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ORIGINAL ARTICLE

Comprehensive bioinformatics study reveals targets and molecular mechanism of hesperetin in overcoming breast cancer chemoresistance

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Abstract

The effectiveness of chemotherapy in breast cancer treatment can be increased using a combinatorial agent. Hesperetin has been reported to increase the sensitivity of doxorubicin in breast cancer cells; however, the underlying molecular mechanism remains unclear. This present study was conducted to identify the potential target and molecular mechanism of hesperetin in circumventing breast cancer chemoresistance using a bioinformatics approach. Microarray data obtained after hesperetin treatment in the NCI-60 cell line panel collection were retrieved from the COMPARE public library. These data were then compared with the list of the regulatory genes of breast cancer resistance obtained from PubMed and further analyzed for gene ontology and KEGG pathway enrichment, as well as protein–protein interaction network. A Venn diagram of COMPARE microarray data and the gene list from PubMed generated 56 genes (potential therapeutic target genes/PTTGs). These PTTGs participate in the biological process of the JAK-STAT cascade and are located in the nucleus, exert a molecular function in protein serine/threonine kinase activity, and regulate the erbB signaling pathway. Drug association analysis demonstrated that both hesperetin and the erbB receptor inhibitors, i.e., monoclonal antibody and tyrosine kinase inhibitor, target the same mRNA expression. Furthermore, results of the molecular docking study revealed that hesperetin is a promising inhibitor that targets ABL1, DNMT3B, and MLH1 due to the similarity of binding properties with its native ligand. In conclusion, the possible pathways and the regulatory genes identified in this study may offer new insights into the mechanism by which hesperetin overcomes breast cancer chemoresistance. A combinatorial therapy with hesperetin targeting ABL1, DNMT3B, and MLH1 may be effective in circumventing chemoresistance in breast cancer.

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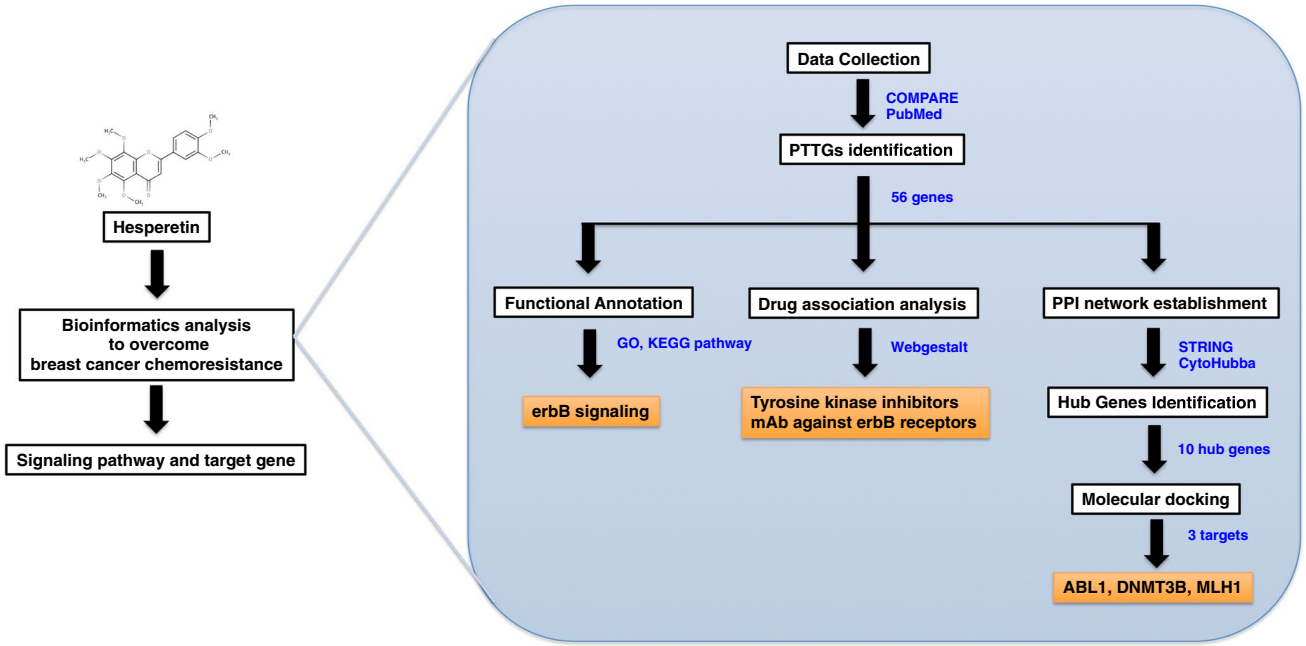
Graphic abstract



Keywords Hesperetin · Breast cancer · Chemoresistance · Bioinformatics · erbB signaling pathway

Introduction

Breast cancer causes the highest mortality rate among women and is also one of the leading causes of death in the world [1]. Conventional treatments for breast cancer include surgery, radiation therapy, chemotherapy, endocrine (hormone) therapy, and targeted therapy [2]. Chemotherapy is used as an adjunct to surgery, radiotherapy, or hormone therapy [3]. However, invasion, metastasis, and drug resistance decrease the effectiveness of chemotherapy [4]. Thus, a combination of drugs can be used to obtain a synergistic effect of therapy, reduce drug toxicity, and reduce or inhibit the development of drug resistance [5]. In this context, a combinatorial agent is required to increase the effectiveness of chemotherapy.

Hesperetin, one of the citrus flavones, has been investigated for its anticancer activities in several cancer cell models, including MDA-MB 231 breast cancer cells [6], SKBR3 breast cancer cells [7], SiHa cervical cancer cells [8], and MCF-7 breast cancer cells [9]. One study reported that hesperetin treatment inhibits cell proliferation and induces cell cycle arrest at the G1 phase in PC3 prostate cancer cells by elevating IL-6 gene expression, IL-6 protein secretion, and the expression of pSTAT3, pERK1/2, and pAKT [10]. Another study found that hesperetin enhances apoptotic cell death and mitochondrial membrane potential loss in H522 lung cancer cells [11]. A recent review conducted by Ferreira de Oliveira demonstrated that hesperetin regulates cell cycle and apoptosis through the regulation of the JNK pathway [12].

