The Role of PAX2 in Breast Cancer: A Study Based on Bioinformatics Analysis and in Vitro Validation

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Research Article

Keywords: breast cancer, PAX2, TCGA, cell growth, migration, invasion

DOI: https://doi.org/10.21203/rs.3.rs-738037/v1

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Abstract

Background: Breast cancer (BC) is the most frequently diagnosed cancer in women and is 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. However, the functions of PAX2 in the BC context are still unclear.

Methods: Transcriptome expression profiles and clinicopathological information of BC were downloaded from the TCGA database. Then the expression level and prognostic value in TCGA database were explored. Gene Set Enrichment Analysis (GSEA) and functional enrichment analysis were performed to investigate the functions and pathways of PAX2. Moreover, RT-qPCR was used to determine the expression of PAX2 in BC tissues, and the predictive value of PAX2 in clinical samples was assessed. CCK-8 assay was used to evaluate cell growth. The migration and invasion capacities of cells were assessed by wound healing assay and Transwell assay.

Results: PAX2 was up-regulated in the TCGA-BC datasets. GSEA analysis suggested that PAX2 might be involved in the regulation of MAPK signaling pathways and so on. Moreover, PAX2 was overexpressed in BC tissues, and PAX2 expression was associated with menopause. PAX2 deficiency could inhibit the growth, migration, and invasion of BC cells.

Conclusion: This study suggested that PAX2 was up-regulated in BC, which inhibited BC cell growth, migration, and invasion. Thus, PAX2 could be a potential therapeutic target for BC.

1. Introduction

Breast cancer (BC) is the most frequently diagnosed cancer in women and ranked second in the cause of cancer-related deaths in women, representing a significant public health problem on a global scale. There were more than 1,600,000 new diagnoses and more than 500,000 cases of deaths related to breast cancer in the world \([1,2]\). Early diagnosis of breast cancer 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 breast cancer is critical in breast cancer research. On the other hand, breast cancer treatment involves a multidisciplinary approach, including surgery, neoadjuvant therapy, and radiation therapy \([5,6]\). In recent years, although widespread uses of hormonal agents and adjuvant chemotherapy have improved the overall survival of breast cancer patients, the prognosis of breast cancer patients with metastasis remains unsatisfactory \([7,8]\). The use of biomarkers in assisting breast cancer treatment and disease monitoring during and after treatment. Thus, it is necessary to identify and apply new biomarkers for BC diagnosis and prognosis prediction.

PAX2 encodes paired box gene 2, one of many human homologs of the Drosophila melanogaster gene prd. It has been reported that PAX2 is a target of transcriptional suppression by the tumor suppressor
gene WT1, and suppression of PAX2 inhibits the growth, drug sensitivity, and metastasis of human ovarian cancer cells [9]. Moreover, Shaolin Chen et al. indicated that PAX2 directly targeted microRNA-744-5p in non-small cell lung cancer. Upregulation of microRNA-744-5p could inhibit non-small cell lung cancer cell proliferation, colony formation, and cell invasion in vitro through targeting PAX2 [10]. Ensaf M Al-Hujaily et al. found that aberrant expression of PAX2 in adult tissues is associated with carcinogenic effects, and PAX2 is highly expressed in low-grade epithelial ovarian tumors [11]. We hypothesized that PAX2 might be involved in breast cancer progression, but the role of PAX2 in breast cancer remains unrevealed.

Bioinformatics is one of the latest fields of biological research, which could process and analyze genomics data to achieve biological understanding and therapeutic progress of diseases [12]. This study investigated the effects of PAX2 in breast cancer by bioinformatics analysis and in vitro experiments.

2. Materials And Methods

2.1 Breast cancer datasets

The transcriptome expression profiles and corresponding clinical information of breast cancer patients (1104 tumor samples and 113 normal samples) were downloaded from the UCSC database (http://xena.ucsc.edu/). Then R packages “affy” and “limma” were used to normalize and summarize the breast cancer datasets. The differentially expressed genes (DEGs) were identified by R package “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 value < 0.05 and | logFC | > 1.

