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ORIGINAL RESEARCH

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Bioinformatics analysis of the diagnostic significance and functions of *RDH5* in breast cancer

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Abstract

Objective: *RDH5* (Retinol dehydrogenase 5) is one of the member of the short-chain dehydrogenase/reductase (SDR) family and plays a critical role in a variety of tumor processes. In this study, we analyzed the expression, diagnostic significance and gene function of *RDH5* in breast cancer. Methods: The Gene Expression Profiling Interactive Analysis (GEPIA) database was used to determine the diagnostic and prognostic value of *RDH5* and the University of Alabama at Birmingham (UALCAN) database analyzed its expression in different subtypes, clinical stages and altered signal pathways in breast cancer. Then we explored the co-expression gene of *RDH5* in breast cancer and constructed its corresponding network through the cBioPortal database. The String database was used to determine its interactions with other proteins. Results: *RDH5* expression was differentiated in various tumours compared to normal tissue, and was down-regulated in breast cancer tissue and different subtypes of breast cancer. There was no correlation between *RDH5* and the survival of breast cancer. *RDH5* expression was significantly associated with different clinical parameters. Additionally, the protein expression of *RDH5* in breast cancer with altered pathways indicated it was closely associated with the SWI-SNF (switching/sucrose non-fermenting) complex status and the mammalian Target of Rapamycin (mTOR), WNT (Wingless/Integrated), MYC/MYCN (Myelocytomatosis oncogene/euroblastoma derived MYC), RTK (receptor Tyrosine kinases), and p53/Rb (tumor protein p53/Retinoblastoma) pathways. Interaction analysis with *RDH5* and its co-expression genes showed that its main related functions were ethanol oxidation, regulation of heart contraction and negative regulation of tumor necrosis factor. Lastly, the Protein-protein interaction (PPI) of *RDH5* showed that the most relevant Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways were retinol metabolism, vitamin digestion and absorption and metabolic pathways. Conclusion: These results implied that *RDH5* might be a larvaceous diagnostic biomarker for breast cancer and was closely associated with its metabolism. However, more studies are needed to confirm our findings and support the clinical importance of *RDH5* in breast cancer.

Keywords

RDH5; Breast cancer; Co-expression network; Metabolism

1. Introduction

Breast cancer is the most commonly diagnosed in women all over the world. According to the data of the 2020 World Health Organization (WHO) International Cancer Study, breast cancer accounts for nearly 2.1 million new cases worldwide and is the first cause of incidence and mortality in women [1]. In China, breast cancer is the most commonly diagnosed cancer in women and the 5th cause of cancer-related death [2]. Although early breast cancer has good prognosis, the later stages of breast cancer have poorer prognoses due to the occurrence of lymph node and bloodstream metastases [3]. Therefore, early breast cancer diagnosis is important to improve patients' treatment outcomes, prognosis and chance of cure. Thus, it is vital to identify novel biomarkers that could improve the early diagnosis, survival prediction and therapy strategies of breast cancer.

RDH5 gene encodes an enzyme named 11-cis-retinol dehydrogenase which is part of the visual cycle. It belongs to the *RDH* enzyme family and catalyzes the oxidation of 11-cis-retinol to 11-cis-retinol [4]. This 32kD membrane-bound enzyme is composed of 318 amino acids and is a key enzyme in the visual cycle [5, 6]. Multiple studies have shown that the *RDH* family has important roles in patients' prognosis, but the role of *RDH5* in tumors, especially breast cancer, remains to be clarified.

In this study, we utilized bioinformatics analysis meth-

ods to assess the function and diagnostic value of *RDH5* in breast cancer. Data from three different databases, The Cancer Genome Atlas (TCGA), Genotype-Tissue Expression (GTEx) and Gene Expression Omnibus (GEO), were used to analyze the expression of *RDH5* in variant cancers. Additionally, the expression of *RDH5* and survival impact on breast cancer patients with different clinical parameters was analyzed. PPI network and co-expression analysis were also conducted to determine genes and proteins co-expressed with *RDH5*.

2. Methods

2.1 Expression analysis of RDH5

GEPIA has a large number of sequence expression data of clinical tumor and normal tissue specimen and combines the TCGA and GTEx databases to allow gene expression analysis in different tumors. The GEPIA database was used to analyze the expression of *RDH5* in different tumors and their corresponding normal tissues. The UALCAN database is a comprehensive, user-friendly and interactive web resource for analyzing cancer omics data [7]. Using the UALCAN database, we determined the expression of *RDH5* in diverse subtypes and pathological stages of breast cancer.

