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Molecular basis of breast cancer with comorbid depression and the mechanistic insights of Xiaoyaosan in treating breast cancer-associated depression

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Abstract

Depression and breast cancer (BC) have been found to have a shared genetic basis, multiple loci of effect, and a presumed causal relationship. The treatment of BC combined with depression poses significant challenges. This study aims to use bioinformatics and network pharmacology to explore the molecular basis of BC combined with depression and to elucidate the potential mechanisms of Xiaoyaosan (XYS) in treating this disease. The molecular background of BC complicated with depression was discovered via data mining and bioinformatics. The molecular mechanism of XYS in the treatment of BC with depression was investigated by network pharmacology. The binding affinity between targets and active compounds was evaluated by molecular docking. The impact of XYS on the gene and protein expression of matrix metallopeptidase 9 (MMP9) in microglial cells was assessed using RT-quantitative PCR and western blot analysis, respectively. Differential expression analysis was conducted to identify genes associated with BC, revealing that 2958 genes were involved, with 277 of these genes also being related to depression. XYS was found to contain 173 active compounds and 342 targets, with 44 of these targets being involved in regulating the progression of BC and depression. Enrichment analysis was performed to identify pathways associated with these targets, revealing that they were related to cell proliferation, catalytic activity, cell communication, and interleukin-18 signaling and LXR/RXR activation. Network analysis was conducted to identify key targets of Xiaoyaosan in treating BC combined with depression, with EGF, interleukin 6, epidermal growth factor receptor, and peroxisome proliferator activated receptor gamma being identified as important targets. Molecular docking was also performed to assess the binding affinity between key targets and active compounds, with puerarin showing the strongest affinity for MMP9. In microglial cells, XYS significantly enhances the gene and protein expression of MMP9. This study elucidated the pharmacological mechanism of co-treatment for BC patients complicated with depression and the pharmacological mechanism of XYS against BC plus depression.

Abbreviations: BC = breast cancer, BDNF = brain derived neurotrophic factor, DEGs = differentially expressed genes, DMEM = Dulbecco's Modified Eagle Medium, FXR = farnesoid X receptor, GR = glucocorticoid receptor, HPA = hypothalamic-pituitary-adrenal, IL = interleukin, MMP9 = matrix metallopeptidase 9, PPARG = peroxisome proliferator activated receptor gamma, PPI = protein–protein interaction, RXR = retinoid x receptor, TCGA = The Cancer Genome Atlas, TCMSP = Traditional Chinese Medicine Systems Pharmacology, XYS = Xiaoyaosan.

Keywords: breast cancer, depression, molecular docking, network pharmacology, Xiaoyaosan

1. Introduction

Breast cancer (BC) is the most frequent malignant disease among women[1] and is the second cause of cancer death after lung cancer.[2] Cytotoxic therapy is connected to significant psychiatric adverse effects, roughly half of cancer patients have different psychiatric/psychological disorders which need optimal diagnostics and therapy.[3] Depression is one of the most common psychiatric disorders among women with BC, complicating their course and outcomes.[4] It has been considered as an independent factor in predicting BC recurrence and survival.[5] Depression could result in decreased immune function and prolonged recovery time in patients with BC, seriously affecting the quality of life of patients.[6] In addition, the depressive behavior has also significant impacts on individual and social performance.[7] Depression in women with BC has long been underreported and undertreated in oncological

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The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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and palliative settings.[8] Although part of patients takes antidepressants after the diagnosis of depression,[9,10] a systematic meta-analysis showed low evidence for the effects of these drugs compared to a placebo.[11]

Accumulating evidence indicated the favorable therapeutic efficacy of traditional Chinese medicine in treating disease-induced depression, such as post-stroke depression,[12] chronic unpredictable mild stress-induced depression-like behaviors,[13] and COVID-19-induced depression.[14] Traditional Chinese medicine prescription is an effective, low-toxicity, multi-target prescription not only in the treatment of depression but also cancers. For example, a Chinese herbal formulation called Xiaoyao Kangai Jieyu Fang could ameliorate cancer-related depression concurrent with BC by promoting hippocampal synaptic plasticity.[15] Quercetin, a key component of the Xiaoyao Kang'ai Jieyu formula, has been demonstrated to effectively alleviate the progression of depression associated with BC.[16] This beneficial effect is attributed to its multifaceted mechanisms, including the inhibition of apoptosis, enhancement of immune response, and amelioration of serum metabolism.[16] In addition, pooled meta-analytic results demonstrated that Traditional Chinese Medicine Five-Element Music Therapy was beneficial in lowing depression levels for cancer patients.[17] Xiaoyaosan (XYS), a classic Chinese medicine compound, was able to ameliorate depression symptoms via multiple pathways including AdipoR1/AMPK/ACC pathway[18] and TLR4/NF-κB pathway[19] or via regulating gut microbiota.[20] Meanwhile, XYS can be used as an adjuvant treatment for BC[21] as well as in the management of long-term survivors of BC to improve the quality of life,[22] including ameliorating depression.[23] However, the underlying molecular mechanisms of XYS in treating BC patients with depression have not been fully elucidated.

Given the increasing attention to cancer survivorship, it is becoming increasingly important for the diagnosis and treatment of cancer patients complicated with depression. Herein, we used the transcriptome data of the TCGA-breast invasive carcinoma (BRCA) cohort and data mining to uncover the common genes that contribute to both BC and depression, and elucidate the potential involved biological processes and pathways via bioinformatic analysis. Subsequently, the pharmacological molecular mechanism of XYS in treating BC along with depression was elucidated using network pharmacology, molecular docking and experimental validation. The workflow of this study is shown in Figure 1.

2. Materials and methods

2.1. Data preparation

The transcriptome data and clinicopathological information of the TCGA-BRCA cohort were downloaded from The Cancer Genome Atlas (TCGA, https://portal.gdc.cancer.gov/) database. The depression-related genes were retrieved from the GeneCards (https://www.genecards.org/) and DisGeNET (https://www.disgenet.org/) databases, and shared genes by these 2 databases were considered as depression-related genes. The chemical components of XYS were obtained from the Traditional Chinese Medicine Systems Pharmacology (TCMSP) database and analysis platform (https://old.tcmsp-e.com/tcmsp.php), and those with oral bioavailability ≥ 20% and drug-likeness ≥ 0.1 were regarded as active compounds. The targets of active compounds were further obtained from the TCMSP database, with the removal of non-human proteins. The gene symbol was unified using the UniProt (https://www.uniprot.org/) database.

2.2. Differential expression analysis

The differentially expressed genes (DEGs) between BC and adjacent normal tissue were identified using the TCGAbiolinks package.[24] Briefly, transcriptomic data of the TCGA-BRCA cohort was downloaded, and the data with the highest value for multiple same genes were retained. The count matrix was normalized using "geneLength" method, and TCGAanalyze_Filtering function was used to filter data using a qut.cut = 0.25. Finally, the DEGs were identified by TCGAanalyze_DEA function with fdr.cut = 0.01, logFC.cut = 1, and a "glmLRT" method. The volcano plot of the DEGs was generated using the TCGAVisualize_volcano function.

2.3. Survival analysis

The survival analysis was performed using the TCGAbiolinks package. The clinical data of the TCGA-BRCA cohort were downloaded using the GDCquery_clinic function. The data matrix of DEGs was extracted and normalized. Kaplan–Meier survival analysis with log-rank test was performed using the TCGAanalyze_SurvivalKM function with a ThreshTop = 0.67 and a ThreshDown = 0.33. DEGs with a P value < .01 were considered as prognosis-related DEGs.