2.2 Weighted gene coexpression network analysis

Weighted gene coexpression network analysis (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) [13]. Firstly, genes were filtered to exclude those detected in few samples. 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 is 0.25. Finally, correlations of modules with clinicopathological information were calculated to explore their clinical significance.

2.3 GSEA analysis

Gene Set Enrichment Analysis (GSEA) was used to extract biological insights from genome-wide RNA expression information. GSEA could explore gene sets that share common chromosomal location, biological function, or regulation. In this study, GSEA analysis was performed to examine potential
functions and molecular mechanisms of PAX2. Breast cancer patients were divided into low and high expression groups using the median value of PAX2 expression level as a threshold. Then transcriptome expression profiles were input into GSEA software (http://www.gsea-msigdb.org/gsea/index.jsp). Those with P < 0.05, and FDR (false discovery rate) q < 0.05 were considered to be statistically significant.

2.4 Functional enrichment analysis

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

2.5 Human tissue samples and cell lines

In this study, breast cancer tissue samples and adjacent normal tissues were obtained from 68 patients at the Fourth Hospital of Hebei Medical University (Shijiazhuang, China). This study followed the Helsinki Declaration guidelines and was approved by the Ethics Committee of the Fourth Hospital of Hebei Medical University. Informed consent was obtained from all patients at the time of sample collection.

Breast cancer cell lines, MCF7 and MB231,[14,15] 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, USA), 50 units/ml penicillin and 50 µg/mL streptomycin (Gibco, Gaithersburg, MD, USA), 2 mM L-glutamine (Gibco) at 37°C in a humidified 5% CO₂ atmosphere.

2.6 RT-qPCR

The expression of PAX2 was in breast cancer tissue samples was measured by RT-qPCR. Total RNA was extracted by TRIzol® reagent (Thermo Fisher Scientific, Waltham, USA), and GAPDH was used as an internal control. The reverse transcription reaction was performed by Reverse Transcription Kit (GeneCopoeia, Guangzhou, China). Quantitative PCR 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’

2.7 Cell transfection

Small interfering RNA targeting PAX2 (si-PAX2, 5’- CCAGGAGACUCAAGGUCCAAUGUUU – 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). Transfection efficiency was evaluated after 24 h.

### 2.8 Cell counting kit-8 (CCK-8) assay

CCK-8 assay was performed to evaluate the effect of overexpression of PAX2 on the proliferation of MCF7 and MB231 cells according to manufacturer 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). Cck-8 solution (10 µL) (Ruisai Biotechnology, Shanghai, China) was added to each well, followed by 10 µL DMSO. OD450 values were read on a spectrophotometer.

### 2.9 Wound healing assay

The wound healing assay was performed to evaluate the effect of PAX2 overexpression on 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. A wound was created by manually scratching the monolayer of cells with the tip of a 200 µL pipette. Cells were imaged 0 and 48h after the wound was created.

### 2.10 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 per 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°C and 5% CO₂. After 24 hours, the upper chamber was cleaned and cells in the lower chamber were mixed with 100% methanol, stained with 0.1% crystal violet and counted.

### 2.11 Statistical analysis

The Kaplan-Meier method was used to estimate the overall survival of breast cancer 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. Each cell culture experiment was performed in triplicate. Quantitative and qualitative variables were expressed as mean ± SD and absolute frequencies/ percentages respectively. The difference between the two groups was analyzed by Student’s t-test. P < 0.05 was considered statistically significant. All statistical analyses were conducted using the R software (version 3.5.3) and SPSS 20.0 (SPSS Inc., Chicago, USA).

