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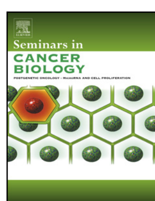

Review

Targeting the IRE1α–XBP1 branch of the unfolded protein response in human diseases

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ABSTRACT

Accumulation of unfolded or misfolded proteins in the endoplasmic reticulum (ER) leads to ER stress, which is characteristic of cells with high level of secretory activity and implicated in a variety of disease conditions. In response to ER stress, the cell elicits an adaptive process called the unfolded protein response (UPR) to support cellular homeostasis and survival. However, prolonged and unsolvable ER stress also induces apoptosis. As the most conserved signaling branch of the UPR, the IRE1α–XBP1 pathway plays important roles in both physiological and pathological settings and its activity has profound effects on disease progression and prognosis. Recently, modulating this pathway with small molecule compounds has been demonstrated as a promising approach for disease therapy. In this review, we summarize a list of current investigational compounds targeting this pathway and their therapeutic features for treating human diseases.

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1. Introduction

Controlling a critical step along the secretory pathway, the endoplasmic reticulum (ER) is the central organelle where newly synthesized proteins mature and are properly folded. A variety of stresses, including increased cellular demands of secretory protein production, glucose deprivation, hypoxia, and redox perturbation, causes accumulation of unfolded or misfolded proteins inside the ER. Collectively, we call these conditions as ER stress. In response to ER stress, the cell initiates a series of adaptive signaling pathways, referred to as the unfolded protein response (UPR), in order to restore protein folding homeostasis. The UPR actively reduces protein translation, increases expression of ER chaperones and enzymes facilitating protein folding, and clears misfolded proteins for degradation [1]. However, under prolonged ER stress, homeostasis cannot be restored and the UPR also induces cell death through apoptosis [2]. A number of specialized secretory cells, such as plasma cells or pancreatic β cells, rely upon the UPR for normal physiologic function because of the increased demand for protein synthesis and secretion [3].

In mammalian cells, the UPR consists of 3 primary signaling pathways. Each pathway initiates with an ER membrane-bound protein that senses the accumulation of unfolded or misfolded proteins and activates a b-ZIP (basic leucine zipper domain) transcription factor. The 3 sensor protein-transcription factor pairs are (i) inositol requiring kinase 1α (IRE1α) and X-box binding protein-1 (XBP1), (ii) eukaryotic translation initiation factor 2-alpha kinase 3 (PERK) and activating transcription factor 4 (ATF4), and (iii) activating transcription factor 6 (ATF6), which serves as both a sensor and transcription factor [1]. Target genes of the IRE1α–XBP1 branch of the UPR are involved in lipid synthesis, ER-associated protein degradation (ERAD), protein folding, translocation to ER and secretion. All of these activities are characteristic of active secretory cells. The PERK–eIF2α pathway regulates a global decrease in protein translation and reduces protein flux into the ER. Paradoxically, activation of PERK and eIF2α phosphorylation also promotes translation of mRNAs with short open reading frames in the 5′-untranslated regions, including ATF4. ATF4 transactivates target genes involved in redox processes, amino acid metabolism, ER chaperones and foldases [4,5]. ATF4 also regulates expression of pro-apoptotic genes like *CHOP* (C/EBP-homologous protein) [6] and *GADD34* (growth arrest and DNA damage-inducible 34) [7]. The transcriptional program regulated by ATF6 is generally geared to increase the protein folding capacity of the ER, but there is considerable overlap between the target genes regulated by the other branches of the UPR [1].

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2. Molecular mechanisms of the IRE1α–XBP1 pathway

