Role of PAX2 in breast cancer verified by bioinformatics analysis and *in vitro* validation

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Background: Breast cancer (BC) is the most frequently diagnosed cancer in women and the second most common cancer among newly diagnosed cancers worldwide. Studies have shown that paired box 2 (PAX2) participates in the tumorigenesis of some cancer cells, but its role in BC is still unclear.

Methods: Transcriptome expression profiles and clinicopathological information of BC were downloaded from The Cancer Genome Atlas (TCGA) database to explore the expression level and prognostic value of PAX2. Gene set enrichment analysis (GSEA) and functional enrichment analysis were performed to investigate the functions and pathways of PAX2. Moreover, real-time reverse transcriptase-polymerase chain reaction (RT-qPCR) was used to determine the expression of PAX2 in BC tissues, and the predictive value of PAX2 in clinical samples was assessed. Cell Counting Kit-8 (CCK-8) assay was used to evaluate cell growth. The migration and invasive capacities of cells were assessed by wound healing assay and Transwell assay.

Results: PAX2 was upregulated in the TCGA-BC datasets. GSEA suggested that PAX2 may be involved in the regulation of signaling pathways such as MAPK. Moreover, PAX2 was overexpressed in BC tissues, and PAX2 expression was associated with tumor size and lymph node metastasis. PAX2 deficiency could promote the growth, migration, and invasion of BC cells.

Conclusions: Upregulation of PAX2 inhibited BC cell growth, migration, and invasion, making PAX2 a potential therapeutic target for BC.

Keywords: Breast cancer (BC); invasion; migration; paired box 2 (PAX2); The Cancer Genome Atlas (TCGA)

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Introduction

Breast cancer (BC) is the most frequently diagnosed cancer in women and ranked second in the causes of cancer-related deaths in women, representing a significant public health problem on a global scale. In 2016, there were more than 1,600,000 new diagnoses and more than 500,000 cases of deaths related to BC worldwide (1,2). Early diagnosis of BC is essential to reduce morbidity and mortality. Although clinical and pathological indicators are generally applied in screening and early diagnosis, their sensitivity and specificity are usually limited (3,4). Thus, discovering new biomarkers for detecting early BC, disease monitoring and prognostication is critical. Treatment of BC is multidisciplinary, including surgery, neoadjuvant therapy, and radiation therapy (5,6). However, despite the widespread use of hormonal agents and adjuvant

chemotherapy, which have improved the overall survival of BC patients, the prognosis of those with metastasis remains unsatisfactory (7,8).

Paired box 2 (PAX2) is a transcription factor that belongs to the paired homeobox domain family. It can bind to nuclear protein containing the BRCT domain, which participates in the methylation of histone H3 at lysine 4 and plays an important role in repairing DNA damage (9-11). Jahangiri *et al.* reported that high expression of PAX2 correlated with better survival in tamoxifen-treated BC patients (12), and Beauchemin *et al.* indicated that overexpression of PAX2 in BC cells reduced their invasive ability (13). Among estrogen receptor (ER)-positive (cancers with 1–100% of cells positive for ER expression are considered ER-positive) patients treated with tamoxifen, PAX2 appears to be a positive prognostic factor in premenopausal patients, and a negative factor in postmenopausal patients (14). However, these studies did not indicate the expression and function of PAX2 in BC.

Bioinformatics is one of the latest fields of biological research, which is the processing and analysis of genomics data to understand the therapeutic progress of diseases (15). In this study, we used it to investigate the effects of PAX2 in BC, in conjunction with *in vitro* validation experiments. Our results of data mining and bioinformatics analysis indicated that PAX2 is overexpressed in BC patients. Furthermore, PAX2 was upregulated in BC tissue samples, and its expression was associated with tumor size and lymph node metastasis. However, downregulation of PAX2 in MCF7 and MB231 cell lines significantly enhanced cell proliferation, migration, and invasion. Our study data suggested that PAX2 is a tumor suppressor gene in BC. These findings have not yet been reported. We present the following article in accordance with the MDAR reporting checklist (available at https://atm.amegroups.com/article/view/10.21037/atm-22-6360/rc).

Highlight box

Key findings

- PAX2 deficiency could promote the growth, migration, and invasion of breast cancer (BC) cells.

What is known and what is new?

- Jahangiri *et al.* reported that high expression of PAX2 correlated with better survival in tamoxifen-treated BC patients.
- We used bioinformatics to investigate the effects of PAX2 in BC, in conjunction with *in vitro* validation experiments.

What is the implication, and what should change now?

- This study has some limitations. First, molecular mechanism verification was not performed. Second, the protein localization and levels were not analyzed because of the difficulty of sample collection and insufficient funds.
- In the future, more basic and clinical research should be performed to explore these aspects.

Methods

BC datasets

The transcriptome expression profiles and corresponding clinical information of BC patients (1,104 tumor samples and 113 normal samples) were downloaded from the UCSC database (http://xena.ucsc.edu/), and the R packages "affy" and "limma" were used to normalize and summarize the datasets. Differentially expressed genes were identified by "limma". The fold change was calculated and P values were adjusted by the Benjamini-Hochberg method. In this study, the cut-off criteria were $P<0.05$ and $|logFC| >1$.

