Xihuang Pill regulates Ferroptosis-Related Genes, contributing to improved prognosis of Breast Cancer

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Research Article

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Abstract

The natural compound Xihuang Pill (XHP) has an anti-cancer effect and was effective for breast cancer (BrCa) prevention and treatment. However, the mechanisms underlying this effect remain poorly characterized. Here, we searched the ferroptosis-related genes (FRGs) regulated by XHP using the HERB and FerrDb databases, and extracted the FRGs' data on expression and pertinent clinical data by way of the TCGA dataset. A single-factor Cox regression analysis was used to confirm FRGs associated with prognosis, and LASSO Cox regression analysis was used for prognostic model building. We then constructed a prognostic model and assessed it based on Kaplan-Meier survival curves and ROC curves. Next, we applied GO, KEGG, and ssGSEA analyses to further investigate FRGs' functions and potential mechanisms. We discovered that eight FRGs from the XHP targets were associated with a higher likelihood of survival. The prognostic model formed by eight genes also possessed good prognostic ability, and the risk score could be a separate risk factor for the BrCa prognosis. The GO, KEGG, and ssGSEA analyses showed risk score-related genes were associated with immune-related activities. Molecular docking showed that quercetin and beta-sitosterol as XHP's active ingredients are effective and promising agents for the treatment of BrCa. Our results provided insights into the underlying molecular mechanisms by which XHP improves BrCa patient prognosis; quercetin and beta-sitosterol may provide a new road for anti-BrCa natural products.

1. Introduction

According to the Global Cancer Statistics report, roughly 2.26 million new instances of breast cancer (BrCa) were diagnosed worldwide in 2020, BrCa overtaking lung cancer as the main factor of female cancer incidence over the world and accounting for 6.9% of cancer deaths worldwide, which extremely threatens women's life and health [1]. Based on the expression of Ki67, the hormonal and HER2 status, BrCa can be divided into five essential subtypes: luminal A, luminal B, triple-negative BrCa (TNBC) and HER2-positive BrCa, of which HER2-positive BrCa and TNBC have the worst prognosis [2]. Until now, the etiology and pathogenesis of BrCa have not been entirely enlightened, combined with the high heterogeneity of BrCa, therefore, developing a novel prognostic model is one of the most serious challenges for BrCa. Recently, ferroptosis is a fairly new type of cell death that is distinct from apoptosis, necrosis, pyroptosis, and autophagy. It is referred to as an iron-dependent kind of programmed cell death caused by lipid peroxides that accumulate [3–4]. Ferroptosis performs a prominent role in tumors and can be used to regulate cancer progression by regulating the ferroptosis [5]. With encouraging efficacy in extending the time of survival, reducing side effects, and enhancing cancer sufferers' quality of life, Traditional Chinese Medicine (TCM) has been widely utilized as a natural remedy for thousands of years to both prevent and cure cancer [6]. Xihuang Pill (XHP), a natural compound prescription of Chinese herbal medicine, contains Niuhuang, Shexiang, Ruxiang and Moyao. As a natural product for the treatment of BrCa with advantages of natural sources, exact efficacy, and less side reactions [7–8]. The mechanism of XHP effect is likely related to the regulation of IFNG, EGFR, TP53 and mTOR protein gene expressions, et al. [9–11]. Many investigations have discovered that these proteins are inextricably linked
to ferroptosis [12]. This suggests that the mechanism by which XHP improves the prognosis of BrCa patients might be associated with the regulation of these ferroptosis genes. The focus of this study was the improvement of the prognosis effect of XHP on BrCa and its underlying mechanism, in order to provide proof of the efficacy of XHP in the therapy of BrCa.

2. Results

2.1. Active ingredients and target prediction of XHP

We obtained 85 active ingredients and 1,351 ingredients-related targets for XHP. Of those, Shexiang had 23 active ingredients and 1007 targets; Niuhuang had 10 active ingredients as well as 273 targets; Ruxiang had 11 active ingredients and a total of 481 targets; and Moyao had 41 active ingredients along with 458 targets.

2.2. Screening of FRGs regulated by XHP

A total of 183 genes were obtained from the FerrDb. We crossed these genes with the targets of XHP and obtained 54 FRGs regulated by XHP (Fig. 1).

2.3. BrCa-related data download

We obtained 1036 BrCa patients from the TCGA database.

