ISSN 2706-6819 Vol.6, Issue 8: 55-59, DOI: 10.25236/IJFM.2024.060808

# Building a Prognostic Model for Breast Cancer Survival Based on Ferroptosis-Related Genes

**Wu Yingying[a], Hao Zhao[b,*]**

*Department of Oncology, The Second Naval Hospital of the Southern Theater Command of PLA, Sanya, China*
*[a]hzhhzhhzhhzhhzh@163.com, [b]18689567234@163.com*
*[*]Corresponding author*

***Abstract:*** *To develop a prognostic model for breast cancer based on the expression of ferroptosis-related proteins combined with clinical variables. Breast cancer data were downloaded from The Cancer Genome Atlas (TCGA) database for this study. The differentially expressed genes (DEGs) between tumor and normal tissues were intersected with ferroptosis-related genes. R language software was used to analyze and visualize the test cohort data to construct the prognostic model.The expressions of EMP1, TF, SLC7A5, and LMO1 were identified as independent risk factors for breast cancer. The predictive model, based on the expression levels of these four genes combined with clinical factors, showed strong predictive power for the survival status of breast cancer patients at 3, 4, and 5 years.*

***Keywords:*** *Breast Cancer, Ferroptosis, Predictive Model*

## 1. Introduction

Ferroptosis, initially proposed in the early 2000s, was formally defined in 2012[1-3]. Distinct from other forms of programmed cell death, ferroptosis cannot be induced by classical apoptosis or necroptosis inhibitors (such as Z-VAD-FMK and necrostatin-1)[3,4]. Cells undergoing ferroptosis exhibit mitochondrial shrinkage, increased mitochondrial membrane density, and reduced mitochondrial cristae[2,5]. The crucial cellular proteins involved in ferroptosis remain unclear, but it is generally believed that the execution of ferroptosis requires oxidized phospholipids (PLs) containing polyunsaturated fatty acids (PUFAs)[6]. Recently, various gene signatures related to ferroptosis have been developed as potential therapeutic targets or prognostic indicators[7-9].

Breast cancer (BC) is a prevalent malignancy among women, with various subtypes. Triple-negative breast cancer (TNBC)[10], which lacks expression of estrogen receptor, progesterone receptor, and human epidermal growth factor receptor 2 (HER2), is more common in younger and obese women, accounting for 15%-20% of all breast cancers[11]. HER2-positive breast cancer, accounting for 20%-25% of cases, is highly aggressive and has a poor prognosis [12]. Although anti-HER2 targeted therapy has significantly improved disease-free survival and overall survival in HER2-positive patients, one-third still experience recurrence, and even responders ultimately develop resistance[13-15]. Given the heterogeneity of breast cancer and the variability in treatment responses, exploring new prognostic predictors and enhancing survival prediction capabilities are crucial. This study aims to screen ferroptosis-related key molecules in breast cancer using public databases, develop a predictive model, and evaluate its prognostic efficacy.

## 2. Materials and Methods

Breast cancer data, including gender, age, pathological stage, TNM stage, survival status, and survival days, were downloaded from the TCGA database (https://portal.gdc.cancer.gov/). RNAseq data from the STAR pipeline were also downloaded and processed to extract TPM-formatted data. After excluding cases with incomplete clinical data, a total of 1086 breast cancer patients were included in the study. Ferroptosis-related genes were collected from the website (http://www.zhounan.org/ferrdb/). This study used R language software (version 4.2.2) for statistical analysis and data visualization.

ISSN 2706-6819 Vol.6, Issue 8: 55-59, DOI: 10.25236/IJFM.2024.060808

## 3. Results

### *3.1 Identification of Ferroptosis-related Genes Associated with Breast Cancer Prognosis*

In this study, 697 ferroptosis-related genes were obtained from the ferroptosis website. Gene sequencing data from 1086 breast cancer tissues and 32 adjacent non-tumor tissues in the TCGA database were screened, and 106 differentially expressed genes related to ferroptosis were identified, including 60 upregulated genes and 46 downregulated genes (Figure 1-A, Figure 1-B). Univariate and multivariate Cox regression analyses were performed on these 106 differentially expressed genes, revealing that the expression of EMP1, TF, SLC7A5, and LMO1 were independent risk factors for breast cancer (Figure 1-C).

