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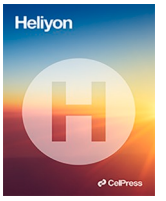

Research article

Screening and identification of potential target of 1′-acetoxychavicol acetate (ACA) in acquired lapatinib-resistant breast cancer

Febri Wulandari [a,*], Ahmad Fauzi [a], Muhammad Da'i [a], Mahmoud Mirzaei [b], Maryati [a], Kun Harismah [c]

[a] Faculty of Pharmacy, Universitas Muhammadiyah Surakarta, Surakarta, Indonesia
[b] Laboratory of Molecular Computations (LMC), Department of Natural and Mathematical Sciences, Faculty of Engineering, Tarsus University, Tarsus, Turkey
[c] Department of Chemical Engineering, Universitas Muhammadiyah Surakarta, Surakarta, Indonesia

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ABSTRACT

1′-Acetoxychavicol acetate (ACA) eliminates breast cancer cells via the HER2/MAPK/ERK1/2 and PI3K/AKT pathways, and it also directly influences endocrine resistance by both enhancing pro-apoptotic signals and suppressing pro-survival molecules. This study utilized bioinformatics to assess ACA target genes for lapatinib-resistant breast cancer. We identified differentially expressed genes (DEGs) using GSE16179 microarray data. DEGs from ACA-treated and lapatinib-resistant cells were analyses using Panther DB, Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analyses, and protein-protein interaction (PPI) network analysis. Genomic mutations, expression levels, prognostic significance, and ROC analysis were examined in selected genes. We used AutoDock Vina to conduct ACA molecular docking with potential target genes. In the PPI network analysis, BCL2, CXCR2, and CDC42 were the three highest-scoring genes. Genetic modification analysis identified PLAU and SSTR3 as the genes most frequently altered in breast cancer samples. The RTK-Ras pathway is likely to be affected by changes in BCL2, CXCR2, CDC42, SSTR3, PLAU, ICAM1, IGF1R, and MET genes. Patients with breast cancer who had lower levels of BCL2, SSTR3, PLAU, ICAM1, IGF1R, and MET had worse overall survival compared to other groups. ACA exhibited moderate binding affinity to BCL2, SSTR3, PLAU, ICAM1, IGF1R, and MET. Overall, ACA might counteract breast cancer resistance to lapatinib by targeting BCL2, SSTR3, PLAU, ICAM1, IGF1R, and MET. Further in vitro studies involving gene silencing could provide more detailed insights into the mechanism by which ACA combats lapatinib resistance.

1. Introduction

Breast cancer remains a serious health concern, with resistance to targeted therapies presenting a substantial barrier to therapeutic success [1]. Lapatinib, a tyrosine kinase inhibitor, has shown effectiveness in human epidermal growth factor receptor 2 (HER2)--positive breast cancer [2]. Nevertheless, the emergence of resistance hinders its therapeutic benefits. Researchers have proposed

* Corresponding author. Faculty of Pharmacy, Universitas Muhammadiyah Surakarta, Sukoharjo, Jawa Tengah, 57169, Indonesia.
E-mail address: fw548@ums.ac.id (F. Wulandari).

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various resistance mechanisms to lapatinib, including mutation of the HER2 tyrosine kinase domain, a key target of lapatinib, and the activation of ligand-independent signaling pathways [3,4]. The interplay between HER2 and estrogen receptor (ER) pathways can activate ligand-independent signaling, affecting apoptotic and survival signaling pathways through several alternative mechanisms [4,5]. Additionally, treatment with trastuzumab or a combination of chemotherapeutic drugs can excerbate lapatinib resistance, further increasing side effects to patients [6].

Despite combining lapatinib with other chemotherapeutic medicines to address limitations, resistance phenomena persist [7]. Resistance to lapatinib may significantly impact therapy outcomes for individuals with HER2-positive breast cancer [8]. Combination chemotherapy and natural agents have been extensively studied for their potential to enhance therapeutic efficacy and reduce side effects in cancer treatment. Understanding the mechanism of lapatinib resistance is crucial for developing strategies to address it and improve patient outcomes.

1'-Acetoxychavicol acetate (ACA) (Fig. 1a) is a natural compound derived from ginger (*Zingiber officinale*) [9]. It has been demonstrated potential anti-cancer properties in various studies [10–14]. ACA has shown significant promise in combating breast cancer by inhibiting cancer cell proliferation, inducing apoptosis, and suppressing migration. Toxicity studies in nude mice have shown that intravenous administration of ACA significantly reduces tumor volume, with a favorable safety profile and no observed side effects [15]. Furthermore, ACA exhibited cytotoxic effects across almost all subtypes of breast cancer cells, including ER+, HER2+, and triple-negative breast cancer (TNBC). ACA also reduces human epidermal growth factor receptors (EGFR) signaling and enhances gefitinib's efficacy in lung cancer [16]. Interactions with EGFR and ER are among the mechanism by which breast cancer cells may develop resistance to chemotherapeutic agents [17]. By targeting the ligand crosstalk of HER2 signaling, we hypothesized that ACA may overcome lapatinib resistance in breast cancer cells.

In this context, investigating novel strategies to overcome lapatinib resistance is essential. This study focuses on using computational analysis to identify possible target genes of ACA to understand its role in overcoming lapatinib resistance in breast cancer. Our research utilizes computational methods to elucidate the molecular pathways that make ACA a promising therapeutic agent for overcoming resistance mechanisms in breast cancer, providing valuable insights for future experiment and improving treatment options.

