Chapter

Targeting Endoplasmic Reticulum and Mitochondrial Dynamics to Combat Triple-Negative Breast Cancer

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Abstract

Triple negative breast cancer (TNBC) is a cancer that is aggressive with short survival rate. In comparison to other breast cancer subtypes, TNBC tumors are bigger, more chemo resistant, highly proliferative, and usually more abundant in stem and immune cells. These modifications are functionally dependent on a high-quality endoplasmic reticulum and mitochondrial pool. Endoplasmic reticulum and mitochondrial health are monitored and enhanced on a regular basis via endoplasmic reticulum and mitochondrial dynamics. The role of endoplasmic reticulum and mitochondrial dynamics in tumor growth and metastasis has been highlighted by recent advances in understanding the endoplasmic reticulum and mitochondrial dynamics in TNBC. This chapter examines the current knowledge of endoplasmic reticulum and mitochondrial dynamics in TNBC.

Keywords: endoplasmic reticulum dynamics, endoplasmic reticulum transmembrane proteins, mitochondrial dynamics, molecular mechanisms, apoptosis, triple-negative breast cancer

1. Introduction

Triple-negative breast cancer (TNBC) is a subgroup of breast tumors that does not have estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER_2) that are commonly found in breast cancer. TNBC cells undergo significant molecular alterations at the cellular level. They are highly proliferative, more chemo resistant, larger in size, and more aggressive than other types of breast cancer (BC) cells. Compared to other BC subtypes, this subtype has a higher concentration of stem cells and lymphocytes. Porporato et al. reviewed that all stages of cancer development, including proliferation, malignant transformation, and progression, require mitochondria [1]. They not only supply sufficient energy but also protect TNBC cells from excessive amounts of reactive oxygen species (ROS). Reactive oxygen species improve TNBC cells metastatic potential in the early stages of development. High amounts of ROS, on the other hand, cause ROS-induced

apoptosis, which is harmful to cancer growth [2]. To survive significant ROS-related damage, TNBC cells must have powerful defensive mechanisms. Mitochondrial dynamics are crucial in maintaining this equilibrium [3].

The endoplasmic reticulum (ER) is an intracellular organelle that is dynamic in both function and structure. It plays a variety of roles in cellular homeostasis, development, and stress tolerance. It is the organelle in charge of protein folding, translocation, and posttranslational modification. Oxidative stress, altered glycosylation, nutrient deprivation, calcium depletion, DNA damage, and energy disturbance are all caused by physiological, biochemical, and pathological stimuli in the ER, resulting in ER stress and the build-up of unfolded or misfolded proteins in the ER. In order to survive, the cells must overcome ER dysfunction and ER stress. Apoptosis can result from unresolved ERS [4].

In TNBC cells, pharmacological aggravation of ER stress causes significant cell death, and this method is even effective in multidrug-resistant forms [5]. The ER stress process has been connected to autophagy, a cellular function that appears to be important for general cell homeostasis, cancer, and chemoresistance. Autophagy and ER stress are linked: several agents that aggravate ER stress cause an increase in autophagic activity; on the other hand, there is evidence that blocking autophagy promotes ER stress [6]. *In vitro* and *in vivo* augmenting of ER stress and simultaneously inhibiting autophagy may result in effective TNBC death [7].

2. Endoplasmic reticulum dynamics and role of ER in TNBC

2.1 Endoplasmic reticulum dynamics

ER stress promotes an increase in transcription of p53 unregulated modulator of apoptosis (PUMA), Bcl2-like11 (BIM), BH3-only proteins, and NADPH oxidase activator (NOXA) due to an imbalance between anti- and pro-apoptotic Bcl-2 proteins. ER stress promotes interactions between Bax and PUMA. This results in the release of cytochrome c and caspase-dependent cleavage of p53 resulting in apoptosis [8]. ER stress in tumor cells may restore homeostasis. It also makes the surrounding environment more conducive to tumor survival and proliferation [9]. Malnutrition, hypoxia, pH fluctuations, and poor vascularization are stressful situations that might inhibit tumor cell development and activate the unfolded protein response (UPR). ER stress is caused by both nutritional deficiency [10] in tumor cells and nutrient excess in normal conditions [11]. High proliferation rates of cancer cells necessitate higher ER protein folding, assembly, and transport activities. This might result in physiological ER stress. This response is cytoprotective and plays a role in tumor development as well as adaptation to harsh conditions [12].

