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RESEARCH ARTICLE

The Mechanism and Potential Targets of Betulinic Acid in the Treatment of Breast Cancer Using Network Pharmacology and Molecular Docking

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Abstract

Betulinic acid (BA) is a natural pentacyclic triterpene in many Chinese herbs, which has effectively anti-tumor biological property. Objective. To explore the anti-breast cancer (BC) mechanism and potential target proteins of BA by using network pharmacology and molecular docking. Methods. Firstly, potential BC-related target proteins of BA were screened by using Super-PRED and DisGeNET databases, and the "BA - potential target proteins against BC" network was summarized by using Cytoscape software. Then, DAVIDA database was used to perform GO and KEGG enrichment analysis to find BC-related targets in core pathways, biological process, cellular components, and the protein-protein interaction (PPI) between target proteins was analyzed by STRING database and Cytoscape software. Finally, the docking analysis of BA to BC-related targets was verified by Ledock software. Results. The results showed that there were 51 BC-related targets to BA. These targets were further mapped to 99 GO biological process, 16 GO cellular component, 17 GO molecular function, and 93 remarkable pathways. In the PPI network analysis and molecular docking verification, 5 key targets were found, including ESR1, STAT3, HSP90AA1, SIRT1, and mTOR. Conclusion. Based on the network pharmacology analysis, it is speculated that BA may not only participate in multiple mechanisms of action on treat breast cancer but may also be involved in worth of BA in clinical application of breast cancer.

Keywords: Betulinic Acid; Breast Cancer; Network Pharmacology

Introduction

Breast cancer (BC) is one of the most common cancers among women in the world. Over 2 million women are diagnosed with breast cancer every year throughout the world [1]. Despite high sensitivity towards surgery, chemotherapy and radiation therapy, but many patients are not benefit from these treatments because of chemo-resistance and poor-prognosis [2]. Hence, it is critical for finding the novel agents to improve the survival rate of BC patients.

Betulinic acid (BA) is a pentacyclic triterpene with a lupine structure, found in birch bark, acuminastissima leaves (Fig. 1A). Many studies have demonstrated that BA has many different biological properties: anti-inflammatory, anti-oxidative, anti-tumor, and so on [3]. Subsequent studies found that BA inhibited proliferation, arrested cell cycle, and induced apoptosis in multiple cancers, including breast cancer, non-small cell lung cancer [4, 5]. However, the underlying molecular pharmacology and potential targets of BA have not been well known.

In this study, we analyzed the potential targets of BA in the treatment of BC. Then, these targets were used to research the target pathways by network pharmacology approach, gene ontology (GO), biological pathway (KEGG) functional enrichment, and protein-protein interaction (PPI) network. Finally, molecular docking technology was used to analyze the BA dock with potential targets to explore the most binding model. This research was carried out to provide a theoretical basis for the molecular mechanisms of BA against BC.

Materials and Methods

Predicting targets of BA

To obtain the putative targets of BA, the Super-PRED (https://prediction.charite.de/subpages/target_prediction.php) databases was used. Then, the name of these identified candidate targets was inputted into UniProt database (www. Uniport.org) for normalization.

Screening targets related to BC

BC-related target genes were collected from DisGeNET database (https://www.disgenet.org/) and Comparative Toxicogenomics database (CTD, http://ctdbase.org/). Potential target genes (overlapping genes) of BA for BC treatment were acquired through the Veeny 2.1 (https://bioinfogp.cnb.csic.es/tools/venny/) intersection.

"BA- BC related genes" network construction

The complex relationship between BA, and BC related genes were visualized using Cytoscape software.

GO and KEGG pathway enrichment analysis

To investigate the effect of BA in treatment BC, the DAVID database was carried out for GO and KEGG pathway enrichment analysis. The enriched GO terms and pathways having a corrected P value of less than 0.01 were selected and subjected to further analyses.

Protein-Protein interaction (PPI) network analysis

The PPI network for the predicted targets of BA was constructed using the STRING database (https://string-db.org). The cut-off criterion of minimum required interaction score was set as ≥0.4 and the other parameters were default. Then, PPI network was visualized by Cytoscape.

