Endoplasmic Reticulum Stress, the Unfolded Protein Response, Autophagy, and the Integrated Regulation of Breast Cancer Cell Fate

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Abstract
How breast cancer cells respond to the stress of endocrine therapies determines whether they will acquire a resistant phenotype or execute a cell-death pathway. After a survival signal is successfully executed, a cell must decide whether it should replicate. How these cell-fate decisions are regulated is unclear, but evidence suggests that the signals that determine these outcomes are highly integrated. Central to the final cell-fate decision is signaling from the unfolded protein response, which can be activated following the sensing of stress within the endoplasmic reticulum. The duration of the response to stress is partly mediated by the duration of inositol-requiring enzyme-1 activation following its release from heat shock protein A5. The resulting signals appear to use several B-cell lymphoma-2 family members to both suppress apoptosis and activate autophagy. Changes in metabolism induced by cellular stress are key components of this regulatory system, and further adaptation of the metabolome is affected in response to stress. Here we describe the unfolded protein response, autophagy, and apoptosis, and how the regulation of these processes is integrated. Central topologic features of the signaling network that integrate cell-fate regulation and decision execution are discussed. Cancer Res; 72(6): 1321–31. ©2012 AACR.

Introduction
Cell fate primarily involves a cell’s decision to live or die. If the decision is to live, the cell must then decide whether to differentiate, arrest growth, or enter the cell cycle. If the decision is to die, the cell must activate a programmed cell death (PCD) pathway such as apoptosis (PCD1), autophagy (PCD2), or necrosis (PCD3). Appropriate regulation of these cell-fate decisions is often critical during normal development, tissue differentiation, and response to stress. The breast provides a useful example of these processes. Normal breast function includes periods of proliferation and differentiation in preparation for lactation, followed by the PCD that occurs during involution as the postlactational breast returns to a resting state. Inappropriate activation/repression of cell-fate decisions can have major consequences, and the loss of regulation of cell cycling, as well as inappropriate cell survival, are common characteristics of neoplasia. How cells integrate complex cell-fate signals, and whether this process differs between normal and neoplastic breast cells, remains unclear. For example, during lactation, the normal breast must balance the extensive production of milk proteins with the risk that an excessive load of these proteins could result in endoplasmic reticulum (ER) stress and induction of the unfolded protein response (UPR). In breast tumors, stress that induces a UPR can arise from the nutrient deprivation and hypoxia induced by inadequate vascularity and from the application of cytotoxic and endocrine therapeutic interventions. Because the UPR can be either prodeath or prosurvival, both the lactating and neoplastic breast must maintain a prosurvival UPR, perhaps using many of the same regulatory mechanisms.

Signaling initiated within the UPR leads to changes in the levels and activities of key regulators of cell survival, with the integration of both prodeath and prosurvival signals and functions determining cell fate. Determinants in this process include signals that cross-talk among the plasma membrane, ER, mitochondria, cytosol, and nucleus, leading to the eventual induction or repression of apoptosis and/or autophagy, and the changes in cellular metabolism that are necessary to enable execution of these decisions. In the breast, the central molecular players in this orchestration include members of the B-cell lymphoma 2 (BCL2) and autophagy-related (ATG) gene families, estrogen receptor-α (ERα, ESR1); nuclear factor κB (NF-κB, RELA), and components of the UPR, such as X-box binding protein-1 (XBP1) and its unconventional splicing.

Precisely how cancer cells die following either endocrine or cytotoxic interventions is unclear; however, several
independent but potentially interrelated cell-death mechanisms are known (Fig. 1). For example, mitotic catastrophe may be important in response to therapies that target microtubules (1). For endocrine therapies, the extent to which necrotic cell death occurs is uncertain (2, 3), but cell death by apoptosis (4–7) and autophagy (8, 9) in vitro are consistently reported.

Emerging evidence is beginning to define a more intimate relationship between apoptosis and autophagy, implying significant communication between these 2 activities. Such communication may reflect the use of similar or related signaling molecules in an integrated or even interdependent manner. For example, events within the mitochondria and ER, and their regulation by BCL2 family members, are areas of commonality in apoptosis and autophagy (10, 11).

**ER stress and the UPR**

The rates of protein synthesis and secretion are tightly linked to the ability of the ER to fold, process, and traffic newly synthesized proteins. Within the ER, nascent proteins are appropriately folded and moved to the Golgi apparatus for...
further trafficking. Folding of the polypeptide chain is achieved through the actions of a series of molecular chaperones and foldases, which keep the polypeptide in solution and facilitate folding of the chain into a thermodynamically favored structure. When this process is incomplete, the cell must deal with any proteins or protein subunits that remain unfolded or misfolded within the ER, which can become characteristically distended (ER stress). If this is unresolved, protein folding becomes further impaired because inappropriately folded proteins continue to sequester molecular chaperones and activate their ATPases. Continual disulfide bond reduction and reformation depletes both energy and reducing molecules such as glutathione, increases the generation or persistence of reactive oxygen species, and creates oxidative stress, further damaging the existing proteins and further limiting their appropriate folding.