2. Methods

2.1. Data mining

Microarray data from GSE16179 were used, including the BT474 cell line, which is HER2-positive and sensitive to lapatinib, and BT474-J4, a cell line that has acquired to be resistant to lapatinib [18]. GEO2R, a web-based software program that uses R as its programming language, was used to analyze the data. differentially expressed genes (DEGs) in breast cancer with lapatinib resistance were identified (classified as upregulated if log fold change >1 and p-value <0.05). Protein target prediction for ACA was obtained from multiple databases, such as TargetNet [19], SwisTargetPrediction [20], and HitPick [21]. Human proteins (Homo sapiens) that were nonduplicative across these databases were considered protein targets for predicting ACA and used in subsequent studies.

To identify proteins predicted to be targets of ACA and those encoded by the DEGs, we used Venny 2.1. A protein classification analysis was performed on the overlapping genes using Panther DB (https://www.pantherdb.org/). The data were then analyzed through the construction of a protein-protein interaction (PPI) network.

2.2. Constructing the gene ontology and PPI network

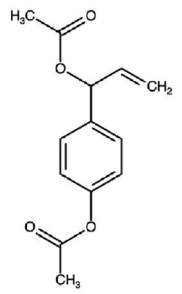
We computed and visually represented the PPI using the Cytoscape and STRING-DB v12.0 tools (https://string-db.org/), respectively [22]. Maximal Clique Centrality (MCC) scores was used to determine the top 20 hub genes in Cyto-Hubba (further mentioned as potential therapeutic targets of ACA (PTCA)). We performed gene ontology (GO) analysis, which encompasses biochemical, cellular components, and molecular functions, as well as KEGG enrichment on significant genes with a p-value less than 0.05. GO and KEGG pathway enrichment data were collected using DAVID 2021 and the WEB-based GEne SeT AnaLysis Toolkit [23].

2.3. Genetic changes in PTCA analysis

Genomic alterations related to PTCA were examined using cBioPortal on query genes from 26 breast cancer studies. The analysis was conducted on breast cancer studies with the most genetic mutations, focusing on oncoprinting, copy number variations, genomic pathways, and mutual exclusivity (p-values <0.05) [24]. The dataset was analyzed using ANOVA with Tukey's post hoc test, while copy number variations were analyzed using a Student's t-test.

2.4. Analysis of gene expression

Using the TCGA dataset, we compared tumor and normal tissues to analyze the expression patterns of BCL2, CXCR2, CDC42, SSTR3, PLAU, ICAM1, IGF1R, and MET using Gene Expression Profiling Interactive Analysis (GEPIA) (http://gepia2.cancer-pku.cn). Tissues from breast cancer patients in Stages I–IV were also employed for gene expression analysis. The criterion for the level of statistical significance was set at $p < 0.01$ using the default option.



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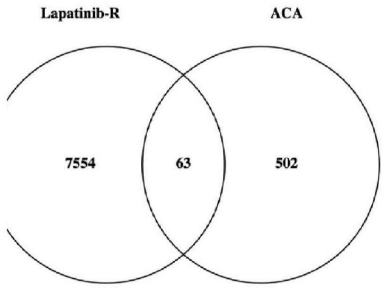

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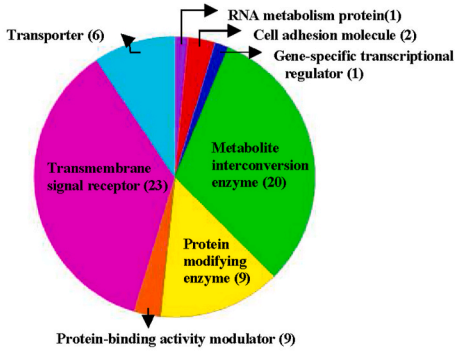

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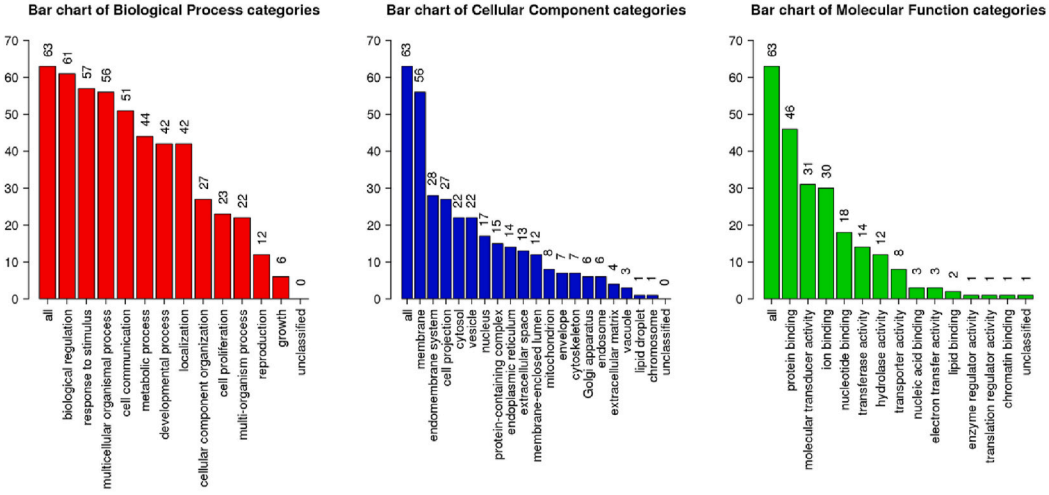

