in vivo *21*: 215-226 (2007)

Review

The Endoplasmic Reticulum: A Target for New Anticancer Drugs

JERINA BOELENS[1*], SOFIE LUST[1*], FRITZ OFFNER[1], MARC E. BRACKE[2] and BARBARA W. VANHOECKE[2]

[1]Department of Hematology and [2]Laboratory of Experimental Cancer Research, Department of Radiotherapy and Nuclear Medicine, Ghent University Hospital, Ghent, Belgium

Abstract. *In eukaryotic cells, the endoplasmic reticulum (ER) is the principal site for the folding and maturation of transmembrane, secretory and ER-resident proteins. Functions of the ER are affected by various intracellular and extracellular stimuli, which include inhibition of glycosylation, reduction of disulfide bonds, calcium depletion from the ER lumen, impairment of protein transport to the Golgi, and expression of mutated proteins in the ER. Under ER stress, unfolded/misfolded proteins accumulate in the ER lumen, which induces conflicting cellular activities: survival and apoptosis. To cope with this stress, cells activate intracellular signalling pathways, such as the unfolded protein response (UPR) and the ER-associated degradation (ERAD). However, under conditions of severe ER stress or when the UPR has been compromised, the cell may be incapable of maintaining ER homeostasis, which may eventually activate programmed cell death (PCD) pathways. Clinical data support the potential of drugs that inhibit the normal functions and homeostasis of the ER and the proteasome in treatment of malignancies like cancer. It is therefore reasonable to assume that manipulation of ER stress might enhance the efficacy of chemotherapeutic drugs and provide new anticancer targets like the ER and the proteasome.*

In order to carry out the folding of transmembrane, secretory and endoplasmic reticulum (ER)-resident proteins, the ER has evolved as a specialized protein-folding machine that promotes folding and prevents aggregation. Co- and posttranslational modifications, including disulfide bond formation and *N*-linked glycosylation, play an important role in the folding and oligomeric assembly of proteins (1). To assist in the folding of nascent polypeptides and to prevent aggregation of folding intermediates, the ER contains a high concentration of chaperones including the glucose-regulated proteins (GRPs), calnexin (CNX), calreticulin (CRT), peptidyl-prolyl isomerases (PPI), and protein-disulphide isomerase (PDI) (2, 3). In addition to their role in folding, some of these chaperones are postulated to act as a quality control system to ensure that only correctly folded proteins proceed to the Golgi for further processing and secretion (4, 5) (Figure 1). There are two major chaperone systems.

The first system comprises of the lectin-like chaperones CNX and CRT, which bind to monoglucosylated *N*-linked glycans and on unfolded regions of nascent glycoproteins. As nascent polypeptides enter the lumen of the ER, they are modified by *N*-linked glycans composed of two *N*-acetylglucosamine, nine mannose and three glucose molecules. The glucoses are almost immediately removed by the action of glucosidase I and II enzymes. When the glycan has been pared down to one single glucose, it becomes a substrate for CNX and CRT. Removal of this last glucose destroys the binding site for these chaperones, allowing the nascent protein to be transported to the Golgi once correctly folded (6, 7). ERp57, a member of the PDI, appears to stabilize protein folding by catalyzing the formation of disulfide bonds. However, when the protein is not folded correctly, it becomes a substrate for UDP-glucose/glycoprotein glucosyltransferase (UGGT), which adds a single glucose to the protein that is recognized as a binding site for CNX and CRT (8). The cycle will continue to repeat itself until the nascent protein is folded correctly; then the UGGT will not rebind and the protein can exit the ER (6). Misfolded proteins are retained in the ER and subsequently targeted for degradation by the ER-associated degradation (ERAD) pathway (9).

The second ER chaperone system is dependent on the presence of unfolded regions of proteins containing hydrophobic residues, which are recognized by the ER chaperone GRP78/BiP (10). Glucose-regulated proteins

*Both authors contributed equally to this work.

Correspondence to: Barbara Vanhoecke, Laboratory of Experimental Cancer Research, De Pintelaan 185, 9000 Ghent, Belgium. Tel: +32 0 9 240 30 69, Fax: +32 0 9 240 49 91, e-mail: barbara.vanhoecke@ugent.be

Key Words: Endoplasmic reticulum, stress, programmed cell death, anticancer drugs, cancer, review.

(GRPs) were first described as a set of proteins whose synthesis was enhanced when cells were deprived of glucose (11). The best studied GRPs are GRP78 and GRP94. GRP78 has been characterized in B-cells as part of the immunoglobulin secretory machinery and is also known as immunoglobulin heavy-chain binding protein (BiP), due to its association with incompletely assembled subunits of antibody molecules (12, 13). It resides primarily within the ER (14-16) but it can also be found in the nucleus, cytoplasm or at the plasma membrane. It can even be released in cell culture medium (17). Although GRP78 is constitutively expressed, its expression is enhanced up to 20-fold in cells under a variety of stressful conditions or when in the presence of agents that interfere with protein glycosylation, folding, transport, and disrupt calcium homeostasis (18, 19).