Up to one third of cellular proteins are synthesized within the ER (12). To address the adverse effects of accumulating unfolded proteins, the cell induces a series of events collectively known as the UPR (ER stress response; Fig. 2A). The primary goal of the UPR is to eliminate inappropriately folded proteins and reduce the load of newly synthesized unfolded proteins within the ER. It accomplishes these actions by reducing the amount of mRNA template for proteins by degrading existing mRNAs, slowing the transcription/translation of new mRNA, and reducing the influx of nascent proteins into the ER lumen (13). Concentrations of protein folding effectors, including molecular chaperones and foldases, are also increased to process the mass of accumulated proteins. The remaining misfolded proteins are eliminated through one of 2 ER-associated degradation (ERAD) pathways (14): a ubiquitin/proteasome pathway known as ERAD(I) or an autophagic/lysosomal pathway known as ERAD(II) (15). Soluble targeted proteins are retrotranslocated into the cytosol, ubiquitinated, and then degraded by the proteasome in ERAD(I) (16, 17). Insoluble misfolded protein aggregates are degraded by autolysosomes in ERAD(II) (15, 18).

Accumulation of unfolded or misfolded proteins is detected by ER transmembrane receptors. The 3 primary molecular sensors are inositol-requiring protein-1α (IRE1α, ERN1), activating transcription factor 6 (ATF6), and protein kinase RNA-like ER kinase [PERK, EIF2AK3 (19)]. In the absence of stress, each is maintained in an inactive state through its association with glucose-regulated protein 78 (GRP78; BiP; HSPA5). As unfolded proteins accumulate, HSPA5 dissociates from the molecular sensors and binds to hydrophobic domains on the surface of these unfolded proteins (20) in an attempt to effect their repair (21). All 3 arms of the UPR can be regulated by changes in the concentration of free HSPA5 (Fig. 2A; ref. 22), but how this leads to stress-specific activation of selected UPR signaling is uncertain (19).

PERK signaling in the UPR

Some UPR-associated signaling may not be unique to the UPR. Three signaling processes have been suggested: (i) signaling through IRE1α/XBP1 and ATF6 that is largely restricted to the UPR; (ii) signaling through PERK and eukaryotic translation initiation factor-2α (eIF-2α, EIF2S3) that can be restricted to the UPR; and (iii) signaling through PERK/eIF-2α and ATF6 that may be specific to the UPR but can also be induced by other stressors (Fig. 2A; ref. 23). Activation of PERK signaling appears to be independent of signaling that involves either ATF6 or IRE1α (23), and may be the least distinctly definitive pathway of the UPR. For example, the primary target of PERK (eIF2α) is also activated by protein kinase RNA-activated (PKR), eukaryotic translation initiation factor-2α kinase 4 (EIF2AK4), and eukaryotic translation initiation factor-2α kinase 1 [EIF2AK1 (24)]. Recent studies indicate that protein kinase B (AKT) phosphorylates and inhibits PERK (25). AKT-mediated inhibition of PERK signaling can inhibit the downstream phosphorylation of eIF2α, preventing the cytoprotective activity of eIF2α. Inhibition of the PERK/eIF2α pathway leads to increased cell death in tumor cells in response to phosphoinositide 3-kinase (PI3K) and AKT inhibitors, indicating a possible role of PERK/eIF2α signaling in PI3K/AKT inhibitor resistance (25). Together, these observations suggest a prosurvival role of PERK/eIF2α signaling in UPR. PERK signaling inhibits translation to reduce the protein load on the ER and increases p53 levels through a PERK-required ribosomal-Hdm2 interaction, preventing Hdm2-mediated p53 ubiquitination (26). Increases in p53 in response to UPR activation lead to cell-cycle inhibition, suggesting another adaptive method for UPR-mediated cell survival.

IRE1 and XBP1 signaling in UPR

Details about how the balance between prodeath and prosurvival UPR outcomes is determined are only beginning to emerge. Using mathematical modeling, Rutkowski and colleagues (27) proposed a model in which the prosurvival outcome is driven by the relative stability of the UPR mRNAs and proteins associated with the restoration of metabolic homeostasis, balanced by the relative instability of molecules that promote apoptosis. Lin and colleagues (28) showed that ER stress activates both prosurvival and prodeath signaling, with the outcome determined by the maintenance (prosurvival) or termination (prodeath) of IRE1α activity.