Inositol-requiring enzyme 1α (IRE1α), pancreatic ER kinase-like ER kinase (PERK), and activating transcription factor 6 (ATF6) localized in the ER, are the three ER stress signaling branches involved in tumorigenesis. X-box binding protein (XBP1) aids in cancer progression and is increased in breast cancer [13]. The ER resident chaperone calreticulin is found on the cell surface of tumor cells and has been linked to immunogenic cell death and calreticulin localization on tumor cell surfaces. This link could be due to ER stress in tumor cells [14]. ER chaperone and UPR components are over-expressed in breast cancers.

The ER stress response, on the other hand, is also directly implicated in pro-apoptotic pathways in both UPR-dependent and UPR-independent ways [15]. To

activate IRE1α, the cytosolic domain of IRE1α interacts with the Bax/Bak apoptotic pathway [16]. EI24/PIG8, an ER-localized Bcl2-binding protein, inhibits breast cancer invasiveness via modulating Bcl-2 function [17]. Bim is also involved in the death of MCF-7 cells generated from breast cancer by activating ER stress-induced apoptosis [18]. Another potential anti-tumor technique is to activate the CHOP-GADD34 axis [19]. In ER stress-exposed cells, CHOP causes cell death by increasing protein synthesis and oxidation [20].

2.2 Unfolded protein response

Depending on the cell state, the UPR can be both cytoprotective and cytotoxic. The UPR's objective is to keep the ER folding environment in check when it is under stress. Tumor cells will die if UPR fails to restore ER equilibrium under prolonged ER stress. In combination with induced tumor dormancy, the UPR can shield tumor cells against apoptosis, allowing for tumor regeneration once favorable conditions have been restored [21].

By dissociating Grp78/binding immunoglobulin protein (Bip), a key chaperone protein, from three membrane-bound ER stress sensors, comprising ATF6, PERK, and IRE1α, cells aim to maintain normal folding processes in the ER [22]. Following the separation of detecting proteins from Grp78/Bip, these sensors are activated in order, with PERK being the first, blocking general protein synthesis by phosphorylating eIF2α [23]. During cellular stress, these activities also cause the transcription factor NF-κB to be inhibited. Another transcription factor triggered by translocation to the Golgi apparatus is ATF6, which is cleaved and the transcription factor in its active form is released to regulate gene expression [24]. Following IRE-1 activation and downregulation, splicing of XBP1 occurs, and the spliced XBP1 protein translocates to the nucleus, where it activates the transcription of chaperones involved in protein folding and secretion or ER-associated protein degradation (ERAD) [25]. Rapid tumor growth and insufficient vascularization occur during carcinogenesis, resulting in microenvironmental stress [26].

2.3 Role of ER in TNBC

Since cancer cells are constantly dividing, they are affected by lack of nutrition and oxygen, as well as by lack of vascularization. As a result, cancer alters the expression patterns of ER resident proteins. On tumors, ER stress has two effects. It has adaptive meaning in the sense that it promotes tumor growth. Second, it possesses cytotoxic properties that cause apoptosis. Cancer cells activate UPR to adapt to their surroundings, and macrophages secrete cytokines, growth factors, and angiogenic substances to generate more favorable microenvironments for cancer cell proliferation and invasiveness [27]. Cancer cells use NF-κB pathways to promote cyclooxygenase-2 expression during ER stress, which has antiapoptotic effects. It also maintains IL-8 production in human epithelial cells and enhances pro-inflammatory NF-κB activation via CHOP [28].

Apoptosis is caused by a variety of pathways. One such pathway is ER stress. The caspase-12 family of proapoptotic cysteine proteases is linked with the ER membrane and plays a key role in ER stress-induced apoptosis, although it is not activated by non-ER stimuli [29]. Vascular endothelial growth factor (VEGF) promotes endothelial cell proliferation and angiogenesis by increasing Grp78 expression on the endothelial surface. Through mitogen-activated protein kinase (MAPK) signaling, Grp78 knockdown reduces endothelial cell growth [30]. The action of P38MAPK keeps cells

in a G_0-like quiescent state [31]. In chicken embryo chorioallantois membrane system and subcutaneous xenograft models, PERK-elf2α also pauses cell development at G_0/G_1 and prevents carcinogenesis [32].