2.4. Identify the differential prognostic genes of the XHP target related to ferroptosis

In the TCGA cohort, 46 DEGs of XHP targets related to ferroptosis were screened; additionally, nine FRGs of XHP targets having prognostic significance were screened. By taking the intersection of these two datasets, eight differential genes of XHP targets related to prognosis were obtained (Fig. 2A) and visualized using a heatmap and a forest plot (Figs. 2B-C). Therefore, these eight genes were included in the prognostic analysis that followed. An analysis of eight genes' interaction networks showed that IFNG, JUN, MAPK9, PIK3CA, and G6PD were the central genes (Fig. 2D). The R package pheatmap was used to visualize the eight genes of the XHP target co-expression networks that were formed based on the correlations (Fig. 2E). We used Cytoscape to visualize the interaction networks between the active compounds in XHP and the eight prognostic-related differentially expressed genes (Fig. 2F and Table 1).

<table>
<thead>
<tr>
<th>Table 1</th>
</tr>
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<tbody>
<tr>
<td>The prognosis FRGs in BrCa corresponding to the ingredients of XHP</td>
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</tbody>
</table>
2.5. Building and validating a prognostic model based on eight XHP target genes.

We employed LASSO Cox regression to establish a predictive model (risk score) using the identified eight prognostic-related genes of XHP targets. Here is the formula for calculating the risk score:

\[
\text{Risk score} = e^{(\text{ALOX15 expression} \times 0.144399 + \text{G6PD expression} \times 0.279826 - \text{IFNG expression} \times 0.500499 - \text{JUN expression} \times 0.093169 + \text{MAPK9 expression} \times 0.051223 + \text{NCOA4 expression} \times 0.063063 + \text{PIK3CA expression} \times 0.482853 + \text{SQLE expression} \times 0.089276)}
\]

Using the median risk score, we separated BrCa patients into two groups: high-risk and low-risk. The Kaplan-Meier analysis revealed that patients with BrCa who were classified into the low-risk group had a better overall survival compared to those in the high-risk group \((p < 0.001)\) (Fig. 3A). In addition, the time-varying ROC curve was plotted to assess the predictive accuracy of the risk score model, and the results showed that the area under the curve (AUC) at 5 years was 0.621, and the 10-year and 15-year AUC were 0.674 and 0.710, respectively. indicating that this model was able to accurately predict BrCa patients’ survival (Fig. 3B). By using the median risk scores, the risk curve can distinguish BrCa patients into high-risk and low-risk groups, indicating that the risk value obtained by this model is related to patient survival; as the risk increases, the number of fatalities rises (Figs. 3C-D).

<table>
<thead>
<tr>
<th>Natural products</th>
<th>Active ingredients</th>
<th>FRGs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shexiang SX1</td>
<td>Androst-4-Ene-3,17-Dione</td>
<td>PIK3CA, G6PD</td>
</tr>
<tr>
<td>SX2</td>
<td>Testosterone</td>
<td>JUN, NCOA4</td>
</tr>
<tr>
<td>SX3</td>
<td>3,5-Dihydroxybenzoic Acid</td>
<td>IFNG, MAPK9, ALOX15</td>
</tr>
<tr>
<td>SX4</td>
<td>17-Beta-Estradiol</td>
<td>NCOA4, JUN</td>
</tr>
<tr>
<td>SX8</td>
<td>5-Cis-Cyclopentadecen-1-One</td>
<td>PIK3CA, G6PD</td>
</tr>
<tr>
<td>SX9</td>
<td>Decamine</td>
<td>SQLE</td>
</tr>
<tr>
<td>SX23</td>
<td>5-Cis-Cyclooctadecen-1-One</td>
<td>PIK3CA, G6PD</td>
</tr>
<tr>
<td>Ruxiang RX5</td>
<td>P-Menth-4-En-3-One</td>
<td>PIK3CA, G6PD</td>
</tr>
<tr>
<td>RX6</td>
<td>O-Methylacetophenone</td>
<td>MAPK9, ALOX15</td>
</tr>
<tr>
<td>RX9</td>
<td>O-Cresol</td>
<td>IFNG</td>
</tr>
<tr>
<td>Moyao MY1</td>
<td>Cumic Acid</td>
<td>MAPK9, IFNG, ALOX15</td>
</tr>
<tr>
<td>MY3</td>
<td>M-Cresol</td>
<td>IFNG</td>
</tr>
<tr>
<td>MY7</td>
<td>P-Cresol</td>
<td>IFNG</td>
</tr>
<tr>
<td>MY35</td>
<td>beta-sitosterol</td>
<td>JUN</td>
</tr>
<tr>
<td>Shexiang MY38</td>
<td>quercetin</td>
<td>JUN, IFNG</td>
</tr>
</tbody>
</table>
2.6. XHP target independent prognostic risk score of eight genes

