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UPR-Activated IRE1 Signaling Pathway Promotes Malignant Growth of Breast Cancer

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Abstract. Initially, it was believed that the unfolded protein response (UPR) was a network signal that synchronized apoptotic and adaptive responses to the buildup of unfolded proteins in the endoplasmic reticulum (ER). The endoplasmic reticulum stress sensor IRE1 is a major player in the UPR, and overactivation of IRE1 can promote a variety of pathological phenomena, such as diabetes, neurodegenerative diseases, and cancer. Under endoplasmic reticulum stress, IRE1 is activated through autophosphorylation and oligomerization, leading to conformational changes in the RNase domain, thereby promoting two signaling pathways: 1) unconventional splicing of XBP1 and 2) regulated IRE1 dependence Decay (RIDD). Research has revealed that aggressive B-type breast cancer cells typically have an overexpressed and amplified IRE1 gene. Through the RIDD mechanism, IRE1 processes and regulates the breakdown of a fraction of tumor-suppressing micro-RNA, which in breast cancer cells results in the Ras oncogene GTPase. RAB3B is elevated. This article reveals the mechanism of action of the IRE1 signaling pathway and the RIE1-RIDD-miRNA pathway in promoting the malignant development of luminal breast cancer.

Keywords: Endoplasmic reticulum stress; UPR; IRE1; RIDD; breast cancer.

1. Introduction

It is commonly known that abnormal buildup of misfolded or unfolded proteins in the endoplasmic reticulum (ER) may be the cause of several human disorders [1], including neurodegeneration, diabetes, cancer, and obesity. The function of the endoplasmic reticulum is critical in maintaining protein quality and homeostasis within cells. This intricate signaling route is intended to control the precision of protein folding in the endoplasmic reticulum lumen of the cell. To modify the cell's capacity for folding protein substances, the UPR not only folds proteins in the ER but also sends data regarding the state of protein folding to the cytoplasm and nucleus [3]. In cases where cells are chronically damaged or have quality control disorders, UPR can also induce apoptosis to maintain cell health. To sense and respond to changes in protein folding states in the ER [2], cells introduce the unfolded protein response (UPR). In the context of cancer, abnormal activation of the UPR is closely associated with rapid tumor growth, increased invasiveness, and the development of treatment resistance. The vast majority of tumors experience endoplasmic reticulum stress and UPR changes during their growth. As a result, more investigation can reveal the UPR sensor IRE1's engagement mechanism as well as the specific link between intraluminal breast cancer malignancy and the IRE1 pathway. It is crucial to investigate this pathway and its connection to luminal breast cancer malignancy to treat breast cancer. In an attempt to offer research directions for breast cancer treatment, this article reviews the advancements made in recent years based on earlier studies.

2. Three pressure sensors of UPR

At least three primary stress sensors found in the endoplasmic reticulum membrane—namely, protein kinase R-like endoplasmic reticulum kinase (PERK) and inositol requiring enzyme 1 (IRE1)—mediate the UPR. and turning on ATF6, the transcription factor [4] (Figure 1). They bind to the molecular chaperone protein glucose-regulated protein 78 (GRP78/BiP) with a relative molecular mass of 78×103 and are inactive under non-endoplasmic reticulum stress conditions. The GRP78/BiP protein is competitively bound by unfolded or misfolded proteins during endoplasmic reticulum stress, which causes GRP78/BiP to separate from IRE1, PRKR, and ATF6 and initiate downstream signaling cascades. Currently, three activation models of ER stress-activated sensors have been proposed. In the first scenario, IRE1 and PERK's luminal domains are bound by the endoplasmic reticulum chaperone Bip, which prevents IRE1 and PERK from oligomerizing and activating [5]. On the other hand, in ATF6, Bip masks two major Golgi localization sequences (GLS) [5]. GLS is responsible for mediating ATF6's translocation to the Golgi complex. It is evident that the molecular chaperone Bip is essential to the stress sensor's activation mechanism. Therefore, when unfolded proteins accumulate, Bip will be isolated from the stress-sensitive domain, so that IRE1 and PERK will oligomerize, and ATF6 will translocate to the Golgi complex. In the second model, the glycosylation status of ATF6 regulates its translocation to the Golgi complex. Divided into two situations, when the ER is in a non-stressed state, ATF6 is fully glycosylated and retained within the ER through interactions with the lectin chaperone (CNX) and calreticulin (CRT). When the endoplasmic reticulum is in a state of stress, the newly synthesized ATF6 is insufficiently glycosylated and does not interact with CNX and CRT, so ATF6 is transported to the Golgi complex. It should be noted that the fully glycosylated ATF6 is degraded at this time to prevent the dimerization of two differently glycosylated bZIP proteins. The third hypothesis is predicated on the yeast IRE1P luminal domain's crystal structure. The conserved areas of the luminal sections of PERK and IRE1 exhibit functional interchangeability with roughly 45% sequence homology and 22% sequence identity [5]. In crystals, IRE1 forms filaments from dimeric units with MHC-like folds. A hallmark of MHC folding is a peptide-binding groove, suggesting that a similar groove in the IRE1 dimer interacts directly with unfolded protein domains.