Weighted gene coexpression network analysis (WGCNA)

WGCNA, a systems biology method to explore the correlation patterns among genes, was used to identify modules of highly correlated genes and relate modules to clinical traits. In this study, WGCNA was performed by R package "WGCNA" (http://www.genetics.ucla.edu/labs/horvath/CoexpressionNetwork/Rpackages/WGCNA) (16). WGCNA has a built-in function to test genes and samples, by which basic filtering can be performed. In order to make the constructed network more in line with the characteristics of the scale-free network, the algorithm can judge from the clustering tree of the samples, and eliminate outliers. In this study, the height calculated by the algorithm was 140. Using the function "hclust" in the R software (17), the clustering method adopted was the average distance (method = "average"). By setting a clustering distance threshold, genes can be clustered into modules. In this study, the function cutreeDynamic in the dynamicTreeCut package was used, and the number of genes contained in the smallest module was set to 30. In order to quantify the coexpression similarity of each module, we calculated the eigengenes of each module. Combining the modules uses the distance matrix calculated by the Pearson correlation coefficient between the eigenvalues of the modules; that is, the correlation between two modules. If sex is >0.75, it will be merged into a new module. An adjacency matrix was calculated to analyze the expression correlation between genes. Subsequently, the

topological overlap matrix (TOM) was constructed based on the adjacency matrix. TOM quantitatively describes the similarity of the nodes. Gene modules were identified by hierarchical clustering (minModuleSize =30), and the threshold to merge similar modules was 0.25. Finally, correlations of modules with clinicopathological information were calculated to explore their clinical significance.

Gene set enrichment analysis (GSEA)

GSEA was used to extract biological insights from genome-wide RNA expression information. GSEA explores gene sets that share common chromosomal location, biological function, or regulation. In this study, GSEA was used to examine the potential functions and molecular mechanisms of PAX2. The BC patients were divided into low and high expression groups using the median value (0.141682) of PAX2 expression level as the threshold. Next, the transcriptome expression profiles were input into GSEA software (http://www.gsea-msigdb.org/gsea/index.jsp, version 4.1.0). Those with P<0.05, and false discovery rate (FDR) P<0.05 were considered to be statistically significant.

Functional enrichment analysis

Gene Ontology (GO) enrichment analysis was used to describe the function of gene and gene products, and Kyoto Encyclopedia of Genes and Genomes (KEGG, www.kegg.jp/kegg/kegg1.html) analysis was used to annotate genes with pathway and functional information. In this study, Metascape (18) (http://metascape.org/gp/index.html, database last update date: 2021-08-01), a web-based database, was used to perform GO and KEGG analyses on PAX2-related modules identified in the WGCNA analysis.

Human tissue samples and cell lines

In this study, BC tissue samples and tumor-adjacent normal tissues were obtained from 68 patients at the Fourth Hospital of Hebei Medical University (Shijiazhuang, China). All patients were female, and the median age was 61.32±12.28 years. The inclusion criteria were pathologically confirmed BC without distant metastasis and complete clinicopathological and survival data. The exclusion criteria were BC with recurrence or distant metastasis at the time of first diagnosis, >2 primary tumors, and male patients. The study protocol was approved by the Ethics Committee of the Fourth Hospital of Hebei Medical University (approval No. 2022KY394), and our study was performed in accordance with the Declaration of Helsinki (as revised in 2013) and International Committee of Medical Journal Editors Privacy and Confidentiality Guidelines. Informed consent was given by all patients at the time of sample collection.

With reference to the literature (19-23), BC cell lines MCF7 (Procell, Wuhan, China, Catalog No. CL-0149) and MB231 (Procell Catalog No. CL-0150B) were obtained from the Pathology Laboratory of the Cancer Institute of the Fourth Hospital of Hebei Medical University. Cell lines were maintained in DMEM-H medium supplemented with fetal bovine serum (FBS, Thermo Fisher Scientific, Waltham, MA, USA), 50 units/mL penicillin and 50 μg/mL streptomycin (Gibco, Gaithersburg, MD, USA), 2 mM L-glutamine (Gibco) at 37 ℃ in a humidified 5% CO_2 atmosphere. Experiments using two cell lines belong to biological replicates.

Real-time reverse transcriptase-polymerase chain reaction (RT-qPCR)

The expression of PAX2 in the BC tissue samples was measured by RT-qPCR. Total RNA was extracted by TRIzol® reagent (Thermo Fisher Scientific, USA, catalogue number: 15596026). With reference to the literature (24,25), GAPDH was used as an internal control. The reverse transcription reaction was performed by a Reverse Transcription Kit (GeneCopoeia, Guangzhou, China, catalogue number: QP007). qPCR was performed by Mastercycler5333 (Eppendorf, Hamburg, Germany). The relative expression of genes was calculated using the 2ΔΔCq method. The primers used in this experiment were as follows:

PAX2 forward, 5'-CTGGTCGTGACATGGC-3', PAX2 reverse, 5'-GGGTTGCACACAAGGG-3';

GAPDH forward, 5'-TGTGTCCGTCGTGGATCTGA-3', GAPDH reverse, 5'-CCTGCTTCACCACCTTCTTGA-3'

Cell transfection

Small interfering RNA targeting PAX2 (si-PAX2, 5'-CCAG GAGACUCAAGGUCCAAUGUUU-3') and the negative control (si-NC, 5'-UUCUCCGAACGUGUCACGUTT-3') were constructed by GenePharma (Shanghai, China). MCF7 and MB231 cells were transfected with si-PAX2 or si-NC by Lipofectamine2000 transfection reagent (Invitrogen, catalogue number: 11668030). Transfection efficiency was

evaluated after 24 h.

Cell Counting Kit-8 (CCK-8) assay

The CCK-8 assay was performed to evaluate the effect of overexpression of PAX2 on the proliferation of MCF7 and MB231 cells according to manufacturer's instructions. Cells were divided into normal control, si-NC, and si-PAX2 groups. MCF7 and MB231 cells were seeded into 96-well plates (6×10^3 cells/well) and the CCK-8 solution (10 µL; Ruisai Biotechnology, Shanghai, China, catalogue number: D002-1) was added to each well, followed by 10 µL DMSO. The optical density (OD) 450 values were read on a spectrophotometer.