GRP78 is responsible for maintaining the permeability barrier of the ER translocon during the early stages of protein translocation. The translocon is the proteinaceous channel in the ER membrane to which a nascent peptide is transferred for folding and assembly in the ER. Once the nascent peptide chains reaches a length of 70 amino acids, GRP78 is released from the translocon, opening the channel to allow translocation of the protein into the luminal space. As the nascent peptide enters the ER, it is often modified by addition of *N*-linked glycans before folding. To ensure appropriate folding, the molecular chaperones CNX, CRT, and GRP78 bind and protect nascent chains until the correct regions interact. GRP78 interacts with a wide variety of unrelated nascent polypeptides that usually show a high degree of hydrophobicity. Depending on the interplay between the ER chaperones, some chaperones will stabilize the nascent proteins, while others have a destabilizing function (20). In addition, it has been established that GRP78 binds to misfolded proteins and mediates their retrograde translocation prior to proteasome degradation (21-23). Recently, it has been shown that GRP78/BiP can form a large multi-chaperone complex with GRP94, PDI, cyclophilin B, Erp72, GRP170, and UGGT but not with components of the CNX/CRT/Erp57. The complex can exist and its organization is thought to maximize the local concentration of chaperones and folding enzymes on unfolded substrates (24).

The functions of the ER can be affected by various intra- or extracellular stimuli, so called ER stress. ER stress can be induced by agents/conditions that interfere with protein glycosylation (*e.g.*, glucose starvation, tunicamycin (TUN), glucosamine), calcium balance (*e.g.*, A23187, thapsigargin (TAPS), EGTA), disulfide bond formation (*e.g.*, DTT, homocysteine), and/or by a general protein overload of the ER (*e.g.*, viral and non-viral oncogenesis) (25, 26, 27). Perturbations that alter ER homeostasis therefore disrupt folding and lead to the accumulation of unfolded proteins and protein aggregates, which are detrimental to cell survival. As a consequence, the cell has evolved an adaptive coordinated response to limit further accumulation of unfolded proteins in the ER. This signalling pathway is termed the unfolded protein response (UPR).

The complex network of physiological responses to ER stress consists of three different mechanisms: (i) translational attenuation to further limit misfolded proteins (28); (ii) transcriptional activation of genes encoding ER-resident chaperones such as GRP78/BiP (2); and (iii) ERAD, which serves to reduce the stress and thereby restores the folding capacity by directing misfolded proteins present in the ER back into the cytosol for degradation by the 26S proteasome (29). This integrated intracellular signalling pathway transmits information about the protein folding status in the ER lumen to the cytoplasm and the nucleus *via* the activation of ER-stress sensor molecules. If the protein folding defect is not corrected, cells undergo apoptosis.

ER-stress Sensors

The three major sensors/transducers of ER stress are the double-stranded RNA-activated protein kinase-like ER kinase (PERK), the inositol-requiring enzyme 1 (IRE1α/β) and activating transcription factor 6 (ATF6). Among these ER-resident transmembrane proteins, IRE1α/β and ATF6 play a dominant role in mediating transcriptional regulation, whereas PERK is mainly responsible for repressing global protein synthesis.

PERK is an ER-resident transmembrane serine/threonine protein kinase which consists of an ER luminal stress-sensing domain, a transmembrane domain and a cytosolic domain with kinase activity that phosphorylates the α subunit of eukaryotic translation initiation factor 2 (eIF2α) in response to ER stress (28, 30 31). Phosphorylation of eIF2α reduces formation of translation initiation complexes which normally lead to translation. This translational control provides an efficient mechanism to reduce the number of unfolded proteins in the ER. On the other hand, phosphorylation of eIF2α can also indirectly control gene transcription by positively regulating the translation of transcription factors (TFs) such as ATF4, which in turn leads to the up-regulation of factors like CHOP/GADD153, GADD34, and ATF3 (32-35).

IRE1 is an ER-transmembrane glycoprotein having 2 isoforms: IRE1α and IRE1β. It consists of a *N*-terminal ER luminal stress-sensing domain, a transmembrane domain and a cytosolic domain with both serine/threonine kinase and *C*-terminal endoribonuclease activities (36, 37). ER stress initiates its dimerization and autophosphorylation to activate its kinase and RNase activities. Two groups have demonstrated that the mRNA of X-box-binding protein 1

(XBP1) is a substrate for the RNase of IRE1 (38, 39). The splicing of the mRNA of XBP1, a basic leucine zipper (bZIP)-containing TF, removes a 26-nucleotide intron leading to a potent TF that binds to the unfolded protein response-responsive element (UPRE) and ER-stress-responsive element (ERSE) sequence of many ER-stress and UPR-target genes such as GRP78 and the ER-degradation-enhancing α-mannosidase-like protein (EDEM). EDEM is an ER-resident transmembrane protein which binds to misfolded glycoproteins with mannose 8 structure and enhances the degradation of misfolded proteins *via* the ERAD pathway (40). Thus, the IRE1-XBP1 pathway is involved not only in the induction of ER chaperones, but also in the capacity control of the ERAD pathway.