Furthermore, studies have also investigated hesperetin in combination with chemotherapeutics. It was found that hesperetin could increase etoposide cytotoxicity and induce G2/M arrest in U2OS human osteosarcoma cells [13], increase the cytotoxicity of cabazitaxel and docetaxel in PCC-1 prostate cancer cells [14], and increase cisplatin sensitivity by elevating the levels of reactive oxygen species in lung adenocarcinoma cells [15]. In breast cancer, hesperetin was shown to increase the sensitivity of MCF-7/Dox breast cancer cells to doxorubicin by inhibiting the expression of P-glycoprotein (PgP) [16]. Nevertheless, the molecular mechanism of hesperetin in overcoming chemoresistance in breast cancer needs to be further investigated.

In the present study, a bioinformatics approach was used to identify the potential target and mechanism of hesperetin in overcoming chemoresistance in patients with breast cancer. Microarray data obtained after hesperetin treatment in the NCI-60 cell line panel collection were retrieved from the COMPARE public library. These data were then compared with the list of the regulatory genes of breast cancer resistance obtained from PubMed and further analyzed for gene ontology and KEGG pathway enrichment, as well as protein–protein interaction (PPI) network. Molecular docking study was performed to identify the potential interaction

between hesperetin and the protein target. We identified a possible specific molecular mechanism of hesperetin using an integrated bioinformatics analysis, which suggested that ABL1, DNMT3, and MLH1 could be developed as novel targets for overcoming chemoresistance in breast cancer.

Materials and methods

Data collection and processing

Cytotoxicity data and mRNA array data were obtained from the NCI-60 DTP Web site (https://dtp.cancer.gov/) [17]. COMPARE analysis with the public library produces a list of drugs that have similarities with hesperetin, as well as a list of gene expressions on the NCI-60 cell line panel [18]. The similarity pattern is expressed as the Pearson correlation coefficient. In this study, the list of compounds and genes was limited to the Pearson correlation coefficients of < -0.5 and > 0.5. Genes associated with breast cancer chemoresistance were obtained from PubMed using the key words "breast cancer resistance."

Gene ontology and KEGG pathway enrichment analysis

Gene ontology (GO) enrichment analysis was conducted using the Database for Annotation, Visualization, and Integrated Discovery v6.8 [19], with $p < 0.05$ considered as the cutoff value. KEGG pathway enrichment was also conducted using the overrepresentation enrichment analysis (ORA) from the WEB-based GEne SeT AnaLysis Toolkit (WebGestalt), with a false discovery rate (FDR) of < 0.05 selected as the cutoff value [20].

Drug association analysis

To identify the potential target genes of hesperetin in breast cancer, drug association analyses were conducted using the ORA from the WebGestalt, with an FDR of < 0.05 considered as the cutoff value [20]. Briefly, the PTTGs were submitted to the ORA from the WebGestalt, with the functional parameter GLAD4U.

Construction of PPI network and hub gene selection

The PPI network was constructed using STRING-DB v11.0 [21]. Confidence scores > 0.4 were considered to be significant. The PPI network was visualized by the Cytoscape software. Genes with the highest degree score of 10, analyzed by cytoHubba plugin, were selected as hub genes.

Molecular docking

Docking simulation was conducted to predict the binding properties of hesperetin on ABL1, DNMT3B, and MLH1. All computational simulations were generated on the Windows 10 Operating System, with Intel Core i5-7th Gen as a processor and 4 GB of RAM. The PDB IDs of 4P7A, 5NR3, and 1FPU were chosen as the crystal structure model of MLH1, DNMT3B, and ABL1 proteins, respectively, based on the presence of the known inhibitor. MOE 2010 (licensed from the Faculty of Pharmacy, UGM) was used for docking simulation, RMSD calculation, and visualization of the interaction. The structure of hesperetin was drawn in the ChemDraw software and subjected to conformational search that was minimized in MOE using the Energy Minimize module. Docking simulation was conducted on the native ligand binding site based on the flexible structure of ligands and rigid receptor. For the docking simulation setting, London dG and Triangle Matcher were chosen for score function and placement setting, respectively. Force field method was used to refine the docking results from 30 retain settings. The default settings were used in each application unless any further explanation was available. The results of the analysis were used to infer which conformation produced the lowest energy state when hesperetin bound to the target protein.

Results and discussion

Data collection and processing

This study investigated the molecular mechanism of hesperetin in breast cancer chemoresistance using a bioinformatics approach. The microarray data revealed that there were 554 genes with a positive Pearson correlation coefficient and 13 genes with a negative Pearson correlation coefficient (Supplementary Table 1). In addition, the genes RHCE, RHD, and FAM65C showed the highest Pearson correlation coefficient values of 0.904, 0.883, and 0.874, respectively. In contrast, the genes TCHH, LHX2, and STS showed negative Pearson correlation coefficient values of -0.658, -0.59, and -0.59, respectively (Table 1).

Using COMPARE, we investigated the gene expression that was affected by treatment with hesperetin in the NCI-60 cell line panel. A correlation analysis was performed between mRNA expression and IC_{50} values of hesperetin in the NCI cell line panel. A positive correlation coefficient indicates a direct correlation, whereas a negative correlation coefficient indicates an inverse correlation. A direct correlation implies that a higher mRNA expression enhances drug resistance, whereas an inverse correlation implies that a higher mRNA expression enhances drug sensitivity [22]. A previous study has demonstrated that a

microarray-based gene expression profiling might indeed be a suitable tool to predict tumor responsiveness to natural products [23]. That study also highlighted that this approach has been confirmed to be successful in breaking down the mechanism of action of new compounds.

A PubMed search using the key words "breast cancer resistance" resulted in 2653 genes associated with breast cancer resistance (Supplementary Table 2). In addition, a Venn diagram of COMPARE microarray data and the gene list from PubMed generated 56 genes that were regulated by hesperetin and were related to breast cancer chemoresistance (Fig. 1b, Supplementary Table 3). These 56 genes were considered as potential therapeutic target genes (PTTGs) and then evaluated in the subsequent experiment.

GO and KEGG pathway enrichment

GO analysis was performed according to the categories of biological process, cellular component, and molecular function. Several PTTGs (Table 2) participated in the biological process of the cytokine-mediated signaling pathway, the JAK-STAT cascade, positive regulation of cell proliferation, and negative regulation of the Notch signaling pathway. These PTTGs are located in the nucleus, nucleoplasm, and cytoplasm. They also exert a molecular function in protein serine/threonine kinase activity, ATP binding, chromatin binding, and single-stranded DNA binding.