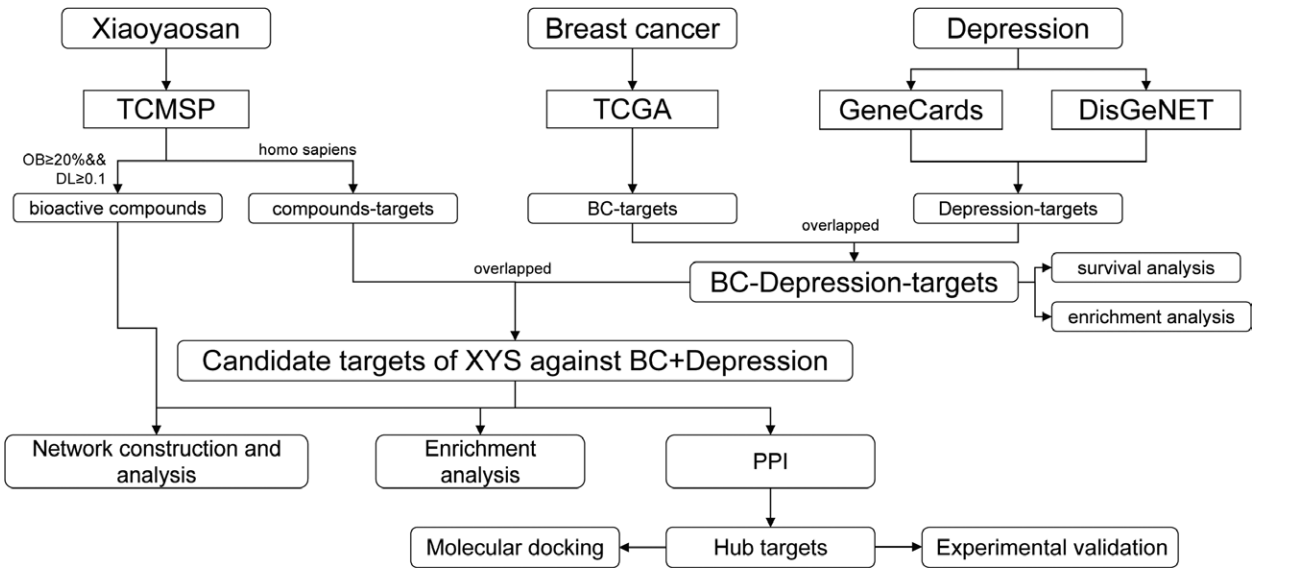

Figure 1. Flow diagram of the present study to investigate the potential mechanism of the XYS in treating breast cancer comorbid with depression. XYS = Xiaoyaosan.

2.4. Enrichment analysis

Enrichment analysis was conducted using the TCGAbiolinks package and Metascape platform (https://metascape.org/). The TCGAanalyze_EAcomplete function was adopted for enrichment analysis and the TCGAvisualize_EAbarplot function was used for the visualization of results. Metascape provides richer functional annotation, enrichment analysis and protein network interpretation of target genes, and relevant genes were submitted into the metascape platform for enrichment analysis using default parameters.

2.5. Protein–protein interaction

The protein–protein interaction (PPI) information of the disease targets and candidate targets were retrieved and downloaded from the STRING (https://cn.string-db.org/) database. The species was set to "Homo sapiens" and the minimum required interaction score was set to high confidence (0.7). The short tabular text file containing the PPI data was downloaded and input into Cytoscape 3.9.1 for visualization.

2.6. Network construction and analysis

The Cytoscape 3.9.1 software was used for network construction and topological analysis. Four networks, including the PPI network of disease targets and candidate targets, the herb-compound network, and the compound-target network. The topological parameters were analyzed using the Analyze Network function, and the hub targets were identified using the cytoHubba plugin.[25] Clusters analysis of the PPI network was conducted using an MCODE plugin.

2.7. Molecular docking

The structural files of hub targets, namely IL6 (1alu), ESR1 (1err), FOS (1fos), MMP9 (1gkc), PPARG (1i7i), EGF (1jl9), EGFR (1m14), STAT1 (1yvl), CCND1 (2w96), and ADIPOQ (4dou), were obtained from the RCSB Protein Data Bank (RCSB PDB, https://www.rcsb.org/) database. The PyMOL 2.5.2 software was utilized to eliminate water molecules, ligands, and other peptide chains from the downloaded structures. The mol2 files of the active compounds were sourced from the TCMSP database. The process of molecular docking, involving the interaction between active compounds and hub targets, was executed via the CB-DOCK2 web server (https://cadd.labshare.cn/cb-dock2/php/index.php) by employing the Auto BlindDock method. This particular method leverages the principles of AutoDock Vina for docking purposes. The ranking of potential binding sites for the query ligands was conducted based on Vina scores expressed in kcal/mol units. Subsequently, the conformation with the lowest score was identified as the optimal conformation for subsequent evaluation and comparative analysis.

2.8. Preparation of XYS extract

The herbal materials, namely Chaihu (Bupleurum chinense), Danggui (Angelica sinensis), Baishao (Cynanchum otophyllum), Baizhu (Atractylodes macrocephala), Fuling (Wolfiporia cocos), Gancao (Glycyrrhiza uralensis Fisch), and Bohe (Mentha haplocalyx Briq.), were meticulously procured and processed in accordance with the guidelines outlined in the Prescriptions of the Pharmacy Bureau. These materials were specifically utilized to prepare the XYS decoction. Subsequently, the prepared decoction was subjected to a purification process using an AB-8 macroporous resin column. The resulting elution fraction, obtained using 80% ethanol as solvent, was carefully collected and subsequently subjected to freeze-drying techniques, ultimately yielding a powdered form. To obtain the XYS extract solution, the freeze-dried powder was accurately weighed and dissolved in Dulbecco's Modified Eagle Medium (DMEM) complete culture medium at a concentration of 0.5 g/L. This solution was then subjected to filtration through a 0.22 μm membrane filter to ensure removal of any particulate matter or impurities.

2.9. Cell culture and treatment

The murine microglial cell line SIM-A9 was cultured at 37 °C with a 6% CO_2 atmosphere in DMEM high glucose supplemented with GlutaMAX (Thermo Fisher, Wilmington, Massachusetts, United States, 10566016). The culture medium was supplemented with 10% fetal bovine serum, 5% horse serum, and 1% antibiotic-antimycotic solution (Thermo Fisher, 15240062).

During the logarithmic growth phase, SIM-A9 cells were seeded into 6-well plates. After a 24-hour incubation period, the cells were allocated into 5 groups: a control group, a low concentration XYS group (LXYS, 0.25 g/L), and a high concentration XYS group (HXYS, 0.5 g/L). The control group received an equal volume of DMEM complete culture medium.

2.10. Real-time quantitative polymerase chain reaction

Total RNA isolation from SIMA-A9 cells was carried out utilizing the RNeasy Mini Kit (Qiagen, Heidelberg, Germany, 74104). The concentration and purity of the extracted RNA were evaluated via NanoDrop 2000C spectrophotometer. Subsequently, 5 μg of total RNA was subjected to reverse transcription using the high-capacity cDNA reverse transcription kit (Thermo Fisher, 4368814). The primer sequences designed for matrix metallopeptidase 9 (MMP9) amplification were as follows: forward primer 5′-TACTCTGCCTGCACCACCGA-3′ and reverse primer 5′-TCTCTCATCATTTCTCAGAT-3′. Quantitative PCR analysis was performed on a Bio-Rad CFX96 system (Bio-Rad Laboratories, Hercules, California, United States) employing the SYBR Green I UltraSYBR Mixture. To determine the expression levels of the target gene, normalization to GAPDH reference genes was conducted using the $2^{-\Delta\Delta Ct}$ method. Each experimental procedure was replicated in triplicate for statistical significance.

2.11. Western blot

Protein extracts were obtained through cellular lysis using a lysis buffer comprising 62.5 mM Tris, 2% SDS, and 10% sucrose. The lysis buffer was supplemented with cocktails of protease inhibitors (Sigma, St. Louis, Missouri, USA, P2714) and phosphatase inhibitors (Roche, Basel, Switzerland, 04906837001). Following protein extraction, samples (30 μg) were subjected to separation via 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis, subsequently transferring the separated proteins onto a polyvinylidene fluoride membrane. To prevent nonspecific binding, the polyvinylidene fluoride membrane was blocked using a solution containing 5% bovine serum albumin. Subsequently, the membrane was incubated overnight at a temperature of 4°C with primary antibodies targeting MMP9 (Abcam, Cambridge, United Kingdom, ab58803) or GAPDH at a dilution ratio of 1:2000. Following thorough washing, the membrane was exposed to secondary antibodies at room temperature for 1 hour. Immunoreactive bands were visualized utilizing an enhanced chemiluminescence detection reagent (Millipore, Darmstadt, Germany, WBKL0500). Grayscale intensity values were quantified using ImageJ software and normalized against the grayscale intensity value of GAPDH as an internal reference control.