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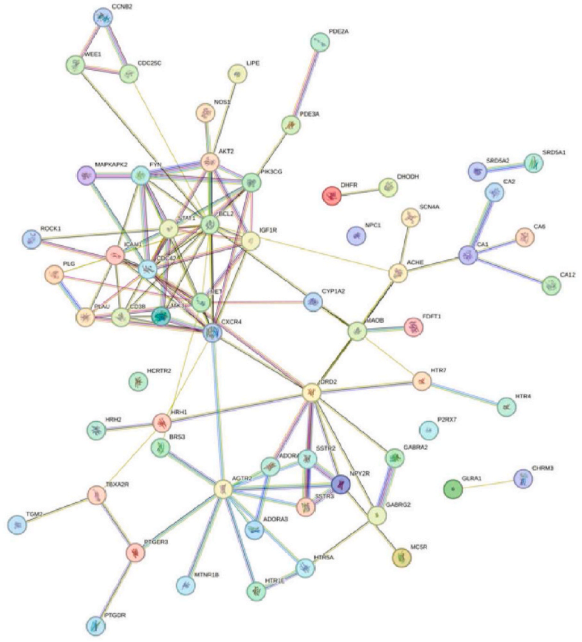

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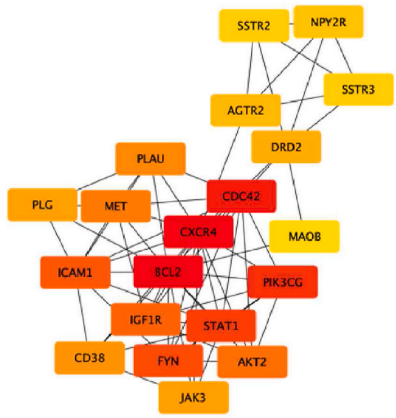

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Fig. 1. (a) Structure of 1′-acetoxychavicol acetate (ACA). (b) Venn diagram of protein target prediction of ACA. (c) Protein class of overlapped genes. (d) Gene ontology (GO) analysis of overlapped genes using WebGestalt. (e) Network interaction using STRING. (f) Top-20 genes as potential therapeutic targets of ACA (PTCA).

2.5. Prognostic implications

The website https://kmplot.com was utilized to evaluate prognostic values for BCL2, CXCR2, CDC42, SSTR3, PLAU, ICAM1, IGF1R, and MET. Multiple parameters ($p < 0.05$) were selected, including HER2 positive status and overall survival (OS) [24].

Table 1
Gene ontology and pathway enrichment analysis.