Wound healing assay

The wound healing assay was performed to evaluate the effect of PAX2 overexpression on cell migration. Cells were divided into normal control, si-NC, and si-PAX2 groups. MCF7 and MB231 cells were plated in 6-well plates with 5×10^5 cells/well. After 12 h, a wound was created by manually scratching the monolayer of cells with the tip of a 200-µL pipette. Cells were imaged at 0 and 48 h after the wound was created.

Transwell assay

The Transwell assay was performed to evaluate the effect of PAX2 overexpression on cell invasion and migration. Cells were divided into normal control, si-NC, and si-PAX2 groups. MCF7 and MB231 cells (1×10^5 cells in 0.2 mL serum-free medium/well) were transferred to the upper Transwell chamber (Corning, NY, USA). Medium supplemented with 20% FBS was added to the lower chamber. Cells were cultured under 37 ℃ and 5% CO_2. After 24 h, the upper chamber was cleaned and cells in the lower chamber were mixed with 100% methanol, stained with 0.1% crystal violet and counted.

Statistical analysis

The Kaplan-Meier method was used to estimate the overall survival of BC patients with high or low PAX2 expression. The log-rank test was used to compare the survival distribution between groups. The performance of PAX2 as a BC biomarker was evaluated by receiver operating characteristic (ROC) curve analysis. The Chi-square test was used to calculate the correlation between PAX2 expression and clinical tumor characteristics. Quantitative and qualitative variables are expressed as mean ± SD and absolute frequencies/percentages respectively. The difference between two groups was analyzed by Student's *t*-test. $P<0.05$ was considered statistically significant. All statistical analyses were conducted using R software (https://www.R-project.org, version 3.5.3) and SPSS 20.0 (SPSS Inc., Chicago, USA). All experiments were performed in triplicate.

Results

Bioinformatics analysis of PAX2

Analysis of the transcriptome expression profiles and corresponding clinical information of BC patients downloaded from the UCSC database revealed that PAX2 was overexpressed in BC patients (P=1.365e-09; *Figure 1A*). For the WGCNA analysis exploring the correlation between PAX2 and BC, a hierarchical clustering tree was first constructed, which obtained 18 modules (*Figure 1B*). The heatmap and dendrogram of all genes and WGCNA-related modules (*Figure 1C,1D*) suggested no significant differences in the interactions among modules. These modules had a high degree of independence. PAX2 was in the green-yellow module, and the correlation between the modules and BC was –0.62 (*Figure 1E*). All genes in the green-yellow module are shown in *Figure 1F*. As shown in *Figure 1G*, the area under the ROC curve was 0.673, indicating that PAX2 could be a diagnostic marker for BC. Because the purpose of this study was to explore the role of PAX2 in BC, the ROC values for other genes are not presented here.

GSEA and functional enrichment analysis

The BC patients were divided into low and high expression groups based on the median value of PAX2 expression level and then compared by GSEA. GO analysis based on GSEA showed that microbody lumen, peroxisome organization, and protein localization to cell junctions were upregulated in BC (*Figure 2A*), while CXCR chemokine receptor binding, negative regulation of innate immune response, and negative regulation of the metaphase anaphase transition of the cell cycle were downregulated (*Figure 2B*). KEGG analysis revealed that the ABC transporters, hedgehog signaling pathway, and MAPK signaling pathways were upregulated

