X-box Binding Protein 1 in Tumor Cell Adaptation and Death: Towards Specific Regulation

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Abstract: - The ability to maintain homeostasis is critical for ensuring proper cell function and organismal viability. Environmental stress disrupts cell homeostasis by triggering molecular and metabolic changes leading to adaptation or death. Cells respond to environmental stress by activating stress- and compartment-specific response pathways. Unfolded protein response (UPR) is one of the stress response pathways that restore endoplasmic reticulum (ER) homeostasis during ER stress by regulation of protein refolding. Transcription factor X- box binding protein 1 (XBP1s) plays a central role in cellular adaptation to ER stress by activation of multiple UPR target genes. Abnormal activity of XBP1s is harmful to cells and has been linked to tumor progression and metastasis. Currently, the targeting of XBP1 is considered a promising strategy for cancer treatment. However, UPR inhibitors are nonselective and decrease the XBP1s activity in normal cells leading to undesired effects of chemotherapy. Besides, the critical accumulation of XBP1s in the nucleus during prolonged ER stress stimulates the expression of transcription factor Krüppel-like factor 9 (KLF9), which induces increases in oxidants and calcium ion concentration and subsequent cell death. Because of differences in XBP1s transcriptional activity between normal and tumor cells, stimulation of UPR in a certain range can enhance oxidative stress and the effect of antitumor drugs in tumor cells and exhibit protective properties in the normal cells. This review discusses the mechanisms of cell adaptive and terminal responses based on transcriptional regulation by XBP1s and describes a biophysical model of dose-dependent biphasic response as a quantitative basis for specific regulation of XBP1s in normal and tumor cells.

Key-Words: - Cell stress response, endoplasmic reticulum stress, unfolded protein response, X- box binding protein 1, Krüppel-like factor 9, tumor cells, biphasic response.

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1 Introduction

Different stressors induce the accumulation of unfolded and misfolded proteins resulting in ER homeostasis disturbances and stress, [1]. For restoring protein folding capacity a multifaceted program termed unfolded protein response is activated. In mammalian cells, three pathways of the UPR have been identified. Each pathway is activated by conformational changes in membrane-bound sensors that include inositol requiring enzyme 1 (IRE1), protein kinase R-like ER kinase (PERK), and activating transcription factor 6 (ATF6), [2]. Activation of these sensors leads to ER-associated protein degradation (ERAD) and transcription of multiple genes involved in protein refolding. Expression of genes that regulate cell survival under ER stress is mainly activated by transcription factor XBP1s, [3], [4].

XBP1s was first characterized as a transcription factor that regulates human major histocompatibility complex class II gene expression in B cells, [5]. Subsequent studies have shown that XBP1s is activated by IRE1 and participates in the most evolutionarily conserved pathway of UPR, [6], [7]. IRE1 is a bifunctional kinase/endoribonuclease (RNase) that induces non-conventional mRNA splicing to transmit the UPR signal. Its ribonuclease function is activated by IRE1 oligomerization in the ER membrane when released chaperone glucose-regulated protein 78 kDa (GRP78), [8]. In the structure of the IRE1 oligomer, phosphates participate in ionic bonds between adjacent monomers, suggesting a role for phosphorylation in IRE1 activation, [9]. Perhaps, the level of IRE1 autophosphorylation can regulate cell outcome depending on ER stress severity, [10], [11]. Low-level ER stress causes transient kinase autophosphorylation and tetramerization that excises 26-nucleotide nonconventional intron from *XBP1* mRNA, leading to subsequent XBP1s translation. In

contrast, high-level IRE1 autophosphorylation during prolonged ER stress leads to higher-order oligomerization of IRE1 and increases *XBP1* splicing activity and non-selective degradation of mRNA species that results in the depletion of ER structural components and cell death activation, [11]. Thus, mild or moderate level of ER stress leads to proteome changes that promote cell adaptation and survival. On the other hand, prolonged ER stress induces cell death. However, it is unclear what mechanism leads to ER stress-induced cell death, [1]. This review describes the possible mechanisms switching the XBP1s-induced cell adaptive and terminal responses and their relationship with cancer. Special emphasis is given to quantitative differences of XBP1s-mediated cell response in tissue-specific gene regulation.

2 XBP1s-regulated Cell Reprogramming in Adaptive and Cytotoxic Response

ER stress activates an adaptive UPR, which reprograms cell functions for further survival, including the activation of gene expression that increases the protein refolding and decrease the synthesis of new proteins, [1], [11]. If the adaptive response fails to restore protein-folding homeostasis, the UPR results in cell death, [12]. The underlying mechanisms that switch from cytoprotective to cytotoxic unfolded protein response are still being investigated.