Term	P-value	Genes
Biological Process		
GO:0007186~G-protein coupled receptor signaling pathway	3.8E-8	CHRM3, HTR1E, PTGER3, CXCR4, HTR4, HTR5A, PIK3CG, HCRTR2, BRS3, HRH1, MTNR1B, TBXA2R, ADORA1, PDE3A, AGTR2, PTGDR, TGM2
GO:0007165~signal transduction	4.9E-5	GABRA2, CHRM3, ROCK1, STAT1, PDE2A, GABRG2, IGF1R, GLRA1, PLAU, ADORA3, AKT2, ADORA1, PDE3A, CD38, MET
GO:0007268~chemical synaptic transmission	4.8E-10	GABRA2, GLRA1, CHRM3, HRH1, HTR7, HTR1E, MTNR1B, HRH2, HTR4, HTR5A, GABRG2, HCRTR2
GO:0007187~G-protein coupled receptor signaling pathway, coupled to cyclic nucleotide second messenger	7.9E-15	CHRM3, HRH1, HTR7, HTR1E, MTNR1B, HRH2, MC5R, HTR4, SSTR2, HTR5A, SSTR3
GO:0009410~response to xenobiotic stimulus	1.6E-8	P2RX7, NPC1, MAOB, SRD5A2, TBXA2R, STAT1, SRD5A1, PDE3A, BCL2, CD38, DRD2
Molecular function		
GO:0005515~protein binding	5.1E-3	ACHE, CHRM3, NPY2R, HTR4, PIK3CG, IGF1R, ICAM1, LIPE, CA1, HTR7, PLAU, CA2, AKT2, ADORA1, NOS1, JAK3, TGM2, PTGDR, SSTR2, CDC25C, SSTR3, GABRG2, NPC1, MTNR1B, MAPKAPK2, SCN4A, AGTR2, MET, MAOB, ROCK1, CXCR4, PLG, CDC42, CCNB2, GLRA1, TBXA2R, FYN, DRD2, FDFT1, GABRA2, HTR1E, SRD5A2, STAT1, PDE2A, HCRTR2, DHODH, P2RX7, WEE1, CYP1A2, MC5R, PDE3A, BCL2
GO:0004930~G-protein coupled receptor activity	1.2E-6	HTR1E, CXCR4, SSTR2, HTR5A, SSTR3, HCRTR2, BRS3, HRH1, HTR7, MTNR1B, ADORA3, MC5R, AGTR2, DRD2
GO:0042802~identical protein binding	9.6E-3	MAOB, STAT1, PDE2A, PIK3CG, IGF1R, P2RX7, CDC42, GLRA1, BCL2, CD38, FYN, DRD2, MET
GO:0005524~ATP binding	2.7E-2	P2RX7, WEE1, ROCK1, AKT2, MAPKAPK2, FYN, JAK3, MET, PIK3CG, IGF1R, TGM2
GO:0030594~neurotransmitter receptor activity	6.3E-12	GABRA2, GLRA1, CHRM3, HRH1, HTR7, HTR1E, HRH2, HTR4, HTR5A, GABRG2
Cellular Component		
GO:0005886~plasma membrane	4.1E-14	ACHE, CHRM3, ROCK1, NPY2R, PTGER3, CXCR4, PLG, HTR4, PIK3CG, IGF1R, BRS3, ICAM1, CDC42, GLRA1, HRH1, HTR7, TBXA2R, HRH2, PLAU, CA2, ADORA3, AKT2, ADORA1, CD38, FYN, NOS1, DRD2, JAK3, TGM2, PTGDR, CA12, GABRA2, HTR1E, PDE2A, SSTR2, HTR5A, SSTR3, GABRG2, HCRTR2, P2RX7, NPC1, MTNR1B, MC5R, SCN4A, AGTR2, MET
GO:0016021~integral component of membrane	6.5E-6	ACHE, MAOB, PTGER3, CXCR4, IGF1R, BRS3, ICAM1, GLRA1, HRH1, HTR7, TBXA2R, HRH2, ADORA3, ADORA1, CD38, DRD2, PTGDR, FDFT1, CA12, GABRA2, SRD5A2, SRD5A1, HTR5A, SSTR3, GABRG2, HCRTR2, DHODH, P2RX7, NPC1, MTNR1B, PDE3A, BCL2, SCN4A, MET
GO:0005887~integral component of plasma membrane	1.3E-16	CHRM3, NPY2R, PTGER3, HTR4, IGF1R, BRS3, ICAM1, GLRA1, HRH1, HTR7, TBXA2R, HRH2, ADORA3, ADORA1, DRD2, GABRA2, HTR1E, SSTR2, HTR5A, SSTR3, GABRG2, HCRTR2, P2RX7, NPC1, MTNR1B, MC5R, SCN4A, AGTR2, MET
GO:0005829~cytosol	9.6E-2	ROCK1, STAT1, PDE2A, SSTR2, CDC25C, PIK3CG, DHODH, CDC42, DHFR, CCNB2, LIPE, HRH1, CA1, CA2, AKT2, MAPKAPK2, PDE3A, BCL2, CA6, FYN, NOS1, JAK3, TGM2
GO:0016020~membrane	4.2E-3	CA12, ACHE, PTGER3, HTR4, PIK3CG, BRS3, ICAM1, IGF1R, P2RX7, CDC42, CCNB2, GLRA1, LIPE, NPC1, PDE3A, BCL2, CD38, JAK3, MET, PTGDR, FDFT1
KEGG Pathway		
hsa04080:Neuroactive ligand-receptor interaction	4.6E-19	CHRM3, NPY2R, PTGER3, PLG, HTR4, BRS3, GLRA1, HRH1, HTR7, TBXA2R, HRH2, ADORA3, ADORA1, DRD2, PTGDR, GABRA2, HTR1E, SSTR2, HTR5A, SSTR3, GABRG2, HCRTR2, P2RX7, MTNR1B, MC5R, AGTR2
hsa04020:Calcium signaling pathway	1.5E-7	CHRM3, PTGER3, CXCR4, HTR4, HTR5A, P2RX7, HRH1, HTR7, TBXA2R, HRH2, CD38, NOS1, MET
hsa04024:cAMP signaling pathway	2.5E-5	LIPE, HTR1E, ROCK1, AKT2, PTGER3, PDE3A, ADORA1, HTR4, SSTR2, DRD2
hsa05200:Pathways in cancer	1.2E-2	CDC42, ROCK1, STAT1, AKT2, PTGER3, BCL2, CXCR4, JAK3, MET, IGF1R
hsa04022:cGMP-PKG signaling pathway	1.1E-3	ROCK1, AKT2, ADORA3, PDE2A, PDE3A, ADORA1, PIK3CG

2.6. ROC Plot

The ROC plotter (http://www.rocplot.org) was used to examine gene expression and lapatinib sensitivity of clinical trials for breast cancer. HER2 positive status, 5-year relapse-free survival rate, overall survival, and lapatinib therapy were included as predefined parameters. A p-value less than 0.05 was considered significant.

2.7. Molecular docking

We searched https://rcsb.org for the protein identifiers (2W3L, 3KID, 5MZA, 2OJ9, and 3DKF) corresponding to the following proteins: BCL2, PLAU, ICAM1, IGF1R, and MET. Binding energy levels were expressed in kcal/mol. The software utilized includes Autodock Vina 1.2.5 for molecular docking, PyMOL 2.5.7 with an Academic License for molecular visualization, LigPlot + v.2.2 with an Academic License for ligand-protein interaction analysis, PLIP for protein-ligand interaction prediction, and Marvin JS online for chemical structure drawing. Default setting were used in molecular docking, including grid box coordinated (x, y, z), number of point 20, spacing 0,375 Å. Grid coordinates were IGF1R (5.350; −6.426; 20.902), BCL2 (39.471; 26.951; −12.626), MET (17.356; 13.008; 138.984), ICAM1 (−29.596; 88.125; −11.25), and PLAU (−0.828; −33.596; −10.66), respectively. We also identified which residues were involved in the interaction [25,26].