Binding capacity between BA and BC-related targets by Molecular Docking

Ledock was used to perform molecular docking analysis on BA and the potential targets to predict their interaction activity. Firstly, the 3D structure of BA (compound CID: 64971) was obtained from PubChem (https://pubchem.ncbi.nlm.nih.gov/), and the mol2 formula of 3D structure of BA was prepared in the Open Babel software. Secondly, the crystal structures of STAT3 (PDB ID: 6NUQ), HSP90AA1 (PDB ID: 5H22), mTOR (PDB ID: 4JT6), ESR1 (PDB ID: 4XI3), and SIRT1(PDB ID: 4I5I) were obtained from the Protein Data Bank (www.rcsb.org). Then, molecular docking was performed by LeDock. All parameters were set as default. PyMol was used to display the molecular docking results.

Results

BC-related target of BA prediction and analysis

159 target genes of BA were predicted from Super-PRED database (Supplementary file 1). By means of the DisGeNET and CTD database, we obtained 1402 BC-related targets (Supplementary file 2). From an intersection from the two categories of targets, 51 overlapping target genes were found as the potential targets of BA in BC treatment (Fig. 1B). As shown in Fig. 1C, the "BA-BC related targets" network was constructed, the interactions between them have 52 nodes and 51 edges.

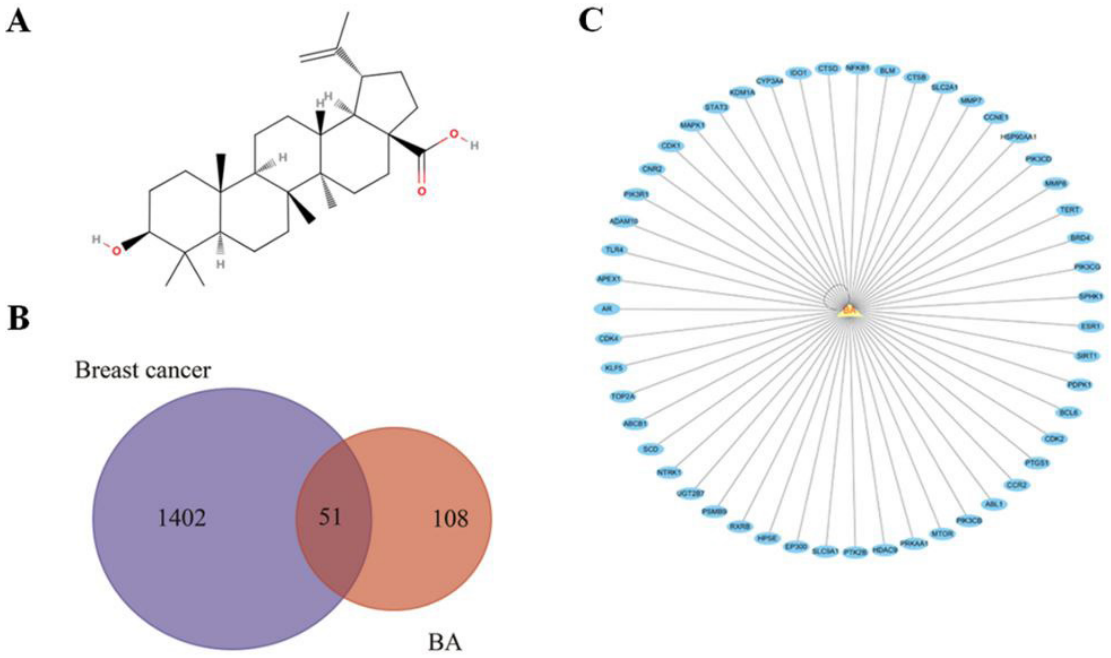

Figure 1: Potential targets of BA therapy for BC. (A) Structural formula of BA. (B) The Venny results of potential targets of BA therapy for BC. (C) The BA-BC related targets network

GO and KEGG analysis of BC-related targets of BA

The results of GO analysis of 51 potential BC-related targets of BA were shown that the inflammatory response, positive regulation of angiogenesis, phosphorylation, and negative regulation of apoptotic process were significantly associated with biological processes; cellular component focuses on response to nucleoplasm, condensed chromosome, cytoplasm, membrane raft, and transcription factor complex; molecular function was mainly concentrated in kinase activity, ATP binding, 1-phosphatidylinositol-4-phosphate 3-kinase activity, protein tyrosine kinase activity, and histone deacetylase binding (Fig. 2A).