p90/p110ATF6 is an ER-resident ATF consisting of a cytosolic *N*-terminal (bZIP) domain, a transmembrane domain, and a C-terminal ER luminal stress-sensing domain (41). Upon ER stress, a mechanism known as regulated intramembrane proteolysis (RIP) is activated (25, 41-45). This mechanism involves TFs and anchoring partners that are localized in one compartment and proteases that are located in a different compartment. Generally, these TFs are inserted into ER membranes and, in response to specific stimuli, they translocate to the Golgi compartment where they are cleaved by resident proteases thereby releasing a N-terminal cytosolic domain which then shuttles to the nucleus to effect transcription of specific target genes (42, 46-48). In case of ATF6, p90ATF6 and p110ATF6 transit to the Golgi compartment where they are cleaved by Golgi site-1 and site-2 proteases (S1P and S2P) to generate p50ATF6 (41, 45, 49, 50). p50ATF6 then translocates to the nucleus where it binds, together with the TF NF-Y, to the ERSE of target genes including *GRP78* and *XBP1*.

Recently, Kondo *et al.* (51) discovered a novel ER-stress sensor molecule, OASIS. It is an ER-resident transmembrane protein containing the bZIP TF of the CREB/ATF family. The molecule is cleaved at the membrane in response to ER stress and its cleaved *N*-terminal cytoplasmic domain translocates to the nucleus where it activates the transcription of target genes that are mediated by ERSE and cyclic AMP-responsive elements. Intriguingly, OASIS is induced at the transcriptional level during ER stress in astrocytes of the central nervous system, but not in other cell types.

GRP78 is the master regulator of the activation of ER-stress sensors. All sensors contain a luminal domain that interacts with GRP78. Under normal conditions, GRP78 serves as a negative regulator of IRE1, PERK and ATF6. Upon ER stress, GRP78 is released from the transducers and binds to unfolded proteins. GRP78 release from IRE1 and PERK permits their homodimerization and activation (30, 52). GRP78 release from ATF6 permits its transport to the Golgi-compartment (44, 53).

Taken together, the signalling from the downstream effectors of IRE1, PERK, ATF6 and OASIS merges in the nucleus to activate transcription of ER-stress target genes coding for molecular chaperones and folding catalysts that increase the folding capacity of the ER, providing a protective effect for cell survival.

ER Stress and the Ubiquitin-proteasome System (UPS)

Cells are equipped with an abundant ubiquitin-proteasome system (UPS) activity (54-56) which enables them to respond to acute proteotoxic stress, reflecting the crucial role of this system in protein quality control and cellular protein homeostasis (57, 58). It is becoming increasingly clear that there is a tight connection between protein quality control in the ER and the UPS (59).

During the ERAD process, proteins that fail the ER quality control are transported back to the cytosol, where they are rapidly ubiquitinated and degraded by the proteasome (59-62). Ubiquitination of substrates is a multi-step process that is dependent on a ubiquitin (Ub)-activating enzyme (E1), a Ub-conjugating enzyme (E2) and a Ub-ligase (E3) enzyme (58). E1 recruits ubiquitin in an ATP-dependent process and adenylates its C-terminus. After E2 has accepted ubiquitin from E1, E3 catalyzes the transfer of ubiquitin from the E2 to the substrate. It is thought that malfolded proteins are recognized by the CNX cycle, allowing multiple rounds of folding to acquire correct folding and that EDEM will extract malfolded proteins from the CNX-cycle and feed them into the downstream ERAD system. Finally, when ER stress persists, the ERAD process is insufficient and programmed cell death (PCD) pathways are activated. Recently, it was found that ER stress causes accumulation of different UPS substrates in the ER, cytosol and nucleus, bringing about a general dysfunction of the ERAD followed by cell death (56).

ER Stress and PCD (Figure 2)

Although the UPR is a cytoprotective response, prolonged ER stress can activate PCD through mitochondria-dependent or mitochondria-independent pathways (63, 64). Apart from the mitochondrium, the ER is now being recognized as an organelle that can regulate ER-stress-induced apoptosis, and even necrosis, which is believed to be associated with several pathologies including diabetes (35), Alzheimer's disease (65), Parkinson's disease (66), and polyglutamine diseases like Huntington's and Machado-Joseph disease (67, 68).

The decision between cell survival and death is presumably made during cell cycle arrest that is also generated in response to ER stress due to decreased

