Prognostic Value and Immunoregulatory Mechanism of BNIP3L/Nix in Breast Cancer

NICOLE XINYU YANG

Gaston day school Shanghai shangde, No. 508 Kangyan Road, Pudong New Area, Shanghai, China

Abstract: BNIP3L/Nix, a crucial receptor for mitochondrial autophagy, is instrumental in the clearance of impaired mitochondria and the regulation of human immune-related disorders, with a notable impact on oncological diseases. Despite its importance, the precise function and underlying mechanisms of BNIP3L/Nix in breast cancer have not been fully elucidated. The objective of this research is to assess the prognostic significance of BNIP3L/Nix in breast cancer and to examine its relationship with the immune system's involvement. Materials and Methods: By leveraging datasets from several public databases, including Kaplan-Meier Plotter, ONCOMINE, GEPIA, and PrognoSan, we dissected the expression patterns and prognostic implications of BNIP3L/Nix in breast cancer. Furthermore, we scrutinized the link between BNIP3L/Nix expression and the presence of immune cells in breast cancer using the TIMER2.0 and GEPIA databases. Results: Our data analysis indicated that breast cancer tissues exhibited a notably higher expression of BNIP3L/Nix than their normal counterparts. Additional findings suggested that patients with elevated BNIP3L/Nix expression had improved survival outcomes and more favorable prognoses. Moreover, a significant positive correlation was identified between the expression of BNIP3L/Nix and the infiltration of diverse immune cell types within breast cancer, encompassing B cells, CD8+ T cells, CD4+ T cells, neutrophils, macrophages, and dendritic cells. These correlations were substantiated by the verification of immune cell-specific molecular markers. Conclusion: The research underscores the prognostic potential of BNIP3L/Nix in breast cancer, with its expression level being intimately connected to the immune cell infiltration. This discovery offers novel perspectives and potential targets for breast cancer immunotherapy.

1. Introduction

Breast cancer, representing one of the most prevalent malignant tumors in the female population, has demonstrated an escalating incidence rate year after year in recent times, posing an immense threat to women's well-being and lives (1, 2). With the rapid advancement of medical science and technology, significant breakthroughs have been made in the diagnosis and treatment of breast cancer. Nevertheless, there are still some patients who experience suboptimal prognoses following treatment and confront the risks of recurrence and metastasis. Consequently, the profound investigation of the pathogenesis of breast cancer and the quest for more precise prognostic markers and therapeutic targets have emerged as pressing issues to be addressed in the field of breast cancer research (3-5).

The role of BNIP3L/Nix, a mitochondrial autophagy receptor, has progressively garnered the attention of researchers (6, 7). Mitochondrial autophagy, an essential process in cell biology, is entrusted with the task of eliminating damaged or dysfunctional mitochondria to preserve the homeostasis of the intracellular environment (8, 9). As a crucial regulator of mitochondrial autophagy, the expression level and activity of BNIP3L/Nix in cells directly influence the efficiency and outcome of mitochondrial autophagy (10, 11). In recent years, an increasing number of studies have started to concentrate on the role of BNIP3L/Nix in tumor development. Notably, BNIP3L/Nix has exhibited its unique biological function in regulating immune responses and tumor immune evasion (12).

However, despite some advancements in cancer research, the specific role and mechanism of BNIP3L/Nix in breast cancer remain obscure. To gain a deeper understanding of the role of BNIP3L/Nix in breast cancer, this study aims to systematically investigate the expression level of BNIP3L/Nix in breast cancer and its predictive value by utilizing data from multiple public databases (13). Initially, Kaplan-Meier Plotter, ONCOMINE, GEPIA, and PrognoSan databases will be employed to comprehensively analyze the expression of BNIP3L/Nix in breast cancer tissues, including its variations in different subtypes, stages, and patient cohorts. Concurrently, the correlation between the expression level of BNIP3L/Nix and the clinicopathological characteristics, treatment efficacy, and prognosis of breast cancer patients will also be explored.

Through the progression of this research, it is anticipated that a deeper comprehension of the biological role of BNIP3L/Nix in breast cancer, as well as its interaction with the immune microenvironment of the

disease, will be achieved. This not only contributes to the delivery of more precise and personalized treatment for breast cancer patients but also holds the potential to introduce novel concepts and methods for breast cancer immunotherapy. As research and technology continue to advance, BNIP3L/Nix, a mitochondrial autophagy receptor, is likely to exhibit a wider range of applications in breast cancer research and treatment.