During the univariate Cox regression analysis, variables such as age (over 65 vs. under 65 years old), BrCa stage (III/IV vs. I/II), and risk level (high vs. low) were significantly associated with overall mortality ($p < 0.001$) (Fig. 4A). To exclude the influence of confounding factors, we performed a multivariate Cox regression analysis, which indicated that age, cancer stage, and risk score were independent prognostic factors for BrCa ($p < 0.001$) (Fig. 4B).

2.7. Function analysis of DEGs related to risk scores

Based on the results of GO enrichment analysis, the DEGs between the high- and low-risk groups were enriched in biological functions associated with immunity (Fig. 5A). These functions include the humoral immune response, B cell-mediated immunity, lymphocyte-mediated immunity, immune response-activating signal transduction, et al. KEGG analysis revealed that the DEGs were significantly enriched in pathways related to the immune system, which included PD-L1 expression, the PD-1 check-point pathway in cancer, Th1 and Th2 cell differentiation, and the T cell receptor signaling pathway, et al.

The ssGSEA results suggested that 14 out of 16 immune cell scores showed a significant increase in the low-risk group, including CD8+ T, B, Th1, Th2, and Treg cells, et al. Moreover, higher scores were obtained for all immune pathways in the low-risk group, which might be the reason why BrCa patients with a low level of risk fared better (Figs. 6A-B).

2.8 Molecular docking analysis validates the binding affinity of XHP active ingredients and prognostic-related genes.

To evaluate the affinity of the active ingredients of XHP for their eight corresponding targets (the screened potential prognostic targets), analyses of molecular docking were conducted. The energy values, interactions, and binding modes were obtained with Autodock Vina v.1.2.2 (Fig. 7 and Table 2). Results showed that each active ingredient of XHP was bound to its targets by hydrogen bonds and electrostatic interactions. Furthermore, the hydrophobic pockets of each target were occupied successfully by active ingredients of XHP. Almost all binding energies of the bioactive ingredients of XHP and eight corresponding target proteins were lower than −5.0, which revealed that XHP active ingredients had high binding activity with prognostic-related proteins, especially the NCOA4-17-Beta-Estradiol complex (-10.405 kcal/mol) and SQLE-Decamine complex (-10.853 kcal/mol). Meanwhile, the natural compound XHP shows the characteristics of an effective multi-target and multi-component drug.

3. Discussion

Increasing evidence reveals that ferroptotic leading to tumour growth inhibition, and targeting ferroptosis is an attractive approach in cancer treatment [13]. Research conducted both in vitro and in vivo has
demonstrated that natural pharmaceutical products have strong anticancer efficacy in BrCa cells [14–16]. In recent years, XHP as a natural product draws increasing attention in the prevention and treatment of BrCa [8–11, 17–18]. However, the relationship between XHP and ferroptotic for BrCa has not been reported. On account of this, we analyzed and discussed the XHP to improve BrCa outcomes by regulating ferroptotic genes by bioinformatics methods. An in-depth functional analysis of the above genes could shed light on the biological mechanism through which XHP improves BrCa patients’ prognoses.

In our study, we identified eight possible ferroptotic-related genes (IFNG, JUN, MAPK9, PIK3CA, G6PD, ALOX15, NCOA4, and SQLE) linked with BrCa patient prognosis, which may be regulated by XHP. We then built an 8-gene BrCa prognostic model that can accurately predict the outcome of BrCa patients. This illustrates that XHP can improve the outcome of BrCa and that the mechanism is connected to the modulation of the levels of expression for eight FRGs.

