Endoplasmic Reticulum Stress and the Unfolded Protein Response: Targeting the Achilles Heel of Multiple Myeloma

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Abstract

Multiple myeloma is characterized by the malignant proliferating antibody-producing plasma cells in the bone marrow. Despite recent advances in therapy that improve the survival of patients, multiple myeloma remains incurable and therapy resistance is the major factor causing lethality. Clearly, more effective treatments are necessary. In recent years it has become apparent that, as highly secretory antibody-producing cells, multiple myeloma cells require an increased capacity to cope with unfolded proteins and are particularly sensitive to compounds targeting proteostasis such as proteasome inhibitors, which represent one of the most prominent new therapeutic strategies. Because of the increased requirement for dealing with secretory proteins within the endoplasmic reticulum, multiple myeloma cells are heavily reliant for survival on a set of signaling pathways, known as the unfolded protein response (UPR). Thus, directly targeting the UPR emerges as a new promising therapeutic strategy. Here, we provide an overview of the current understanding of the UPR signaling in cancer, and outline its important role in myeloma pathogenesis and treatment. We discuss new therapeutic approaches based on targeting the proteostasis machinery and particularly the IRE1α/XBP1 axis of the UPR. Mol Cancer Ther; 12(6); 1–13. ©2013 AACR.

The Unfolded Protein Response

The endoplasmic reticulum (ER) is the primary cellular Ca2⁺ store and the site of biosynthesis of secreted and transmembrane proteins, both of which enter the ER cotranslationally. Inside the ER proteins are folded and undergo glycosylation or lipidation. The correct folding and trafficking of these proteins are dependent on chaperones within the ER, which require Ca2⁺ and ATP, and on an oxidizing environment to facilitate the formation of disulfide bonds between protein chains. Therefore, these processes are sensitive to nutrient deprivation, to changes in Ca2⁺ homeostasis and in the cellular redox state. Such conditions, as well as a high load of secreted proteins, the presence of folding-deficient mutant proteins, impairment of glycosylation, of vesicular trafficking or of protein degradation will lead to an accumulation of misfolded or unfolded proteins in the ER. These conditions are collectively referred to as “ER stress”. As a consequence, the cell triggers a set of signaling pathways termed the unfolded protein response (UPR) that initially aims to restore homeostasis, but can also induce apoptosis if the stress cannot be resolved (1).

The initial phase of the UPR aims at resolving the stress by expanding the secretory apparatus, increasing ER volume, decreasing the load of newly synthesized proteins, enhancing the removal of unfolded proteins from the ER by a process termed ER-associated degradation (ERAD; ref. 2), and by inducing autophagy (3). Thus, the UPR serves as an important physiologic adaptation mechanism of particular importance in secretory cell types. However, when these attempts to overcome the stress fail, cell death ensues. ER stress-induced apoptosis proceeds primarily via the mitochondrial pathway, which is controlled by the BCL-2 family of proteins (4).

UPR signaling pathways

In mammals, the 3 major ER stress sensors are the ER transmembrane proteins inositol-requiring enzyme 1 (IRE1, ERN1), PKR-like ER kinase (PERK, EIF2AK3), and activating transcription factor 6 (ATF6). The ER luminal domains of these proteins interact with the ER chaperone 78 kDa glucose-regulated protein [GRP78, or immunoglobulin binding protein (BiP)]. As unfolded proteins compete for binding with GRP78, their accumulation leads to dissociation of GRP78 from the luminal domains of the ER stress sensors, allowing their activation (Fig. 1).

PERK is a serine/threonine protein kinase that phosphorylates eukaryotic initiation factor 2α (eIF2α) to inhibit the initiation step of mRNA translation, thus lowering overall protein load of the ER. However, eIF2α phosphorylation promotes increased translation of activation...
transcription factor 4 (ATF4), which induces a set of genes involved in apoptotic and in adaptive responses during ER stress (5, 6). A second target of PERK is the transcription factor, nuclear factor-erythroid 2–related factor 2 (NRF2), whose phosphorylation liberates it from its inhibitor Kelch-like ECH-associated protein 1 (KEAP1), allowing the expression of genes involved in oxidative stress/redox signaling (7).

ATF6 is synthesized as a transmembrane protein and is occluded from the nucleus by tethering to the ER membrane. Dissociation of GRP78 allows for transport of ATF6 to the Golgi where it is cleaved from its transmembrane domain, allowing for nuclear translocation (8). ATF6 regulates the expression of a set of genes involved in protein quality control and ERAD (9) and stimulates expression of the X-box binding protein 1 (XBP1) gene whose transcript is a target of IRE1α (10).