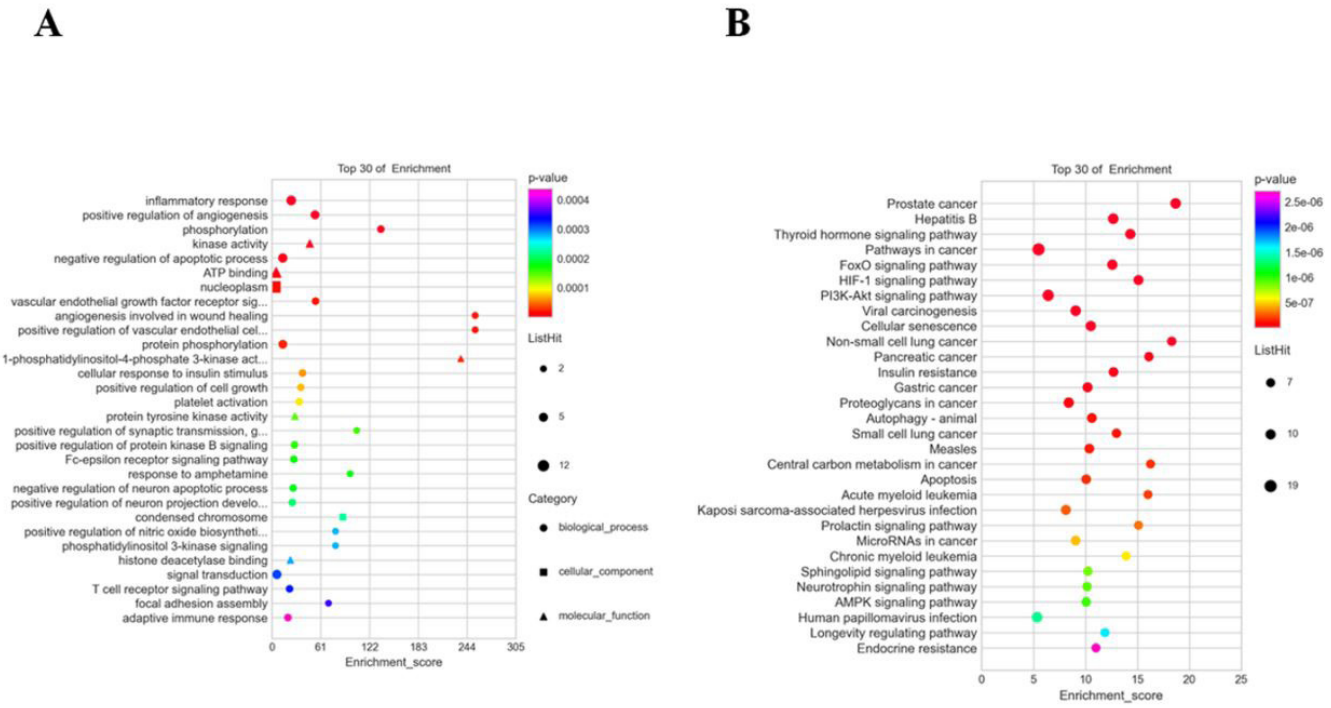

Figure 2: GO and KEGG pathway analysis of the 51 potential BC-related targets of BA. (A) Bubble diagram of GO enrichment analysis. (B) Bubble diagram of KEGG pathway analysis

In Fig. 2B, the KEGG enrichment analysis demonstrated that the KEGG pathway mainly manifested in Thyroid hormone signaling pathway, FoxO signaling pathway, HIF-1 signaling pathway, PI3K-AKT signaling pathway, and AMPK signaling pathway. Taken together, these results of GO and KEGG pathway enrichments suggested that 51 predicted BC-related targets had the key role in BA treatment for BC.

PPI network of BC-related targets of BA

PPI network of 51 potential BC-related targets of BA was constructed using STRING database. As shown in Fig. 3, the top targets with the high degree (the number of lines linked to a given node) were HSP90AA1, STAT3, ESR1, SIRT1, mTOR.