### 3. Results
3.1 Bioinformatics analysis of PAX2

In this study, the transcriptome expression profiles and corresponding clinical information of breast cancer patients were downloaded from the UCSC database and analyzed. As shown in Fig. 1A, the PAX2 was overexpressed in breast cancer patients ($P = 1.365e-09$). WGCNA analysis was used to explore the correlation between PAX2 and breast cancer. Firstly, a hierarchical clustering tree was constructed. As shown in Fig. 1B, 18 modules were obtained. The heatmap and dendrogram of all genes and WGCNA related modules, shown in Fig. 1C and D, suggested no significant differences in the interactions among modules. These modules had high degrees of independence. PAX2 was in the greenyellow module, and the correlation between the modules and breast cancer was $-0.62$ (Fig. 1E). All genes in the greenyellow module were shown in Fig. 1F. As shown in Fig. 1G, the area under the ROC curve was 0.673, indicating that PAX2 could be a diagnostic marker for breast cancer patients.

3.2 GSEA and functional enrichment analysis

In this study, GSEA and functional enrichment analysis were used to explore potential functions of PAX2 and its role in breast cancer progression. Breast cancer 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, protein localization to cell junction were upregulated in breast cancer (Fig. 2A), while CXCR chemokine receptor binding, negative regulation of innate immune response, and negative regulation of metaphase anaphase transition of the cell cycle were downregulated (Fig. 2B). KEGG analysis revealed that the ABC transporters, hedgehog signaling pathway, and MAPK signaling pathways were upregulated (Fig. 2C), whereas the apoptosis, cell cycle, and chemokine signaling pathway were downregulated in breast cancer (Fig. 2D).

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

3.3 The expression of PAX2 in clinical breast cancer samples

The expression of PAX2 in 68 pairs of breast cancer samples and matched adjacent normal tissues was measured by RT-qPCR. As shown in Fig. 3A, PAX2 overexpression was frequently observed in breast cancer. The correlations of PAX2 expression with clinicopathological information (age, menopausal state, tumor diameter, clinical stage, histological grade, metastasis of lymph nodes, pathological type,
molecular subtypes) were summarized in Table 1. PAX2 expression was significantly associated with menopause in breast cancer (P < 0.05, Table 1).
Table 1
The relationships between the expression of PAX2 protein and the clinical-pathological features in breast cancer tissues

| Groups                     | n  | Low expression | Over expression | p   |
|----------------------------|----|----------------|-----------------|-----|
| **Age**                    |    |                |                 | 0.1113 |
| <50                        | 32 | 26             | 6               |     |
| ≥ 50                       | 36 | 23             | 13              |     |
| **Menopausal state**       |    |                |                 | 0.0332 |
| pre-menopause              | 34 | 28             | 6               |     |
| post-menopause             | 34 | 20             | 14              |     |
| **T(cm)**                  |    |                |                 | 0.8012 |
| ≤ 2                        | 41 | 30             | 11              |     |
| >2                         | 27 | 19             | 8               |     |
| **Clinical stage**         |    |                |                 | 0.3310 |
| I                          | 19 | 16             | 3               |     |
| II                         | 33 | 23             | 10              |     |
| III                        | 16 | 10             | 6               |     |
| **Histological grade**     |    |                |                 | 0.3575 |
| I~II                       | 58 | 43             | 15              |     |
| III                        | 10 | 6              | 4               |     |
| **Metastasis of lymph nodes** |   |                |                 | 0.5586 |
| Negative                   | 25 | 18             | 7               |     |
| 1 ≤ N+ ≤ 3                 | 27 | 21             | 6               |     |
| N+>3                       | 16 | 10             | 6               |     |
| **Pathological type**      |    |                |                 | 0.9190 |
| infiltrating ductal carcinoma | 56 | 40       | 16              |     |
| infiltrating lobular carcinoma | 7  | 5         | 2               |     |
| Other carcinoma            | 5  | 4              | 1               |     |
| **Molecular subtypes**     |    |                |                 | 0.7132 |