IRE1 is a type I ER transmembrane protein. It has both a kinase activity and an endoribonuclease activity. There are two IRE1 isoforms; IRE1α is ubiquitously expressed, whereas IRE1β expression seems to be restricted to gastrointestinal epithelial cells. Dissociation of GRP78 during
ER stress leads to the activation and autophosphorylation of the cytoplasmic kinase domain of IRE1α, followed by oligomerization that activates the RNase activity. The kinase domain of IRE1α recruits the E3 ubiquitin ligase, TNF receptor-associated factor 2 (TRAF2) that mediates activation of c-jun-NH2-kinase (JNK; ref. 11), and of NF-κB (12) signaling pathways, which may be involved in cell death induction or expression of prosurvival genes and/or cytokines, respectively. The RNase activity in conjunction with a RNA ligase removes an intron from the XBP1 mRNA (13). The unspliced mRNA encodes an unstable protein, XBP1u, which expresses a DNA binding domain, but is mainly cytoplasmic. XBP1 splicing results in a shift in the open reading frame of the XBP1 protein (XBP1s), therefore, is a potent transcription factor that controls genes involved in ER membrane biosynthesis, protein import, chaperoning, ERAD, and cell type-specific genetic programs (14).

The RNase activity of IRE1α has also been implicated in the degradation of specific mRNAs, mostly encoding ER-synthesized proteins, a process termed regulated IRE1-dependent decay (RIDD; ref. 15). The roles of RIDD in ER stress and cell death are not fully understood but may comprise adaptive functions arising from lessening protein load at the ER or proapoptotic functions due to the degradation of transcripts encoding proteins important for cell survival.

How these specific activities of IRE1α are coordinated to launch adaptive, cytotoxic, and inflammatory responses is currently not well understood. As a component of a complex protein platform, referred to as the UPRosome (Fig. 2), IRE1α activity is modulated by several interacting proteins (e.g., BAX, BAK, BI-1, HSP90, HSP70, and RACK; ref. 16). The interaction with the BCL-2 family proteins BAX and BAK is reported to be crucial for IRE1α activation during ER stress (16). This interaction is counteracted by the ER-resident transmembrane protein BAX Inhibitor-1 (BI-1) whose overexpression inhibits IRE1α activity, and its deficiency increases XBP1 splicing and increases secretory activity of B cells (16). Interestingly, BI-1 abundance is regulated at the level of protein stability by the ER-associated RING type E3 ligase bifunctional apoptosis regulator, BAR, which mediates ubiquitination of BI-1, thus initiating proteasomal degradation and removing the block of IRE1α activation imposed by BI-1 (17).

Regulation of proliferation, autophagy, and apoptosis

Beyond restoring ER homeostasis, the UPR impacts on the proliferation and apoptosis equilibrium, thus helping cells or tissues to cope with the consequences of ER stress.

The main mechanism of PERK-induced apoptosis is thought to be through increased expression of the transcription factor C/EBP-homologous protein (CHOP; ref. 18), which is mediated by ATF4/ATF3 (5). The exact mechanism by which CHOP can induce apoptosis has not yet been delineated.

Apart from increasing the folding capacity of the ER, little is known about how IRE1α/XBP1s signaling exerts its prosurvival function during ER stress at the level of the apoptotic machinery. Recently, it was reported that XBP1s overexpression leads to increased BCL-2 expression in a breast cancer cell line (19). In a hematopoietic cell line that undergoes apoptosis upon interleukin (IL)-3 withdrawal, overexpression of XBP1s was cytoprotective and attenuated induction of the proapoptotic BCL-2 family member BIM (20). However, there is no evidence that BCL-2 family members are direct transcriptional targets of XBP1s. Since IRE1α interacts with BCL-2 family members at the ER membrane (21), it is possible that the activation status of IRE1α might influence their pro- or antiapoptotic activities.

ER stress can also activate autophagy as a mechanism for removing unfolded proteins or damaged ER. Autophagy can be induced by both the PERK and the IRE1α arm of the UPR (22).