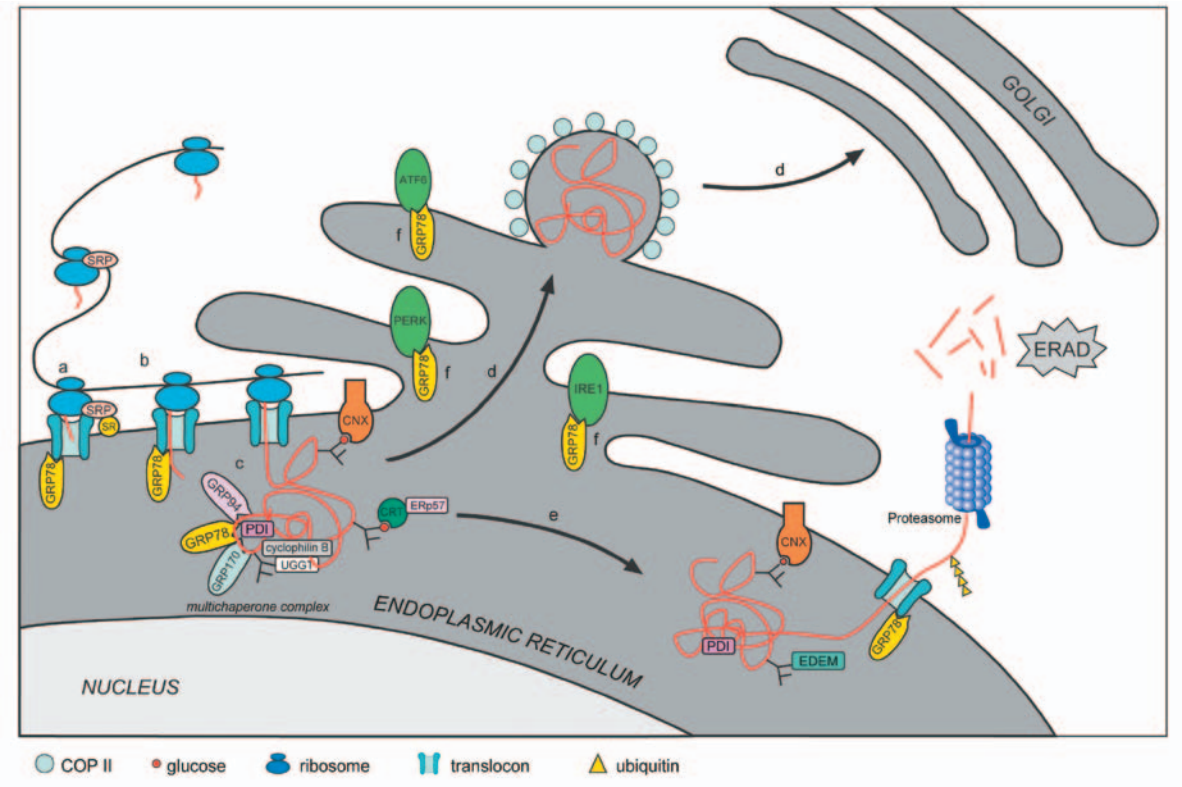

Figure 1. *Schematic of ER functions under non-stress conditions. Partially adapted from Kaufman et al. (25), and Ma and Hendershot (119). a. Proteins that are destined for synthesis in the ER are recognized, through signal peptides, by the signal recognition particle (SRP), which associates with the ribosome. The complex diffuses to the translocon, where the SRP docks with the SRP receptor (SR). b. Passage of the growing peptide through the translocon is coupled with the ATPase activity of GRP78. c. In the extremely crowded, calcium-rich, oxidizing environment of the ER lumen, resident chaperones like GRP78, GRP94, GRP170, CNX, CRT, PDI, and ERp57 serve to facilitate proper folding of the nascent protein by preventing its aggregation, monitoring the processing of the highly branched glycans, and forming disulfide bonds to stabilize the folded protein. d. Once correctly folded and modified, the protein will exit the ER through coat protein (COP) II-coated vesicles and move on through the secretory pathway. e. Misfolded proteins can associate with GRP78, CNX, PDI and EDEM for retrotranslocation to the cytosol, ubiquitination and digestion by the proteasome. GRP78 is the master regulator of the activation of three ER-stress sensors - IRE1, PERK and ATF6. In non-stress conditions, the ER-stress sensors are associated with GRP78, which keeps them in their inactive state.*

transcription of cyclin D1 (69). This delay may allow the cell to determine whether adaptation is feasible or whether to commit suicide. Several PCD pathways are activated during ER stress. While attenuating global protein synthesis, phosphorylation of eIF2α by PERK promotes preferential translation of ATF4 mRNA, which results in transcriptional induction of CHOP (C/EBP homologous protein-10, also known as GADD153) (32, 35, 37, 70). CHOP is a bZIP transcription factor that contains an ERSE in its promotor and is transcriptionally induced by ATF4 (71) and ATF6 (70-72). It can form heterodimers with members of the C/EBP and fos-jun families (73, 74) and controls the expression of stress-induced genes. Apart from its transcriptional induction by ATF4/ATF6, CHOP is also activated by p38 mitogen-activated protein kinase (p38MAPK) (75). Together with C/EBP family members, CHOP is involved in mitochondrion-dependent cell death pathways. It is able to transcriptionally down-regulate the levels of the anti-apoptotic bcl-2 and up-regulate DR5, a member of the death receptor protein family (76, 77). In addition, CHOP leads to a depletion of the cellular glutathione levels (78) and increases the levels of reactive oxygen species (ROS) in the cell (79). This results in leakage of mitochondrial cytochrome c, activation of cytosolic apoptotic protease activating factor 1 (APAF-1) and stimulation of the caspase-9 and caspase-3 cascade.