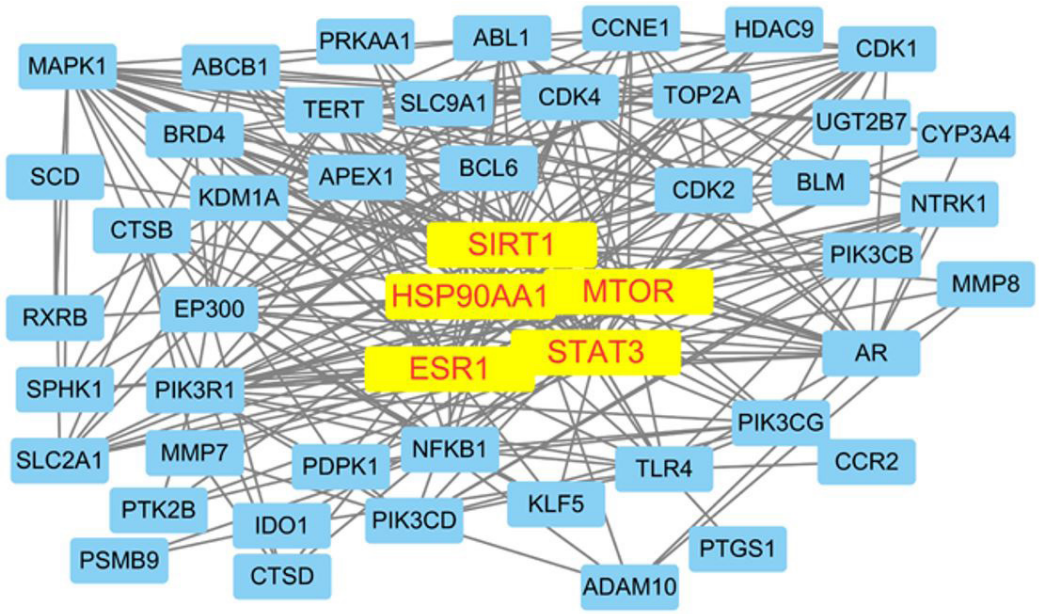

Figure 3: PPI network of the 51 BC-related targets of BA

Molecular docking analysis

To verify the reliability of BA and BC-related targets, HSP90AA1, STAT3, ESR1, SIRT1, mTOR were selected as receptor for molecular docking based on the above results. The docking scores for each target, shown in Table 2, suggested that there was a strong interaction between BA and the 5 targets.

NO.	Target Name	Gene Symbol	NO.	Target Name	Gene Symbol
1	Bloom syndrome protein	BLM	27	Sodium/hydrogen exchanger 1	SLC9A1
2	Nuclear factor NF-kappa-B p105 subunit	NFKB1	28	Protein tyrosine kinase 2 beta	PTK2B
3	Cathepsin D	CTSD	29	Histone deacetylase 9	HDAC9
4	Indoleamine 2,3-dioxygenase	IDO1	30	AMP-activated protein kinase, alpha-1 subunit	PRKAA1
5	Cytochrome P450 3A4	CYP3A4	31	Serine/threonine-protein kinase mTOR	MTOR
6	LSD1/CoREST complex	KDM1A	32	PI3-kinase p110-beta subunit	PIK3CB
7	Signal transducer and activator of transcription 3	STAT3	33	Bcr/Abl fusion protein	ABL1
8	MAP kinase ERK2	MAPK1	34	C-C chemokine receptor type 2	CCR2
9	Cyclin-dependent kinase 1/cyclin B1	CDK1	35	Cyclooxygenase-1	PTGS1
10	Cannabinoid CB2 receptor	CNR2	36	Cyclin-dependent kinase 2/cyclin A	CDK2
11	PI3-kinase p110-alpha/ p85-alpha	PIK3R1	37	B-cell lymphoma 6 protein	BCL6
12	ADAM10	ADAM10	38	3-phosphoinositide dependent protein kinase-1	PDPK1
13	Toll-like receptor 4	TLR4	39	NAD-dependent deacetylase sirtuin 1	SIRT1
14	DNA-(apurinic or apyrimidinic site) lyase	APEX1	40	Estrogen receptor alpha	ESR1
15	Androgen Receptor	AR	41	Sphingosine kinase 1	SPHK1
16	Cyclin-dependent kinase 4/cyclin D1	CDK4	42	PI3-kinase p110-gamma subunit	PIK3CG
17	Kruppel-like factor 5	KLF5	43	Bromodomain-containing protein 4	BRD4
18	DNA topoisomerase II alpha	TOP2A	44	Telomerase reverse transcriptase	TERT