2.12. Statistical analysis

The data were reported as the mean ± standard deviation and subjected to statistical analysis using the SPSS 21.0 software package (IBM, Armonk, NY). Group differences were evaluated

through *t* tests, with a predetermined significance level of $P < .05$ denoting statistical significance.

3. Results

3.1. The molecular background of BC and depression

In order to find out the molecular background of co-treatment of BC and depression, we used the transcriptome data of the TCGA-BRCA cohort to conduct differential analysis and identify DEGs. As a result, 113 normal tissue and 1106 tumor tissue were included, and 2958 DEGs between tumor tissue and normal tissue (Fig. 2A). Meanwhile, 1210 depression-related genes were obtained by intersecting the results from the GeneCards (7774) and DisGeNET (1719) databases (Fig. 2B). Consequently, the common region with 277 targets between DEGs and depression-related genes was regarded as the molecular basis of co-treatment of BC and depression (Fig. 2B). Figure 2C illustrated the PPI network of the common genes, and the top 3 targets with a higher degree value were interleukin (IL)-6, peroxisome proliferator activated receptor gamma (PPARG), and epidermal growth factor receptor, indicating the important role of these targets in preventing BC plus depression. Metascape enrichment analysis revealed that these common targets were significantly associated with behavior, positive regulation of protein phosphorylation, response to hormone, etc. (Fig. 2D) In addition, these common targets were significantly enriched in multiple types of nerve cells (Fig. 2E), such as HNBGABA, HRGL2B, HOPC. As shown in Figure 2F, it was revealed that the common targets were significantly associated with neurological diseases, such as unipolar depression, mental disorders, and presenile dementia, etc.

3.2. Molecular signature of prognosis-related DEGs

It was believed that DEGs related to cancer prognosis provide favorable therapeutic targets for cancer treatment. Therefore, we further evaluate the prognostic value of DEGs identified

Figure 2. The molecular basis of co-treatment for BC patients complicated with depression. (A) The volcano plot of DEGs between tumor samples and normal samples in the TCGA-BRCA cohort. (B) The Venn diagram of BC-related genes and depression-related genes. (C) The PPI network of the common targets between BC and depression. (D) The enrichment analysis of the common targets in terms of biological processes and pathways. (E and F) The top 20 terms that were significantly associated with common targets in terms of cell types and diseases, respectively. BC = breast cancer, BRCA = breast invasive carcinoma, DEGs = differentially expressed genes, PPI = protein–protein interaction, TCGA = the Cancer Genome Atlas.

before. As a result, 165 DEGs were significantly associated with the prognosis of BC patients, and 18 of them overlapped with common disease targets (Fig. 3A), indicating the potential value as therapeutic targets against BC complicated with depression. Among the 18 targets, overexpression of 12 genes was beneficial for BC prognosis, whereas the overexpression of the rest

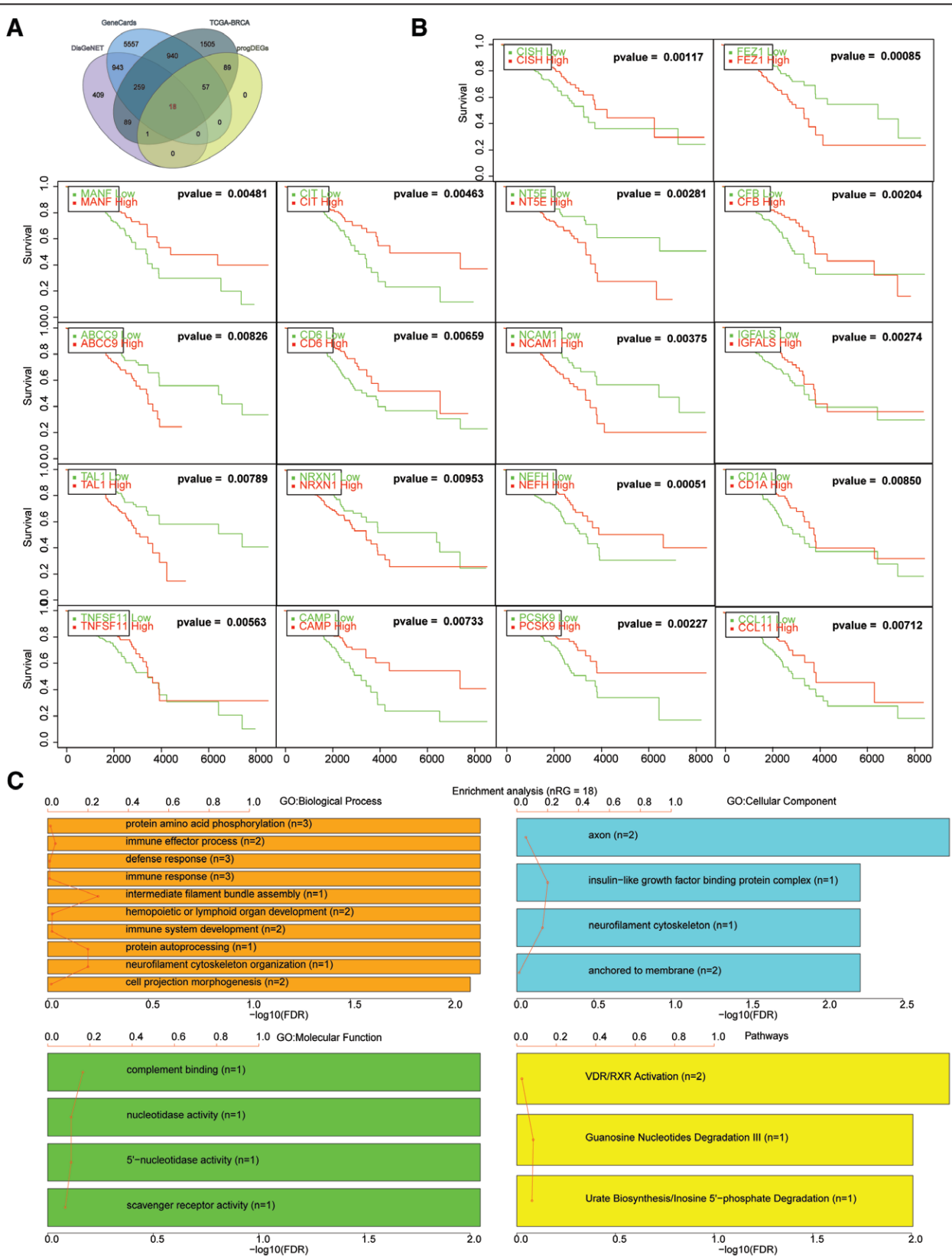

Figure 3. The identification and analysis of prognosis-related common targets. (A) The Venn diagram of the progDEGs and diseases-related targets. (B) The forest plot of the 18 common progDEGs. (C) GO and KEGG enrichment analysis of the 18 common progDEGs. DEGs = differentially expressed genes, GO = Gene Ontology, KEGG = Kyoto Encyclopedia of Genes and Genomes.

6 targets was unfavorable to the prognosis (Fig. 3B). To reveal the biological processes and pathways in that these targets were involved, we conducted an enrichment analysis. The results showed that the therapeutic targets were significantly associated with biological processes involved in the immune system and protein processing. In addition, these therapeutic targets were mainly involved in VDR/RXR activation, guanosine nucleotide degradation, and urate biosynthesis/inosine 5'-phosphate degradation, as shown in Figure 3C.

3.3. Identification of candidate targets of XYS against BC plus depression

A comprehensive analysis was conducted to identify active compounds within the 7 herbs of XYS, based on established criteria of oral bioavailability ≥ 20% and drug-likeness ≥ 0.1. As a result, a total of 252 active compounds were successfully identified (Fig. 4A). Furthermore, these compounds were mapped to 342 human targets, thereby establishing them as potential candidates for therapeutic drug targeting. A Venn diagram of the disease targets and candidate drug targets found an intersected region with 44 targets, which were regarded as candidate targets of XYS against BC plus depression (Fig. 4B). Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis demonstrated that the candidate targets were mainly involved in the regulation of cell proliferation, catalytic activity, and cell communication, and located in extracellular regions or membranes. In addition to cancer-related pathways, these targets are also associated with IL-18 signaling, LXR/RXR activation, aryl hydrocarbon receptor signaling, etc., as shown in Figure 5A–D. These data provide novel clues of molecular basis and pathways for the co-treatment of BC and depression.