The KEGG pathway enrichment analysis of the PTTGs revealed regulation in several pathways (Fig. 2). Based on an FDR of < 0.05 and the highest enrichment ratio, the PTTGs were found to regulate the prolactin signaling

Table 1 Top ten mRNA with the highest and lowest Pearson correlation coefficients

No	Pearson correlation coefficient	Target vector ID	Gene symbol	Gene name
1	0.904	MoltId:GC389686	RHCE	Blood group Rh(CE) polypeptide
2	0.883	MoltId:GC387983	RHD	Rh blood group D antigen
3	0.874	MoltId:GC411521	FAM65C	Family with sequence similarity 65, member C
4	0.847	MoltId:GC256471	PDE4DIP	Myomegalin
5	0.824	MoltId:GC412460	CPED1	Cadherin-like and PC-esterase domain containing 1
6	0.815	MoltId:GC406846	FHL3	Four and a half LIM domains 3
7	0.813	MoltId:GC98129	GYPB	Glycophorin-B
8	0.813	MoltId:GC400125	RELN	Reelin
9	0.811	MoltId:GC174924	PKLR	Pyruvate kinase
10	0.807	MoltId:GC168891	UNC13D	Protein unc-13 homolog D
11	−0.658	MoltId:GC16253	TCHH	Trichohyalin
12	−0.59	MoltId:GC10812	LHX2	LIM/homeobox protein
13	−0.59	MoltId:GC12548	STS	Steryl-sulfatase
14	−0.576	MoltId:GC15206	DNAJB5	DnaJ heat shock protein family member B5
15	−0.56	MoltId:GC11797	POLR3GL	DNA-directed RNA polymerase III subunit RPC7-like
16	−0.55	MoltId:GC405690	C10orf76	UPF0668 protein
17	−0.539	MoltId:GC265602	HIF1AN	Hypoxia-inducible factor 1-alpha inhibitor
18	−0.539	MoltId:GC12356	SLC6A2	Sodium-dependent noradrenaline transporter
19	−0.538	MoltId:GC18892	ID1	DNA-binding protein inhibitor ID-1
20	−0.529	MoltId:GC16118	SFXN3	Sideroflexin-3

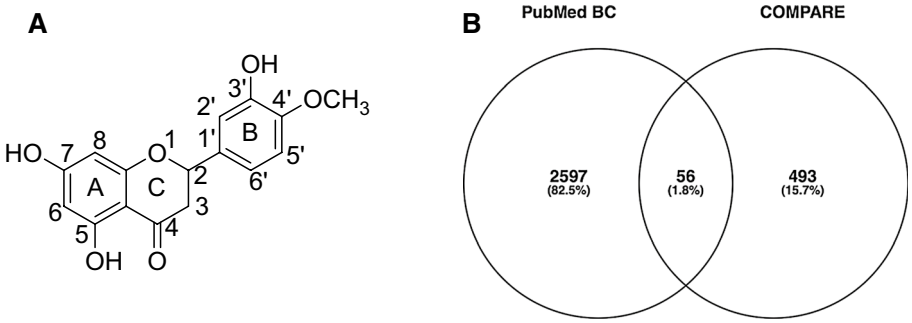


Fig. 1 **a** Chemical structure of hesperetin and **b** a Venn diagram of breast cancer chemoresistance regulatory gene from PubMed and mRNA from COMPARE

Table 2 Top five of gene ontology of the PTTGs

Term	*P* value	Genes
Biological process		
GO:0048538 ~ thymus development	3.56E−04	MAPK1, CRKL, BRAF, ABL1
GO:0019221 ~ cytokine-mediated signaling pathway	8.75E−04	STAT5A, SOCS1, STAT5B, JAK2, CISH
GO:0050731 ~ positive regulation of peptidyl-tyrosine phosphorylation	9.74E−04	GATA1, STK11, ANGPT1, ABL1
GO:0007259 ~ JAK-STAT cascade	0.001054172	STAT5A, SOCS1, STAT5B
GO:0008284 ~ positive regulation of cell proliferation	0.002722429	CRKL, STAT5A, HIPK2, STAT5B, IGF1, TET1
GO:0045648 ~ positive regulation of erythrocyte differentiation	0.002784055	GATA1, GATA2, STAT5B
GO:0046627 ~ negative regulation of insulin receptor signaling pathway	0.00305511	SOCS1, CISH, PRKCB
GO:0045746 ~ negative regulation of Notch signaling pathway	0.00305511	GATA2, HIF1AN, DLK1
GO:2000352 ~ negative regulation of endothelial cell apoptotic process	0.00305511	BRAF, ANGPT1, ABL1
GO:0042104 ~ positive regulation of activated T cell proliferation	0.00305511	STAT5A, STAT5B, IGF1
Cellular component		
GO:0005634 ~ nucleus	0.023278228	STK11, STAT5A, STAT5B, PIM1, PRKAB1, ESR2, CBFA2T3, TET1, RAD51, PRKCB, GATA1, GATA2, MAPK1, HIST2H2BE, RASSF1, NOS3, JAK2
GO:0005654 ~ nucleoplasm	0.029201016	GATA2, MAPK1, HIF1AN, HIST2H2BE, ID1, STAT5A, MLH1, ELAVL1, ABL1, RAD51
GO:0005737 ~ cytoplasm	0.045236474	STAT5A, STAT5B, SOCS1, PIM1, CISH, RAD51, PRKCB, MAPK1, HIST2H2BE, RASSF1, HIPK2, EEF2 K, NOS3, RELN, JAK2, TUBB1, CBS
Molecular function		
GO:0004674 ~ protein serine/threonine kinase activity	1.69E−04	MAPK1, BRAF, STK11, HIPK2, PIM1, EEF2 K, PRKCB
GO:0005524 ~ ATP binding	0.002142091	BRAF, STK11, PIM1, MLH1, HK1, PRKCB, RAD51, MAPK1, HIPK2, RRM1, EEF2 K, JAK2, ABL1
GO:0003682 ~ chromatin binding	0.00675883	GATA1, GATA2, STAT5B, MLH1, RAD51, PRKCB
GO:0020037 ~ heme binding	0.011353025	JAK2, NOS3, HBB, CBS
GO:0005506 ~ iron ion binding	0.017794072	HIF1AN, NOS3, TET1, HBB
GO:0003697 ~ single-stranded DNA binding	0.022091386	SSBP4, MLH1, RAD51
GO:0001085 ~ RNA polymerase II transcription factor binding	0.053191354	GATA1, GATA2