When the key activity within prosurvival UPR signaling is the duration of IRE1α activation (28), cell-fate outcome is substantially mediated by the unconventional splicing of XBP1 (19, 29), one of the primary regulators of the transcription network activated by the UPR (30). Conventional mRNA splicing generally occurs within spliceosomes in the nucleus. Non-spliceosomal extranuclear splicing can occur when essential components of the spliceosome are present, such as in the cytoplasm of platelets (31). Unconventional splicing occurs in the cytoplasm and is largely independent of spliceosomal components. For XBP1, this splicing is accomplished by the endoribonuclease activity of IRE1α. Splicing removes a 26 bp sequence (Fig. 2B), creating a frameshift that encodes a larger protein, XBP1(S), that can now act as a transcription factor. Regulation of transcription by XBP1(S) is a consequence of its ability to activate specific cyclic AMP response elements (CRE) with a conserved ACGT core sequence (32, 33). XBP1(S) can also regulate transcription from ER stress response elements [ERSE1, consensus sequence CCAAT-N9-CCACG (34)]. The unspliced mRNA protein product, XBP1(U), has a molecular
Figure 2. UPR and cross-talk between apoptosis and metabolism. A, the UPR is an adaptive signaling pathway in which the proximal activators of each of its 3 arms (PERK, ATF6, IRE1α) are normally activated following their dissociation from HSPA5 (GRP78, BiP). When released from HSPA5, the N-terminal luminal domains of 2 PERK proteins bind together (113). The resulting dimer undergoes an activating autophosphorylation, and phosphorylation of PERK tyrosine-615 is a key event (114). PERK is a type I transmembrane protein that phosphorylates eIF2α (115). Phosphorylation of eIF2α at serine-51 blocks translational initiation (116) because, as a dominant negative inhibitor of eIF2α, the eIF2 recycling required for further protein biosynthesis is blocked and the rate of protein biosynthesis is reduced (114). Downstream events include induction of ATF4, which then regulates the expression of several genes, including the proapoptotic DD13 [also known as CHOP or GADD153 (117)]. The 2 mammalian ATF6 alleles (ATF6α and ATF6β) encode a type II transmembrane bZIP transcription factor. HSPA5 blocks 2 Golgi localization signals that are exposed upon its dissociation from ATF6 (118). Following translocation to the Golgi, regulated intramembrane proteolysis by S1P and S2P cleaves, ATF6α to its active p50 form (ATF6β plays only a minor role in the UPR). ATF6 p50 then enters the cytosol, translocates to the nucleus, and activates transcription in cooperation with the general transcription factor NF-Y (119, 120). The key genes regulated by ATF6 p50 include XBP1 [spliced in the IRE1α pathway (121)], DD13 [also induced in the PERK pathway (122)], and HSPA5 [regulates all 3 pathways (123)]. Activation of IRE1α and splicing of HAC1 (yeast) and XBP1 represent the oldest and most conserved pathway for UPR signaling. Downstream targets of XBP1(S) include p58IPK and several UPR chaperones (124). p58IPK represses PERK activity (125). Thus, persistent XBP1(S) production in the face of continued ER stress could shift UPR signaling from PERK to favor IRE1α and/or the integration of ATF6 (though increased XBP1(S) production) and IRE1α (through increased XBP1 splicing) signaling. B, 2 stem-loop structures, each containing a highly conserved CNGNNG motif, are cleaved. How the 2 exons are ligated in mammalian cells remains unclear, and this function likely differs from that described for yeast (126). XBP1 splicing may not be exclusively cytosolic, but this remains controversial (127). Other nucleotide substrates may exist for IRE1α, but none are known to possess the stem-loop structures evident in XBP1 and HAC1. C, UPR modulates cross-talk between autophagy and apoptosis through various mechanisms. Stimulation of UPR results in an increase in CHOP that promotes apoptosis. Moreover, IRE1α activation promotes apoptosis by phosphorylation of JNK, directly and indirectly inactivating ant apoptotic BCL2 proteins. UPR release of ER Ca2+ also directly promotes apoptosis. UPR signaling also stimulates autophagy, Activation of PERK and the resulting phosphorylation of eIF2α promote autophagy through ATF4-mediated Atg12 transcription. Furthermore, IRE1α-mediated activation of JNK and the subsequent phosphorylation of BCL2 result in dissociation of the BCL2/BECN1 complex, promoting autophagy. D, low intracellular glucose concentrations result in the accumulation of unfolded proteins, stimulating the release of the 3 UPR signaling arms (PERK, IRE1, and ATF6) by HSPA5 and activating the UPR. UPR signaling can activate autophagy, resulting in increased degradation of cellular material and the release of peptides, amino acids, and fatty acids. PPARs likely play a major role during metabolic stress by ensuring adequate turnover of peroxisomes to manage the greater metabolic requirement for release of the energy stored in the longer-chain fatty acids. Autophagy degradation byproducts (amino acids, carbohydrates, and short-chain fatty acids) promote the tricarboxylic acid cycle and the corresponding generation of ATP. Formation of ATP by mitochondria, using the raw material provided by autophagy, enables the cell to cope with low glucose levels and promotes survival. IGF, insulin-like growth factor; S1P, site-1 protease; S2P, site-2 protease.
mass of ~33 kDa and can act as a dominant negative of the spliced XBP1(S) mRNA product that encodes a protein of ~54 kDa (35, 36). Activation of both ATF6 (induces XBP1 transcription) and IRE1α (splices XBP1) can be coordinated by their respective dissociation from HSPA5. This coordinated activation, and the eventual balance between the relative production of XBP1(U) versus XBP1(S), could have significant consequences for UPR activation, function, and cell fate.

