Implications of aging and the endoplasmic reticulum unfolded protein response on the molecular modality of breast cancer

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The endoplasmic reticulum (ER) is an important subcellular organelle that is involved in numerous activities required to achieve and maintain functional proteins in addition to its role in the biosynthesis of lipids and as a repository of intracellular Ca2+. The inability of the ER to cope with protein folding beyond its capacity causes disturbances that evoke ER stress. Cells possess molecular mechanisms aimed at clearing unwanted cargo from the ER lumen as an adaptive response, but failing to do so navigates the system towards cell death. This systemic approach is called the unfolded protein response. Aging insults cells through various perturbations in homeostasis that involve curtailing ER function by mitigating the expression of its resident chaperones and enzymes. Here the unfolded protein response (UPR) cannot protect the cell due to the weakening of its protective arm, which exacerbates imbalanced homeostasis. Aging predisposed breast malignancy activates the UPR, but tumor cells maneuver the mechanistic details of the UPR, favoring tumorigenesis and thereby eliciting a treacherous condition. Tumor cells exploit UPR pathways via crosstalk involving various signaling cascades that usher tumor cells to immortality. This review aims to present a collection of data that can delineate the missing links of molecular signatures between aging and breast cancer.

INTRODUCTION

Breast cancer, like other cancers, results from interactions of various factors, such as genetic predisposition and the environment. The scientific repertoire has an enormous amount of data on the mechanisms of breast cancer development, its diagnosis, prevention and treatment. The most important aspect of any cancer study is the risk factors. When we discuss breast cancer, among all other risk factors, getting older, that is, aging, is known to be a condition that promotes the disease. The American Cancer Society has previously shown that most invasive breast cancer cases are reported among women aged 55 years or more.1 Hence, there is evidence suggesting that malignancies are a direct function of aging.2,3 Although aging accompanies the degeneration and loss of function of various tissues in the body, especially the skeletal muscles, the development of a tumor and its progression in aging is blamed on the accumulated mutations in oncogenes and tumor suppressors.4

Breast cancer has multiple molecular classifications: luminal A and B, triple-negative/basal-like (TNBC; tumors that do not express genes for the estrogen receptor, progesterone receptor and human epidermal growth factor receptor 2 (HER2)) and HER2-positive subtypes.5 Aging does lead to changes in the expression pattern of the transcriptome, as shown by previous studies in various models of breast cancer.6–9 The field of geroscience is still in its infancy and is evolving to understand the paradigm of breast cancer.

Environmental factors constantly affect the cellular microenvironment. There are a number of adverse effects posed by environmental factors, such as radiation and mutagens, that lead to intracellular damage, whereby cellular deterioration substantially contributes to aging and tumorigenesis. This damage primarily involves important components of cells: the proteins in their proper folded, functional form. Under normal conditions, the endoplasmic reticulum (ER) controls the damaged/misfolded protein overload by activating rescue
pathways that upregulate the expression of resident chaperones to properly fold the misfolded proteins, halting its translational function and appropriately diverting the unwanted cargo towards a degradation pathway, the endoplasmic reticulum-associated degradation (ERAD).10

The overall aim is to restore a healthy cellular microenvironment, directing the cell towards homeostasis. The overload of misfolded and accumulated proteins invariably disturbs the quality control process in the ER lumen, thereby presenting a condition of 'ER stress.'11 The molecular machinery in the ER lumen tackles this stress via a multifarious response cascade called the unfolded protein response (UPR). The UPR is an orchestration of complex events comprising an increased rate of protein folding by upregulating resident molecular chaperones and the diversion of the extra misfolded proteins to degradation pathways aimed at rescuing the cell from the imposed stress.

ONSET OF THE ENDOPLASMIC RETICULUM UNFOLDED PROTEIN RESPONSE

The ER lumen harbors important molecular chaperones, folding sensors and enzymes that are engaged in protein quality control, that is, proper folding of nascent polypeptides. These include glucose regulated proteins 78 (GRP78)/immunoglobulin-binding protein, GRP94, calnexin, calreticulin, protein disulfide isomerase, thiol-disulfide oxidoreductase and ERp57.12,13 Studies have shown that aging leads to a condition in which there is downregulation in the expression pattern of resident molecular chaperones and enzymes in the ER across several tissues.14 This in turn lowers the folding efficiency of the ER, thereby increasing the bulk of misfolded proteins. This instability in protein homeostasis results in ER stress that consequently stimulates the UPR.15 Stressors arise from the tumor microenvironment, such as diminishing levels of available oxygen and nutrient supplies, leading to UPR activation in malignant cells.16–18

