Non-canonical Endoplasmic Reticulum Stress Transducers CREB3 family in cancer and other diseases

Yaqi Han[1], Ziwei Li[2], Zijian Cao[3], Jirui Sun[4], and Jinku Zhang[4]

[1]Hebei Medical University
[2]Chengde Medical University
[3]Institute of Translational Medicine
[4]Baoding No 1 Central Hospital

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Abstract

Background When unfolded proteins accumulate in the endoplasmic reticulum (ER), they cause ER stress and activate the pathways of unfolded protein response (UPR), constituted by a set of canonical ER stress transducing proteins. While the classical UPR is well-studied, the functions of non-canonical ER stress transducers are emerging. Findings The CREB3 (cyclic AMP responsive element binding protein 3) family, which contains five members including CREB3, CREB3L1, CREB3L2, CREB3L3, and CREB3L4, is the most important non-canonical ER stress response factor sensing and modulating unfolded protein homeostasis. As novel ER stress transducers, the CREB3 family plays important roles in regulating protein folding, modification, and secretion, contributing to biological events, including cell proliferation, differentiation, and migration in diverse contexts, especially in cancer. Conclusion This review summarized the roles of the CREB3 family in development and disease progression, with an aim to provide references for further research and clinical translation.

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Ziwei Li[a,c*], Yaqi Han [b,c*], Zijian Cao[e*], Jirui Sun[c,d] and Jinku Zhang[c,d]

[a] Hebei Medical University, Shijiazhuang, Hebei Province, China; [b] Chengde Medical University, Chengde, Hebei Province, China; [c]Department of Pathology, Baoding No.1 Central Hospital, Baoding, Hebei Province, China;[d] Key Laboratory of Molecular Pathology and Early Diagnosis of Tumor in Hebei Province, Baoding, Hebei province, China;[e] Zhejiang University School of Medicine, Hangzhou

Correspondence:

Jinku Zhang, zjkblk@sina.com, Department of Pathology, Baoding No.1 Central Hospital, Baoding, Hebei province 071030, China and Key Laboratory of Molecular Pathology and Early Diagnosis of Tumor in Hebei Province, Baoding, Hebei province 071030, China

*Equal contribution.

These authors declare no conflict of interest.

Background

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proteins. While the classical UPR is well-studied, the functions of non-canonical ER stress transducers are emerging.

Findings

The CREB3 (cyclic AMP responsive element binding protein 3) family, which contains five members including CREB3, CREB3L1, CREB3L2, CREB3L3, and CREB3L4, is the most important non-canonical ER stress response factor sensing and modulating unfolded protein homeostasis. As novel ER stress transducers, the CREB3 family plays important roles in regulating protein folding, modification, and secretion, contributing to biological events, including cell proliferation, differentiation, and migration in diverse contexts especially in cancer.

Conclusion

This review summarized the roles of the CREB3 family in development and disease progression, with an aim to provide references for further research and clinical translation.

Keywords : CREB3; unfolded protein response; endoplasmic reticulum stress; cell proliferation; cell differentiation; cancer progression

Introduction

1. Canonical ER stress transducing proteins

The endoplasmic reticulum (ER) is a vital organelle in eukaryotic cells, responsible for modifying newly synthesized proteins before they're transported to the Golgi apparatus and other organelles. This ensures the proper functioning of cellular processes and tissue homeostasis. In many physical or pathological situations, various triggers, whether endogenous or exogenous, can interrupt the normal folding process of proteins, generating unfolded or misfolded proteins in the cells. When these illy-processed proteins accumulate in the ER, they cause ER stress. The occurrence of ER stress could be detrimental to cells and tissues. To reduce the damage caused by ER stress, eukaryotes have evolved the unfolded protein response (UPR) [1-3]. Canonically, the UPR pathway is mediated by three classical ER stress transducers, including inositol requiring 1 (IRE1), PKR-like endoplasmic reticulum kinase (PERK), and activating transcription factor 6 (ATF6), which can sense the protein stress signal from the ER and transmit it to the nucleus to promote the transcription of UPR related target genes, thus relieving the protein stress from the ER by reducing protein synthesis, promoting protein folding, and degrading misfolded proteins. However, if the load of misfolded proteins surpasses the capacity of ER, the activated UPR pathway can initiate apoptosis, aiding in removing cells unable to reestablish ER equilibrium, thereby preserving overall cellular homeostasis[4-6].