2.3.1 Glucose-regulated protein 78/binding immunoglobulin protein

Grp78, an ER chaperone protein, is one of the cancer cells' most active components and is overexpressed in a variety of malignancies [33]. It is been considered as a chaperone protein that helps cancer cells adapt to hypoxic conditions and as a resistance protein to anti-cancer drugs [34]. In cancer systems, Grp78 affects cell apoptosis, proliferation, invasion, inflammation, and immunity [35]. It has recently been discovered to play a role in carcinogenesis, metastasis, and angiogenesis [36]. Grp78, through physical and functional interactions with BIK in the ER, suppresses BIK-mediated apoptosis and confers resistance to estrogen starvation-induced apoptosis in human breast cancer cells [37]. Grp78 is primarily found inside the ER, although it may be translocated to the surface of tumor cells during ER stress [38]. In addition to the ER, some Grp78 is found in the cytosol, nucleus, and mitochondria during ER stress [39]. A noteworthy prospective anticancer treatment is to block Grp78 translocation. Grp78, present on the surface of cell was found in receptor-positive BT474 breast cancer cells but not in triple-negative MDA-MB-468 cells. The absence of ER, PR, and HER2 receptors in this TNBC was linked to Grp78 negative expression on tumor cells of breast cancer [40]. GRP78 expression was also found to be low in TNBC cell lines, such as MDA-MB-231, by others [41]. Grp78 expression was found to be substantially linked to TNBC invasiveness, distant metastasis, and proliferation. Also, patients with Grp78 expression were found to have shorter overall survival (OS) and disease-free survival (DFS). Furthermore, elevated Grp78 expression was linked to disease-free survival (DFS) in TNBC patients [42].

3. Role of ER transmembrane proteins in TNBC

3.1 Pancreatic endoplasmic reticulum kinase-like endoplasmic reticulum kinase

PERK/eIF2 regulates tumor initiation and survival, making it easier for cells to adapt to varied circumstances including hypoxia and oxidative stress [43]. Tumor cells multiply quickly, resulting in the creation of new blood vessels and, eventually, a link to the microenvironment and nutrition restriction. Cytotoxic circumstances result from increased demand for glucose and oxygen. Reactive oxygen species (ROS) are produced when the generation of ATP and NADPH in a reducing equivalent form is disrupted. ROS build up in the mitochondria, causing ER stress to activate [44]. A cellular stress sensor in the ER responds to changes in nutritional shortage, which has been linked to cancer. PERK is a trans-ER membrane serine/threonine protein kinase with an ER luminal domain at the N-terminus and a cytoplasmic protein kinase domain at the C-terminus [45]. The transcription factors Nrf2 transcription factor [46] and elF2 are phosphorylated by PERK. The phosphorylation of elF2 suppresses most transcript translation while promoting the translation of a few mRNAs, such as the transcription factor ATF4 [47]. PERK phosphorylates Nrf2, which is then liberated from an inhibiting E3 ligase complex and translocated into the nucleus, where it generates enzymes that reduce intracellular ROS [48]. When hypoxia occurs in tumors, the transcription regulator HIF1 is stabilized, and the whole branch of

the UPR, namely PERK, is fully activated, resulting in the phosphorylation of elf2, ATF4, and GADD34. Although elf2 phosphorylation reduces overall protein synthesis, ATF4, a transcription factor, is linked to cancer cell growth and survival despite food restriction via amino acid synthesis [49]. TNBC viability and proliferation were reduced *in vivo* by CCT020312, a specific eIF2/PERK activator that activated the PERK/eIF2/ATF4/CHOP pathway while inactivating the AKT/mTOR pathway [50]. In luminal androgen receptor (LAR), TNBC ER stress reduced androgen receptor (AR) expression at the transcriptional level via PERK/eIF2/ATF4 signaling. ATF4 also inhibits AR promoter activity by binding to the promoter region of AR from −2813 to −2486 nt and from −2084 to −1742 nt [51]. In MDA-MB-468 and T47D cell lines, PERK-dependent signaling is involved in tumor initiation and expansion to maintain redox homeostasis and drive tumor growth [52].

