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Introduction of hub genes and herbal treatment of breast cancer through bioinformatics

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ABSTRACT

Background: Breast cancer (BC) is a prevalent form of endocrine cancer that affects women globally, and their incidence and mortality rates are predicted to rise significantly in the coming years. As a result, breast cancer continues to pose a significant health issue and is a top priority for biomedical research.

Methods: We used bioinformatics and reverse pharmacology techniques to identify herbal medicines that could be effective in treating breast cancer. To do this, we analyzed 121 genes from a dataset (GSE42568) containing both cancer and normal samples. Through this analysis, we identified differentially expressed genes (DEGs) and then used the protein-protein interaction (PPI) network to identify 19 hub genes. To pinpoint hub genes, we utilized the widely-used bioinformatics tool, Search Tool for Reciprocal Genes (STRING). To conduct a more detailed analysis, subnetworks were identified using the molecular complex detection (MCODE) algorithm.

Results: The hub genes identified in our research are involved in various functions, including positive regulation of cold-induced thermogenesis, patched binding, and the Peroxisome Proliferator-Activated Receptor (PPAR) signaling pathway, as revealed by Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analyses. We understood that the herbs Ginkgo biloba seeds, Polygoni Cuspidati Rhizoma Et Radix, Smilacis Glabrae Rhizoma, Capsici Fructus, Cyathulae Radix, Puerariae Flos, and Ardisiae Japonicae Herba can target hub genes such as PPARG, CCNB1, CAV1, CDH1, ADIPOQ, LEP, IGF1, LPL, DGAT2, ACSL1, and PCK1. Using nine ingredients, these herbs were identified as key in targeting hub genes. This study provides insights into potential therapeutic targets and drugs for treating breast cancer.

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Abbreviations

BC, Breast Cancer; DEGs, Differentially expressed genes; PPI, Protein-protein interaction; STRING, Search Tool for Retrieving Interacting Genes; PPAR, Peroxisome Proliferator-Activated Receptor; GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; CAM, Complementary and alternative medicine; GEO, Gene Expression Omnibus; TCMSP, Traditional Chinese medicine systems pharmacology; HPA, Human Protein Atlas; OS, Overall survival; OB, Oral bioavailability; DL, Drug-likeness; BP, Biological process; MF, Molecular function; HER, Human epidermal growth factor receptor, MCODE, Molecular complex detection

Introduction

Breast cancer is a common and serious health concern for women globally, and its incidence has been increasing over the past few decades (1). Current approaches for treating breast cancer include various methods such as surgery, chemotherapy, radiotherapy, hormone therapy, and targeted therapy (2). While breast cancer in situ is not typically fatal, advanced cases with lymph nodes and distant metastasis can be life-threatening (3). Despite advances in understanding the causes and characteristics of breast cancer, predicting metastasis, and recurrence remains challenging in clinical practice (4), and further research is needed to identify potential mechanisms and biomarkers for monitoring the recurrence of the disease. As biological research technologies, particularly sequencing technologies and bioinformatic algorithms, continue to advance, there has been a remarkable increase in the amount of genomic information being generated. This accumulation of information is happening at an exponential rate (5). In recent years, innovative therapeutic methods have emerged as promising approaches for improving breast cancer treatment outcomes. These methods encompass a range of modalities, including biological therapies, small molecule inhibitors, and stromal cell therapy. Biological therapies, such as immunotherapy, have shown significant promise in the treatment of breast cancer. Immunotherapeutic approaches, such as immune checkpoint inhibitors and CAR-T cell therapy, aim to harness the body's immune system to selectively target and eliminate cancer cells. By enhancing the immune response against breast cancer cells, these therapies have the potential to induce durable responses and improve overall survival rates in patients (6,7). Small molecule inhibitors have also demonstrated efficacy in breast cancer treatment. These inhibitors target specific molecular pathways involved in cancer progression and can disrupt aberrant signaling mechanisms. By inhibiting key molecules involved in tumor growth and survival, small molecule inhibitors provide a targeted approach to breast cancer therapy (8,9). Furthermore, stromal cell therapy has emerged as a potential strategy for enhancing treatment efficacy in breast cancer. Stromal cells, including cancer-associated fibroblasts and immune cells, play a crucial role in the tumor microenvironment and contribute to tumor growth and metastasis. Targeting the interactions between stromal cells and cancer cells offers the opportunity to disrupt tumor-promoting mechanisms and improve treatment outcomes (10,11). In addition to these innovative therapeutic methods, complementary and alternative medicine (CAM) approaches, such as herbal medicine and acupuncture, have shown promise in symptom control and improving the quality of life in breast cancer patients. These therapies can be used in palliative care and as adjuvants in breast cancer treatment. By integrating these innovative therapeutic methods with traditional treatment approaches, there is potential for improved outcomes in breast cancer management. Studying genes that are expressed abnormally in breast cancer (BC) could help shed light on the molecular mechanisms involved in the disease (12). By combining molecular biology and web servers and bioinformatics tools, potential biomarkers for diagnosis, prognosis, and treatment can be identified (12–14). In this study, we aimed to identify key BC-specific genes and investigate their potential as prognostic biomarkers. To do this, gene expression data from BC and normal samples were analyzed and bioinformatic analyses were performed to identify key genes with prognostic value. However, despite advances in BC treatment, the limited therapeutic range and side effects of current drugs remain major barriers to their effectiveness. Complementary and alternative medicine (CAM), such as herbal medicine and acupuncture, has demonstrated that those hold a crucial function in symptom control and improving the quality of life in BC patients. This review focuses on the use of these therapies in palliative care and as adjuvants in BC treatment. The review summarizes the use of herbal medicines and acupuncture, their mechanisms of action, and the acu-points used in acupuncture. The purpose of this review is to provide a better understanding of the performance of medicinal plants in the treatment of breast cancer.