ER stress can also activate general regulators of apoptosis, including the bcl-2 and caspase families of proteins. As there is endogenous bcl-2 present in the ER membrane, there is evidence that this pool influences homeostasis and apoptosis from the ER (80, 81). Conversely, ER stress itself can activate several BH3-only pro-apoptotic members of the bcl-2 family, including bim

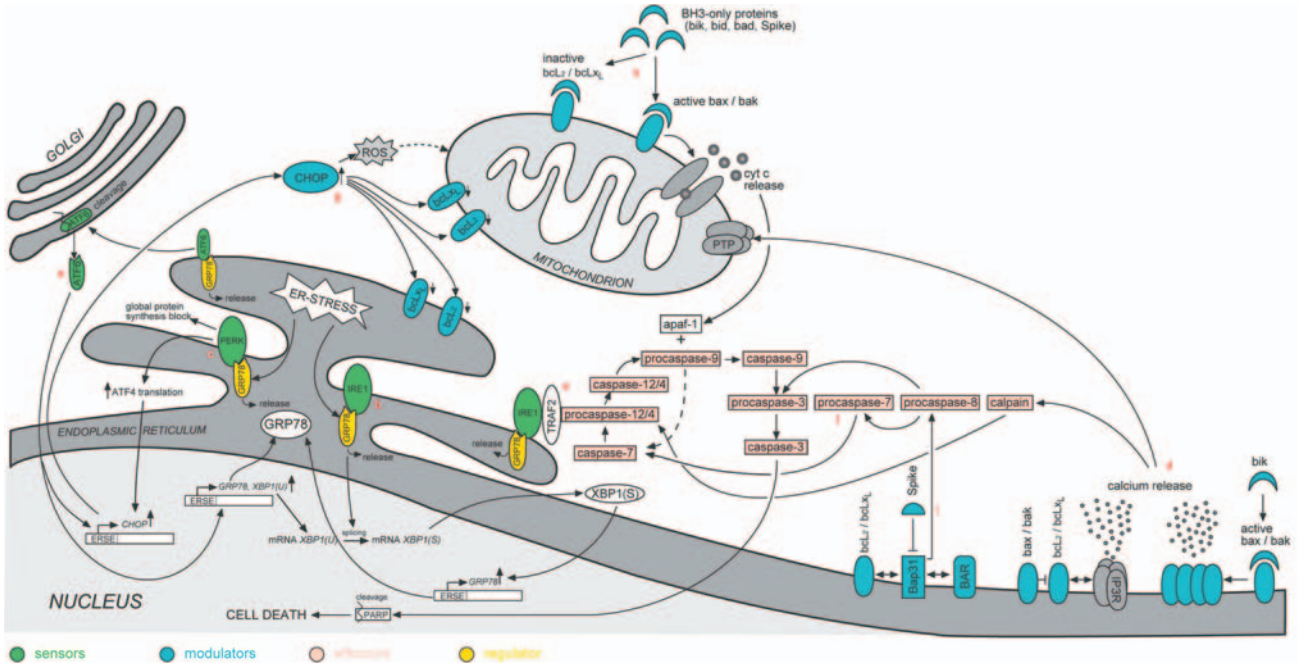

Figure 2. *Schematic of ER functions under stress conditions. Partially adapted from Ma and Hendershot (119). a. On accumulation of unfolded proteins in the ER lumen, GRP78 releases from p90/p110ATF6. ATF6 moves to the Golgi compartment, where it is cleaved by the S1P and S2P proteases to yield a cytosolic fragment. This p50ATF6 fragment migrates to the nucleus to activate the transcription of responsive genes, including XBP1, GRP78 and CHOP. b. In parallel, IRE1 dimerizes and activates its endoribonuclease activity after its release from GRP78. It splices its substrate XBP1 mRNA to remove a small intron, which changes the translational reading frame of XBP1 to yield a potent transcriptional activator that activates the transcription of responsive genes, including GRP78. c. At the same time, PERK is activated to phosphorylate eIF2 on the α-subunit at Ser51. As phosphorylation of eIF2α reduces the functional level of eIF2α, the general rate of translation initiation is reduced. However, selective mRNAs can be preferentially translated under these conditions. Translational up-regulation of ATF4 yields a potent transcriptional activator that activates the transcription of responsive genes, including CHOP. d. While most BH3-only proteins are found in the cytosol or at the mitochondrion, bik is somewhat unique in that it is primarily localized to the ER. Bik was identified as a binding partner for bcl-2 and its expression was found to be dependent of p53. It is suggested that p53 induces and activates bik, followed by bax/bak signalling to release calcium from the ER as an upstream signal form apoptosis. Furthermore, it is thought that the bik-induced release of ER calcium is taken up by mitochondria which leads to fission, cristae remodeling, and cytochrome c release. Also, bcl-2 and bcl-xL can modulate ER calcium levels by binding the IP3 receptor. This interaction can be inhibited by bax/bak, consistent with the observation that bax/bak can also regulate ER calcium levels. Perturbations of the calcium pools activate calpains in the cytosol, whose substrates include bax and bid (which are activated), bcl-2 and bcl-xL (which are inhibited) and several caspases. ER-localized procaspase-12/-4 is converted to caspase-12/-4. Activated caspase-12 then initiates a caspase cascade through cleavage of procaspase-9 and -3. This pathway is independent of Apaf-1 and mitochondrial cytochrome c (cyt c) release. Calcium released from the ER is rapidly taken up by the mitochondria, where it may lead to collapse of the inner membrane potential, and subsequent initiation of apoptosis. e. By sequestering IRE1, TRAF2 promotes clustering of and release from procaspase-12 upon ER stress. f. Furthermore, procaspase-12 can be activated by caspase-7 after its relocation from the cytosol to the ER. g. Up-regulation of CHOP during ER stress is mediated by ATF6 and the translational up-regulation of ATF4 and results in the down-regulation of the anti-apoptotic proteins bcl-2 and bcl-xL and in ROS accumulation. Eventually, the disturbed balance between pro-apoptotic proteins, e.g. bad, bak and bax, and the anti-apoptotic bcl-2 proteins and the increase in oxidative stress activate the intrinsic apoptotic pathway. h. Functionally, BH3-only proteins can be separated into: 1) activators that bind to anti-apoptotic proteins or activate bax and bak to trigger cytochrome c release, and 2) sensitizers that only bind and antagonize anti-apoptocic bcl-2 members to sequester them away from interfering with the activation of bax and bak. i. The ER resident proteins Bap31 and BAR can both interact with bcl-2/bcl-xL via a domain separate from their caspase-interacting domain. Bap31 contains 3 transmembrane domains, a leucine zipper and a death effector domain-like region that associates with certain isoforms of procaspase-8 modulating apoptosis. The BH3-only protein Spike promotes Bap31 cleavage, presumably to the pro-apoptotic form.*