Role of the UPR in Cancer

UPR pathways are frequently activated and play crucial roles in tumorigenesis and therapy response (23). Evidence suggests that the UPR is of particular importance for adaptation of cancer cells to hypoxic conditions. For example, PERK was shown to be involved in growth and hypoxia resistance of tumors derived from transformed mouse embryonic fibroblasts inoculated into mice (6). XBP1 splicing by IRE1α has also been implicated in adaptation to hypoxia (24). As oxygen is the preferred terminal electron acceptor in the redox relay required for disulphide bond formation, hypoxia leads to an increase in misfolded proteins triggering IRE1α activation and XBP1 splicing (25). In fact, a XBP1 splicing reporter transgene revealed activation of IRE1α at sites of hypoxia within tumors in a transgenic breast cancer model (26). Furthermore, all 3 arms of the UPR have been shown to at least partially control VEGF levels in hepatoma cell lines and fibroblasts, respectively (27). Thus, UPR signaling in tumors seems to be important for switching on angiogenesis in response to local hypoxia.

Remarkably, IRE1α is one of the most frequently mutated kinases in cancer (28). Because at least some of the mutations result in loss of kinase and RNase activity (29), this would suggest that in certain cancers, IRE1α signaling counteracts tumorigenesis, possibly via JNK activation or via degradation of essential mRNAs by RIDD. Mutations in XBP1 have been found in a number of cancers including multiple myeloma (30); however, neither their relevance nor functional consequences have been shown thus far.

Other studies, in contrast, point towards a protumorigenic role of IRE1α, in particular of XBP1 splicing activity. Intriguingly, XBP1 splicing seems to play a driving role in the pathogenesis of multiple myeloma (31).
How Myeloma Cells Deal with Protein Load and ER Stress

Multiple myeloma is a malignancy of post-germinal center B lymphocytes in the bone marrow that are classified by a number of chromosomal abnormalities and genetic mutations. Multiple myeloma cells share phenotypical characteristics with long-lived plasma cells and express extensively hypermutated immunoglobulin genes. As multiple myeloma cells actively produce and secrete immunoglobulin, they are prone to ER stress and therefore require strict regulation of ER stress for survival.

Cellular strategies to maintain ER homeostasis include activation of the UPR, induction of chaperones, and autophagy, all of which have been shown to play important roles in myeloma pathogenesis (32). A significant number of genes involved in protein synthesis as well as the UPR are frequently mutated in patients with multiple myeloma (30).

The UPR is highly active in multiple myeloma cells, and this activity increases in advanced disease stages (32). The expression of UPR genes such as XBP1 may be a selection factor during the progression of multiple myeloma by...
promoting cell survival during ER stress (32). At the same time, multiple myeloma cells are exceptionally sensitive to ER stress-induced apoptosis caused by the proteasome inhibitor, bortezomib, compared with other cancer cells (33). This could be explained by the high immunoglobulin production leading to high basal ER stress before treatment (Fig. 3). Indeed, a correlation between the levels of immunoglobulin production and sensitivity of multiple myeloma cells to ER stress was observed (34). Although the UPR promotes survival of multiple myeloma cells and disease progression, the balance between prosurvival and proapoptotic signaling of the UPR is easily tipped towards death when multiple myeloma cells are exposed to exogenous ER stress. Thus, drug-induced ER stress by targeting the protein quality control machinery as well as inhibition of prosurvival signaling pathways of the UPR emerge as promising strategies for treatment of multiple myeloma.

Central role of XBP1s in multiple myeloma

In response to antigenic stimuli, mature B cells in the germinal center differentiate into antibody producing plasma cells, a process mediated by a complex transcriptional program involving the coordinated expression of a number of transcription factors. This involves upregulation of the transcriptional repressor B-lymphocyte-induced maturation protein 1 (BLIMP-1), which suppresses expression of genes maintaining earlier developmental stages and proliferation, and of XBP1, which is a critical transcription factor for plasma cell differentiation and immunoglobulin production (35). In line with this important role in normal plasma cell biology, XBP1 is frequently overexpressed in multiple myeloma (36). Interestingly, mutations in XBP1 have been found in a small percentage of patients with myeloma, further suggesting a potential causative role in disease pathogenesis at least in some patients (30).