Many studies have been done on various Chinese mechanisms and their combinations in the treatment of diseases. In this study, the gene expression omnibus database (GEO, https://www.ncbi.nlm.nih.gov/geo/) and the pharmacology analysis platform of traditional Chinese medicine systems (TCMSP, https://tcmspw.com/tcmsp.php were used to identify hub genes that are linked to breast cancer and to explore the underlying mechanisms through enrichment analysis. In addition, these databases were used to predict the potential anti-breast cancer effects of some medicinal plants and their active ingredients.

Materials and methods

Data Collection

To detect differentially expressed genes (DEGs) in breast cancer (BC), a gene expression dataset GSE42568 (GPL570 platform) was retrieved from the GEO database (http://www.ncbi.nlm.nih.gov/geo), which is a global repository for microarray/gene data submitted by scientists and researchers. The dataset comprised 104 BC tissue samples and 17 normal breast tissue samples.

Data Preprocessing

The raw gene expression data were processed and normalized using the R software, which is an open-source graphical and statistical computing platform (15). Background adjustment, quantitative normalization, and data processing for all raw files were performed using a strong multiarray averaging RMA algorithm in the Affy package (16).

Identification of DEGs

To determine differentially expressed genes (DEGs) between breast cancer (BC) samples and normal tissue samples, the limma R package, which implements linear models for microarray data, was used (17). Adjusted P values less than 0.05 and absolute log2 fold change |(log2FC)| greater than 2 were considered threshold values for DEG identification.

PPI Network Construction and Hub Gene Validation

The STRING online tool (Search Tool for Retrieving Interacting Genes, available at (https://string-db.org/) was applied to construct a protein-protein interaction (PPI) network. To determine the hub gene, we used a criterion with a degree higher than 20. In addition, a cutoff criterion of an MCODE score greater than 6 was used to identify subnetworks.

GO and KEGG Pathway Enrichment Analyses

GO enrichment analysis is a technique that helps determine the biological function and corresponding mechanism of genes based on the three aspects of molecular function (MF), biological process (BP), and cellular component (CC). Microarray data are commonly used to extract biological function information (18). The KEGG database is an extensive collection of pathways, genes, compounds, drugs, and diseases used to annotate the biological functions of genes and genomes at the molecular level (18). GO and KEGG enrichment analyses were performed on hub genes using Enrichr, an online analysis tool for gene function annotation accessible at https://maayanlab.cloud/Enrichr/. $P< 0.05$ was defined as significant.

Validation of Gene Expression in the UALCAN Database

An online cancer transcriptome database called UALCAN, accessible at http://ualcan.path.uab.edu/, was used to compare the expression levels of hub genes identified in normal breast tissue and breast cancer samples. UALCAN was created specifically to facilitate access to publicly available cancer transcriptome data, including transcriptome sequencing data from TCGA and MET500 (19). We accessed this database to perform our analysis.

Human Protein Atlas

It has been recognized for some time that immunohistochemistry can provide valuable additional information beyond morphology and that protein expression patterns in cancer may provide important diagnostic and prognostic insights. To detect the protein expression profiles in both tumor and normal tissue, we used the Human Protein Atlas, available at www.proteinatlas.org (20).

Kaplan-Meier Plotter

The possible role of hub genes and specific subgroups with different expression levels (DEGs) in predicting patient outcomes was investigated. The Kaplan-Meier plotter database containing expression profiles and survival information of cancer patients was used (21). This allowed us to divide breast cancer patient samples into high and low -expression groups based on mean mRNA levels. Then we analyzed the duration of life (overall survival or OS) of the patients. A log-rank P-value below 0.05 was considered statistically significant, indicating a possible prognostic value.

Screening of Active Ingredients

The TCMSP database requires protein names as inputs to retrieve drug information about specific target genes. However, the gene expression data from previous steps used gene names, not protein names. To enable comparison with the TCMSP database, the gene names first had to be converted to the corresponding protein names. This was achieved using the UniProt (https://www.uniprot.org/help/about)database, a freely accessible repository of protein sequences and annotations. After obtaining the protein names for the key genes, active ingredients of Chinese herbal plants were selected in TCMSP based on oral bioavailability (OB) and drug similarity (DL).

Screening and Annotation of Key Herbs

After determining herbal plants with active substances to treat breast cancer, a study examined the connection between plants and target genes. If multiple components in a plant were related to a target gene, only one association between that plant and the gene was counted. The relevance of herbal medicines for breast cancer treatment increased with the number of target genes linked to them. We defined plants that interact with multiple target genes as key plants for breast cancer treatment.

Statistical Analysis

To determine differentially expressed genes (DEGs) between breast cancer samples and normal tissue samples, the limma R package was used. The statistical test implemented in limma is a linear model for microarray data analysis. The adjusted p-values were calculated to assess the significance of differential expression. The Kaplan-Meier plotter database was used to analyze patient outcomes based on the expression levels of hub genes. The log-rank test was used to assess the statistical significance of the differences in overall survival (OS) between high and low-expression groups. GO and KEGG enrichment analyses on hub genes were part of the statistical tests of this study.

During the study Adjusted P values and P values <0.05 were considered significant.

Results

Identification of DEGs

The analysis focused on a dataset GSE42568 that included 17 normal samples and 104 samples of breast cancer. Genes that met the criteria of an adjusted P-value less than 0.05 and a |log2FC| greater than 2 were classified as differentially expressed genes (DEGs). A total of 355 DEGs were identified, with 99 being up-regulated and 256 being down-regulated, as demonstrated in the volcano plot (Fig. 1 and Supplementary Table S1). The volcano plot displays the log2 (fold change) value on the horizontal axis and the log10 (p-value) on the vertical axis.