Mammalian IRE1 has two isoforms – IRE1α and IRE1β, which are encoded by different genes (*ERN1* and *ERN2* in humans, respectively) [8]. While IRE1α is ubiquitously expressed, expression of IRE1β is limited to the epithelial cells of the gastrointestinal track [8,9]. At the molecular level, IRE1α is a type I transmembrane protein with dual enzymatic activities, consisting of an N-terminal ER luminal domain (IRE1-LD) and a serine/threonine kinase domain plus a C-terminal ribonuclease (RNase) domain located on the cytosolic side of the protein. Upon accumulation of unfolded/misfolded proteins in the ER, IRE1α dimerizes and oligomerizes while stimulating *trans*-autophosphorylation, leading to activation of the RNase domain [10,11]. The exact mechanism of IRE1 activation by unfolded/misfolded proteins is not entirely clear. In one model, activation of IRE1 is mediated through competitive binding of unfolded proteins to ER-resident chaperone binding immunoglobulin (BiP), which in the absence of unfolded proteins associates with the IRE1-LD to keep it in an inactive state. When levels of unfolded proteins increase, BIP dissociates from IRE1-LD and associates with unfolded proteins, freeing IRE1 to dimerize/oligomerize, which leads to the activation of IRE1 [12–14]. Paradoxically, however, the deletion of the KAR2/BiP binding site from yeast IRE1-LD did not result in constitutive activation of IRE1 [14]. Furthermore, crystal structure analyses of both yeast and mammalian IRE1 revealed a similarity to the peptide binding domain of MHC-I molecule [13,15]. The subsequent study showed that IRE1-LD could bind directly to certain peptides [16,17]. These experiments suggested the presence of an additional activation step(s) such as binding of unfolded proteins themselves to IRE1-LD. Once activated, IRE1 becomes an active kinase and autophosphorylates themselves. At this point, however, no other substrates of the IRE1 kinase have been identified. Autophosphorylation of the kinase domain and binding of ADP (or ATP *in vivo*) allosterically regulates dimerization/oligomerization and leads to activation of IRE1 RNase domain [18,19].

Activated IRE1α, through the RNase domain, excises an intron from the *XBP1* mRNA in metazoans (and *HAC1* mRNA in yeast), which causes a translational frame shift that results in the production of the spliced/activated form of XBP1 protein in metazoans (and HAC1 in yeast), an active transcription factor responsible for the induction of a specific set of target genes [20]. Ligation of the spliced intron is mediated through tRNA ligase in yeast [21] and the RTCB/archease complex in metazoans [22].

The unconventional cleavage of an intron from the inactive form of *XBP1/HAC1* mRNA happens at a stem-loop structure [23–26]. Activated IRE1 also degrades ER-bound mRNAs through cleavage at both stem-loop sites and non-stem-loop sites, a process referred to as regulated IRE1-dependent decay (RIDD). RIDD may help to reduce the folding load of nascent proteins entering the ER and thus, further alleviating ER stress [27–29]. Using *in vitro* evidence, a recent study revealed that while oligomerization is required for *XBP1/HAC1* mRNA cleavage, RIDD activity is retained with the IRE1 monomer/dimer [30]. This differential substrate preference may be translated into cell fate determination, as activation of *XBP1* splicing by IRE1 promotes cell survival while activation of RIDD leads to cell death *in vivo*. However, depending upon different cell types or on the kinetic relationships and magnitude of activations between *XBP1* splicing and RIDD in different tissues, ultimate output of either XBP1s or RIDD might differ.

Activated IRE1α also promotes apoptosis by activating apoptosis signal-regulating kinase 1 (ASK1) and JUN N-terminal kinase (JNK) through interaction with tumor necrosis factor (TNF) receptor-associated factor 2 (TRAF2) [31–33].

3. The IRE1α–XBP1 pathway in human diseases

Studies in animal models have revealed that the IRE1α–XBP1 pathway is involved in various human pathological conditions, including neurodegenerative diseases, inflammation, metabolic disorders, liver dysfunction, brain and heart ischemia, and cancer. Targeting this pathway has emerged as a promising therapeutic strategy against these diseases [3]. As more mechanistic data regarding the regulation of this pathway emerges, modulating this pathway through inhibition or activation will likely confer different clinical benefits depending on the context of the disease state. While this is not intended to be a comprehensive review, in the disease conditions described below we will highlight relevant human diseases in which modulation of the IRE1α–XBP1 pathway may lead to the development of novel therapies.