In addition to increased XBP1 transcription by the UPR (30), XBP1 is also rapidly induced in breast cancer cells following 17β-estradiol (E2) stimulation (37, 38). Upregulation of XBP1 by activation of the UPR or by a UPR-independent mechanism confers antiestrogen resistance and implicates XBP1 function as an important component of breast cancer signaling (39). Moreover, expression of XBP1 mRNA is strongly associated with ESR1 positivity in breast tumors (40), and XBP1 can bind to and activate ESR1 in a ligand-independent manner (41). XBP1(S) expression is associated with acquired endocrine resistance (42). Overexpression of XBP1 cDNA in breast cancer cells produces primarily XBP1(S) and is sufficient to confer both E2 independence and antiestrogen cross-resistance (39). Expression of XBP1(S) is elevated in breast tumors that respond poorly to tamoxifen (43).

**UPR and the regulation of autophagy and apoptosis**

The UPR regulates multiple signals in an attempt to restore metabolic homeostasis, a process that could be fruitless if the cell did not concurrently attempt to block cell-death signaling long enough to determine whether the stress could be adequately resolved. The most effective means of accomplishing both tasks would be to integrate their respective signals. This integration can be initiated within the UPR and yet concurrently regulate both autophagy and apoptosis.

Autophagy (macroautophagy) is a lysosomal degradation process in which cellular components are encapsulated within autophagosomes and degraded by lysosomal hydrolases [see Cook and colleagues (10) for a recent review]. The signaling network topology associated with autophagy is complex and only beginning to emerge (44). Autophagy is generally characterized by the presence of cytoplasmic vacuoles and autophagosomes, the absence of margined nuclear chromatin (45, 46), an increase in cleavage of microtubule-associated protein 1 light chain 3 (LC3), and a reduction in p62/sequestosome-1 (p62/SQSTM1) protein levels (47). LC3 cleavage, which may require eIF2α phosphorylation by PERK within the UPR (48), may not occur with noncanonical ATG5/ATG7-independent autophagy (49). Under normal conditions, basal autophagy removes long-lived proteins and damaged organelles, releasing the degradation products into the cytosol as intermediate metabolites. Autophagic removal of specific organelles [e.g., pexophagy (peroxisomes), mitophagy (mitochondria), cromophagy (Golgi), ribophagy (ribosomes), and reticulophagy (ER)] is uniquely identified.

The level and duration of autophagy can vary significantly, and, like the UPR, autophagy is associated with both cell survival and cell death (33). Prosurvival autophagy likely depends on recycling of cellular contents to feed the cell’s basal metabolic machinery at a level sufficient for survival. An induction or persistence of autophagy, such that the minimum subcellular machinery necessary for survival is no longer maintained, could result in autophagic and/or apoptotic cell death. Prodeath outcomes may reflect the need to eliminate cells that cannot function normally due to the absence of key proteins, have failed to secrete correctly folded proteins (including essential hormones and growth factors), and/or have been subjected to excessive or irreversible oxidative stress and DNA damage (33).