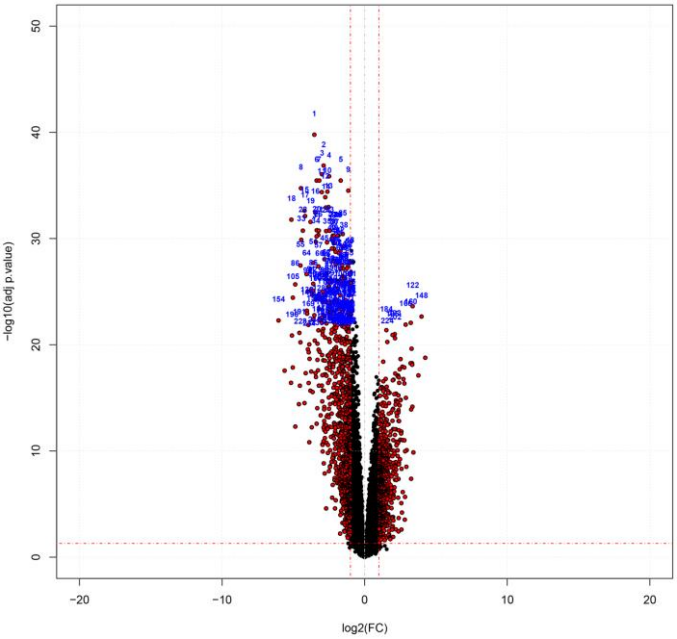

Figure 1. Volcano plot to identify diferentially expressed genes (DEGs). Panel a illustrate volcano plots of DEGs GSE42568. Differential gene expression profiles are shown according to the negative logarithm to base 10 of Adj. P-value and and |log2FC|>1 were considered as cutoff criteria.

Analysis of protein-protein interaction (PPI) networks and identification of hub genes

To identify hub genes and modules within the protein-protein interaction (PPI) network, we utilized Cytoscape software, which is a tool for integrating, analyzing, and visualizing network data. We used a cutoff criterion of degree >20 to identify hub genes, resulting in the identification of 19 hub genes including PPARG, CDH1, GF2, ADIPOQ, EPCAM, EZH2, FABP4, LEP, LPL, CCNB1, SCD, IGF1, DGAT2, PLIN1, ACSL1, KRT19, CAV1, FOXA1, and PCK1. Were reported subnets with MCODE scores <6 are depicted in Fig. 2.

GO Enrichment Analysis of Hub Genes:

To determine pathways and functions associated with differentially expressed genes (DEGs), KEGG pathway enrichment analysis and Gene Ontology (GO) were performed. The analysis indicated that the DEGs were primarily enriched in the PPAR signaling pathway, upregulation of cold-induced thermogenesis, and tight junctions. These results are shown in Figure 3.

Hub Gene Validation through UALCAN:

The UALCAN database was used to confirm the expression status of the 19 hub genes identified in breast cancer compared to normal breast tissue. The analysis showed that six up-regulated hub genes (CDH1, EPCAM, EZH2, CCNB1, KRT19, and FOXA1) identified from the GSE database were expressed at higher levels in breast cancer (as shown in Figure 4 with p <0.05), while 13 down-regulated hub genes (PPARG, FGF2, ADIPOQ, FABP4, LEP, LPL, SCD, IGF1, DGAT2, PLIN1, ACSL1, CAV1, and PCK1) were expressed at lower levels (as shown in Figure 4 with p < 0.05).

Prognosis in Patients with Protein Expression:

To determine whether abnormal expression of hub genes affects the overall survival of breast cancer patients, we conducted a survival analysis using the Human Protein Atlas (HPA). We evaluated the relationship of hub genes to each other in the PPI network and overall survival (OS). The results showed that high expression of six up-regulated hub genes (CDH1, EPCAM, EZH2, CCNB1, KRT19, and FOXA1) was associated with decreased patient survival, while low expression of eight down-regulated hub genes (PPARG, FGF2, ADIPOQ, FABP4, SCD, PLIN1, CAV1, and PCK1) was significantly linked to improved patient survival. These findings are presented in Figure 5.

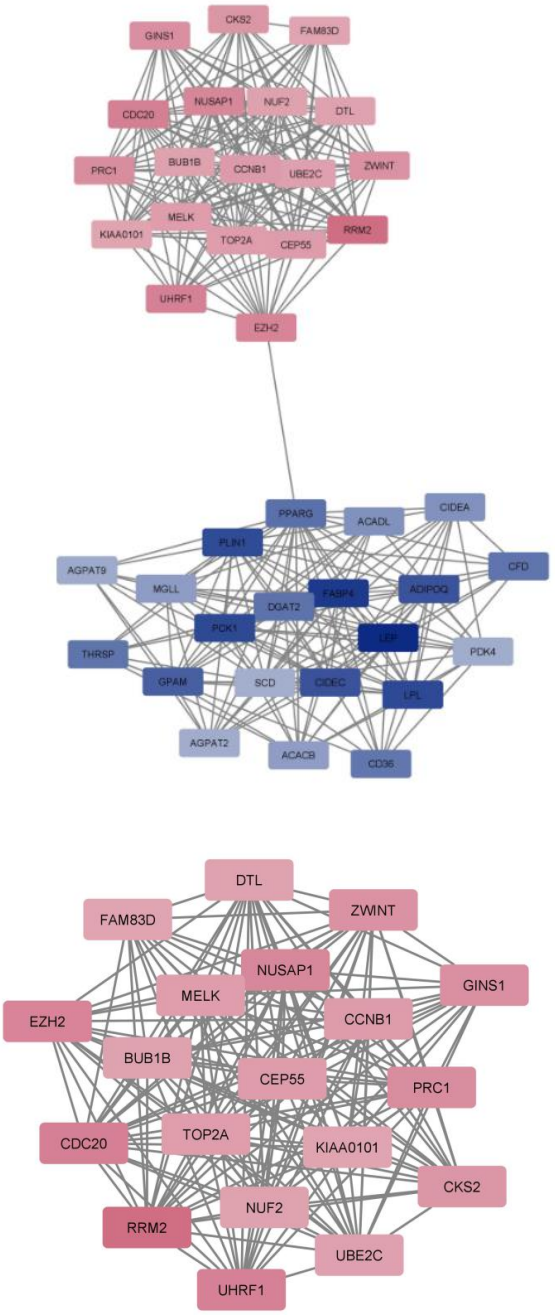

Figure 2. Subnetwork of MCODE score<6 from the protein–protein interaction (PPI) network.