2.2 Survival analysis

The GEPIA database was used to evaluate the association between *RDH5* expression and the prognosis of breast cancer patients. According to the expression of *RDH5*, the patients were divided into a low-expression group or a high-expression group. The overall survival (OS) and recurrence-free survival (RFS) of both groups were obtained with a 95% confidence interval.

2.3 RDH5 and clinicopathologic features in breast cancer

The breast cancer gene-expression miner is a statistical mining tool that evaluates various breast cancer genes for prognostic assessment [8, 9]. It was used to analyze the association of *RDH5* with patients' age, estrogen receptor (ER; ESR1), progesterone receptor (PR), triple-negative breast cancer, Prediction Analysis of Microarray 50 (PAM50) subtype and robust Single sample predictor classification (RSSPC) subtype.

2.4 Signal pathway analysis

The UALCAN database was developed to analyze *RDH5* protein expression levels in breast cancer with the SWI-SNF complex status and the mTOR, WNT, MYC/MYCN, RTK and p53/Rb pathways.

2.5 Gene co-expression network and protein-protein interaction (PPI) analysis

The cBioPortal database is a comprehensive web-based resource of genomic data visualization and multidimensional analysis of database sources such as TCGA, International Cancer Genome Consortium (ICGC), GEO, *etc.* [10]. It was used to identify genes co-expressed with *RDH5* (Spearman's Correlation >0.6), which were entered into the Coexpedia database to obtain the gene co-expression network map of *RDH5*. The PPI network for *RDH5* was obtained by using the String database.

2.6 Statistical analysis

IBM SPSS Statistics version 26 (IBM SPSS Inc., Chicago, USA) was used for statistical analysis, using Student's *t*-test and one-way analysis of variance. $p < 0.05$ indicated that there was a statistically significant difference.

3. Result

3.1 Pan-cancer differential expression of RDH5

GEPIA was used to analyze the expression level of *RDH5* in 33 tumors and their matched normal tissues (Fig. 1), in which we found that it was obviously and differentially expressed in 20 cancer types. Among them, *RDH5* was significantly down-regulated in adrenocortical carcinoma (ACC), bladder urothelial carcinoma (BLCA), breast invasive carcinoma (BRCA), cervical squamous cell carcinoma and endocervical adenocarcinoma (CESC), colon adenocarcinoma (COAD), esophageal carcinoma (ESCA), kidney chromophobe (KICH), lung adenocarcinoma (LUAD), lung squamous cell carcinoma (LUSC), prostate adenocarcinoma (PRAD), rectum adenocarcinoma (READ), skin cutaneous melanoma (SKCM), testicular germ cell tumors (TGCT), uterine corpus endometrial carcinoma (UCEC) and uterine carcinosarcoma (UCS). Adversely, *RDH5* was highly expressed in glioblastoma multiforme (GBM), kidney renal clear cell carcinoma (KIRC), kidney renal papillary cell carcinoma (KIRP), acute myeloid leukemia (LAML) and pancreatic adenocarcinoma (PAAD). Altogether, the expression of *RDH5* was relatively low in various tumor tissues (15 kinds) compared with normal tissues ($p < 0.001$), indicating that *RDH5* may be a potential diagnostic marker for these tumors.

3.2 Clinical diagnosis and prognostic significance of RDH5

To determine the relationship between *RDH5* and breast cancer, we inspected the expression level of *RDH5* in different breast cancer tissues in the UALCAN database. The results demonstrated that the expression of *RDH5* in breast cancer tissues was significantly lower than in normal tissues (Fig. 2A). Next, we analyzed the expression of *RDH5* in breast cancer subtypes and found that it was significantly down-regulated in the luminal type and HER-2 positive and triple-negative breast cancer. Significant expression differences between normal tissues and these three breast cancer subtypes were observed (Fig. 2B). However, the *RDH5* expression was not significantly associated with different clinical stages of breast cancer (Fig. 2C,D). We also analyzed the prognostic value of *RDH5* and found no significant association with the overall survival and disease-free survival (DFS) of the patients (p value > 0.05). Of note, survival curve analysis showed that before