**Bcl-1 and BCL2 interactions determine activation of autophagy**

Two primary regulatory activities have been reported to initiate autophagosome production in canonical autophagy signaling (50, 51). Bcl-1 (BECN1) acts through its ability to form the BECN1 complex, which includes PI3K class 3 (PI3KC3), Vps34, and Vps15, and activating molecule in BECN1-regulated autophagy (AMBRA1; Fig. 1). Alternatively, derepression of ULK1 (ATG1) by suppression of mTOR (10) or phosphorylation by AMP kinase [AMPK (52)] enables the formation of a protein scaffold to build the preautophagosomal structure. Signaling initiated within the UPR can affect both of these autophagy-initiating mechanisms. BECN1 binds to and is inhibited by BCL2, BCL-X₇ (BCL2L1), BCL-W (BCL2L2), and MCL1. Proteins that regulate the expression and/or interact with these BCL2 family members affect their ability to inhibit BECN1’s proautophagic function. Thus, competitive interactions by BAD, BID, BIK, BIM (BCL2L11), BNIP1, BNIP3, NOXA (PMAIP1), and PUMA (BBC3) can promote autophagy by effectively sequestering BECN1 inhibitors and releasing free BECN1 to act elsewhere (10). Phosphorylation of BECN1 by death-associated protein kinase (DAPK) reduces BECN1’s affinity for BCL-X₇ (53), also releasing BECN1. Subcellular localization is critical. BCL2 inhibition of BECN1 is evident in the ER, but not when this interaction occurs at mitochondria (54). The apparent ability of BCL2 to sequester AMBRA1 at mitochondria can prevent formation of the BECN1 complex at the ER, whereas BCL2 cannot bind AMBRA1 when they are localized in the ER (55). Once autophagy is initiated, AMBRA1 can cause BCL2 to dissociate from BECN1 (55), perhaps reflecting the binding of BCL2 and BECN1 at distinct sites on AMBRA1 (56). The relative importance of location for the action of other BECN1-interacting proteins requires further clarification.

Other key regulatory events can be initiated within the UPR and directly affect autophagy, including the ability of XBP1(S) to transcriptionally induce BCL2 expression (39). Given the importance of IRE1α in affecting UPR prodeath/prosurvival outcomes (28), and by implication the importance of XBP1 splicing, the ability of XBP1(S) to regulate BCL2 expression may be one of several essential downstream activities that integrate UPR and autophagy signaling. For example, endogenous XBP1(S) is overexpressed in antiestrogen-resistant breast cancer cells (42), and its overexpression increases BCL2 expression and induces antiestrogen resistance in sensitive cells (39). BCL2 inhibition can partly reverse XBP1-induced antiestrogen resistance, but a greater effect is seen when both BCL2 and
BCL-W are inhibited, and a further improvement is seen when BECN1 is also inhibited by either 3-methyladenine or anti-BECN1 shRNA (57). XBP1 can bind ESR1 and increase its transcriptional potency (39, 41). Because ESR1 can also induce BCL2 expression, XBP1 can potentially drive BCL2 through 2 independent mechanisms (directly through ACTG-CRE sites in the BCL2 promoter or indirectly through ESR1), providing redundancy for XBP1 regulation of BCL2 (58).

NF-kB has multiple functions, including regulation of the inflammatory response and apoptosis. Because ER stress is associated with increased production of reactive oxygen species and oxidative stress, it is logical that NF-kB and its signaling would be activated. In the context of UPR, NF-kB can be activated by PERK through the action of phosphorylated eIF2α and its regulation of IκBα translation (59). In some cells, NF-kB can induce BECN1 expression (60). NFκB can inhibit CHOP (GADD153) and prevent ER-induced cell death, establishing a link between NFκB and UPR regulation (61). Of importance, endogenous NFκB expression is increased in antiestrogen-resistant breast cancer cells, in part through the increased expression of p65/RELA and IKKγ [IκBKG (42, 62)]. Activation of PERK may contribute to increased NF-kB activity, and overexpression of XBP1(S) also increases endogenous NF-kB transcription and activation in breast cancer cells (R. Hu, et al.; unpublished data). Activation of NFκB increases BCL2 expression, and inhibition of either NF-kB (62) or BCL2 (57) can partly restore antiestrogen sensitivity in resistant cells. c-Jun-NH2-kinase (JNK, MAPK8) is activated following the binding of IRE1α and TNF receptor-associated factor 2 (TRAF2), a process that often requires signal-regulating kinase-1 [ASK1, MAP3K5 (63)]. ASK1 is strongly implicated in ER stress-induced autophagy, a process that is accompanied by IRE1α activation (64). Phosphorylation of BCL2 by JNK does not affect BCL2 binding to AMBRA1 (55), but it can disassociate BCL2 from BECN1, potentially freeing BECN1 to initiate autophagosome formation. Although JNK has roles in both intrinsic and extrinsic apoptotic pathways (65), basal levels of JNK and phospho-JNK expression are increased in antiestrogen-resistant cells (66), suggesting that JNK plays a dominant role in prosurvival UPR/autophagy rather than in apoptosis. These activities may reflect the release of BCL2 (antia apoptotic) and BECN1 (prosurvival autophagy) from each other.

