Breast Cancer: Targets and Therapy

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ORIGINAL RESEARCH

IDO1, IL4I1: Novel Immune Checkpoints in Breast Cancer Tumor-Associated Macrophages

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Purpose: Increasing evidence supports the critical role of immune cell infiltration in breast cancer progression. Tumor-associated macrophages (TAMs) undergo metabolic reprogramming during polarization, which is vital for immune responses. Here, we screened for genes associated with TAMs infiltration and involved in tumor metabolism, and investigated their expression and effects in breast cancer.

Patients and Methods: Bioinformatics analysis was used to screen genes related to immune cell infiltration and involved in tumor metabolism. Immunohistochemistry (IHC) was performed to validate the expression of selected target genes. Multiplex immunohistochemistry (mIHC) was applied to investigate the localization and expression relationship between target genes and TAMs. Real-time quantitative PCR (qRT-PCR) was used to detect the expression of target genes in TAMs. The Human Protein Atlas was utilized for single-cell clustering analysis of breast cancer to assess the expression patterns of target genes, while also evaluating the correlation between target genes and immune checkpoint expression.

Results: Database analysis revealed that Indoleamine2,3-Dioxygenase1 (IDO1) and Interleukin 4 Induced 1 (IL4I1) are highly expressed in breast cancer, with their expression closely associated with immune cell infiltration, particularly macrophage infiltration, which exhibited the highest infiltration rate. IHC analysis revealed that both IDO1 and IL4I1 were expressed in breast cancer, which were mostly located in TAMs. mIHC co-localization demonstrated that IDO1 and IL4I1 were both expressed in TAMs, and qRT-PCR results confirmed increased expression of IDO1 and IL4I1 in TAMs. Single-cell analysis of breast cancer revealed that IDO1 and IL4I1 were most highly expressed in c-19 macrophages, and their expression was positively correlated with most immune checkpoints.

Conclusion: This study suggests that IDO1 and IL4I1, which are involved in tumor metabolism, may play an important role in regulating TAMs immune infiltration in breast cancer. Therefore, IDO1 and IL4I1 are potential therapeutic targets for breast cancer.

Keywords: breast cancer, immune cell infiltration, metabolic reprogramming, IDO1, IL4I1, tumor microenvironment

Introduction

Breast cancer remains one of the most common malignant tumors and the leading cause of tumor-related deaths for females worldwide.[1] Triple negative breast cancer (TNBC), which is the highest malignancy among breast cancer subtypes, accounts for 10–20% of all breast cancer cases. Due to the lack of estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor (HER2), there are still a lack of standard therapeutic strategies available for TNBC.[2]

Tumor microenvironment (TME) is a complex ecosystem containing tumor cells, immune cells, stromal cells, and multiple signaling molecules. This intricate network coordinates key malignant biological behaviors including tumor progression, metastasis, and immune escape.[3] In the breast cancer microenvironment, with restricted local availability of oxygen and nutrients, these environmental changes allow for the accumulation of metabolites and alteration in pH. Tumor progression is profoundly influenced by metabolic stress in the local microenvironment.

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Increasing evidence has indicated that immune cells infiltration plays a pivotal role in the response of breast cancer patients to standardized therapies, and differences in the degree of infiltration by distinct immune cells are closely correlated with tumor progression and prognosis.[4–6] Consequently, developing immunotherapeutic approaches that target the immune microenvironment may significantly improve survival outcomes for breast cancer patients. Simultaneously, immune cells undergo metabolic reprogramming during proliferation, differentiation, and effector function execution, and metabolic disorders have a significant impact on immune cells infiltration.[7] Reprogramming-mediated immunosuppression of TME plays a critical role in tumor immune escape. Among the various mechanisms involved, dysregulated amino acid metabolism in immune cells is a key factor contributing to impaired anti-tumor immunity functions.[8] Therefore, elucidating the interactions between immune regulation and metabolic reprogramming in breast cancer is essential for developing targeted therapeutic strategies.

In this study, we use bioinformatics analysis to identify key genes Indoleamine2,3-Dioxygenase1 (IDO1) and Interleukin 4 Induced 1 (IL4I1) expressed in tumor associated macrophages (TAMs), which are involved in amino acid metabolic reprogramming and regulate immune infiltration. These findings may provide novel insights for targeted therapy and clinical prognosis of breast cancer.

Materials and Methods

Data Collection

Transcriptome gene expression data of 1222 breast samples (tumor samples: 1109/normal samples: 113) were obtained from the Cancer Genome Atlas (TCGA) database (accessed on 13 October 2023). Documents pertaining to human tumor metabolic genes obtained from the GSEA website (www.gsea-msigdb.org) (accessed on 21 November 2023).

Metabolism-Related Differentially Expressed Gene Analysis

The files of human tumor metabolism-related genes were obtained through the GSEA website. The expression levels of these metabolic genes in 1222 breast samples were downloaded from the TCGA database, processed and analyzed using Perl programming language. The difference analysis was conducted using limma package of R language software (Rx64 4.0.2 version), and the paired difference analysis was carried out using the ggpubr package to merge, filter and analyze the data, and then screened out metabolism-related differentially expressed genes. The thresholds of metabolism-related differentially expressed genes were set as $|\log2FC| > 0.5$ and p value< 0.05.

Analysis of Characteristic Genes in Immune Cells

Using the ESTIMATE algorithm to search immune infiltration in breast cancer, we extracted differentially expressed genes associated with immune cell characteristics from gene expression data of 1222 breast samples in the TCGA database. The ESTIMATE algorithm is a method that uses gene expression features to infer the proportion of immune cells in tumor samples. It can estimate the content of immune cells and stromal cells in malignant tumor tissues by analyzing transcriptomic data, predicting immune scores and stromal scores, and consequently extract immune cell-specific genes and stromal cell-specific genes.[9] The thresholds of immune cell-specific genes were set as $|\log2FC| > 1$ and p value< 0.05.

Functional Enrichment Analysis

GSEA enrichment analysis was performed using GSEA software (version GSEA4.0.3) to analyze potential signaling pathway enrichment in the high-expression and low-expression groups.