NO.	Target Name	Gene Symbol	NO.	Target Name	Gene Symbol
19	P-glycoprotein 1	ABCB1	45	Matrix metalloproteinase 8	MMP8
20	Acyl-CoA desaturase	SCD	46	PI3-kinase p110-delta subunit	PIK3CD
21	Nerve growth factor receptor Trk-A	NTRK1	47	Heat shock protein HSP 90-alpha	HSP90AA1
22	UDP-glucuronosyl transferase 2B7	UGT2B7	48	Cyclin-dependent kinase 2/cyclin E1	CCNE1
23	Proteasome subunit beta type-9	PSMB9	49	Matrix metalloproteinase 7	MMP7
24	Retinoid X receptor beta	RXRB	50	Glucose transporter	SLC2A1
25	Heparanase	HPSE	51	Cathepsin B	CTSB
26		EP300			

Table 1: The potential targets of BA against breast cancer

PDB ID	Protein Name	Scores (kcal/mol)	Ledock pocket center		
			x	y	z
5H22	HSP90AA1	-5.01	-39.638, 22.275	-19.987, 3.005	-32.917, 19.119
4JT6	mTOR	-5.28	-27.128, 8.585	-39.165, 27.439	-64.943, 47.536
4XI3	ESR1	-4.51	-14.901, 5.472	-16.795, 0.558	22.208, 40.621
4I5I	SIRT1	-4.48	41.402, 56.183	-40.801, -11.523	11.388, 27.396
6NUQ	STAT3	-4.31	3.7, 23.5	38.7, 69.3	-11.1, 10.9

Table 2: Molecular docking parameters and results of five BC-related targets binding with BA

As showed in Fig. 4, Leu346, Thr347, Ala350, Asp351, located in functional domain, were the amino acid residues in the binding sites of BA to ESR1(Fig. 4A); while Asn-51, Asp-54, Ile-91, Gly-97, Met-98, and Leu-107 were responsible for BA binding to HSP90AA1 (Fig. 4B); For analyzing the binding sites between BA and mTOR, Ser-2165, Gln-2167, Pro-2169, Trp-2239, Val-2240, Cys-2243 were involved in stabilizing binding poses (Fig. 4C); The amino acid residue of SIRT1, interacted with BA were Phe-297, Ile-316, Phe-413, Phe-414, Gly-440, and Ser-441 (Fig. 4D); The bonds between BA and residues of STAT3 (Tyr-640, Gln-644, and Met-648) may be vital in BA binding to STAT3, the worth of Ser-613, Trp-623, Val-637, Glu-638, Asn-647, and Tyr-657 could not be ignored (Fig. 4E).

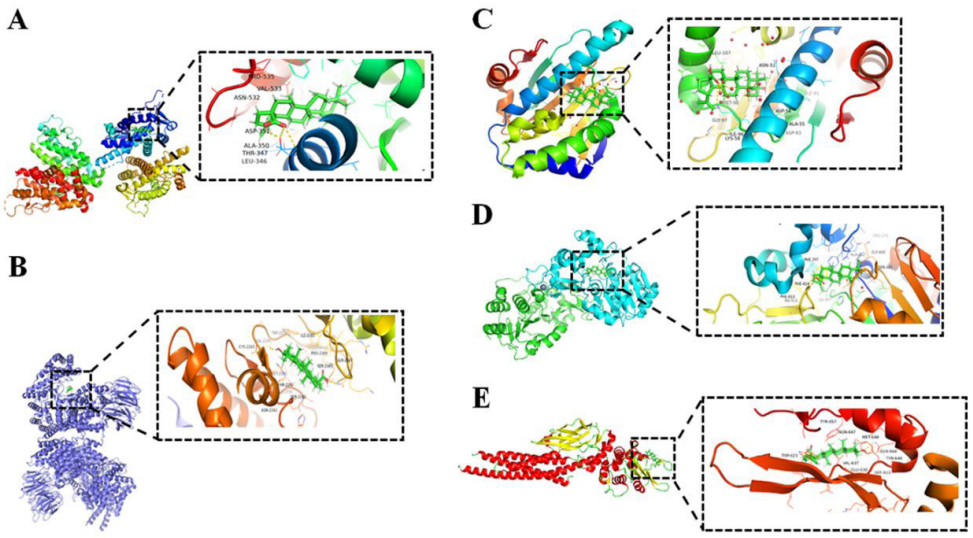

Figure 4: Molecular docking of BA with the core BC-related targets. (A) Molecular docking of BA with ESR1. (B) Molecular docking of BA with mTOR. (C) Molecular docking of BA with HSP90AA1. (D) Molecular docking of BA with SIRT1. (E) Molecular docking of BA with STAT3