UPR signaling involves three resident transmembrane ER proteins, PERK (the PKR-like ER kinase), IRE-1 (the inositol requiring element 1) and ATF-6 (the activating transcription factor 6), which act in a complex multifaceted cascade aimed at restoring proteostasis in the ER lumen. A diagrammatic representation of the events showing activation of UPR transducers and the subsequent downstream signaling effectors is presented in Figure 1. Activation of PERK and IRE-1 is marked by their homo-dimerization followed by trans-autophosphorylation of their cytoplasmic components, while ATF-6 activation leads to its translocation to the Golgi apparatus where two serine proteases (site-1 protease, S1P and site-2 protease, S2P) cleave it.

GRP78 has a vital role in protecting the cell during ER stress. During normal cell functions, GRP78 is associated with the luminal domains of the UPR transducers. The association of GRP78 sterically prevents the homo-dimerization of PERK and IRE-1 and also inhibits the translocation of ATF-6 to the Golgi apparatus. When the concentration of misfolded proteins increases in the ER lumen, GRP78 dissociates from the transmembrane stress transducers and is recruited to protein folding, a marked step that signals ER stress in the cell. Apart from this, the GRP78 prevents cell death triggered by cytosolic accumulation of Ca2+ during ER stress by withholding luminal Ca2+ in the ER, thereby preventing its leakage into the cytosol.19

ERAD focuses on diverting the aberrant and misfolded protein cargo towards a systematic degradation pathway. The process invariably starts with tagging unwanted bulk misfolded proteins with molecular chaperones and their subsequent retro-translocation back into the cytosol, where poly-ubiquitination ensues ending in 26S proteasomal degradation.10 Thus, ERAD functions to direct the cell towards adaptive UPR.20,21 Autophagy is another process that clears the misfolded protein accumulation from the cell.22

After the occurrence of ER stress in the cell, UPR signaling first triggers an adaptive cascade through programming of a series of events, which are pro-survival. But if the stress condition is not alleviated, UPR commits to the activation of a pro-death cascade.

PERK-stimulated phosphorylation of eIF2α and the attenuation of cellular mRNA translation

During the UPR, GRP78 detached PERK gets homodimerizes and transduces the signal through autophosphorylation of its cytoplasmic domain. This dissipates its effect, causing phosphorylation of cytoplasmic eukaryotic initiation factor alpha (eIF2α), whereby the global protein synthesis comes to an immediate halt.23 The phosphorylated eIF2α lowers the levels of the active eIF2α-GTP pivotal for the association of Met-tRNA^Met with the 40S ribosome in the formation of the ternary complex of the translational apparatus.24,25 This action is aimed at reducing the amount of fresh proteins in the ER lumen. However, this does not stop the translation of certain selected proteins, such as activating transcription factor-4 (ATF-4) and GRP78 by exploiting their internal ribosome-entry site (IRES) elements in mRNA.26,27 The human and mice ATF-4 mRNA are characterized by two upstream open reading frames (uORFs) in the 5′ non-coding region of the transcript. uORF1 codes for a short 3 amino-acid tripeptide, whereas the uORF2, which overlaps the first 83 nucleotides of the ATF-4 coding region, encodes a polypeptide with 59 amino acids.28,29

In normal cells, eIF2-GTP-Met-tRNA^Met bound to the 40S ribosome machinery starts its scan from the 5′-end of ATF-4 mRNA and translates the uORF1, exerting a positive effect by facilitating the retention of the translational apparatus for immediate reinitiation of the upcoming uORF2. The translational machinery scans past the overlapping, now out-of-frame AUG of ATF-4 ORF in uORF2, impeding ATF-4 translation. While during ER stress, under diminishing levels of eIF2α-GTP due to phosphorylated eIF2α, the 40S translational machinery spends more time on the reinitiation of translation of uORF2; this delayed time interval selects for the translation of ATF-4 ORF (delayed reinitiation model).29

This bypassed translational upregulation of ATF-4 is called the integrated stress response (ISR).30 ATF-4 is a transcription factor that controls the expression of genes involved in amino-
acid metabolism, the antioxidant response and apoptosis.\textsuperscript{28,30,31} The transcription of the C/EBP (CCAAT/enhancer-binding protein) homologous protein, CHOP (also known as growth arrest and DNA damage 153, GADD153), is activated by ATF-4, which is pro-death.\textsuperscript{26,32–34}