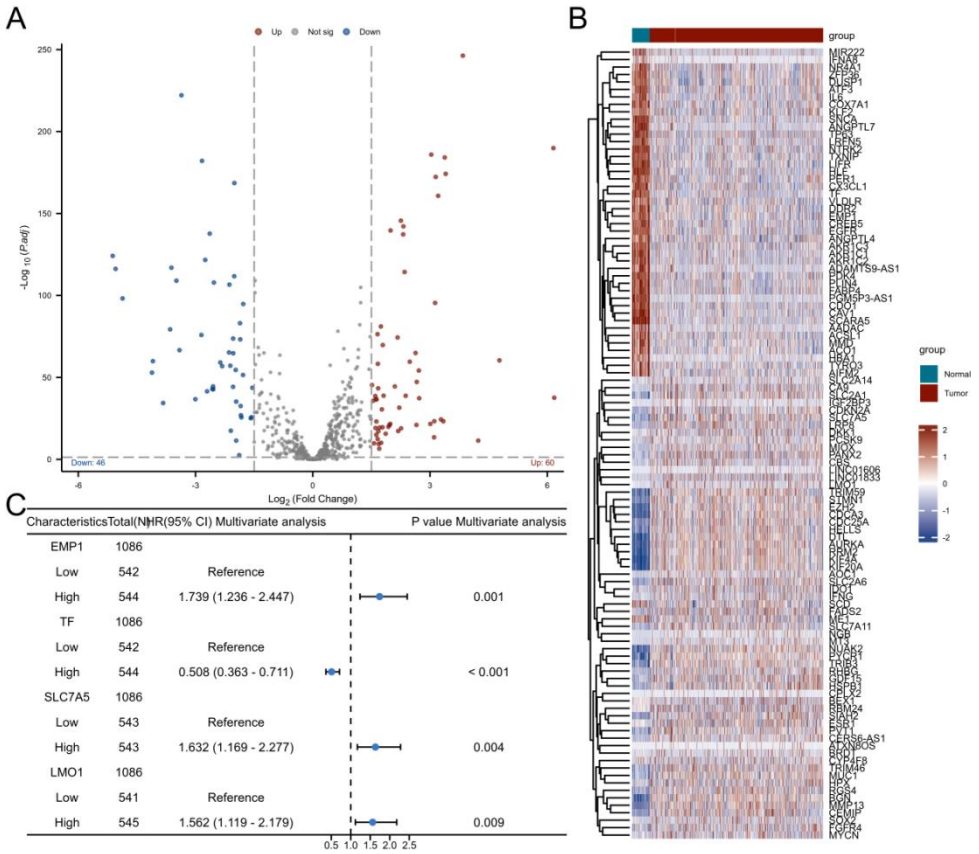


*Figure 1-A: Volcano plot of breast cancer-related ferroptosis genes. Figure 1-B: Heatmap of differentially expressed ferroptosis genes related to breast cancer. Figure 1-C: Forest plot of four independent risk ferroptosis genes for breast cancer.*

### *3.2 Establishment of a Prognostic Model Based on Differentially Expressed Ferroptosis Genes*

Firstly, risk coefficients for the four prognostic genes were derived from the multivariate Cox regression results. Then, according to the PI formula: Prognostic Index (PI) = (0.553391065 × EMP1 expression) + (-0.67700237 × TF expression) + (0.489677459 × SLC7A5 expression) + (0.445648149 × LMO1 expression), the PI value for each patient was calculated. A significant difference in survival rate was observed between the two groups ($P < 0.001$) (Figure 2-A). ROC analysis demonstrated that PI had strong predictive ability for the 3/4/5-year prognosis of breast cancer patients, with AUC values of 0.683, 0.711, and 0.680, respectively (Figure 2-B).

ISSN 2706-6819 Vol.6, Issue 8: 55-59, DOI: 10.25236/IJFM.2024.060808

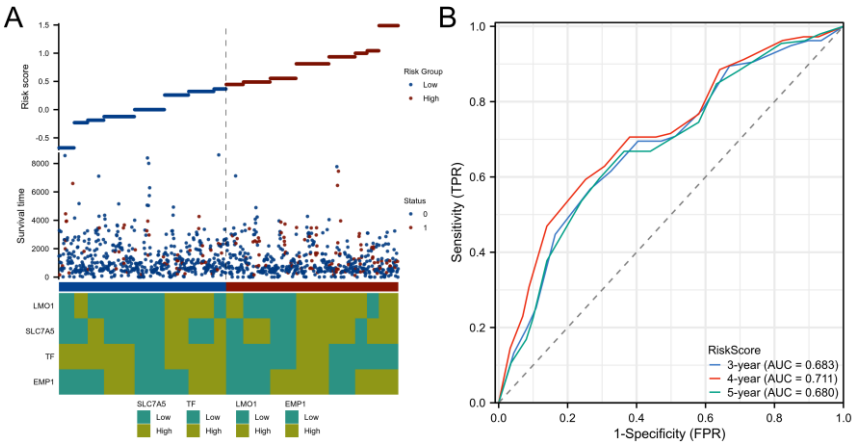


*Figure 2-A: Risk factor plot for the four independent risk ferroptosis genes in the test cohort. Figure 2-B: Receiver Operating Characteristic (ROC) curves for 3-year, 4-year, and 5-year prognosis grouped by PI values in the test cohort.*