3.4. Metascape enrichment analysis of candidate targets

To further explore the molecular signature of the candidate targets, we performed additional enrichment analysis based on the Metascape platform. It was observed that the candidate targets were significantly enriched in 587 GO terms, 91 KEGG pathways, 112 WikiPathways, 34 Canonical Pathways, and 74 Reactome Gene Sets. The top terms with lower *P* value included the response to hormone, nuclear receptors meta-pathway, pathway in cancer, PID AP1 pathway, etc., as shown in Figure 6A. Cell type enrichment analysis revealed that the candidate targets were enriched in various brain neurotypes (HRGL2B and HRGL2A), megakaryocyte erythroid progenitor, megakaryocyte progenitor, and so on (Fig. 6B). Clustering analysis of the candidate targets revealed 3 stable clusters, which contain 8, 3, and 3 proteins, respectively (Fig. 6C), respectively. The top 3 terms related to the clusters were illustrated in Table 1. It showed that cluster1 participated in the PID AP1 pathway, pathways in cancer, and reactive oxygen sepsis, whereas cluster2 and cluster3 were related to cell cycle control and metabolism of tyrosine and drugs, respectively.

3.5. Identification and validation of Hub targets

The PPI network of the candidate targets was visualization in Figure 7A, which contains 37 target nodes and 94 edges. Some target nodes have more connections in the network than others, indicating that they play a more important role in the network. Therefore, a central network containing 10 hub targets was identified using cytoHubba and was extracted (Fig. 7B). The binding affinity between the hub targets and active compounds was assessed and compared, as shown in Figure 7C. MMP9 had the lowest average binding energy with active compounds. Figure 7D illustrated the best binding conformation of MMP9 and puerarin, which had the lowest binding energy. Moreover, we conducted additional experiments to confirm the impact of XYS on the genetic and protein expression of MMP9 in microglial cells. Our findings revealed a substantial elevation in both the gene and protein expression levels of MMP9 within microglial cells upon XYS treatment (Fig. 7E and F). Consequently, this upregulation facilitated the conversion of proBDNF into its mature form, mBDNF.[26] Such molecular transformation is known to induce neuroplastic changes and ameliorate symptoms associated with depression.

4. Discussion

Depression is a serious complication in women with BC, which exerts a significant influence on cancer outcomes. Research on

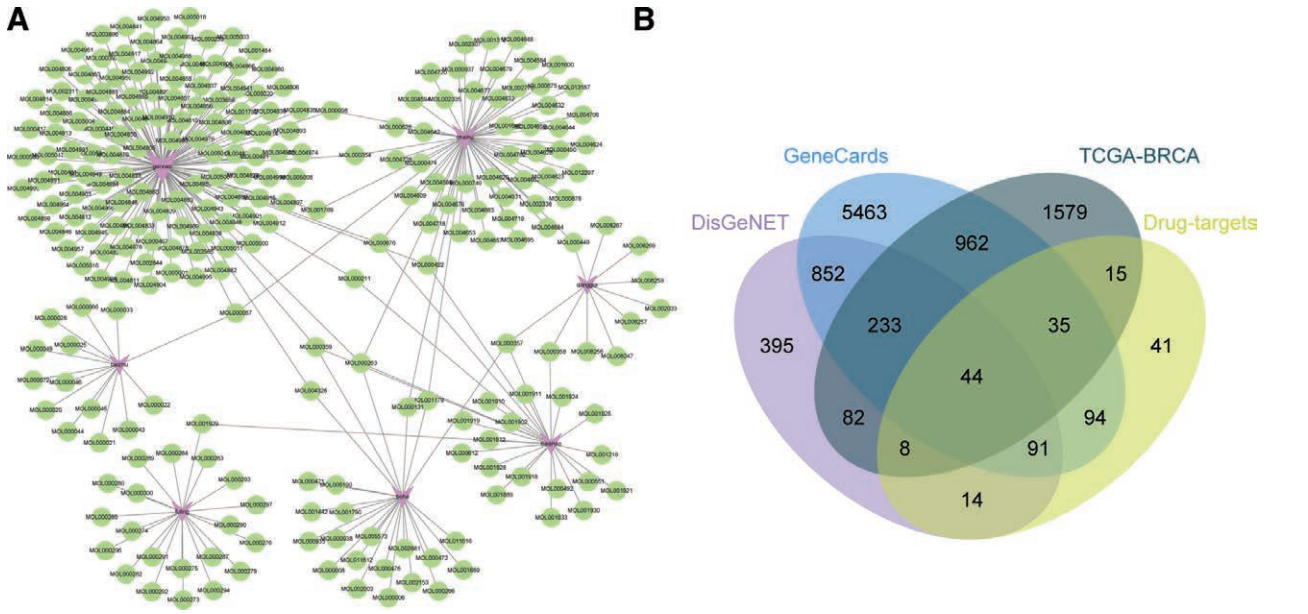

Figure 4. The identification of candidate targets of XYS in the treatment of BC plus depression. (A) The network of herbs and active compounds of XYS. (B) Venn diagram of XYS's targets and BC-related genes to identify candidate targets. BC = breast cancer, XYS = Xiaoyaosan.

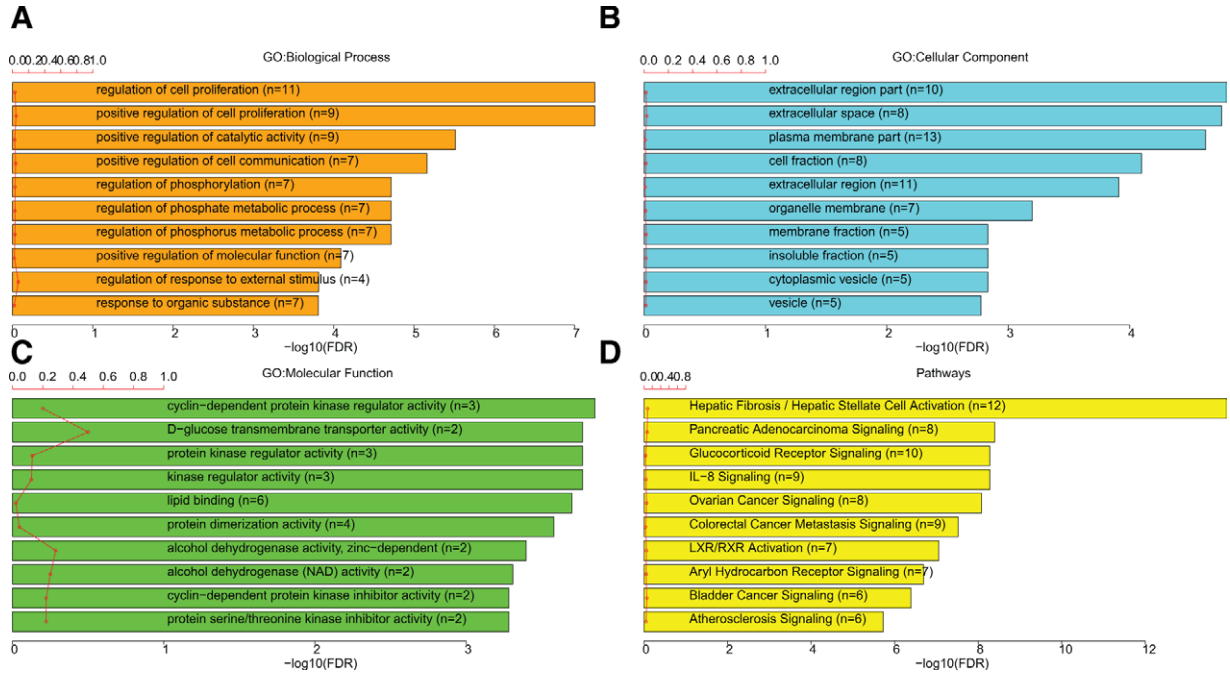

Figure 5. Enrichment analysis of the candidate targets of XYS in treating BC plus depression. The top 10 enriched terms in biological process (A), cellular component (B), molecular function (C), and pathway (D). BC = breast cancer, XYS = Xiaoyaosan.

the diagnosis and treatment of BC patients complicated with depression is still limited, and there is insufficient evidence to support the efficacy of antidepressants in treating the comorbidity of BC patients. Herein, we thought to discover the identical molecular basis that is involved in the pathogenesis of BC and depression, not only to lay the foundation for subsequent drug development but also to reveal the pharmacological mechanism of XYS against BC-related depression.