3.2 Inositol-requiring enzyme 1α/X-box binding protein

In cells and tissues, an ER transmembrane sensor called IRE1α protects against ER stress. IRE1 is activated during ER stress by autophosphorylation and oligomerization, which causes its endoribonuclease to cleave and start splicing the XBP1 mRNA [53]. IRE1α-dependent mRNA decay (RIDD), which is different from XBP1 splicing, aids in the restoration of ER homeostasis. This is done by targeting mRNAs encoding secretory proteins. IRE1α RNase activity controls the activity of RIDD [54]. In the UPR, the IRE1-XBP1 pathway has also been suggested to play a pro-survival role [53]. The UPR, on the other hand, causes cellular death in the presence of persistent and uncompensated stress. [55]. IRE1-TRAF2-ASK is another pathway that has been proposed. Phosphorylation of IRE1 causes it to bind to tumor-necrosis factor receptor-associated factor 2 (TRAF2) and activate apoptosis signal-regulating kinase (ASK1), resulting in JNK and p38 activation and ER-stressed caused cell death [56]. By directly activating procaspase-4, the IRE1 and TRAF2 pathways are also engaged in mitochondria-independent apoptosis [57]. TNBCs have an elevated basal level of endoplasmic reticulum stress. There is activation of the XBP1 branch of the UPR, a significant cellular stress response system in the tumor microenvironment [58].

3.3 Inositol 1,4,5 triphosphate receptors

Calcium ions play a key in the development of metastases. The cytosolic Ca^{2+} concentration increases by a factor of 5 to 10 when Ca^{2+} channels activate (from 100 nM to 500–1000 nM). Part of this calcium signaling is generated inside the cell by inositol (1,4,5)-triphosphate (IP3), which has three identified subtypes: IP3R1, IP3R2, and IP3R3. Each subtype has its own calcium release signature: IP3R2 produces strong Ca^{2+} oscillations, IP3R1 produces milder oscillations, while IP3R3 produces monophasic transients [59].

The ER is the cell's principal intracellular Ca^{2+} storing organelle. Ca^{2+} is rapidly transported between the two intracellular organelles due to the tight connection between mitochondria and ER membranes [60]. The IP3R, along with the ryanodine receptors, has been identified as the primary ER Ca^{2+} channel [61]. Massive and long-term Ca^{2+} overload in the mitochondria can open the permeability transition pore [62]. As a result, proapoptotic and caspase-activating substances in mitochondria, such as cytochrome c, are released into the cytoplasm. By binding to IP3R, cytochrome c in the cytoplasm exacerbates Ca^{2+} release, avoiding Ca^{2+} −dependent regulation of the receptor and increasing caspase activation to promote apoptosis [63].

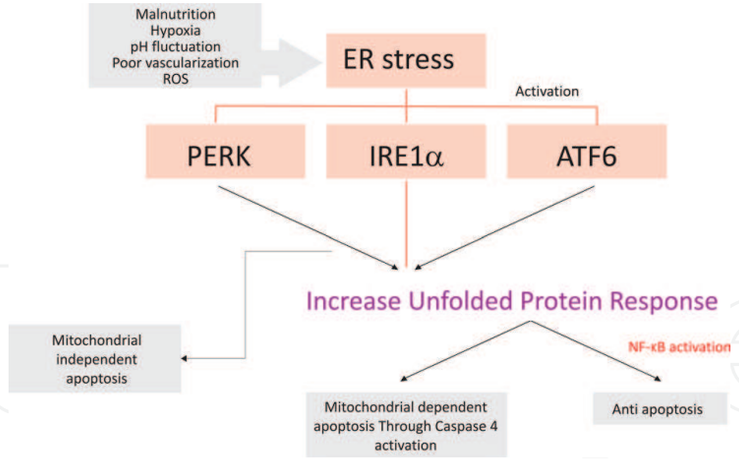

Figure 1.
Endoplasmic reticular stress activates the ER stress signaling branches that cause an increase in unfolded protein response. Depending on the cell status, UPR leads to either apoptosis or anti-apoptosis.

In breast cancer tissue, IP3R3 is the most highly expressed subtype. Furthermore, invasive breast cancer tissue, such as TNBCs, has much higher levels of IP3R3 and IP3R1 expression than non-tumor tissue. Because its expression is linked to the severity of BC, IP3R3 can be regarded as a sign of aggressiveness in BC [59].

3.4 STAT 3

STAT3 is constitutively activated and overexpressed in TNBC cells. By regulating the expression of its downstream target genes, it contributes to cell survival, cell cycle progression, migration, invasion, anti-apoptosis, chemoresistance, immunosuppression, and stem cell self-renewal and differentiation [64]. STAT3, an oncogenic transcription factor, has been found to be abundant in ER and MAM lately. Reduced ER-mitochondria Ca^{2+} transport and IP3R3 degradation mediated by the IP3R3/STAT3 association increase cell resistance to apoptosis in constitutively active STAT3 (**Figure 1**) [65].