### 3.4 The functions of PAX2 in breast cancer

To understand the functions of PAX2 in breast cancer, we transfected MCF7 and MB231 cells with si-PAX2. As shown in Fig. 3B, transient transfection with si-PAX2 significantly decreased PAX2 expression in MCF7 and MB231 cells. Cell viability of MCF7 and MB231 cell was significantly enhanced at 48, 72, and 96 hours after si-PAX2 transfection (Fig. 3C, D). The effect of downregulation of PAX2 on cell invasion and migration was explored by wound healing assay and Transwell assay. Wound healing assay suggested that the downregulation of PAX2 significantly increased the percentage of wound closure of MCF7 and MB231 cells, indicating that PAX2 downregulation could reduce the migratory capacity in MCF7 and MB231 cells (Fig. 4). In the Transwell assay, the downregulation of PAX2 also markedly increased the amount of crystal violet-stained cells of MCF7 and MB231 cells (Fig. 5). These data 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, up-regulating PAX2 could be a novel strategy for treating breast cancer.

### 4. Discussion

Breast cancer is the most frequently diagnosed cancer in women. It has been a significant health problem and represents a top biomedical research priority \cite{16,17}. With increases in breast cancer incidence and mortality in recent years \cite{18,19}, it is essential to diagnose and treat breast cancer in the early stages. Magnetic resonance imaging, ultrasound, mammography, positron emission tomography, and biopsy are currently available for breast cancer diagnosis \cite{20,21}. However, these methods have some limitations include expensive, low sensitivity, and time consuming. Biomarkers are promising diagnostic targets for rapid and cost-effective early breast cancer detection. Breast cancer patients are currently available for multimodal treatment, including surgery, radiotherapy, chemotherapy, and biological therapy \cite{22}. Although many efforts have been made to treat breast cancer, the survival rate remains low over the past few years. Thus, it is necessary to explore novel targets for early diagnosis and optimal therapies for breast cancer.

PAX2 encodes paired box gene 2, one of many human homologs of the Drosophila melanogaster gene prd. It has been reported that PAX2 is commonly overexpressed in epithelial tumors of the kidney and female genital tract, which may be a diagnostic biomarker for renal epithelial neoplasms and epithelial
tumors of the female [23]. Naoto Kuroda et al. indicated that the combined panel of PAX2 and PAX8 is a good marker for diagnosing metastatic renal cell carcinoma [24]. Moreover, Yan Feng et al. suggested that PAX2 may promote ovarian cancer progression through fatty acid metabolic reprogramming [25]. Hence, we hypothesized that PAX2 might play a regulatory role in breast cancer.

In this study, the expression and function of PAX2 in breast cancer were explored by data mining, bioinformatics analysis, and cell culture experiments. The result of data mining and bioinformatics analysis indicated that the PAX2 was overexpressed in breast cancer patients. Furthermore, PAX2 was upregulated in breast cancer tissue samples, and its expression was associated with menopause. The downregulation of PAX2 in MCF7 and MB231 cells significantly enhanced cell proliferation, migration and invasion. Hence, PAX2 might be an early diagnostic marker and a therapeutic target for breast cancer. Our study suggested that PAX2 is a tumor suppressor gene in breast cancer. Intriguingly, overexpression of PAX2 has also been described in breast cancer. Further experiments are needed to elucidate when and why PAX2 overexpression occurs.

In our study, WGCNA analysis and genes in the greenyellow module were obtained. These genes have 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. It has been reported that downregulating HAS3 could inhibit gastric cancer cell proliferation, colony-forming, migration, and invasion [26]. Moreover, Uma Thanigai Arasu et al. indicates that the overexpression of HAS3 could induce extracellular vesicle shedding, which could cause cell proliferation and epithelial-to-mesenchymal transition in cancer cells [27]. Thus, we hypothesized that PAX2 could be involved in breast cancer development by targeting HAS3, but the 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 chemokines 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 could manipulate the immune response through TSLP and subsequently change the ability of immune cells to recognize and effectively remove tumors [28, 29]. Shadmehr 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. TSLP is also expressed in breast tumor cells to block breast cancer progression [30]. Thus, we also hypothesized that PAX2 might have a tumor suppressor role in breast cancer through the TSLP genes. However, additional studies are needed to elucidate the molecular mechanisms.