Discussion

The network pharmacology has been significantly promoted an understanding of the complex interactions between drugs and their targets and the potential mechanisms of action. It is usually applied for studying the novel targets of Traditional Chinese Medicine, and drug repurposing [6-8]. In this work, we explored the potential mechanism and targets of BA against BC by using reverse-docking analysis, GO, KEGG enrichment analysis, and PPI analysis. The results demonstrated that 5 targets including HSP90AA1, ESR1, SIRT1, mTOR, and STAT3 which have a well combination with BA were selected as potential targets for BA in BC therapy.

Previous studies have revealed that BA display anti-tumor biological effects in BC cells. Jiao et al. found that BA inhibited aerobio glycolysis activity for reducing proliferation via caveolin-1/NF-κB/c-Myc pathway in BC cell lines MCF-7 and MDA-MB-231[9]. Zheng et al. suggested that BA could attenuate migration and invasion of highly aggressive breast cancer cells by targeting GRP78 mediated glycolysis and ER stress apoptotic pathway [10]. In addition, BA increases expression of p53 and p21, which are in cell cycle regulation, and arrest MCF-7 in G1 phase [11]. BA mediated inhibition of topoisomerase I and II in MDA-MB-231 cells induces cell cycle arrest and apoptosis [12, 13]. Other research found that BA down-regulates sp1, ROS, and VEGFR pathways [14-16].

In this study, we predicted 149 pharmacophore candidates via Supra- and found 51 potential targets of BA against BC. Moreover, analysis of GO and KEGG enrichment results for 51 targets suggested that 134 biological functions and 98 signaling pathways are directly involved in the occurrence and development of BC, suggesting that these gene function and signaling pathways may be the mechanism by which BA can treat BC. Subsequently, the results of PPI analysis and molecular verification demonstrated that BA could directly bind to HSP90AA1, ESR1, SIRT1, mTOR, and STAT3 for regulating thyroid hormone, FoxO, HIF-1, PI3K-AKT, AMPK signaling pathway.

Heat shock protein 90-alpha (HSP90AA1), an essential molecular chaperon that is highly conserved in evolution, is efficiently expressed under the stimulatory conditions of trauma, infection, and tumors [17-19]. In breast cancer, HSP90AA1 is required for the stabilization of many proteins in pathways that play key roles in cancer growth and survival, such as estrogen receptor (ER), progesterone receptor (PR), essential components of HER2 signaling (HER2, AKT, c-SRC, RAF and HIF-1α), and EGFR [20, 21]. Hence, HSP90AA1 has been acted as a candidate marker for diagnosis, prognosis, and therapy of BC.

Estrogen receptor alpha gene (ESR1) regulates the expression of estrogen receptors, involved in the regulation of gene expression, and affect proliferation and differentiation in target tissues [22]. In BC cells, it has been suggested that ESR1 can mediates PI3K-AKT signaling pathway to promote cell growth [23].

Numerous contributions support the notion that SIRT1, a type III histone deacetylase, activities influence hormone receptors (HR) actions, expressly those mediating long-term estrogenic effects in the mammary gland, namely the classic ERs, for which a relevant degree of interdependence between these factors has been described with apparent importance for BC onset and development. Moreover, SIRT1 shows a convoluted role in the regulation of the epithelial-mesenchymal transition (EMT) process, with apparent relevance in the case of reproductive tumors such as BC [24]. Moreover, Xu et al found that FoxO family members were recruits into active ERα and SIRT1 transcriptomic complexes [25]. Additionally, SIRT1 up-regulates PI3K-AKT, MEK-ERK, and GPER pathway [26,27].

Signal transducer and activator of transcription 3 (STAT3), phosphorylated by IL-6 and JAK2 at Tyr 705, has been shown to play an important role in BA cells by inducing EMT and angiogenesis [28]. VEFGR, PKC, PI3K also could mediate constitutive STAT3 activation [29-31].