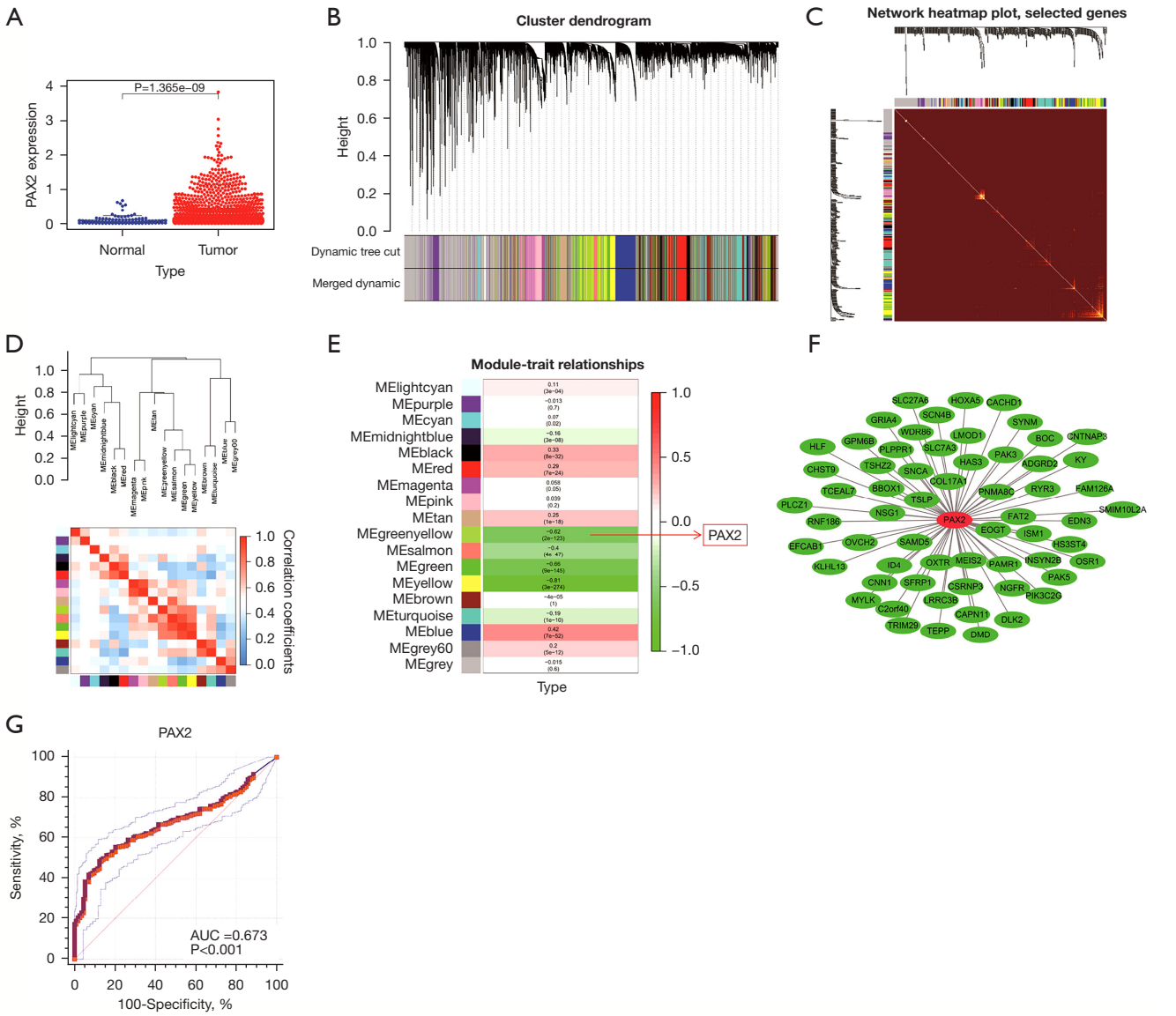

Figure 1 Differential expression analysis and WGCNA analysis of the genes in the TCGA database. (A) Box diagram of PAX2 showing differential expression between BC and normal groups. (B) Repeated hierarchical clustering tree of all genes, and PAX2 is in the green-yellow module. (C) Dendrogram and heatmap of all genes. (D) Interactions between these modules. (E) Associations between clinical traits and the modules; the correlation between the green-yellow module and BC is –0.62. (F) Interrelationships between PAX2 and the genes in the green-yellow module. (G) ROC curve of PAX2. PAX2, paired box 2; AUC, area under the curves; WGCNA, weighted gene coexpression network analysis; TCGA, The Cancer Genome Atlas; BC, breast cancer; ROC, receiver operating characteristic.

(*Figure 2C*), whereas apoptosis, cell cycle, and the chemokine signaling pathway were downregulated in BC (*Figure 2D*).

GO and KEGG analyses of genes in the green-yellow module were also performed to explore the potential functions and molecular mechanisms of PAX2. GO analysis showed that genes in the green-yellow module were mainly enriched in epidermis development, muscle system process and muscle structure development, while KEGG analysis showed that these genes were primarily enriched in focal adhesion, regulation of the actin cytoskeleton and Hippo signaling pathway (*Figure 2E-2H*). The P values of the GO and KEGG terms are shown in *Figure 2E,2F*.

