Stress (ER Stress)

The endoplasmic reticulum (ER) is a multifunctional organelle consisting of the nuclear envelope and the rough and smooth ER [1]. It performs a variety of cellular processes, including sterol and lipid biosynthesis, Ca^{2+} storage, and folding of newly synthetized proteins. Disruption of these processes negatively impacts the ER homeostasis, leading to the accumulation of misfolded/unfolded proteins (proteotoxicity) in the ER lumen, a condition called ER stress [2][3][4]. Upon sensing ER stress, the cell activates signaling pathways known as the unfolded protein response (UPR) to restore ER homeostasis [5].

The ER plays a key role in lipid membrane biogenesis. This involves the synthesis of enzymes required for the production of neutral lipids, such as triglycerides, cholesterol esters, and sphingolipids, that are incorporated into lipid membranes [6]. ER homeostasis is disturbed by factors such as the accumulation of exogenous saturated fatty acids, deficiency of desaturase enzymes, or diet-induced lipid depletion. These disruptions can cause lipotoxicity, followed by misfolded protein accumulation in the ER, and consequently, ER stress [7][8]. In addition, changes in the lipid composition of the ER membrane (saturated fatty acyl chains) increase the stiffness of the ER membrane, causing lipid bilayer stress [9]. This can directly activate the UPR without the involvement of unfolded proteins [10].

The ER serves as a reservoir for Ca^{2+} and regulates Ca^{2+} signaling through inositol 1,4,5-triphosphate (IP_3) receptors (IP_3R) and ryanodine receptors. These receptors are located in regions enriched with signaling proteins that are in contact with mitochondria, called mitochondria-associated ER-membrane (MAM) [3][4][11][12]. A constant level of Ca^{2+} in the ER lumen is essential for keeping Ca^{2+} receptors in a sensitive state [4] and supporting protein folding through Ca^{2+}-dependent chaperones, such as calnexin (CANX), calreticulin (CALR), and heat shock protein family A member 5 (HSPA5), also known as immunoglobulin heavy chain-binding protein (BiP/GRP-78) [3]. Hence, a decrease in ER luminal Ca^{2+} may result in the accumulation of misfolded proteins, inducing ER stress.

Folding and assembly of newly synthesized proteins take place in the ER. This process ensures that only properly folded polypeptides proceed through the secretory pathway to their final cellular destinations [13]. In contrast, incompletely folded polypeptides are transported back to the cytosol for subsequent ubiquitylation and degradation by the 26S proteasome [14][15]. In conditions where there is an increased demand for protein synthesis, whether due to physiological needs or pathological conditions, the ER may accumulate misfolded proteins within its lumen, leading to ER stress [16].

Interruption of protein transport between organelles can also cause the accumulation of misfolded proteins in the ER, thus inducing ER stress. An example of this can be observed in brefeldin A-mediated inhibition of guanine nucleotide-exchange factors and vesicle trafficking [17][18]. Vesicle trafficking between the ER and Golgi is primarily regulated by seven transmembrane KDEL receptors (KDELRs 1–3) [19]. ER chaperones possesses a carboxyl-terminal Lys-Asp-Glu-Leu (KDEL) retrieval signal that binds to KDELRs in intermediate compartments and cis-Golgi, enabling their return to the ER via coat protein complex I (COPI)-coated vesicles [20][21]. Upon binding to chaperones and other KDEL-containing proteins in the Golgi, KDELRs become activated. This leads to the activation of heterotrimeric G proteins such as Gαq, which

targets phospholipase C (PLC) for the generation of IP_3 and diacylglycerol, and Gαs, which stimulates adenylate cyclase [22]. This process activates protein kinase A and Src family tyrosine kinases that mediate the phosphorylation of transport proteins to maintain homeostasis of the membrane transport apparatus [23][24][25]. Additionally, KDELR signaling activates cAMP response element binding protein 1, a transcription factor that upregulates genes involved in vesicle transport [26]. Through these mechanisms, KDELR activation helps maintain cell homeostasis by integrating transduction cascades with membrane trafficking, cytoskeleton reorganization, invadopodia (actin-based structures that facilitate extracellular matrix degradation and cancer cell invasion) formation, and remodeling of the extracellular matrix [25][27]. Conversely, impaired KDELR-mediated recycling of chaperones can cause their secretion into the extracellular medium and subsequent shortage in the ER [28]. This imbalance can result in increased accumulation of misfolded proteins in the ER, aggravating the ER stress and the UPR, which can have detrimental effects. For example, cells stably expressing a non-functional transport mutant KDELR (D193N) restricted reverse transport of COPI from the Golgi to the ER and became sensitive to ER stress. Transgenic mice expressing this mutant KDELR developed myocardial cell death and cardiac hypertrophy, and ultimately died due to heart failure [29]. These findings illustrate the consequences of disturbing the recycling of proteins between the ER and the Golgi complex, leading to the accumulation of misfolded protein and ER stress. Similarly, gene deletion or homozygous mutation of chaperone genes (HSPA5, CALR) affected heart physiology and were lethal [30][31][32].