Table 2 Binding Energy for prognostic targets with the active ingredients of XHP
<table>
<thead>
<tr>
<th>Prognostic Targets</th>
<th>Active Ingredients</th>
<th>Binding Energy (kcal/mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALOX15 (PDB ID: 2SBL)</td>
<td>Dihydroxybenzoic Acid (CID: 7424)</td>
<td>-6.175</td>
</tr>
<tr>
<td></td>
<td>O-Methylacetophenone (CID: 11340)</td>
<td>-5.861</td>
</tr>
<tr>
<td></td>
<td>Cumic Acid (CID: 10820)</td>
<td>-6.722</td>
</tr>
<tr>
<td></td>
<td>Androst-4-Ene-3,17-Dione (CID: 6128)</td>
<td>-8.579</td>
</tr>
<tr>
<td>G6PD (PDB ID: 7SNF)</td>
<td>5-Cis-Cyclopentadecen-1-One (CID: 5316264)</td>
<td>-6.977</td>
</tr>
<tr>
<td></td>
<td>5-Cis-Cyclotetradecen-1-One (CID: 5316269)</td>
<td>-6.716</td>
</tr>
<tr>
<td></td>
<td>P-Menth-4-En-3-One (CID: 107372)</td>
<td>-5.35</td>
</tr>
<tr>
<td></td>
<td>3,5-Dihydroxybenzoic Acid (CID: 7424)</td>
<td>-5.163</td>
</tr>
<tr>
<td></td>
<td>O-Cresol (CID: 335)</td>
<td>-4.773</td>
</tr>
<tr>
<td>IFNG (PDB ID: 1FG9)</td>
<td>M-Cresol (CID: 342)</td>
<td>-4.664</td>
</tr>
<tr>
<td></td>
<td>P-Cresol (CID: 2879)</td>
<td>-4.583</td>
</tr>
<tr>
<td></td>
<td>Quercetin (CID: 5280343)</td>
<td>-6.613</td>
</tr>
<tr>
<td></td>
<td>Testosterone (CID: 6013)</td>
<td>-5.359</td>
</tr>
<tr>
<td></td>
<td>17-Beta-Estradiol (CID: 5757)</td>
<td>-5.227</td>
</tr>
<tr>
<td></td>
<td>beta-sitosterol (CID: 222284)</td>
<td>-5.578</td>
</tr>
<tr>
<td>JUN (PDB ID: 1JNM)</td>
<td>3,5-Dihydroxybenzoic Acid (CID: 7424)</td>
<td>-5.514</td>
</tr>
<tr>
<td></td>
<td>O-Methylacetophenone (CID: 11340)</td>
<td>-5.603</td>
</tr>
<tr>
<td></td>
<td>Cumic Acid (CID: 10820)</td>
<td>-6.428</td>
</tr>
<tr>
<td></td>
<td>Testosterone (CID: 6013)</td>
<td>-7.374</td>
</tr>
<tr>
<td>MAPK9 (PDB ID: 3E7O)</td>
<td>17-Beta-Estradiol (CID: 5757)</td>
<td>-10.405</td>
</tr>
<tr>
<td></td>
<td>Androst-4-Ene-3,17-Dione (CID: 6128)</td>
<td>-6.546</td>
</tr>
<tr>
<td></td>
<td>5-Cis-Cyclopentadecen-1-One (CID: 5316264)</td>
<td>-4.947</td>
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<tr>
<td></td>
<td>5-Cis-Cyclotetradecen-1-One (CID: 5316269)</td>
<td>-5.115</td>
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<tr>
<td>NCOA4 (PDB ID: 1T5Z)</td>
<td>P-Menth-4-En-3-One (CID: 107372)</td>
<td>-4.342</td>
</tr>
<tr>
<td></td>
<td>Decamine (CID: 5351247)</td>
<td>-10.853</td>
</tr>
</tbody>
</table>

The association of ferroptosis and tumor-immune cell infiltration and immunotherapy are closely related in BrCa according to the reported literature [19]. XHP has been reported to modulate the IL-2 and IFN-γ
levels and the content of B7-1 cells, Treg cells, CD3 + T cells, and CD8 + T cells, increase the function of the mononuclear phagocytic cells, and to further enhance immune function and play an antitumor effect \[10, 17, 20\]. So, whether \textit{XHP} is involved in the regulation of immune responses by eight ferroptosis genes related to the \textit{BrCa} patient's prognosis still has to be elucidated. Analyses of GO and KEGG have revealed that the DEGs separating a high-risk category and a low-risk category for \textit{BrCa} in the prognosis model for eight genes of the \textit{XHP} target that were enriched in biological functions and pathways relevant to immune responses. The ssGSEA results indicated that scores were higher among the low-risk group for immune cells and immunological pathways than the high-risk group. Those findings revealed that mechanism by which \textit{XHP} improves the prognosis of \textit{BrCa} patients perhaps connected to the regulation of ferroptosis genes and antitumor immune responses.