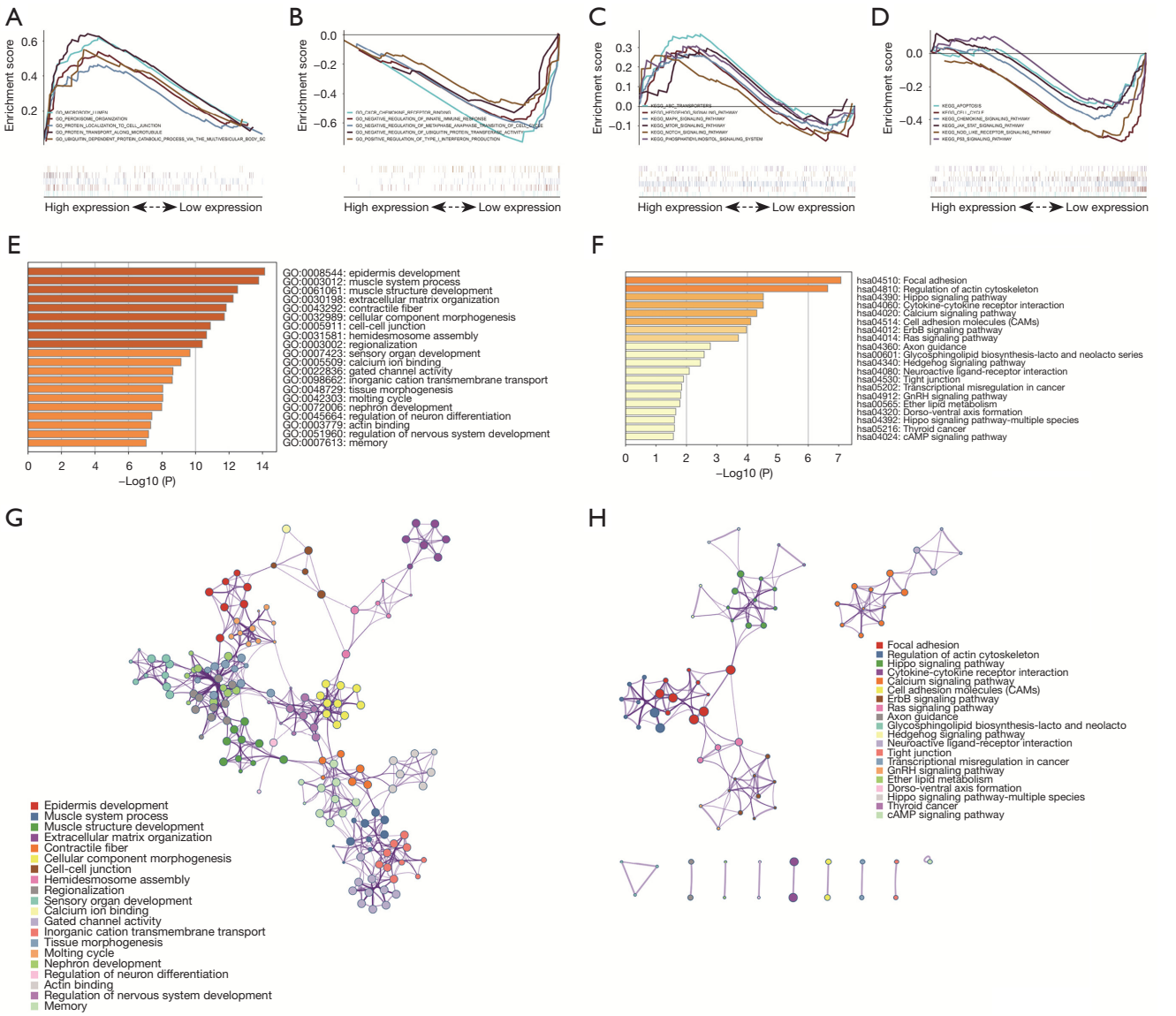

Figure 2 Gene functional enrichment analysis of PAX2. (A,B) GO analyses by GSEA. (C,D) KEGG analyses of GSEA. (E) GO enrichment analysis of the green-yellow model genes by Metascape. (F) KEGG analysis. (G) Heatmap of GO analyses by Metascape. (H) Heatmap of KEGG analyses by Metascape. PAX2, paired box 2; GO, gene ontology; GSEA, gene set enrichment analysis; KEGG, Kyoto Encyclopedia of Genes and Genomes.

Expression of PAX2 in clinical BC samples

RT-qPCR measurement of the expression of PAX2 in 68 pairs of BC samples and matched adjacent normal tissues revealed frequent overexpression in BC (*Figure 3A*). The correlation of PAX2 expression with clinicopathological information (age, menopausal state, tumor diameter, clinical stage, histological grade, metastasis of lymph nodes, pathological type, molecular subtypes) is summarized in *Table 1*. PAX2 expression was significantly associated with tumor size and metastasis of lymph nodes in BC (P<0.05, *Table 1*).

Functions of PAX2 in BC

To understand the functions of PAX2 in BC, we transfected MCF7 and MB231 cells with si-PAX2. As shown in *Figure 3B*, transient transfection with si-PAX2 significantly decreased

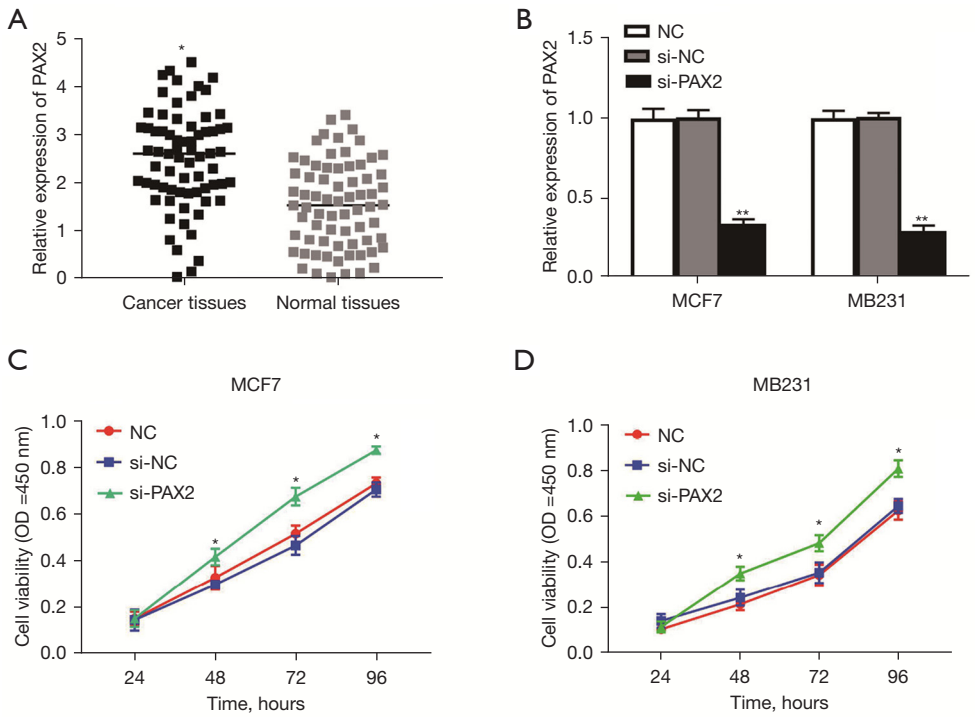

Figure 3 CCK-8 analysis is used to study the effect of down regulated expression of PAX2 on cell proliferation in the cell line. (A) The expression levels of PAX2 in the Clinical BC samples which included 68 BC tissues (mean ± SD: 2.543±0.986) and 68 paired tissues from the adjacent normal tissues (mean ± SD: 1.55±0.9022). (B) The expression of PAX2 is detected by qRT-PCR in down regulated expression transfected BC cell lines. (C) Cell proliferation was measured by CCK-8 assay in MCF7 cells. (D) Cell proliferation was measured by CCK-8 assay in MB231 cells. Data are expressed as the mean ± SD from three independent experiments. *, P<0.05; **, P<0.01. NC, normal control; OD, optical density; CCK-8, Cell Counting Kit-8; PAX2, paired box 2; SD, standard deviation; qRT-PCR, quantitative real time polymerase chain reaction.