2. The Unfolded Protein Response (UPR)

The UPR serves as a safeguard mechanism designed to adapt to physiological or pathological demands in protein synthesis [33] via the generation of effector molecules that control gene transcription, mRNA translation, and degradation of misfolded proteins [34]. The UPR is initiated by three highly conserved signal transduction machineries held within the ER membrane upon sensing ER stress. They include two type I transmembrane kinases—the ER transmembrane inositol-requiring enzyme 1α and 1β (IRE1α and IRE1β) [35] and the eukaryotic translation initiation factor 2 alpha kinase 3 (EIF2AK3/PERK) [36]—as well as one unprocessed transcription factor: activating transcription factor 6 (ATF6) [37].

2.1. IRE1α/β

IRE1α and IRE1β are encoded by *ERN1* and *ERN2* genes, respectively. IRE1α is ubiquitously expressed, whereas IRE1β is predominantly found in the mucosal epithelium [38]. IRE1 has an endoribonuclease (RNase) domain and a serine/threonine kinase domain, both of which participate in the UPR. The RNase activity of IRE1 mediates the splicing of a 26-basepair intron from the mRNA encoding X-box-binding protein 1 (XBP1), generating the spliced form (XBP1s). XBP1s activates the transcription of genes required for energy expenditure, metabolism, ER function (e.g., chaperones and KDELRs) [28], cell survival, and differentiation in a cell type specific manner [39]. Importantly, XBP1s mitigates ER stress and promotes ER function by controlling the transcription of ER factors required for protein folding (e.g., protein disulfide isomerase, PDI), secretion, and factors involved in degradation of misfolded proteins, which is known as the ER-associated degradation (ERAD) machinery. XBP1s controls cell differentiation, adaptation, survival, and cell identity of highly secretory cells such as hepatocytes, pancreatic acinar and β-cells, and intestinal goblet cells [36][39][40], and the increased levels of protein synthesis and secretion make these cell types vulnerable to ER stress.

IRE1-mediated degradation of mRNAs, known as regulated IRE1-dependent decay, helps reduce ER stress by decreasing the abundance of mRNAs and synthesized proteins arriving to the ER for protein folding, including degradation of transcripts that may promote apoptosis such as death receptor 5 [41][42]. However, during unmitigated ER stress, the phosphorylated IRE1α appears to favor a switch from homodimers to higher oligomers, increasing the affinity of its RNase domain to additional RNA substrates. This leads to depletion of ER protein folding components, which further exacerbates the ER stress [43]. Under conditions of persistent UPR activation, IRE1α RNase also degrades microRNAs (miRs-17, -24a, -96, and -125b) that normally repress translation of caspase 2 mRNA, promoting caspase 2 activation and cell death [44].

Activation of IRE1 is not restricted to ER stress and the UPR. Through its cytoplasmic domain, IRE1 located at MAMs can be activated by docking signaling competent factors, independently of ER stress. This activation helps in regulating the redistribution of IP_3Rs and the local transfer of Ca^{2+} from the ER to the mitochondria matrix [11]. Subcellular distribution of IRE1 and EIF2AK3/PERK at MAMs has been suggested to optimize Ca^{2+} signaling and the crosstalk between these organelles [12]. In addition, the physical interaction of IRE1 with TNFα receptor-associated factor (TRAF2) activates NF-κB and c-Jun N-terminal kinase (JNK), thereby inducing inflammatory mediators. Through ER stress, inflammatory stimuli, or engagement of pattern recognition receptors (PRRs), IRE1-XBP1s signaling in myeloid cells controls eicosanoid metabolism, biosynthesis of prostaglandins (e.g., PGE2), and the resultant pain from tissue injury [45]. Finally, IRE1 can physically interact with proapoptotic proteins such as Bcl-2-associated X protein (BAX/BCL2L4) and Bcl-2-antagonist/killer

1 [46]. These interactions may alter the ER-mitochondria Ca^{2+} balance and subsequently induce mitochondrial-dependent cell death [3][46]

