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# Phosphorylated protein kinase R (PKR)-like endoplasmic reticulum kinase (PERK) expression in breast cancer is correlated with malignant proliferation and histological grading

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**Summary.** This study aims to detect the expression of phosphorylated PERK in breast cancer using immunohistochemistry and explore its significance. We examined 134 cases of formalin-fixed and paraffin-embedded breast cancer tissues. It was found that the expression of phosphorylated PERK in ductal carcinoma was higher than that in lobular carcinoma, and the difference between them was statistically significant, suggesting that phosphorylated PERK played different roles in the occurrence and development of different types of breast cancer. Compared with Ki-67-negative breast cancer tissues, phosphorylated PERK has higher expression in Ki-67-positive tissues and is positively correlated with Ki67 expression, indicating that phosphorylated PERK plays an important role in breast cancer's malignant proliferation and progression. We also found a positive correlation between phosphorylated PERK expression and the histological grading of invasive ductal carcinoma, indicating that phosphorylated PERK plays an important role in the differentiation of invasive ductal carcinoma. Our study revealed the differential expression of phosphorylated PERK in subtypes of breast cancer. It contributed to the malignant proliferation of breast cancer and tissue differentiation of invasive ductal carcinoma of the breast.

**Key words:** Breast cancer, Immunohistochemistry, Protein kinase R (PKR)-like endoplasmic reticulum kinase, Phospho-PERK, Ki67

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## Introduction

Multiple stressors, such as hypoxia, nutritional deficiency, and various other factors that lead to the accumulation of unfolded or misfolded proteins in the endoplasmic reticulum, can cause endoplasmic reticulum stress (Chen and Cubillos-Ruiz, 2021; Mandula et al., 2022), triggering a series of cascade signaling reactions known as the unfolded protein response (UPR). As one of the most important adaptive systems for tumor cells, the UPR can adapt to external stimuli by integrating multiple signal transduction pathways, alleviating endoplasmic reticulum stress, restoring endoplasmic reticulum homeostasis, promoting tumor cell survival, and being involved in tumorigenesis, progression, metastasis, immune escape, inflammatory response, and apoptosis (Lin et al., 2019; Nie et al., 2021). Endoplasmic reticulum stress plays an important role in regulating cell plasticity. It is involved in regulating the plasticity of vascular smooth muscle cells in aortic aneurysm dissection (Clement et al., 2019). The role of endoplasmic reticulum stress in driving T-cell plasticity has also been reported (Franco et al., 2010). Under stress conditions, the repositioning of GRP78 from the endoplasmic reticulum to the cell surface enhances CRIPTO signaling and promotes cell plasticity (Balcioglu et al., 2020).

Protein kinase R (PKR)-like endoplasmic reticulum kinase (PERK) is a type I transmembrane receptor located in the endoplasmic reticulum membrane. Serine/threonine residues in the cytoplasmic region are phosphorylated for activation. PERK is an important component of the UPR and is a key regulatory factor in protein synthesis during endoplasmic reticulum stress.

The plasticity of tumors enables cells to exhibit different phenotypes to adapt to constantly changing conditions, leading to tumor heterogeneity. The epithelial-mesenchymal transition (EMT) is an important

example of tumor cell plasticity. Yuan et al. reported that, in squamous cell carcinoma, ectopic expression of transmembrane and tetratricopeptide repeat (TPR) containing protein 3 (TMTC3) during endoplasmic reticulum stress disrupts the interaction between PERK and GRP78, activates the PERK pathway, causes ATF4 nuclear translocation, increases the transcriptional activity of interleukin-like EMT inducer (ILEI), and promotes the expression of EMT markers (Yuan et al., 2022). EMT cells display constitutive activation of the PERK–eIF2α axis. PERK activation is also required for EMT cell invasion and metastasis. In human tumor tissues, EMT gene expression is strongly correlated with both ECM and PERK–eIF2α genes, PERK–eIF2α signaling is required to maintain endoplasmic reticulum homeostasis and is indispensable for EMT cells to invade and metastasize (Feng et al., 2014).

Breast cancer is a malignant tumor with the highest incidence rate among female tumors. It is highly heterogeneous at both the morphological and molecular levels, including various biological subtypes with different molecular and clinicopathological characteristics (Sung et al., 2021).

In this study, immunohistochemistry was used to identify the expression of phosphorylated PERK (phospho-PERK) in 134 breast cancer tissues. This study aimed to investigate the relationship between phospho-PERK expression and clinicopathological characteristics of breast cancer, including tumor size, pathological type, histological grading, lymph node metastasis, and the expression of ERα, PR, and Ki67.

## Materials and methods

### *Study subjects*

A total of 134 breast cancer tissue samples were collected from the Anqing Municipal Hospital, Anhui Province, People's Republic of China, between January 2018 and December 2022. The samples included 84 cases of invasive ductal carcinoma (IDC), 22 of invasive lobular carcinoma (ILC), and 28 of ductal carcinoma *in situ* (DCIS). All patients were females diagnosed with breast cancer for the first time and aged from 27 to 80 years. All specimens were collected from the central region of the tumor without extensive necrosis or bleeding. All cases were confirmed as breast cancer according to the WHO Classification of Tumors, 5th Edition. None of the patients had received chemotherapy, radiotherapy, immunotherapy, or endocrine therapy before surgery. Furthermore, none of the patients had any other organic disease or malignant tumor.