In mammalian cells, it exists as two distinct multiprotein complexes: mTORC1 and mTORC2. mTORC1 is sensitive to Rapamycin and promotes cell growth and survival by stimulating nutrient uptake and metabolism. It also stimulates cell growth by promoting ribosome production and protein synthesis and by inhibiting protein degradation [32]. mTORC1 may be activated through differ-

ent pathways, but mainly through the PI3P/AKT pathway, which is activated by extracellular growth factors and nutrients [33]. Inhibitors of mTOR such as rapamycin (sirolimus) and the rapalogs temsirolimus (CCI-779), everolimus (RAD001), and deforolimus (AP23573) have been extensively evaluated in ER-positive, and HER2-overexpressing breast cancer. hematological malignancies, renal cancer, and as treatment for transplant rejection [34]. The results of molecular docking analysis, suggested that BA could bind the functional domain of these targets, and validated the reliability of interaction between BA and BC related targets screened by network pharmacology.

In summary, this study used a network pharmacology approach to construct a network to display the interactions between BA and BC-related target genes. The results indicate that BA may exert anti-breast cancer effect by acting on SIRT1, ESR1, STAT3, HSP90AA1, and mTOR, and also speculated that BA may participate in multiple mechanisms of action to treat breast cancer. Therefore, it is considered that BA can be a candidate drug in the anti-breast cancer therapy.

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References

1.Siegel RL, Miller KD, Fuchs HE, et al. (2021) Cancer statistics, 2021. CA: A Cancer Journal for Clinicians. 71: 7-33.

2. Zhang SW, Sun KX, Zhang RS, et al. (2020) Cancer incidence and mortality in China, 2015. Journal of the National Cancer Center. 1: 2-11.

3. Lou H, Li H, Zhang S, et al. (2021) A Review on Preparation of Betulinic Acid and Its Biological Activities. Molecules 26: 5583.

4.Gheorgheosu D, Duicu O, Dehelean C, et al. (2014) Betulinic acid as a potent and complex antitumor phytochemical: a minireview. AntiCancer Agents Med Chem. 14: 936-45.

5.Luo R, Fang D, Chu P, et al. (2016) Multiple molecular targets in breast cancer therapy by betulinic acid. Biomed Pharmacother. 84: 1321-30.

6.Berger SI, Iyengar R. (2009) Network analyses in systems pharmacology. Bioinformatics (Oxford, England). 5: 2466-72.

7.Zhang R, Zhu X, Bai H, et al. (2019) Network Pharmacology Databases for Traditional Chinese Medicine: Review and Assessment. Front Pharmacol. 10: 123.

8.Parvathaneni V, Kulkarni NS, Muth A, et al. (2019) Drug repurposing: a promising tool to accelerate the drug discovery process. Drug Discov Today. 24: 2076-85.

9.Jiao L, Wang S, Zheng Y, et al. (2019) Betulinic acid suppresses breast cancer aerobic glycolysis via caveolin-1/NF-κB/c-Myc pathway. Biochem Pharmacol. 161: 149-62.

10.Zheng Y, Liu P, Wang N, et al. (2019) Betulinic Acid Suppresses Breast Cancer Metastasis by Targeting GRP78-Mediated Glycolysis and ER Stress Apoptotic Pathway. Oxid Med Cell Longev 8781690.

11.Foo JB, Yazan LS, Tor YS, et al. (2015) Induction of cell cycle arrest and apoptosis by betulinic acid-rich fraction from Dillenia suffruticosa root in MCF-7 cells involved p53/p21 and mitochondrial signalling pathway. J Ethnopharmacol. 166: 270-8.

12.Bar F, Khanfar MA, Elnagar AY, et al. (2009) Rational Design and Semisynthesis of Betulinic Acid Analogues as Potent Topoisomerase Inhibitors. Journal of Natural Products. 72: 1643-50.

13.Dillon LW, Pierce L, Lehman CE, et al. (2013) DNA Topoisomerases Participate in Fragility of the Oncogene RET. PLoS ONE. 8: e75741.

14.Laszczyk MN (2009) Pentacyclic Triterpenes of the Lupane, Oleanane and Ursane Group as Tools in Cancer Therapy. Planta Med. 75: 1549-60.