Survival Analysis:

The Kaplan-Meier plotter was utilized to examine the relation of the selected DEGs to overall survival (OS) in breast cancer (BC) patients. The researchers analyzed the association of identified key DEGs and OS in all BC patients and found that 11 out of the 19 hub genes showed a noticeable connection with OS, as shown in Figure 6, High expression of PPARG, FGF2, ADIPOQ, LPL, IGF1, DGAT2, PLIN1, and CAV1 was associated with better OS in BC patients. Conversely, high expression levels of EPCAM, EZH2, and CCNB1 were significantly correlated with poor OS in BC.

Screening of Active Ingredients:

We converted the 19 hub genes to their protein names on the UniProt website so that those could be identified through the TCMSP site. This site allows us to identify effective plants in the treatment treating of breast cancer. After removing genes that were not present in the databases or those that did not have relevant components, 11 genes were identified for further investigation, namely PPARG, CCNB1, CAV1, CDH1, ADIPOQ, LEP, IGF1, LPL, DGAT2, ACSL1, and PCK1. These genes corresponded to 10 substances including chenodeoxycholic acid, naringenin, resveratrol, nicotine, aloe-emodin, daidzein, taxifolin, sucrose, quercetin, and myristin, which showed sufficient human oral bioavailability (OB) and drug-likeness (DL) values listed in Supplementary Table S2.

Introducing the Main Plants in the Treatment of BC:

The databases contained 913 herbs with active ingredients, out of which the top 7 herbs were selected based on their association with more DEGs (related genes=12) and were considered critical in the study. These herbs included Ginkgo Semen, "Polygoni Cuspidati Rhizoma Et Radix", Smilacis Glabrae Rhizoma, Capsici Fructus, Cyathulae Radix, Puerariae Flos, and Ardisiae Japonicae Herbae.

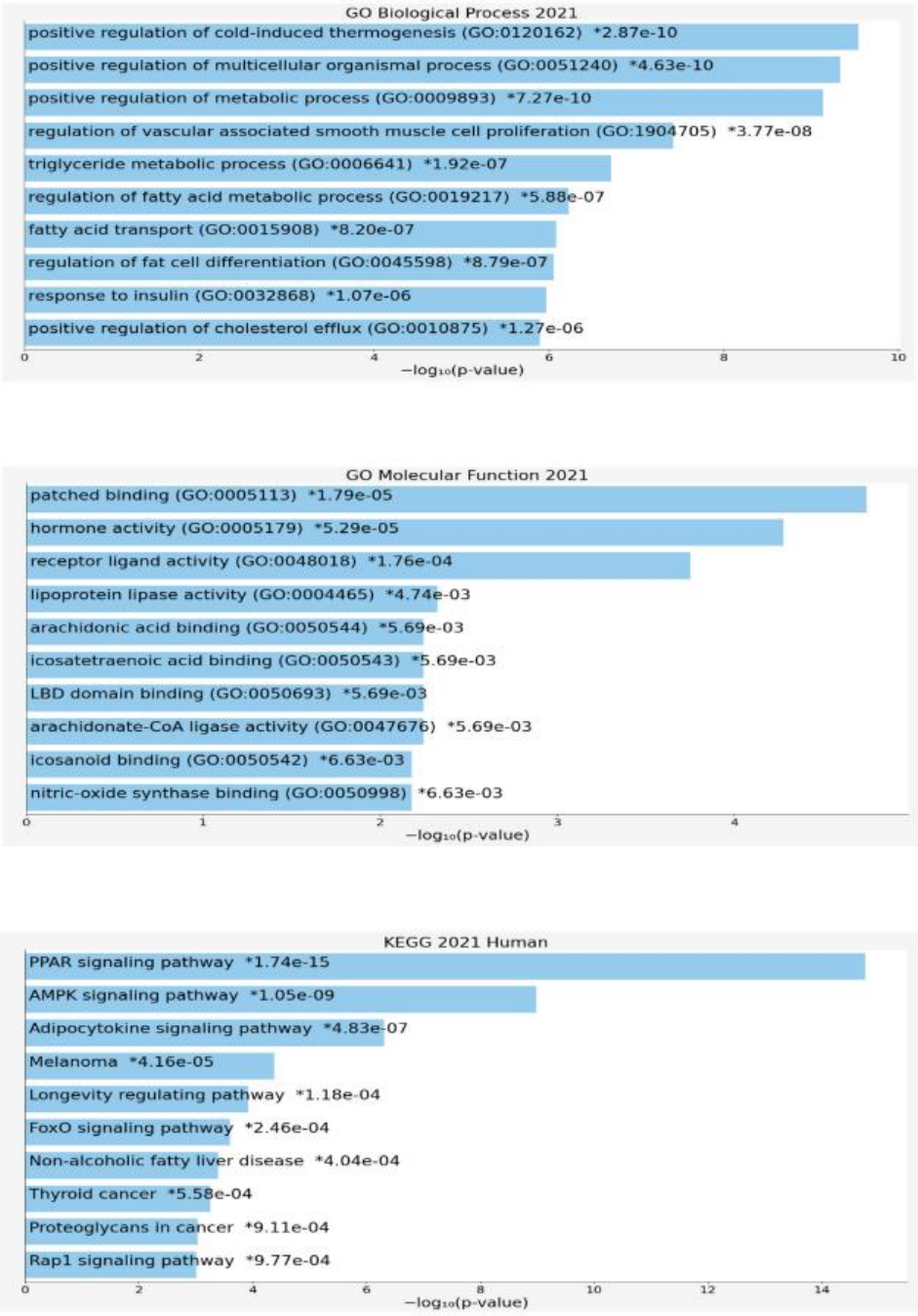

Figure 3. Biological functions based on Gene Ontology (GO) analysis of breast cancer-related hub genes. Advanced bar chart shows signifcance in GO enrichment items of hub genes in two functional groups: biological process (BP) and molecular function (MF). Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis of hub genes.