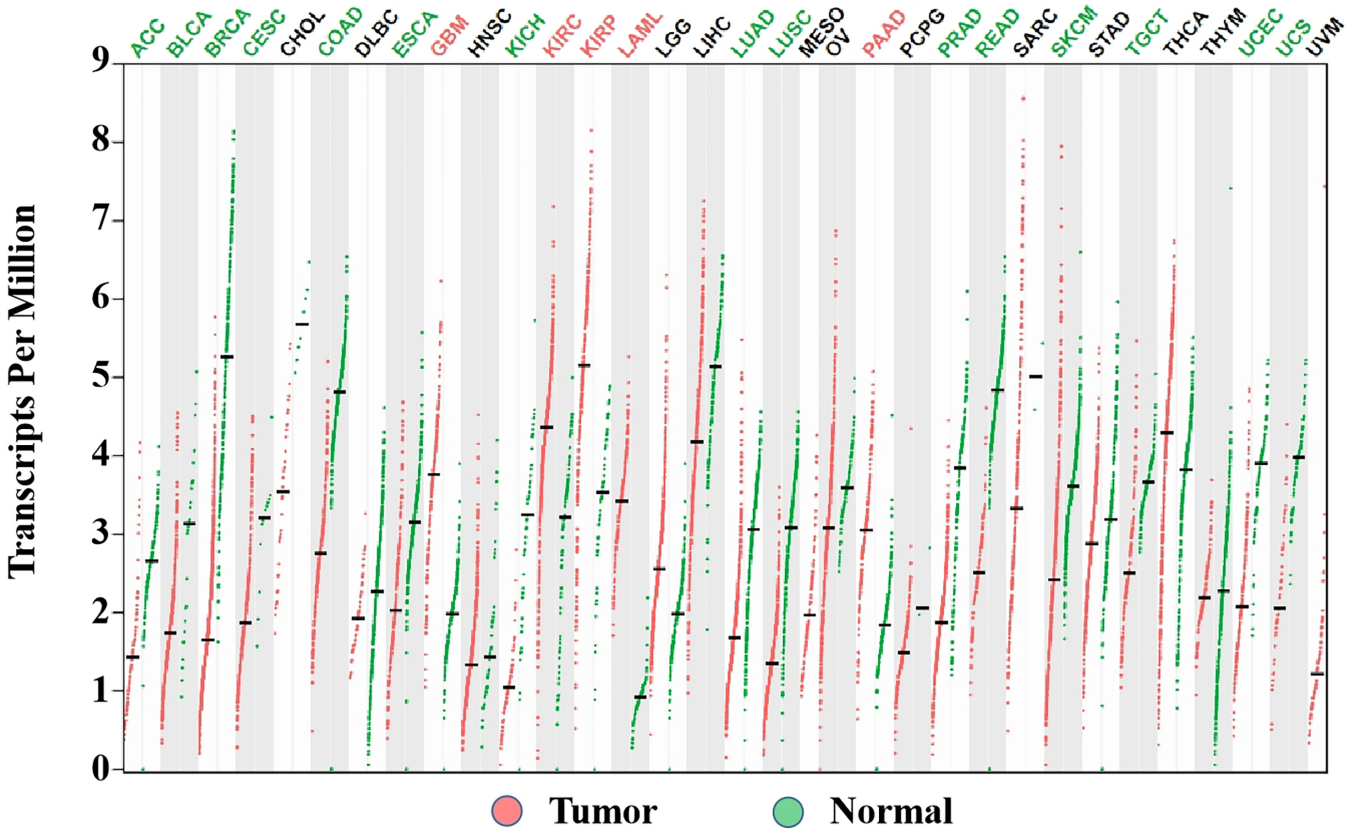

FIGURE 1. Pan-cancer expression of *RDH5*.

150 months, the overall survival of patients with low *RDH5* expression tended to be better than those with high *RDH5* expression (Fig. 2E,F).

3.3 RDH5 expression in breast cancer and association with clinical characteristics

There are many different subtypes of breast cancer, such as Luminal A, Luminal B, human epidermal growth factor receptor 2 (HER-2) positive and triple-negative (TNBC) molecular subtypes [11]. The classification of these subtypes is on the basis of the expression of ER, PR and HER-2. The ER and PR are considered predictive and prognostic markers in the National Society of Clinical Biochemistry Guidelines [3]. HER-2 belongs to the EGFR family, which plays a vital role in cell signaling and is an important regulator of breast cancer cell growth, differentiation and survival. The bc-GenExMiner database was used to analyze *RDH5* expression differences in breast cancer with different clinical parameters. *RDH5* expression did not differ in age groups of $\leq$ 51 years old and >51 years old, but we observed significant differences in different breast cancer subtypes. Fig. 3A shows that the mRNA expression of *RDH5* in ER-positive (ER+) patients was higher than in ER-negative (ER−) patients. A similar situation was found in PR status (Fig. 3B). Moreover, we found that the mRNA expression of *RDH5* was the lowest in double negative status (ER−/PR−) compared with ER+/PR+, ER+/PR− and ER−/PR+ (Fig. 3C). However, the mRNA expression of *RDH5* in HER2 positive (HER2+) patients was lower than in HER2 negative (HER2−) patients (Fig. 3F). The differential expression of *RDH5* was also found to be statistically significant with the PAM50 subtype, RSSPC subtype, Nottingham Prognostic Index (NPI), Scarff-Bloom-Richardson (SBR) and triple-negative status (Fig. 3D,E,G,H,I).