3. Results

3.1. Data mining

We began by using microarray data from BT474-J4, a cell line that has become resistance to lapatinib, in GSE16179 to investigate the genes that ACA targets to overcome lapatinib resistance in breast cancer. We identified DEGs and found that they prevented BT474 cells from becoming resistant to lapatinib. GSE16179 provided us with 7617 genes (Supplementary Table 1). ACA's protein prediction targets were derived from several databases, selected by genes expressed in human without duplication, yielding 565 genes (Supplementary Table 2). A Venn diagram was to show the overlap between DEGs from GSE16179 and ACA prediction targets. The diagram revealed 63 common DEGs (Fig. 1b and Supplementary Table 3). These 63 genes were analyzed for their classification and network interactions.

3.2. Gene ontology and pathway analysis

As shown in Fig. 1c, we categorized the 63 obtained genes into various groups, including transmembrane signal receptors (23 genes), metabolite interconversion enzymes (20 genes), protein modifying enzymes (9 genes), protein-binding activity modulators (9 genes), transporters (6 genes), cell adhesion molecules (2 genes), RNA metabolism proteins (1 gene), and gene-specific transcriptional regulators (1 gene). We classified the GO results into biological processes, cellular components, and molecular functions. Several DEGs listed in Table 1 associated with various biological reactions, such as the G-protein-coupled receptor signaling pathway, signal transduction, chemical synaptic transmission, GPCR signaling pathway, and response to xenobiotic stimuli. DEGs were found in multiple cellular locations, including the plasma membrane, the cytosol, and the integral component membrane. According to Table 1,

Table 2
The top 20 hub genes ranked by Maximal Clique Centrality (MCC) score, assessed using CytoHubba.

Rank	Name	Score
1	BCL2	824.0
2	CXCR2	797.0
3	CDC42	750.0
4	PIK3CG	481.0
5	STAT1	408.0
6	FYN	362.0
7	ICAM1	288.0
8	IGF1R	264.0
9	AKT2	243.0
10	MET	170.0
11	PLAU	144.0
12	CD38	48.0
13	JAK3	24.0
13	PLG	24.0
15	DRD2	17.0
16	AGTR2	14.0
17	NPY2R	13.0
18	SSTR2	12.0
18	SSTR3	12.0
20	MAOB	8.0