The immunohistochemical results for ERα, PR, and Ki67 were evaluated based on the percentage of cells that stained positively and were independently determined by two senior pathologists. For ERα and PR, a positive cutoff point of 10% was used (Sleightholm et al., 2021; Muller et al., 2022), while a cutoff value of 14% was set for Ki67 (Cheang et al., 2009).

The results of HER2 immunohistochemistry were evaluated according to ASCO/CAP guidelines (2018) (Ahn et al., 2020). The score ranges from 0 to 3, where 0 indicates no cell staining or incomplete or weak membrane staining in ≤10% of the tumor cells; 1 indicates incomplete, weak, or almost imperceptible membrane staining in more than 10% of tumor cells; 2 indicates weak to moderate complete membrane staining in >10% of tumor cells; and 3 indicates complete and strong circumferential membrane staining in more than 10% of tumor cells. A score of 0 or 1 was considered negative, whereas a score of 2 or 3 was considered positive.

### *Immunohistochemistry of phospho-PERK*

Three-μm tumor sections of formalin-fixed tissues were deparaffinized in xylene and rehydrated using a series of graded ethanol solutions. Endogenous peroxidase activity was blocked by immersing sections in 3% $H_2O_2$ for 5 min. Antigen retrieval involved boiling the sections in Tris-EDTA buffer (pH 9.0) for 15 minutes. The sections were blocked with 10% fetal bovine serum for 10 min at room temperature. The primary antibody, phospho-PERK (Thr982) (rabbit polyclonal, Affinity, 1:100, DF7576), was incubated at 4°C overnight. The sections were incubated with secondary antibodies to goat anti-rabbit IgG (H+L) HRP (rabbit polyclonal, Affinity, 1:200, S0001) for 1h at room temperature after washing with PBS. Slides were developed using diaminobenzidine (DAB) and counterstained with hematoxylin. Finally, the slides were dehydrated and mounted.

### *Evaluation of phospho-PERK immunoreactivity*

Two senior pathologists evaluated the staining patterns of tumor tissue samples using a blind and random method and provided the same scores. Based on cytoplasmic staining, five random areas on each slide were selected using an Olympus BX53 microscope and evaluated, with a percentage of less than 10% positive cells indicating negative results. For cells with a percentage of positive cells greater than 10%, the integration method was used to calculate the proportion of positive cells and cell staining intensity; the percentage of positive cells ≤30% indicated 1 point, 31% to 50% indicated was 2 points, >51% indicated 3 points, and the positive staining intensity was calculated as 1, 2, and 3 points in the order of yellow, brown yellow, and dark brown, respectively. After adding these two points, 1-2 points were (+), 3-4 points were (++), and 5-6 points were (+++).

### *Statistical analysis*

Statistical analyses were conducted using SPSS 27.0 version software (IBM Corporation, Armonk, NY,

USA). The $\chi^2$ test was used to compare the expression of phospho-PERK for the different factors. The correlation between phospho-PERK levels and clinicopathological characteristics was analyzed using the kappa test. All statistical tests were two-sided and the statistical significance threshold was set at $p<0.05$.

## Results

### *Expression of phospho-PERK in breast cancer*

Of the 134 breast cancer cases, 70 had a positive expression of phospho-PERK, and the positive rate was 72.4% (97/134). For different sources of breast cancer, the positive expression rate of phospho-PERK in lobular carcinoma was 27.3% (6/22), and in ductal carcinoma was 81.3% (91/112). Immunohistochemistry results for different subtypes of breast cancer are shown in Figure 1.

A statistically significant difference was observed between lobular carcinoma and ductal carcinoma ($\chi^2=26.804$, $p<0.01$), but no statistical difference was found in the expression of phospho-PERK between IDC and DCIS. In invasive carcinoma, the expression of phospho-PERK was significantly different between the ILC and IDC groups ($\chi^2=34.84$, $p<0.01$). The statistical results are presented in Table 1.