Several lines of evidence argue that IRE1 can function as a molecular rheostat capable of regulating cell fate, [12], [13]. As previously discussed, the differences in autophosphorylation and oligomerization of IRE1 could be used in the activation mechanisms of opposing cellular programs. Additional ER stress-mediated mechanisms of cell death may include phosphorylation of apoptotic-signaling kinase-1 (ASK1) by IRE1, which causes activation of stress kinases Jun-N-terminal kinase (JNK) and p38 mitogen-activated protein kinase (p38 MAPK), [14]. Among the apoptosis-related substrates of JNK are pro-apoptotic Bax and anti-apoptotic Bcl-2, which are activated and inhibited, respectively, by JNK phosphorylation, [15], [16]. p38 MAPK is reported to promote apoptosis by phosphorylation of transcription factor CHOP (CCAAT/enhancer-binding proteins-homologous protein), which causes changes in apoptotic gene expression, [17]. Similarly, other UPR effectors, such as ATF6 and PERK, can also activate CHOP-dependent apoptosis during prolonged ER stress, [18].

Another attractive mechanism is that the switch from adaptive to terminal UPR can be controlled by the same transcriptional factor, XBP1s (Figure 1). Upon ER stress XBP1s is translocated into the nucleus to initiate expression of UPR-associated genes that encode folding enzymes, chaperones and ER-associated protein degradation components, [7], [19]. In mammals, XBP1s regulate the transcription of UPR-associated genes by binding to specific sites, such as ER stress response element (ERSE), ERSE-II, and unfolded protein response element (UPRE), [20]. The CCACG section of the consensus sequence in ERSE (CCAAT-N9-CCACG) or ERSE-II (ATTGG-N-CCACG) is specific for the binding of XBP1s and ATF6, while the consensus sequence of TGACGTG(G/A) in UPRE is preferentially bound by XBP1s, [21].

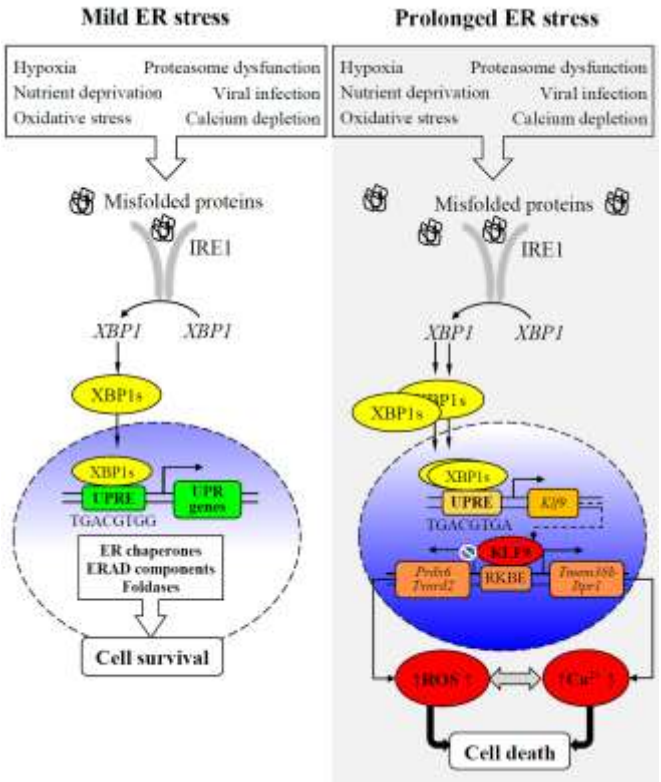

Fig. 1: Regulation of the switch from adaptive to terminal cell response by XBP1s

Subsets of XBP1s-regulated genes depend on the cell types and signals. UPR-associated genes include chaperones (*Dnajb9*, *Dnajb11*, *Dnajc3*, *Pdia3*, *Grb78*, *Grp94*, and *Calr*), ER-associated protein degradation components (*Edem1*, *Herpud1*, and *Hrd1*), foldases (*Pdia6*), and translocon (*Sec61a1*), [11], [22]. Additionally, XBP1s activate the gene expression of transcription factor Mist1,

disintegrin, and metalloproteinase 10, [23], [24]. Moreover, XBP1s regulate the transcription of diverse genes involved in lipid and glucose metabolism and immune responses, [25], [26].