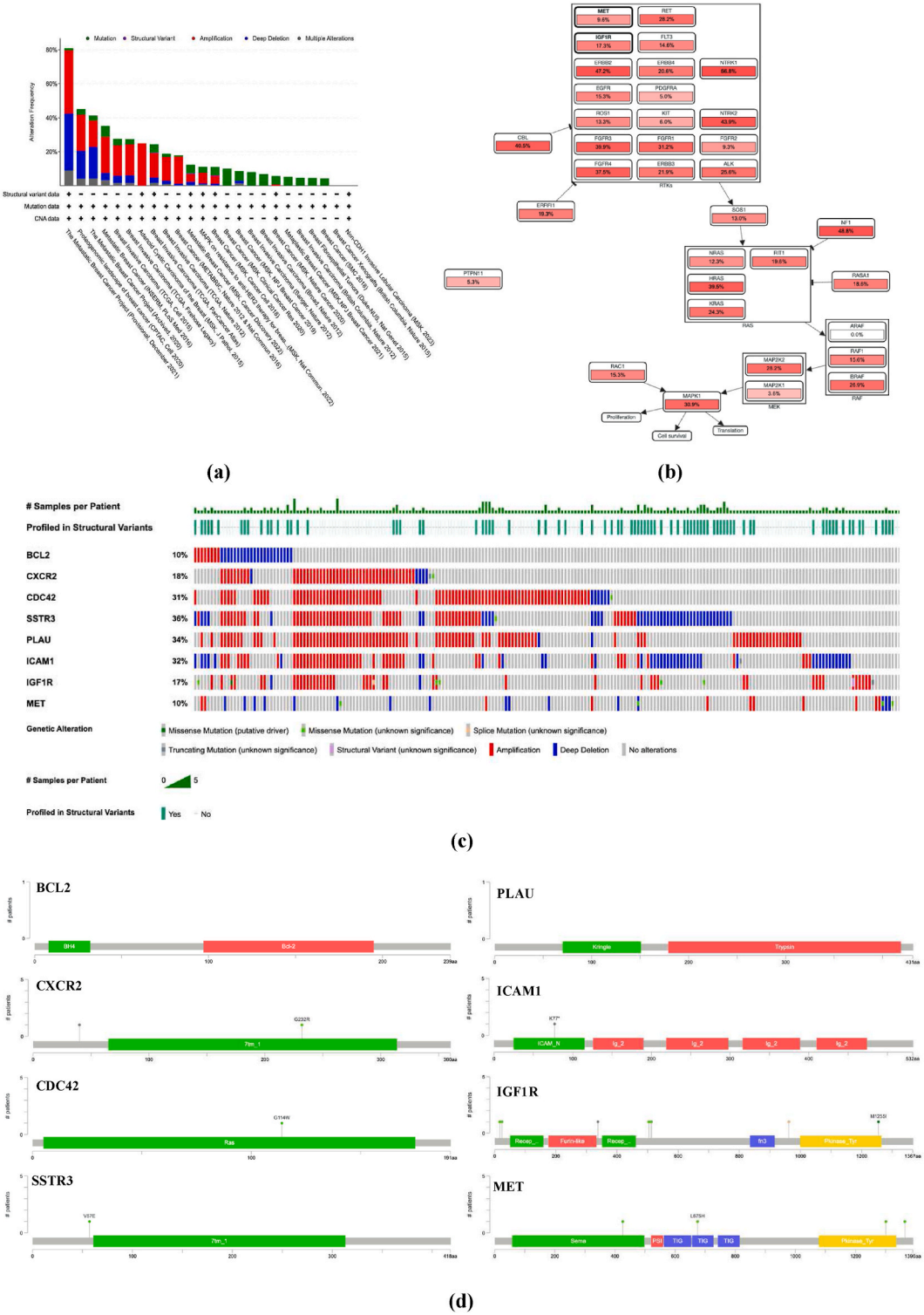
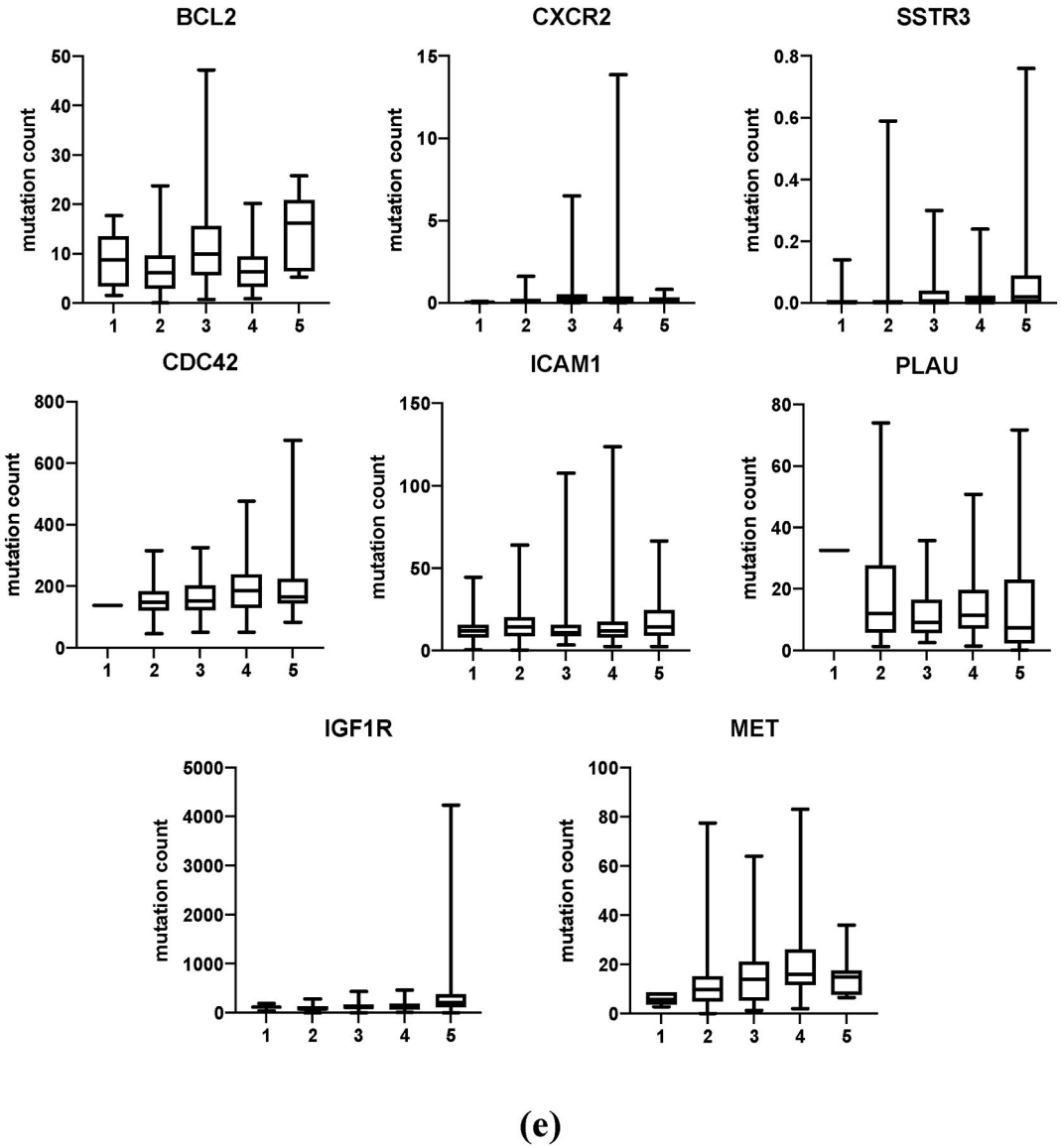


Fig. 2. cBioPortal examination for genetic alterations and pathway of PTCA. (a) Genetic changes in 26 studies on breast cancer. (b) RTK-RAS signaling pathway of PTCA. (c) Summary of genetic alterations in BCL2, CXCR2, CDC42, SSTR3, PLAU, ICAM1, IGF1R, and MET in The Metastatic Breast Cancer Project (Provisional, December 2021) samples. (d) Mutations of genes BCL2, CXCR2, CDC42, SSTR3, PLAU, ICAM1, IGF1R, and MET in breast cancer samples. Green dots represent missense mutations, yellow dots represent splice mutations, and gray dots represent truncating mutations. (e) Copy number of alterations of BCL2, CXCR2, CDC42, SSTR3, PLAU, ICAM1, IGF1R, and MET across breast cancer samples. 1: deep deletion, 2: shallow deletion, 3: diploid, 4: gain, and 5: amplification.



(e)

Fig. 2. (*continued*).