(82), bik (83-86) and PUMA (87, 88). Also bax and bak have been shown to regulate ER stress-induced apoptosis from both the mitochondrial and ER membranes. In the ER membrane, they regulate the level of lumenal calcium through an interaction with the inositol triphosphate (IP3) receptor (89-93). Accumulation of these pro-apoptotic members may antagonize ER-membrane-resident anti-apoptotic members such as bcl-xL and bcl-2, resulting in structural changes of the ER, ER calcium release and/or caspase-12 activation (63, 93). A recent study demonstrated that bak mediates swelling and restructuring of the ER in a bcl-xL-dependent manner (94).

Another BH3-only protein that is anchored in the ER and is involved in the regulation of apoptosis at the ER is Spike (95). Although the mechanism of its pro-apoptotic effect is yet unknown, it is likely that dimerization with partners among the bcl-2/bax family proteins integrated with the ER, like mcl-1 (96) and bik (85), is one of the possibilities. Another ER-resident protein, BI-1, has been shown to associate with bcl-2 family members thereby protecting cells from insults known to trigger ER stress (97, 98). Although the ER-resident protein, Bap31, lacks homology with bcl-2/bax family proteins and contains no BH3 dimerization domain, it binds bcl-2 and bcl-xL thereby regulating apoptosis. Bap31 contains 3 transmembrane domains, a leucine zipper and a death effector domain-like (DED-L) region that associates with certain isoforms of procaspase-8 (99). Depending on whether its cytosolic tail is removed by caspases, it is associated with a pro-or anti-apoptotic activity. The DED-L domain of Bap31 binds a homologous DED-L domain in another ER-associated protein, BAR (100). It is thought that both Bap31 and BAR could promote caspase-8 activation, if induced to aggregate in the ER membranes, through the formation of an ER-associated 'apoptosome'. Apart from its DED-L domain, BAR contains a separate region that binds bcl-2 and bcl-xL, so it is speculated this separate binding domain might supply a mechanism for preventing caspase activation (101).

Caspases are required for apoptosis and certain members of this family of cysteine proteinases associate with the ER. Murine caspase-12 is primarily localized on the cytoplasmic side of the ER membrane. A number of mechanisms have been suggested as being involved in the cleavage and processing of caspase-12. Upon ER stress, procaspase-12 might autoactivate through a direct association with IRE1 and TNF-receptor-activating factor 2 (TRAF2), although how ER stress regulates the formation of this complex is yet unknown (102, 103). Once activated, the catalytic subunit of caspase-12 is released in the cytosol where it cleaves procaspase-9 in a cytochrome c-independent manner (104). However, caspase-12 can also be processed indirectly *via* calpains, activated by elevated cytoplasmic calcium levels during ER stress (102). Calpains are a family of calcium-dependent cysteine proteases of which two isozymes, μ- and m-calpain, are ubiquitously expressed (105). Unlike the caspases, which function only during apoptosis, calpains have been implicated in several processes involved in normal cellular metabolism and physiology (106, 107), such as remodelling of the actin cytoskeleton during cell motility (106). It is believed that, under non-apoptotic conditions, calpain activity is controlled by its inhibitor, calpastatin. However, during apoptosis, a massive and sustained influx of calcium into the cytoplasm might result in uncontrolled calpain activation that, together with caspase activation, can lead to excessive proteolysis of many substrates. Procaspase-12 can also be cleaved by caspase-7. Rao *et al.* (108) demonstrated that ER stress induces translocation of cytosolic caspase-7 to the ER surface followed by cleavage of pocaspase-12.