UPR, autophagy, and apoptosis pathway crosstalk

Many of the UPR signaling outputs associated with autophagy are also associated with the regulation of apoptosis (Fig. 2C). For example, NF-kB and JNK activation contribute to the regulation of apoptosis. Both caspase-8 and apoptosis are activated when NF-kB activity is inhibited in antiestrogen-resistant breast cancer cells, whereas autophagy is not (66). NF-kB can directly regulate BCL2 expression, which partly explains NF-kB’s ability to influence both autophagy and apoptosis. Antia apoptotic BCL2 action in the mitochondria is well known, and the binding between AMBRA1 and BCL2 at mitochondria is reduced during apoptosis (55). Association of IRE1α with BAK and BAX likely also affects apoptosis (67), and the loss of IRE1α activation enables the induction of apoptosis (28). Indeed, many members of the BCL2 family, including those implicated above in sequestering BECN1-interacting proteins, are intricately involved in the functional regulation of apoptosis (11).

Antiestrogens induce both apoptosis and an apparently prodeath autophagy in sensitive cells (68). However, resistant cells that are resensitized to antiestrogens by inhibition of BCL2 and/or BCL-W do not die through apoptosis but through an autophagy-associated necrosis (57). When BECN1 is then also inhibited, necrosis (PCD-3) is no longer a dominant cell-death mechanism and the cells recover the ability to die through apoptosis. Thus, the cell-fate decisions associated with regulation of BCL2 family members and BECN1 are differentially regulated depending on the cellular contexts in endocrine-sensitive and -resistant breast cancer cells (57). Death receptor-5 (DR5, TNFRSF10B), a major component of the extrinsic apoptosis pathway, is regulated by CHOP [GADD153, DNA damage-inducible transcript (DDIT)] that is activated by both PERK and ATF6 (69). CHOP also regulates BCL2 expression (70), which likely concurrently affects its role in both apoptosis and autophagy.

Although p53 is strongly implicated in the regulation of apoptosis, its role in UPR-associated signaling is unclear. Limited evidence suggests a dual role for p53 with respect to autophagy. Genomic stress can induce an apparent p53-dependent autophagy and stimulate the transcription of autophagy-related genes. Conversely, deletion or inhibition of p53 can also activate autophagy (71). Currently, a definitive mechanistic link among antiestrogens, autophagy, and p53 remains to be established. Studies exploring the role of antiestrogen therapies and autophagy using both MCF7 (p53 wild type) or T47D (p53 null) breast cancer cell lines show a broadly similar activation of autophagy in response to endocrine therapy. For example, inhibition of autophagy using either RNA interference or chemical inhibitors potentiates antiestrogen-mediated cell death (72), suggesting that p53 may not play a central role in mediating antiestrogen-induced autophagy.

Changes in intracellular Ca2+ and activity of the Ca2+-binding protein calmodulin are implicated in responsiveness to antiestrogens (73). Increased cytosolic Ca2+ induces a BECN1/ATG7-dependent, BCL2-sensitive autophagy by activating calcium/calmodulin-dependent protein kinase II beta (CAMK2B) and AMPK, which then inhibits mTOR (74). An AMPK-independent pathway involving the protein phosphatase WIP1 (PPM1D) and LC3 is also implicated in Ca2+-mediated autophagy (75). JNK phosphorylation of BCL2 and its consequent release from BECN1 allows BCL2 to bind and inhibit the function of inositol 1,4,5-triphosphate receptor [IP3R, ITPR1 (76, 77)]. IP3R controls the release of Ca2+ from the ER into the cytosol, and a decrease in Ca2+ can delay or reduce apoptosis. This activity may be unrelated to its role in autophagy (78); rather, the concurrent release of BECN1 is likely to be the regulator of autophagy. Cleavage of ATG5 by the calcium-dependent, nonlysosomal cysteine protease calpain can also cause a transition from autophagy to apoptosis (79). Although the precise role of Ca2+-mediated signaling may be complex and cell-context–dependent, these observations provide further evidence of how components common
to UPR, apoptosis, and autophagy may coordinately affect their relative activation.

**Coordination of cellular metabolism and cell fate**

An appropriately activated UPR can eliminate ER stress, restore correct protein folding, and allow a cell to function normally (the prosurvival function). UPR activation of ERAD may support the recycling of material recovered from the degradation of misfolded proteins, which could also allow cells to survive when extracellular nutrient sources are limited. A link between cell fate and UPR is consistent with the use of ERAD(II) to eliminate insoluble misfolded proteins through an autophagic process. The eventual dissolution of autolysosomes during autophagy releases the degraded or partially degraded macromolecules from damaged or unnecessary organelles and cytosolic contents for subsequent reuse. Autophagy can be initiated by several stressors, including the persistent nutrient deprivation that may arise from inadequate vascularization and/or loss of stimulation by growth factors [e.g., insulin-like growth factor (IGF)]. However, precisely how nutrient deprivation is sensed is not entirely clear. mTOR can integrate signaling from insulin, growth factors such as IGF-I and IGF-II, and amino acids (80, 81). Nutrient/energy deprivation-regulated signaling may also include activation of AMPK by means of an increased AMP:ATP ratio (ATP depletion), or induction of REDD by HIF1 in response to hypoxia/ oxidative stress, which can lead to inactivation of the TORC1 complex and release its repression of autophagy (82, 83).