DEGs were involved in protein binding, GPCR activity, identical protein binding, and neurotransmitter receptor activity. The DEGs' KEGG pathway enrichment analysis indicated their involvement in controlling interactions between neuroactive ligands and receptors, the calcium signaling pathway, the cAMP signaling pathway, cancer pathways, and the cGMP-PKH signaling pathway (Table 1).

3.3. Network interaction and top genes

The PPI network complexity comprised of 63 genes with a confidence level of 0.4. The network included 63 nodes and 121 edges, with an average node degree of 3.84 and a local clustering coefficient of 0.649. The PPI enrichment p-value, less than 1.0e-16 (Fig. 1C), highlighted the complex structure of the networks and the significance of protein interactions. Combating lapatinib resistance via ACA may involve numerous PPI network proteins that participate in specific molecular pathways. We selected hub genes using the MCC score to determine the most crucial PPI network protein, as MCC captures essential proteins in the top ranked list, regardless of their degree. BCL2, CXCR4, CDC42, PIK3CG, STAT1, FYN, ICAM1, IGF1R, AKT2, MET, PLAU, CD38, JAK, PLG, DRD2, AGTR2, NPY2R, SSTR2, SSTR3, and MAOB are the 20 genes with the highest scores (Table 2).

3.4. Genetic alteration in BCL2, CXCR2, CDC42, SSTR3, PLAU, ICAM1, IGF1R, and MET

We examined the mutations, structural variations, amplifications, deep deletions, and numerous modifications of the top 20 genes using cBioPortal. The Metastatic Breast Cancer Project (Provisional, December 2021) exhibited the most genomic changes and was examined using Oncoprint (Fig. 2a). cBioPortal was utilized in multiple breast cancer studies to assess genetic alterations in eight specific genes, namely BCL2, CXCR2, CDC42, SSTR3, PLAU, ICAM1, IGF1R, and MET. The genes BCL2, CXCR2, and CDC42 were chosen based on their top scores, while SSTR3, PLAU, ICAM1, IGF1R, and MET were selected based on KEGG pathway enrichment analysis findings, which indicated their regulatory role in ligand-receptor interaction. Pathway enrichment analysis of individual genes linked RTK-Ras to genetic alterations in BCL2, CXCR2, CDC42, SSTR3, PLAU, ICAM1, IGF1R, and MET (Fig. 2b).

The Metastatic Breast Cancer Project demonstrated the most significant genetic changes compared to other breast cancer studies, and these changes were further analyzed using Oncoprint. Genetic mutations in the specified target genes were as follows: 10 % for BCL2, 18 % for CXCR2, 31 % for CDC42, 36 % for SSTR3, 34 % for PLAU, 32 % for ICAM1, 17 % for IGF1R, and 10 % for MET (Fig. 2c). Additionally, most of the gene modifications were amplification, as shown in Fig. 2e. An investigation of mutual exclusivity revealed substantial co-occurrence of certain gene pairs (SSTR3-ICAM1; CXCR2-ICAM1; CXCR2-PLAU, CXCR2-SSTR3, and PLAU-IGF1R) in breast cancer research conducted by the Metastatic Breast Cancer Project (Table 3) with a p-value of less than 0.05. The findings highlighted the important roles of ICAM1, SSTR3, CXCR2, and PLAU in ACA therapy. Subsequent examinations of copy number modifications yielded noteworthy findings.

3.5. Gene expression of BCL2, CXCR2, CDC42, SSTR3, PLAU, ICAM1, IGF1R, and MET in breast cancer samples

Breast cancer tissues have much higher PLAU and MET levels than normal tissues (Fig. 3a). Using GEPIA, we investigated the relationship between mRNA levels of BCL2, CXCR2, CDC42, SSTR3, PLAU, ICAM1, IGF1R, MET, and tumour stages in breast cancer. We found that CXCR2, SSTR3, and CDC42 levels remained constant throughout. In stage IV, BCL2 and IGF1R levels decreased, remained stable in stages I–III, and increased in stage X. ICAM1 and MET levels declined in stage X after being stable in stages I–IV.

3.6. Prognostic value

We examined how mRNA levels of BCL2, CXCR2, CDC42, SSTR3, PLAU, ICAM1, IGF1R, and MET could predict overall survival (OS) and found a stronger survival association in breast cancer compared to other groups ($p < 0.05$) (Fig. 3c). Individuals with breast cancer who had low levels of CXCR2, SSTR3, ICAM1, and MET mRNA had longer overall survival rates. Analyzed data indicate that breast cancer patients with low BCL2, CDC42, PLAU, and IGF1R mRNA levels had substantially worse overall survival compared to other groups.