PAX2 expression in MCF7 and MB231 cells. The viability of MCF7 and MB231 cells was significantly enhanced at 48, 72, and 96 h after si-PAX2 transfection (*Figure 3C*,*3D*). The effect of downregulation of PAX2 on cell invasion and migration was explored by wound healing assay and Transwell assay. Wound healing assay results suggested that downregulation of PAX2 significantly increased the percentage of wound closure of MCF7 and MB231 cells, indicating that PAX2 downregulation could increase the migratory capacity of MCF7 and MB231 cells (*Figure 4*). In the Transwell assay, the downregulation of PAX2 also markedly increased the amount of crystal violet-stained MCF7 and MB231 cells (*Figure 5*). These findings suggested that downregulation of PAX2 could enhance MCF7 and MB231 cell invasion and migration. Taken together, PAX2 inhibited MCF7 and MB231 cell proliferation, migration, and aggression. Therefore, upregulating PAX2 could be a novel strategy for treating BC.

Discussion

BC is a significant health problem and a top biomedical research priority (26,27). There are many genes that regulate BC. There are many genes that regulate BC. In our previous research work, we found that PAX2 plays an important role in BC through literature reading, and the expression and molecular function of PAX2 have not been studied. Bioinformatics analysis showed that PAX2 was abnormally expressed in BC, so we chose PAX2 as the target for subsequent studies. It has been reported that overexpression of PAX2 is a diagnostic biomarker for renal epithelial neoplasms and epithelial tumors of the female (28), and studies have shown the participation of PAX2 in the progression of BC (12-14). Hence, we hypothesized that PAX2 plays a regulatory role in BC.

Our results of data mining and bioinformatics analysis indicated that PAX2 is overexpressed in BC patients.

Table 1 The relationships between the expression of PAX2 protein and the clinical-pathological features in breast cancer tissues

Groups	n	Expression of PAX2		P
		Low expression	Over expression	
Age (years)				0.1113
<50	32	26	6	
≥50	36	23	13	
Menopausal state				0.8012
pre-menopause	41	30	11	
post-menopause	27	19	8	
Tumor size (cm)				0.0225
≤2	33	28	5	
>2	35	21	14	
Clinical stage				0.3310
I	19	16	3	
II	33	23	10	
III	16	10	6	
Histological grade				0.3853
I	28	19	9	
II	30	24	6	
III	10	6	4	
Metastasis of lymph nodes				0.0109
Negative	21	10	11	
1≤ N+ ≤3	25	21	4	
N+ >3	22	18	4	
Pathological type				0.9190
infiltrating ductal carcinoma	56	40	16	
infiltrating lobular carcinoma	7	5	2	
Other carcinoma	5	4	1	
Molecular subtypes				0.5917
Luminal A	10	9	1	
Luminal B	25	17	8	
HER2 positive	26	18	8	
Triple negative	7	5	2	

The definition of menopause: meet any of the following, (I) patients aged 60 years or older; (II) in patients younger than 60 years old, natural menopause is greater than or equal to 12 months, FSH and E2 levels are within the postmenopausal range if they have not received other drugs in the past year. PAX2, paired box 2; FSH, follicle-stimulating hormone; E2, estradiol; HER2, human epidermal growth factor receptor 2.