Molecular Docking Analysis showed that \textit{XHP} active ingredients had high binding activity with eight ferroptosis and prognostic-related targets, among them, quercetin and beta-sitosterol have been reported to exert \textit{BrCa}-preventive effects. Quercetin is a flavonoid that exhibits various anti-\textit{BrCa} activities, such as antioxidant \[21\], antiproliferation \[22\], cell cycle arrest \[23\], apoptotic activities \[24\], and antimetastatic \[25\]. According to one recent study, quercetin increases nuclear transcription factor EB (TFEB) expression and nuclear transcription, promotes ferroptotic cell death, and is thus able to kill \textit{BrCa} cells \[26\]. Beta-sitosterol has anti-tumor activity in \textit{BrCa} via cell cycle arrest, apoptosis induction, and tubulin-targeting action \[27–29\]. Taken together, \textit{XHP} has important natural active compounds such as quercetin and beta-sitosterol, which are effective and promising agents for the treatment of \textit{BrCa}.

4. Materials And Methods

4.1. Mining of active ingredients and targets of \textit{XHP}

The BenCaoZuJian dataset (http://herb.ac.cn/) was searched for the active ingredients of \textit{XHP} \[30\]. Swiss ADME was employed to screen the active ingredients for high oral availability and drug-like properties (http://www.swissadme.ch/index.php), and Swiss Target Prediction was used to acquire the target protein for the active ingredients of \textit{XHP} (http://www.swisstargetprediction.ch/) \[31–32\]. All of the above-mentioned targets were converted to their appropriate gene symbols using the UniProt dataset (https://www.uniprot.org/).

4.2. \textit{XHP}-regulated ferroptosis-related genes (FRGs) screening

We obtained \textit{FRGs} from the ferroptosis dataset (FerrDb) (http://www.zhounan.org/ferrdb/), which is a dataset of regulators and indicators of ferroptosis associated with illnesses that have been empirically verified \[12\]. The intersection of \textit{XHP} active ingredient targets and \textit{FRGs} was obtained, and the Venn diagram was made with the Venny 2.1 tool.

4.3 \textit{BrCa}-related data download
The gene expression and clinical characteristics for BrCa were taken out from the TCGA dataset (https://portal.gdc.cancer.gov/). Our study was exempt from ethical review because we used publicly released data and TCGA access policies and publication guidelines were followed.

4.4. Building a prognostic model using XHP-regulated FRGs

Using the R software's limma package, we analyzed the differently expressed genes (DEGs) in BrCa tissues and their surroundings. Using the intersection of DEGs and XHP-regulated FRGs, we identified XHP-regulated FRGs in BrCa. Analyzing overall survival (OS) using univariate Cox regression and looking for prognostic FRGs of XHP-regulated.

A protein-protein interaction (PPI) network of XHP-regulated FRGs with prognostic value was generated using String online tools (https://www.string-db.org/). A LASSO-COX regression model analysis was carried out using the glmnet package in R software. Meanwhile, we evaluated the effectiveness of our prognostic model using ROC curves.

4.5 Independent prognostic analysis of XHP target genes

In order to determine if the risk score of the screen genes of the XHP target could be used as an independent predictive predictor of OS, univariate and multivariate Cox regression analyses were conducted on the provided clinical indicators.

4.6. GO and KEGG analyses of the DEGs

We utilized the Limma package of programs based on R to assess the DEGs between groups at high and low risk and to screen out risk-differential genes. Then, DEGs were mapped to the GO and KEGG databases, and analyses were carried out in R using the “cluster Profiler” package. The scores relating to the infiltration of immune cells and immune pathways in BrCa patients in a high-risk group and a low-risk group were analyzed using the "ssGSEA' R package.