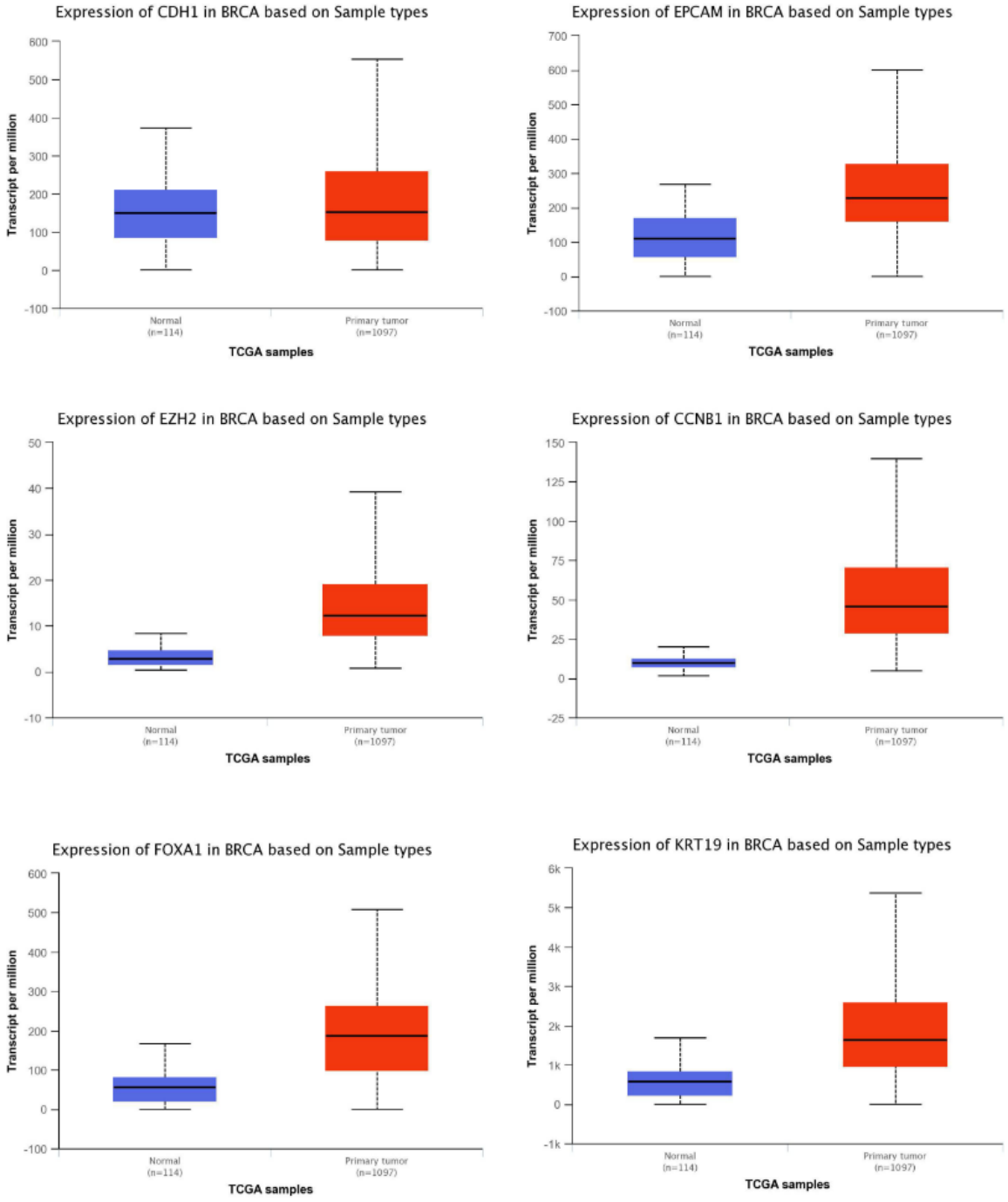

Figure 4. Validation of expression in the UALCAN database for up-regulated hub genes in tomour.