3.1. Neurodegenerative diseases

In mouse models of amyotrophic lateral sclerosis (ALS), XBP1 deficiency leads to augmented autophagy, which enhances clearance of the mutant superoxide dismutase-1 (SOD1) protein and decreases its toxicity [34]. Similarly, in a mouse model of Huntington's disease, XBP1 deficiency also stimulates degradation of the mutant Huntington protein through autophagy and delays disease progression [35]. The same study revealed that XBP1 deficiency promotes autophagy by induction of *FOXO1* expression, which encodes a key transcription factor regulating autophagy in neurons. In contrast, XBP1 is required for locomotor recovery after spinal cord injury (SCI) [36]. However, a study modeling prion-related disorders in mice showed that XBP1 is dispensable for disease progression [37]. In addition, *XBP1* splicing was detected in mice experiencing cerebral ischemia [38]. In both drosophila and mammalian cell culture models of Alzheimer's disease, *XBP1* splicing was found to have neuroprotective effects [39]. In support of this concept, both elevated *XBP1* splicing and IRE1α phosphorylation were detected in disease tissues of patients with Alzheimer's disease [40,41]. In another study using cell culture and mouse model of Parkinson's disease, overexpression of spliced XBP1 demonstrated cytoprotection against 1-methyl-4-phenylpyridinium (MPP+) and 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced cell death [42]. Moreover, elevated levels of XBP1 were also detected in multiple sclerosis demyelinated lesions [43].

3.2. Inflammatory diseases

In a mouse model of inflammatory bowel disease induced by dextran sodium sulfate, IRE1β was identified as a critical protein that mitigates perturbations of ER function during disease progression [44]. Toll-like receptor 2 (TLR2) and TLR4 signaling induce IRE1α activation and *XBP1* splicing, which is required for optimal production of proinflammatory cytokines in macrophages [45]. XBP1 is also essential for dendritic cell differentiation and survival [46].

3.3. Metabolic diseases

In liver cells, XBP1 regulates the expression of genes involved in fatty acid synthesis, including stearoyl-CoA desaturase-1 (*SCD1*), acetyl-CoA carboxylase 2 (*ACC2*) and diacyl glycerol acyl transferase 2 (*DGAT2*). Accordingly, mice with liver-specific deficiency of XBP1 have lower levels of triglyceride and cholesterol production and are free of hepatic steatosis (fatty liver) when fed high carbohydrate diet [47]. XBP1 deficiency has also been linked to induction of insulin resistance, and mice haploinsufficient for *XBP1* develop hyperinsulinemia, hyperglycemia, impaired glucose and

insulin tolerance, and increase in body weight [48]. Through direct interaction with FOXO1, activated XBP1 (XBP1s) alleviates hepatic insulin resistance [49]. In addition, XBP1 interacts with the regulatory subunits of phosphoinositide 3-kinase (PI3K), which increases XBP1 nuclear translocation, ER stress resolution, and insulin sensitization [50,51].

3.4. Cancer

Several genomic screens have identified common mutations associated with IRE1α in human cancers [52–54]. The IRE1α–XBP1 pathway plays an indispensable role in tumor growth, metastatic progression and chemo-resistance [55]. Expression and activation of XBP1 correlates with clinical outcome in breast cancer [56,57] and angiogenesis in pancreatic cancer [58]. Furthermore, tumor growth and survival under hypoxic conditions are severely compromised when XBP1 expression is blocked [58]. In mouse models of glioblastoma, IRE1α is required for upregulation of pro-inflammatory cytokines and angiogenic factors, which contributes to tumor growth, angiogenesis and invasiveness [59,60]. XBP1 is crucial for development of terminally-differentiated plasma cells [61,62] and is overexpressed in multiple myeloma (MM) [63,64], a plasma cell malignancy. Several lines of evidence demonstrate that the IRE1α–XBP1 pathway is involved in the pathogenesis of multiple myeloma [65,66]. Moreover, XBP1 has been implicated in the development of resistance to chemotherapy [64,66]. However, more recent evidence suggests that XBP1 inactivation may contribute to bortezomib resistance [67], underscoring the complexity of its role in regulating this function.

4. IRE1α–XBP1 inhibitors and activators

Recently, several groups have identified small molecule inhibitors that selectively block IRE1α–XBP1 activation [68–73]. Two major sites on IRE1α have been identified as targets for developing inhibitors: the catalytic core of the RNase domain and the ATP binding site of the kinase domain. Small molecules targeting the RNase domain include salicylaldehydes [70], 4μ8C [71], MKC-946 [69], STF-83010 [68], toyocamycin [72], and hydroxyl-aryl-aldehydes (HAA) [73]. Many of these compounds were identified utilizing different chemical screening strategies. The only compound reported to inhibit IRE1α–XBP1 by directly interfering with ATP binding in the IRE1α kinase domain is “Compound 3” [74]. Although there has not been an extensive effort to identify compounds that specifically activate IRE1α–XBP1, the flavonol quercetin was reported to activate the RNAase activity of IRE1α [19].