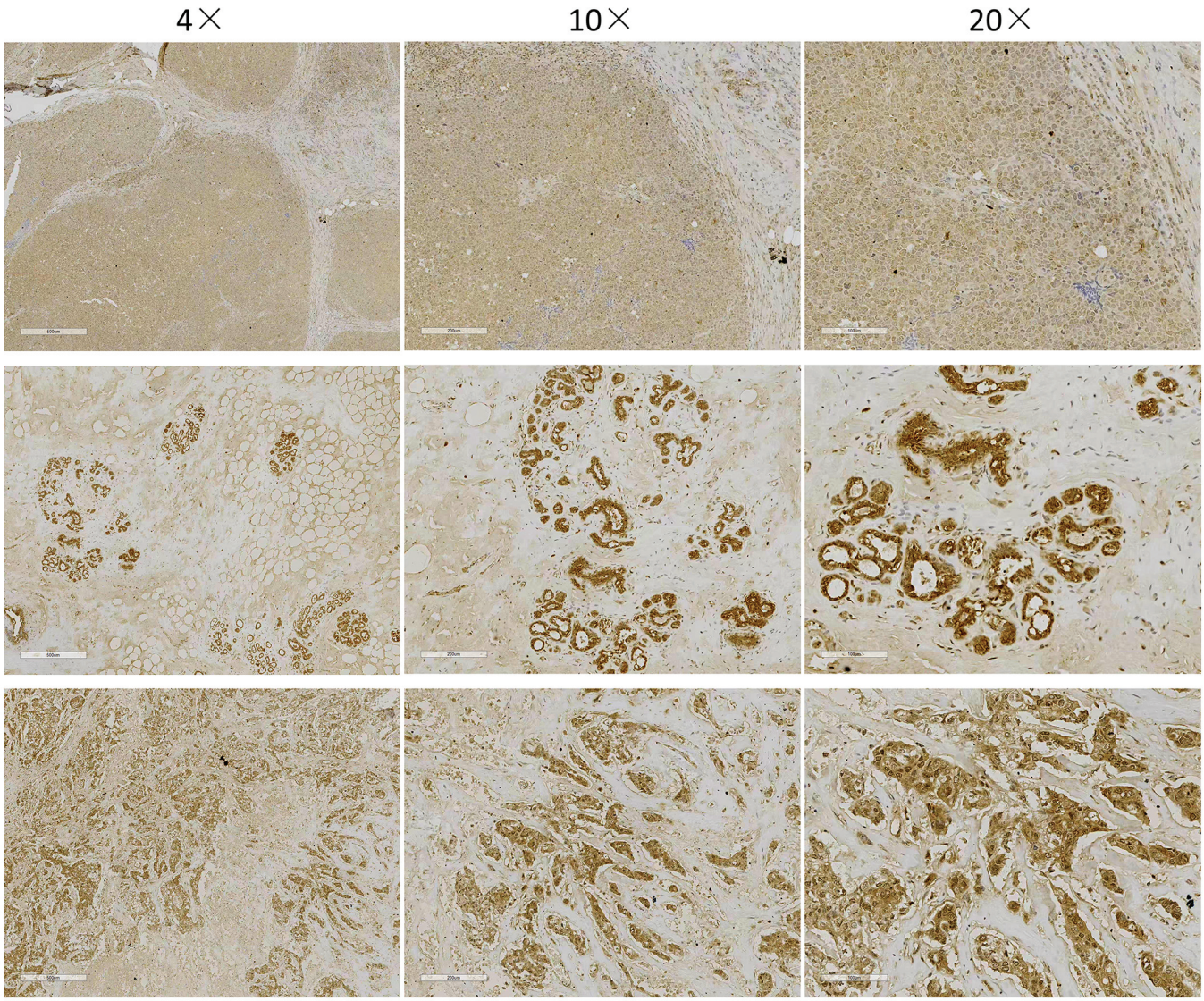


**Fig. 1.** Immunohistochemical analysis of phospho-PERK in breast cancer tissues. **A.** Invasive lobular carcinoma, more than 51% of tumor cells had yellow cytoplasmic staining, staining evaluation: ++; **B.** Ductal carcinoma *in situ*, more than 51% of tumor cells had dark brown cytoplasmic staining, staining evaluation: +++; **C.** Invasive ductal carcinoma, more than 51% of tumor cells had brown-yellow cytoplasmic staining, staining evaluation: +++. The scale sizes in the images are 4x, 500 $\mu$m; 10x, 200 $\mu$m and 20x, 100 $\mu$m.

### *Correlation between clinicopathological characteristics and phospho-PERK in 134 cases of breast cancer*

In Ki67-negative breast cancer, 47.8% (22/46) of tissue samples showed positive expression of phospho-PERK, in Ki67-positive breast cancer, it was 85.2% (75/88). There was a statistically significant difference between the two groups ($\chi^2$=21.142, $p$<0.01), and a linear correlation was observed (Z=20.984, $p$<0.01). The expression of phospho-PERK in breast cancer was correlated with the expression of Ki76 (κ=0.392, $p$<0.01). Immunohistochemistry for Ki67 and phospho-PERK in the same case of IDC is shown in Figure 2.

No statistical differences were found between the expression of phospho-PERK and the age ($\chi^2$=0.739, $p$=0.39), tumor diameter ($\chi^2$=1.121, $p$=0.933), and lymph node metastasis ($\chi^2$=0.279, $p$=0.597) subgroups. Regarding the molecular phenotype of breast cancer, there was no statistical difference in phospho-PERK expression among the ERα ($\chi^2$=0.371, $p$=0.543), PR ($\chi^2$=0.107, $p$=0.744), and HER2 ($\chi^2$=0.028, $p$=0.866) subgroups, in addition to the Ki67 subgroup mentioned above (Table 2).