2. The CREB3 family as non-canonical ER stress transducing proteins

Two canonical ER stress transducing proteins, IRE1 and PERK, are protein kinases, while ATF6 is a transcriptional factor located on the ER membrane before its activation. As an ER-resident transmembrane protein, ATF6 contains a basic leucine zipper (bZIP) motif in the cytoplasmic domain to drive gene transcription when activated[7-9]. Similar to the structure of ATF6, a set of five proteins belonging to the CREB3 (cyclic AMP responsive element binding protein 3) family, including CREB3, CREB3L1, CREB3L2, CREB3L3, and CREB3L4,has been identified as important players in the ER stressing and signaling[10,11].

CREB3, alternatively termed LZIP or Luman[12], was pinpointed using yeast-two hybrid assays due to its interaction with the transcriptional co-activator HCF[13]. While CREB3 mRNA is found in various rat and human tissues, its expression is most pronounced in the liver and nervous system[14]. CREB3L1, initially known as OASIS (old astrocyte specifically induced substance), was discovered during a gene screening process targeting long-term cultured astrocytes (old astrocytes) derived from the brains of newborn mice. These cultured astrocytes served as an in vitro model for investigating gliosis, a condition characterized by glial cell activation and proliferation[15]. Further investigation using Northern blot assays on various human tissues revealed that CREB3L1 expression levels were notably higher in many tissues, including heart, placenta, pancreas, prostate, lung, and colon, compared to the brain, testis, and skeletal muscle[16]. CREB3L2

was initially identified in a study of low-grade fibromyxoid sarcoma, where the bZIP domain and the C-terminal fragment of the CREB3L2 gene were fused with the FUS gene due to chromosomal translocation[17]. This pattern of genomic abnormality suggested that CREB3L2 could be an oncoprotein in sarcoma, but actually, very little was known about this fused protein for years, not to mention the wild-type CREB3L2. CREB3L3, also referred to as CREB-H, was initially identified as a liver-specific transcription factor[18]. In addition to the liver, CREB3L3 is expressed in the stomach and small intestine. This transcription factor plays various roles, including involvement in liver triglyceride metabolism[19], reduction of cholesterol absorption[19], regulation of glucose and lipid metabolism[20], activation of acute phase response, and mediation of hepcidin-dependent iron metabolism[21]. CREB3L4, also known as AIbZIP, CREB4, or TISP40, was initially characterized in the year of 2002. The CREB3L4 cDNA was initially isolated from LNCaP human prostate cancer cells treated with the synthetic androgen R1881. Notably, the cDNA analysis revealed a region exhibiting significant similarity to the bZIP domain found in CREB/ATF transcription factors. Consequently, the protein was designated AIbZIP (Androgen-Induced bZIP protein)[22]. AIbZIP, a novel bZIP gene located on chromosome 1q21.3 that is highly expressed in prostate tumors and of which the expression is up-regulated by androgens in LNCaP human prostate cancer cells.

The multifaced roles of the CREB3 non-canonical UPR proteins in cancer and other diseases

1. Structural characteristics and its implications for the CREB3 family

The members of the CREB3 family possess specific functional domains, arranged from the N- to C-terminus as shown in Figure 1. These include the transactivation domain (TAD) for sequence-specific DNA binding, a unique domain of about 30 residues known as ATB (Adjacent to bZIP), a basic region (Basic) that is contiguous with the leucine zipper domain (Zip)—collectively referred to as the bZIP DNA-binding domain—and a transmembrane domain (TMD). Notably, the ATB domain is a distinctive feature of this family and may suggest specialized functions for these proteins. Due to their structure features and specialties, the mechanism of activation of ATF6 and the CREB3 family members are strikingly different than IRE1 and PERK. Upon challenging the ER homeostasis, or induction of ER stress, ATF6 and these CREB3 family members will undergo regulated intramembrane proteolysis (RIP) to be activated, during which these proteins are transported to the Golgi apparatus and cleaved into two parts, the N-terminal and C-terminal fragments. The cleaved forms of proteins have different functions in various contexts, depending on tissue types, developmental stages, and disease progression status[10,11,23-26].