pathway, non-small-cell lung cancer, type II diabetes mellitus, and the erbB signaling pathway. Several genes participated in the biological process of the JAK-STAT cascade. The PTTGs are located in the nucleus. The PTTGs play a molecular function in protein serine/threonine kinase activity. The KEGG pathway enrichment analysis of the PTTGs revealed regulation in the erbB signaling pathway. The erbB signaling pathway regulates signaling in breast cancer cells, including proliferation, survival, angiogenesis, metastasis [24], migration, and invasion [25]. The erbB receptor family, which is a receptor tyrosine kinase, includes epidermal growth factor receptor (EGFR), erbB2 (HER2), erbB3 (HER3), and erbB4 (HER4) [26]. Binding of the ligand to the erbB receptor family triggers dimerization and activation of the intracellular tyrosine kinase domain, followed by the activation of the kinase signaling pathway involving mitogen-activated protein kinase (MAPK), PI3 K/Akt, mTOR, and JAK-STAT [27, 28].

The erbB signaling pathway regulates chemoresistance in breast cancer through overexpression, mutation, and deregulation of the downstream signaling molecules. It has been reported that overexpression of c-erbB-2/neu increased the resistance of breast cancer cells to paclitaxel [29]. In contrast, the downregulation of HER-2 was found to increase the sensitivity of breast cancer cells to adriamycin and paclitaxel [30]. Studies have shown that patients with triple-negative breast cancer [31] and those with primary breast cancer [32] exhibit mutation in the EGFR kinase domain. Moreover, mutation in the erbB2 kinase domain also occurs in patients with breast cancer [33]. In addition to the overexpression of the receptor, regulation of chemoresistance also occurs through the deregulation of the downstream signaling of

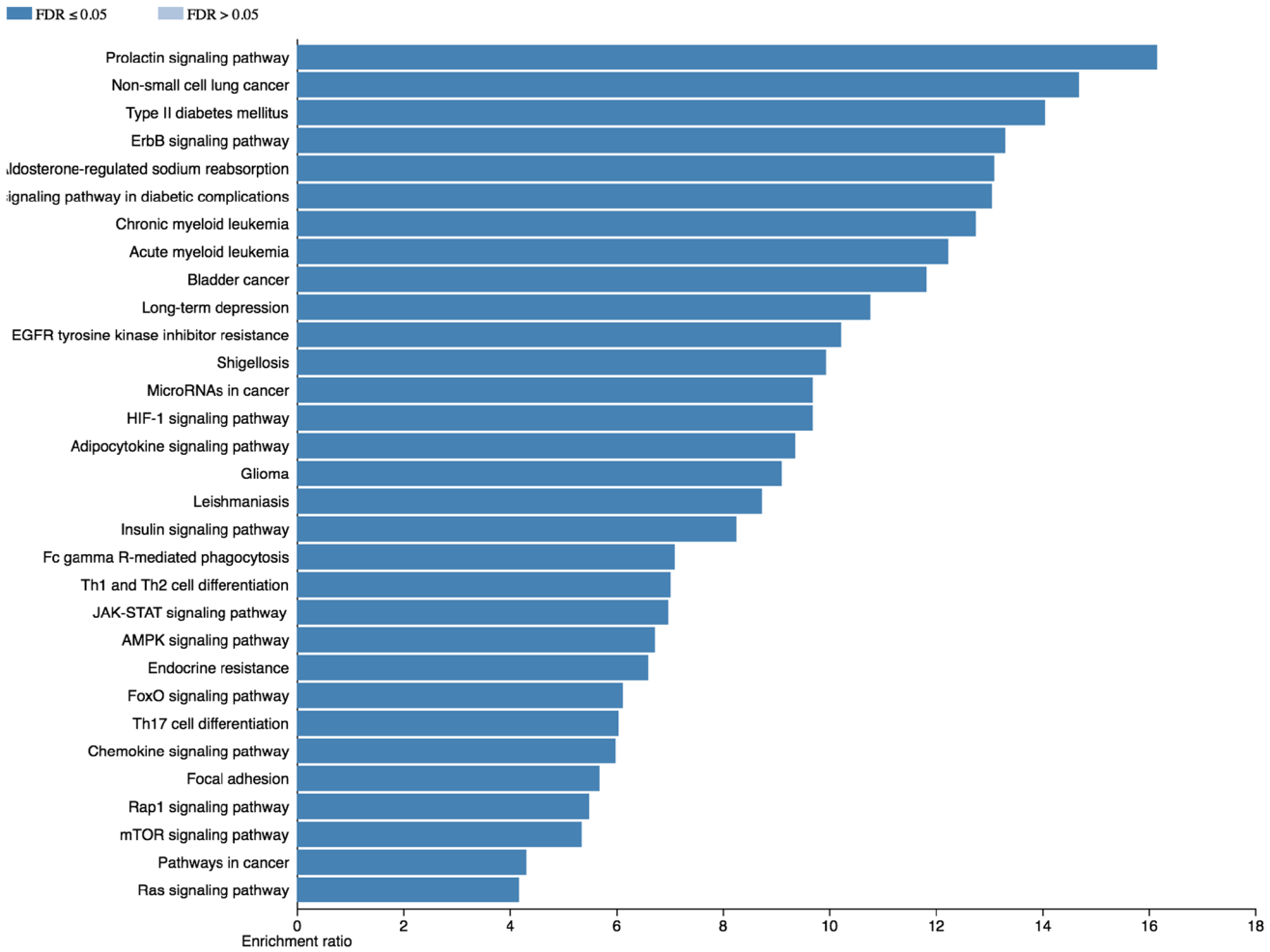


Fig. 2 KEGG pathway enrichment analyzed using the ORA, WebGestalt

the erbB receptors, e.g., JAK-STAT and MAPK. The JAK/STAT signaling pathway is an important signal transduction pathway in cytokine and growth factor signaling that regulates cell proliferation, differentiation, migration, and survival [34]. A proteomics study showed that breast cancer chemoresistance is associated with the activation of JAK-STAT signaling [35]. Deregulation of JAK-STAT signaling regulates migration and metastasis in breast cancer cells by targeting GRAMD1B expression [36]. Activation of erbB2 signaling through STAT3 increases the resistance of breast cancer cells to paclitaxel through the upregulation of p21 [37]. Therefore, targeting erbB signaling is an important strategy to overcome chemoresistance in breast cancer.