4.7. Validation of affinity of the active ingredients of XHP and prognostic-related genes by protein-ligand docking analysis

We obtained the molecular structures of XHP's active ingredients in .sdf format from PubChem (https://pubchem.ncbi.nlm.nih.gov/), while the 3D structures of the target proteins were retrieved from the Protein Data Bank (http://www.rcsb.org/) and saved in .pdb format. Subsequently, we performed docking calculations using Autodock Vina version 1.1.2 program.

4.8. Statistical analysis

Statistical analysis was conducted using R version 4.1.1. A t-test was used to analyze DEGs among BrCa tissues and neighboring noncancerous tissues; the Kaplan-Meier method was utilized to estimate OS rates among groups at high- and low-risk; and the OS prognostic factors were determined using univariate and multivariate Cox regression analysis. The p value under 0.05 was considered statistically significant.
5. Conclusions

Finally, we identified eight ferroptotic-related genes that are regulated by XHP and associated with the prognosis of patients with BrCa, and we built a BrCa prognostic risk model that performed well using these identified prognostic genes. BrCa immunity was linked to the risk score. These findings describe a novel mechanism for using XHP as a natural product to treat BrCa. However, more research will be required to explain this.

Declarations

Supplementary Materials: The following supporting information can be downloaded at: www.mdpi.com/xxx/s1, Table and Figure: Table 1. The prognosis FRGs in BrCa corresponding to the ingredients of XHP. Table 2. Binding Energy for prognostic targets with the active ingredients of XHP. Figure 1. Venn diagrams of XHP targets and FRGs. Figure 2. Identify differential prognostic genes of XHP target related to ferroptosis. Figure 3. Analysis of the prognostic model including eight prognostic genes of XHP target in BrCa patients. Figure 4. Independent prognostic analysis of eight genes of the XHP target for BrCa. Figure 5. Function analysis of DEGs related to risk scores. Figure 6. Comparison of the ssGsEA scores between high and low-risk group. Figure 7. Binding mode of the active ingredients of XHP and prognostic-related genes molecular docking.

Author Contributions: Dehui Li designed the research, Dehui Li, Chenglin Mu and Xukuo Liu performed the research, Dehui Li and Guanjun Wang analyzed the data, and Dehui Li and Tiegang Li wrote the essay. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: Our study was exempt from ethical review because we used publicly released data and TCGA access policies and publication guidelines were followed.

Data Availability Statement: On reasonable request, the data will be acquired from the corresponding authors.

Conflicts of Interest: All authors state that they have no conflicting interests in this research.

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References


Figures

![Venn diagram](image)

**Figure 1.** Venn diagrams of XHP targets and FRGs.

**Figure 1**

See image above for figure legend.
Figure 2. Identify differential prognostic genes of XHP target related to ferroptosis.  
A. Venn diagrams of the DEGs and prognostic FRGs of XHP targets.  
B. Heatmap of the DEGs of XHP target associated with OS.  
C. Forest plot of univariate Cox regression analysis for FRGs of XHP targets expression and OS.  
D. PPI protein interaction network of eight prognostic-related genes of XHP targets.  
E. The correlations among eight prognostic-related genes of XHP targets.  
F. Interaction network between the active ingredients and eight prognostic-related genes of XHP.  

Figure 2  
See image above for figure legend
Figure 3. Analysis of the prognostic model including eight prognostic genes of XHP target in BrCa patients.

A. Kaplan-Meier survival curve of BrCa patients in the high and low-risk group.
B. Prognostic model for evaluating the area under the curve (AUC).
C. Risk score distribution and median in TCGA-BrCa cohort.
D. OS status and risk score scatter plots in TCGA-BrCa cohort.

Figure 3

See image above for figure legend
Figure 4. Independent prognostic analysis of eight genes of the XHP target for BrCa.
A. Results of single-factor regression analysis.
B. Results of multivariate regression analysis.

Figure 4

See image above for figure legend
Figure 5. Function analysis of DEGs related to risk scores.
A. GO enrichment analysis of DEGs between high and low-risk group.
B. KEGG analysis of DEGs between high and low-risk group.

Figure 5
See image above for figure legend
Figure 6. Comparison of the ssGSEA scores between high and low-risk group.
A. The scores of 16 immune cells.
B. The scores of 13 immune pathways.
(ns: No significant difference; *p<0.05; **p<0.01; ***p<0.001)

Figure 6

See image above for figure legend
Figure 7. Binding mode of the active ingredients of XHP and prognostic-related genes molecular docking.

Figure 7

See image above for figure legend