**IRE-1-induced unconventional splicing of XBP-1**

The second transducer of ER stress, IRE-1, upon losing its association with GRP78, dimerizes and trans-autophosphorylates, marking IRE-1 activation. The activated IRE-1 has RNase activity. The substrate for this endoribonuclease is the intron of mRNA that codes for X-box-binding protein-1 (XBP-1), a transcription factor specific to the UPR. This unconventional splicing removes the 26 bp nucleotide, yielding a spliced variant called XBP-1(S), which can alleviate ER stress by acting as a transcriptional activator of genes involved in ER expansion, protein maturation and secretion, as well as clearing the misfolded protein overload via degradation.\textsuperscript{35,36} Cells
overexpressing XBP-1 inhibit CHOP expression, which is pro-survival.\textsuperscript{37} However, there are compelling reports that challenge this idea of IRE-1-XBP-1 being pro-survival during acute ER stress.\textsuperscript{38} Additionally, reports indicate that IRE-1 binds directly to unfolded proteins, thereby activating an acting ligand itself.\textsuperscript{39,40}

**ATF-6 translocation to the Golgi and its activation**

The third transmembrane sensor, ATF-6, is a 90 kDa protein. Upon disassociating from GRP78, as a part of regulated intramembrane proteolysis, this ER membrane-resident transcription factor translocates to the Golgi apparatus, where it is cleaved by serine proteases (site-1 protease, S1P and site-2 protease, S2P), releasing a 50 kDa cytosolic fragment called p50ATF-6, the functional isoform.\textsuperscript{41} This transcription factor is imported into the nucleus where it targets the cis-acting ER stress response elements.\textsuperscript{42} ATF-6 controls the expression of genes coding for ER-resident molecular chaperones and enzymes involved in protein folding.\textsuperscript{43,44} Most importantly, ATF-6 accentuates the expression of GRP78 to ameliorate the built-up stress.\textsuperscript{45} Studies confirm the interaction of XBP-1 and ATF-6 during UPR.\textsuperscript{44}

**ER STRESS-INDUCED APOPTOSIS**

The orchestrated functioning of the three transmembrane ER stress transducers, PERK, IRE-1 and ATF-6, is aimed at mitigating the stress and the restoration of proteostasis, and thereby cellular homeostasis. However, prolonged stress, and burdening factors such as aging may lead the cell towards apoptosis.\textsuperscript{46} Two pathways govern the cellular control of apoptosis with molecular crosstalk: the intrinsic or mitochondrial pathway and the extrinsic or death receptor pathway. The stimuli for the onset of the intrinsic pathway, such as DNA damage, suppression of growth hormones and cytokines, lead to changes in the inner mitochondrial membrane causing the exposure of pro-apoptotic proteins.\textsuperscript{47} There is efflux of ER luminal Ca\textsuperscript{2+} into the cytosol, which dissipates its effect on caspase-12.\textsuperscript{48} In two separate studies, the activation of caspases is shown during ER stress-induced cell death. Nakagawa et al.\textsuperscript{49} demonstrated that mice lacking caspase-12 genes were partially resistant to pharmacological inducers of ER stress, such as tunicamycin and thapsigargin. Rao et al.\textsuperscript{50} demonstrated that caspase-7 may activate caspase-12 by translocating from the cytosol to the ER. The ER stress succumbing cell activates CHOP at various levels. PERK phosphorylates the alpha subunit of eIF2α but paradoxically activates ATF-4, which affects the CHOP promoter.\textsuperscript{51} CHOP is also a component of the downstream effectors of ATF-6. These factors collectively stimulate the expression of pro-apoptotic proteins such as growth arrest and DNA damage inducible protein 34 (GADD34), TRB3 (tribbles-related protein 3) and so on.\textsuperscript{33,52} The activation of GADD34 by CHOP leads to ER luminal protein synthesis by dephosphorylation of eIF2α aimed at increasing protein load in the ER.\textsuperscript{33,53} CHOP potentiates the upregulation of pro-apoptotic proteins belonging to the BCL2 family such as BAK/BAD and the downregulation of anti-apoptotic proteins, leading to the release of cytochrome c from the mitochondrial membrane into the cytosol, an event that further activates cytosolic apoptotic protease activating factor1 (APAF1). This further activates caspase-3- and caspase-9-dependent signaling.\textsuperscript{54,55} CHOP is also positively regulated by p38 kinase.\textsuperscript{56}