4.1. Salicylaldehydes

Through *in vitro* fluorescence quenching (FQ)-based high throughput screening strategy detecting the cleavage of Cy5-labeled XBP1 stem-loop RNA substrate by purified recombinant human IRE1α-cytosolic domain (hIRE1α-cyto, amino acids 462–977), salicylaldimines and their hydrolysis products, salicylaldehydes, were identified as inhibitors of the endoribonuclease activity of IRE1α [70]. These compounds inhibited yeast IRE1α, but not RNase L or the unrelated RNase A and T_1. They also blocked chemically-induced *XBP1* splicing and prevented induction of known XBP1 target genes in cultured cell lines. One potent non-competitive inhibitor of XBP1 activation, 3-ethoxy-5,6-dibromosalicylaldehyde, binds to IRE1α in a specific, reversible and dose-dependent fashion revealed by surface plasmon resonance analysis. Additional analysis revealed that these compounds do not inhibit autophosphorylation of IRE1α.

4.2. 4μ8C

High throughput screening using an *in vitro* fluorescent-based FRET-derepression assay with purified recombinant human IRE1α-cytosolic domain (amino acids 464–977) identified compound CB5305630, an 8-formyl-7-hydroxy-4-methylcoumarin conjugated to 2-aminopyridine *via* an aldimine. This compound was shown to be a noncompetitive inhibitor of the endoribonuclease activity of IRE1α, with an IC_{50} of 60 nM in the FRET-derepression assay [71]. In an aqueous environment, CB5305630 is hydrolyzed to generate the active component 8-formyl-7-hydroxy-4-methylcoumarin, which is referred to as 4μ8c. Interestingly, 4μ8c can also be categorized as a salicylaldehyde derivative and potentially has a similar mechanism of action as those described previously. Through HPLC and MALDI-TOF mass spectrometry, 4μ8c was shown to bind to K^{599} (in the kinase domain) and K^{907} (in the RNase domain) of IRE1α protein *via* Schiff-base formation, thus inhibiting both the kinase and RNase activity of IRE1α. 4μ8c was shown to inhibit chemically-induced *XBP1* splicing by IRE1α and induction of XBP1 target genes, but not ATF4 regulated genes. Furthermore, 4μ8c did not block RNase L activity *in vitro*. 4μ8c blocked RIDD activity both *in vitro* and in cultured cells. However, inhibition of IRE1α activity by 4μ8c did not sensitize mammalian cells to chemically-induced ER stress, but this compound did affect the expansion of the cell's secretory capacity. Recently, the structure and mechanism of action of this class of salicylaldehyde derivatives were further characterized through protein-compound co-crystallization studies, highlighting the interaction between these compounds and a shallow pocket around K^{907} of the IRE1α protein [73].

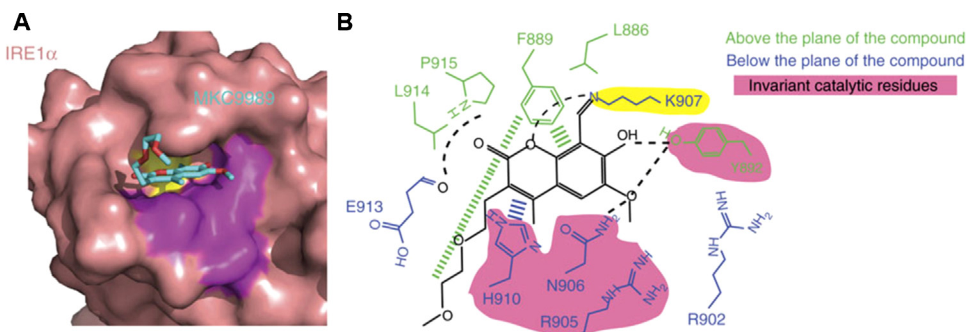

Fig. 1. Surface and schematic view of the interaction between murine IRE1α and MKC9989 adapted from Sanches et al. [73]. (A) Surface view of the IRE1α–MKC9989 complex. Lysine 907 is colored yellow and invariant active site residues are colored purple. (B) Schematic view of the contact residues of IRE1α and notable interactions between IRE1α and MKC9989.