2. Related work

Breast cancer, a prevalent malignant tumor among women, has seen an increasing incidence rate, posing a significant threat to women's health. Recent advances in medical science have led to significant breakthroughs in the diagnosis and treatment of breast cancer, yet challenges remain in achieving optimal prognoses for some patients and preventing recurrence and metastasis. Therefore, understanding the pathogenesis of breast cancer and identifying more precise prognostic markers and therapeutic targets are urgent issues in breast cancer research [1-5].

BNIP3L/Nix, a mitochondrial autophagy receptor, has gained researchers' attention for its role in tumor development and immune response regulation [6-16]. However, its specific role and mechanism in breast cancer are not well understood. This study aims to systematically investigate the expression level of BNIP3L/Nix in breast cancer and its predictive value using data from multiple public databases [13].

Studies have shown that BNIP3L/Nix mediates mitophagy, which protects against glucocorticoid-induced synapse defects [6] and is implicated in mitophagy deficiency in ischemic brains [7]. The molecular mechanisms and implications of BNIP3L/Nix-mediated mitophagy in human diseases have been reviewed, highlighting its potential as a therapeutic target [10].

Moreover, research has indicated that BNIP3L/Nix regulates both mitophagy and pexophagy [11], and its degradation could lead to mitophagy deficiency, affecting neuronal survival in cerebral ischemia [12-16]. These findings underscore the importance of BNIP3L/Nix in cellular processes and its potential role in disease pathology.

3. Methods

Selection of databases. To thoroughly examine the predictive significance of BNIP3L/Nix in breast cancer and its correlation with immune infiltration, four authoritative databases were chosen: Kaplan-Meier Plotter, ONCOMINE, GEPIA, and PrognoSan. These databases compile a vast array of breast cancer clinical data, gene expression profiles, and immuno-infiltration information, thereby laying a solid foundation for further exploration of the function of BNIP3L/Nix in breast cancer.

Progress of data analysis. Analysis of expression level of BNIP3L/Nix in breast cancer. Data source and collation: The gene expression data of BNIP3L/Nix in breast cancer samples were retrieved from the ONCOMINE and GEPIA databases, encompassing information on the expression profiles of both normal breast tissue and various breast cancer subtypes. After data cleaning and sorting, standardized data suitable for analysis was obtained.

Expression difference analysis: It involved comparing the expression level of BNIP3L/Nix in normal breast tissue and breast cancer tissue, and evaluating the significance of the observed difference using statistical analysis methods, such as the t test and Mann-Whitney U test. The prognostic value: The prognostic value of BNIP3L/Nix expression in breast cancer patients was assessed using the Kaplan-Meier Plotter database. This analysis categorized patients into high and low expression groups based on their BNIP3L/Nix expression levels. Furthermore, a multivariate Cox regression model was employed to examine the interaction between BNIP3L/Nix expression and other prognostic factors, such as age, tumor size, and lymph node metastasis.

Research of the relationship between BNIP3L/Nix and breast cancer. Data source and collation: The datasets of BNIP3L/Nix expression and immune cell infiltration in breast cancer specimens were retrieved from TIMER2.0 and GEPIA databases. Correlation analysis: The correlation between the expression level of BNIP3L/Nix and the degree of immune cell invasion, such as the Pearson correlation coefficient and Spearman rank correlation coefficient, was calculated to investigate the association between BNIP3L/Nix expression and breast cancer immune invasion. Furthermore, regression analysis was employed to delve into the causal relationship between BNIP3L/Nix expression and immune cell infiltration. Immune cell type analysis: An investigation on the interrelation between distinct immune cell types and BNIP3L/Nix expression was conducted.

Statistical analysis method: We used indicators such as, mean, median, mode, range, variance, standard deviation, skewness, kurtosis and so on to complete descriptive statistics. Regression analysis mainly consist of descriptive statistics (the frequency of the data, central tendency, and degree of dispersion). Hypothesis testing predominantly adopted t-value, Z-value or chi-square value. Analysis of variance was completed via ANOVA.