Immunohistochemistry (IHC)

The archived paraffin-embedded tissue sections from 50 patients diagnosed with invasive breast cancer at the Affiliated Hospital of Inner Mongolia Medical University between 2018 and 2020, including 30 cases of non-triple negative breast cancer and 20 cases of triple negative breast cancer, were retrospectively collected for immunohistochemical staining using monoclonal antibodies. IDO1 primary antibody (1:400, Cell Signaling Technology, USA, Catalog No. 86630) and

IL4I1 primary antibody (1:1600, Abcam, USA, Catalog No. ab222102). EDTA antigen retrieval solution (Catalog MVS-0099), animal non-immune serum (Catalog SP KIT-B3), ready-to-use immunohistochemistry kit (Catalog number KIT-9921), and DAB color development kit (Catalog DAB-0031) were all purchased from Maixin Company, Fuzhou. Experimental procedures were performed in accordance with the instructions provided by each reagent. Immunohistochemically stained slides were evaluated by experienced pathologists using a standardized scoring scheme (staining showing cytoplasmic brownish-yellow color and stained cell count exceeding 10% were judged positive results) to ensure repeatability and minimize observer bias. (The scoring method we used was the CPS, which was calculated as follows. CPS= (number of positive tumor cells + number of positive lymphocytes + number of positive macrophages) / total number of tumor cells × 100).

Multiplex Immunohistochemical (mIHC)

Tissue sections were subjected to dewaxing and rehydration, antigen retrieval, blocking, and incubation with differentially labeled fluorescent primary antibodies. IDO1 primary antibody (1:100, Cell Signaling Technology, USA, Catalog No. 86630) and IL4I1 primary antibody (1:50, Abcam, USA, Catalog No. ab222102). CD163 primary antibody (1:300, Abcam, USA, Catalog No. ab182422). The sections were incubated with the secondary antibodies provided by the four-color mIHC staining kit (Aibixin Biotechnology, Shanghai, Catalog No. ABS50012-100T). Add fluorescence staining signal amplifier and incubate DAPI working solution for 5 minutes then drop the anti-fluorescence quencher in the kit and seal the slide.

Cell Culture

MDA-MB-231 breast cancer cell line and THP-1 human monocyte macrophage cell line were purchased from Procell Life Science Technology, Wuhan. RPMI-1640 medium, fetal bovine serum (FBS), penicillin-streptomycin and trypsin were obtained from Gibco, USA. MDA-MB-231 breast cancer cells and THP-1 cells were cultured in complete medium containing 89% RPMI-1640 medium+10% FBS+1% penicillin-streptomycin, and placed in cell culture incubator (37°C, 5% CO_2).

Cell Co-Culture

When MDA-MB-231 breast cancer cells reached 80% to 90%, we switched to the medium containing 1% serum and cultured them for 24 hours. The medium from MDA-MB-231 breast cancer cells was collected after centrifugation at 500*g for 20 minutes, and then filtered, mixed with double volume of medium containing 10% FBS to prepare the conditioned medium. Meanwhile, THP-1 cells were cultured with PMA for 36 hours to induce differentiation into M0 macrophages. Subsequently, the M0 macrophages were cultivated in the conditioned medium for 7 days (with medium changes every 3 days) to differentiate into TAMs.

Quantitative Real-Time PCR (qRT-PCR)

The processed M0 macrophages and TAMs were collected in 2 mL enzyme-free EP tubes for detecting the mRNA expression levels of TAMs markers. Total RNA was extracted using 2 mL of TransZol Up reagent and subsequently reverse transcribed into cDNA. The reaction system was prepared according to the PerfectStart Green qRT-PCR SuperMix kit (TransGen Biotech, Beijing, Catalog No. AQ601), and amplification was performed on the qRT-PCR system. Reaction conditions: 94°C for 5 seconds, 55°C for 15 seconds, 72°C for 10 seconds, repeated for 50 cycles. The relative mRNA expression levels were normalized to the internal control gene GAPDH and expressed as $2^{-\Delta\Delta Ct}$. $\Delta Ct = Ct_{IDO1/IL4I1} - Ct_{GAPDH}$, $\Delta\Delta Ct = \Delta Ct_{sample} - \Delta Ct_{control\ sample}$. The sequences of the primer are listed below: The sequences of the primer are listed below:

IDO1: 5′-AGACTGCTGGTGGAGGACATGCT-3′ (forward) and 5′-TTCTCCTTTGGCTGCTGGCTTGC-3′ (reverse); IL4I1: 5′-GCCTCAGCGACAGACTCCAGTA-3′ (forward) and 5′-GCCTTCAGCACCTTCAGATTCCG-3′ (reverse); CD163: 5′-TGGTAGATGGAGTCACTGAATGT-3′ (forward) and 5′-CCCTGGCAAGAAACGCTGTC-3′ (reverse); GAPDH: 5′-GGAGCGAGATCCCTCCAAAAT-3′ (forward) and 5′-GGCTGTTGTCATACTTCTCATGG-3′ (reverse).

Statistical Methods

Statistical analysis was conducted using SPSS 25.0, GraphPad Prism 8.0.2, and R 4.0.2 software. (SPSS 25.0 was used for immunohistochemical (IHC/mIHC) data GraphPad Prism 8.0.2 was applied for the visualization and statistical analysis of experimental data (qRT-PCR results). R 4.0.2 software was used for differential expression analysis of metabolism-related and immune-related genes). All experiments were repeated three times. P value < 0.05 was considered statistically significant. Measurement data are presented as mean ± standard deviation (mean ± SD). Data between two groups were compared using *t*-test, and data among multiple groups were compared using two-way analysis of variance (ANOVA).

Results

In Breast Cancer, IDO1 and IL4I1 are Genes Involved in Tumor Metabolism and Associated with Immune Infiltration

After analyzing the expression levels of metabolic genes in breast samples (tumor/normal) from the TCGA database, 428 differentially expressed tumor metabolism-related genes were identified (Supplementary Table 1). Using the ESTIMATE algorithm, 1437 differentially expressed genes associated with immune features were obtained (Supplementary Table 2). The intersection of these two groups was taken, with selection criteria of logFC > 1.5 and *p* value< 0.05, resulting in IDO1 and IL4I1 as the final target genes (Figure 1). (The supplementary table is large, so it is uploaded as a file).