3.4 Protein expression of the RDH5 gene in breast cancer with altered pathways

Some signaling pathways govern the formation, maintenance and expansion processes of breast cancer, and further understanding of the relationship between *RDH5* and these signaling pathways may help clarify the biological function of *RDH5* in breast cancer. The mTOR plays key roles in various biological processes, including cell proliferation, survival, metabolism, autophagy and immunity [12]. Through several mechanisms, up-regulation of the mTOR signaling pathway was shown to promote tumor growth and progression, such as the boosting of the growth factor receptor signaling, angiogenesis, cancer cell migration, and suppression of autophagy [13]. The WNT pathway was reported to be associated with cancer stem cell properties, metastasis and immune surveillance [14]. Up-regulation RTKs may increase breast cancer invasion and decrease the OS and DFS rate of breast cancer patients [15]. The p53/Rb pathway is mainly related to cell cycle and apoptosis [16]. SWI/SNF chromatin remodeling complexes are considered prototypes of epigenetic regulators of gene expression involved in tumor suppression [17]. Using the UALCAN database, we found that *RDH5* expression was apparently lower in breast cancer tissues with altered signaling pathways, including the SWI-SNF complex status and the mTOR, WNT,

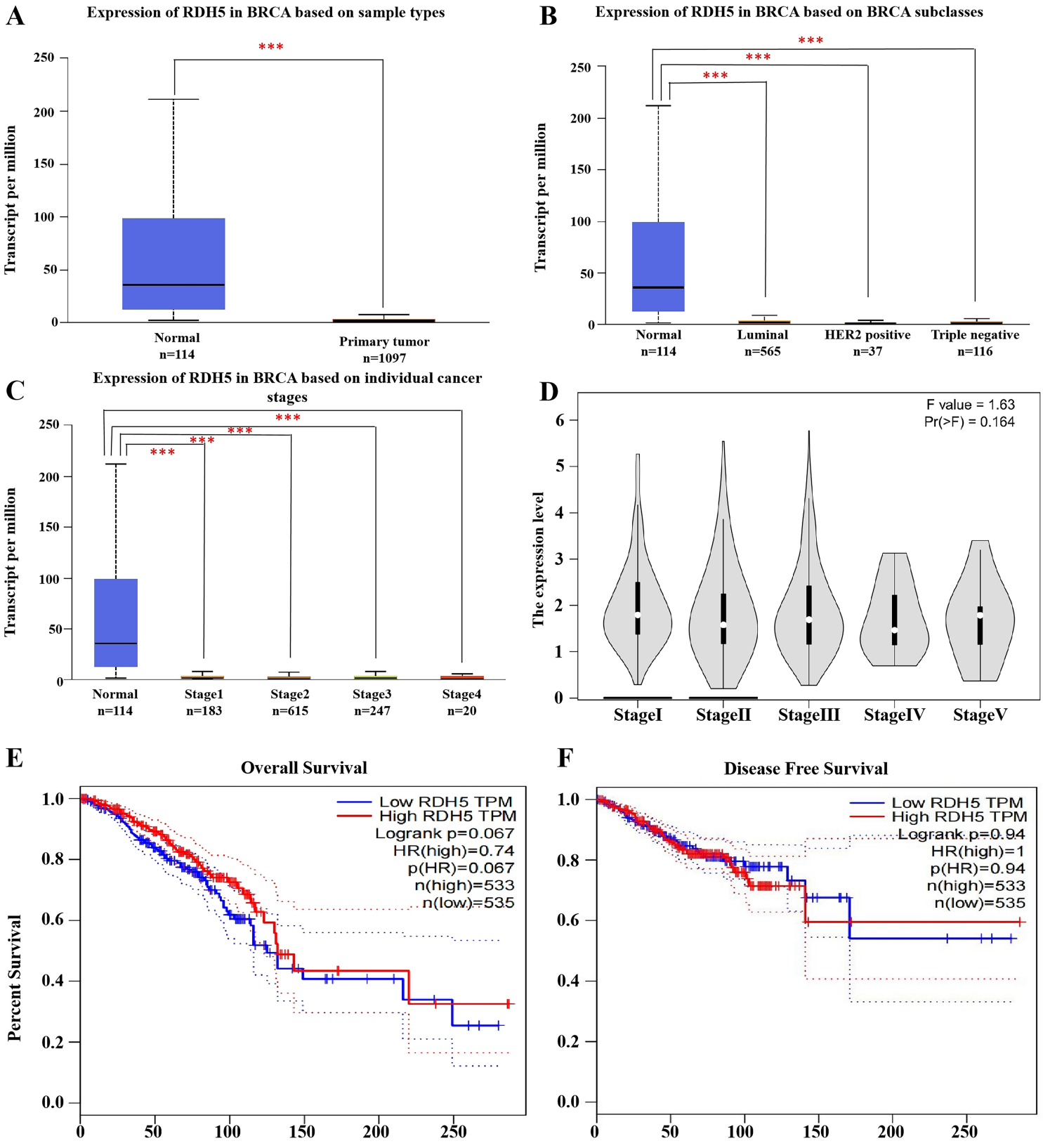

FIGURE 2. Expression levels of *RDH5* in various subtypes and different clinical stages of breast cancer. (A) The expression of *RDH5* in breast cancer. (B) Expression of *RDH5* in three subtypes of breast cancer. (C,D) The expression of *RDH5* in breast cancer at different clinical and pathological stages analyzed in the UALCAN and GEPIA databases. (E,F) OS and DFS curve of patients with high (red) and low (blue) *RDH5* expression. *RDH5*: Retinol dehydrogenase 5; BRCA: breast invasive carcinoma; TPM: Transcript per million; HR: Hazard Rate.