Table 1
Summary of known IRE1α–XBP1 modulators.

Name	Structure	RNase activity	Kinase activity	RIDD	Oligomerization	IRE1α binding	Cell culture	*In vivo*	Refs.
Salicylaldehydes (3-methoxy-6-bromosalicylaldehyde)		Inhibition (*In vitro* cleavage of Mini-XBP-1 stem-loop substrate)	No effect on thapsigargin-induced IRE1α phosphorylation in MM cells	Inhibition	Not tested	Binding confirmed by surface plasmon resonance assay	Inhibits *XBP1* splicing in HEK293 cells	Inhibits tunicamycin-induced *XBP1* splicing in CB17 SCID mice	[1]
4μ8C		Inhibition (*In vitro* cleavage of RNA substrates)	Inhibition *in vitro* but not on thapsigargin-induced phosphorylation in MEFs.	Inhibition	Not tested	Binding to K^{599} and K^{907} of IRE1α detected by HPLC–MALDI-TOF	Inhibits *XBP1* splicing in MEFs cells, induces cytotoxicity in MM cells	Not tested	[2]
MKC-3946		Not tested	No effect on IRE1α phosphorylation in MM cells	Not tested	Not tested	Not tested	Inhibits *XBP1* splicing, induces cytotoxicity, enhances cytotoxicity of bortezomib and 17-AAG in MM cells	Inhibits tunicamycin-induced *XBP1* splicing in SCID mice and MM xenograft growth alone or in combination with bortezomib	[3]
STF-083010		Inhibition (*In vitro* cleavage of RNA substrates)	No effect on IRE1α phosphorylation in MM cells	Inhibits RIDD activity induced by ADP but not quercetin	Not tested	Not tested	Inhibits endogenous and thapsigargin-induced *XBP1* splicing in MM cells	Inhibits *XBP1* splicing in luciferase reporter mice and MM xenograft growth	[4,5]
Compound 2		Not tested	Not tested	Not tested	Not tested	Not tested	Inhibits *XBP1* splicing in U373 glioblastoma cells	Synergizes with oncolytic virus in reducing tumor burden in an OVCAR-4 orthotopic xenograft tumor model	[6]

Table 1 (*Continued*)

Name	Structure	RNase activity	Kinase activity	RIDD	Oligomerization	IRE1α binding	Cell culture	*In vivo*	Refs.
MKC9989		Inhibition (*In vitro* cleavage of RNA substrates)	Have minor effect on IRE1α auto-phosphorylation *In vitro* at highest tested concentration (100 μM)	Inhibition	Not tested	Binds to the RNase domain of murine IRE1α revealed by X-ray crystallography	Inhibits *XBP1* splicing in RPMI8226 MM cells	Not tested	[7]
OICR464		Inhibition (*In vitro* cleavage of RNA substrates)	No effect on IRE1α auto-phosphorylation *In vitro* at highest tested concentration (100 μM)	Not tested	Not tested	Binds to the RNase domain of murine IRE1α revealed by X-ray crystallography	Not tested	Not tested	[7]
OICR573		Inhibition (*In vitro* cleavage of RNA substrates)	No effect on IRE1α auto-phosphorylation *In vitro* at highest tested concentration (100 μM)	Not tested	Not tested	Binds to the RNase domain of murine IRE1α revealed by X-ray crystallography	Not tested	Not tested	[7]
Toyocamycin		Inhibition (*In vitro* cleavage of RNA substrates)	No effect on IRE1α phosphorylation in HEK293T and MM cells	Not tested	Not tested	Not tested	Inhibits thapsigargin-, tumicamycin- and 2-DG-induced *XBP1* splicing in HeLa cells and endogenous *XBP1* splicing in MM cells, induces cytotoxicity and enhances cytotoxicity by bortezomib in MM cells	Inhibits MM xenograft growth alone and enhances the effects of bortezomib	[8]