Gene Expression Analysis of IDO1 in Breast Cancer

The differential analysis of normal breast tissue samples and tumor samples from the TCGA database demonstrated that IDO1 was significantly expressed in tumor samples, with $p = 0.010$, indicating a statistically significant difference (Figure 2A). Paired differential analysis showed that there were significant differences in IDO1 expression between normal and cancer tissues from the same samples, with IDO1 higher expression observed in cancerous tissues, $p<0.001$ (Figure 2B). GSEA functional enrichment analysis indicated that high IDO1 expression was predominantly associated with immune-related functions, including “antigen processing and presentation”, “T-cell receptor signaling pathway”,

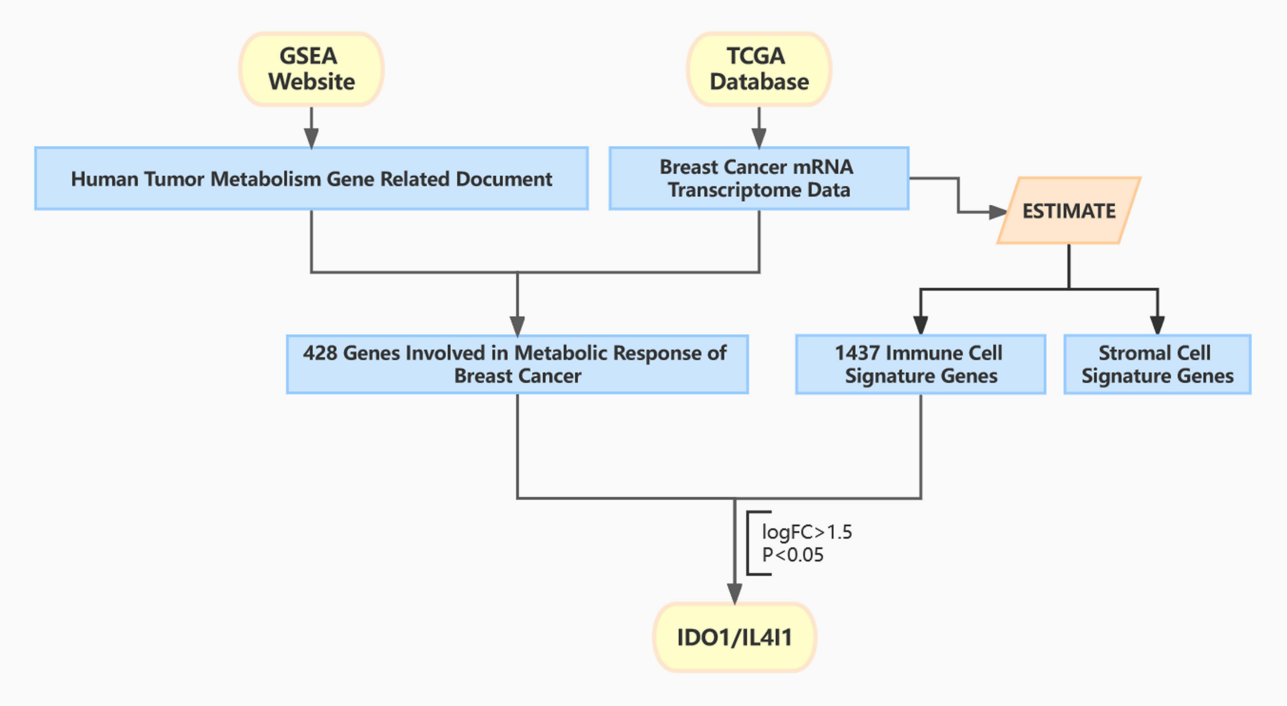

Figure 1 A schematic diagram of bioinformatics methods for screening target genes.

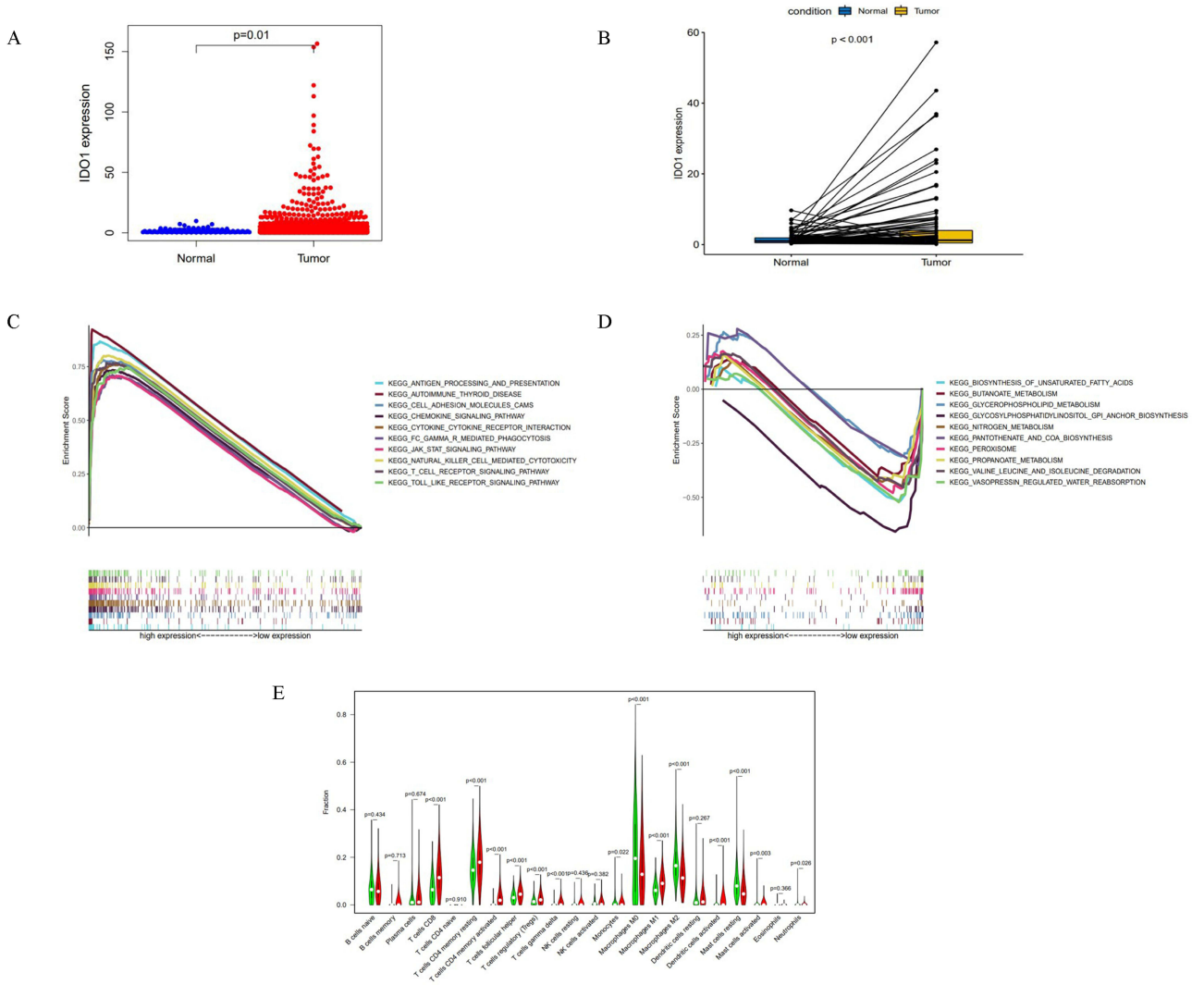

Figure 2 Gene expression analysis of IDO1 in Breast Cancer (**A**) Difference analysis (**B**) Paired difference analysis (**C**) GSEA enrichment analysis at high expression (**D**) GSEA enrichment analysis at low expression (**E**) correlation analysis with immune cell infiltration (In the figure, the green group represents the low expression group and the red group represents the high expression group).