The prognostic role for XBP1s overexpression has recently been recognized in the clinic. High levels of spliced XBP1 mRNA were consistently detected in all samples from a group of 253 newly diagnosed patients, and high ratios of spliced versus unspliced XBP1 mRNA directly correlated with lower median overall survival, which was independent of other previously known
prognostic factors (37). Patients treated with thalidomide-based regimes on the MRC Myeloma IX trial had inferior outcome in the presence of increased XBP1s transcripts (37). Accordingly, XBP1s was proposed as an independent prognostic marker and a predictor of thalidomide response (37). In a separate study, analysis of samples from 22 patients showed increased levels of spliced XBP1 mRNA in stage III patients compared with stage I and II patients (32). Thus, high levels of XBP1s are correlated with advanced disease stages and poor prognosis for treatment response and disease outcome. In contrast to the findings in thalidomide-treated patients, a recent study found that low XBP1 mRNA levels predicted poor response to bortezomib, both in vitro and in patients (38). In this study, the ratio of spliced versus unspliced XBP1 transcripts did not correlate with bortezomib sensitivity. These findings suggest that XBP1 levels are a correlate of UPR induction in cells and, as shown by others (33), the UPR renders myeloma cells sensitive to proteasome inhibition. Therefore, the prognostic value of XBP1 levels may depend on the type of therapy and how this influences the UPR.

A clear indication that XBP1 might play a causal role in myeloma pathogenesis is a transgenic mouse, in which XBP1s is overexpressed under the control of the Eμ-promoter in the B-cell lineage (31). At only 40 weeks of age, Eμ-XBP1s mice developed monoclonal gammopathy of undetermined significance (MGUS), which resembles multiple myeloma in the secretion of paraprotein and can result in the development of multiple myeloma, and 26% of mice spontaneously developed multiple myeloma within 2 years (31). This study shows that XBP1 overexpression alone can drive transformation of plasma cells and promote multiple myeloma pathogenesis. B cells from these mice showed an enhanced proliferation rate and increased secretion of immunoglobulin compared with control mice. Microarray analysis identified more than 1,000 genes that were differentially expressed in Eμ-XBP1s myeloma cells compared with B cells from young neoplasm-free mice, including genes involved in the regulation of cell-cycle progression and proliferation. This strongly suggests a role of XBP1s in the expression of these genes. In particular, XBP1s may play an important role in the regulation of IL-6, a cytokine essential for the survival of plasma and myeloma cells (39).

Thus, XBP1s has been identified as a driving survival factor of multiple myeloma cells and may, in fact, determine selective survival of more resilient tumor cells during the progression of multiple myeloma (31, 32, 37).

Although there is growing evidence implicating XBP1s in the pathogenesis of multiple myeloma, very little is known about how the IRE1α/XBP1 pathway is regulated in this disease. We recently reported that the 70 kDa HSP (HSP70, HSP72, or HSPA1) protects cells from ER stress-induced apoptosis by prolonging XBP1 splicing (40). HSP70 is an important survival factor in myeloma and has been implicated in drug resistance (41). Adherence of myeloma cells to bone marrow stromal cells or fibronectin results in integrin-dependent upregulation of HSP70, inducing resistance to treatment with melphalan (41). HSP70 is also associated with bortezomib resistance (42). HSF70-mediated drug resistance may be, at least in part, due to the enhanced XBP1 splicing.

Recent studies suggest that the bone marrow microenvironment in multiple myeloma is hypoxic, with a majority of multiple myeloma cells residing in a hypoxic niche (43). Moreover, multiple myeloma cell lines grown in a hypoxic environment show activation of IRE1α with increased XBP1 splicing (44). IRE1α activation secondary to hypoxia may play an important role in the survival of multiple myeloma cells in the hypoxic bone marrow niche. As such sites may also be the location of tumor-initiating cells, IRE1α activation may play a role in their survival during therapy.

Targeting Protein Turnover and Quality Control

Proteasome inhibition

Currently, the single most important class of antimyeloma therapeutics is the proteasome inhibitors. The dipeptide boronic acid analogue bortezomib (Velcade, PS-341; Fig. 4) is a potent, highly selective, and reversible inhibitor of the 26S proteasome complex and induces apoptosis in multiple myeloma cells (45). Many different mechanisms of action may account for this activity of bortezomib in multiple myeloma. One mechanism may involve blockade of ERAD, resulting in accumulation of unfolded proteins and induction of ER stress. In fact, bortezomib treatment rapidly activates PERK and eIF2α phosphorylation in multiple myeloma cells, followed by induction of ATF4 and of CHOP (Table 1; ref. 33).