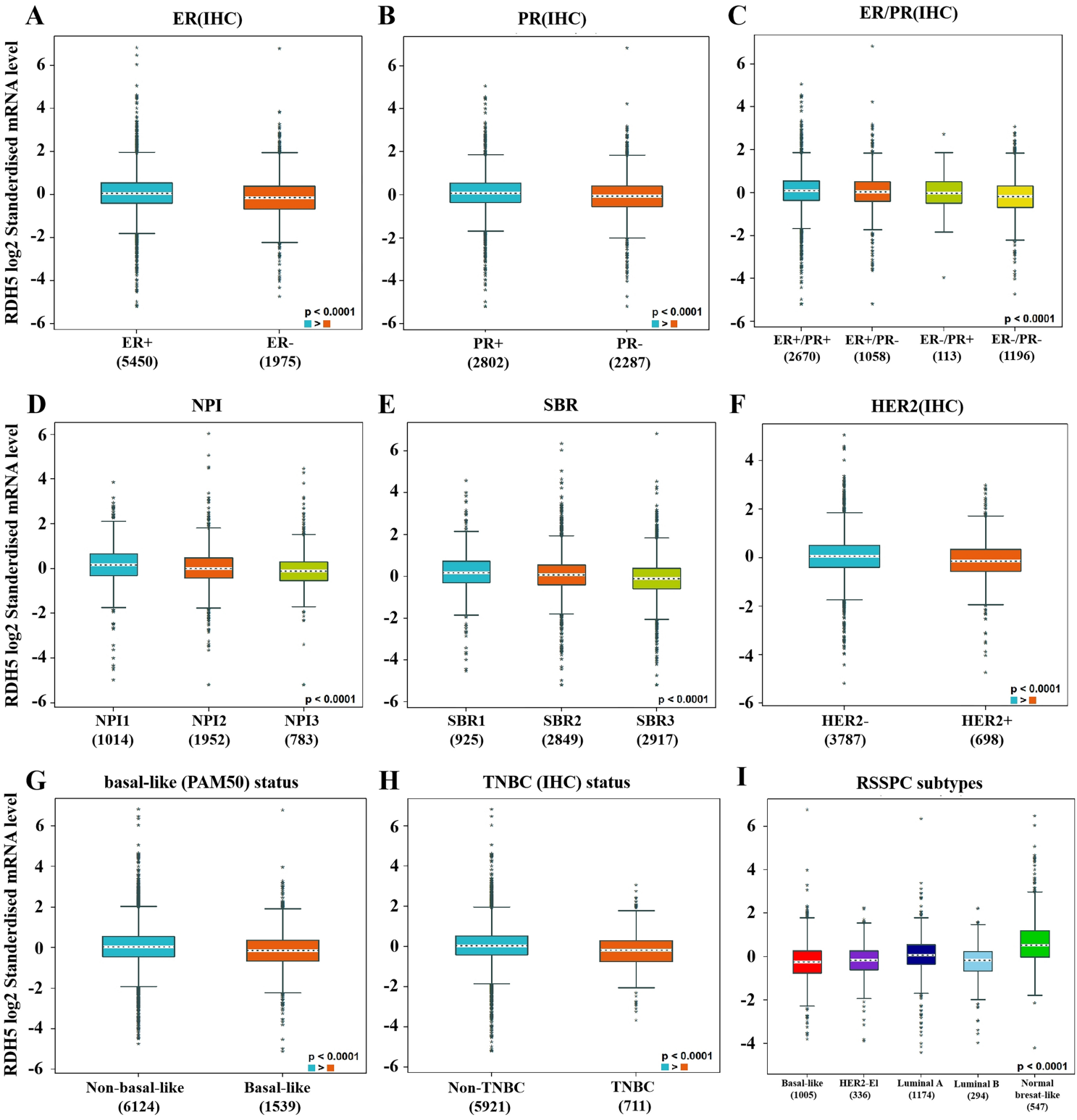

FIGURE 3. Relationship between *RDH5* and clinicopathologic features of breast cancer. Box plot estimated *RDH5* expression among different groups of patients on the basis of clinical parameters using the bc-GenExMiner software. (A) ER: estrogen receptor; (B) PR: progesterone receptor; (C) ER/PR; (D) NPI: Nottingham Prognostic Index; (E) SBR: Scarff-Bloom-Richardson; (F) HER2: human epidermal growth factor receptor 2; (G) basal-like status; (H) triple-negative breast cancer; (I) RSSPC subtypes. IHC: immunohistochemistry.