XBP1s is a key transcription factor that regulates the homeostasis of endoplasmic reticulum and cell survival. However, the critical accumulation of XBP1s under prolonged ER stress leads to the transcriptional upregulation of KLF9, [27]. The consensus sequence of *Klf9* in UPRE (TGACGTG**A**) differs from the sequence (TGACGTG**G**) of other XBP1s targets by a single nucleotide therefore forms a complex with XBP1s at higher concentrations than other XBP1s-regulated genes. Transcription factor KLF9 regulates the expression of Ca^{2+} storage regulator transmembrane protein 38B *(Tmem38b*) and inositol 1,4,5-trisphosphate receptor type 1 (*Itpr1*) genes resulting in an increase of cellular Ca^{2+} concentration by Ca^{2+} release from ER.

KLF9 also participates in the regulation of different biological functions, including oncogenesis, cell proliferation, and stress responses, [28], [29]. KLF9 operates as an activator or a repressor depending on the number of GC elements present in the promoter of target genes, [28], [30]. Gene promoters with multiple GC-boxes are activated by KLF9, while promoters with a single GC-box are repressed by KLF9. Importantly, KLF9 is an inducible transcriptional factor that is stimulated by various stressors. It has been shown, that overexpression of *Klf9* increases oxidative stress, [31]. High level of KLF9 represses antioxidant defense genes, such as mitochondrial thioredoxin reductase 2 (*Txnrd2*) and peroxiredoxin 6 (*Prdx6*) genes, resulting in increased reactive oxygen species (ROS) levels and ROS-induced cell death, [32], [33]. Importantly, the expression of *Klf9* is also stimulated by excessive nuclear erythroid 2 p45-related factor 2 (NRF2), a key transcriptional regulator of cellular redox homeostasis, [33]. Under normal conditions, cytoplasmic Kelch-like-ECH-associated protein 1 (KEAP1) noncovalently binds NRF2 which results to the degradation of complex KEAP1-NRF2 in the 26S proteasome. Moderate oxidative stress and electrophilic agents disrupt the KEAP1-NRF2 interaction and NRF2 activates the expression of genes involved in cell protection and adaptation to oxidative stress, [34]. Besides oxidative stress, dissociation of the KEAP1-NRF2 complex can be also induced as a result of NRF2 phosphorylation by PERK, [35]. Moreover, PERK indirectly supports the level of NRF2 via regulation of activating transcription factor 4, which increases *Nrf2* expression by binding to *cis*-regulatory C/ebp-Atf response element (CARE) within the NRF2 promoter, [36]. Due to the key role of the KEAP1-NRF2 system in cell adaptation under stressful conditions, it has been considered a potential target for the treatment of a wide range of diseases, [37], [38]. However, the critical accumulation of NRF2 under prolonged ER or oxidative stress stimulates the expression of transcription factor KLF9, resulting in further KLF9-dependent increases in oxidants and subsequent cell death, [27], [32]. Cells expressing an increased level of KLF9 have high sensitivity to oxidative stress, [32], [33]. Instead, depletion of the *Klf9* gene enhances the *Prdx6* expression that increases the resistance of transfectants to ROS-induced cell death, [39]. Moreover, depletion of *Klf9* decreases endoplasmic reticulum stress, [27].

Based on the key role of the ER redox state in protein folding, it should be no surprise that oxidative stress and endoplasmic reticulum stress are closely associated processes, [40]. Increased level of ROS activates inositol-1,4,5-trisphosphate receptors and ryanodine receptors on the ER membrane and triggers the Ca^{2+} release from ER, [41], [42]. Ca^{2+}, in turn, stimulates mitochondrial ROS production, which further increases Ca^{2+} release and ER stress. On the other hand, the transcription factor CHOP can regulate the expression of endoplasmic reticulum oxidoreductase 1 (ERO1) leading to an increase in H_2O_2 production, [43]. Taken together, these findings suggest that the overexpression of KLF9 amplifies both endoplasmic reticulum stress and oxidative stress through elevated Ca^{2+} release and suppression of antioxidant genes, thus resulting in cell death. Probably, differences in the affinity of KLF9 promoter region and other target genes allow XBP1s to transform a continuous stress signal into qualitatively different output cell response – cytoprotective adaptation or death, [27], [44]. These findings suggest that the switch from high affinity to lower affinity targets is a general paradigm of how the same transcription factor can drive different patterns of cell functioning in response to quantitative changes in an incoming signal. Further, a simple biophysical model of dose-dependent biphasic response regulated by XBP1s was used to see how quantitative differences in UPR response in normal and tumor cells can define an output signal.