"cell adhesion molecules (CAMs)", "chemokine signaling pathway", "natural killer cell-mediated cytotoxicity", "Toll-like receptor signaling pathway", and "JAK-STAT signaling pathway" (Figure 2C). These findings suggest that IDO1 contributes to regulating immune response in breast cancer. In contrast, when IDO1 is expressed at normal or low levels, its functions are primarily involved in metabolic pathways such as "amino acid metabolism" and "lipid metabolism" (Figure 2D). This indicates that IDO1, as a gene involved in metabolic reactions, also participates in regulating immune signaling pathways in breast cancer, exerting a dual role. Furthermore, the correlation between IDO1 expression levels and immune cell infiltration was assessed. The results showed that IDO1 expression was significantly associated with the infiltration of various immune cells, including $CD8^+$ T cells, $CD4^+$ T cells, and macrophages, with the most pronounced association observed for macrophages whether IDO1 was highly expressed or lowly expressed (Figure 2E).

Gene Expression Analysis of IL4I1 in Breast Cancer

IL4I1 was also found highly expressed in breast cancer, with $p < 0.001$ (Figure 3A). Paired analysis revealed that IL4I1 expression was markedly higher in breast cancer tissue compared to normal tissue from the same sample, with $p < 0.001$, indicating a statistically significant difference (Figure 3B). GSEA enrichment analysis revealed that high expression of IL4I1 was associated with functional pathways such as "antigen processing and presentation", "T-cell receptor signaling

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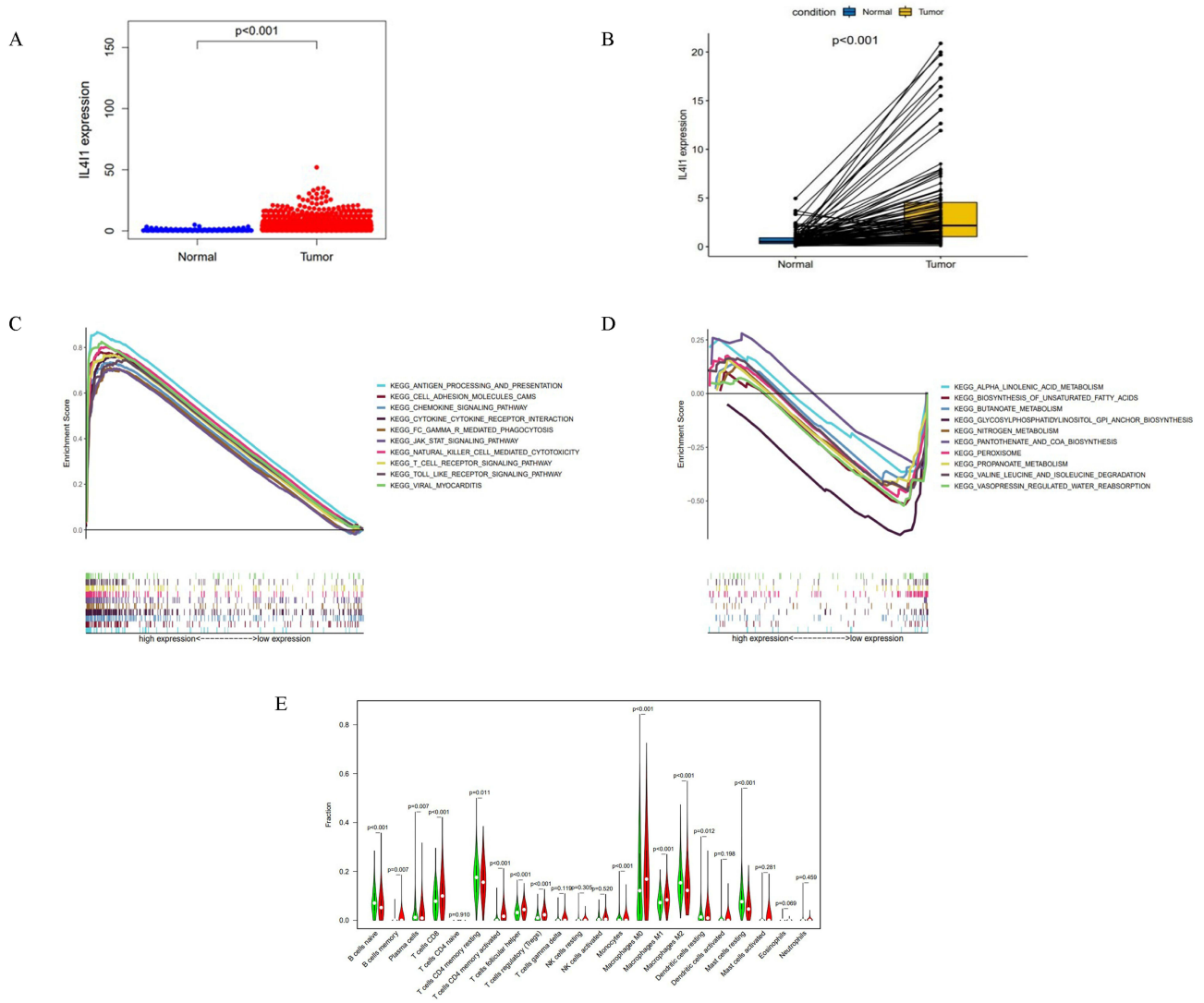

Figure 3 Gene expression analysis of IL4I1 in Breast Cancer (**A**) Difference analysis (**B**) Paired difference analysis (**C**) GSEA enrichment analysis at high expression (**D**) GSEA enrichment analysis at low expression (**E**) correlation analysis with immune cell infiltration (In the figure, the green group represents the low expression group and the red group represents the high expression group).

pathway", "natural killer cell-mediated cytotoxicity", and "Toll-like receptor signaling pathway" (Figure 3C). In contrast, when IL4I1 was expressed at low or normal levels, its functional enrichment was primarily observed in metabolic pathways, such as "amino acid metabolism" (Figure 3D), suggesting a functional similarity between IL4I1 and IDO1. Furthermore, correlation analysis of immune cell infiltration indicated that IL4I1 expression was significantly associated with the infiltration of various immune cells, including B cells, $CD8^{+}$T cells, $CD4^{+}$T cells, and macrophages, among which macrophage infiltration accounts for the highest proportion (Figure 3E).