Extracellular stimuli, such as transmembrane receptor-mediated interactions, lead to the onset of the extrinsic pathway of apoptosis. Members of the tumor necrosis factor receptor gene superfamily, such as death receptors, are involved in this process.\textsuperscript{37,58} Reports point to the activation of c-Jun N-terminal kinase (JNK), a family of signal transducers activated by exogenous stimuli, during ER stress.\textsuperscript{39} The tumor necrosis factor receptor-associated factor2 (TRAF2), which is bound to the cytoplasmic component of IRE-1 and transduces signals from IRE-1, activates JNK.\textsuperscript{59} TRAF2 also invariably promotes caspase-12 activation.\textsuperscript{59} Reports suggest that kinases, such as apoptotic signal regulating kinase-1, are activated, causing further stimulation of JNK and p38 MAPK.\textsuperscript{50}

**ACTIVATION OF THE INFLAMMATORY SIGNALING CASCADE DURING THE UPR**

Increasing evidence suggests that the inflammatory signaling cascade responds to the cellular insults imposed during ER stress. The production of reactive oxygen species, leakage of ER luminal Ca\textsuperscript{2+} into the cytosol and the activation of nuclear factor-κB (NF-κB), the master transcriptional regulator of pro-inflammatory signaling, are some examples that point to the connection between the UPR and inflammation.\textsuperscript{31,61,62} A schematic of these events is presented in Figure 2 and described as follows. In a normally functioning cell, NF-κB is primarily maintained in its inactive form through binding with its constitutively expressed inhibitors, inhibitors of NF-κB (IκB). The imposed stress stimulates the activation of NF-κB, after which it translocates into the nucleus to upregulate inflammatory genes. The stressful condition in the ER lumen due to high oxidative stress created by increased protein folding and the leakage of luminal Ca\textsuperscript{2+} plays a role in NF-κB activation.\textsuperscript{63} Additionally, PERK-eIF2α-directed translation attenuation activates NF-κB. The half-life of IκB is low, so the translational attenuation frees NF-κB.\textsuperscript{54,65}

IRE-1 also integrates the UPR signal to an inflammatory response. NF-κB can also be activated after the formation of the IRE-1–TRAF2 complex during ER stress.\textsuperscript{65} Studies on IRE-1 knockdown in mouse embryonic fibroblasts support the activation of NF-κB by UPR and production of inflammatory cytokine tumor necrosis factor-α (TNFα).\textsuperscript{65} As mentioned previously, the IRE-1–TRAF2 complex also activates JNK, which upregulates the expression of inflammatory genes through the phosphorylated activator protein 1 (API).\textsuperscript{66} Studies on subtilase cytotoxin (SubAB), a toxin produced by Shiga toxigenic *E. coli*, show that ATF-6 also accentuates the NF-κB during UPR.\textsuperscript{67}

The mammalian target of rapamycin (mTOR), a serine/threonine protein kinase of the phosphatidylinositol-3-OH kinase (PI(3)K)-related family, plays a pivotal role in the
regulation of proliferation and metabolism in various cellular processes. There are two multiprotein complexes of mTOR, mTOR complex 1 (mTORC1) and 2 (mTORC2). Akt, also known as protein kinase B or PKB, is an important molecule involved in the regulation of cellular processes and is suppressed by mTORC1, whereby apoptosis is induced through the IRE-1–JNK pathway of UPR. Reports suggest the hyperactivation of mTOR during the inflammatory response.

THE CROSSTALK BETWEEN THE UPR AND AUTOPHAGY

Autophagy pathways are highly inducible under stress conditions and are mediated by a series of well-coordinated genes, autophagy-related genes. ER stress-induced autophagy can be cytoprotective or cytotoxic. The conserved recycling pathway that helps the cell to maintain its ‘quality control’ system represents the cytoprotective facet of autophagy, whereas stress-induced mitgation of autophagy has been implicated in several diseases, including cancer.