Although caspase-12 may play an important role during ER stress-induced apoptosis, it has recently been shown that its expression is not required for apoptosis, as cells lacking caspase-12 were not protected from apoptosis after treatment with ER-stress agents (109). Furthermore, it has been reported that caspase-12 can also be activated under other apoptotic conditions, such as Fas-triggering (110). In this regard, the relevance of caspase-12 to ER-induced apoptosis has been questioned because of an absence of caspase-12 in most humans. Human caspase-4, one of the closest paralogs of rodent caspase-12, may associate with the ER (111), raising the possibility that it can perform the functions of rodent caspase-12 in the context of ER stress.

Signalling from the ER-stress transducers to the cell nucleus can also be coupled to the activation of stress-activated protein kinases such as JNK and p38MAPK (112, 113). Activated IRE1 is reported to recruit Jun *N*-terminal inhibitory kinase (JIK) and TRAF2 during ER stress. This leads to activation of apoptosis-signalling kinase 1 (ASK1), JNK and mitochondria/APAF-1-dependent caspases. Apart from IRE1, Liang *et al.* (114) demonstrated recently that PERK may be required for activation of JNK and p38MAPK and expression of immediate early genes like *c-myc* and *egr-1*, which have been shown to be transcriptionally up-regulated during TAPS-induced ER stress (35, 115-118).

Another pathway that may initiate cell death due to ER stress is the ER-overload response (EOR), which results from over-accumulation of proteins in the ER membrane increasing calcium-permeability and activation of the NFκB-pathway. However, this response will not be discussed in this review.

ER Stress and Cancer

Having accumulated mutations that overcome cell-cycle and apoptotic checkpoints, the main obstacle to survival faced by a cancer cell is the restricted supply of nutrients and oxygen (119). Cancer cells respond by producing pro-angiogenic factors to initiate formation and attraction of new blood vessels to the tumour. However, this is often not sufficient to provide optimal oxygen and nutrients to the tumour or to dispose of wastes (120). As a result, a range of cellular stress response pathways, including the UPR, are activated, regulating the balance between cancer cell death, dormancy, and aggressive growth.

Many aspects of the UPR are cytoprotective and several studies indicate that activation of the UPR might have a crucial role in tumour growth. Indeed, increased expression of XBP1, ATF6, CHOP, GRP78/BiP, GRP94, and GRP170 have been reported in breast tumours, (121), hepatocellular carcinomas (122), gastric tumours (123), and oesophageal

adenocarcinomas (124). However, prolonged activation of the UPR can initiate apoptosis, which could serve to protect the host (119).

At present, it is unclear where the balance between cancer cell death, dormancy and aggressive growth lies in tumour development. Recent studies revealed a role for the UPR during angiogenesis and dormancy. It was shown that the pro-angiogenic glycoprotein, vascular endothelial growth factor (VEGF) is up-regulated during ER stress thereby promoting survival. Translation of VEGF mRNA is stimulated during ER stress through an ATF4-dependent pathway (125) and controlled by GRP170, an ER-resident Hsp 70 family member up-regulated during ER stress and hypoxia (126). Alternatively, if a tumour fails to induce angiogenesis, it can exit the cell cycle and become dormant. There are data indicating that the relative activation levels of two mitogen-activated protein kinase (MAPK) family members – the extracellular-signal-regulated kinases (ERKs) and p38MAPK – can affect tumour progression (127, 128). Although activation of p38MAPK during ER stress has not been thoroughly examined, one study reported that treatment of cells with ER-stress inducers like azetidine also activated p38MAPK (112). A second characteristic of the UPR that can contribute to dormancy is the G1 arrest in response to inhibition of cyclin D1 translation, which is downstream of PERK (69). As mutations in cancer cells often inactivate their apoptotic potential, persistent activation of the UPR in tumour cells could function to promote cell-cycle arrest and dormancy instead of apoptosis. Therefore, during early stages of tumour development, ER stress could either benefit the tumour by increasing angiogenesis or protect the host by inducing dormancy. On the other hand, the induced dormant state can also protect tumour cells from apoptosis and allow them a second chance for tumour growth if conditions change. It seems likely that the interplay between various signalling pathways within the tumour and the microenvironment around the tumour will dictate whether apoptosis, growth arrest or proliferation will occur (119).

Although the UPR is generally viewed as a cytoprotective response, prolonged ER stress can activate apoptosis through both mitochondria-dependent and -independent pathways (63, 64). It would seem that prolonged ER stress should render cancer cells more vulnerable to apoptotic cell death. However, it is unclear how tumour cells adapt to long-term ER stress *in vivo*. It is known that some of the mitochondria-dependent pro-apoptotic components used during ER stress are mutated or their expression levels are altered in cancer cells. Therefore, in spite of the evidence for activation of apoptotic components by the UPR pathway, they might be ineffective in tumours, allowing them to benefit from the long-term cytoprotective effects of the UPR (119).