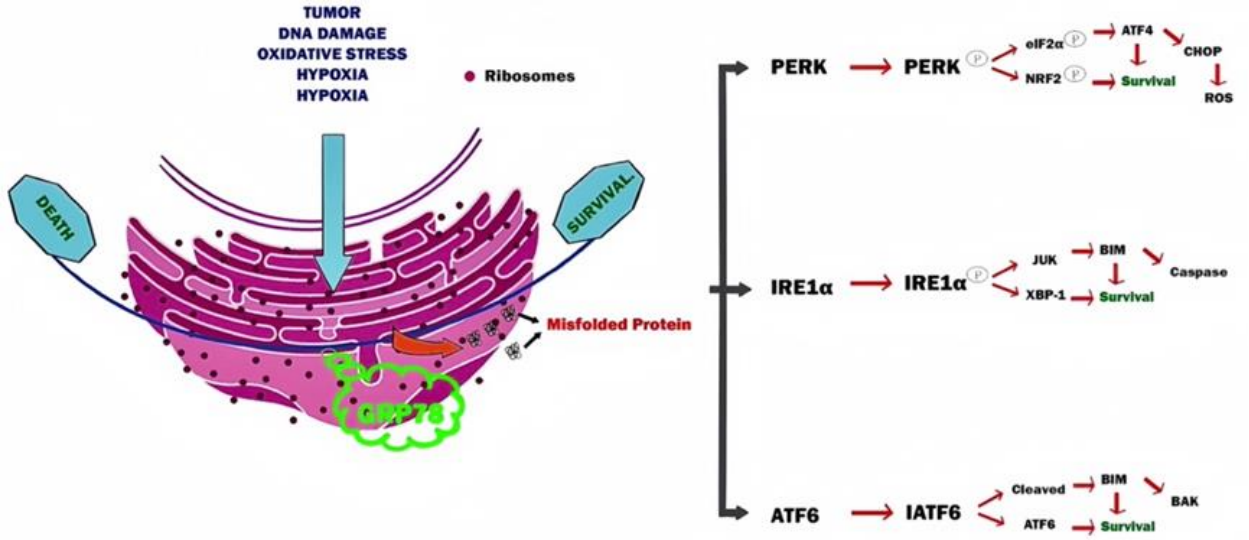

Figure 1. Three stress sensors of UPR [4]

3. IRE1

IRE1 is an important type 1 transmembrane protein kinase receptor activated in the endoplasmic reticulum rescue state [6]. The IRE1 gene includes 22 exons and 21 introns. In addition to having a kinase active site, it also has A specific endoribonuclease active site. Once activated, this enzyme cleavage site will initiate abnormal splicing reactions to relieve cellular stress. IRE1 includes 4 domains (N-terminal luminal domain, NLD), linker domain, kinase domain (kinase), and ribonuclease domain (RNase) (Figure 2), each domain having an important role [7]. Under normal