**Table1.** Different expression of phospho-PERK in breast cancer subtypes.

| Breast cancer subtypes | phospho-PERK | | $\chi^2$ | $p$ |
|---|---|---|---|---|
| | Negative | Positive | | |
| Invasive carcinoma | 10 | 53 | | |
| ILC | 16 | 6 | | |
| IDC | 10 | 74 | 34.84[a] | $p$<0.01 |
| Ductal carcinoma *in situ* | 4 | 24 | 26.804[b] | $p$<0.01 |

[a]: $\chi^2$ tests on phospho-PERK expression in IDC and ILC; [b]: $\chi^2$ tests on phospho-PERK expression in lobular carcinoma and ductal carcinoma.

### *Correlation between histological grading and phospho-PERK in invasive ductal carcinoma*

The relationship between histological grading and phospho-PERK expression in IDC was analyzed using the kappa test. The results are shown in Table 3. The expression of phospho-PERK was positively correlated with histological grading ($\chi^2$=31.555, $p$<0.01; Z=17.304, $p$<0.01; κ=0.266, $p$<0.01) (Table 3). HE staining for IDC and immunohistochemistry for phospho-PERK are shown in Figure 3.

## Discussion

Malignant proliferation is a characteristic of tumors

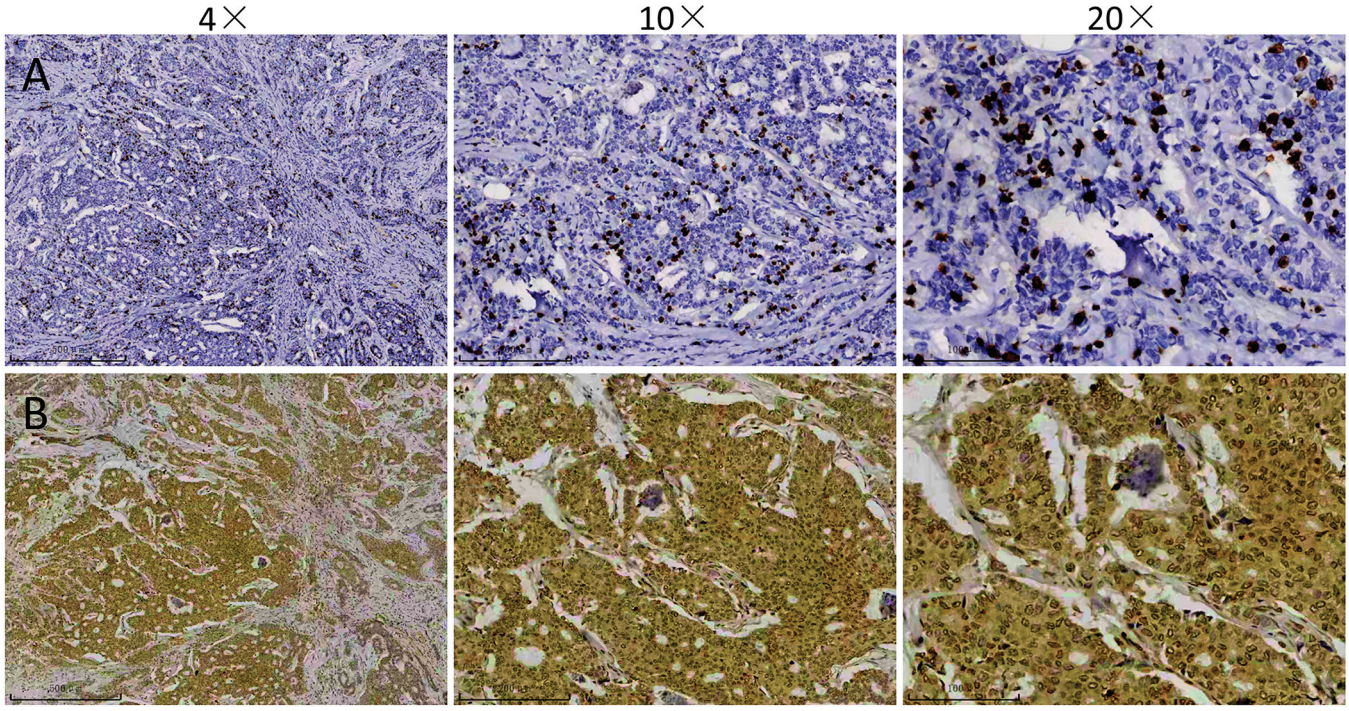


**Fig. 2.** Immunohistochemical analysis of Ki67 and phospho-PERK in breast cancer tissue. **A.** Immunohistochemistry for Ki67 in invasive ductal carcinoma. More than 10% of tumor cells had nuclear staining, staining evaluation: +; **B.** Immunohistochemistry for phospho-PERK showed brown-yellow cytoplasmic staining in over 51% of tumor cells in the same ki67 invasive ductal carcinoma case shown in A, staining evaluation: +++.The scale sizes in the images are 4x, 500 μm; 10x, 200 μm and 20x, 100 μm.

that requires a sufficient supply of nutrients. However, the newly formed vascular network in tumor tissue may fail to mature and prune, and cannot be divided into arterioles, capillaries, and venules, resulting in a mismatch between the supply of blood vessels and the nutritional and metabolic needs of tumor cells. Tumor blood vessels are insufficient to meet the needs of growing tumor cells (Lugano et al., 2020; Bai et al., 2022).