Transcriptome Data Analysis of Breast Cancer: IDO1 and IL4I1 are Highly Expressed in TNBC

From the 1109 breast cancer samples in the TCGA database, 100 samples with negative ER, PR, and HER2 expression (TNBC) were selected for validation. The expression levels of IDO1 and IL4I1 in these samples were analyzed and compared with those of the remaining 1009 non-TNBC. The results showed that both IDO1 and IL4I1 were highly expressed in TNBC, with statistical significance ($P < 0.001$) (Figure 4).

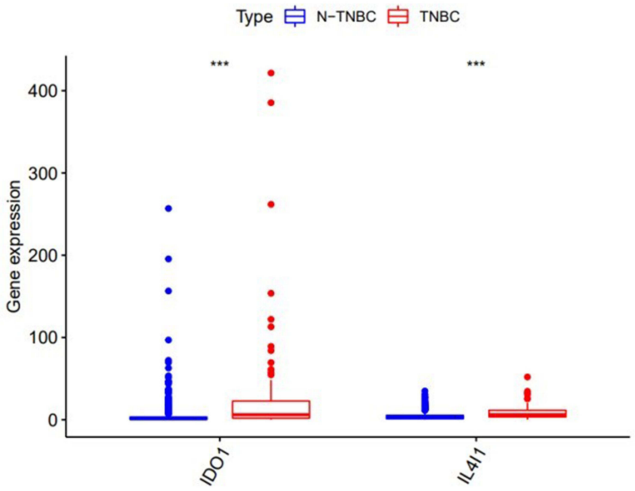

Figure 4 Transcriptome data analysis of breast cancer: Differential expression of IDO1 and IL4I1 in TNBC and non-TNBC (*** indicates P<0.001).

IDO1 and IL4I1 Expression in Breast Cancer

Based on IHC staining results, IDO1 and IL4I1 were both predominantly expressed in immune cells infiltrating the TME, particularly in TAMs and a small proportion detected in lymphocytes, with both IDO1 and IL4I1 locating in the cytoplasm. When comparing IHC staining results between 30 cases non-TNBC and 20 cases TNBC, it was found that IDO1 and IL4I1 were both expressed at higher levels in TNBC (Figure 5). Although the difference was not statistically significant, this trend aligns with the results of bioinformatics analysis.

Expression of IDO1 and IL4I1 in TAMs

Correlation analysis of IDO1 and IL4I1 expression in breast cancer with immune infiltration revealed that both were most highly expressed in macrophages, and the majority of immune cells infiltrated around the tumor were TAMs. The

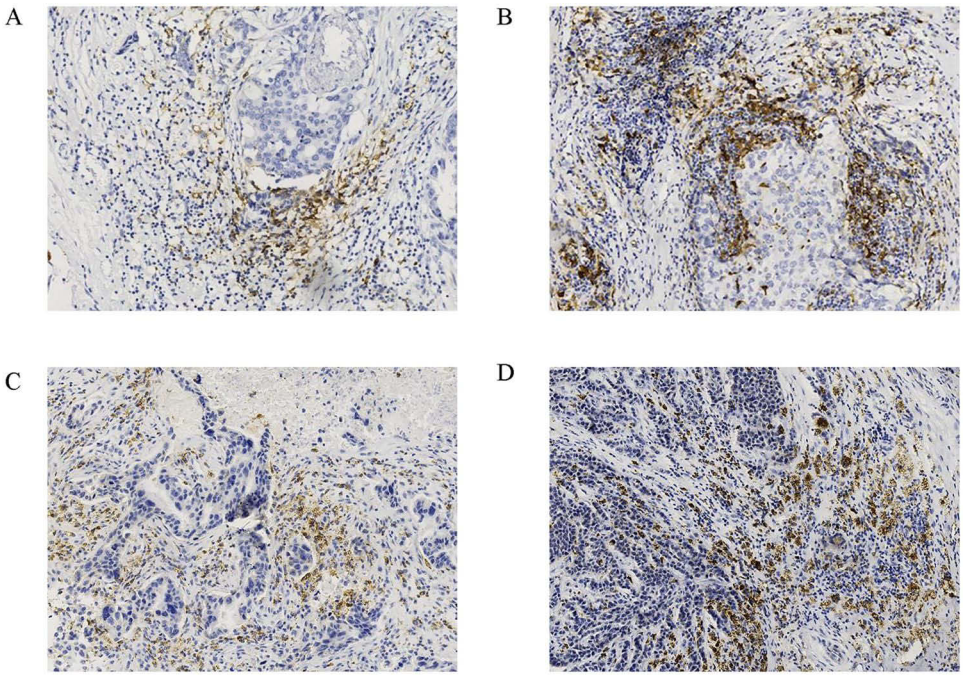

Figure 5 IDO1 and IL4I1 expression in IHC Magnification×20 (**A**) IDO1 expression in non-TNBC (**B**) IDO1 expression in TNBC (**C**) IL4I1 expression in non-TNBC (**D**) IL4I1 expression in TNBC.

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expression of IDO1 and IL4I1 in TAMs was assessed using mIHC, with TAMs specifically identified by the marker CD163.[10] The results demonstrated that both IDO1 (Figure 6A) and IL4I1 (Figure 6B) were expressed in TAMs within the TME.

mRNA Expression of IDO1 and IL4I1 in TAMs

qRT-PCR was applied to detect the mRNA expression of IDO1 and IL4I1 in TAMs. The results demonstrated that the mRNA expression of IDO1 and IL4I1 in TAMs induced by tumor-conditioned medium increased to varying degrees, and the differences were statistically significant (Figure 7).

The Human Protein Atlas Breast Cancer Single Cell Clustering Analysis of IDO1 and IL4I1 Expression

It was observed that both IDO1 and IL4I1 exhibited the highest expression levels in c-19 macrophages of breast cancer single cell analysis (Figure 8A and B). The normalized transcripts per million (nTPM) value of IDO1 in c-19 macrophages was measured at 64.5, with a z-score of 2.11 (Figure 8C). Similarly, the nTPM value of IL4I1 in the same cell population was 40.7, with a z-score of 2.23 (Figure 8D). Meanwhile, CD163 had the highest expression in c-19Macrophages, with an nTPM value of 114.1 and a zScore of 3.05 (Figure 8E and F). These findings confirm that IDO1 and IL4I1 are most highly expressed in c-19 macrophages, which also exhibit the highest CD163 content, indicating that in breast cancer, IDO1 and IL4I1 primarily function within $CD163^{+}$ macrophages.