4. Experiments

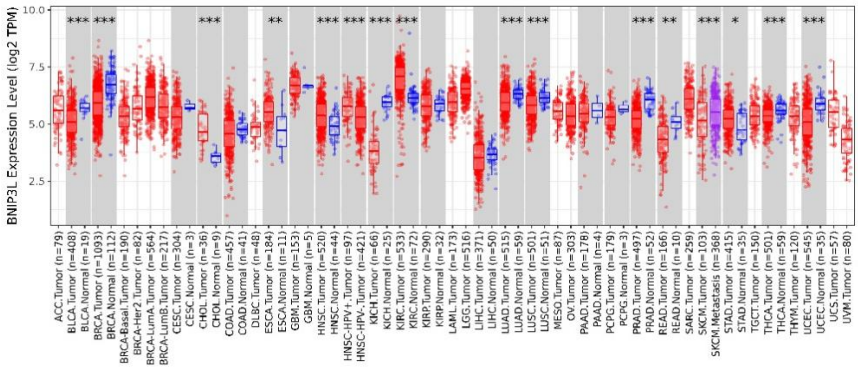

Figure 1. The expression level of BNIP3L/Nix

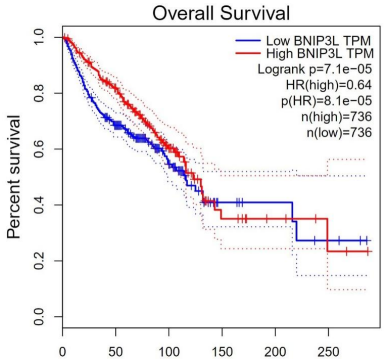

Figure 2. BNIP3L/Nix and prognosis in patients with breast cancer

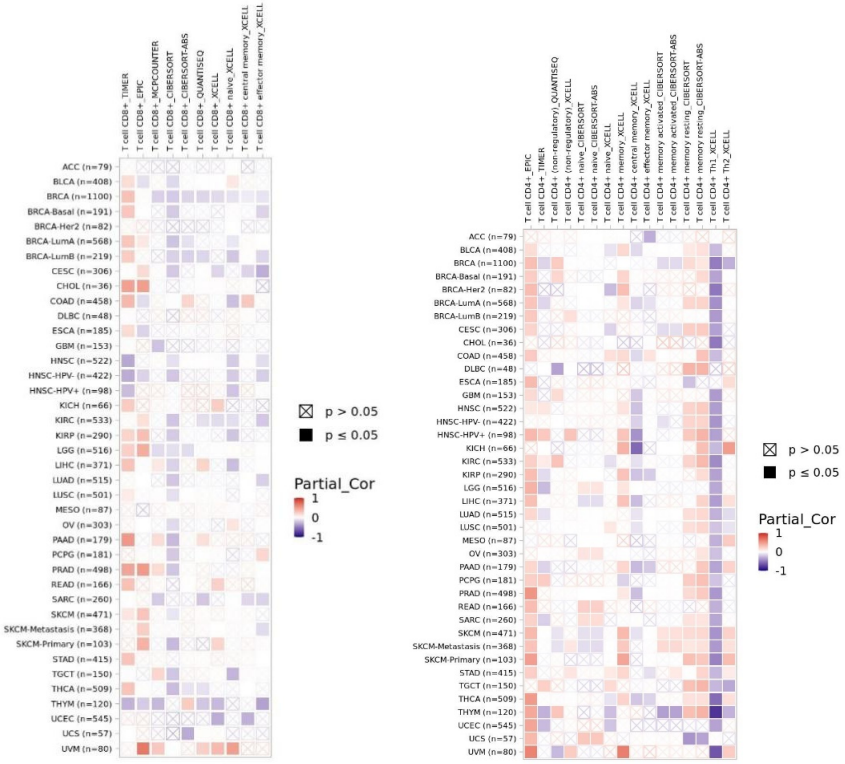

Figure 3. The heatmap between BNIP3L/Nix and immune cell infiltration

4.1. Results

Difference in the expression level of BNIP3L/Nix. The gene expression data of BNIP3L/Nix in breast cancer samples and normal breast tissue were retrieved using ONCOMINE and GEPIA databases. The data included information on the expression profiles of normal breast tissue as well as different breast cancer subtypes. The red showed the BNIP3L/Nix expression level in breast cancer

sample, while the blue represented the BNIP3L/Nix expression level in normal breast tissue. Obviously, the expression level of BNIP3L/Nix in breast cancer tissue was significantly higher than that in normal breast tissue (Figure 1). In addition, the P values are labeled in Figure 1, *, ** and *** indicate P values less than 0.05, 0.01, 0.001, respectively. These results suggest that BNIP3L/Nix may play a crucial role in the pathogenesis of breast cancer.

Relationship between BNIP3L/Nix and prognosis of breast cancer patients. Using Kaplan-Meier Plotter database, breast cancer patients were divided into high expression group and low expression group according to the expression level of BNIP3L/Nix. By conducting survival analysis, the survival rates and recurrence rates, among other prognostic indicators, of both groups were compared to evaluate the impact of BNIP3L/Nix expression on breast cancer prognosis. Further analysis revealed that breast cancer patients with elevated expression levels of BNIP3L/Nix exhibit significantly longer survival durations and improved prognoses (Figure 2). This indicates that BNIP3L/Nix could serve as a vital prognostic marker for breast cancer patients.