Autophagy may be one of the mechanisms by which tumor cells balance the demand and supply of nutrients and energy when they cannot obtain them from the blood (He, 2022; Debnath et al., 2023; Assi and Kimmelman, 2023). Under stress conditions, autophagy can function as a protective mechanism that promotes tumor survival and growth (Qi et al., 2019; Romine et al., 2019). An insufficient supply of blood vessels leads to relative hypoxia in tumor cells, initiating oxidative stress in the endoplasmic reticulum.

Rapid and extensive proliferation of tumor cells may lead to abnormal accumulation of unfolded or misfolded proteins in the endoplasmic reticulum, competing with PERK for the GRP78/Pip binding site and allowing PERK to be released from its binding state, leading to homodimerization and autophosphorylation activation (Lin et al., 2019; Romine et al., 2019). Phospho-PERK affects the expression of autophagy-related genes *Atg12* and *LC3* by phosphorylating eIFα, promoting cellular autophagy, which can provide a certain amount of energy and nutrition for the rapid and massive proliferation of tumor cells (Qi et al., 2019).

Ki67 is a proliferation index that indicates the growth rate of breast cancer cells. High expression of Ki67 suggests rapid proliferation of breast cancer cells. The results of this study showed a strong correlation between the expression of phospho-PERK and ki67 ($\chi^2$=21.142, $p$<0.01; κ=0.392, $p$<0.01), suggesting that the expression of phospho-PERK is related to the rapid proliferation of breast cancer cells.

Endoplasmic reticulum stress participates in the regulation of vascular endothelial cells and angiogenesis in tumor tissue by altering the expression and activity of vascular growth factors (Takayanagi et al., 2015), thereby affecting the nutritional and metabolic requirements of tumor cell proliferation. The activation of PERK promotes the survival effect of vascular endothelial growth factor (VEGF) on endothelial cells by positively regulating mTORC2-mediated AKT phosphorylation at Ser473 and participating in regulating tumor angiogenesis (Karali et al., 2014). The PERK signaling pathway is involved in the malignant proliferation of breast cancer, mediating angiogenesis

**Table 2.** Correlation between clinicopathological characteristics and phospho-PERK expression.

| | | | phospho-PERK | | Kappa | *p* | $\chi^2$ | *p* |
|---|---|---|---|---|---|---|---|---|
| | | | Negative | Positive | | | | |
| All cases | | 134 | 37 | 97 | | | | |
| Age (years) | ≤50 | 68 | 21 | 47 | | | | |
| | >50 | 66 | 16 | 50 | | | | |
| Tumor diameter (cm) | <2 | 31 | 11 | 20 | | | | |
| | 2-5 | 83 | 21 | 62 | | | | |
| | >5 | 20 | 5 | 15 | | | | |
| Metastatic lymph nodes | Negative | 88 | 23 | 65 | | | | |
| | Positive | 46 | 14 | 32 | | | | |
| ERα | Negative | 54 | 15 | 45 | | | | |
| | Positive | 80 | 22 | 52 | | | | |
| PR | negative | 61 | 16 | 45 | | | | |
| | Positive | 73 | 21 | 52 | | | | |
| HER2 | Negative | 74 | 20 | 54 | | | | |
| | Positive | 60 | 17 | 43 | | | | |
| Ki-67 | Negative | 46 | 24 | 22 | 0.392 | <0.01 | 21.142 | <0.01 |

**Table 3.** Correlation between phospho-PERK expression and histological grading of IDC.

| Histological grading | phospho-PERK | | | | $\chi^2$ | *p* | Z | *p* | κ | *p* |
|---|---|---|---|---|---|---|---|---|---|---|
| | - | + | ++ | +++ | | | | | | |
| 1 | 6 | 4 | 1 | 1 | | | | | | |
| 2 | 2 | 18 | 6 | 5 | | | | | | |
| 3 | 2 | 9 | 16 | 14 | 31.555 | <0.01 | 17.304 | <0.01 | 0.266 | <0.01 |