Much different than the well-studied canonical ER stress response proteins IRE1, PERK, and ATF6, the CREB3 family members are non-canonical ER stress transducing proteins and much less studied[1,2,23,24]. For the canonical ER stress transducing proteins, they mainly regulate the protein folding capacity of the ER. For example, both the IRE1-XBP1 and ATF6 pathways transcriptionally promote the expression of chaperone proteins. At the same time, the PERK-ATF4 can trigger cell death if the enhanced ER folding capacity cannot meet the requirement of fixing the damaged ER[4,5]. A common feature of the non-canonical ER stress transducing proteins is that they regulate the capacity of protein secretion of cells[27,28]. For example, CREB3L1 regulates the expression of essential proteins involved in the protein secretion pathway by directly transcribing the secretory capacity genes. It has been established that the CREB3 non-canonical ER stress transducing proteins have been implicated in regulating ER homeostasis and UPR signaling. For example, several studies have shown that the expression of CREB3L1 and CREB3L2 is significantly induced in the process of ER stress[27-29]. In the presence of ER stress, the CREB3 members, are cleaved by proteolytic enzymes site-1 protease (S1P) and site-2 protease (S2P) in the transmembrane domain, like ATF6. The N-terminus containing the transcription activation domain and bZIP domain after protein cleavage can enter the nucleus to promote the expression of target genes. At the same time, the C-terminus can be secreted into the extracellular space. The C-terminal fragment of CREB3L2 can activate the Hedgehog pathway of adjacent cells, promoting cell proliferation[27-29]. Upon activation, many of the CREB3 family members are closely related to various development and disease processes, including chondrocyte differentiation, chondrocyte proliferation, tumorigenesis, and tumor proliferation[17,27-29].Therefore, in this review, we summarized the research progress on the CREB3 non-canonical ER stress transducing proteins in cancer and other diseases,

providing references for further research on the mechanisms of related diseases.

2. Sensing protein folding status and regulating ER stress

As the non-canonical components of the UPR pathway, the CREB3 family can also sense the accumulation of unfolded or misfolded proteins in the ER and transmit the signal from the ER to the cytoplasm and nucleus, similar to the classical UPR sensors IRE1, PERK, and ATF6[4-6,11,23]. In fact, all members of the CREB/ATF family, including CREB3L1, CREB3L3, CREB3L4, and CREB3, are important proteins in sensing and regulating ER stress[11,23-26]. From a structural perspective, CREB3L2 is highly similar to the ATF6 protein, containing an ER transmembrane domain, a transcription activation domain, and a bZIP domain[30]. With these classical elements, CREB3L2 could act like other UPR proteins to sense ER stress. Like other UPR proteins, in normal physiological conditions, CREB3L2 is unstable and can be degraded by the ubiquitin protease pathway. In the presence of ER stress, on the contrary, the stability of the CREB3L1 protein is increased[10,31], and subsequently activated, transported from the ER to the Golgi apparatus, where the regulated intramembrane proteolysis (RIP) occurs[32]. Upon cleavage, the N-terminal fragment of CREB3L2 undergoes nuclear translocation and binds to the cyclic AMP-responsive element (CARE) site. This binding activates the transcription of UPR pathway genes, mitigating ER stress from accumulating unfolded proteins in stressed and damaged cells[10]. Like other UPR proteins, CREB3L2 exhibits a dual function in response to ER stress caused by the heightened synthesis and secretion of collagen and extracellular matrix proteins in proliferating chondrocytes. It not only alleviates ER load by enhancing protein secretion and transport but also suppresses apoptosis triggered by ER stress[29,33].