p53 is altered in 20% to 40% of all breast carcinomas (84) and was recently implicated in the regulation of metabolism. For example, decreased oxygen consumption and increased glycolytic activity occur in p53−/− mutant mice, with no overall change in total ATP production. Altered metabolism is linked to p53-mediated transcriptional regulated targets, such as mitochondrial cytochrome oxidase c (COX)-complex, with an observed increase in lactate accumulation (85). Low pH1 can stimulate AMPK and p53 expression, resulting in a high glycolytic flux and inhibiting apoptosis through increased expression of BCL2 and p53 (TP53)-induced glycolysis and apoptosis regulator [TIGAR (86)]. p53-induced TIGAR expression protects cells against oxidative stress and regulates glycolysis (87). Given the high frequency of p53 mutations observed in breast cancer, the role of p53 in the possible coordination of UPR signaling, antiestrogen resistance, and metabolism clearly requires further study.

In cancer cells, insufficient glucose or other energy substrates may create low intracellular ATP concentrations. Moreover, as intracellular glucose levels fall, members of the glucose-regulated protein family are activated (88). This family includes HSPA5, and low glucose can result in the release of HSPA5 from the UPR sensors proteins and activation of the UPR. Thus, activation of glucose-regulated proteins provides another general means of sensing nutrient insufficiency and inducing a UPR-regulated autophagy. Whatever the upstream activation, once autophagy is initiated, it can enable metabolite recycling and contribute to the restoration of metabolic homeostasis.

Further study is needed to determine precisely how the contents released from autolysosomes feed into a cancer cell’s energy metabolism, which generally has a high glycolytic demand due to the Warburg Effect, or into its intermediate metabolism to maintain or replace basic cellular components. Intermediate metabolism may be largely intact, and the reuse of amino acids, peptides, carbohydrates, and small fatty acids may ultimately feed into the tricarboxylic acid (TCA) cycle in adequately functional mitochondria. Larger fatty acids are probably metabolized in peroxisomes, as would also be the case in most cells. PPARs may play a major role during metabolic stress by ensuring adequate turnover of peroxisomes to manage the greater metabolic requirement for release of the energy stored in the longer-chain fatty acids that are provided by autophagy (Fig. 2D). This is also likely to be a dominant role for PPARs during stress. Similarly, the primary roles of insulin and the IGFs may be to affect autophagy and basal survival metabolism, including regulation of glucose metabolism. However, they may only be able to increase proliferation if cellular metabolism permits. Whether growth factors or other mitogens activate proliferation is probably a secondary concern for a cancer cell, because the ability to survive, even in an essentially dormant (nonreplicative) state, is likely preferable to death. Thus, it is not surprising that growth factors, hormones, and other mitogenic signals involve a coordinated regulation of metabolism, cell survival, and cell cycling. We propose that this regulation is often hierarchical, or at least appears to be so. Because both survival and PCD mechanisms are energy-dependent, and the choice to live or die may be determined by metabolic status, the hierarchical importance for cellular decision-making may be ordered as follows: signaling to regulate metabolism (highest priority) → survival → proliferation (lowest priority). As such, by focusing on efforts to therapeutically target replication, investigators may miss the potential of targeting metabolism, provided that can be done in a manner that does not also adversely affect noncancer cells and induce excessive toxicity.

**UPR and the tumor microenvironment**

In addition to the role played by UPR-mediated control of autophagy and apoptosis in regulating tumor cell fate, as clearly highlighted in this review, recent studies suggest that UPR signaling also affects interactions within the tumor microenvironment. A transgene-induced mammary tumor model in HSPA5 heterozygous knockout mice exhibited decreased angiogenesis and tumor microvessel density (89). In a syngeneic breast tumor model, wild-type tumor cells implanted into a HSPA5 heterozygous mouse showed decreased angiogenesis in early- but not late-phase tumor growth, and the number of metastatic lesions was also reduced in the HSPA5 heterozygous animals (90). Knockdown of HSPA5 in endothelial cells decreased their proliferation, survival, and migration, implicating UPR in angiogenesis within the tumor microenvironment (90). In contrast, increased expression of HSPA5, GRP94 (HSP90B1), and protein disulfide isomerase (PDI) was detected in the
Conclusions and Future Prospects