4. Mitochondrial dynamics in TNBC

Mitochondrial dynamics is a mechanism that regulates the quality of the mitochondrial pool. In simple terms, it is a dynamic flow of mitochondria that includes divisions and assembly, allowing for the destruction or repair of malfunctioning parts. Mitochondrial dynamics thus serve as the first line of defense against ROS-induced mitochondrial damage. Given that TNBC cells have a high quantity of ROS, it is assumed that this process is critical for TNBC survival.

Changing mitochondrial dynamics helps cancer cells survive because they regulate a delicate balance between delivering energy and apoptosis. Many cancer tissues, including lung, glioma, neuroblastoma, colorectal, pancreatic, and melanoma, contain fragmented mitochondria [66]. Similarly, mitochondrial fission is significantly enhanced in TNBC clinical samples and is associated with poorer TNBC patient survival. Drp1 was upregulated and Mfn1 was downregulated in TNBC tumor tissues, according to immunohistochemistry (IHC) labeling. The results are further supported by cancer cell labeling and the relative mRNA expression ratio of tumor/

peritumor determined by qRTPCR [67]. Hypoxia also increases Drp1 expression in TNBC MDA-MB-231 cells, but not in ER-positive MCF7 cells [68]. TNBC tumors are more hypoxic than other BC subtypes, therefore this pattern is particularly intriguing [69]. A mutation in the tumor stressor gene TP53 is also found in the majority of TNBC tumors [70].

Increase in fission offers TNBC tumors with an optimal environment for development, given that knockout mice without p53 (Trp53) are able to survive longer in hypoxia environments because tumorgenesis levels are lower than in normoxic situations. Fission has been identified as a critical stage in the release of cytochrome c and, as a result, apoptosis progression. Fission-dependent apoptosis, on the other hand, appears to be context and cell-type dependent. Mitochondrial fission boosted TNBC cell growth *in vitro* and *in vivo* by suppressing apoptosis and enhancing proliferation through a notch-dependent mechanism [71].

4.1 Mitochondrial fusion and fission

Mitochondria are double membrane organelles consisting of an outer mitochondrial membrane (OMM), an inner mitochondrial membrane (IMM), and an intermembrane space between the two membranes. They are not static organelles, but rather a dynamic pool that is constantly undergoing fusion and fission. This dynamic process is essential for mitochondrial health and cellular adaptation to environmental changes.

Mitochondrial fusion occurs when two mitochondria fuse to form one mitochondrion. Mitofusin 1 (Mfn1) and mitofusin 2 (Mfn2), as well as optic atrophy protein 1 (OPA1) are critical proteins in mitochondrial fusion. Mfn1 and 2 are homodimers and heterodimers that are found near the OMM. The mitofusins are in control of OMM fusion, while OPA1 is in charge of IMM fusion. Furthermore, OPA1 interacts with Mfn2 during fusion, and interruption of this mechanism results in mitochondrial fission. ROS induce mutations in mtDNA, which results in altered respiratory functions in mitochondria. As a result, mitochondrial fusion is critical because it allows for the interchange of metabolites and gene products between fusing mitochondria, improving overall respiratory performance. Similarly, mitochondrial fusion impediment is linked to a reduction in mitochondrial function [72]. Fusion of mitochondrion and promotion of docking is the primary function of mitofusins. The oligomerization of the GTPase domains of MFNs is essential for the tethering of two OMMS, this oligomerization also requires GTP hydrolysis. When GTP is bound and hydrolyzed, the GTPase domains undergo a conformational shift that causes them to oligomerize, allowing the two mitochondria to dock at the two outer membranes and fuse. Ubiquitination, deacetylation, and phosphorylation regulate MFN 1's activity. The extracellular signal-regulated kinase (ERK) phosphorylates Mfnl in the HRI domain, which inhibits mitochondrial fusion and causes death. In situations of glucose deprivation, however, deacetylation of Mfn1 by histone deacetylase 6 (HDAC6) leads to its activation and facilitation of fusion. JNK phosphorylates MFN2 in response to cellular stress, which recruits E3 ubiquitin ligase, which ubiquitinates MFN2 and causes its proteasomal destruction. MFN2 degradation leads to mitochondrial fragmentation and an increase in apoptotic cell death. S-OPA1 and L-OPA1 combination is essential for IMM fusion. Similarly, discovered that L-OPA1 and S-OPA1 collaborate to promote fusion activity in liposomes, resulting in efficient and rapid membrane pore opening. Other research, on the other hand, has found that only L-OPA1 is required to stimulate IMM fusion [73]. A phospholipid cardiolipin, IMM component is required for IMM fusion.