3. Regulating protein trafficking and secretion

To accommodate the diverse functions of different cells, sufficient and timely protein synthesis is indispensable. Especially in secretory cells, there's an elevated rate of protein synthesis. If the increased secreted protein cannot be transported out of the cells in a regulatory and timely manner, it may post a high pressure on the ER homeostasis, therefore leading to ER stress. Thus, ensuring the proper balance of protein synthesis and secretion causing ER stress is pivotal for maintaining a normal physiological internal cellular environment. Many studies have found that CREB3 family is instrumental in directing protein transport from the ER to the Golgi apparatus and regulating protein secretion.

Expression of the active form of CREB3L1 in pancreatic β-cell line can induce expression of genes involved in protein transport and extracellular matrix production[34]. It was also indicated that the expression level of CREB3L1 is up-regulated by thyrotropin in thyroid cells and CREB3L1 is sufficient to increase transport proteins levels and induce Golgi enlargement[35]. Liver fibrosis is marked by the transformation of hepatic stellate cells (HSCs) into myofibroblast-like cells, representing the HSC activation process. Once activated, HSCs exhibit elevated protein synthesis and secretion. During this transition, CREB3L2 augments the expression of Sec23A and Sec24D proteins that comprise the coat protein complex II (COPII), bolstering protein transport and secretion[36]. Antibody-secreting cells handle a substantial protein synthesis and secretion workload, necessitating the role of the ER stress transducer for optimal function. It has been reported that CREB3L2 is induced and activated during the differentiation of human B cells into plasma cells, possibly vital for sustaining the function of adaptive antibody-secreting cells (ASCs)[37]. CREB3L2 autonomously amplifies translation and secretion capabilities in pituitary cells, while a decline in CREB3L2 expression is significantly correlated with diminished secretion of growth hormone[38,39]. The Sec23a pathway, steered by CREB3L2, is integral in guiding protein transport from the ER to the Golgi apparatus in skin fibroblasts, providing support for collagen synthesis and secretion, and facilitating extracellular matrix protein production during chondrocyte development[29,40,41]. During the embryonic development of Xenopus laevis, CREB3L2 mRNA is predominantly found in the notochord, impacting the expression of genes involved in the secretion pathway[42].

Similarly, in the early stage of embryogenesis in Oryzias latipes, CREB3L2 orchestrates an array of COPII-related genes, including Sec23a/24d/13/31a, in collagen synthesis and secretion[43]. A missense mutation in CREB3L2's DNA binding domain reduces sec23a and sec24d gene expression. This gene expression change

can result in defects of protein secretion, leading to atypical head and notochord development in Brachydanio rerio[44].

4. Regulation of tumorigenesis and cancer progression

In human osteogenic sarcoma cells (HOS), CREB3 binds to the CC chemokine receptor 1 (CCR1) and contribute to Leukotactin-1-induced cell migration by enhancing the NF-ϰB activation pathway[45]. In human breast cancer cells, a specific binding relationship exists between CREB3 and Histone Deacetylase 3 (HDAC3). In this context, HDAC3 acts as a co-repressor of CREB3-mediated CXCR4 gene expression[46]. The CXCR4 gene encodes a chemokine receptor that is targeted by CREB3 and plays a critical role in cell migration for both leukocytes and tumor cells. Notably, high expression levels of CXCR4 have been observed in primary and metastatic human breast cancer cells. CREB3L1 may act as a suppressor of both metastasis and proliferation, potentially operating similarly to p53[47]. However, another group suggested that that in a metastatic variant of triple-negative breast cancer cells with activated PERK signaling and the epithelial-to-mesenchymal transition process, CREB3L1 is actually up-regulated. In these cancer cells, increased CREB3L1 expression enhances invasion by activating extracellular matrix genes like Col1A1 and FN1 (50).