Signaling initiated from within the UPR actively participates in autophagy and both intrinsic and extrinsic apoptosis pathways. The latter is logical because ER stress can result from internal or external stressors. Inappropriate activation of the UPR, whether the effect on cell fate is prodeath or prosurvival, can be problematic. Failure to eliminate stressed cells, particularly in cells with damaged DNA, could lead to cancer. UPR activation leading to a prosurvival outcome in preexisting cancer cells would clearly be detrimental to the host. Activation of the UPR may be more likely for cancers that arise from normal cells with a significant secretory function, where UPR activation may be a common occurrence. Cancers of the breast, prostate, immune system, and pancreas are among the most common cancers and are strong candidates to exhibit a central role for UPR activation as a cell-survival mechanism. Nonetheless, this general function is conserved in evolution and may be active in most cancers. Cancer cells generally experience multiple cellular stressors associated with the UPR, including nutrient deprivation from inadequate vascularization (92) or exposure to endogenous and/or treatment-induced oxidative stress (93, 94).

Signaling that is initiated within the UPR, or is external to the UPR but uses some of its signaling components, can influence the initiation of both apoptosis and autophagy and contribute to the cell-fate decision process. Integration of this signaling is critical if the cell is to use UPR and autophagy first to determine whether it should or can survive. It would be pointless to initiate a stress-response pathway to resolve the stress if an irreversible cell death signal were concurrently activated. Thus, cell signaling appears to be wired so that the same molecules, such as BCL2 family members, can concurrently repress one function (such as prodeath) while activating an opposing activity (prosurvival). For example, the association of IRE1α with the proapoptotic BAK and BAX affects the UPR (67), suggesting one mechanism by which apoptosis could be inhibited while the cell tries to use a UPR-mediated autophagy to recover.

Given that UPR and autophagy have integrated and perhaps interdependent functions, it is not surprising that both can be associated with prodeath and prosurvival outcomes. How these interactions differ between cancer and normal phenotypes, or between drug-sensitive and drug-resistant phenotypes, is an area for research. Moreover, although we chose to use the widely described PDC2 for autophagy in the context of cell death, in a recent study of autophagic flux in response to chemically induced stress, Shen and colleagues (95) suggested that the process we usually think of as autophagic cell death may actually be a very rare cell-fate outcome. This intriguing observation requires small topologic features of the network that controls endocrine responsiveness have been identified from within gene expression microarray data additional study, but it may also require a revision in how we think of autophagy as a mechanism for executing cell death (95).

Regardless of whether autophagic cell death occurs, the plasticity of the cell-fate decision and the importance of cellular context are already evident. Plasticity and context can each exist within one integrated signaling network, each being explained by the presence of an adaptive network topology. For example, the nodes of the signaling network that determines cell fate may be largely maintained even though the frequency, strength, and direction of their interactions (edges) are changed (58). Such topologic changes could be further modified by perturbations in the set-points required to activate irreversible decisions (96). Also, the relative importance of a node or edge could be modified by a change in sequence (e.g., mutation or splicing), transcription, translation, post-translational modification, and/or subcellular localization.

Cellular signaling occurs in the context of interactive networks (58, 97), and a considerable degree of integration and communication occurs among the signals associated with the UPR, autophagy, and apoptosis. It is unlikely that we will be able to represent, understand, and explore such complex processes by attempting to capture information in static wiring diagrams such as we have used here to illustrate some signaling transduction. Such diagrams are necessarily simple, and many potential nodes are already available for inclusion in a model that might explain cell-fate decisions, such as those activated in response to endocrine therapies in breast cancer (98, 99). Instead, we may need to use a systems approach involving both computational and mathematical modeling to construct hypotheses that will better identify the most important and informative experiments, and ultimately enable the testing of predictions about how the system responds to stress and makes irreversible cell-fate decisions (58).

Despite the many challenges of working in high-dimensional data spaces (97), small topologic features of the network that controls endocrine responsiveness have been identified from within gene expression microarray data (44, 100). A framework for mathematical modeling of cell-fate decision-making in the context of responsiveness to endocrine therapies in breast cancer was recently proposed (101). The model incorporates modules for the cell cycle, apoptosis, autophagy, and the UPR. Models for some individual modules, including the UPR, have also been proposed (27). However, the current models are generally high level, and there is a notable paucity of data we can use to define the parameters and construct informative and sufficiently robust mathematical models for any of these critical functions and their regulatory components. Finally, it is evident that studies involving therapy responsiveness and cell-fate decisions require careful consideration of the integrated role of UPR, autophagy, apoptosis, and necrosis. It also seems likely that novel therapeutic targets reside within this network (102). It remains to be seen how these opportunities can be identified and used to good effect in the attempt to eradicate cancer of the breast and other cancers.
Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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