The process of autophagy is initiated with the formation of a double membrane structure, the autophagosome, which engulfs the targeted molecule in the cytosol destined for the lysosome (Figure 3). LC3, the microtubule-associated protein 1 light chain 3, existing as a soluble protein, LC3-I, conjugates with phosphatidylethanolamine and is transformed into membrane-associated LC3-II protein, which is recruited to autophagosomal membranes. This key step is followed by a series of events, leading to the fusion of autophagosomes with lysosomes possessing integral membrane proteins, the lysosome-associated membrane proteins (LAMPS), which constitute a family represented by glycosylated type I transmembrane proteins. The members LAMP-1 and LAMP-2 are found in the lysosome and late endosomes, and they make up half of the total integral membrane protein.
LAMP-3 is expressed only in specific cell types and is under temporal and spatial regulation. LAMP-3 invariably contains more sites for glycosylation than LAMP-1 and LAMP-2. Evidence favoring the involvement of the UPR in the regulation of autophagy is accumulating. Studies have shown the involvement of the PERK/eIF2α arm of the UPR, in inducing the transcription of essential autophagy genes, which focuses on cell survival under hypoxic conditions. Studies have reported the atypical localization of LAMP-2 on the cell surface during human diseases such as cancer. Also, the expression of LAMP-3 surges in tumor cells, which are metastatic in nature. Mujcic et al. have shown that the PERK/eIF2α/ATF-4 arm of the UPR systemically accentuates LAMP-3 induction under hypoxic conditions. The sustained activation of IRE-1 downstream and spliced XBP-1 mRNA triggers autophagy through the marker LC3.

Autophagy also intersects with the inflammatory responses in the cell during the UPR. NF-κB activation is terminated during the induction of autophagy. Studies have corroborated the antagonistic relationship between mTOR and autophagy. Conditions of starvation and a lack of growth factors lead to the induction of autophagy in the cell by the inactivation of mTOR.

**AGING AND THE UPR**

Resistance to stress decreases with aging. Maintenance of proteostasis in cells declines with the process of aging, leading to protein toxicity that can cause various age-related diseases and disorders. Aging causes weakening of the UPR and drives the mechanism towards cell death. Studies on aged mice have shown that a decline in protein folding due to the oxidation of molecular chaperones in the ER lumen results in increased proteotoxicity. Not only are the existing molecular
chaperones destroyed, but the expression levels of GRP78 have also been reported to decline significantly. Aging also eclipses the ER stress transducers of the UPR. Paz Gavilan et al. showed using RT-PCR that the mRNA expression of PERK significantly diminishes in aged rats compared with younger rats. Aging-accelerated failure of protective responses by the tripartite arms of the UPR further exacerbates the condition by the activation of apoptotic effectors of the UPR. The levels of CHOP and pro-apoptotic caspase-12 are reported to increase in aged mice during sustained ER stress, steering the cell toward death. Further studies support that the increase in CHOP expression sensitizes the cell to the ill effects of oxidation. Aging further shortens the threshold of apoptosis by activating the kinase-directed apoptotic pathway in ER stress-sensitized cells. INK kinases are upregulated through the IRE-1 kinase activity involving apoptotic signal regulating kinase-1 (ASK1). Adler et al. reported the cis-regulatory motifs associated with aging through microarray-based analysis and found that NF-kB was the strongest candidate enforcing aging-related features. mTOR, a mediator of stress responses, extends lifespan when suppressed. Autophagy works in coherence with the UPR in normal cells in which stress is overcome by clearing the aggregated cargo of misfolded proteins, but aging further deregulates the fusion of autophagosomes with lysosomes. The inhibitory effect of mTOR on autophagy is well documented in the case of aging. Aging-associated diseases show diminishing levels of the LC3 marker, that mark the enfeeblement of autophagy.

A correlation between aging and a reduced UPR has been implicated in several age-related conditions such as diabetes and a number of neurodegenerative disorders.

STATUS OF THE UPR IN BREAST TUMORIGENESIS

Cells may endure various environmental and pathophysiological insults that can occasionally lead to the development and progression of malignancies. In a normal scenario, the immunity of a mammalian system tries to withstand such abuses and fights back to restore homeostasis. However, these actions can lead to cell transformation, resulting in malignancies. There is accumulating evidence supporting the role of the UPR in the development and progression of cancer cells. The conundrum behind activation of the UPR during tumorigenesis is compelling. The increased proliferation of tumor cells is a burden on nutrient and oxygen requirements. The tumor cells trigger angiogenesis, resulting in hypoxia and nutrient depletion. Cancer cells extensively exploit the molecular machinery of the ER, leading to ER stress involving meticulous alterations of UPR transducers and effectors, so as to turn them immortal by preventing apoptosis. The ER-resident molecular chaperone GRP78 is overexpressed in cases of common malignancies such as breast cancers. The physiology of the breast presents a paradigm in which ER stress and the ensuing UPR control the normal functioning of the system. During lactation, normally ER is under increased pressure for milk protein production, but the UPR arising due to this ER stress navigates the cell toward survival (the pro-survival response is activated). Also, during the normal menstrual cycle, the breast responds to various hormonal surges, thereby establishing proper homeostasis through the tripartite arms of the UPR. In cases of the initiation of breast malignancies, stressors such as hypoxia, nutrient deprivation, and cytotoxic and endocrine therapeutic interventions lead to the activation of a range of stress responses, including the UPR.