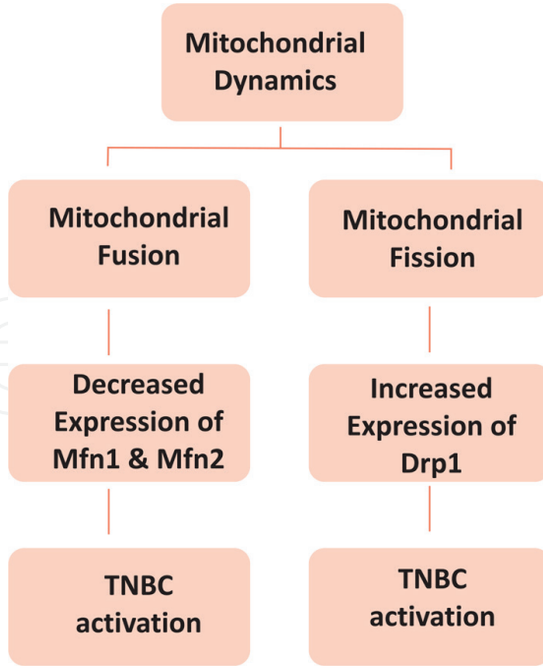

Figure 2.
Mfn1 and Mfn2 are the membrane proteins involved in mitochondrial fusion, its downregulation activates TNBC cells. Drp1 is the main membrane protein involved in mitochondrial fission. Its upregulation activates TNBC cells.

Mitochondrial fission occurs when one mitochondrion splits into two daughter mitochondria. Dynamin-related proteins (DRPs) and cofactors mediate mitochondrial fission. Constriction of mitochondria occurs where ER binds to mitochondria. At the ER–mitochondrial interaction point, Drp1 is recruited to OMM. Drp1 binding site and constriction are indicated by contact between ER tubules and mitochondria. Drp1 binds to the ER binding site on the OMM and interacts with adaptor proteins such as mitochondrial fission factor (MFF), mitochondrial dynamics protein, and cardiolipin (CL) once activated. Drpl undergoes oligomerization when the fission machinery is assembled, forming a ring around the OMM. Drp1, MFF, Mid49/51, and Mdv1-dependent GTP hydrolysis provides energy for scission completion and constriction [73].

Various cellular stresses, ROS, hypoxia, posttranslational modification, etc., regulate mitochondrial dynamics.

When these spatiotemporal events are disrupted, the consequence is either a fragmented network with many small round mitochondria or a hyperfused network with elongated mitochondria (**Figure 2**).

5. Mitochondria-associated ER membranes

Mitochondria-associated ER membranes (MAMs) function as signaling hubs that regulate ER and mitochondrial activity. They regulate lipid metabolism, Ca^{2+} homeostasis, mitochondrial function and cell death. Bcl2, p53 tumor suppressor, etc., are the cell-death pathway protein involved in MAM [74]. Also, proteins like Mfn2 and Drp1 are enriched in MAM. Mfn2 is present on both OMM and ER. Mfn2 along with Mfn1 on OMM establish ER–mitochondrial interactions [75]. Rapid Ca^{2+} exchange between mitochondria and ER also determines mitochondrial bioenergetics and cell fate. An oxidizing enzyme endoplasmic reticulum oxidoreductin 1-α (ERO1-α) is enriched in

MAM. ERO1-α affects ER redox homeostasis, ER Ca^{2+} flow, and consequent mitochondrial Ca^{2+} build-up. It also modulates oxidative folding. ERO1-α expression is correlated with programmed cell death ligand 1 (PD-L1) in TNBC [76]. It is also an indicator of poor prognosis in breast cancer.

When MAMs are disrupted, a variety of cellular processes, such as apoptosis, inflammation, and autophagy, become dysfunctional. These cellular processes are very important in the pathophysiology of TNBC [74].

6. Conclusion

The significance of the ER stress response and dynamic transformations of mitochondria, which regulate cell fate decisions in carcinogenesis and cancer resistance, is being clarified by increasing evidence. Understanding how these events are controlled from not only a molecular perspective but also a biological perspective is essential to comprehending a variety of human disorders. The search for novel medications to control these occurrences is an ongoing process.

Conflict of interest

The authors declare no conflict of interest.

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