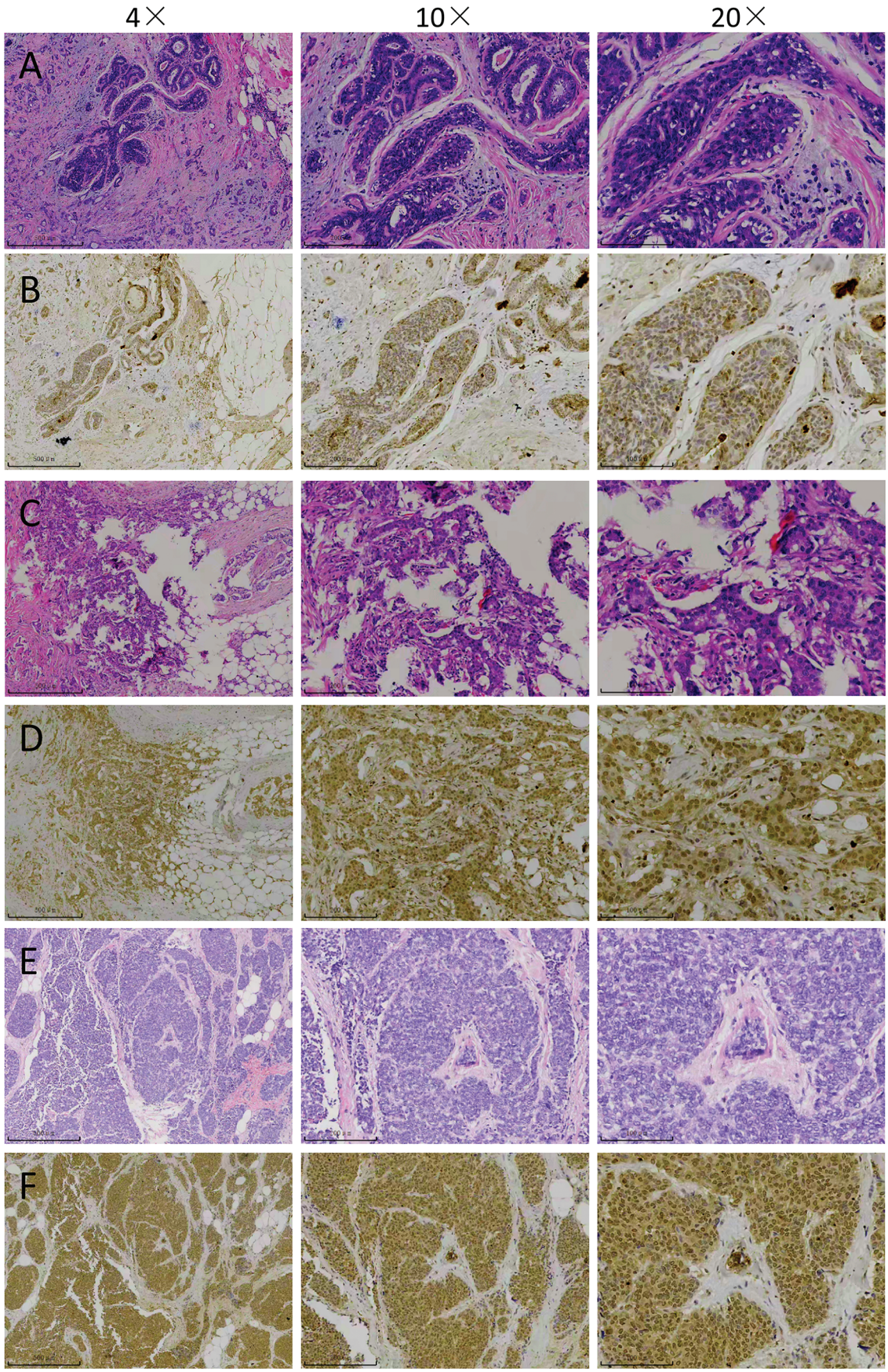


**Fig. 3.** phospho-PERK HE staining of invasive ductal carcinoma and immunohistochemistry. **A.** HE staining of invasive ductal carcinoma, grading 1. **B.** Immunohistochemistry for phospho-PERK showed yellow cytoplasmic staining in < 30% tumor cells in the same HE staining invasive ductal carcinoma case, grading 1, as shown in A, staining evaluation: +. **C.** HE staining for invasive ductal carcinoma, grading 2. **D.** Immunohistochemistry for phospho-PERK showed brown-yellow cytoplasmic staining in > 50% tumor cells in the same HE staining invasive ductal carcinoma case, grading 2, as shown in C, staining evaluation: +++. **E.** HE staining for invasive ductal carcinoma, grading 3. **F.** Immunohistochemistry for phospho-PERK showed brown-yellow cytoplasmic staining in > 50% tumor cells in the same HE staining invasive ductal carcinoma case, grading 3, as shown in E, staining evaluation: +++. The scale sizes in the images are 4x, 500 $\mu$m; 10x, 200 $\mu$m and 20x, 100 $\mu$m.

and autophagy contributing to the survival and rapid growth of cancer cells.