2.2. PERK

Upon ER stress, the EIF2AK3/PERK phosphorylates the eukaryotic translation initiation factor 2 alpha subunit (eIF2α) at serine 51, inhibiting protein synthesis. This action mitigates the ER stress while maintaining the translation of mRNA molecules that favor the UPR [15][47]. In this manner, the EIF2AK3/PERK-eIF2α pathway upregulates activating transcription factor 4 (ATF4), which appears to have dual functions. On one hand, it increases the biosynthesis of amino acids, chaperones, foldases, and components of the ERAD machinery to enhance ER function, mitigate the ER stress, and maintain cellular homeostasis [47]. ATF4 also upregulates protein phosphatase 1 (PP1) and the growth arrest and DNA damage-inducible protein (GADD34), which dephosphorylate and activate eIF2α, restoring protein synthesis [12]. On the other hand, EIF2AK3/PERK-eIF2α-ATF4 induces the transcription of CHOP (CCAAT/enhancer-binding protein homologous protein), leading to apoptosis during prolonged or unmitigated UPR. Of note, under established ER stress, restoration of mRNA translation by GADD34, or the expression of death receptor 5 by CHOP [42] can aggravate the dysfunctional UPR, leading to apoptosis [10][48][49]. EIF2AK3/PERK is found at MAMs, where it regulates reactive oxygen species (ROS) propagation under ER stress [11], supporting the idea that persistent EIF2AK3/PERK activation and Ca^{2+} release from the ER promotes mitochondrial damage and cell apoptosis [50].

CHOP enhances the expression of the ER oxidase 1α, which induces ROS-mediated oxidative damage and Ca^{2+} release from the ER by activating IP_3Rs. Ca^{2+} released from the ER-storage proteins into the cytosol reaches the mitochondrial membrane, promoting oxidative damage and resulting in the release of c-cytochrome and the assembly of the apoptosome [4]. Additionally, ROS, apart from activating Ca^{2+} release from the ER, are also considered to act as signaling molecules by regulating the activity of protein kinases and protein phosphatases. For instance, ROS can activate NF-κB and JNK and subsequently promote inflammatory and apoptotic signaling in the UPR [4]. CHOP activates proapoptotic proteins Bim (BCL2L11), telomere repeat binding factor 3, and death receptors, while inhibiting the prosurvival factor, Bcl-2 [51]. The promotion of apoptosis by CHOP aligns with the low level of both apoptosis and inflammation in the colon of dextran sulfate sodium (DSS)-treated $CHOP^{-/-}$ mice [52].

EIF2AK3/PERK also phosphorylates the nuclear factor-erythroid-2-related factor 2 (NRF2) [53], which, together with ATF4, controls the expression of antioxidant proteins (e.g., oxidoreductases, glutathione-S-transferase, and phenolic sulfotransferases). These proteins counteract the effects of ROS and promote cell survival [53][54].

Moreover, eIF2α can also be phosphorylated by eukaryotic translation initiation factor 2 alpha kinase 2 (EIF2AK2), also known as double-stranded RNA-activated protein kinase (PKR) [55]. This kinase is usually activated by viral infection and pathogen-associated molecular patterns (PAMPs), such as lipopolysaccharide (LPS) and inflammatory cytokines (e.g., TNFα). A role of EIF2AK2 in activating the adaptive UPR, prosurvival signaling, and proliferation of intestinal epithelial cells was reported in DSS-colitic $EIF2AK2/PKR^{-/-}$ mice [56]. However, EIF2AK2/PKR-mediated activation of eIF2α was also linked to apoptosis [57][58]. Thus, moderate activation of the EIF2AK3/PERK branch of the UPR can be protective, whereas prolonged activation may promote apoptosis [34].

2.3. ATF6α

ATF6α, a type II transmembrane protein within the ER membrane, undergoes proteolytic processing to generate the active bZIP transcription factor ATF6αp50. Under normal conditions, ATF6α stably binds to HSPA5/BiP. However, ER stress stimulates the ATPase activity of HSPA5/BiP, leading to its dissociation from ATF6α [59]. This liberates ATF6α monomers, which then relocate to the Golgi apparatus to be cleaved by site-1 and site-2-proteases in the luminal and transmembrane domains, respectively [37][60][61]. This processing results in the generation of the ATF6αp50 transcription factor.

ATF6αp50 binds to the CCAAT consensus sequence known as the cis-acting ER stress response element in the DNA, initiating the transcription of ER and ERAD-associated genes to expand the ER organelle and its protein folding capacity, including the expression of XBP1, CHOP, and ER chaperones [62]. ATF6αp50 can form heterodimers with XBP1s and synergistically enhance the UPR response [63], favoring the synthesis of proteins essential for folding and degradation, which, in turn, confers cytoprotection.

Both XBP1s and ATF6p50 regulate the transcription of genes encoding ER chaperones (e.g., HSPA5/BiP, HSP90B1/GRP94 and DNAJC3/p58IPK), foldases such as PDI, growth factors including mesencephalic astrocyte-derived neurotrophic factor, and enzymes vital for lipid membrane biogenesis, as well as the modification, translocation, and

secretion of proteins [12]. Taken together, ATF6α enacts various effector mechanisms essential for cytoprotection, membrane biogenesis, proper protein folding, and protein secretion to maintain ER-homeostasis.

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