Correlation between BNIP3L/Nix and immune cell infiltration. The expression data of BNIP3L/Nix and infiltration data of immune cells in breast cancer samples were retrieved using TIMER2.0 and GEPIA databases. The data covered the expression levels of various types of immune cells in breast cancer tissues, the degree of infiltration, and the correlation with BNIP3L/Nix expression. A positive correlation was observed between the expression level of BNIP3L/Nix and the degree of infiltration of various immune cells, including B cells, CD8+ T cells, CD4+ T cells, neutrophils, macrophages, and dendritic cells, in breast cancer tissues (Figure 3). This finding establishes a strong connection between BNIP3L/Nix and the immune microenvironment of breast cancer.

Verification of immune cell molecular markers. The expression data of BNIP3L/Nix and infiltration data of immune cells in breast cancer samples were retrieved using TIMER2.0 and GEPIA databases. The data covered the expression levels of various types of immune cells in breast cancer tissues, the degree of infiltration, and the correlation with BNIP3L/Nix expression. To further validate the association between BNIP3L/Nix and immune cell infiltration, immune cell molecular markers, such as Th1, Th2, were employed for confirmation . The findings indicated a significant positive correlation between the expression level of BNIP3L/Nix and the extent of immune cell infiltration, thereby corroborating the initial results.

In a nutshell, the expression level of BNIP3L/Nix in breast cancer tissues is notably elevated, and it is intimately linked to the prognosis of breast cancer patients and the degree of immune cell infiltration. These results furnish novel insights and avenues for further exploration into the role of BNIP3L/Nix in breast cancer progression and its interaction with the immune system.

4.2. Discussion

Breast cancer ranks among the most prevalent malignant tumors in women globally, involving a multitude of intricate biological processes in its onset and progression (14-16). As a component of the Bcl-2 family, BNIP3L/Nix has garnered increasing attention in breast cancer research in recent years, with its role in breast cancer prognosis appraisal and immune regulation capturing the spotlight (17). The objective of this investigation was to explore the prognostic significance of BNIP3L/Nix in breast cancer and unravel its potential immunomodulatory mechanisms.

The investigation into the correlation between BNIP3L/Nix and breast cancer prognosis revealed a significant association between the expression level of BNIP3L/Nix and the survival prospects of breast cancer patients (18-20). Breast cancer patients with elevated expression of BNIP3L/Nix tend to exhibit a dismal prognosis, potentially due to the ability of BNIP3L/Nix to stimulate the proliferation, migration, and invasion of breast cancer cells (21, 22). Moreover, BNIP3L/Nix may also impact the prognosis of patients by influencing the apoptosis process of breast cancer cells. As such, monitoring the expression level of BNIP3L/Nix could aid clinicians in more accurately determining the prognosis of breast cancer patients.

The expression of BNIP3L/Nix is intimately linked to the immune status of breast cancer patients. This research discovered that BNIP3L/Nix has the potential to influence the immune microenvironment of breast cancer by regulating the functionality and quantity of immune cells. Specifically, BNIP3L/Nix could potentially diminish the immune function of patients by inhibiting the activation and proliferation of immune cells, thereby fostering the occurrence and progression of breast cancer. Furthermore, BNIP3L/Nix may also impact the recruitment and infiltration of immune cells, enabling tumor cells to evade the surveillance and elimination of the immune system (23).

The role of BNIP3L/Nix in breast cancer immunomodulation was preliminarily investigated in this study. We discovered that BNIP3L/Nix potentially alters immune cell functionality by regulating specific key signaling pathways. For instance, BNIP3L/Nix could affect the immune microenvironment of breast cancer by activating signaling pathways such as NF-κB, thereby stimulating the secretion of inflammatory factors by immune cells (24). These findings offer significant insights for further exploration of the mechanism underlying BNIP3L/Nix's role in breast cancer immunomodulation.

However, there are certain constraints to this research. Firstly, the primary emphasis of this study was on the predictive value and immunomodulatory effects of BNIP3L/Nix in breast cancer; however, the specific molecular mechanisms require further exploration. Secondly, the sample size of this research is relatively modest and might not comprehensively represent the broader landscape of BNIP3L/Nix in breast cancer. Future investigations can enhance the credibility of findings by increasing the sample size and implementing multi-center studies.