physiological conditions, IRE1 exists in monomeric form. At this time, NLD interacts with the molecular chaperone Bip on the endoplasmic reticulum. Once endoplasmic reticulum stress occurs, NLD, as a sensor of endoplasmic reticulum stress, depolymerizes and binds to Bip. The unfolded protein binds, and the dissociated IRE1 forms a homodimer [7]. The dimer form of IRE1 activates the kinase and RNase regions located on the cytoplasmic lumen. Kinase and RNase are closely related. Endoplasmic reticulum stress induces autophosphorylation of the kinase and RNase regions. At the same time, the kinase region binds to inhibitors or inhibitor analogs to inhibit kinase activity [8] and activates RNase at the same time. The activated RNase the region splices the mRNA of the active XBP1S and is transcribed. (IRE1 activates XBP1 by cleaving a 26-nucleotide intron from XBP1 mRNA.) IRE1 can degrade certain mRNAs or pre-miRNAs in addition to XBP1 mRNA; this process is referred to as Regulated IRE1-dependent decay (RIDD) [9]. IDD is a conserved mechanism that allows the reduction of protein load in the ER through the degradation of many mRNAs, thereby maintaining proteostasis. Through the RIDD mechanism, IRE1 processes and mediates the degradation of a subset of tumor suppressor miRNAs, such as miR-3607, miR-374a, and miR-96. The RAS oncogene GTPase RAB3B is elevated in breast cancer cells as a result of IRE1-dependent degradation of the tumor suppressor miR-3607. Tumor suppressors include miRNA. The primary enzyme in these continuous enzymatic activities, DICER RNase, is necessary for the occurrence of miRNA. This enzyme is responsible for cleaving pre-miRNA into mature miRNA. By causing the degradation of the target mRNA and interfering with the machinery involved in protein translation, miRNA controls the expression of genes. By directly causing RIDD to degrade mRNA and miRNA, the RNase activity of IRE1 controls their stability. In other words, the UPR sensor IRE1, as an oncogenic factor, inhibits the tumor suppressor miRNA through the RIDD mechanism, causing tumor cells to proliferate.