In this study, BC-related genes were identified by analyzing the transcriptome data of the TCGA-BRCA cohort. By integrating with depression-related genes obtained from data mining of public databases, we eventually included 277 candidate overlapped genes that might contribute both to the occurrence and/or progression of BC and depression. The PPI network of these candidate targets highlighted the crucial role of several targets, such as IL6 and PPARG. Increased peripheral or central cytokine IL6 levels were observed in depressive disorders, and have impacts on the prognosis and therapeutic response.[27] IL6-like cytokines are involved in the regulation of immunity and homeostasis in various diseases, including cancer and depression.[28] Hence, developing drugs targeting IL6 activity may hold great promise for BC comorbid with depression. Sufficient evidence supported that PPARG augmentation has a positive impact on multiple significant pathological processes in depression.[29] It was demonstrated that PPARG was a potential biomarker of BC and its gene expression was associated with BC prognosis,[30] although our prognosis analysis did not reach consistent results due to the stricter screening thresholds. Blocking PPARG interaction facilitates nuclear receptor subfamily 4 group A member 1 interdiction of fatty acid uptake and suppresses BC progression.[31] Taken together, PPARG agonists could potentially exert anticancer and antidepressant activity simultaneously. Besides, other candidate targets also provided novel clues for the drug development of depression in BC patients.

It is difficult to control and improve complex diseases with a single therapeutic target. However, the synergistic regulation of intracellular multiple pathways based on specific cell types may have unexpected effects. Therefore, we enriched the candidate targets to explore relevant biological processes and pathways that might serve as therapeutic targets against BC plus depression. Our data pointed out the potential involvement of multiple identical pathways including VDR/RXR or LXR/RXR or FXR/RXR activation, glutamate receptor signaling, NF-κB signaling etc., in the pathogenesis of BC and depression. Vitamin D receptor and retinoid × receptor (RXR) were suggested to potentially evolve as interesting markers or even targets in hereditary BC.[32] LXR/RXR pathway signaling associated with triple-negative BC in African American women.[33] The farnesoid X receptor (FXR) is expressed in BC and regulates apoptosis and aromatase expression.[34] Activation of the FXR in BC cell lines results in cytotoxicity but not increased migration potential.[35] Activation of FXR impairs the tumor-promoting function of BC-associated fibroblasts.[36] These data support the continued examination of FXR agonists as a novel class of therapeutics for the treatment of BC. The RXR agonist MSU42011 is effective for the treatment of preclinical HER2+BC.[37] Accumulating evidence has associated RXR-mediated signaling with depression.[38,39] These findings provide support for drug development based on RXR-mediated signal pathway for BC comorbid with depression. In terms of glutamate receptors, a meta-analytic study suggested that glutamate receptor modulators including ketamine and esketamine were efficacious on unipolar depression.[40] Metabotropic glutamate receptor 1 was suggested to be associated with poor outcomes in ER-negative and triple-negative BC.[41] NF-κB signaling is involved in various crucial cellular processes and is associated with the neurobiology of depression. Ariel Caviedes et al[42] proposed a potential beneficial effect of a positive feedback loop between brain derived neurotrophic factor (BDNF) and NF-κB-activated pathways in antidepressant action. Meanwhile, it has been well-known that the NF-κB signaling was associated with the proliferation, metastasis and drug resistance of BC. Therefore, these findings in pathways might be potential as therapeutic targets against BC comorbid with depression.

As an effective prescription in treating BC and depression, it is necessary to elucidate the underlying mechanism of XYS. Network pharmacology discovered a bunch of potential targets, which were significantly correlated with multiple pathways including glucocorticoid receptor (GR) signaling, IL-8 signaling, LXR/RXR activation, aryl hydrocarbon receptor

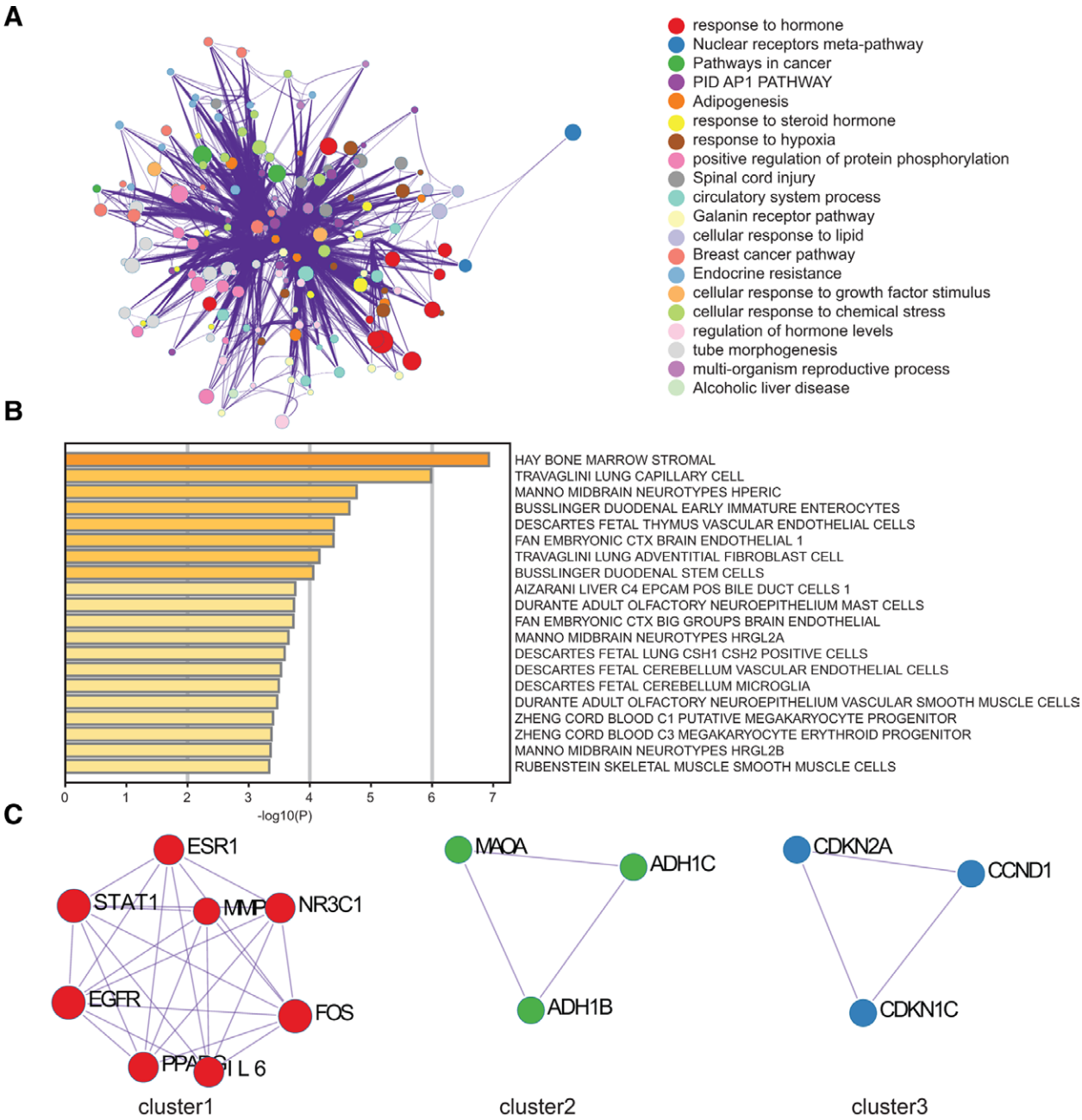

Figure 6. The enrichment and clustering analysis of the candidate targets via the Metascape platform. (A) The PPI network of the candidate targets and the top 20 enriched terms. (B) The top 20 enriched cell types of the candidate targets. (C) The 3 clusters were generated from the PPI network of candidate targets using MCODE methods. PPI = protein–protein interaction.