Role of PERK in breast malignancy

Regarding the tripartite arms of the UPR, Bobrovnikova-Marjon et al. reported in their work on breast cancer cells that PERK was necessary for tumor proliferation. Studies have shown that hypoxic parts of the tumor microenvironment activate the translational control program, the integrated stress response, which helps the malignant cells to adapt to the imposed stress of hypoxia. The integrated stress response target ATF-4 is highly expressed in the hypoxic cores of tumors supporting tumor immortalization. Harding et al. suggested that the activation of the integrated stress response provokes a cohort of genes that defend the tumor cells against oxidative bursts, selectively inducing glutathione biosynthesis.

The nuclear factor erythroid-derived 2-related factor (NRF2), a cytoplasmic transcription factor, is a novel, second substrate for the PERK arm of the UPR, which is phosphorylated and subsequently imported into the nucleus to support cell survival as a protective answer to the UPR. Under normal circumstances, NRF2 remains in an inactive state in the cytosol through its association with the actin-binding protein, Kelch-like ECH-associated protein (Keap1). ER-stress-induced PERK activation leads to the dissociation of this NRF2–Keap1 complex, thereby aiding the translocation of NRF2 into the nucleus to act on its target, antioxidant response element (ARE). This event activates pathways for antioxidants, detoxifying enzymes, trafficking proteins and degrading them to alleviate stress in the cell. Cullinan et al. have shown that the expression of CHOP, which is activated through the PERK arm of the UPR favoring apoptosis, is attenuated in NRF2 overexpressing cells. These data demonstrate that the PERK arm is pro-survival in cells with the accrual of NRF2 during the UPR. NRF2 has also been reported to promote cell survival in cases of malignancies. The work of Bobrovnikova-Marjon et al. supports the inactivation of NRF2 in cancer cells with PERK deletion. PERK regulates homeostasis in cancer cells through the prevention of oxidative DNA damage checkpoints. For therapeutic intervention of breast malignancies, loss of the PERK arm of the UPR can result in the suppression of tumor onset. Studies show that NRF2 plays a role in the survival of breast tumors under a limiting microenvironment via chemoresistance.

In a separate study by Nagelkerke et al., the PERK/ATF-4 arm of the UPR activates the expression of LAMP-3, which supports migrating breast malignancies. An analysis published by Sawada et al. corroborates that this migration characteristic of malignant cells is due to the overexpression of LAMP-1 on their surface, which is responsible for the resilient adhesion to surrounding E-selectin (cell adhesion molecule) expressing...
cells. Studies of the knockdown of LAMP-3 have shown a decline in the invasion capacity of cancer cells. Investigations of the knockdown of the PERK/ATF-4/LAMP-3 pathway of the UPR has demonstrated the role of PERK arm in the radio resistance of breast malignancies. In extracellular matrix containing detached mammary epithelial cells, PERK has been shown to be pro-survival for malignant cells, as it induces the shield of autophagy. Thus, the PERK arm of the UPR in alliance with autophagy supports the survival attempt of tumor cells under stressful conditions.

Role of IRE-1 in breast malignancy

There are reports of increased XBP-1 expression and their unconventional splicing in breast cancer, which is a downstream effector of IRE-1. Lin et al. demonstrated that in stressed cells, the ability of the UPR to navigate the cells through pro-survival or pro-death was largely a mandate of IRE-1 activity. The stimulation of breast cancer cells by 17β-estradiol (E2) treatment specifically upregulates XBP-1. In a recent report by Xi Chen et al., the levels of XBP-1 expression were easily detected in several breast cancer cell lines with triple-negative breast cancer (TNBC; tumors not expressing genes for estrogen receptor, progesterone receptor and human epidermal growth factor receptor 2), where they form an axis in tumor development and progression. In cases of anti-estrogen-resistant breast malignancies, XBP-1 expression levels are high with co-expression of the estrogen receptor alpha (ER-α) protein, a nuclear receptor protein expressed in known aspects of breast malignancy.