### *3.3 Construction and Validation of a Nomogram Based on Four Ferroptosis-related Genes*

Age, T stage, N stage, M stage, and the expression of the four genes EMP1, TF, SLC7A5, and LMO1 were selected as variables for univariate and multivariate Cox regression analyses in the test cohort. The results indicated that age, N stage, and the expression of the four genes EMP1, TF, SLC7A5, and LMO1 were independent risk factors for breast cancer (Figure 3-A). A nomogram was constructed using the results of the multivariate Cox regression analysis (Figure 3-B). The prognostic calibration curve (Figure 3-C) showed that the model's confidence intervals aligned well with the standard curve, indicating a high concordance between predicted probabilities and actual probabilities.

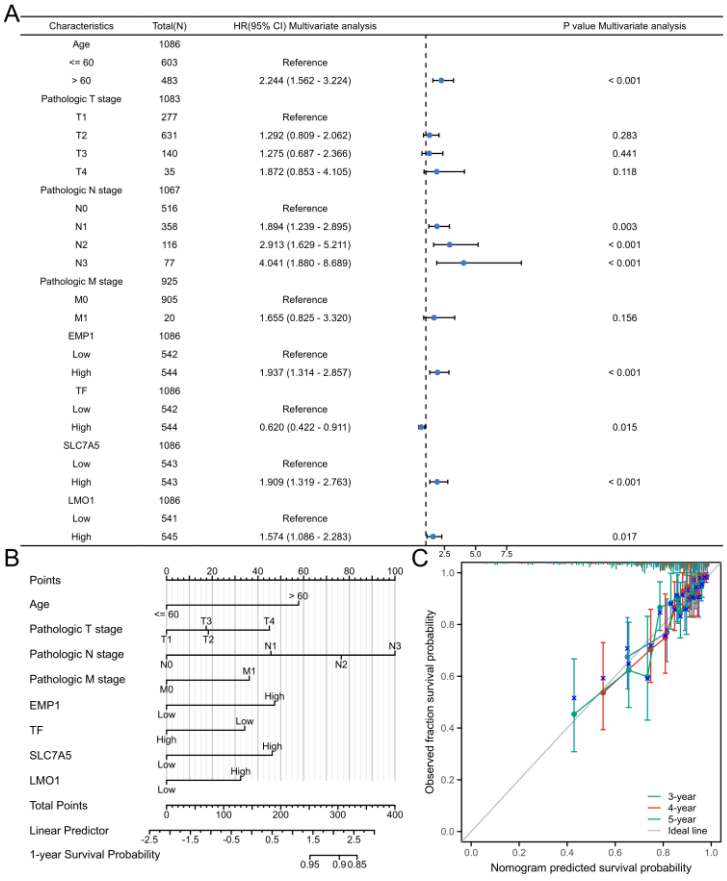


*Figure 3-A: COX regression forest plot for the test cohort. Figure 3-B: Nomogram constructed for the test cohort.*

ISSN 2706-6819 Vol.6, Issue 8: 55-59, DOI: 10.25236/IJFM.2024.060808

## 4. Discussion

According to the analysis of the prevalence of malignant tumors in China in 2016, published in 2023, breast cancer ranks third in incidence, following lung cancer and colorectal cancer. In terms of mortality, it ranks third after lung cancer and liver cancer [16]. This underscores the importance of seeking a broader predictive model based on molecular markers to guide breast cancer treatment and open up new avenues of thought. Ferroptosis, a newly established form of programmed cell death in 2012, is characterized by iron-dependent lipid peroxidation and is tightly regulated at multiple levels[17]. Ferroptosis has been elucidated to play a crucial role in some malignancies, for instance, protecting lymphoma tissue tumor cells from ferroptosis and promoting melanoma metastasis[18]. However, the relationship between ferroptosis and breast cancer remains understudied.