Table 1

The top 3 terms that were enriched by the drug candidate targets against BC+depression.

Cluster	Category	Description	LogP	Hits
cluster1	Canonical pathways	PID AP1 PATHWAY	−11	ESR1|FOS|NR3C1|IL6|MMP9
cluster1	KEGG pathway	Pathways in cancer	−11	EGFR|ESR1|FOS|IL6|MMP9|PPARG|STAT1
cluster1	GO biological processes	Response to reactive oxygen species	−9.5	EGFR|FOS|IL6|MMP9|STAT1
cluster2	Reactome gene sets	G1 phase	−8.5	CCND1|CDKN1C|CDKN2A
cluster2	Reactome gene sets	Cyclin D associated events in G1	−8.5	CCND1|CDKN1C|CDKN2A
cluster2	WikiPathways	G1 to S cell cycle control	−8	CCND1|CDKN1C|CDKN2A
cluster3	KEGG pathway	Tyrosine metabolism	−8.8	ADH1B|ADH1C|MAOA
cluster3	KEGG pathway	Drug metabolism – cytochrome P450	−7.9	ADH1B|ADH1C|MAOA
cluster3	Reactome gene sets	Phase I – functionalization of compounds	−7.4	ADH1B|ADH1C|MAOA

GO = Gene Ontology, KEGG = Kyoto Encyclopedia of Genes and Genomes.

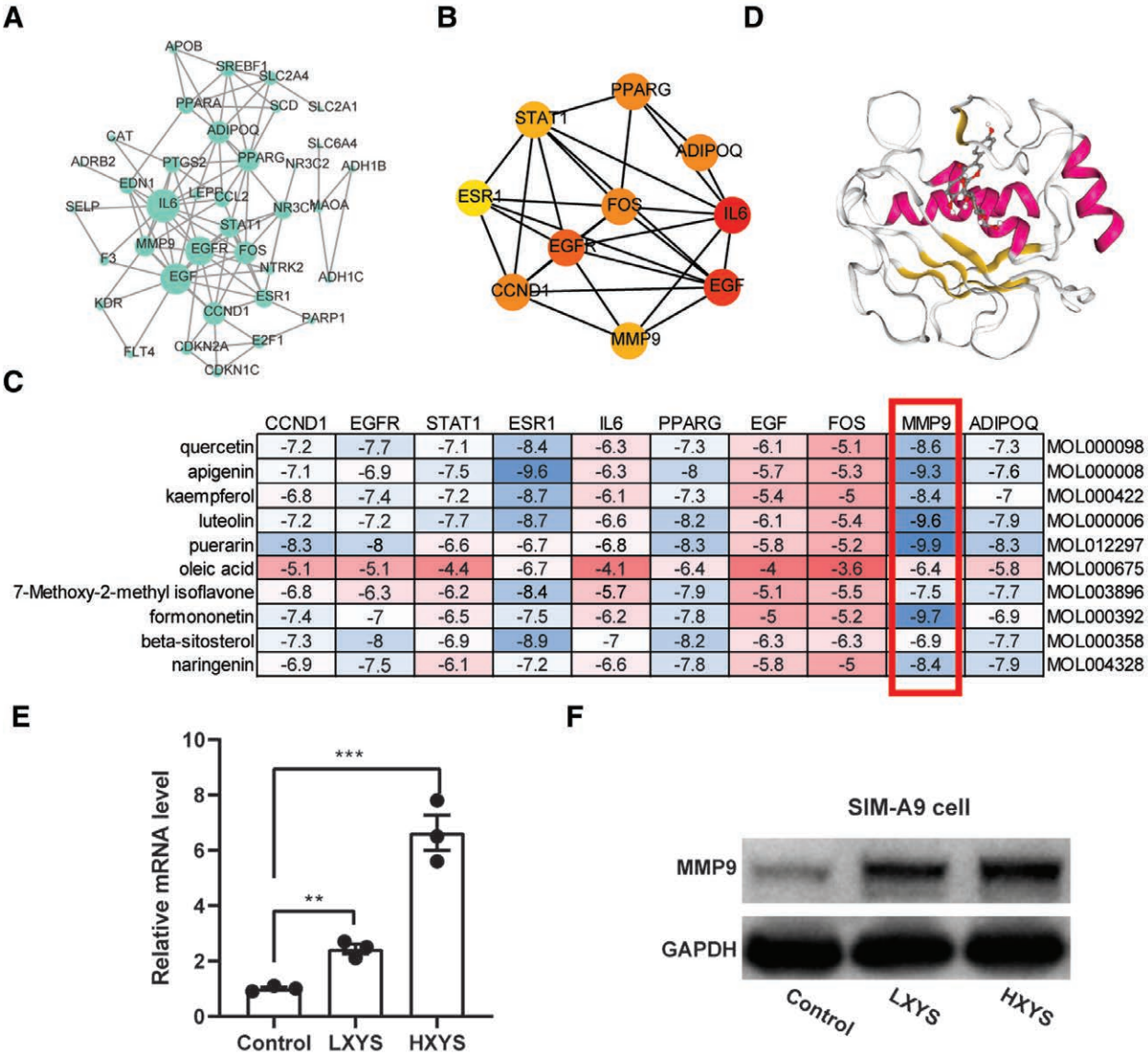

Figure 7. Evaluation of the binding affinities between hub candidate targets and active compounds. (A) The PPI network of the candidate targets using the data obtained from the STING database. (B) The central network contains top 10 hub targets. (C) The binding energy between 10 hub targets and 10 active ingredients. (D) The 3D diagram of the best conformation of MMP9 and puerarin. (E) Relative expression levels of MMP9 mRNA in microglial cells after treatment with XYS compared to the control group. (F) Protein expression levels of MMP9 in microglial cells after treatment with XYS detected by Western blot. $^{**}P < .01$, $^{***}P < .001$. PPI = protein–protein interaction, XYS = Xiaoyaosan.

signaling, etc. In addition to some pathways overlapping with disease targets, other pathways have sufficient evidence as targets of combined therapy. For example, repeated findings in biological psychiatry has confirmed the pathogenetic role of the hypothalamic-pituitary-adrenal (HPA) axis in depression, and restrained GR feedback lead to increased levels of glucocorticoid hormones and the dysregulation of HPA axis.[43] Sufficient data showed that antidepressants inhibited the hyperactive HPA axis and alleviate depressive symptoms by modulating GR activity.[44] Meanwhile, GR is an important marker in BC and was associated with the prognosis of BC patients,[45,46] and the activation of GR increased BC heterogeneity and metastasis.[47] BC cells are known to secrete high levels of IL-8, which promote angiogenesis and contribute to poor prognosis.[48] Drugs targeting IL-8 have been shown to be effective against BC.[49,50] Emerging evidence suggested that aryl hydrocarbon receptor suppressed mammary tumor development and could be of great value as therapeutic targets for BC.[51] Aryl hydrocarbon receptor has been implicated in major depressive disorder and is also considered as a therapeutic target for depression.[52]

The binding potential between key targets and active compounds was assessed through molecular docking, with particular emphasis on MMP9 as the most promising target. Microglial cells play a vital role in maintaining neural tissue homeostasis, providing structural support, and participating in immune responses. Prior investigations have demonstrated that upregulated expression and secretion of MMP9 in microglial cells facilitate the maturation of extracellular BDNF.[26] The signaling cascade involving BDNF and its receptor tropomyosin receptor kinase B holds significant importance in both the pathophysiology of depression and the therapeutic mechanisms of antidepressant medications.[53] Our findings indicate that XYS exerts a substantial influence on the gene and protein expression levels of MMP9 in microglial cells, thereby suggesting its potential as an antidepressant agent by modulating the BDNF-tropomyosin receptor kinase B signaling pathway.