Compound 3		Inhibition (*In vitro* cleavage of Mini-XBP-1 stem-loop substrate)	Inhibition *in vitro* and on T-Rex 293 and INS-1 cells	Not tested	Inhibition *in vitro*	Interacts with the ATP-binding site of IRE1α detected by ICAT footprinting	Inhibits *XBP1* splicing in T-Rex 293 and thapsigargin-induced *XBP1* splicing in INS-1 cells	Not tested	[9]
Quercetin		Activation (*In vitro* cleavage of Mini-XBP-1 stem-loop substrate)	Weak inhibitor	Promotes RIDD	Stimulates dimerization *in vitro*	Binds to the "Q site" in the RNase domain of IRE1α	Induces *XBP1* splicing in IRE1-null MEFs overexpressing a chimeric hyIRE1 protein	Not tested	[5,10]

4.3. MKC-3946

Through chemical optimization of the salicylaldehydes identified from [70], a more potent and soluble inhibitor, MKC-3946, was synthesized [69]. MKC-3946 inhibits chemically-induced *XBP1* splicing in a dose dependent manner from a MM cell line and from patient derived samples. Furthermore, endogenous *XBP1* splicing was blocked in patient derived MM cells and an MM tumor xenograft model, without affecting IRE1α phosphorylation in this context. MKC-3946 also demonstrated selective cytotoxicity against MM cell lines without cytotoxicity to normal mononuclear cells. Furthermore, in MM cell lines, MKC-3946 inhibited *XBP1* splicing induced by bortezomib (a proteasome inhibitor) and 17-AAG (an HSP90 inhibitor) and enhanced cytotoxicity induced by these compounds. Mechanistically, combined treatment with MKC-3946 and bortezomib/17-AAG activates the PERK–eIF2α-ATF4 branch of the UPR and subsequently increases expression of pro-apoptotic factor CHOP, leading to apoptosis in MM cell lines. Interestingly, in this context, binding of IRE1α to TRAF2 and IRE1α/JNK phosphorylation was also enhanced by treatment of MKC-3946 alone or in combination with bortezomib. In this pre-clinical setting, MKC-3946 alone or in combination with bortezomib significantly inhibited xenograft MM cell growth *in vivo*.

4.4. STF-083010

As a result of a high throughput chemical screening strategy using HT1080 human fibrosarcoma cells stably expressing a luciferase-based XBP1 reporter construct [75], STF-083010 was identified as a small molecule inhibitor of *XBP1* splicing [68]. Subsequently, in an *in vitro* cell-free IRE1α RNase reaction, this compound also inhibited *XBP1* mRNA splicing by directly inhibiting IRE1α RNase activity. A later study showed that this compound forms a carbaldehyde in water and selectively binds to K^{907} of IRE1α [71]. STF-083010 inhibits both endogenous and chemically-induced *XBP1* splicing in an MM cell line, without affecting IRE1α phosphorylation. This compound also inhibits bortezomib-induced *XBP1* splicing in reporter mice expressing the same luciferase construct as a transgene, without causing detectable toxicity in the normal tissue of these mice. STF-083010 further demonstrated cytotoxicity against a panel of MM cell lines in a dose- and time-dependent manner, and selectively killed $CD138^{+}$ cells from MM patients but not normal hematopoietic cells. And finally, it inhibited *in vivo* growth of xenograft MM tumors.

4.5. HAA (hydroxy-aryl-aldehydes) inhibitors

Stemming from the salicylaldehyde derivatives, the combination of the adjacent hydroxy-aldehyde motif and a few dual-ring structures form a class of compounds called HAA (hydroxy-aryl-aldehydes), including MKC-3946 [69], 4μ8C [71] and an experimental IRE1α inhibitor ("Compound 2") developed by Mannkind Corporation [76]. Recently, the crystal structures of murine IRE1α in complex with three HAA inhibitors, MKC9989, OICR464 and OICR573, were solved to elucidate the mechanism of action of this group of inhibitors [73]. These inhibitors blocked XBP1 splicing in a cell-free assay while having minimum effect on the kinase activity of IRE1α. Furthermore, MKC9989 inhibits XBP1 splicing as well as the RIDD activity in RPMI8226 multiple myeloma cells. The three compound-protein co-structures reveal that these HAA inhibitors bind to a shallow pocket at the RNase-active site of IRE1α through pi-stacking interactions with His910 and Phe889 and a hydrogen bond with Tyr892. Consistent with previous studies [71], the essential interaction is a Schiff base interaction between the HAA aldehyde group and the amine group of Lys907. Surface and schematic view of the interaction between

IRE1α and MKC9989 are shown in Fig. 1. This study provides the only X-ray crystallographic data for the interaction between chemical compounds and the RNase active-site of IRE1α. The revealed structures not only give insight into the molecular functions of the IRE1α protein, but also suggest new strategies in designing IRE1α inhibitors.