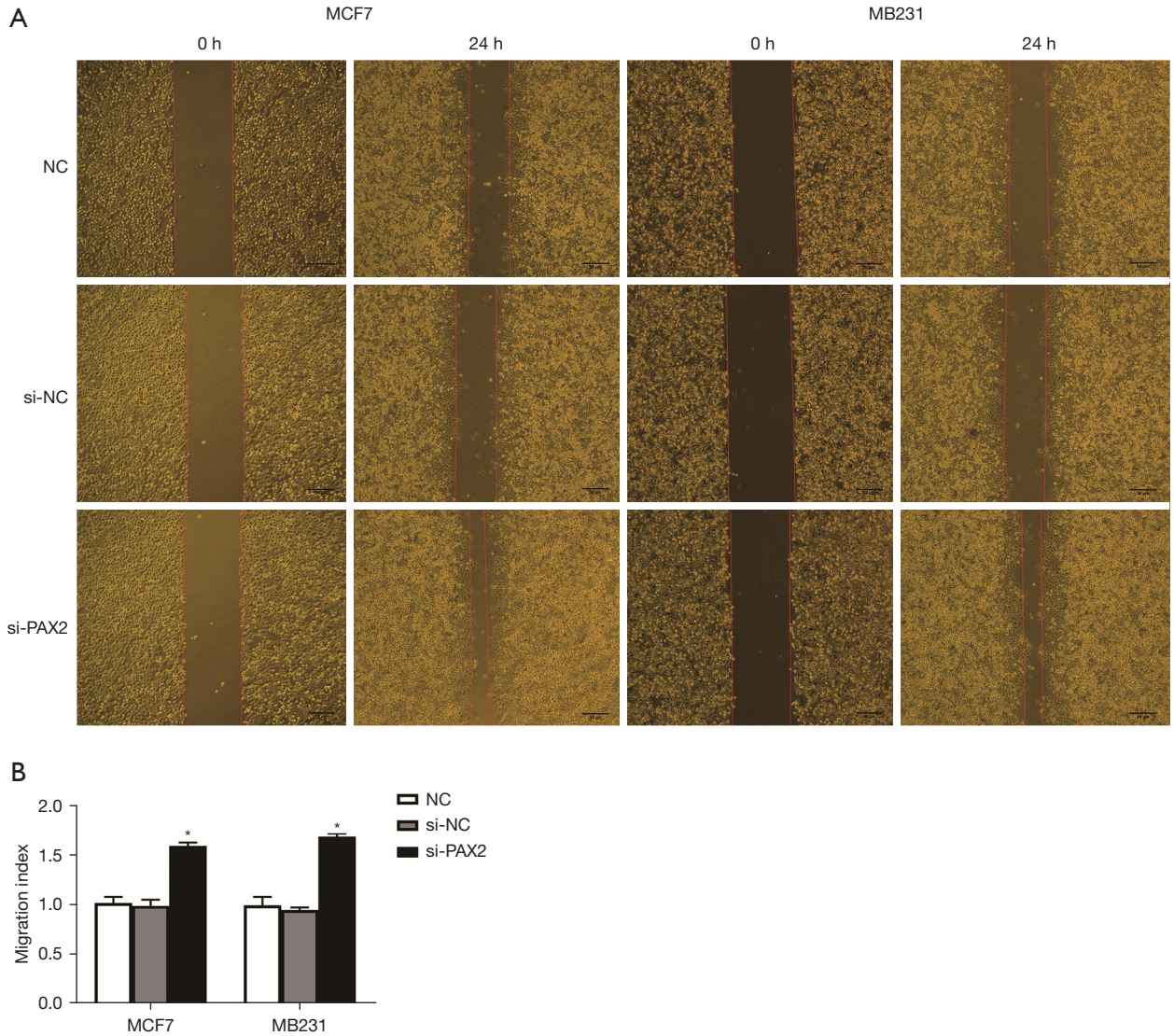

Figure 4 Effect of downregulated expression of PAX2 on migration of BC cell lines MCF7 and MB231 in the wound healing assay (A). (B) As is indicated in the bar chart, downregulated expression of PAX2 can increase the percentage of wound closure. The experimental results were observed under an optical microscope at a factor of 200. *, P<0.05. NC, negative control; PAX2, paired box 2; si-PAX2, small interfering RNA targeting PAX2; BC, breast cancer.

Furthermore, PAX2 was upregulated in BC tissue samples, and its expression was associated with tumor size and lymph node metastasis. However, downregulation of PAX2 in MCF7 and MB231 cell lines significantly enhanced cell proliferation, migration, and invasion. Our study data suggested that PAX2 is a tumor suppressor gene in BC. Intriguingly, overexpression of PAX2 in BC has also been described. Therefore, we believe that PAX2 may play different roles in different stages of BC tumorigenesis and development. In the early stages of tumor development, PAX2 is a potent growth inhibitor and aberrant expression of PAX2 induces carcinogenesis. In addition to its tumor suppressor function, PAX2 may also act as an oncogenic factor (29). However, further research is needed to elucidate when and why PAX2 overexpression occurs.

Our WGCNA analysis obtained genes in the green-yellow module that had similar gene expression patterns as PAX2. Hyaluronan synthase 3 (HAS3) is involved in the synthesis of an unbranched glycosaminoglycan, hyaluronan, a main component of the extracellular matrix.