5. Conclusion

Breast cancer ranks among the most prevalent malignant tumors in women. BNIP3L/Nix, an essential regulator of apoptosis, exhibits aberrant expression in various tumors. This study provides a new perspective and idea for the research and treatment of breast cancer by deeply exploring the prognostic value and immunomodulatory mechanism of BNIP3L/Nix in breast cancer. The future research can further expand and deepen on this basis, in order to provide more beneficial enlightenment for the diagnosis and treatment of breast cancer.

Acknowledgments

This study was supported by Scientific and technological innovation project of China Academy of Chinese Medical Sciences (CI2023E002), the Central Public Welfare Research Institutes (Grant Nos.: ZZ15-YQ-065).

References

1. Kawiak A: Molecular Research and Treatment of Breast Cancer. Int J Mol Sci 23(17): 9617, 2022. DOI: 10.3390/ijms23179617.
2. Jagsi R, Mason G, Overmoyer BA, Woodward WA, Badve S, Schneider RJ, Lang JE, Alpaugh M, Williams KP, Vaught D, Smith A, Smith K, Miller KD; Susan G. Komen-IBCRF IBC Collaborative in partnership with the Milburn Foundation Inflammatory breast cancer defined: proposed common diagnostic criteria to guide treatment and research. Breast Cancer Res Treat 192(2):235-243, 2022. DOI: 10.1007/s10549-021-06434-x.
3. Shan L, Wang W, Lv K, et al. Class-incremental Learning for Semantic Segmentation in Aerial Imagery via Distillation in All Aspects[J]. IEEE Transactions on Geoscience and Remote Sensing, 2021.
4. Li M, Shan L, Li X, et al. Global-local attention network for semantic segmentation in aerial images[C]//2020 25th International Conference on Pattern Recognition (ICPR). IEEE, 2021: 5704-5711.
5. Chen L C, Zhu Y, Papandreou G, et al. Encoder-decoder with atrous separable convolution for semantic image segmentation[C]//Proceedings of the European conference on computer vision (ECCV). 2018: 801-818.
6. Shan L, Li X, Wang W. Decouple the High-Frequency and Low-Frequency Information of Images for Semantic Segmentation[C]//ICASSP 2021-2021 IEEE International Conference on Acoustics, Speech and Signal Processing (ICASSP). IEEE, 2021: 1805-1809.
7. Shan L, Li M, Li X, et al. UHRSNet: A Semantic Segmentation Network Specifically for Ultra-High-Resolution Images[C]//2020 25th International Conference on Pattern Recognition (ICPR). IEEE, 2021: 1460-1466.
8. Shan L, Wang W, Lv K, et al. Boosting Semantic Segmentation of Aerial Images via Decoupled and Multi-level Compaction and Dispersion[J]. IEEE Transactions on Geoscience and Remote Sensing, 2023.
9. Wu W, Zhao Y, Li Z, et al. Continual Learning for Image Segmentation with Dynamic Query[J]. IEEE Transactions on Circuits and Systems for Video Technology, 2023.
10. Shan L, Zhou W, Zhao G. Incremental Few Shot Semantic Segmentation via Class-agnostic Mask Proposal and Language-driven Classifier[C]//Proceedings of the 31st ACM International Conference on Multimedia. 2023: 8561-8570.
11. Shan L, Zhao G, Xie J, et al. A Data-Related Patch Proposal for Semantic Segmentation of Aerial Images[J]. IEEE Geoscience and Remote Sensing Letters, 2023, 20: 1-5.
12. Zhao G, Shan L, Wang W. End-to-End Remote Sensing Change Detection of Unregistered Bi-temporal Images for Natural Disasters[C]//International Conference on Artificial Neural Networks. Cham: Springer Nature Switzerland, 2023: 259-270.
13. Shan L, Wang W, Lv K, et al. Boosting Semantic Segmentation of Aerial Images via Decoupled and Multi-level Compaction and Dispersion[J]. IEEE Transactions on Geoscience and Remote Sensing, 2023.
14. Shan L, Wang W, Lv K, et al. Class-Incremental Semantic Segmentation of Aerial Images via Pixel-Level Feature Generation and Task-Wise Distillation[J]. IEEE Transactions on Geoscience and Remote Sensing, 2022, 60: 1-17.
15. Shan L, Wang W. DenseNet-Based Land Cover Classification Network with Deep Fusion[J]. IEEE Geoscience and Remote Sensing Letters, 2021, 19: 1-5.
16. Shan L, Wang W. MBNet: A Multi-Resolution Branch Network for Semantic Segmentation of Ultra-High Resolution Images[C]//ICASSP 2022-2022 IEEE International Conference on Acoustics, Speech and Signal Processing (ICASSP). IEEE, 2022: 2589-2593.