Role of ATF-6 in breast malignancy

Prolonged ATF-6 activation is related to other UPR transducers under the condition of extended stress to activate the transcription of CHOP, thereby acting as a pro-death factor. Research conducted by Okada et al. in HeLa cells suggests a connection between the ATF-6 and the PERK arms of the UPR by converging at the activation of CHOP. Michallet et al. showed using knockdown studies in myeloma cells that the targeted loss of ATF-6 was pro-death. The ATF-6 arm of the UPR, when expressed at low levels, plays anticipatory roles in cell destruction. In one study on quiescent disseminated cancer cells, ATF-6 is identified as a pivotal transcription factor for their resilience to chemotherapy. This pitfall is created by ATF-6 through the upregulation of Rheb and mTOR activation. We still lack scientific data on the role of ATF-6 in cancer.

Inflammatory responses in breast cancer

Numerous documents note the escalating activity of NF-κB in breast malignancies. Studies show that the activation of NF-κB might be one of the early events during breast malignancy development. CD44, a unique cell surface glycoprotein, is upregulated in tumor cells. Smith et al. have shown that the activity of a cis-acting element, conserved region 1, of CD44 is modulated by NF-κB.

AGING DECREASES THE UPR WHILE BREAST MALIGNANCY POTENTIATES IT: THE UPR HELPS WHOM?

Breast cancer is a variegated malignancy displaying diversity within and between tumors, among patients and the age of the individual. There is an intricate alliance forged between age-related frailty and breast malignancy. There are many reports on cases in which the incidence of breast cancer rises exponentially with aging. Aging unequivocally mitigates the overall density of the breast and at the same time increases the cellular mass of adipose tissue, thereby causing a change in the microenvironment of the breast. The current review is an attempt to underline the molecular signatures inside the breast cancer cell that can help us to understand the relationship established between UPR and aging. However, there is a conundrum: aging-implicated ER stress is not decreased by the UPR; rather, the burdened cell is driven toward the treacherous path of apoptosis. We have discussed numerous cases in this review that support the UPR-driven activation of pro-death transcription factors engendered by aging. When we discuss the involvement of the UPR in the escalation of breast malignancy with the progression of aging, there is a sudden dearth of data. The UPR does not abrogate malignancy; rather, the cancer cells exploit the UPR transducers to become immortal by activating pro-survival signals. Therefore, it is plausible to discuss the crucial roles of aging and the UPR in unlocking the lesser-known aspects of breast malignancy.

The first conundrum worth mentioning here relates to the status of GRP78. Aging leads to the diminished expression pattern of GRP78, whereas in breast malignancy there is a surge in its expression. GRP78 is not only localized with the ER transmembrane UPR transducers, but there is mounting evidence of a subfraction of GRP78 being localized on the cell surface of tumor cells. Cases support the protective role of GRP78 for human breast cancer cells, where GRP78 confers endocrine resistance to the breast tumor. Some of the mechanistic details of GRP78 as an agent to keep cancer cells fertile come from its involvement in inhibiting apoptosis by preventing the cleavage of pro-caspase-7, inhibition of pro-apoptotic BCL2-family proteins and decreasing ER stress by maintaining low levels of misfolded proteins in the ER lumen, which again marks the inhibition of a pro-apoptotic UPR. The overexpression of GRP78 in breast cancer can be measured in its prognosis. Additionally, GRP78 has been implicated in chemoresistance to anticancer treatments; therefore, the levels of this ER chaperone may serve as a novel biomarker for chemoresponsiveness in breast malignancy.

The novel substrate for PERK, NRF2, has been designated the master regulator of cytoprotective genes. Aging abates the interactions of NRF2 with its target sequence, antioxidant
response element, thereby inhibiting the role of NRF2 in responding to stressors, which favors age-related frailty.\textsuperscript{162} In breast cancer, NRF2 supports tumorigenesis and chemoresistance by affecting several molecular targets in the cell.\textsuperscript{163,164} A plausible explanation for this paradoxical behavior of NRF2 would be the selective activation of the PERK arm of UPR by tumor cells to sustain the limiting microenvironment of malignancy.

The UPR signal transducer, ATF-6, upregulates the mTOR pathway in cancer cells.\textsuperscript{142} Studies have proven that the mTOR pathway is fueled up during aging.\textsuperscript{165,166} This pathway and its target, the phosphoinositid 3 kinase (PI3K)/Akt/mammalian pathway, have also been well documented to promote tumor proliferation in endocrine-resistant breast cancer.\textsuperscript{167} The mTOR pathway is therefore a key target of crosstalks between the UPR and aging that sustains breast malignancy. Hence, a reduction in aging-linked inflammation by affecting mTOR could be a potential mechanism for interfering with aging-related malignancy.\textsuperscript{165,166} More evidence of molecular cross-linking involves NF-kB, which is a hallmark of inflammatory responses and is activated through all three arms of the UPR: PERK, IRE-1 and ATF-6. There are accumulating reports that support the unabated transcriptional activity of NF-kB in different tissues with aging.\textsuperscript{104,168–170} The work of Smith et al.\textsuperscript{151} in triple-negative breast cancer cells clearly shows that the inhibition of NF-kB can repress the expression of CD44 thereby mitigating the cell proliferation and invasiveness of breast cancer cells.