The role of CREB3L2 in tumorigenesis primarily stems from a specific chromosomal translocation that results in the fusion of the CREB3L2 gene's C-terminus with the FUS gene, which was the very first time this protein caused attention from the field. This chimeric FUS-CREB3L2 gene has been closely associated with the onset of low-grade fibromyxoid sarcoma (LGFMS)[17]. Another form of protein fusion was found in some patients with thyroid carcinoma. It was observed that a fused protein containing the 5-prime sequence of the CREB3L2 gene (N-terminal fragment) and the PPARg gene was found in patients. The product of the CREB3L2-PPARg fusion gene triggers thyroid cell proliferation, ultimately contributing to the initiation or progression of thyroid carcinoma[48,49]. In malignant glioma, the RAS/MAPK or PI3K signaling pathways can activate CREB3L2, which in turn upregulates the anti-apoptotic factor ATF5, promoting cell survival and leading to the onset of malignant glioma[50]. Upon RIP, the N-terminus formed by CREB3L2 cleavage, as a transcription factor, enters the nucleus to promote the expression of genes involved in the ER-Golgi transport and plays a vital role in the differentiation of chondrocytes[29], while the C-terminus of CREB3L2 can be secreted to the extracellular environment of the cells and acts on adjacent chondrocytes to promote cell proliferation during chondrogenesis[27,28]. Therefore, it is currently proposed that both the N- and C-fragments of the cleaved CREB3L2 could be functional in promoting cancer cell proliferation. In addition, CREB3L2 is required for the proliferation and survival of chondrocytes by inhibiting cell apoptosis caused by ER stress[33,50]. Further studies have found that the C-terminus secreted following CREB3L2 cleavage in tumor cells can also activate the proliferation of adjacent tumor cells dependent on the Hedgehog pathway[28]. Considering the wild-spread functions of the Hedgehog pathway in cancer and other diseases, it is possible that CREB3L2 may generally contribute to the development of human diseases when the Hedgehog pathway is involved.

CREB3L3 and CREB3L4 have been also reported in cell proliferation of cells of different origins. CREB3L3 was found to play important roles in proliferation of hepatocytes. For CREB3L2, it has been shown that this factor was involved in the proliferation of prostate cancer cells promoted by the Androgen Receptor (AR) and IRE1α[51]. Mechanistically, the action of CREB3L4 is found associated with CREB3L1, resulting in suppression of p21 and, consequently, promoting cell proliferation[52].

5. The role of CREB3 family in development and other human diseases

Skeletal tissue homeostasis and dysfunction

Depending on the tissue context, different organs have specific signal transduction pathways to cope with the protein synthesis stress in the ER to maintain tissue homeostasis. Bone is a special organ in the human body, which requires very active extracellular protein synthesis and turnover to facilitate its normal functions. CREB3L2 is shown to have high expression in chondrocytes, and the N-terminus and the C-terminus formed by cleavage have different physiological functions in bones[27-29].

During chondrocyte differentiation, the heightened need for collagen and extracellular matrix protein synthesis leads to protein accumulation within the ER. Like its family member CREB3L1, CREB3L2 can directly bind to the promoter region of *Sec23a* —a gene essential for protein transport and secretion—to activate the latter's transcription. This regulation is pivotal for chondrocyte differentiation and soft bone and cartilage morphogenesis. CREB3L2 deficient mice exhibit pronounced cartilage dysplasia and often succumb to suffocation shortly after birth due to underdeveloped thoraxes[29]. Consistent with the important role of CREB3L2, Sox9, a well-established master regulator of chondrogenesis, orchestrates cartilage development by modulating the CREB3L2-Sec23a axis[41]. In the meantime, the C-terminus of CREB3L2 is found in the extracellular space during cartilage formation, likely due to the cleavage and secretion of CREB3L2. Upon secretion, it binds to the Patched-1 receptor on neighboring chondrocytes, stimulating the Hedgehog pathway, which fosters chondrocyte proliferation[27]. Therefore, the cleavage of CREB3L2 in the transmembrane region, guided by proteolytic enzymes S1P and S2P, is integral to its role in skeletal development. Clinical evidence suggests that S1P deficiency might cause human skeletal dysplasia[53].

On the other hand, CREB3L2 mitigates cell apoptosis triggered by ER stress by engaging the ATF5-MCL1 pathway in growing chondrocytes, thereby facilitating chondrocyte proliferation[33]. The differential proteomics-based method, DiPIUS, identified that CREB3L1 and CREB3L2 can be targeted and downregulated by Fbxw7 for ubiquitin-based protein degradation, therefore regulating both osteogenesis and cartilage formation[54]. Investigations into how human immunodeficiency virus type 1 enhancer-binding protein 3 (Hivep3) influences osteogenesis and chondrogenesis revealed that Hivep3-induced Alg2 upregulation is vital for modulating CREB3L2 expression, thereby advancing chondrogenesis[55]. Thus, The CREB3 proteins appears as a signaling hub to integrate multiple regulation mechanisms in skeletal development.