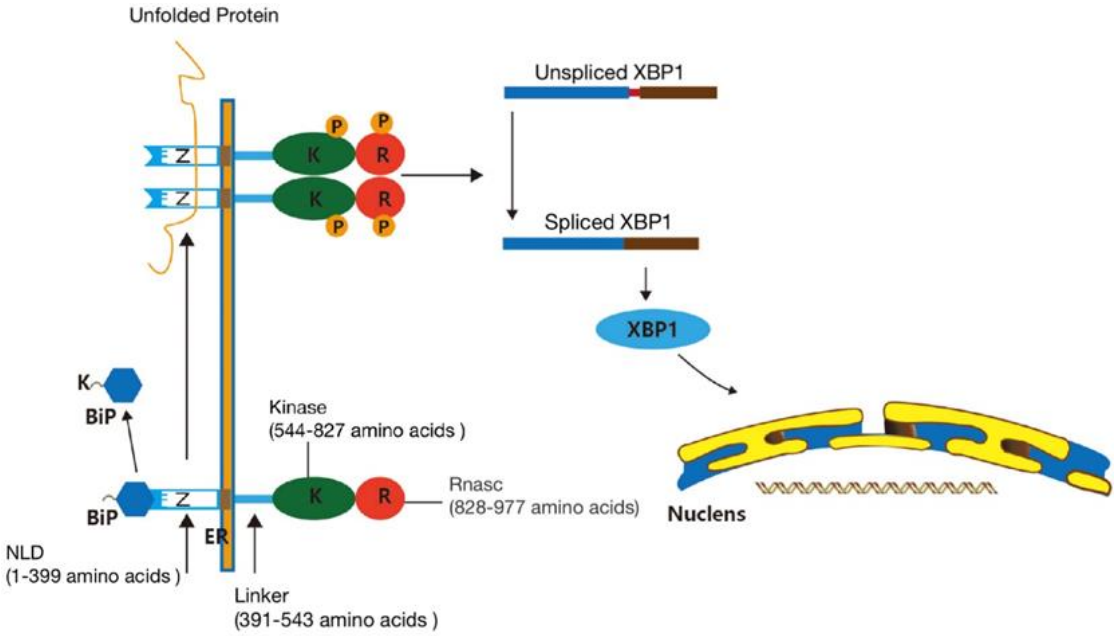

Figure 2. Four domains of IRE1

4. IRE1-IRDD-breast Cancer Pathway

Specific to breast cancer, a disease that seriously endangers women's health, activation of UPR has been proven to be closely related to tumor development and treatment resistance. Depending on different breast cancer subtypes, the UPR signaling pathway may play different roles in the malignant growth and development of cancer. Therefore, an in-depth study of the mechanism of the UPR-activated IRE1 signaling pathway in breast cancer has become a crucial research area. Although IRE1 plays a role in many types of tumors, IRE1 gene amplification is most frequently observed in breast cancer. Five molecular subtypes of breast tumors have been identified through research: basal-like (triple-negative), normal-like, HER2+, estrogen receptor-positive luminal A and B, and basal-like

(triple-negative) breast cancer [10]. In the final analysis, it turned out that the highest rate of IRE1 gene amplification occurred in cavity B cancer of the breast. A member of the extensive Ras superfamily of small GTPases is RAB3B [11]. In luminal B breast cancer, RAB3B production increases and exhibits a positive correlation with elevated IRE1 mRNA expression. demonstrated that IRE1 may be more important for RAB3B elevation than the amplification of genes. Consequently, IRE1-RIDD-miRNAs are crucial for the growth and spread of tumors [12], setting the stage for the targeted reduction in IRE1's RNase activity in the therapy of breast cancer. (Figure 3)

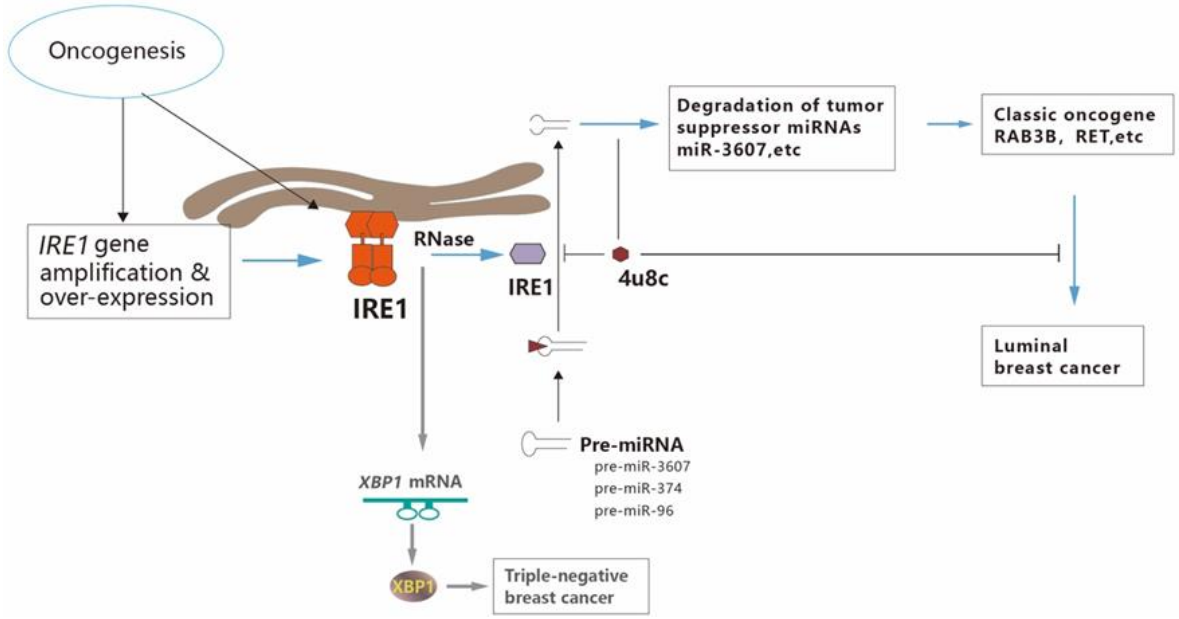

Figure 3. IRE1-RIDD-miRNA pathway diagram [13]

There have been few papers on the potential involvement of the IRE1-mediated RIDD pathway in breast cancer malignancy, and previous research on the role of IRE1 in breast cancer has mainly investigated the activity of processing XBP1 mRNA. Studies from the past showed that through the oncogenic processes of HIF1 and MYC, activated XBP1 facilitates the advancement of triple-negative breast cancer. For this instance, it is examined how the RNase activity of IRE1 might specifically cleave precursor miRNAs (tumor suppressors) through the RIDD process, halting the maturation and synthesis of miRNAs and ultimately increasing oncogenic factors in type B breast cancer.