Drug association analysis

To identify the potential target genes of hesperetin in breast cancer, drug association analyses were conducted in this study. We screened drugs based on genes that are associated with the individual drug, i.e., hesperetin. The WebGestalt database was used for identifying suitable drug molecules that may be used for the treatment of disease caused by 56 genes. We predicted the mechanism of hesperetin based on the similarity of the gene associated with certain drugs.

A total of 56 genes were analyzed using the ORA on the WebGestalt database, with the functional database GLAD4U. All the 56 genes were significantly associated with 76 drugs (FDR < 0.05). The drugs anagrelide, afutuzumab, panitumumab, pimozide, tetrahydrofolic acid, bepridil, pemetrexed, raltitrexed, ruxolitinib, and selumetinib had the highest enrichment ratio, which indicates that these drugs and hesperetin are associated with the same gene (Fig. 3).

In addition, these 76 drugs primarily target erbB receptor signaling. Panitumumab is a fully human monoclonal antibody that blocks the EGFR in the treatment of patients with metastatic triple-negative breast cancer [38], metastatic colorectal cancer [39], primary HER2-negative inflammatory breast cancer [40], and pancreatic cancer [41]. Pemetrexed is a multitarget antifolate that is used in combination with

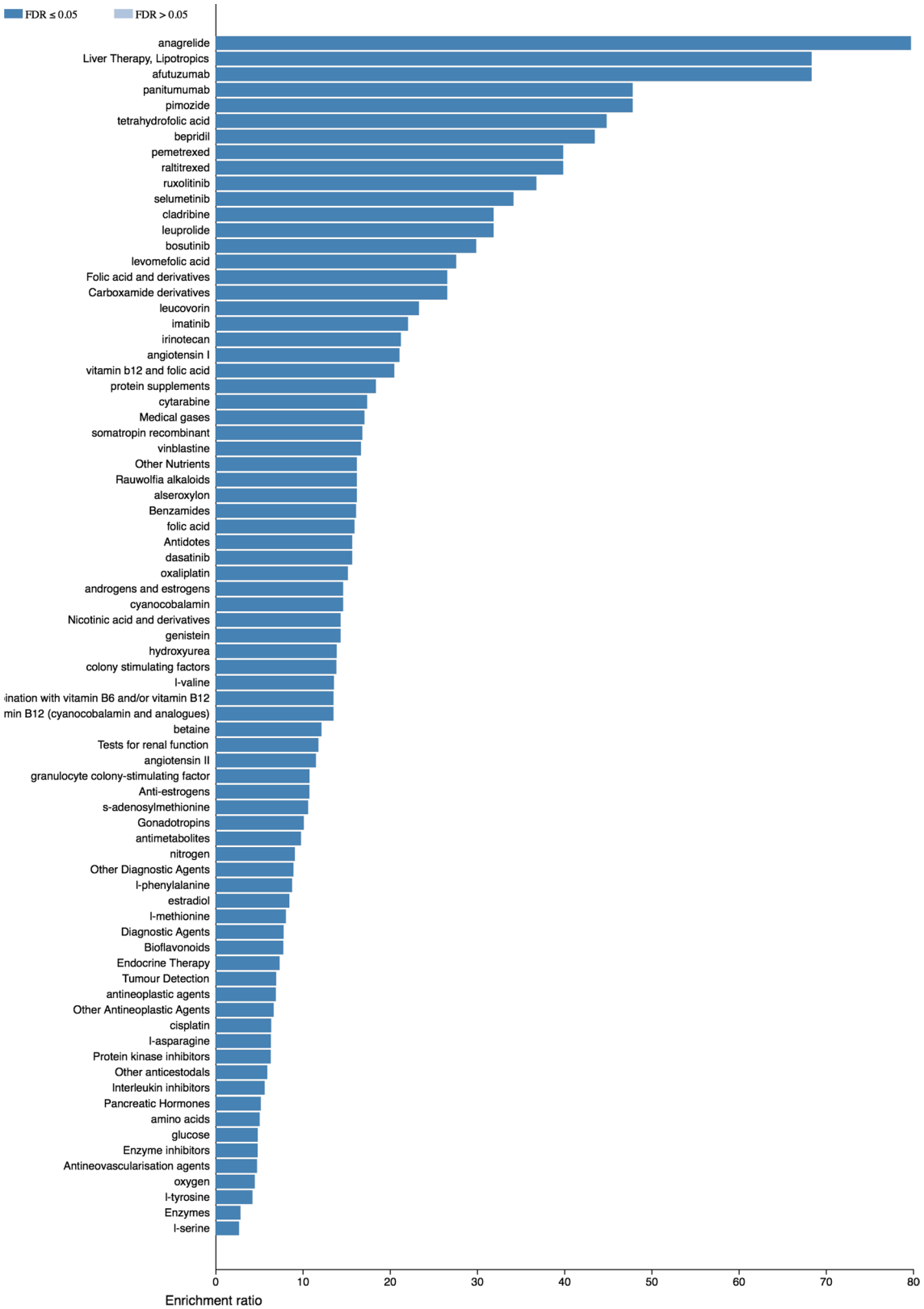


Fig. 3 Drug association analysis analyzed using the ORA, WebGestalt

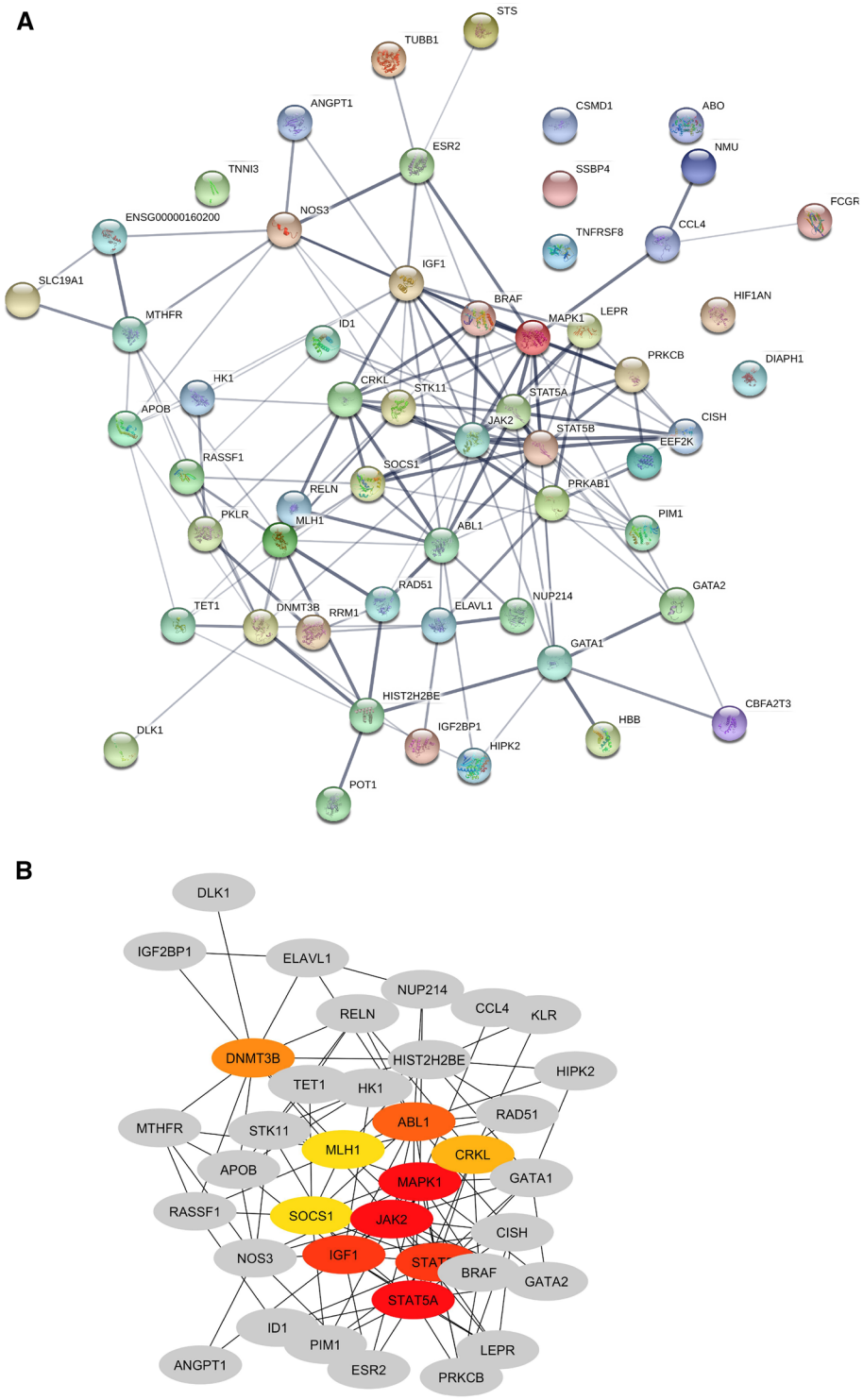


Fig. 4 **a** Protein–protein interaction networks of PTTGs analyzed using STRING-DB and Cytoscape and **b** Top ten hub genes with the highest degree score analyzed using Cytoscape

classical chemotherapy or mAb for the treatment of patients with non-small-cell lung cancer [42–46], advanced breast cancer [47], and metastatic breast cancer [48–50]. Ruxolitinib is a potent and selective oral inhibitor of JAK1 and JAK2 used for the treatment of patients with myelofibrosis [51, 52]. Selumetinib is a MAPK inhibitor used for the treatment of patients with neurofibromatosis [53], advanced non-small-cell lung cancer [54], and metastatic KRAS wild-type or unknown non-squamous non-small-cell lung cancer [55].

Table 3 Top ten hub genes based on score degree

No	Gene symbol	Full name	Degree score
1	JAK2	Tyrosine-protein kinase JAK2	15
2	STAT5A	Signal transducer and activator of transcription 5A	15