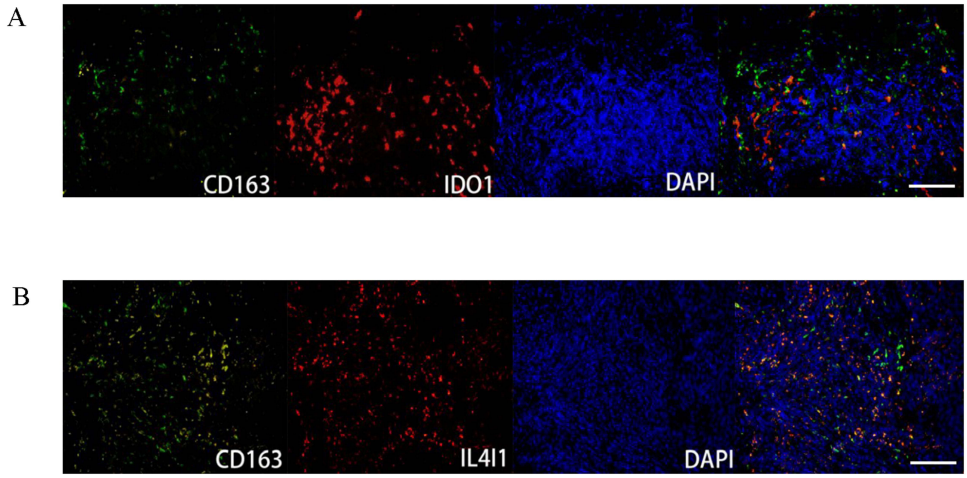

Figure 6 Expression of IDO1 and IL4I1 in mIHC (**A**) Co-localization of IDO1 and CD163. (**B**) Co-localization of IL4I1 and CD163. (Green indicates CD163+ cells; red indicates the target antibody staining; blue represents cell nucleus; yellow indicates the co-localization regions. Magnification×200; Scale bar=100 μm).

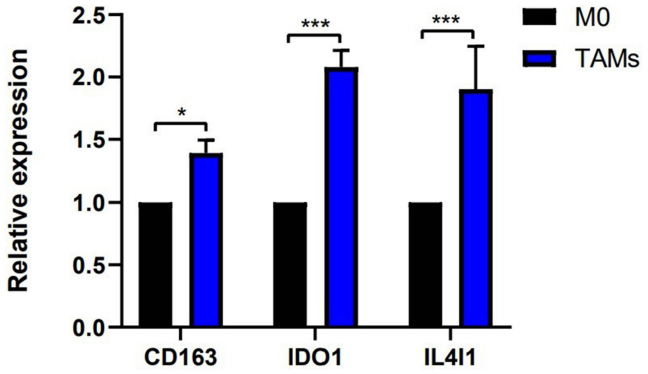

Figure 7 Relative mRNA expression of CD163, IDO1 and IL4I1 in M0 macrophages and TAMs (* represents P<0.05; *** represents P<0.001).

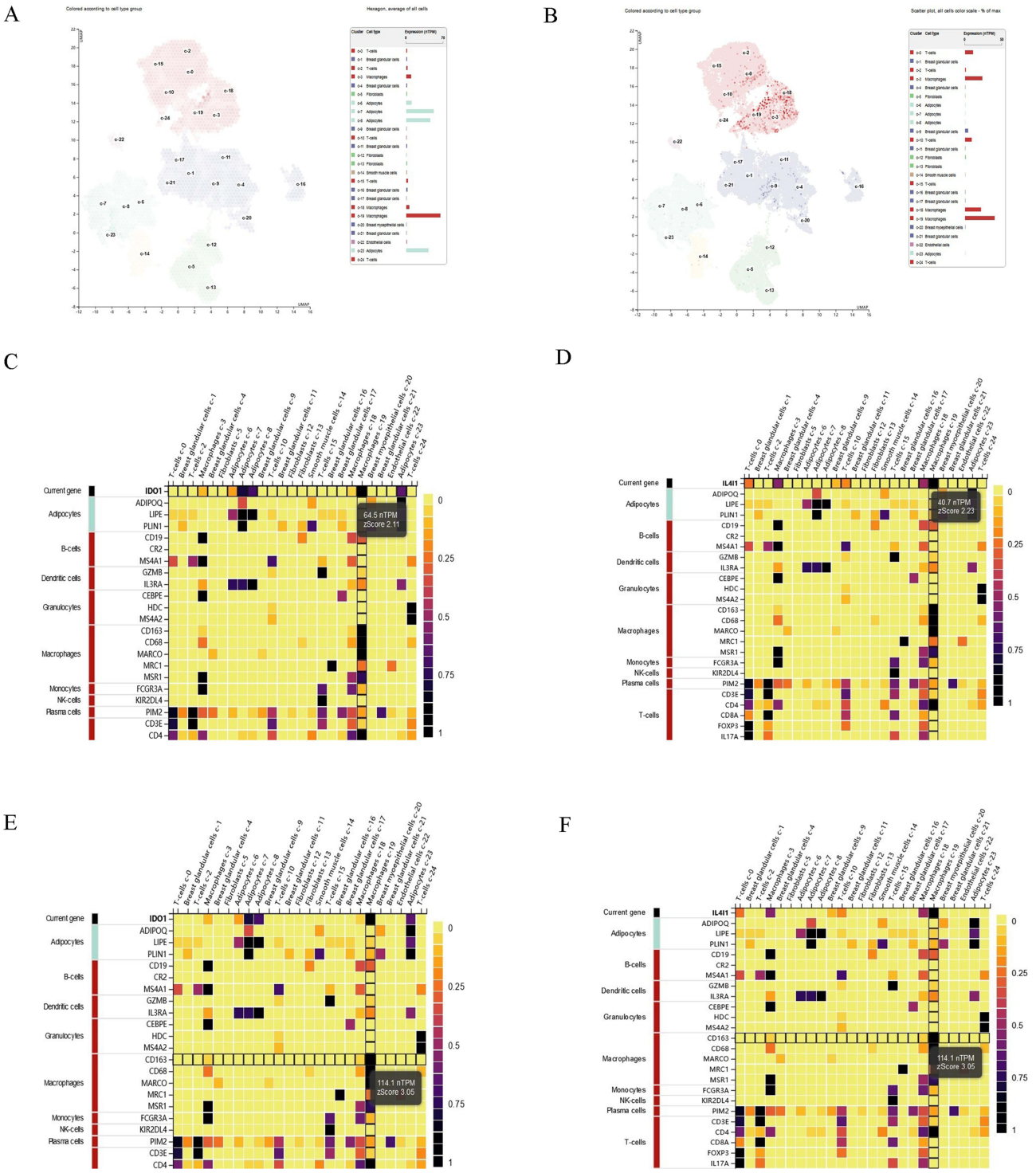

Figure 8 The Human Protein Atlas Breast Cancer Single Cell Clustering Analysis of IDO1 and IL4I1 expression (**A**) Content expression of IDO1 in single cell cluster analysis of breast cancer (**B**) Content expression of IL4I1 in single cell cluster analysis of breast cancer (**C**) Specific expression of IDO1 in c-19 macrophages (**D**) Specific expression of IL4I1 in c-19 macrophages (**E**) Specific expression of CD163 in c-19 macrophages (**F**) Specific expression of CD163 in c-19 macrophages.