To confirm that in luminal breast cancer cells, IRE1 induces the expression of RAB3, a member of the RAS oncogene family. In this study, the IRE1 RNase inhibitor 4m8C was utilized to treat the SUM52 cell line. RNA sequencing (RNA-seq) analysis was performed on SUM52 cells in both the inhibitor group and the control group. RNA sequencing study revealed that RAB3B was positioned higher. In the inhibitory group SUM52, 98 genes were found to be frequently up- or down-regulated. Most of the mRNAs that are up-regulated following IRE1 inhibition, as indicated by RNA sequencing information and pathway analysis, contain regulators or enzymes of endoplasmic reticulum stress reaction or protein folding homeostasis, such PDIA4, DERL2, SEC61B, and several. Particularly, IRE1 RIDD has been found to attach to PDIA4 mRNA. Furthermore, the majority of the decreased genes that IRE1 inhibits are related to cell proliferation, invasion, and cancer-causing pathways. These genes include RET proto-oncogene and RAB3B, members of the RAS oncogene family. This study used western blot and PCR to identify the main oncogenic factors that were downregulated in SUM52 cells treated with the IRE1 RNase inhibitor 4m8C, to validate the findings of the RNA sequencing analysis. Among them, this study demonstrated that 4m8C-treated SUM52 cells had much lower levels of the RAS oncogene RAB3B's mRNA and protein than did the control SUM52 cells. This suggests that IRE1 RNase activity in breast cancer has RAB3B as a downstream target.

To investigate IRE1's capacity to directly digest pre-miRNA in order to block miRNA creation via the RIDD pathway. In wild-type (WT) or IRE1 knockout (KO) mouse embryonic fibroblasts, an expression vector expressing pre-miRNA was overexpressed. The quantities of mature miRNA were then assessed. According to the experimental findings, IRE1-KO had a much greater amount of miRNA than WT. This suggests that pre-miRNA can be processed by IRE1 to prevent the creation of mature miRNA. Prior research has demonstrated that by activating XBP1, which forms a transcription complex with hypoxia-induced factor (HIF) to promote carcinogenesis, IRE 1 enhances the aggressive phenotype of triple-negative breast cancer [14]. However, by increasing XBP1's transcriptional activity, IRE1 only appears to support the aggressive phenotype of triple-negative breast cancer—not luminal breast cancer. The IRE1-XBP1 pathway in luminal breast cancer cells may be less active, as this study's use of the RE1 RNase inhibitor 4m8C did not significantly alter the expression of spliced XBP1 mRNA in SUM52 cells [15]. These findings point to IRE1 possibly controlling RAB3B expression through a mechanism that is independent of XBP1. This study employed IRE1 inhibitor 4m8C technology to block IRE1 activity in luminal breast cancer cell lines to investigate the regulatory mechanism of IRE1 in regulating oncogenic variables (especially RAB3B) in luminal breast cancer cells and to ascertain whether IRE1 regulates IRE1 through miRNA. controls the RAB3B oncogene. After administering 4m8C for two days to the SUM52 line with elevated IRE1 expression, miRNA microarray analysis revealed a shift in the miRNA expression profile landscape in IRE1-inhibited luminal breast cancer cells. Five miRNAs were found to be upregulated in IRE1-inhibited breast cancer cells in this study, suggesting that IRE1 inhibits miRNAs to control the expression of oncogenic factors.