In this study, we found that PAX2 overexpression significantly suppressed cell proliferation. Cell proliferation is an integral part of cancer development and progression. The constitutive activation of many signaling pathways could stimulate the cell proliferation [31, 32]. It was worth noting that the MAPK signaling pathway was significantly enriched in the GSEA analysis. Many studies have shown that the MAPK signaling pathway is downstream of many growth factor receptors, and its activation plays key roles in cancer cell proliferation [33, 34]. Xinying Zhu et al. indicates that MicroRNA-188-5p could inhibit cell
proliferation of breast cancer cells via the MAPK signaling pathway \cite{35}. Thus, we hypothesized that PAX2 suppressed breast cancer cell proliferation by regulating the MAPK signaling pathway. However, the results need to be examined by further investigations with larger samples. We also found that PAX2 overexpression could inhibit the migration and invasion of breast cancer cells. Migration and invasion of cancer cells are important initial steps in metastasis modulated by multiple signaling pathways\cite{36,37}. The Hippo signaling and MAPK signaling pathways were significantly enriched in the GSEA analysis. It has been reported that Sushi repeat containing protein X-linked 2 could promote cell migration and invasion in osteosarcoma through regulating Hippo Signaling Pathway \cite{38}. G M Sharif et al. also indicated that Hippo Signaling Pathway could modulate cancer cell invasion \cite{39}. Moreover, studies have shown that the activated MAPK signaling pathway could promote cell migration and invasion\cite{40,41}. Thus, we hypothesized that PAX2 inhibited the migration and invasion of breast cancer cells by regulating the Hippo signaling pathway and MAPK signaling pathway.

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

**Declarations**

**Ethics approval and consent to participate**

The study protocol was approved by the Ethics Committee of the Fourth Hospital of Hebei Medical University. Informed consent was obtained from all patients at the time of sample collection.

**Author contributions:**

SNL conceived the study, WG collected the data, HQW and XZ conducted data analysis and statistics. SY,YZM and SNL performed the experiments. YWD prepared figures. YS,SNL and CZG wrote the manuscript, commented on drafts and approved the final version. All authors read and approved the manuscript to publish.

**Competing interests**

The authors declare that they have no competing interests.

**Acknowledgements**

Not available.

**References**

1. Fan L, Strasser-Weippl K, Li JJ, et al. Breast cancer in China. *Lancet Oncol*. 15(7): e279-89(2014).
2. Harbeck N, Gnant M. Breast cancer. *Lancet*. 389(10074): 1134-1150(2017).
3. M Braden A, V Stankowski R, M Engel J, A Onitilo A. Breast cancer biomarkers: risk assessment, diagnosis, prognosis, prediction of treatment efficacy and toxicity, and recurrence. *Curr Pharm Des.* 20(30): 4879-98(2014).

4. Apantaku LM. Breast cancer diagnosis and screening. *Am Fam Physician.* 62(3): 596-602, 605-6(2000).

5. Maughan KL, Lutterbie MA, Ham PS. Treatment of breast cancer. *Am Fam Physician.* 81(11): 1339-46(2010).

6. Rossi L, Mazzara C, Pagani O. Diagnosis and Treatment of Breast Cancer in Young Women. *Curr Treat Options Oncol.* 20(12): 86(2019).

7. de la Mare JA, Contu L, Hunter MC, et al. Breast cancer: current developments in molecular approaches to diagnosis and treatment. *Recent Pat Anticancer Drug Discov.* 9(2): 153-75(2014).

8. Castaneda SA, Strasser J. Updates in the Treatment of Breast Cancer with Radiotherapy. *Surg Oncol Clin N Am.* 26(3): 371-382(2017).

9. Yang S, Yang R, Lin R, Si L. MicroRNA-375 inhibits the growth, drug sensitivity and metastasis of human ovarian cancer cells by targeting PAX2. *J BUON.* 24(6): 2341-2346(2019).

10. Chen S, Shi F, Zhang W, Zhou Y, Huang J. miR-744-5p Inhibits Non-Small Cell Lung Cancer Proliferation and Invasion by Directly Targeting PAX2. *Technol Cancer Res Treat.* 18: 1533033819876913(2019).