3	MAPK1	Mitogen-activated protein kinase 1	15
4	STAT5B	Signal transducer and activator of transcription 5B	14
5	IGF1	Insulin-like growth factor I	14
6	ABL1	Tyrosine-protein kinase ABL1	13
7	DNMT3B	DNA (cytosine-5)-methyltransferase 3B	11
8	CRKL	Crk-like protein	10
9	SOCS1	Suppressor of cytokine signaling 1	9
10	MLH1	DNA mismatch repair protein Mlh1	9

PPI network construction and hub gene selection

A total of 56 genes were constructed to the PPI network complex containing 56 nodes and 142 edges, with an average node degree of 5.07, an average local clustering coefficient of 0.415, and a PPI enrichment p value of $< 1.0e{-}16$ (Fig. 4a). The top ten genes with the highest degree score were identified, i.e., JAK2, STAT5A, MAPK1, STAT5B, IGF1, ABL1, DNMT3B, CRKL, SOCS1, and MLH1 (Fig. 4b, Table 3).

These top ten genes with the highest degree score are involved in erbB signaling. Insulin-like growth factor I (IGF1) is the ligand that binds to the insulin-like growth factor-1 receptor (IGF-1R), a member of the erbB family of receptors that play a vital role in cancer [56]. Tyrosine-protein kinase ABL1 is a proto-oncogene that forms a fusion with BCR to become an active form of oncogene and is found abundantly in patients with leukemia [57]. ABL is involved in the regulation of endocytosis of EGFR in human tumors [58]. Furthermore, constitutively active ABL increases the invasion of breast cancer cells [59]. DNA (cytosine-5)-methyltransferase 3B (DNMT3B), an enzyme that catalyzes the methylation of the 5′ position of cytosine of DNA, plays an important role in cancer development [60]. Activation of EGFR has been reported to increase the activity of DNA methyltransferase in ovarian cancer [61]. The CRKL, an adaptor protein that activates SOS1-RAS-RAF-ERK and SRC-C3G-RAP1 signaling in the downstream of EGFR activation, promotes the resistance of human non-small-cell lung cancers to the EGFR inhibitor [62]. The suppressor of cytokine signaling 1 (SOCS1) is also involved in erbB signaling pathway. A study showed that SOCS1 is important for the negative regulation of the IL-6R/Janus-activated kinase (JAK)-mediated activation of STAT3 in head and neck squamous cell carcinomas [63]. The DNA mismatch repair protein MLH1 promotes cisplatin sensitivity of human endometrial carcinoma cells [64]. Polymorphism in MLH1 has been shown to be associated with the poor response of lung adenocarcinoma to EGFR tyrosine kinase inhibitors [65].