The induction of the proapoptotic BH3-only protein NOXA may be critical for the proapoptotic effect of bortezomib following induction of ER stress in myeloma cells (46) and may be mediated by the activation of ATF3 and ATF4 (47). JNK is activated downstream of IRE1α and facilitates apoptosis by its ability to regulate BCL-2 family proteins. Phosphorylation of BCL-2 by JNK suppresses its antiapoptotic activity, whereas phosphorylation of the proapoptotic BH3-only member BIM enhances its proapoptotic potential. Bortezomib has previously been shown to induce the phosphorylation of JNK and its downstream targets c-JUN and ATF2 in myeloma cells. Inhibition of JNK activity reduced bortezomib-induced apoptosis (48). These findings suggest that treatment with bortezomib induces ER stress, leading to activation of the proapoptotic arm of the IRE1α signaling pathway while simultaneously suppressing prosurvival XBP1s signaling, leading to cell death.

Several mechanisms of bortezomib resistance have been described. These include mutation or overexpression of proteasome subunits (49), induction of HSPs (42), the formation of aggresomes, induction of autophagy and finally, a reduction in protein biosynthesis (in particular immunoglobulins). In the case of acquired resistance at the level of the proteasome, this may be overcome...
with alternative proteasome inhibitors, such as carfilzomib (PR-171), marizomib (NPI-0052), or ixazomib (MLN9708; Fig. 4), which, unlike bortezomib, irreversibly block the proteasome (50). However, resistance despite adequate proteasome inhibition may require different approaches, including the use of HSP inhibitors and histone deacetylase (HDAC) inhibition to overcome aggresome-mediated resistance and inhibition of autophagy. Aggresomes are inclusion bodies formed by the accumulation of misfolded proteins when the capacity of the intracellular protein folding and degradation machinery is exceeded, such as with proteasome inhibition. The formation of aggresomes leading to clearance of aggregated proteins is cytoprotective, enabling myeloma cells to overcome an otherwise toxic load of protein aggregates. The formation of aggresomes involves transport by the microtubule network, a process that is dependent on HDAC6 (51). There is evidence of a potent in vitro synergy

Figure 4. Chemical structures of small molecular compounds targeting protein quality control and the UPR in multiple myeloma.
between HDAC6 inhibitors and bortezomib (52). In one study, the HDAC6 inhibitor Tubacin markedly augmented both JNK phosphorylation and caspase activity in multiple myeloma cells leading to synergistic cell death (53). The *Myc* oncogene, which is frequently deregulated in multiple myeloma, has been shown to regulate aggresome formation (54). Myc activation leads to an increase in protein translation in multiple myeloma cells, while at the same time, upregulates HDAC6, favoring aggresome formation (54). Bortezomib and HDAC inhibitor combination studies are currently the subjects of clinical trials and patients with multiple myeloma with elevated levels of Myc activity may be particularly sensitive to this approach.

Table 1. Therapeutic agents that affect UPR signaling in multiple myeloma

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<th>Therapeutic agent</th>
<th>Molecular target</th>
<th>Effect on UPR</th>
<th>In vitro antimyeloma activity</th>
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<th>Phase of trial for multiple myeloma</th>
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<tr>
<td>Bortezomib (PS-341)</td>
<td>26S proteasome</td>
<td>Induction of PERK, ATF4, CHOP</td>
<td>Induction of apoptosis</td>
<td>33, 60</td>
<td>U.S. Food and Drug Administration–approved</td>
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<td>17-AAG (Tanespimycin)</td>
<td>HSP90</td>
<td>Induction of CHOP and ATF6, less induction of XBP1 splicing</td>
<td>Induction of apoptosis</td>
<td>60</td>
<td>Phase III clinical trials</td>
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<tr>
<td>Radicicol</td>
<td>HSP90</td>
<td>Induction of CHOP and ATF6, less induction of XBP1 splicing</td>
<td>Induction of apoptosis</td>
<td>60</td>
<td>Preclinical studies</td>
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<tr>
<td>MAL3-101</td>
<td>HSP70</td>
<td>Induction of XBP1 splicing</td>
<td>Inhibition of proliferation, induction of apoptosis, enhanced effects of proteasome and HSP90 inhibitors</td>
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<tr>
<td>CHR-2797 (Tosedostat)</td>
<td>M1 aminopeptidases</td>
<td>Induction of CHOP, ATF4, and ATF6; no effect on XBP1</td>
<td>Inhibition of proliferation and survival in bone marrow microenvironment, induction of apoptosis</td>
<td>69</td>
<td>Phase II clinical trial</td>
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<td>Reolysin (Reovirus)</td>
<td>Whole cell</td>
<td>Induction of XBP1 splicing</td>
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**Targeting UPR signaling pathways**