11. Al-Hujaily EM, Tang Y, Yao DS, Carmona E, Garson K, Vanderhyden BC. Divergent Roles of PAX2 in the Etiology and Progression of Ovarian Cancer. *Cancer Prev Res (Phila).* 8(12): 1163-73(2015).

12. Tao Z, Shi A, Li R, Wang Y, Wang X, Zhao J. Microarray bioinformatics in cancer- a review. *J BUON.* 22(4): 838-843(2017).

13. Langfelder P, Horvath S. WGCNA: an R package for weighted correlation network analysis. *BMC Bioinformatics.* 9: 559(2008).

14. Fakai MI, Abd Malek SN, Karsani SA. Induction of apoptosis by chalepin through phosphatidylserine externalisations and DNA fragmentation in breast cancer cells (MCF7). *Life Sci.* 220: 186-193(2019).

15. Nehdi A, Ali R, Alhallaj A, et al. Nuclear Receptors Are Differentially Expressed and Activated in KAIMRC1 Compared to MCF7 and MDA-MB231 Breast Cancer Cells. *Molecules.* 24(11)(2019).

16. Woolston C. Breast cancer. *Nature.* 527(7578): S101(2015).

17. Liang Y, Zhang H, Song X, Yang Q. Metastatic heterogeneity of breast cancer: Molecular mechanism and potential therapeutic targets. *Semin Cancer Biol.* 60: 14-27(2020).

18. Li T, Mello-Thoms C, Brennan PC. Descriptive epidemiology of breast cancer in China: incidence, mortality, survival and prevalence. *Breast Cancer Res Treat.* 159(3): 395-406(2016).

19. DeSantis CE, Bray F, Ferlai J, Lortet-Tieulent J, Anderson BO, Jemal A. International Variation in Female Breast Cancer Incidence and Mortality Rates. *Cancer Epidemiol Biomarkers Prev.* 24(10): 1495-506(2015).

20. Wang L. Early Diagnosis of Breast Cancer. *Sensors (Basel).* 17(7)(2017).
21. Sitt JC, Lui CY, Sinn LH, Fong JC. Understanding breast cancer screening–past, present, and future. *Hong Kong Med J.* 24(2): 166-174(2018).

22. Lee K, Kruper L, Dieli-Conwright CM, Mortimer JE. The Impact of Obesity on Breast Cancer Diagnosis and Treatment. *Curr Oncol Rep.* 21(5): 41(2019).

23. Ordóñez NG. Value of PAX2 immunostaining in tumor diagnosis: a review and update. *Adv Anat Pathol.* 19(6): 401-9(2012).

24. Kuroda N, Tanaka A, Ohe C, Nagashima Y. Recent advances of immunohistochemistry for diagnosis of renal tumors. *Pathol Int.* 63(8): 381-90(2013).

25. Feng Y, Tang Y, Mao Y, et al. PAX2 promotes epithelial ovarian cancer progression involving fatty acid metabolic reprogramming. *Int J Oncol.* 56(3): 697-708(2020).

26. Bai F, Jiu M, You Y, et al. miR-29a-3p represses proliferation and metastasis of gastric cancer cells via attenuating HAS3 levels. *Mol Med Rep.* 17(6): 8145-8152(2018).

27. Arasu UT, Deen AJ, Pasonen-Seppänen S, et al. HAS3-induced extracellular vesicles from melanoma cells stimulate IHH mediated c-Myc upregulation via the hedgehog signaling pathway in target cells. *Cell Mol Life Sci.* 77(20): 4093-4115(2020).

28. Corren J, Ziegler SF. TSLP: from allergy to cancer. *Nat Immunol.* 20(12): 1603-1609(2019).

29. Kuan EL, Ziegler SF. A tumor-myeloid cell axis, mediated via the cytokines IL-1α and TSLP, promotes the progression of breast cancer. *Nat Immunol.* 19(4): 366-374(2018).