Molecular docking

Inhibition of erbB signaling can be used as a strategic method to overcome the resistance of breast cancer cells. In this study, we performed a molecular docking investigation to predict the possible inhibitory activity of hesperetin in erbB signaling. Docking simulation and ligand–protein binding visualization were generated by the MOE software. The protein targets ALB1, DNMT3B, and MLH1 were selected from the top ten genes with the highest degree score based on their uniqueness as a drug target. Native ligands were embedded into ABL1, DNMT3B, and MLH1 complexes consisting of STI-571, ethambutol, and ADP, respectively. On ABL1, hesperetin demonstrated a slightly lower docking score with STI-571 (Table 4). The lower the docking score, the more potent the binding affinity of the ligand, suggesting that ABL-1 binds and reacts preferentially with hesperetin. Hesperetin also formed an H-bond with Glu316 with a bonding distance of 1.83, which was shorter than the H-bond distance of STI-571 with Met318 (Fig. 5). The binding of hesperetin was also stabilized through arene bonding between the aromatic cage of hesperetin with the hydrogen atom of Lys271 (Fig. 5). The higher docking score of hesperetin that was found on DNMT3B and MLH1 suggested the lower binding affinity than that of native ligands (Table 4). This result could possibly be due to Trp239 and Trp236, which interacted with hesperetin on DNMT3B through the arene-H bond (Table 4). This was in contrast to ethambutol, which not only formed an arene-H bonding with Trp239 but also formed an H-bond with Asp266 (Table 4). In the case of MLH1, although hesperetin formed only an H-bond with Ala103 and Asn38, the binding distance was slightly shorter than that of the H-bond formation of ADP (Table 4). Overall, hesperetin exhibited a promising inhibitory activity on ABL1, DNMT3B, and MLH1 with similar binding properties as those of the native ligand.

Table 4 Molecular docking results of hesperetin against the protein targets ABL1, DNMT3B, and MLH1

Protein	Ligand native						Hesperetin					
	Docking Score	RMSD (Å)	Ligand atom	Amino acid	Binding type	Distance	Docking score	RMSD (Å)	Ligand atom	Amino acid	Binding type	Distance
ABL1 (1FPU)	−14.24	1.356	N	Met318	H-bond	2.23	−17.38	0.857	H	Glu286	H-bond	1.99
			C	Leu248	Arene-H				C	Lys271	Arene-H	
			C	Thr315	Arene-H				C	Leu370	Arene-H	
DNMT3B (5NR3)	−10.88	1.948	H	Asp266	H-bond	1.83	−11.15	1.131	H	Trp263	Arene-H	
			H	Asp266	H-bond	1.93			H	Trp239	Arene-H	
			H	Phe236	Arene-H							
			H	Phe236	Arene-H							
			H	Trp239	Arene-H							
MLH1 (4P7A)	−33.96	1.194	O	Ala103	H-bond	2.20	−14.04	1.24	O	Ala103	H-bond	2.14
			O	Leu104	H-bond	1.78			O	Asn38	H-bond	1.6
			O	Asn38	H-bond	1.73			C	Asn38	Arene-H	
			O	Thr82	H-bond	1.46			C	Ile68	Arene-H	
			O	Ser83	H-bond	2.15			C	Leu104	Arene-H	
			O	Ser83	H-bond	1.60						
			H	Asp63	H-bond	2.23						
			C	Ile68	Arene-H							