The EMP1 gene belongs to the same transmembrane family as peripheral myelin protein-22 (PMP-22) and plays a significant role in cell growth, proliferation, differentiation, and apoptosis, with suggested associations in tumorigenesis and progression[19]. The TF gene encodes transferrin (Tf), an iron-binding protein that facilitates cellular iron uptake. Iron-loaded Tf first binds to the Tf receptor (TfR) and enters the cell through clathrin-mediated endocytosis. Within the cell, Tf is transported to early endosomes, where it delivers iron and is subsequently recycled back to the cell surface[19]. SLC7A5 is an amino acid transporter that plays a vital role in glutamine metabolic reprogramming in TNBC cells. Knockdown of SLC7A5 significantly inhibits proliferation, migration, and invasion of human and mouse TNBC cells. Additionally, downregulation of SLC7A5 increases CD8 T cell infiltration[20]. LMO1 belongs to a family of transcriptional co-factors that act as bridges, connecting master transcription factors to control cell states during development, forming large enhancers that further amplify through interactions with MYC family proteins[21].

We established a prognostic model based on ferroptosis-related proteins combined with clinical variables using public databases and validated the model. The results showed that this model could effectively predict patient prognosis by integrating molecular expression levels and clinical factors. We found that TF expression levels were negatively correlated with breast cancer prognosis, while EMP1, SLC7A5, and LMO1 expression levels were positively correlated. Combining the TCGA database, the expression of these four ferroptosis-related genes, age, and N stage were all independent prognostic factors for breast cancer.

In conclusion, the expression levels of the four ferroptosis-related genes EMP1, TF, SLC7A5, and LMO1 are closely related to the prognosis of breast cancer. This study provides insights into the pathogenesis and progression mechanisms of breast cancer and opens up avenues for identifying potential therapeutic targets and predictive molecules in breast cancer research.

## Acknowledgement

Funding: This work was supported by Natural Science Foundation of Hainan Province (824MS174)

## References

*[1] Li YN, Xie B, Zhang Y, He MH, Xing Y, Mu DM, Wang H, Guo R. Advances and key focus areas in gastric cancer immunotherapy: A comprehensive scientometric and clinical trial review (1999-2023) World J Gastroenterol. 2023; 29:5593–5617.*

*[2] Yang WJ, Zhao HP, Yu Y, Wang JH, Guo L, Liu JY, Pu J, Lv J. Updates on global epidemiology, risk and prognostic factors of gastric cancer. World J Gastroenterol. 2023; 29:2452–2468.*

*[3] Dixon S.J., Lemberg K.M., Lamprecht M.R., Skouta R., Zaitsev E.M., Gleason C.E., Patel D.N., Bauer A.J., Cantley A.M., Yang W.S., et al. Ferroptosis: An Iron-Dependent Form of Nonapoptotic Cell Death. Cell. 2012; 149:1060–1072. doi: 10.1016/j.cell.2012.03.042.*

*[4] Yagoda N., von Rechenberg M., Zaganjor E., Bauer A.J., Yang W.S., Fridman D.J., Wolpaw A.J., Smukste I., Peltier J.M., Boniface J.J., et al. RAS–RAF–MEK-Dependent Oxidative Cell Death Involving Voltage-Dependent Anion Channels. Nature. 2007;447: 865–869. doi: 10.1038/nature05859.*

*[5] Chen X., Comish P.B., Tang D., Kang R. Characteristics and Biomarkers of Ferroptosis. Front. Cell Dev. Biol. 2021; 9:637162. doi: 10.3389/fcell.2021.637162.*

*[6] Yang W.S., Stockwell B.R. Synthetic Lethal Screening Identifies Compounds Activating Iron-Dependent, Nonapoptotic Cell Death in Oncogenic-RAS-Harboring Cancer Cells. Chem. Biol. 2008; 15:234–245. doi: 10.1016/j.chembiol.2008.02.010.*

ISSN 2706-6819 Vol.6, Issue 8: 55-59, DOI: 10.25236/IJFM.2024.060808

*[7] Hu D., Zhou Z., Wang J., Zhu K. Screening of Ferroptosis-Related Genes with Prognostic Effect in Colorectal Cancer by Bioinformatic Analysis. Front. Mol. Biosci. 2022;9:979854. doi: 10.3389/fmolb. 2022. 979854.*

*[8] Liu C., Liu Y., Yu Y., Zhao Y., Yu A. Comprehensive Analysis of Ferroptosis-Related Genes and Prognosis of Cutaneous Melanoma. BMC Med. Genom. 2022;15:39. doi: 10.1186/s 12920-022-01194-z.*

*[9] Wang N., Gu Y., Li L., Chi J., Liu X., Xiong Y., Jiang S., Zhang W., Zhong C. Identification of Novel Prognostic Risk Signature of Breast Cancer Based on Ferroptosis-Related Genes. Sci. Rep. 2022; 12:13766. doi: 10.1038/s41598-022-18044-8.*