Considering that there was no significant difference in the expression of PERK between IDC and DCIS, but the expression was higher in IDC than in ILC ($\chi^2$=34.84, $p$<0.01), it can be considered that PERK pathway-mediated cell proliferation played a more important role in ductal carcinoma than in lobular carcinoma. Different pathological types of breast cancer may involve different proliferation pathways. The PERK signaling pathway is involved in cell differentiation and has been reported to play a key role in the maintenance and differentiation of myoblasts, as well as in the differentiation of osteoblasts and osteoclasts (Zhang et al., 2019; Guo et al., 2020; Tan et al., 2021). The PERK-eIF2α axis is involved in T helper cell differentiation and plays a major role in peripheral Treg cell differentiation (Scheu et al., 2006; Franco et al., 2010). Bobrovnikova-Marjon et al. reported that PERK is necessary for the functional maturation of milk-secreting mammary epithelial cells and that PERK-dependent signaling contributes to lipogenic differentiation in the mammary epithelium (Bobrovnikova-Marjon et al., 2008). Mandula et al. reported that PERK controlled the differentiation of myeloid precursors into monocytic-lineage inflammatory dendritic cells in tumor cells (Mandula et al., 2022).

Histopathological grading refers to the degree of differentiation of the tumor cells. Higher histological grading indicates worse differentiation and faster growth of tumor cells. Our results indicated that the expression of phospho-PERK was correlated with the histological grading of IDC (κ=0.266, $p$<0.01), indicating that the expression of phospho-PERK was related to cell differentiation in IDC.

### *Conclusions*

In summary, the histological grading of IDC is a well-established prognostic factor for breast cancer and is an independent predictor of survival (Wang et al., 2022). A higher histological grading indicates a worse prognosis. Our experimental findings indicated that phospho-PERK expression in IDC was positively correlated with histological grading. Second, ILC is believed to have a better prognosis than IDC (Danzinger et al., 2021), and our study found that the expression of phospho- PERK was higher in IDC than in ILC. These observations suggest that phospho-PERK may serve as a potential prognostic indicator for breast cancer.

Our study provides evidence that phospho-PERK is associated with the clinicopathological characteristics of breast cancer. Phospho-PERK is involved in malignant proliferation and differentiation of breast cancer cells.

*Conflicts of Interest.* The authors declare no conflict of interest.

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## References

Ahn S., Woo J.W., Lee K. and Park S.Y. (2020). HER2 status in breast cancer: changes in guidelines and complicating factors for interpretation. J. Pathol. Transl. Med. 54, 34-44.

Assi M. and Kimmelman A.C. (2023). Impact of context-dependent autophagy states on tumor progression. Nat. Cancer 4, 596-607.

Balcioglu O., Heinz R.E., Freeman D.W., Gates B.L., Hagos B.M., Booker E., Mirzaei M.E., Diesen H.T., Bhakta K., Ranganathan S., Kachi M., Leblanc M., Gray P.C., Spike B.T. (2020). CRIPTO antagonist ALK4$^{L75A}$-Fc inhibits breast cancer cell plasticity and adaptation to stress. Breast Cancer Res. 22, 125.

Bai R., Li Y., Jian L., Yang Y., Zhao L. and Wei M. (2022). The hypoxia-driven crosstalk between tumor and tumor-associated macrophages: mechanisms and clinical treatment strategies. Mol. Cancer 21, 177.

Bobrovnikova-Marjon E., Hatzivassiliou G., Grigoriadou C., Romero M., Cavener D.R., Thompson C.B. and Diehl J.A. (2008). PERK-dependent regulation of lipogenesis during mouse mammary gland development and adipocyte differentiation. Proc. Natl. Acad. Sci. USA 105, 16314-16319.

Cheang M.C., Chia S.K., Voduc D., Gao D., Leung S., Snider J., Watson M., Davies S., Bernard P.S., Parker J.S., Perou C.M., Ellis M.J. and Nielsen T.O. (2009). Ki67 index, HER2 status, and prognosis of patients with luminal B breast cancer. J. Natl. Cancer Inst. 101, 736-750.

Chen X. and Cubillos-Ruiz J.R. (2021). Endoplasmic reticulum stress signals in the tumour and its microenvironment. Nat. Rev. Cancer 21, 71-88.

Clement M., Chappell J., Raffort J., Lareyre F., Vandestienne M., Taylor AL., Finigan A., Harrison J., Bennett M.R., Bruneval P., Taleb S., Jorgensen H.F. and Mallat Z. (2019). Vascular smooth muscle cell plasticity and autophagy in dissecting aortic aneurysms. Arterioscler. Thromb. Vasc. Biol. 39, 1149-1159.

Danzinger S., Hielscher N., Izso M., Metzler J., Trinkl C., Pfeifer C., Tendl-Schulz K. and Singer C.F. (2021). Invasive lobular carcinoma: clinicopathological features and subtypes. J. Int. Med. Res. 49, 3000605211017039.

Debnath J., Gammoh N. and Ryan K.M. (2023). Autophagy and autophagy-related pathways in cancer. Nat. Rev. Mol. Cell Biol. 24, 560-575.

Feng Y.X., Sokol E.S., Del V.C., Sanduja S., Claessen J.H., Proia T.A., Jin D.X., Reinhardt F., Ploegh H.L., Wang Q. and Gupta P.B. (2014). Epithelial-to-mesenchymal transition activates PERK-eIF2α and sensitizes cells to endoplasmic reticulum stress. Cancer Discov. 4, 702-715.