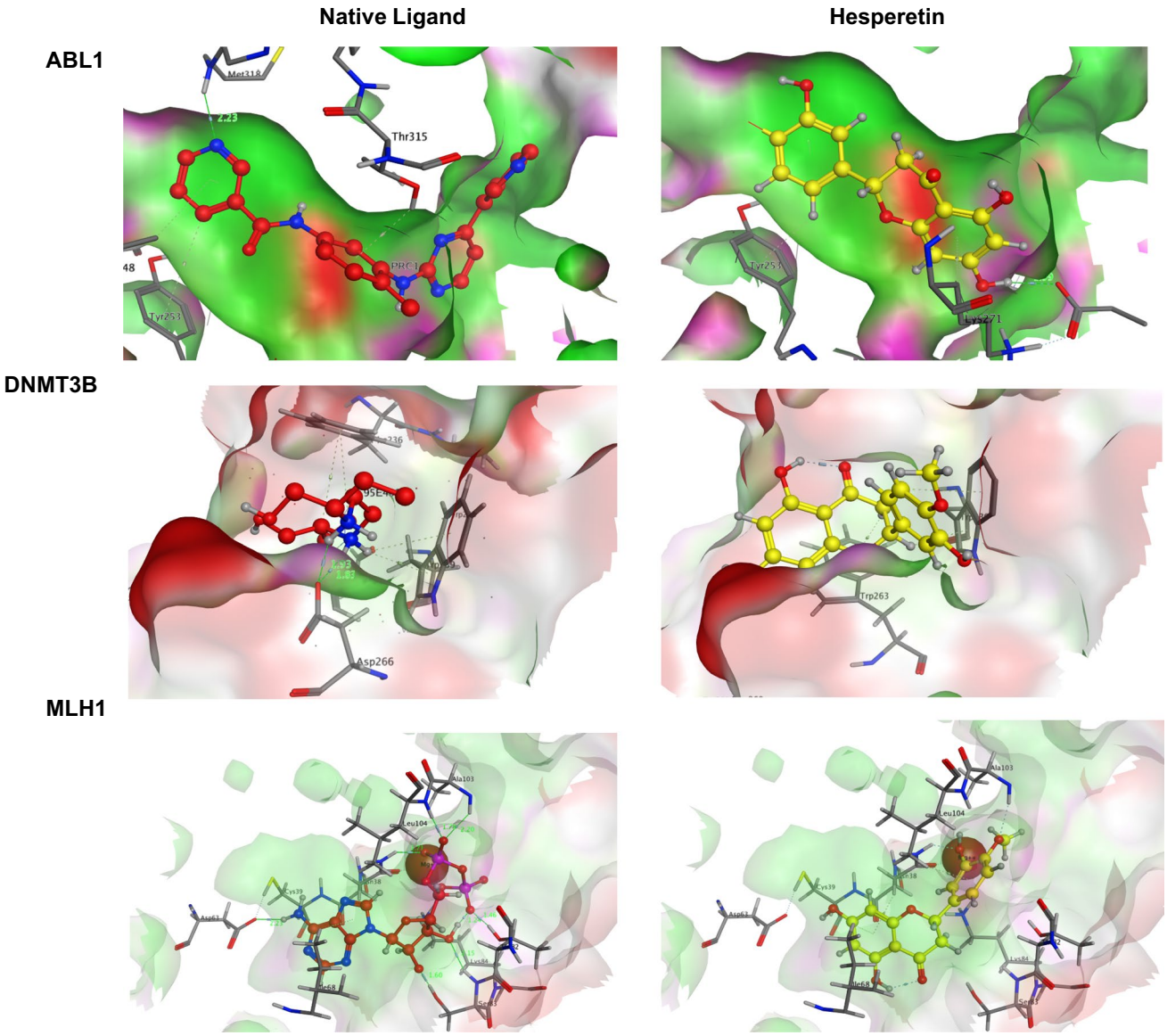


Fig. 5 Visualization of ligand interaction to ABL1, DNMT3B, and MLH1 using MOE

The molecular docking study provided adequate information from the binding interaction to the potential inhibitory activity of hesperetin on ABL1, DNMT3B, and MLH1. One of the potent ABL1 inhibitors, STI-571, bound to the ATP binding site of ABL1. The docking simulation on ABL1 demonstrated that hesperetin exhibited a different binding pattern from that of STI-571 but similar to that of ST013616 and DB04200, two designed ABL1 inhibitors that were stronger than imatinib and ponatinib. The OH group of ring A of hesperetin forms an H-bond with Glu316, which closely correlated with the inhibition of the ATP binding site on ABL1 [66]. The binding affinity was stabilized through the arene-H bond formed between ring B of hesperetin with Lys271, one of the protein kinase disruption characters [67]. These results indicate the significance of ring A and ring B of hesperetin for its binding affinity. Altogether, hesperetin has the potential to bind to the ATP binding site and thus inhibit the kinase activity of ABL1.

DNMT3B, one of the proteins involved in de novo methylation, was also used as a protein target of hesperetin. A previous study of hesperetin demonstrated the inhibition of DNMT1 activity in KYSE-510 human esophageal squamous cells [68]. In the present study, although hesperetin had a higher score than ethambutol, the presence of a similar binding site could indicate the potential of binding affinities to DNMT3B. Binding studies on the DNMT3B PWWP domain in combination with the epigenetic mark H3K36me3 (H332–38K36me3) have revealed that Trp236 and Trp239

formed van der Waals and p-cation interactions with the trimethylated side chain of Lys3 on H3K36me3 [69]. Our results demonstrated that ring A of hesperetin exerts its function by forming the arene bond between the OH group and the CH group with Trp263 and Trp239, respectively. Overall, the binding of hesperetin could possibly interfere with the methylation process of DNMT3B on several epigenetic marks.

Hesperetin also exhibited its binding potential to MLH1, a DNA mismatch repair protein. An earlier research showed that ADP binding was required for the interaction of MutLα and MutSα with MLH1 to promote mismatch repair [70]. According to the results, we have once again confirmed the important role of rings A and B in hesperetin binding affinities. Ring A provides the arene-H bonding with Ile68 and Asn38. Mutated Ile68 is associated with the activity of MLH1, while mutated Asn38 on MLH1 correlates with the marker of Lynch syndrome [71, 72]. On the other hand, ring B accommodates the arene binding with Leu104 and H-bond with Asn38 and Ala103 through its methoxy and hydroxyl groups, respectively. ADP also binds to Asn38, which forms a coordinate with Mg^{2+}, a stabilizing agent of the secondary and tertiary structure of MLH1 [69]. Thus, it can be suggested that hesperetin interacts with MLH1 on the ADP binding site.

Targeted therapies for erbB family receptors include monoclonal antibodies that target the extracellular domain and tyrosine kinase inhibitors that target the intracellular kinase domain [73]. However, resistance to therapy can occur due to changes in the conformation of the extracellular domain, as well as mutations in the tyrosine kinase domain [74].

Previous studies on hesperetin have demonstrated an inhibition of the erbB signaling pathway. Hesperetin exhibited a strong interaction with the ATP binding site of HER2 and thus has the potential to be used as a candidate of HER2 inhibitors [7]. Hesperetin was also shown to exhibit synergism with irinotecan CPT-11 by suppressing STAT3 activity in colon cancer [75]. Furthermore, hesperetin inhibits MAPK signaling in osteoclastogenesis [76] and lipopolysaccharide-induced acute lung injury [77]. Hesperetin derivative-12 (HDND-12) regulates macrophage polarization by modulating the JAK2/STAT3 signaling pathway [78]. In addition, the combination of hesperetin and sunitinib, an oral tyrosine kinase inhibitor against renal cancer, was found to be more effective than sunitinib alone in the treatment of corneal neovascularization [79]. Therefore, further in vitro and in vivo studies are required to investigate the combinatorial effect of hesperetin in overcoming chemoresistance in breast cancer, especially in the erbB signaling pathway.

Conclusions

This study has demonstrated that hesperetin targets erbB signaling in overcoming chemoresistance in breast cancer. Both hesperetin and the erbB receptor signaling inhibitors, i.e., monoclonal antibody and tyrosine kinase inhibitor, target the same mRNA expression. More importantly, results of the molecular docking study revealed the potential target of hesperetin against the regulator of the erbB signaling pathway. Overall, the results of this study could be beneficial for the research on accelerating and directing the screening of potential targets and delineating the molecular mechanism of hesperetin in overcoming breast cancer chemoresistance. Further in vitro and in vivo studies are required to validate the results of the present study.

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Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

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