**THE UPR AND HEXOSAMINE BIOSYNTHETIC PATHWAY IN AGING AND BREAST CANCER: A PROMISE AGAINST AGONY**

Increasing evidence suggests an increase in life span coupled with a decrease in cancer pathology by a considerable percentage under calorie restriction (CR), that is, restricting the intake of calories without prompting malnutrition.\textsuperscript{171–173} The effect of CR is effectively sensed at the cellular level through a well-studied metabolic pathway, the hexosamine biosynthetic pathway (HBP).\textsuperscript{174}

The HBP produces the metabolite nucleotide sugar uridine monophosphate-N-acetylglucosamine (UDP-GlcNAc), which acts as the substrate for the process of N-glycosylation and O-glycosylation (O-GlcNAc), a mandatory step in protein folding within the ER lumen.\textsuperscript{175} Tumor progression demands extra glucose and glutamine to meet the needs of the tumor under conditions of nutritional deprivation. As studied in cases of breast cancer, the resulting hyperglycosylation in stressful ER conditions imposes a demand for substrate requirements on the HBP, leading to an increase in HBP flux.\textsuperscript{176–178} The resulting UPR shows an alliance with the HBP, in which the transcription of the first and important rate-limiting enzyme of the HBP, glutamine:fructose-6-phosphate aminotransferase 1 (GFAT-1), is directly stimulated by XBP-1. XBP-1 acts as an upstream activator of several genes encoding important enzymes in the HBP, thereby leading to a rise in O-GlcNAc protein modification during ER stress.\textsuperscript{179} In a separate study in chemoresistant basal-like/triple-negative breast cancer, higher levels of O-GlcNAC protein modification have been observed.\textsuperscript{180} Chaveroux et al.\textsuperscript{181} showed in their study that the PERK/eIF2α/ATF-4 arm of the UPR also regulates HBP. They demonstrated the involvement of ATF-4 in controlling the abundance of GFAT-1, which further evokes protein modification under conditions of nutritional stress.\textsuperscript{182} Together, these findings strongly corroborate the cytoprotective aspect of the UPR–HBP alliance. RNA interference studies in breast cancer cells report that the downregulation of GFAT-1 has anti-progression and anti-invasion effects.\textsuperscript{176}

Aging induces rise in nutrient sensing, leading to increased HBP flux which is reasoned for the onset of insulin resistance and age-related maladies.\textsuperscript{183} The exhausted ER protein homeostasis during aging can be ameliorated by modulating GFAT-1, as shown in a study on *C. elegans*, where a gain-of-function mutation in GFAT-1-induced ERAD and autophagy, resulting in better health and longevity.\textsuperscript{184}

The role of CR in impeding aging-associated pathologies has been demonstrated in young calorie-restricted rats, where insulin sensitivity escalated with decreasing hexosamine levels, proving the participation of the HBP in features of aging.\textsuperscript{185} A recent report by Busti et al.\textsuperscript{186} advocates CR in relieving ER stress. Thus, CR can be translated into a strategy, which is an effective and reproducible intervention against the alarmingly increasing cases of aging-driven cancers.

**CONCLUSION**

Until now, the cause of increasing risk of breast cancer in women over 50 years of age was a conundrum. Some explanations have come through studies that support the secretions from aging stromal cells supporting pre-cancer cells; also, aging potentiates pro-inflammatory pathways that provide selective advantages to cancer cells.\textsuperscript{187–190} The data on the direct involvement of the UPR in aging-driven breast cancer cases are seemingly feeble. There is a need to study this aspect of the story. Aging-linked upregulation of the UPR drives cells toward pro-death. Breast malignancy-linked activation of the UPR mitigates the pro-death attempt and supports the immortality of cancer cells.

There are numerous reports that suggest that therapeutic interventions of UPR transducers and their effectors can diminish the supportive effect on tumors.\textsuperscript{191} We need a more systemic approach that is not reductionist, but at the same time, we should focus on various aspects of aging, breast malignancy and the molecular mechanism of the UPR so that human intervention can utilize new findings in breast cancer.

**CONFLICT OF INTEREST**

The authors declare no conflict of interest.

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