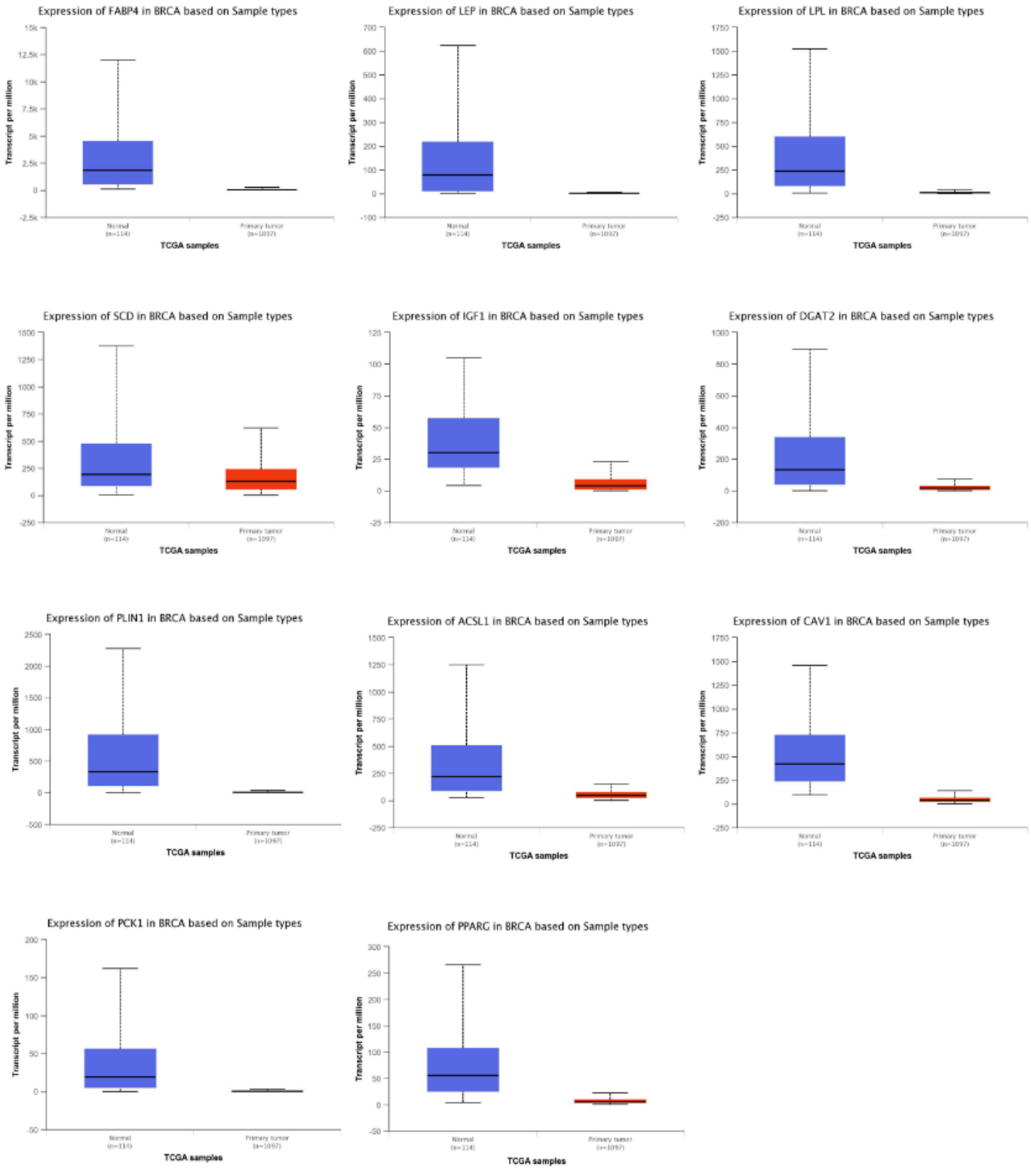

Figure 5. Validation of expression in the UALCAN database for down-regulated hub genes in tomour

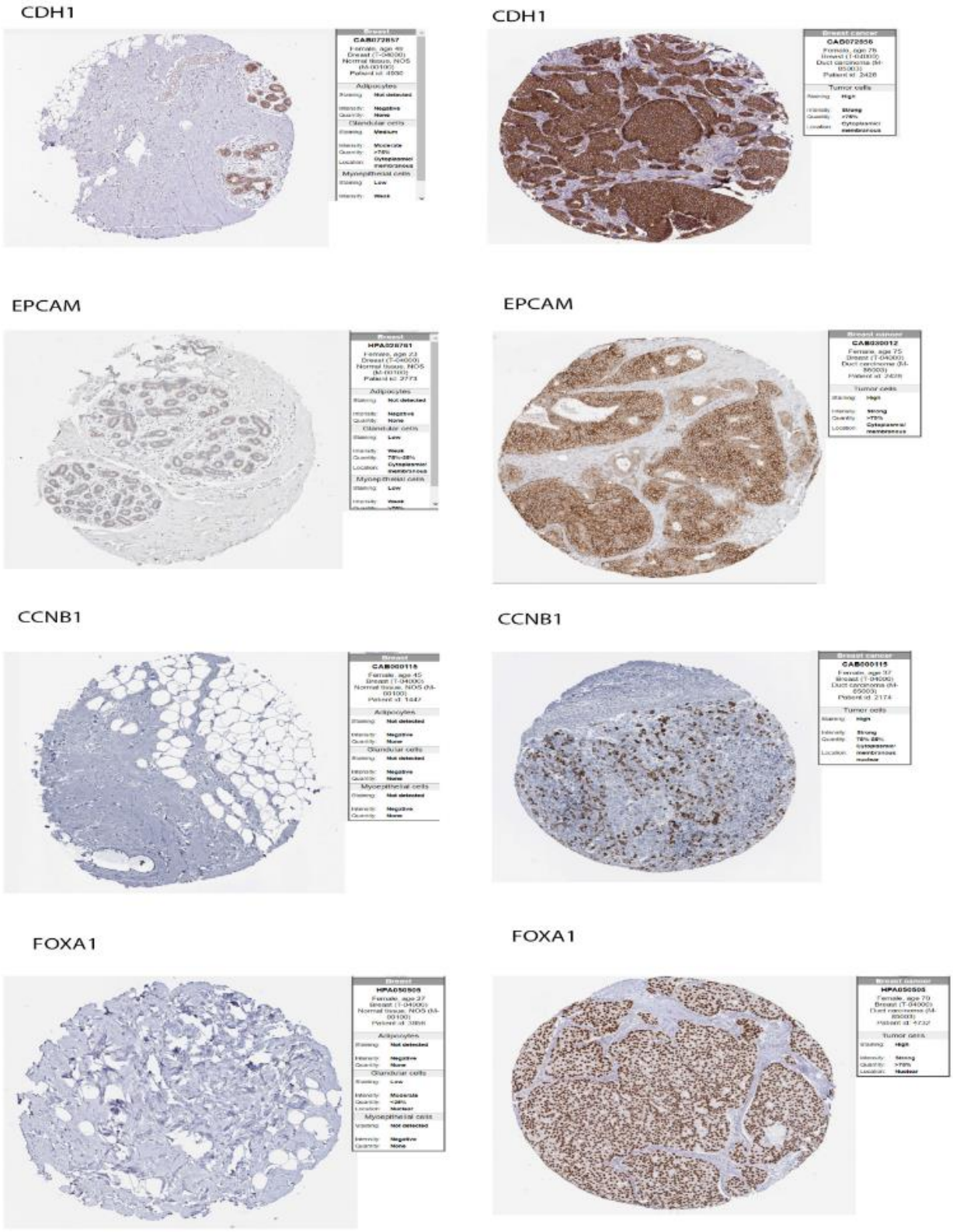

Figure 6. Immunohistochemistry of the four hub genes based on the Human Protein Atlas.