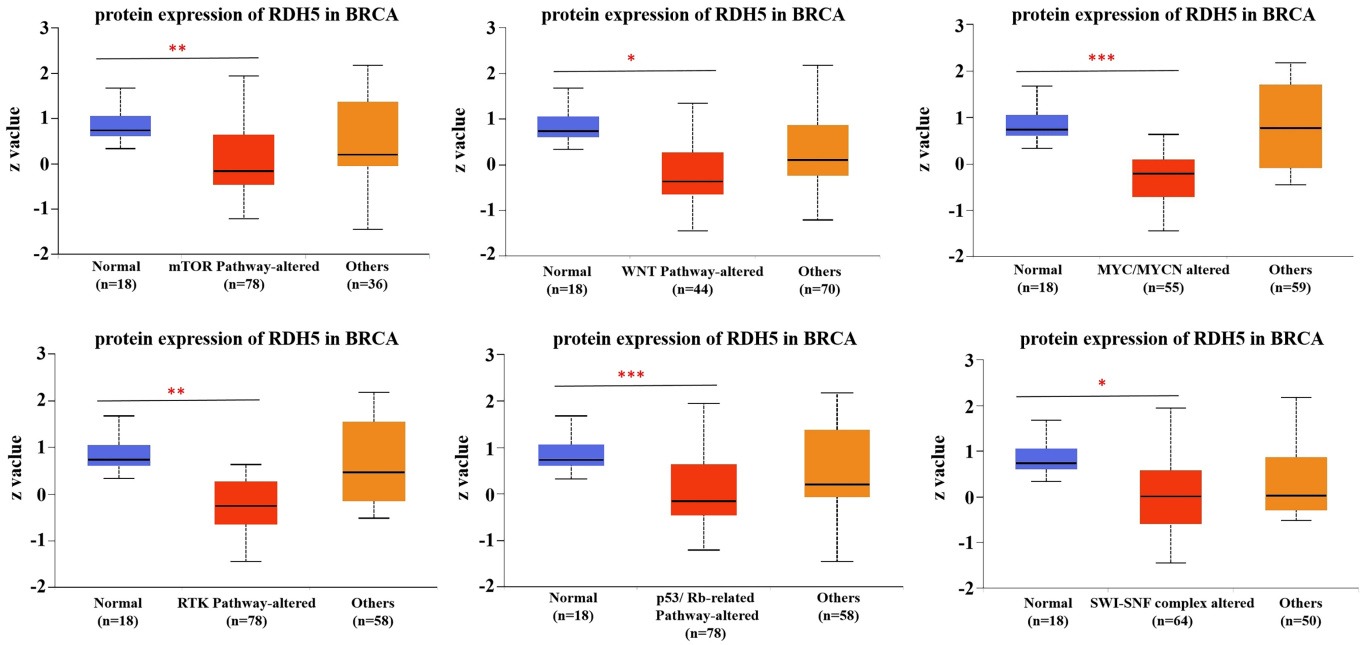

FIGURE 4. Relationship between *RDH5* expression and pathways in breast cancer. *RDH5*: Retinol dehydrogenase 5; BRCA: breast invasive carcinoma.

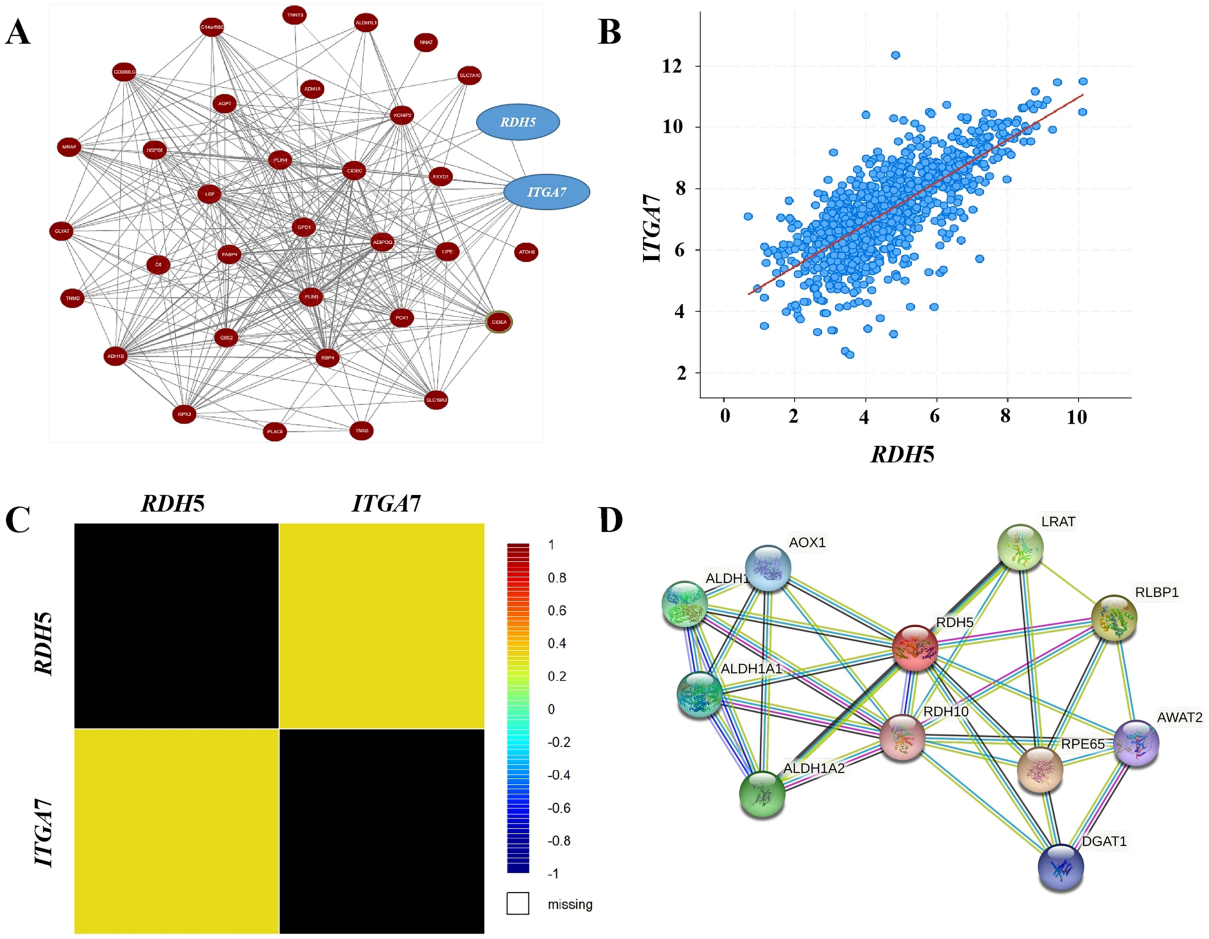

FIGURE 5. Co-expression of the *RDH5* gene. (A) Co-expression of Retinol dehydrogenase 5 (*RDH5*) gene. (B,C) Correlation of *RDH5* and integrin subunit alpha 7 (*ITGA7)* expression in breast cancer by the bc-GenExMiner software; (D) String database showing the PPI (Protein-protein interaction) of *RDH5*.