On the basis of network analysis, we could infer that some of the active ingredients in XYS are pivotal in the concurrent treatment of BC and depression, based on existing evidence. Quercetin is a polyphenol with multiple biological activities, including anti-cancer,[54] anti-inflammation,[55] and anti-oxidation.[56] In addition, it was observed to alleviate lipopolysaccharide-induced depression-like behavior in rats or reverse chronic unpredictable mild stress-induced depression-like behavior.[57,58] Wang et al[59,60] reported that apigenin could reverse chronic corticosterone-induced depression-live behavior in mice, which might be attributed to its anti-inflammation activity via Nlrp3, and Tlr4. Besides, apigenin also exerts cytotoxic activity in BC cells[61] and the chemotherapeutic effects of apigenin in BC have been demonstrated.[62] Luteolin is a representative of a natural flavonoid that has been proven to modulate various signaling pathways involved in cancer and depression development. It was revealed to be beneficial for BC and depression.[63,64] Several other active compounds including kaempferol,[65] puerarin,[66,67] etc., had also been proposed as candidate drugs for BC and depression, indicating their potential in the treatment of BC patients complicated with depression.

There are several limitations in this study. Further experimental validation of the target genes and pathways identified through bioinformatics analysis is required, as we have only validated a single target in this study; although a preliminary understanding of molecular background for BC comorbid with depression, it's remains unclear whether these molecules are responsible for suppressuing depression or promoting it, which should be taken into account in future studies; and this study only conducted enrichment analysis in GO, KEGG, cell types and diseases, and the current methodology cannot incorporate tissue and organ-specific enrichment analysis, which is very useful for understanding and exploring the principles of traditional Chinese medicine formulation.

5. Conclusion

In conclusion, this article provides a comprehensive analysis of the potential therapeutic targets and pathways for managing depression in BC patients. Notably, it establishes MMP9 as a pivotal target for XYS treatment. Nevertheless, additional rigorous in vitro and in vivo investigations are imperative to corroborate alternative targets and pathways. This study engenders novel perspectives on the rational research approach to investigating complications and unraveling the pharmacological mechanisms underlying traditional Chinese medicine interventions for cancer-related complications.

Author contributions

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References

[1] DeSantis CE, Ma J, Goding SA, et al. Breast cancer statistics, 2017, racial disparity in mortality by state. CA Cancer J Clin. 2017;67:439–48.
[2] Bray F, Ferlay J, Soerjomataram I, et al. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2018;68:394–424.
[3] Massie MJ. Prevalence of depression in patients with cancer. J Natl Cancer Inst Monogr. 2004:32:57–71.
[4] Refaee SN, Aghamohammadian SH, Asghari EM, et al. Psychological consequences of breast cancer in Iran: a meta-analysis. Iran J Public Health. 2019;48:816–24.
[5] Wang X, Wang N, Zhong L, et al. Prognostic value of depression and anxiety on breast cancer recurrence and mortality: a systematic review and meta-analysis of 282,203 patients. Mol Psychiatry. 2020;25:3186–97.
[6] Karakoyun-Celik O, Gorken I, Sahin S, et al. Depression and anxiety levels in woman under follow-up for breast cancer: relationship to coping with cancer and quality of life. Med Oncol. 2010;27:108–13.
[7] Cvetković J, Nenadović M. Depression in breast cancer patients. Psychiatry Res. 2016;240:343–7.
[8] Carreira H, Williams R, Müller M, et al. Associations between breast cancer survivorship and adverse mental health outcomes: a systematic review. J Natl Cancer Inst. 2018;110:1311–27.
[9] Suppli NP, Deltour I, Damkjaer LH, et al. Factors associated with the prescription of antidepressive medication to breast cancer patients. Acta Oncol. 2011;50:243–51.
[10] Mitchell AJ, Chan M, Bhatti H, et al. Prevalence of depression, anxiety, and adjustment disorder in oncological, haematological, and palliative-care settings: a meta-analysis of 94 interview-based studies. Lancet Oncol. 2011;12:160–74.
[11] Ostuzzi G, Matcham F, Dauchy S, et al. Antidepressants for the treatment of depression in people with cancer. Cochrane Database Syst Rev. 2018;4:CD011006.
[12] Yang Y, Zhang M, Zhao J, et al. Effect of traditional Chinese medicine emotional therapy on post-stroke depression: a protocol for systematic review and meta-analysis. Medicine (Baltim). 2021;100:e25386.
[13] Qu W, Liu S, Zhang W, et al. Impact of traditional Chinese medicine treatment on chronic unpredictable mild stress-induced depression-like behaviors: intestinal microbiota and gut microbiome function. Food Funct. 2019;10:5886–97.
[14] Da XL, Yue LF, Li XJ, et al. Potential therapeutic effect and methods of traditional Chinese medicine on COVID-19-induced depression: a review. Anat Rec (Hoboken). 2021;304:2566–78.
[15] Meng P, Han Y, Yang Q, et al. Xiaoyao Kangai Jieyu Fang, a Chinese Herbal Formulation, Ameliorates Cancer-Related Depression Concurrent with Breast Cancer in Mice via Promoting Hippocampal Synaptic Plasticity. Evid Based Complement Alternat Med. 2018;2018:3967642.
[16] Zhu Q, Yang L, Yang H, et al. Quercetin alleviates the progression of breast cancer-related depression via inhibiting the pyroptosis and promoting the immune response. Mediators Inflamm. 2022;2022:8011988.
[17] Tao WW, Jiang H, Tao XM, et al. Effects of Acupuncture, Tuina, Tai Chi, Qigong, and traditional Chinese medicine five-element music therapy on symptom management and quality of life for cancer patients: a meta-analysis. J Pain Symptom Manage. 2016;51:728–47.
[18] Tang KR, Mo XW, Zhou XY, et al. Xiaoyao San, a Chinese herbal formula, ameliorates depression-like behavior in mice through the AdipoR1/AMPK/ACC pathway in hypothalamus. J Integr Med. 2022;20:442–52.
[19] Guo R, Qin WS, Zhang SY, et al. [Effects of modified Xiaoyao San on TLR4/NF-κB pathway in hippocampal microglia of LPS-induced depression model rats]. Zhongguo Ying Yong Sheng Li Xue Za Zhi 2020;36:354–7.
[20] Yang Y, Zhong Z, Wang B, et al. Xiaoyao San ameliorates high-fat diet-induced anxiety and depression via regulating gut microbiota in mice. Biomed Pharmacother. 2022;156:113902.
[21] Chao C, Huafeng L, Hongling X. Study on the attenuation and sensitization of Xiaoyao San in adjuvant radiotherapy of breast cancer patients with liver depression and spleen deficiency (in Chinese). Chin General Pract. 2017;15:1956–8.
[22] Wenjing G, Xiaoying P, Ping H. Effect of Xiaoyao San on PFS score, sex hormone level and sleep quality in patients with climacteric syndrome after endocrine therapy for breast cancer (in Chinese). Sichuan Trad Chin Med. 2022;40:163–5.
[23] Shiling S, Liping Y, Hongrui Z, et al. Effect of Modified Xiaoyao San on Depression in Patients with breast cancer Undergoing Postoperative Chemotherapy (in Chinese). Chin J Trad Chin Med. 2016;31:1499–502.
[24] Colaprico A, Silva TC, Olsen C, et al. TCGAbiolinks: an R/Bioconductor package for integrative analysis of TCGA data. Nucleic Acids Res. 2016;44:e71.
[25] Chin CH, Chen SH, Wu HH, et al. cytoHubba: identifying hub objects and sub-networks from complex interactome. BMC Syst Biol. 2014;8(Suppl 4):S11.