Correlation Analysis of IDO1 and IL4I1 Expression with Immune Checkpoints in Breast Cancer

Considering the potential tumor-promoting role of IDO1 and IL4I1 in breast cancer, we summarized the important immune checkpoints involved in tumor immune escape and assessed their correlation with each immune checkpoint. The results showed that IDO1 was significantly positively correlated with CD274 (R=0.67) and IL4I1 was significantly

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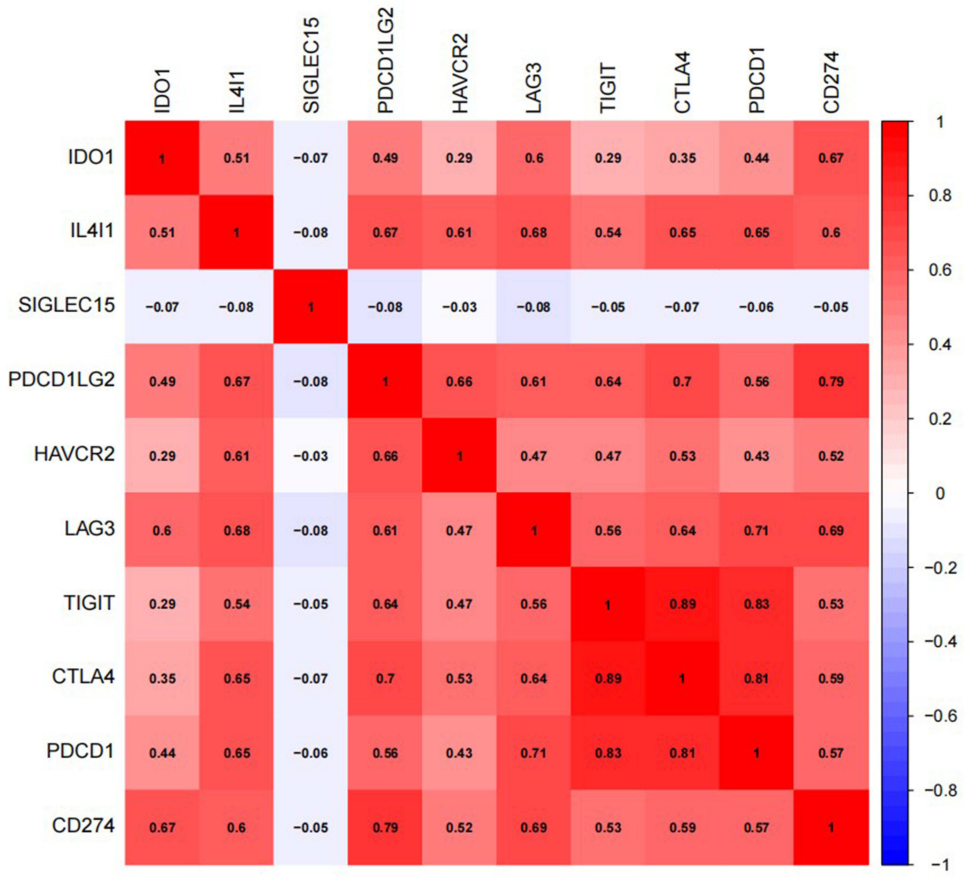

Figure 9 Correlation analysis between IDO1, IL4I1, and immune checkpoints.

positively correlated with Lymphocyte activation gene-3 (LAG3) (R = 0.68) (Figure 9). CD274, also known as programmed death-ligand 1 (PD-L1), has been demonstrated to exert a role in regulating immune responses. Activation of the PD-1/PD-L1 signaling pathway facilitates tumor immune escape, while blocking this pathway enhances the body's intrinsic antitumor immune effects.[11] LAG3 is also a co-inhibitory receptor expressed on activated $CD4^+$ and $CD8^+$ T cells, which promotes tumor immune escape.[12] The positive correlations between IDO1 and IL4I1 expression and multiple immune checkpoints suggest that these genes may contribute to immune evasion in breast cancer.

Discussion

The composition and abundance of immune cells in the TME significantly influence tumor progression and the efficacy of immunotherapy.[13] A variety of immune cells infiltrating the TME and the cytokines they secrete can promote tumor growth, invasion, and metastasis, which participate in tumorigenesis and tumor development. The composition of immune cells in the TME is highly complex, and they play diverse roles at different stages of tumor progression. Among these, TAMs are the most abundant innate immune cell population.[14] Both M1 and M2-polarized TAMs coexist within the same tumor. M1-type TAMs exert antitumor effects by recognizing and eliminating tumor cells, whereas M2-type TAMs promote tumor progression by facilitating tumor growth, invasion, and metastasis.[8] In the microenvironment, M2-type TAMs predominate. Unlike functionally normal macrophages, M2-type TAMs highly express CD163 and secrete anti-inflammatory cytokines such as IL-10 and TGF-β, promoting Th2 cell differentiation and participating in immune regulation, angiogenesis, and other processes that facilitate tumor progression while mediating immunosuppressive effects in the TME.[15,16] The polarization of TAMs is regulated by multiple factors, including amino acids and their metabolic products.[17]