Development and disease progression of the neural system

The neural system is another type of tissue that mainly requires the CREB3 family. The neuron development and nerve maturation process is highly dependent on active protein secretion. Therefore, neural tissue is highly susceptible to ER stress. Interestingly, during the recovery process post-nerve injury, the demand for protein synthesis and secretion is also elevated, accompanied by activation of the UPR pathways.

It has been demonstrated that both CREB3L1 and CREB3L2 exhibits widespread expression in the central nervous system and is notably upregulated in injured nerves. The N-terminal fragment of CREB3L2 is highly expressed in the injured neural tissues, likely in response to the ER stress that occurred in the damaged nerves[10]. Moreover, this N-terminus specifically binds to the promoter region of the brain-derived neurotrophic factor (BDNF) gene within injured neurons, stimulating its transcription and expression and consequently playing a critical role in neuronal protection[56,57]. In addition, during axon development, the C-terminal fragment of CREB3L2 can be secreted into the dorsal root ganglion (DRG) axon and acts as a promoting factor for axon growth. From a mechanical perspective, the C-terminal CREB3L2 binds to Patched-1 receptors on developing axons with Shh ligands, facilitating axon growth[58]. Additional findings suggest that CREB3L2 plays a significant regulatory role in modulating neuron differentiation induced by nerve growth factor, as well as oligodendrocyte maturation and apoptosis[59-61]. In addition to the well-studied N-terminal and C-terminal fragments, another fraction of CREB3L2, the so-called BBF2H7-derived small peptide (BSP), which was a short peptide located between the S1P and S2P splice sites and a product upon RIP during ER stress, was found in many neural tissues. This BSP peptide tends to aggregate like amyloid β (Aβ) proteins. The production of aggregated peptides may be closely associated with the pathogenesis of neurodegenerative diseases[32].

Emerging functions of the CREB3 proteins in other contexts

The IRE1-XBP1 and ATF6 branch of the UPR has been implicated in the development of B cells and certain sub-linages of T cells. Interestingly, in T helper 2 (Th2) cells, the role of CREB3L2 was identified by unbiased analysis on transcriptomic datasets of immune cells to identify important transcription factors regulated by transcription activator 6 (STAT6), which is an essential determinant of cell fate of Th2 cells. It was found that CREB3L2 is one of the most significant direct target genes positively regulated by STAT6,

implying an unreported function of CREB3L2 in the differentiation of Th cells[62]. These findings further suggested that CREB3L2, a UPR factor, may also contribute to cell fate determination in specific cell types. In addition to T cells, CREB3L1 and CREB3L2 are also functionally involved in the transdifferentiation of hepatic stellate cells (HSCs) and impacts the progression of liver fibrosis[36,63]. Interestingly, CREB3L2 appears to be elevated upon alcohol intake, suggesting additional potential functions of CREB3L2 in alcohol metabolism and even the pathological conditions caused by alcohol abuse[64-66].

Conclusion

As non-canonical ER stress transducing proteins, the CREB3 family members have been implicated in diversified settings of developmental processes and diseases. While it is of great interest to further explore the new functions of the CREB3 family in additional contexts, it would be even more critical to accurately dissect the underlying mechanisms of how these proteins are fine-tuned to modulate different biological events and pathways in distinct cell types[67]. An in-depth understanding of this family and its mode of regulation will shed light on how the ER stress-sensing machinery orchestrates multiple cellular components to govern tissue homeostasis and affect disease progression.

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Figures Legends

Figure 1 Schematic of the structure and mode of activation of the CREB family

(A) Structure of the CREB3 family protein.

(B) The process of activation of the CREB3 family

TAD: Transactivating domain; TM: Transmembrane domain; C-ter: C-terminal domain; N-ter: N-terminal domain

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Figure 1