15.Jutooru I, Chadalapaka G, Sreevalsan S, et al. (2010) Arsenic trioxide downregulates specificity protein (Sp) transcription factors and inhibits bladder cancer cell and tumor growth. Exp Cell Res. 316: 2174-88.

16.Karna E, Szoka L, Palka JA (2010) Betulinic acid inhibits the expression of hypoxia-inducible factor 1α and vascular endothelial growth factor in human endometrial adenocarcinoma cells. Molecular and Cellular Biochemistry 340: 15-20.

17.Condelli V, Crispo F, Pietrafesa M, et al. (2019) HSP90 molecular chaperones, metabolic rewiring, and epigenetics: impact on tumor progression and perspective for anticancer therapy. Cells. 8: 532.

18.Chehab M, Caza T, Skotnicki K, et al. (2015) Targeting Hsp90 in urothelial carcinoma. Oncotarget. 6: 8454-73.

19.Wu J, Liu T, Rios Z, et al. (2017) Heat shock proteins and cancer. Trends Pharmacol. Sci. 38: 226-56.

20.Neckers L, Ivy SP. (2003) Heat shock protein 90. Curr Opin Oncol. 15: 419-24.

21.Zhang H, Burrows F. (2004) Targeting multiple signal transduction pathways through inhibition of Hsp90. J Mol Med (Berl). 82: 488-99.

22.Stein B, Yang MX (1995) Repression of the interleukin-6 promoter by estrogen receptor is mediated by NF-kappa B and C/EBP beta. Mol Cell Biol.15: 4971-9.

23.Engel JA (2009) Targteting PI3K signaling in cancer: opportunities, challenges and limitations. Nat Rev Cancer. 9: 550-62.

24.Simic P, Williams EO, Bell EL, et al. (2013) SIRT1 suppresses the epithelial-to-mesenchymal transition in cancer metastasis and organ fibrosis. Cell Rep 3: 1175-86.

25.Xu Z, Yang Y, Li B, et al. (2018) Checkpoint suppressor 1 suppresses transcriptional activity of ERalpha and breast cancer cell proliferation via deacetylase SIRT1. Cell Death Dis 9: 559.

26.Revankar CM, Cimino DF, Sklar LA, et al. (2005) A transmembrane intracellular estrogen receptor mediates rapid cell signaling. Science. 307: 1625-30.

27.Filardo EJ (2002) Epidermal growth factor receptor (EGFR) transactivation by estrogen via the G-protein-coupled receptor, GPR30: a novel signaling pathway with potential significance for breast cancer. J Steroid Biochem Mol Biol. 80: 231-8.

28.Johnson DE, O'Keefe RA, Grandis JR (2018) Targeting the IL-6/JAK/STAT3 signalling axis in cancer. Nat Rev Clin Oncol. 15: 234-48.

29.Zhao D, Pan C, Sun J, et al. (2015) VEGF drives cancer-initiating stem cells through VEGFR-2/Stat3 signaling to upregulate Myc and Sox2. Oncogene. 34: 3107-19.

30.Aziz MH, Hafeez BB, Sand JM, et al. (2010) Protein kinase Cvarepsilon mediates Stat3 Ser727 phosphorylation, Stat3-regulated gene expression, and cell invasion in various human cancer cell lines through integration with MAPK cascade (RAF-1, MEK1/2, and ERK1/2). Oncogene. 29: 3100-9.

31.Dunkel Y, Ong A, Notani D, et al. (2012) STAT3 protein up-regulates Galpha-interacting vesicle-associated protein (GIV)/Girdin expression, and GIV enhances STAT3 activation in a positive feedback loop during wound healing and tumor invasion/metastasis. J Biol Chem. 287: 41667-83.

32.Wei X, Luo L, Chen J (2019) Roles of mTOR signaling in tissue regeneration. Cells. 2019, 8: 1075.

33.Kakumoto K, Ikeda JI, Okada M, et al. (2015) mLST8 promotes mTOR-mediated tumor progression. PLoS One. 10: e0119015.

34.Guerrero-Zotano A, Mayer IA, Arteaga CL (2016) PI3K/AKT/mTOR: role in breast cancer progression, drug resistance, and treatment. Cancer Metastasis Rev. 35: 515-24.