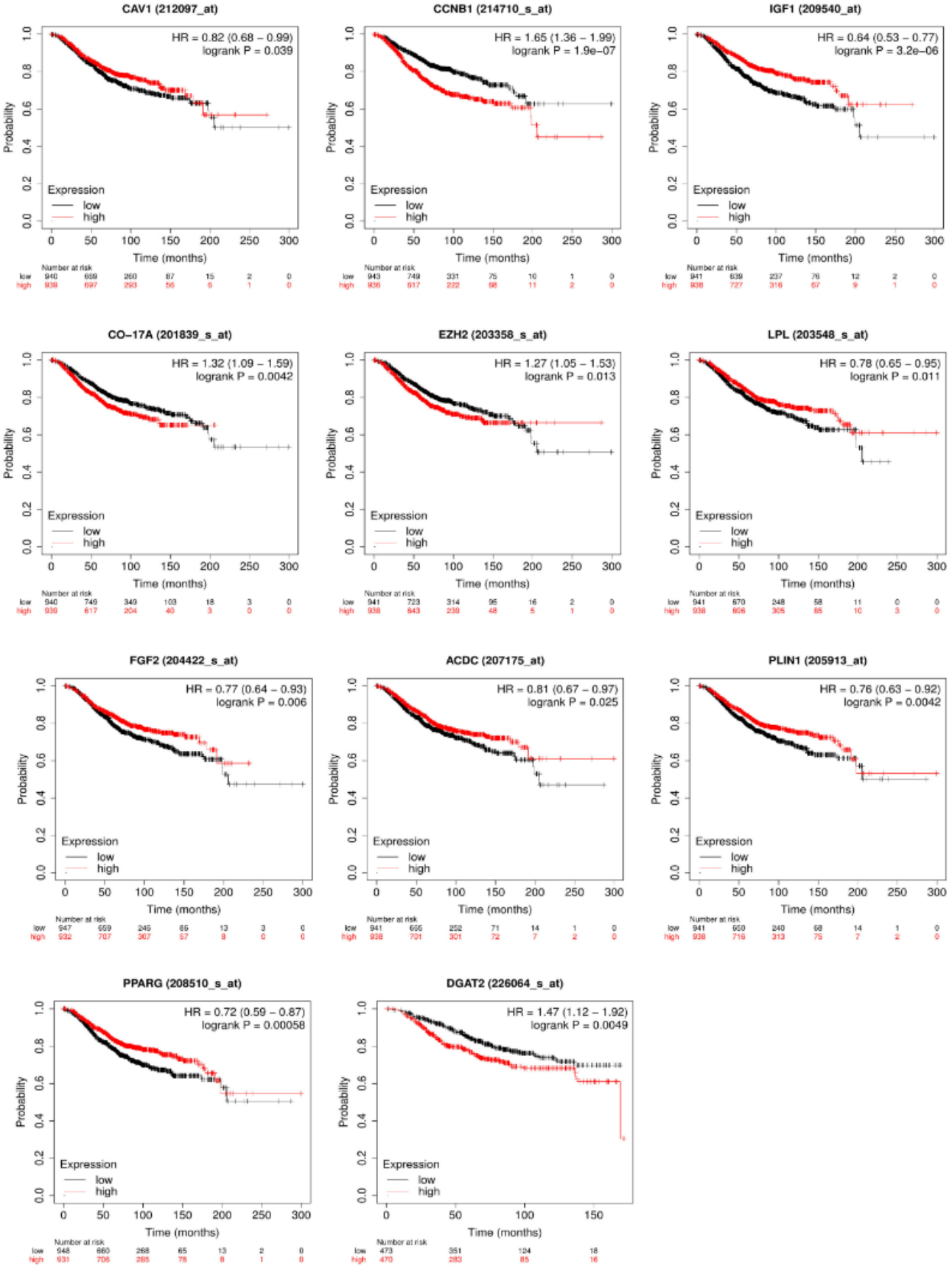

Figure 7. Overall survival (OS) of hub genes in breast cancer based on Kaplan Meier-plotter.

Discussion

Breast cancer is a serious issue, with high incidence and mortality. Understanding its molecular basis can help develop better treatments and outcomes. We analyzed genes in Cytoscape and identified 19 key genes, 11 related to breast cancer prognosis. One of these genes, PPARG, encodes a nuclear receptor activated by fatty acids and lipids. This receptor may play a role in breast cancer (22). The discovery of PPARG's association with breast cancer prognosis is in agreement with the finding that lipid metabolic processes were among the top three pathways identified through KEGG and GO enrichment analyses. This suggests that PPARG, as well as lipid metabolic processes, may play a crucial role in the development of breast cancer. Although PPARG is primarily known for its role in adipocyte differentiation and diabetes, several studies have demonstrated that it also has a significant impact on the growth of various types of cancer (23–26). We obtained reliable evidence that PPARG expression levels are significantly reduced in BRCA tumors. We used TCGA, GEO, and UALCAN to show that PPARG mRNA expression in tumor tissues is lower than in normal tissues. In addition, UALCAN showed a slightly lower protein level than PPARG in tumor tissues. PPARG is predicted to be specifically downregulated in breast cancer. In addition, PPARG overexpression showed longer overall survival (OS) in breast cancer subjects.

ADIPOQ, a hormone released by fat tissue in breast tumors, impedes cancer cell growth. Higher ADIPOQ levels are linked to slower breast cancer progression. ADIPOQ markedly suppresses breast cancer proliferation and triggers cell suicide, in experiments and animals. These actions involve autophagy, a process where cells degrade unwanted parts, which seems key for ADIPOQ's cancer-killing ability. Additionally, ADIPOQ increases the effectiveness of chemotherapeutic agents. ADIPOQ was specifically, decreased in breast cancer. Importantly, high expression of ADIPOQ is significantly associated with improved overall survival in breast cancer patients who have undergone chemotherapy (27).