[26] Martinelli S, Anderzhanova EA, Bajaj T, et al. Stress-primed secretory autophagy promotes extracellular BDNF maturation by enhancing MMP9 secretion. Nat Commun. 2021;12:4643.
[27] Ting EY, Yang AC, Tsai SJ. Role of interleukin-6 in depressive disorder. Int J Mol Sci. 2020;21:2194.
[28] Martínez-Pérez C, Kay C, Meehan J, et al. The IL6-like cytokine family: role and biomarker potential in breast cancer. J Pers Med. 2021;11:1073.
[29] Gold PW. The PPARg system in major depression: pathophysiologic and therapeutic implications. Int J Mol Sci . 2021;22:9248.
[30] Wu J, Luo M, Chen Z, et al. Integrated analysis of the expression characteristics, prognostic value, and immune characteristics of PPARG in breast cancer. Front Genet. 2021;12:737656.
[31] Yang PB, Hou PP, Liu FY, et al. Blocking PPARγ interaction facilitates Nur77 interdiction of fatty acid uptake and suppresses breast cancer progression. Proc Natl Acad Sci U S A. 2020;117:27412–22.
[32] Heublein S, Mayr D, Meindl A, et al. Vitamin D receptor, retinoid X receptor and peroxisome proliferator-activated receptor γ are overexpressed in BRCA1 mutated breast cancer and predict prognosis. J Exp Clin Cancer Res. 2017;36:57.
[33] Torres-Luquis O, Madden K, N'Dri NM, et al. LXR/RXR pathway signaling associated with triple-negative breast cancer in African American women. Breast Cancer (Dove Med Press). 2019;11:1–12.
[34] Swales KE, Korbonits M, Carpenter R, et al. The farnesoid X receptor is expressed in breast cancer and regulates apoptosis and aromatase expression. Cancer Res. 2006;66:10120–6.
[35] Alasmael N, Mohan R, Meira LB, et al. Activation of the Farnesoid X-receptor in breast cancer cell lines results in cytotoxicity but not increased migration potential. Cancer Lett. 2016;370:250–9.
[36] Barone I, Vircillo V, Giordano C, et al. Activation of Farnesoid X receptor impairs the tumor-promoting function of breast cancer-associated fibroblasts. Cancer Lett. 2018;437:89–99.
[37] Leal AS, Moerland JA, Zhang D, et al. The RXR agonist MSU42011 Is effective for the treatment of preclinical HER2+ breast cancer and Kras-driven lung cancer. Cancers (Basel). 2021;13:5004.
[38] Krzyżosiak A, Podleśny-Drabiniok A, Vaz B, et al. Vitamin A5/X controls stress-adaptation and prevents depressive-like behaviors in a mouse model of chronic stress. Neurobiol Stress. 2021;15:100375.
[39] Wray NR, Ripke S, Mattheisen M, et al. Genome-wide association analyses identify 44 risk variants and refine the genetic architecture of major depression. Nat Genet. 2018;50:668–81.
[40] Dean RL, Hurducas C, Hawton K, et al. Ketamine and other glutamate receptor modulators for depression in adults with unipolar major depressive disorder. Cochrane Database Syst Rev. 2021;9:CD011612.
[41] Bastiaansen A, Timmermans AM, Smid M, et al. Metabotropic glutamate receptor 1 is associated with unfavorable prognosis in ER-negative and triple-negative breast cancer. Sci Rep. 2020;10:22292.
[42] Caviedes A, Lafourcade C, Soto C, et al. BDNF/NF-κB signaling in the neurobiology of depression. Curr Pharm Des. 2017;23:3154–63.
[43] Farrell C, O'Keane V. Epigenetics and the glucocorticoid receptor: a review of the implications in depression. Psychiatry Res. 2016;242:349–56.
[44] Anacker C, Zunszain PA, Carvalho LA, et al. The glucocorticoid receptor: pivot of depression and of antidepressant treatment? Psychoneuroendocrinology. 2011;36:415–25.
[45] He L, Yuan L, Sun Y, et al. Glucocorticoid receptor signaling activates TEAD4 to promote breast cancer progression. Cancer Res. 2019;79:4399–411.
[46] Sorrentino G, Ruggeri N, Zannini A, et al. Glucocorticoid receptor signalling activates YAP in breast cancer. Nat Commun. 2017;8:14073.
[47] Obradović M, Hamelin B, Manevski N, et al. Glucocorticoids promote breast cancer metastasis. Nature. 2019;567:540–4.
[48] Singh JK, Simões BM, Howell SJ, et al. Recent advances reveal IL-8 signaling as a potential key to targeting breast cancer stem cells. Breast Cancer Res. 2013;15:210.
[49] Alraouji NN, Aboussekhra A. Tocilizumab inhibits IL-8 and the proangiogenic potential of triple negative breast cancer cells. Mol Carcinog. 2021;60:51–9.
[50] Papadimitropoulou A, Vellon L, Atlas E, et al. Heregulin drives endocrine resistance by altering IL-8 expression in ER-positive breast cancer. Int J Mol Sci . 2020;21:7737.
[51] Baker JR, Sakoff JA, McCluskey A. The aryl hydrocarbon receptor (AhR) as a breast cancer drug target. Med Res Rev. 2020;40:972–1001.
[52] Aguiniga LM, Yang W, Yaggie RE, et al.; MAPP Research Network Study Group. Acyloxyacyl hydrolase modulates depressive-like behaviors through aryl hydrocarbon receptor. Am J Physiol Regul Integr Comp Physiol. 2019;317:R289–300.
[53] Zhang JC, Yao W, Hashimoto K. Brain-derived neurotrophic factor (BDNF)-TrkB signaling in inflammation-related depression and potential therapeutic targets. Curr Neuropharmacol. 2016;14:721–31.
[54] Ezzati M, Yousefi B, Velaei K, et al. A review on anti-cancer properties of Quercetin in breast cancer. Life Sci. 2020;248:117463.
[55] Li Y, Yao J, Han C, et al. Quercetin, inflammation and immunity. Nutrients. 2016;8:167.
[56] Tang J, Diao P, Shu X, et al. Quercetin and quercitrin attenuates the inflammatory response and oxidative stress in LPS-induced RAW264.7 cells: in vitro assessment and a theoretical model. Biomed Res Int. 2019;2019:1–8.
[57] Fang K, Li HR, Chen XX, et al. Quercetin alleviates LPS-induced depression-like behavior in rats via regulating BDNF-related imbalance of Copine 6 and TREM1/2 in the hippocampus and PFC. Front Pharmacol. 2019;10:1544.
[58] Guan Y, Wang J, Wu X, et al. Quercetin reverses chronic unpredictable mild stress-induced depression-like behavior in vivo by involving nuclear factor-E2-related factor 2. Brain Res. 2021;1772:147661.
[59] Bijani S, Dizaji R, Sharafi A, et al. Neuroprotective effect of apigenin on depressive-like behavior: mechanistic approach. Neurochem Res. 2022;47:644–55.
[60] Weng L, Guo X, Li Y, et al. Apigenin reverses depression-like behavior induced by chronic corticosterone treatment in mice. Eur J Pharmacol. 2016;774:50–4.
[61] Imran M, Aslam GT, Atif M, et al. Apigenin as an anticancer agent. Phytother Res. 2020;34:1812–28.
[62] Adel M, Zahmatkeshan M, Akbarzadeh A, et al. Chemotherapeutic effects of Apigenin in breast cancer: preclinical evidence and molecular mechanisms; enhanced bioavailability by nanoparticles. Biotechnol Rep (Amst). 2022;34:e00730.
[63] Fasoulakis Z, Koutras A, Syllaios A, et al. Breast cancer apoptosis and the therapeutic role of luteolin. Chirurgia (Bucur). 2021;116:170–7.
[64] Achour M, Ferdousi F, Sasaki K, et al. Luteolin modulates neural stem cells fate determination: in vitro study on human neural stem cells, and in vivo study on LPS-induced depression mice model. Front Cell Dev Biol. 2021;9:753279.
[65] Silva DSJ, Gonçalves CJ, de Oliveira CP, et al. The pharmacological action of kaempferol in central nervous system diseases: a review. Front Pharmacol. 2020;11:565700.
[66] Gao LN, Yan M, Zhou L, et al. Puerarin alleviates depression-like behavior induced by high-fat diet combined with chronic unpredictable mild stress via repairing TLR4-induced inflammatory damages and phospholipid metabolism disorders. Front Pharmacol. 2021;12:767333.
[67] Xu H, Hu M, Liu M, et al. Nano-puerarin regulates tumor microenvironment and facilitates chemo- and immunotherapy in murine triple negative breast cancer model. Biomaterials. 2020;235:119769.