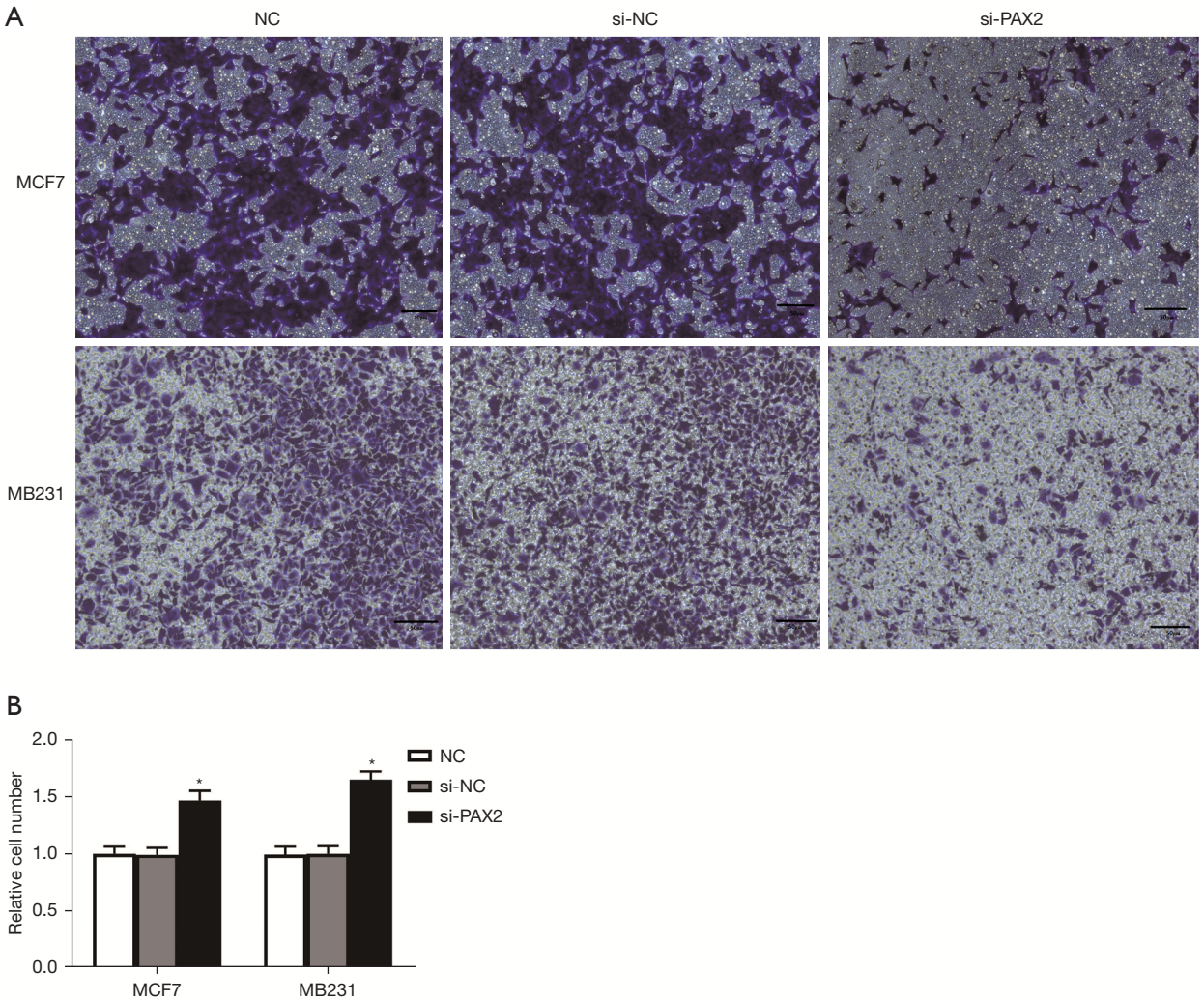

Figure 5 Effect of downregulated expression of PAX2 on cell invasion in BC cell lines MCF7 and MB231 in the Transwell assay, crystal violet-staining (A). (B) Histogram showed that downregulated expression of PAX2 can increase cell invasiveness. The experimental results were observed under an optical microscope at a factor of 200. *, P<0.05. NC, negative control; PAX2, paired box 2; si-PAX2, small interfering RNA targeting PAX2; BC, breast cancer.

It has been reported that downregulating HAS3 could inhibit gastric cancer cell proliferation, colony formation, migration, and invasion (30). Moreover, Arasu *et al.* report that overexpression of HAS3 induces extracellular vesicle shedding, which could cause cell proliferation and epithelial-to-mesenchymal transition in cancer cells (31). Thus, we hypothesize that PAX2 is involved in BC development by targeting HAS3, but these results need to be examined by further experiments. Thymic stromal lymphopoietin (TSLP) is a hemopoietic cytokine acting on myeloid cells to induce T cell-attracting chemokine release from monocytes and enhance CD11c (+) dendritic cell maturation. TSLP also plays essential roles in maintaining immune homeostasis and regulating inflammatory responses. It has been reported that cancer cells manipulate the immune response through TSLP and subsequently change the ability of immune cells to recognize and effectively remove tumors (32,33), Demehri *et al.* indicated that genetic and chemical induction of TSLP at a distant site could result in antitumor immunity against breast carcinogenesis in mice, and that TSLP is also expressed in BC cells to block progression (34). Thus, we also hypothesized that PAX2 has a tumor suppressor role in BC through *TSLP*. However, additional studies are needed to elucidate the molecular

mechanisms.

We found that PAX2 overexpression significantly suppressed cell proliferation, which is an integral part of cancer development and progression. The constitutive activation of many signaling pathways could stimulate cell proliferation (35,36). It was worth noting that the MAPK signaling pathway was significantly enriched in the GSEA. Many studies have shown that the MAPK signaling pathway is downstream of many growth factor receptors, and its activation plays a key role in cancer cell proliferation (37,38). Zhu *et al.* indicated that MICRORNA-188-5p inhibited the proliferation of BC cells via the MAPK signaling pathway (39). Thus, we hypothesized that PAX2 suppresses BC cell proliferation by regulating the MAPK signaling pathway. However, the results need to be examined in further investigations with larger samples. We also found that PAX2 overexpression could inhibit the migration and invasion of BC cells. Migration and invasion are important initial steps in the metastasis of cancer cells, and are modulated by multiple signaling pathways (40,41). The Hippo and MAPK signaling pathways were significantly enriched in our GSEA. Wu *et al.* reported that Sushi repeat containing protein X-linked 2 promoted cell migration and invasion in osteosarcoma through regulation of the Hippo signaling pathway (42). Sharif *et al.* also indicated that the Hippo signaling pathway modulated cancer cell invasion (43). Moreover, studies have shown that the activated MAPK signaling pathway promotes cell migration and invasion (44,45). Thus, we hypothesized that PAX2 inhibits the migration and invasion of BC cells by regulating the Hippo and MAPK signaling pathways.

This study has some limitations. Although we explored the role of PAX2 in BC using bioinformatics and *in vitro* validation methods, molecular mechanism verification was not performed. Therefore, more basic and clinical research needs to be performed to validate the molecular mechanisms and pathways. Moreover, because we were focused on the differential expression of PAX2 in cancerous and precancerous tissues, analyses regarding subtype, age, stage, and treatment were not performed. Furthermore, to explore the role of PAX2 in BC, patients were divided into low and high expression groups based on a median value of PAX2 expression level and then compared by GSEA and Metascape. However, due to algorithmic limitations (18,46-50), the correlations between these pathways and the molecular functions and stage-specific gene regulation of known subtypes cannot be calculated and compared. Finally, the protein localization and levels were not analyzed because of the difficulty of sample collection and insufficient funds. In the future, more basic and clinical research should be performed to explore these aspects.

In conclusion, our study revealed a tumor suppressing effect of PAX2 in BC, which might provide a novel therapeutic approach and early diagnosis for BC.

Conclusions

In conclusion, PAX2 was upregulated in the TCGA datasets and BC tissues, which associated with tumor size and lymph node metastasis. Moreover, PAX2 deficiency could promote the growth, migration, and invasion of BC cells. PAX2 could be a potential therapeutic target for BC.

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Footnote

Reporting Checklist: The authors have completed the MDAR reporting checklist. Available at https://atm.amegroups.com/article/view/10.21037/atm-22-6360/rc

Data Sharing Statement: Available at https://atm.amegroups.com/article/view/10.21037/atm-22-6360/dss

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://atm.amegroups.com/article/view/10.21037/atm-22-6360/coif). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study protocol was approved by the Ethics Committee of the Fourth Hospital of Hebei Medical University (approval No. 2022KY394). Informed consent was given by all patients at the time of sample collection. The study was performed in accordance with the Declaration of Helsinki (as revised in 2013).

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