<table>
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<th>Molecular target</th>
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<td>MKC-3946</td>
<td>IRE1α RNase</td>
<td>Inhibition of XBP1 splicing</td>
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<td>4μ8C</td>
<td>IRE1α RNase</td>
<td>Inhibition of IRE1-mediated XBP1 splicing</td>
<td>Inhibition of multiple myeloma cell growth</td>
<td>73</td>
<td>Preclinical studies</td>
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</table>

*NOTE:* A range of therapeutic agents with antimyeloma activity affect UPR signaling. The table summarizes the stage of trials for multiple myeloma only. Some of the agents have already been approved or tested in clinical studies for other types of cancer but have so far been used for the treatment of multiple myeloma in preclinical studies only.
Proteasome inhibition may increase the requirement for molecular chaperones to maintain accumulating proteins in a soluble state and vice versa inhibition of molecular chaperones may lead to an increase in misfolded proteins that require degradation by the proteasome. In line with this, HSPI90 inhibition showed synergistic antilymoma activity in combination with bortezomib in multiple myeloma cell lines as well as in mouse models (55–58). A combination of bortezomib with the HSPI90 inhibitor tanespimycin (Fig. 4) has recently been tested in a phase I/II study in patients with relapsed multiple myeloma with promising result as it showed activity even in patients that had previously been treated with bortezomib (59). Furthermore, the combination was well tolerated and seemed to be safer than other combinational treatments with bortezomib (59).

Acquired bortezomib resistance in multiple myeloma has recently been associated with low levels of XBP1 mRNA and of ATF6 protein (38). At the same time, acquired bortezomib resistance was accompanied by increased levels of phosphorylated eIF2α and reduced immunoglobulin production. This may suggest that the acquired bortezomib resistance is due to a dampened immunoglobulin synthesis leading to a reduction in ER protein load, basal ER stress, and UPR activity. Intriguingly, the acquired bortezomib resistance made cells more sensitive to the melphalan and the ER stress inducer tunicamycin (38). However, as experimentally manipulating XBP1 levels in multiple myeloma cell lines only slightly affected bortezomib sensitivity (38). These studies therefore lend support to a therapeutic approach involving the combination with an ER stress-inducing agent to enhance the effectiveness of proteasome inhibition and to overcome bortezomib resistance.

HSP inhibitors

Inhibition of HSPs also leads to a disruption in protein processing and induces ER stress. Indeed, both HSPI70 and HSPI90 inhibitors, which show significant antilymoma activity, have been shown to induce the UPR in multiple myeloma cells (Table 1; refs. 60, 61). HSPs are molecular chaperones that can bind client proteins and mediate protein folding, refolding, stability, degradation, activation, and trafficking. Increased expression of prosurvival HSPs is observed in many types of cancers and their inhibition has emerged as a promising anticancer strategy (62). The HSPI90 inhibitors 17-AAG (tanespimycin) and radicicol (Fig. 4) induce apoptosis in multiple myeloma cell lines (60). Cell death induced by these HSPI90 inhibitors is associated with the induction of the UPR in multiple myeloma cells. Intriguingly, it was observed that ATF6 cleavage and CHOP expression downstream of PERK signaling were stimulated by both compounds to a higher extent than XBP1 splicing (Table 1), as compared with the effects of common pharmacologic ER stress inducers (60). The early effects (within 2 hours) on XBP1 splicing were similar but the HSPI90 inhibitors failed to induce further splicing on prolonged incubation (up to 24 hours; ref. 60). Since HSPI90 is involved in the regulation and stability of IRE1α, it is conceivable that inhibition may limit the degree of XBP1 splicing (63). The net effect of HSPI90 inhibition is a dominantly proapoptotic UPR response. Stress-inducible HSPI70, a chaperone of HSPI90 was strongly upregulated as a survival response following HSPI90 inhibition in vitro, enabling myeloma cells to fold greater quantities of damaged proteins (60). In recent clinical trials of HSPI90 inhibitors in multiple myeloma, consistent induction of HSPI70 has been observed and HSPI70 induction is now considered a biomarker of in vivo HSPI90 inhibition (64).