5. Conclusion

This article clarifies the UPR sensor IRE1's activation mechanism and the causal connection between intraluminal breast cancer malignancy and the IRE1 pathway. This study discovered that, mechanistically, IRE1 can process and mediate the RIDD mechanism-mediated degradation of a subset of tumor suppressor miRNAs, which in turn stimulates the expression of the RAS oncogene GTPase RAB3B in IRE1-amplified luminal breast cancer cells. The proliferation of luminal breast cancer cells and aggressive cancer phenotypes are suppressed by the pharmacological inhibition of IRE1. Lastly, by mediating the degradation of precursor miRNA, IRE1 plays an essential driving role in breast tumorigenesis. This study has revealed the regulatory mechanism of tumor suppressor miRNA in breast cancer cells. Recognizing the RAS oncogene RAB3B's regulatory mechanism will have an important effect on the current prognosis of breast cancer and future targeted treatments. Overall, the IRE1-RIDD-miRNA pathway in luminal breast cells was identified by the present investigation using biochemical techniques. It is essential to bear in mind that additional research on the pathophysiological significance of this pathway in luminal breast cancer will require clinical trials in the future. It is still unknown how the IRE1-mediated signaling pathway is differently activated in various breast cancer subtypes, even though earlier research has shown that the IRE1-mediated XBP1 pathway fails to encourage the growth of cancer cells in luminal breast cancer cells. Future research on this question is necessary

Authors Contribution

All the authors contributed equally and their names were listed in alphabetical order.

References

[1] McLaughlin, T., et al., Cellular stress signaling and the unfolded protein response in retinal degeneration: mechanisms and therapeutic implications. Mol Neurodegener, 2022. 17 (1): p. 25.

[2] Henkel, A. and R.M. Green, The unfolded protein response in fatty liver disease. Semin Liver Dis, 2013. 33 (4): p. 321 - 9.

[3] Zhang, S.X., et al., Endoplasmic reticulum stress and the unfolded protein responses in retinal degeneration. Exp Eye Res, 2014. 125: p. 30 - 40.

[4] Han, C.C. and F.S. Wan, New Insights into the Role of Endoplasmic Reticulum Stress in Breast Cancer Metastasis. J Breast Cancer, 2018. 21 (4): p. 354 - 362.SHI Biao, LI Yu Xia, YU Xhua, YAN Wang. Short-term load forecasting based on modified particle swarm optimizer and fuzzy neural network model. Systems Engineering-Theory and Practice, 2010, 30 (1): 158 - 160.

[5] Schröder, M., The unfolded protein response. Mol Biotechnol, 2006. 34 (2): p. 279 - 90.

[6] Zhou, J., et al., The crystal structure of human IRE1 luminal domain reveals a conserved dimerization interface required for activation of the unfolded protein response. Proc Natl Acad Sci U S A, 2006. 103 (39): p. 14343 - 8.

[7] Li, H., et al., Mammalian endoplasmic reticulum stress sensor IRE1 signals by dynamic clustering. Proc Natl Acad Sci U S A, 2010. 107 (37): p. 16113 - 8.

[8] Martín-Pérez, R., et al., Activated ERBB2/HER2 licenses sensitivity to apoptosis upon endoplasmic reticulum stress through a PERK-dependent pathway. Cancer Res, 2014. 74 (6): p. 1766 - 77.

[9] Wang, J.M., et al., IRE1α prevents hepatic steatosis by processing and promoting the degradation of select microRNAs. Sci Signal, 2018. 11 (530).

[10] Jääskeläinen, A., et al., High Parity Predicts Poor Outcomes in Patients with Luminal B-like (HER2 Negative) Early Breast Cancer: A Prospective Finnish Single-Center Study. Front Oncol, 2020. 10: p. 1470.

[11] Ye, F., et al., miR-200b as a prognostic factor in breast cancer targets multiple members of the RAB family. J Transl Med, 2014. 12: p. 17.

[12] Chalmers, F., et al., The multiple roles of the unfolded protein response regulator IRE1α in cancer. Mol Carcinog, 2019. 58 (9): p. 1623 - 1630.

[13] Zhang K, Liu H, Song Z, et al., The UPR Transducer IRE1 Promotes Breast Cancer Malignancy by Degrading Tumor Suppressor microRNAs. iScience., 2020. 23 (9).

[14] Wang, M., et al., The unfolded protein response as a target for anticancer therapeutics. Crit Rev Oncol Hematol, 2018. 127: p. 66 - 79.

[15] Keenan, M. M., C.K. Ding, and J.T. Chi, an unexpected alliance between stress responses to drive oncogenesis. Breast Cancer Res, 2014. 16 (6): p. 471.