3 Biophysical Model of Dose-Dependent Biphasic Response Regulated by XBP1s

To describe the output response, it is assumed that the cytoprotective activity of the cell is proportional to the number of XBP1s (L) bound to specific sites (S) of DNA. The most general mechanism of specific protein-site binding can be represented as a reaction of kinds:

$$L+S \underset{k_{-1}}{\overset{k_{+1}}{\rightleftarrows}} LS \tag{1}$$

Using a steady-state approximation and the law of mass-action, we obtain:

$$k_{+1}[L]([S]_0-[LS])=k_{-1}[LS] \tag{2}$$

where k_{+1} is the association rate constant, k_{-1} is the dissociation rate constant, $[L]$ is the concentration of unbound XBP1s, $[S]_0$ is the total concentration of specific binding sites, $[LS]$ is the concentration of bound XBP1s. From equation (2) the fraction of bound XBP1s will be determined by the equation:

$$\frac{[LS]}{[S]_0}=\frac{[L]}{k_d+[L]} \tag{3}$$

where $k_d = k_{-1}/ k_{+1}$ is the dissociation constant. This equation is similar to the Hufner, Langmuir and Michaelis-Menten equations, since it describes a similar physical process. One of the most evident theoretical limitations of these equations is predicting of hyperbolic response approaching asymptotically to maximum. However, in the case of a molecular switch like XBP1s, the system is characterized by a non-monotonic biphasic response, which is described by a U-shape curve. According to experimental data, when a certain threshold is reached, part of XBP1s molecules will bind to another site, resulting in the activation of specific gene expressions that contribute to cell death. This fact can be taken into account by introducing an additional equation that describes the inactivation of the complex at high concentrations:

$$L+S \underset{k_{-1}}{\overset{k_{+1}}{\rightleftarrows}} LS+S \underset{k_{-2}}{\overset{k_{+2}}{\rightleftarrows}} LS_2 \tag{4}$$

where LS_2 is the inactive complex. Using a definition of the dissociation constant for inactivate complex as $k_i = k_{-2}/ k_{+2}$ we obtain the next equation:

$$\frac{[LS]}{[S]_0}=\frac{[L]}{k_d+[L]\left(1+\frac{[L]}{k_i}\right)} \tag{5}$$

Steady-state values of the fraction of bound XBP1s as a function of the concentration of unbound XBP1s are shown in Figure 2. The dependence described by equation (5) characterizes a biphasic response since it has a rising phase and a decreasing phase.

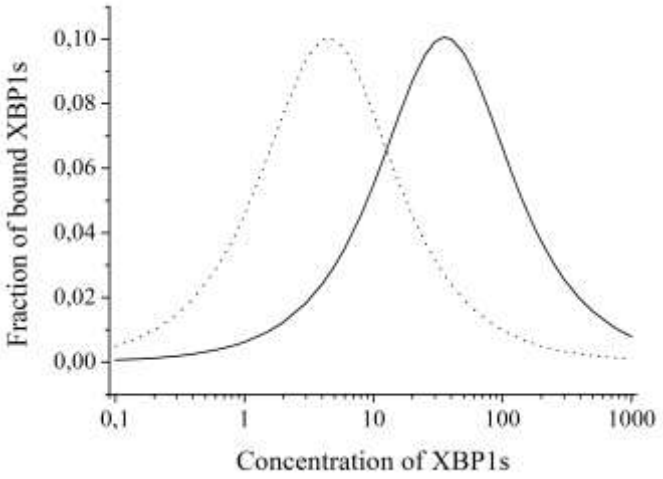

Fig. 2: Model predictions

The bold line represents the dose-dependent response of normal cells. The dotted line represents the dose-dependent response when the affinity of L for S is increased, which can appear in tumor cells (Parameters values for equation (5) are set to: k_d=160, k_i=8 (for bold line); k_d=20, k_i=1 (for dotted line))

Despite the simplicity of this two-step reaction model, it allows one to make an important assumption. If, due to mutations, the dissociation constants of XBP1s are lower in tumor cells (dotted line) than in normal ones (bold line), then as a result of a shift in the biphasic response maximum, there is a concentration range in which the normal cells response is cytoprotective, while the tumor cells response is cytotoxic (Figure 2). These differences could be involved in the new strategy for cancer treatment.