Inhibition of HSPI70 sensitizes cells to induction of apoptosis by HSPI90 inhibition (61, 65). The HSPI70 inhibitor MAL3-101 (Fig. 4) causes growth arrest and apoptosis not only in human multiple myeloma cell lines, but also in primary multiple myeloma cells, without toxicity in healthy control cell populations (61). MAL3-101 induces XBP1 splicing at concentrations around 4-fold higher than its IC50 value and showed synergistic effects in combinations with proteasome inhibitors MG-132 and bortezomib (61).

Oncolytic virotherapy

Oncolytic viral therapy may represent another novel therapeutic approach that induces ER stress in multiple myeloma. The reovirus-based therapeutic Reolysin is well tolerated in clinical trials for several cancers and showed potent anticancer activity (66). It has been proposed that Reolysin has a potential in targeting multiple myeloma cells as reovirus replication may promote ER stress-induced apoptosis via the accumulation of viral proteins (67). Indeed, Reolysin was shown to have antilymoma activity in cell lines, in ex vivo patient tumor specimens, and in in vivo mouse models of multiple myeloma (67, 68). Furthermore, Reolysin induced NOXA-mediated apoptosis in multiple myeloma cells and significantly increased the antilymoma activity of bortezomib (67). Interestingly, Reolysin induced ER stress in multiple myeloma cells as determined by increased XBP1 splicing, ER swelling, and increased intracellular calcium levels (Table 1; ref. 67). This effect was significantly increased by cotreatment with bortezomib. Reolysin represents an attractive therapeutic strategy as reovirus was shown to selectively target multiple myeloma cells but not hematopoietic stem cells in a mouse model of multiple myeloma (68).

Aminopeptidase inhibitors

Aminopeptidases have been identified as a target for cancer therapy, as their inhibition leads to a disruption of protein turnover. The small-molecule inhibitor CHR-2797 (tosedostat; Fig. 4) targets the M1 family of aminopeptidases and is currently in phase II clinical trials (Table 1). CHR-2797 has been shown to inhibit the proliferation of multiple myeloma cells, induce apoptosis, and overcome the protective effect of the bone marrow stroma.
microenvironment on multiple myeloma cells (69). CHR-2797 increased the levels of ATF4 and CHOP and stimulates the activation of ATF6 in the myeloma cell line H929, without affecting XBP1 splicing (69), thus primarily inducing proapoptotic UPR signaling. Similarly to the effect of proteasome inhibition, inhibition of aminopeptidases by CHR-2797 leads to the upregulation of HSPA7 (69).

Targeting IRE1α as a Therapeutic Strategy in Multiple Myeloma

Given the importance of the IRE1α/XBP1 axis in myeloma, it is not surprising that this has become an attractive target for drug development.

The kinase inhibitor sunitinib has antimyeloma activity (70) and is currently in phase II clinical trials. Sunitinib was shown to reduce XBP1 splicing in multiple myeloma cells, which shows the potential of developing specific kinase inhibitors of IRE1α as a means of modulating the UPR in human cells (71).

However, if IRE1α kinase inhibitors also prevent JNK activation, this could be counterproductive. Several groups have recently shown the potential of compounds that specifically target the RNase activity of IRE1α and selectively reduce XBP1 splicing, STF-083010 (Fig. 4) was recently reported as a novel small-molecule inhibitor of IRE1α RNase activity in multiple myeloma (72). STF-083010 inhibits XBP1 splicing in multiple myeloma cell lines treated with a range of ER stress-inducing agents (Table 1), while not affecting the kinase activity, and also inhibited XBP1 splicing in a transgenic mouse expressing an XBP1-luciferase reporter gene. STF-083010 showed cytotoxic activity in multiple myeloma cell lines and was selectively toxic to transformed cells isolated from patients with multiple myeloma as compared with healthy plasma cells, showing the therapeutic potential of targeting XBP1 splicing (72). Another recent study reported the aldehyde 8-formyl-7-hydroxy-4-methylcoumarin (4μ8C; Fig. 4) as an inhibitor of IRE1α RNase activity, inhibiting both XBP1 splicing and RIDD, but not affecting IRE1α autophosphorylation (73). This study showed that 4μ8C acts by covalently and selectively binding to a lysine residue (K907) within the RNase domain inhibiting its activity in a noncompetitive fashion, and elucidated the mechanism of action of other reported IRE1α inhibitors. STF-083010 selectively binds to K907, whereas the compounds described by Volkmann and colleagues (74) were shown to bind to K907 and K599 within IRE1α kinase domain. These findings are consistent with the observation that STF-083010 selectively inhibits IRE1α endoribonuclease activity without affecting its kinase activity, whereas the compounds described by Volkmann and colleagues (74) inhibited RNase activity but could, at higher concentrations, also inhibit autophosphorylation.