Franco A., Almanza G., Burns J.C., Wheeler M. and Zanetti M. (2010). Endoplasmic reticulum stress drives a regulatory phenotype in human T-cell clones. Cell. Immunol. 266, 1-6.

Guo J., Ren R., Sun K., Yao X., Lin J., Wang G., Guo Z., Xu T. and Guo F. (2020). PERK controls bone homeostasis through the regulation of osteoclast differentiation and function. Cell Death Dis. 11, 847.

He C. (2022). Balancing nutrient and energy demand and supply via autophagy. Curr. Biol. 32, R684-R696.

Karali E., Bellou S., Stellas D., Klinakis A., Murphy C. and Fotsis T. (2014). VEGF Signals through ATF6 and PERK to promote endothelial cell survival and angiogenesis in the absence of ER stress. Mol. Cell 54, 559-572.

Lin Y., Jiang M., Chen W., Zhao T. and Wei Y. (2019). Cancer and ER stress: Mutual crosstalk between autophagy, oxidative stress and

inflammatory response. Biomed. Pharmacother. 118, 109249.

Lugano R., Ramachandran M. and Dimberg A. (2020). Tumor angiogenesis: causes, consequences, challenges and opportunities. Cell. Mol. Life Sci. 77, 1745-1770.

Mandula J.K., Chang S., Mohamed E., Jimenez R., Sierra-Mondragon R.A., Chang D.C., Obermayer AN., Moran-Segura C.M., Das S., Vazquez-Martinez J.A., Prieto K., Chen A., Smalley K., Czerniecki B., Forsyth P., Koya R.C., Ruffell B., Cubillos-Ruiz J.R., Munn D.H., Shaw T.I., Conejo-Garcia J.R. and Rodriguez P.C. (2022). Ablation of the endoplasmic reticulum stress kinase PERK induces paraptosis and type I interferon to promote anti-tumor T cell responses. Cancer Cell 40, 1145-1160.

Muller K., Jorns J.M. and Tozbikian G. (2022). What's new in breast pathology 2022: WHO 5th edition and biomarker updates. J. Pathol. Transl. Med. 56, 170-171.

Nie Z., Chen M., Wen X., Gao Y., Huang D., Cao H., Peng Y., Guo N., Ni J. and Zhang S. (2021). Endoplasmic reticulum stress and tumor microenvironment in bladder cancer: The missing link. Front. Cell Dev. Biol. 9, 683940.

Qi Z. and Chen L. (2019). Endoplasmic reticulum stress and autophagy. Adv. Exp. Med. Biol. 1206, 167-177.

Romine I.C. and Wiseman R.L. (2019). PERK signaling regulates extracellular proteostasis of an amyloidogenic protein during endoplasmic reticulum stress. Sci. Rep. 9, 410.

Scheu S., Stetson D.B., Reinhardt R.L., Leber J.H., Mohrs M. and Locksley R.M. (2006). Activation of the integrated stress response during T helper cell differentiation. Nat. Immunol. 7, 644-651.

Sleightholm R., Neilsen B.K., Elkhatib S., Flores L., Dukkipati S., Zhao R., Choudhury S., Gardner B., Carmichael J., Smith L., Bennion N., Wahl A. and Baine M. (2021). Percentage of hormone receptor positivity in breast cancer provides prognostic value: A single-institute study. J. Clin. Med. Res. 13, 9-19.

Sung H., Ferlay J., Siegel R.L., Laversanne M., Soerjomataram I., Jemal A. and Bray F. (2021). Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J. Clin. 71, 209-249.

Takayanagi T., Kawai T., Forrester S.J., Obama T., Tsuji T., Fukuda Y., Elliott K.J., Tilley D.G., Davisson R.L., Park J.Y. and Eguchi S. (2015). Role of epidermal growth factor receptor and endoplasmic reticulum stress in vascular remodeling induced by angiotensin II. Hypertension 65, 1349-1355.

Tan Y.Y., Zhang Y., Li B., Ou Y.W., Xie S.J., Chen P.P., Mei S.Q., Huang Q.J., Zheng L.L. and Qu L.H. (2021). PERK signaling controls myoblast differentiation by regulating MicroRNA networks. Front Cell Dev. Biol. 9, 670435.

Wang Y., Acs B., Robertson S., Liu B., Solorzano L., Wahlby C., Hartman J. and Rantalainen M. (2022). Improved breast cancer histological grading using deep learning. Ann. Oncol. 33, 89-98.

Yuan H., Zhao Z., Guo Z., Ma L., Han J. and Song Y. (2022). A novel ER stress mediator TMTC3 promotes squamous cell carcinoma progression by activating GRP78/PERK signaling pathway. Int. J. Biol. Sci. 18, 4853-4868.

Zhang K., Wang M., Li Y., Li C., Tang S., Qu X., Feng N. and Wu Y. (2019). The PERK-EIF2α-ATF4 signaling branch regulates osteoblast differentiation and proliferation by PTH. Am. J. Physiol. Endocrinol. Metab. 316, E590-E604.

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