MYC/MYCN, RTK and p53/Rb pathways (Fig. 4).

3.5 Analysis of RDH5 gene co-expression network and protein interaction

The similarity of gene expression can be used to analyze the possible interaction between genes to determine the relationship between genes and search for core genes. Using the cBioPortal database, 42 genes co-expressed with *RDH5* were identified. Then, a co-expression network (Fig. 5A) was plotted using the Coexpedia database, and its main related functions were identified to be ethanol oxidation, regulation of heart contraction and negative regulation of tumor necrosis factor. Furthermore, it was found that integrin subunit alpha 7 (*ITGA7)* was most strongly associated with *RDH5* (Fig. 5B,C). Lastly, using the String database, we analyzed the protein interaction network of *RDH5* (Fig. 5D) and found that *RDH5* interacted with the remaining 10 proteins, including retinol dehydrogenase 10 (RDH10), retinaldehyde binding protein 1 (RLBP1), retinoid isomerohydrolase (RPE65), and others. The most relevant KEGG pathways were retinol metabolism, vitamin digestion and absorption and metabolic pathways.

4. Discussion

Breast cancer is one of the most common malignancies and the top cause of death in women [18]. Although great progress has been made in treating breast cancer, the prognoses of advanced-stage patients are poor due to its invasive and metastatic characteristics, high malignancy and rapid progression [19]. Thus, screening and early diagnosis of breast cancer are key to reducing morbidity and mortality, and it is urgently needed to look for more and more effective biomarkers [20, 21]. The *RDH5* gene is mapped on chromosome 12q13–q14 and encodes a 32 kda protein containing 318 amino acids [6]. *RDH5* expression, nonsense mutation and DNA methylation disorder were reported to contribute to night blindness [22]. In addition, *RDH5*'s mutation was found in early gastric cancer, suggesting that *RDH5* might be involved in the process of early carcinogenesis [23]. mRNA levels were significantly reduced in colorectal cancer, resulting in reduction of all-trans-retinoic acid biosynthesis, which promotes colorectal cancer progression through affecting cell growth and differentiation [24]. Compared to para-cancer tissues, *RDH5* is hypomethylated and highly expressed in papillary thyroid cancer [25]. However, the exact roles and underlying mechanisms of *RDH5* in breast cancer patients remain under investigated.

In this paper, we analyzed the *RDH5* expression and found that *RDH5* was differentially expressed in various tumors and had a low expression in breast cancer. In particular, *RDH5* was significantly down-regulated in the luminal, HER-2 positive, and triple-negative subtypes of breast cancer. Using the bcGenExMiner database, we found no significant difference in *RDH5* among breast cancer patients aged $\leq$51 and $>$51 years, but significant differences in PR, HER-2, NPI, SBR, PAM50 subtype, RSSPC subtype and triple-negative status. The overall and disease-free survival curves showed no significant association between *RDH5* expression and the survival of breast cancer patients. The UALCAN database showed that the mTOR, WNT, MYC/MYCN, RTK and p53/Rb pathways and the SWI-SNF complex were closely associated with the protein expression of the *RDH5* gene in breast cancer. Lastly, we also analyzed the gene co-expression network of *RDH5* and found that its main related functions were associated with ethanol oxidation, regulation of heart contraction and negative regulation of tumor necrosis factor. We also analyzed the PPI network of *RDH5* and found 10 proteins interacting with it.

5. Conclusions

The expression of *RDH5* in breast cancer indicates that it could be used as a biomarker for the early diagnosis of breast cancer and might have some indication for its treatment and prognosis. However, further investigations, including exploring the mechanism of *RDH5 in vitro* and *in vivo* settings, are required to confirm its significance in breast cancer.

AUTHOR CONTRIBUTIONS

LLW—Data curation, Formal analysis, Writing–Review & Editing; WWZ—Conceptualization & Methodology; CY—Designing the study & conceptualization, HC—concepted, supervised, reviewed & edited the draft. All authors were engaged in the editorial revision of the manuscript. All authors have read and the final manuscript has been approved.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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