The activity of another compound specifically targeting the endoribonuclease domain of IRE1α has recently been reported (44). MKC-3946 (Fig. 4) was shown to inhibit growth of multiple myeloma cell lines but not of normal mononuclear cells. Furthermore, MKC-3946 inhibited XBP1 splicing after treatment with bortezomib or 17-AAG. This sensitized multiple myeloma cells to cytotoxicity of these compounds and overcame the protection provided by bone marrow stromal cells and IL-6 treatment (Table 1). MKC-3946 also inhibited XBP1 splicing in vivo and significantly inhibited growth of RPMI8226 plasmacytoma in a xenograft murine model (44). Treatment of multiple myeloma cells with MKC-3946 did not affect the phosphorylation state of IRE1α (44). In fact, binding of IRE1α to TRAF2 and phosphorylation of JNK were both enhanced by MKC-3946 treatment.

However, given that IRE1α is involved in the UPR in other cell types, particularly highly secretory cells, systemic targeting of IRE1α may have undesired side effects and caution will be required when IRE1α inhibitors enter clinical development (75).

Conclusion

A hallmark of multiple myeloma is the high level of production and secretion of immunoglobulin putting a heavy load on the secretory machinery and perturbing proteostasis (protein homeostasis) within the ER. The proteostasis network includes all pathways involved in protein synthesis, folding, trafficking, and degradation. Disturbances of proteostasis lead to accumulation of misfolded proteins and induction of cellular stress responses such as the UPR in case of proteostatic stress within the ER. Because of their high secretory activity, multiple myeloma cells experience persistent high levels of ER stress and are dependent on the UPR for maintenance of proteostasis. Consequently, multiple myeloma cells are characterized by a high basal UPR activity, and multiple myeloma cells may be addicted to the UPR for survival. Thus, multiple myeloma cells are highly sensitive to compounds that target proteostasis, such as proteasome inhibitors, and in particular, such that directly target ER proteostasis such as IRE1α inhibitors. These compounds act by shifting the balance between prosurvival and proapoptotic signaling of the UPR, pushing the cells beyond the point of no return. Considering these, multiple myeloma cells are particularly sensitive to agents that further disturb proteostasis. Furthermore, synergistic effects have been observed when targeting several pathways of the proteostasis network such as the proteasome and HSPs. Interestingly, bortezomib resistance has been linked to reduced UPR signaling, which indicates that reduced basal ER stress, possibly due to a downregulation of immunoglobulin production, may play a role in resistance to proteasome inhibition. Thus, treatment with ER stress-inducing agents might be a promising strategy to re-sensitize multiple myeloma cells to proteasome inhibition. The UPR-induced transcription factor XBP1s has been identified as a driving survival factor of multiple myeloma and inhibitors specifically targeting XBP1 mRNA splicing by IRE1α show antimyeloma activity.
Furthermore, NF-κB, an important survival factor in multiple myeloma, may be induced by the UPR. Several antimyeloma compounds target NF-κB and directly targeting NF-κB is a promising antimyeloma therapy. Targeting prosurvival signaling of the UPR is a novel promising strategy for myeloma therapy. Taken together, we describe two therapeutic strategies targeting this Achilles heel of multiple myeloma cells; first, compounds that disturb ER proteostasis by targeting components of the protein quality control machinery, such as the proteasome or HSPs, leading to ER stress; and second, compounds directly targeting the prosurvival signaling arms of the UPR, in particular IRE1α.

Targeting proteostasis is the most powerful strategy for the treatment of multiple myeloma. In particular, the IRE1α/XBP1 pathway seems to play an important role in multiple myeloma pathogenesis and therapeutic responses. Targeting ER stress responses emerges as a promising strategy to overcome resistance of multiple myeloma cells to current treatment modalities.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

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Conception and design: L. Vincenz, R. Jäger, M. O’Dwyer, A. Samali.
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Analysis and interpretation of data (e.g., statistical analysis, bioinformatics, computational analysis): M. O’Dwyer.
Writing, review, and/or revision of the manuscript: L. Vincenz, R. Jäger, M. O’Dwyer, A. Samali.
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): L. Vincenz.
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