New Anticancer Drugs that Target the ER

For many years, two major pathways of PCD were believed to induce apoptosis namely the intrinsic pathway, mainly controlled at the level of the mitochondria, and the extrinsic pathway, regulated by the binding of specific death ligands to their receptors on the cell surface. However, a number of recent studies have provided convincing evidence that PCD cascades can also be initiated at other sites within the cell, in particular, at organelles such as the ER and the Golgi-apparatus. Chemical ER-stress inducers like tunicamycin, thapsigargin and brefeldin A are reported to induce cell-death *via* a disturbance of ER homeostasis, although in distinct ways. Tunicamycin induces an accumulation of unfolded proteins due to a blockage of the formation of *N*-glycosidic linkages by the inhibition of the transfer of *N*-acetylglucosamine 1-phosphate to dolichol monophosphate. Thapsigargin is a potent, cell-permeable, IP3-indepentent intracellular calcium releaser that inhibits the calcium-ATPase, thus disrupting intracellular free calcium levels and disturbing the ER (129-131). Brefeldin A is a fungal metabolite produced by *Penicillium brefeldianum* and known to inhibit protein secretion in mammalian and other eukaryotic cells by interterfering with the function of the Golgi-apparatus. It blocks the secretory pathway and induces ER stress by disrupting the movement of material from the ER to the Golgi-apparatus (132-135). The effect of brefeldin A was tested by Carew *et al.* on B-chronic lymphocytic leukemia (B-CLL) cells and on multiple myeloma (MM) cells after they observed that B-CLL cells appeared to have a far more developed ER network than normal B-lymphocytes (136, 137). This made them suggest that the ER may be of great importance for the survival of B-CLL cells. As MM cells are the malignant counterparts of plasma cells having great secretory activities, they also contain a well developed ER-Golgi network which makes them attractive cells to target with ER-stress-inducing agents. Firstly, they observed an induction of apoptosis with brefeldin A in MM cell lines and primary B-CLL cells. In B-CLL cells, the observed effect was comparable in fludarabine-refractory and non-refractory cells which indicates that the mechanisms of resistance to fludarabine and brefeldin A do not overlap. Apoptosis was associated with activation of the Golgi-resident caspase-2 and the caspases-8, -9 and -3, a blocked secretion of the pro-survival proteins VEGF (Vascular Endothelial Growth Factor) and APRIL (a proliferation-inducing ligand) and a severe dilation of the ER.

The importance of the ER-Golgi secretory machinery as a therapeutic target in B-cell malignancies is also suggested by the role of the UPR in the B-cell development. Already in the pro-B-cell stage, IRE1 is needed for successfull VDJ-rearrangement (137). During

plasma cell differentiation, both the IRE1 and ATF6/XBP1 arm of the UPR are activated and necessary (138, 139). These observations made targeting of the ER in B-cell malignancies very attractive and the induction of a terminal UPR a logical pathway to cell death. This terminal UPR-induction can be considered as an alternative way to induce cell death apart from the classical intrinsic and extrinsic apoptotic pathways especially in B-CLL cells which have a defective apoptosis. However, although the reported results with brefeldin A on B-CLL and MM are offering new therapeutic perspectives, its clinical relevance should still be evaluated in future trials.

Apart from chemical inducers of ER stress, we recently discovered that plant-derived chemicals like the hop-derived flavonoid xanthohumol (X) can also induce ER stress and apoptosis. *Via* a proteomic approach (2-D gel electrophoresis and MALDI-TOF-MS/MS), we identified GRP78 and demonstrated an up-regulation of its transcription and expression in X-treated compared to solvent-treated breast cancer cells (unpublished data). The X-induced ER stress involved that the activation of IRE1, ATF6 and PERK and was associated with apoptotic events like processing of caspases, down-regulation of anti-apoptotic Bcl-xL and Mcl-1, and PARP cleavage. Interestingly, the effect seems to be selective for human breast cancer cells as human primary normal breast epithelial cells were not affected. Moreover, susceptibility to the X-induced apoptosis appeared to correlate with the GRP78 expression levels. The next challenge is to evaluate if these promising chemicals are suitable clinical candidates.

Conclusion

In higher organisms, ER-stress signalling is crucial for the development or maintenance of differentiated tissues that are specialized in secretion. It is therefore an import issue in the development of new anticancer strategies to determine the role of ER stress and its mediators in normal cell development and malignancies. Furthermore, an important question is if ER stress plays a critical role in the acquisition of apoptosis-resistance to chemotherapy. Further characterization of ER-stress markers in cancer models and ER-stress inducers in the clinic will help us to determine the physiological roles of UPR and ER stress during tumour development and its therapeutic potential.

Acknowledgements

The authors thank J. Roels van Kerckvoorde for technical assistance. B. Vanhoecke is a postdoctoral fellow supported by the Flemish Foundation against Cancer and the FOD Public Health FYTOES project (B/069501/01).

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Received February 2, 2007
Accepted February 6, 2007