4.6. Toyocamycin

Through the application of a similar XBP1-luciferase reporter construct overexpressed in HeLa cells, toyocamycin, a nucleoside-type antibiotic analog of adenosine, was identified as an inhibitor of ER stress-induced XBP1 activation [72]. Toyocamycin blocked chemically-induced *XBP1* splicing as well as XBP1 target gene expression in HeLa cells, without affecting PERK and ATF6 activation or IRE1α phosphorylation. Toyocamycin also prevented the splicing of an *XBP1* RNA substrate by purified recombinant human IRE1α-cytosolic domain (amino acids 467–977) *in vitro*. Toyocamycin induced profound apoptosis in MM cell lines, including those resistant to bortezomib treatment, in a dose-dependent manner, the magnitude of which correlated with extent of XBP1 activation in the different cell lines. Toyocamycin was synergistic with bortezomib in inducing apoptosis in a MM cell line. In addition, toyocamycin inhibited *XBP1* splicing in primary MM cells and demonstrated cytotoxicity to primary MM but not healthy PBMC cells. This compound showed antitumor activity in a xenograft MM tumor model, either alone or in a synergistic manner with bortezomib.

4.7. Compound 3

Compound 3 is a type II kinase inhibitor that competes for ATP-binding to IRE1α and stabilizes an inactive form of the protein [74]. This compound blocked autophosphorylation, oligomerization and *XBP1* splicing capacity of IRE1α *in vitro*, as well as chemically-induced IRE1α autophosphorylation and *XBP1* splicing in cultured INS-1 insulinoma cells.

4.8. Quercetin

Through an *in vitro* fluorescence quenching (FQ)-based screening strategy, quercetin was identified as a compound that activates the RNase activity of IRE1α on XBP1 splicing [19]. The co-crystal structure of IRE1α, ADP and quercetin revealed that quercetin binds to the “Q site” in the RNase domain of IRE1α, defined by S984, K985, E988, K992, P1077, I1108, and F1112 of yeast IRE1α. In IRE1-null mouse embryonic fibroblasts (MEFs) expressing a chimeric human-yeast IRE1α protein (hyIRE1, with lumenal, transmembrane, and juxtamembrane domains of human IRE1α fused with the kinase and RNase domains of yeast IRE1α), quercetin induces XBP1 splicing. Additional evidence indicates that quercetin stimulates IRE1α dimerization *in vitro*.

In summary, multiple modulators of IRE1α–XBP1 have been described. The specific activities of these compounds and the corresponding references are summarized in Table 1. Collectively, these data indicate the tremendous enthusiasm and promise for developing therapeutic drugs targeting this pathway in human disease.

5. Conclusions and future perspectives

Recapitulating the complex nature of protein folding homeostasis, the UPR displays variable physiological outputs depending on the cellular context. Selective activation of or inhibition of IRE1α may be desirable depending on the disease state. Thus, therapeutic intervention based on targeting the UPR pathways should be optimized to differentially modulate the adaptive pro-survival or pro-apoptotic activity of the UPR according to the specific disease context. Within the three major branches of the UPR, the IRE1α–XBP1 pathway reveals multiple roles in neurodegenerative and metabolic diseases, while mainly serving as a pro-survival pathway in multiple human cancers. The complexity of IRE1α biology was demonstrated through a recent study suggesting that modulation of IRE1α RNase activity was possible through an allosteric mechanism using ATP-competitive kinase inhibitors APY29 and sunitinib [74]. Furthermore, a peptide derived from the IRE1α kinase domain [77] was shown to stimulate IRE1α oligomerization while inhibiting the JNK activation and RIDD activity of IRE1α. Another yet unexplored aspect of targeting IRE1α is the specificity on *XBP1* splicing and RIDD activity. Finally, as RIDD activity is implicated in certain physiological and pathological settings, including lipid metabolism [78,79], acetaminophen toxicity [80] and cell migration [81], IRE1α modulators specific for either *XBP1* splicing or RIDD activity may be clinically useful depending on the therapeutic intent.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

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