*[10] Lebert, J. M., Lester, R., Powell, E., Seal, M., & McCarthy, J. (2018). Advances in the systemic treatment of triple-negative breast cancer. Current oncology (Toronto, Ont.), 25(Suppl 1), S142–S150.*

*[11] Caulfield, S. E., Davis, C. C., & Byers, K. F. (2019). Olaparib: A Novel Therapy for Metastatic Breast Cancer in Patients with a BRCA1/2 Mutation. Journal of the advanced practitioner in oncology, 10(2), 167–174.*

*[12] Lehmann, B. D., Bauer, J. A., Chen, X., Sanders, M. E., Chakravarthy, A. B., Shyr, Y., & Pietenpol, J. A. (2011). Identification of human triple-negative breast cancer subtypes and preclinical models for selection of targeted therapies. The Journal of clinical investigation, 121(7), 2750–2767.*

*[13] Jiang, H., Li, M., Du, K., Ma, C., Cheng, Y., Wang, S., Nie, X., Fu, C., & He, Y. (2021). Traditional Chinese Medicine for adjuvant treatment of breast cancer: Taohong Siwu Decoction. Chinese medicine, 16(1), 129.*

*[14] Robert, M., Patsouris, A., Frenel, J. S., Gourmelon, C., Augereau, P., & Campone, M. (2018). Emerging PARP inhibitors for treating breast cancer. Expert opinion on emerging drugs, 23(3), 211–221.*

*[15] Marcus, L., Donoghue, M., Aungst, S., Myers, C. E., Helms, W. S., Shen, G., Zhao, H., Stephens, O., Keegan, P., &Pazdur, R. (2021). FDA Approval Summary: Entrectinib for the Treatment of NTRK gene Fusion Solid Tumors. Clinical cancer research: an official journal of the American Association for Cancer Research, 27(4), 928–932.*

*[16] Zheng RS, Zhang SW, Sun KX, Chen R, Wang SM, Li L, Zeng HM, Wei WW, He J. [Cancer statistics in China, 2016]. Zhonghua Zhong Liu Za Zhi. 2023 Mar 23;45(3):212-220. Chinese. doi: 10.3760/cma.j.cn112152-20220922-00647. PMID: 36944542.*

*[17] Zhou, Q., Meng, Y., Li, D., Yao, L., Le, J., Liu, Y., Sun, Y., Zeng, F., Chen, X., & Deng, G. (2024). Ferroptosis in cancer: From molecular mechanisms to therapeutic strategies. Signal transduction and targeted therapy, 9(1), 55. https://doi.org/10.1038/s41392-024-01769-5*

*[18] Ubellacker JM, Tasdogan A, Ramesh V, Shen B, Mitchell EC, Martin-Sandoval MS, et al. Lymph protects metastasizing melanoma cells from ferroptosis. Nature. 2020;585:113–8. doi: 10.1038/s41586-020-2623-z.*

*[19] Nagpal A, Redvers RP, Ling X, Ayton S, Fuentes M, Tavancheh E, et al. Neoadjuvant neratinib promotes ferroptosis and inhibits brain metastasis in a novel syngeneic model of spontaneous HER2+ve breast cancer metastasis. Breast Cancer Res. 2019; 21:94. doi: 10.1186/s13058 -019-1177-1.*

*[20] Huang R, Wang H, Hong J, Wu J, Huang O, He J, Chen W, Li Y, Chen X, Shen K, Wang Z. Targeting glutamine metabolic reprogramming of SLC7A5 enhances the efficacy of anti-PD-1 in triple-negative breast cancer. Front Immunol. 2023 Sep 4; 14: 1251643. doi: 10.3389/fimmu. 2023. 1251643.*

*[21] Zhu S, Zhang X, Weichert-Leahey N, Dong Z, Zhang C, Lopez G, Tao T, He S, Wood AC, Oldridge D, Ung CY, van Ree JH, Khan A, Salazar BM, Lummertz da Rocha E, Zimmerman MW, Guo F, Cao H, Hou X, Weroha SJ, Perez-Atayde AR, Neuberg DS, Meves A, McNiven MA, van Deursen JM, Li H, Maris JM, Look AT. LMO1 Synergizes with MYCN to Promote Neuroblastoma Initiation and Metastasis. Cancer Cell. 2017 Sep 11;32(3):310-323.e5. doi: 10.1016/j.ccell.2017.08.002. Epub 2017 Aug 31.*