30. Demehri S, Cunningham TJ, Manivasagam S, et al. Thymic stromal lymphopoietin blocks early stages of breast carcinogenesis. *J Clin Invest.* 126(4): 1458-70(2016).

31. Kroemer G, Pouyssegur J. Tumor cell metabolism: cancer’s Achilles' heel. *Cancer Cell.* 13(6): 472-82(2018).

32. Feitelson MA, Arzumanyan A, Kulathinal RJ, et al. Sustained proliferation in cancer: Mechanisms and novel therapeutic targets. *Semin Cancer Biol.* 35 Suppl(Suppl): S25-S54(2015).

33. Peng WX, Huang JG, Yang L, Gong AH, Mo YY. Linc-RoR promotes MAPK/ERK signaling and confers estrogen-independent growth of breast cancer. *Mol Cancer.* 16(1): 161(2017).

34. Yin H, Zhao J, He H, et al. Gga-miR-3525 Targets PDLIM3 through the MAPK Signaling Pathway to Regulate the Proliferation and Differentiation of Skeletal Muscle Satellite Cells. *Int J Mol Sci.* 21(15) (2020).

35. Zhu X, Qiu J, Zhang T, et al. MicroRNA-188-5p promotes apoptosis and inhibits cell proliferation of breast cancer cells via the MAPK signaling pathway by targeting Rap2c. *J Cell Physiol.* 235(3): 2389-2402(2020).

36. VanderVorst K, Dreyer CA, Konopelski SE, Lee H, Ho HH, Carraway KL 3rd. Wnt/PCP Signaling Contribution to Carcinoma Collective Cell Migration and Metastasis. Cancer Res. 2019. 79(8): 1719-1729.

37. Ghasemi A, Saeidi J, Azimi-Nejad M, Hashemy SI. Leptin-induced signaling pathways in cancer cell migration and invasion. *Cell Oncol (Dordr).* 42(3): 243-260(2019).
38. Wu Z, Wang C, Chen Y, Sun Z, Yan W. SRPX2 Promotes Cell Proliferation and Invasion in Osteosarcoma Through Regulating Hippo Signaling Pathway. *Onco Targets Ther.* 13: 1737-1749(2020).

39. Sharif GM, Schmidt MO, Yi C, et al. Cell growth density modulates cancer cell vascular invasion via Hippo pathway activity and CXCR2 signaling. *Oncogene.* 34(48): 5879-89(2015).

40. Zhang YP, Liu KL, Yang Z, et al. The involvement of FBP1 in prostate cancer cell epithelial mesenchymal transition, invasion and metastasis by regulating the MAPK signaling pathway. *Cell Cycle.* 18(19): 2432-2446(2019).

41. Wu Y, Tan X, Liu P, et al. ITGA6 and RPSA synergistically promote pancreatic cancer invasion and metastasis via PI3K and MAPK signaling pathways. *Exp Cell Res.* 379(1): 30-47(2019).

**Figures**
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 greenyellow module. (C) The dendrogram and heatmap of all genes. (D) Interactions between these modules. (E) The associations between clinic traits and the modules and the correlation between greenyellow module and BC is -0.62. (F) Interrelationships between PAX2 and the genes in the greenyellow module. (G) ROC curve of PAX2.
Figure 2

Gene functional enrichment analysis of PAX2. (A-B) GO analyses by GSEA. (C-D) KEGG analyses of by GSEA. (E) GO enrichment analysis of the greenyellow model genes by Metascape. (F) KEGG analysis (G) Heatmap of GO analyses by Metascape. (H) Heatmap of KEGG analyses by Metascape.
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 and 68 paired tissues from the adjacent normal tissues. (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.

**Figure 4**

Down regulated expression of PAX2 can more effectively increase the migration of BC cell lines. (A) Wound healing assay. (B) Wound healing assay demonstrated that down regulated expression of PAX2 can increase the percentage of wound closure.
Figure 5

Down regulated expression can inhibit the cell invasion in BC cell lines. (A) Transwell assay. (B) The transwell assay suggested that down regulated expression of PAX2 can increase cell invasion in BC cell lines.