IDO1 is an intracellular heme-dependent oxidase that converts tryptophan into kynurenine (Kyn).[18] Kyn, as an endogenous ligand of the aryl hydrocarbon receptor (AHR), can activate AHR transcription, thereby boosting the expression of the immunosuppressive cytokine IL-10, promoting the activation of Tregs, and suppressing the proliferation and function of effector T cells.[19] Furthermore, the Kyn-AHR axis has been shown to upregulate PD-1 expression on $CD8^+$ T cells and recruit TAMs that exert immunosuppressive effects.[20,21] Recent studies have identified another enzyme, IL4I1, as an L-type amino acid oxidase that generates bioactive metabolites during tryptophan consumption. IL4I1 promotes tumor progression and suppresses adaptive immunity by activating the AHR through the production of indole metabolites and kynurenic acid.[22] In the TME, IL4I1 expression is often upregulated and is thought to be involved in immunosuppressive mechanisms, impairing the function of anti-tumor T cells. CB-668, a highly selective small-molecule inhibitor of IL4I1 enzymatic activity, exhibits anti-tumor efficacy primarily dependent on CD8+ T cells, and its combination with anti–PDL1 immune checkpoint inhibitors (ICIs) further enhances the anti-tumor response.[23] In a clinical study characterizing and quantifying IL4I1-expressing immune cells in peripheral blood and tissue samples from patients with cutaneous melanoma to evaluate the prognostic value of IL4I1, preclinical evidence has suggested that IL4I1 expression may promote melanoma progression and immune evasion, highlighting its potential as an important biomarker for assessing patient prognosis and predicting the efficacy or resistance to (ICIs). In the TME, IDO1 and IL4I1 exhibit overlapping expression patterns, particularly in myeloid cells, where both enzymes regulate the tryptophan metabolic network and are intricately involved in immune modulation and various tumor biological behaviors.[18] Research indicates that IL4I1 derived from TAMs can directly inhibit the anti-tumor immune response by suppressing $CD8^+$ T cell proliferation and function, while simultaneously promoting Treg activation which indirectly facilitates tumor immune escape.[24] Collectively, IDO1 and IL4I1 not only contribute to amino acid metabolic regulation but also play pivotal roles in immune cell infiltration and tumor immune escape, positioning them as key metabolic immune checkpoints.

At present, breast cancer remains a major threat to women's health and well-being, and current methods for predicting prognosis and identifying treatment targets have certain limitations, particularly for patients with TNBC, who generally exhibit poorer outcomes.[25] Therefore, identifying novel biomarkers to predict prognosis and improve individualized treatment strategies is of critical significance. In this study, we utilized the TCGA database to analyze the expression of IDO1 and IL4I1 in breast cancer and found that the expression of IDO1 and IL4I1 in breast cancer was higher than that in normal tissues. Furthermore, IHC experiments revealed that the expression of IDO1 and IL4I1 were higher in TNBC patients than in non-TNBC patients, consistent with the transcriptomic data. Previous studies have shown that IDO1 and IL4I1 are tryptophan metabolic enzymes predominantly expressed in immune cells that participate in receptor regulation and are associated with immune response regulation.[18] Meanwhile, the study by Xu et al found that in TNBC, IL4I1 expression is also correlated with marker genes of M2-type TAMs. GO and pathway enrichment analyses indicate that IL4I1 may serve as a potential target to enhance the efficacy of immune checkpoint blockade (ICB) therapy by reprogramming the TME.[26] GSEA enrichment analysis also confirmed that IDO1 and IL4I1 are involved in immune cell infiltration, which plays a crucial role in the TME. mIHC results demonstrated that IDO1 and IL4I1 are primarily expressed on TAMs, in accordance with single-cell clustering analysis of breast cancer from The Human Protein Atlas. Additionally, the mRNA expression levels of IDO1 and IL4I1 were elevated on $CD163^+$ TAMs. We speculate that the expression of these two genes may promote the polarization of TAMs toward the M2 type, thereby facilitating tumor immune escape. Correlation analyses between IDO1 and IL4I1 expression and immune checkpoint molecules in breast cancer further supports this hypothesis.

The Phase III clinical trial of the IDO1 inhibitor failed, and in the analysis of failure, it was found that AHR may possess alternative activation pathways leading to resistance against IDO1 inhibition.[27] Studies have revealed that IL4I1 exhibits the highest correlation with AHR-related modules, surpassing even IDO1 and tryptophan-2,3-dioxgenase (TDO2), suggesting that IL4I1 is the most strongly associated with AHR activity in various human cancer. It has been proposed that the failure of the clinical trial may be attributed to IL4I1, as IDO1 inhibitors are incapable of suppressing its activity.[28] IL4I1 is considered a promising therapeutic target, and the potential clinical benefits of dual inhibition of IDO1 and IL4I1 warrant further investigation. Our experimental results demonstrate that both IDO1 and IL4I1 are expressed at higher levels in TNBC. We hypothesize that tryptophan metabolism mediated by IDO1 and IL4I1 in TNBC

enhances tumor immunosuppression and facilitates immune escape. Therefore, simultaneously inhibiting the expression of IDO1 and IL4I1 may serve as a therapeutic target for TNBC.

Although our study provides new insights into the expression of IDO1 and IL4I1 on TAMs among breast cancer patients, there are still several limitations. Firstly, this research only involved a single dataset, which may introduce selection bias. Secondly, the correlation between IDO1 and IL4I1 expression and tumor-infiltrating immune cells within the breast cancer microenvironment still needs to be elucidated through carefully designed experiments. Additionally, the biological functions and underlying mechanisms of IDO1 and IL4I1 in breast cancer TAMs require further investigation.

Conclusions

In summary, this research utilizes bioinformatics analysis to integrate the study of genes involved in tumor metabolism and the regulation of immune cell infiltration in breast cancer. Our results indicate that IDO1 and IL4I1 in breast cancer participate in immune regulatory signaling pathways that influence immune cell infiltration and are related to tumor immune escape, potentially promoting tumor growth. Given the emerging evidence on the immunoregulatory roles of IDO1 and IL4I1, further retrospective and prospective studies are warranted to determine their potential as predictive biomarkers for immunotherapy response. Comparative analyses with established markers such as PD-L1 will be particularly valuable to clarify whether IDO1 and IL4I1 can provide complementary or superior predictive value in identifying patients most likely to benefit from ICIs. In conclusion, our study suggests that in breast cancer, IDO1 and IL4I1 not only serve as genes involved in tumor metabolism but also influence immune cell infiltration and immune escape within the TME, providing new potential targets for personalized treatment and improved outcomes in breast cancer.

Data Sharing Statement

All data generated or analyzed during this study are included in this published article (and its Supplementary Information files). The datasets used and analyzed during the current study are available from the Corresponding Authors (Yongfeng Jia or Xia Liu) on reasonable request.

Ethics Approval

This study has been approved by the Institutional Review Board of Inner Mongolia Medical University (No. YKD202101117). The need for informed consent was waived owing to the retrospective design of the study. This study strictly adheres to the principles of the Declaration of Helsinki. During the research process, the privacy and personal data of the participants were strictly protected. All identifiable personal information has been deleted, and the data was analyzed anonymously to ensure that they were used solely for research purposes and not disclosed under any circumstances.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

Aorong Shi and Yanrong An are co-first authors for this study. The authors report no conflicts of interest in this work.

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