Research shows CAV1, plays a role in tumor progression (28). High CAV1 expression can promote cancer by inhibiting cell suicide, growth without anchors, and metastasis (29–31). In pancreatic cancer, higher CAV1 expression correlates with worse cachectic (32). Though low CAV1 can slow cancer, CAV1's role is complex. For instance, less CAV1 sped up lung cancer cell growth (33). Studies show CAV1's dual role in breast cancer. While CAV1 can hinder breast cancer, loss of CAV1 predicts a poorer outlook (34). Also, high CAV1 advanced breast cancer cell spread, suggesting a cancer-causing role. More research is needed to clarify CAV1's dual role in breast cancer (35).

The Kaplan-Meier survival analysis showed that breast cancer patients with high expression levels of CCNB1 had lower survival rates. CCNB1 is a crucial member of the cyclin family and plays a vital role in the regulation and initiation of mitosis. During the S phase, CCNB1 levels increase and reach their peak during mitosis, after which they are rapidly degraded as the cell cycle progresses from metaphase to anaphase. CCNB1's role in cancer has been well-studied, including its involvement in breast cancer progression (36).

The proteins DGAT1 and DGAT2 control lipid synthesis by catalyzing the last step in triglyceride formation. Research shows that DGAT2-mediated production of 1-O-acyl ceramide from ceramide and fatty acids at the ER-lipid droplet junction influences how glycerolipids and sphingolipids interact. This process sequesters pro-apoptotic ceramide, which has been associated with chemotherapy resistance in colon cancer cells. Disruption of genes that normally regulate the cell, like DGAT proteins, can fuel uncontrolled growth and metabolism in cancer cells. Finding ways, cancer cells upregulate lipid storage pathways, such as overexpressing DGAT proteins, may uncover new drug targets (37).

EpCAM is a cell surface molecule that is highly expressed in colon and other epithelial carcinomas, and it plays a role in cell-to-cell adhesion. Some clinical trials have targeted EpCAM using antibody therapy. This study found that EpCAM is significantly overexpressed in both primary and metastatic breast cancer tissues. Furthermore, silencing the EpCAM gene decreases the proliferative capacity and invasive potential of breast cancer cells (38).

Endocrine resistance in breast cancer can result from increased growth factor signaling. Fibroblast growth factor 2 (FGF2) exists in low and high-molecular-weight forms, including HMW-FGF2. High levels of intracellular FGF2 have been associated with aggressive behavior in breast carcinomas. To overcome endocrine resistance and reduce breast cancer progression, developing new therapies that target intracellular components of the FGF2 pathway in addition to blocking membrane FGFR may be more effective (39).

EZH2 is an enzymatic epi-protein involved in various essential cell functions. It has been suggested that EZH2 is dysregulated in certain types of breast cancer, particularly in advanced stages. Growing evidence indicates that EZH2 overexpression or dysfunction can affect the pathophysiology of breast cancer (40). Numerous studies over the past twenty years have highlighted the role of insulin-like growth factor-1 (IGF-1) in various pathophysiological processes and the development of solid tumors, including breast cancer.

Both preclinical and clinical data have demonstrated that the IGF-1 receptor is overexpressed and hyper-phosphorylated in various subtypes of breast cancer. The importance of the IGF pathway in tumor cell proliferation and metastasis has made it a significant therapeutic target. The IGF system has been implicated in the oncogenesis of most solid tumors, including breast cancer, and has been linked to resistance to standard breast cancer therapies such as hormonal agents, human epidermal growth factor (HER) receptor targeting agents, and cytotoxic chemotherapy (41).

Breast cancers meet their energy demand in part through the β-oxidation of fatty acids obtained from external sources. These fatty acids also play a role in cell signaling and the construction of new membranes required for the rapid proliferation of tumor cells. Lipoprotein lipase (LPL) hydrolyzes lipoprotein triacylglycerols and phospholipids to produce a significant amount of fatty acids. The lipids obtained through LPL in the microenvironment of breast tumors may promote the growth and development of breast tumors (42). The results of this study, show that the mRNA expression of perilipin-1 (PLIN1) is significantly decreased in human breast cancer. Furthermore, Kaplan-Meier analysis revealed that patients with lower PLIN1 expression had a worse overall metastatic relapse-free survival (43). In this study, we identified 19 hub genes linked to breast cancer progression, and analyzed their associated signaling pathways using public databases. We also used the TCMSP platform to predict several possible natural compounds, and Chinese herbs that target these genes. Based on our findings, we selected the top 7 herbs: ginkgo seeds, knotweed rhizome, and root, Chinese yam rhizome, chili fruit, Cyathula root, kudzu flower, and Ardisia leaf. These were related to more differently expressed genes (11 related genes) and were considered important in our study. These compounds, have the potential to effectively treat breast cancer.

Conclusion

The study identified Ginkgo biloba seeds, Polygoni Cuspidati Rhizoma Et Radix, Smilacis Glabrae RhizomaRhixoma, Capsici Fructus, Cyathulae Radix, Puerariae Flos, and Ardisiae Japonicae Herba as herbs that can target hub genes such as PPARG, CCNB1, CAV1, CDH1, ADIPOQ, LEP, IGF1, LPL, DGAT2, ACSL1, and PCK1. These findings suggest that these herbs, particularly when combined with the nine identified ingredients, have the potential to be used as therapeutic targets or in the development of drugs for the treatment of breast cancer.

Declaration

Funding
Not available.

Conflicts of interest/Competing interests
The authors declare no conflict of interest.

Authors' contributions
AA and FVE designed the study concept, collected and interpreted the data and drafted the manuscript.

Ethics approval
Not applicable.

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