XBP1s is known to play an important role in cancer development and progression, which has been reviewed many times, [11], [45], [46], [47]. In general, XBP1s are required for tumor growth, tumor cells immune evasion, tumor angiogenesis, invasion, and metastasis. Moreover, XBP1 regulates tumor cells' response to hypoxic and acidic environments, promoting malignant tumor metabolism, and improving their chemoresistance and resistance to oxidative stress, [48].

Expression of mutant XBP1s has been detected in various cancer cells. Mutations in XBP1s can result in a change in transcriptional factor activity and DNA binding affinity. For example, several studies indicate that frameshift mutations XBP1s likely produce highly active transcription factors independent of the RNase activity of IRE1 in breast

cancer, [49]. Although mutations in XBP1s that alter its binding ability have not yet been identified, it is assumed that mutations increasing the binding affinity may cause a raise of cell survival and growth in several cancer types, [50]. Given that the high XBP1s activity is needed for tumorigenesis it should be no surprise that targeting XBP1s has become a new approach for cancer treatment, [51], [52], [53].

Several lines of evidence show that XBP1 knockdown significantly enhances the sensitivity of glioma cells to oxidative stress, [54]. Downregulation of XBP1 also increases ROS production and sensitivity to oxidative stress in serous ovarian cancer cells, [55]. Recently, several low-molecular compounds that selectively block *XBP1* mRNA splicing have been identified, [56], [57]. Inhibitors of IRE1-dependent XBP1 activation also exhibit anticancer potential and can be used in antitumor therapy. It was shown that toyocamycin, an antibiotic analog of adenosine, selectively inhibits IRE1 endoribonuclease activity and induces apoptosis in multiple myeloma cell lines, [58]. In addition to toyocamycin, antitumor activity is also found in other inhibitors of *XBP1* mRNA splicing, [56], [57]. However, UPR inhibitors are non-selective and decrease the XBP1s activity in normal cells leading to the undesired effects of chemotherapy. On the other hand, as previously discussed, the stimulation of UPR in a certain range can enhance oxidative stress and the effect of antitumor drugs in tumor cells and exhibit protective properties in normal cells.

One of the possible IRE1 endoribonuclease activators that induce opposite responses in normal and tumor cells can be a plant flavonoid quercetin. As was shown this compound binds to “Q site” of the endoribonuclease domain and activates *XBP1* mRNA splicing, [59]. In endothelial cells, quercetin has been shown to reduce tunicamycin-induced ER stress, [60].

Quercetin enhances the antitumor action of paclitaxel towards prostate cancer through ER stress induction and ROS production, [61]. As was shown quercetin increases apoptosis through induction of ER stress in human prostate cancer cells, [62]. In addition, it was shown that a synthetic derivative of quercetin (5,30-dihydroxy-3,7,40-trimethoxyflavone or TEF) increases the levels of IRE1α and XBP-1, and induces apoptosis colon cancer cells, [63]. Moreover, it is shown that quercetin inhibits proliferation and induces apoptosis in various cancer cells, [64], [65], [66]. Although flavonoids can influence different signaling pathways, the proposed model of biphasic response shows that different cell outcomes may be achieved by the activation of the same pathway. Future studies are needed to see how broadly this model is used in nature and whether it can account for the opposite properties of the same compound.

4 Conclusion

ER is involved in multiple fundamental biological processes including lipid and protein synthesis, folding, and Ca^{2+} signaling. In response to stress, the ER triggers the unfolded protein response via three stress sensors on the ER membrane that activate the expression of genes involved in protein refolding. Among the transcription factors activated during unfolded protein response, XBP1s is a key transcription factor that regulates the homeostasis of endoplasmic reticulum and cell survival. However, the high level of XBP1s accumulation above a critical threshold leads to the expression of a gene encoding transcription factor KLF9 and triggers cell death. Unlike other XBP1s targets, the KLF9 promoter contains a lower-affinity binding site that forms a complex at higher concentrations of XBP1s. A quantitative description of this process by a two-step reaction model predicts a non-monotonic biphasic response, which is described by a U-shape curve. Although the quantitative parameters that describe the switch between life-to-death decisions are not fully understood, the model of biphasic response allows us to make an important assumption according to which differences in XBP1s transcriptional activity between normal and tumor cells can determine the activation of opposing cellular programs. These findings open new opportunities in the development of therapeutic approaches to anticancer therapy. Given the dichotomy in outcomes of XBP1s activation, it is clear that the selective cell response can be induced not only by different substances but also by differences in concentration. Future studies are needed to develop approaches that make it possible to quantitatively characterize the level of cellular ER stress in different cells.

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