3.7. The ROC Plot demonstrates the substantial prognostic capability of IGF1R expression

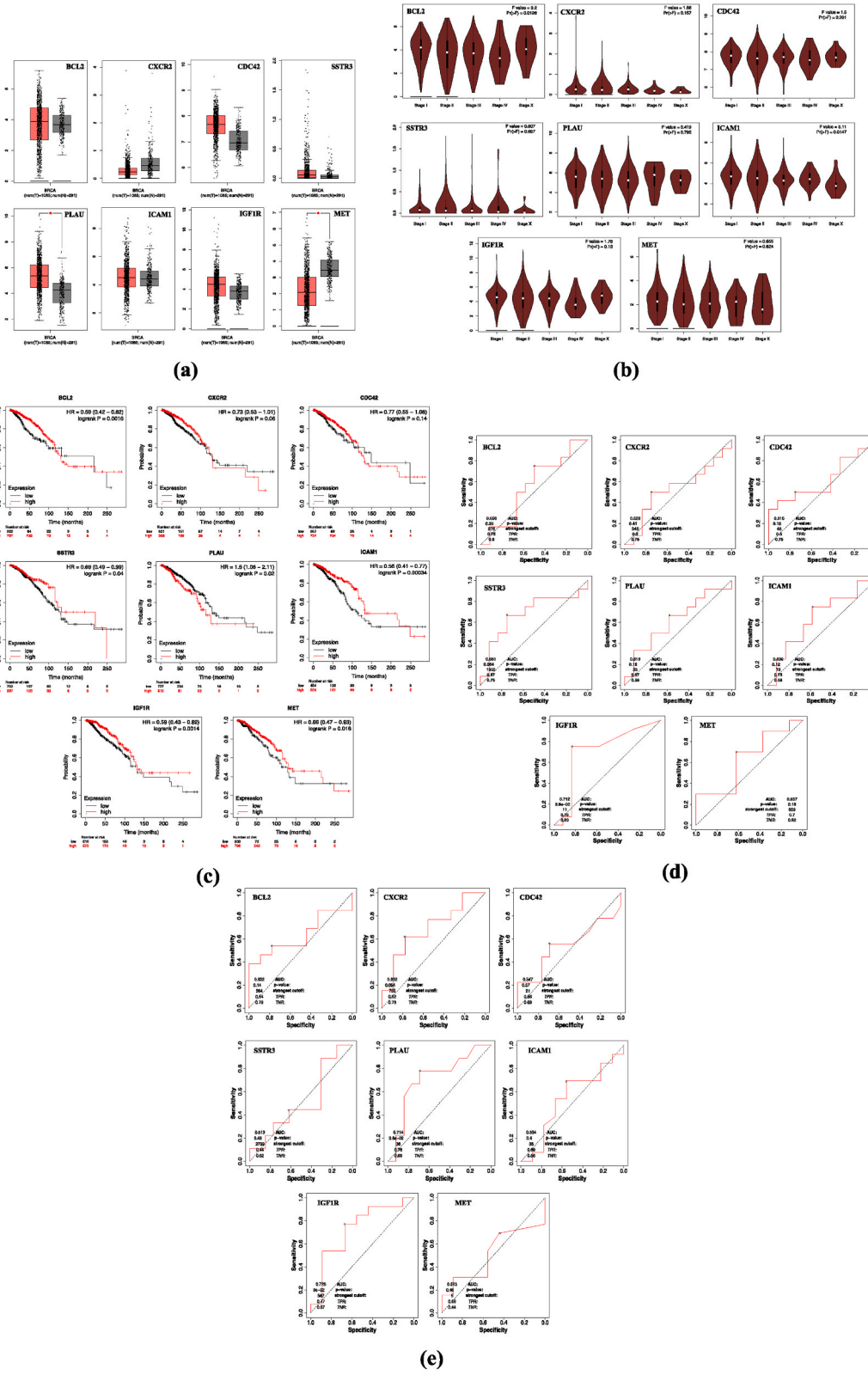
Based on transcriptome data from breast cancer patients, gene expression levels were correlated with lapatinib response by relapse-free survival (RFS) and pathological complete response (PCR). AUC values of 0.712 were strongly linked with IGF1R expression (Fig. 3d). Other gene expression levels did not correlate wirh RFS in lapatinib-treated patients. CXCR2, PLAU, and IGF1R expression levels had high predictive power using the PCR parameter, with AUC values of 0.692, 0.714, and 0.726 (Fig. 3e).

3.8. Molecular docking

Molecular docking studies for ACA with BCL2, PLAU, ICAM1, IGF1R, and MET were performed (Fig. 4). BCL2, SSTR3, PLAU, ICAM1, IGF1R, and MET were selected based on the outcomes of the earlier step. The docking score of ICAM1 with ACA was the only one stronger than that of its native ligand compared to other proteins. Indeed, the other protein and ACA had comparable docking score with their native ligands (Table 4). An RMSD <2 indicated the validity of the docking method (Table 5). The docking results showed that ACA could bind to BCL2, SSTR3, PLAU, ICAM1, IGF1R, and MET, indicating potential for further exploration to determine the binding properties and molecular interaction. The amino acid interaction and detailed ligand interaction should be further explored to establish a comprehensive result.

Table 3
The mutual exclusivity analysis.

A	B	Log2 Odds Ratio	p-Value	Tendency
SSTR3	ICAM1	>3	<0.001	Co-occurrence
CXCR2	ICAM1	>3	<0.001	Co-occurrence
CXCR2	PLAU	>3	<0.001	Co-occurrence
CXCR2	SSTR3	>3	<0.001	Co-occurrence
PLAU	IGF1R	2.404	0.008	Co-occurrence



(caption on next page)

Fig. 3. Analysis of BCL2, CXCR2, CDC42, SSTR3, PLAU, ICAM1, IGF1R, and MET expression in breast cancer. (a) mRNA expression BCL2, CXCR2, CDC42, SSTR3, PLAU, ICAM1, IGF1R, and MET in normal and breast cancer tissues. (Normal = 291, Tumor = 1085, and $p < 0.01$). (b) The mRNA levels and tumor stages in breast cancer patients ($p < 0.01$). (c) Survival prediction of BCL2, CXCR2, CDC42, SSTR3, PLAU, ICAM1, IGF1R, and MET, as analyzed by KMPlotter.

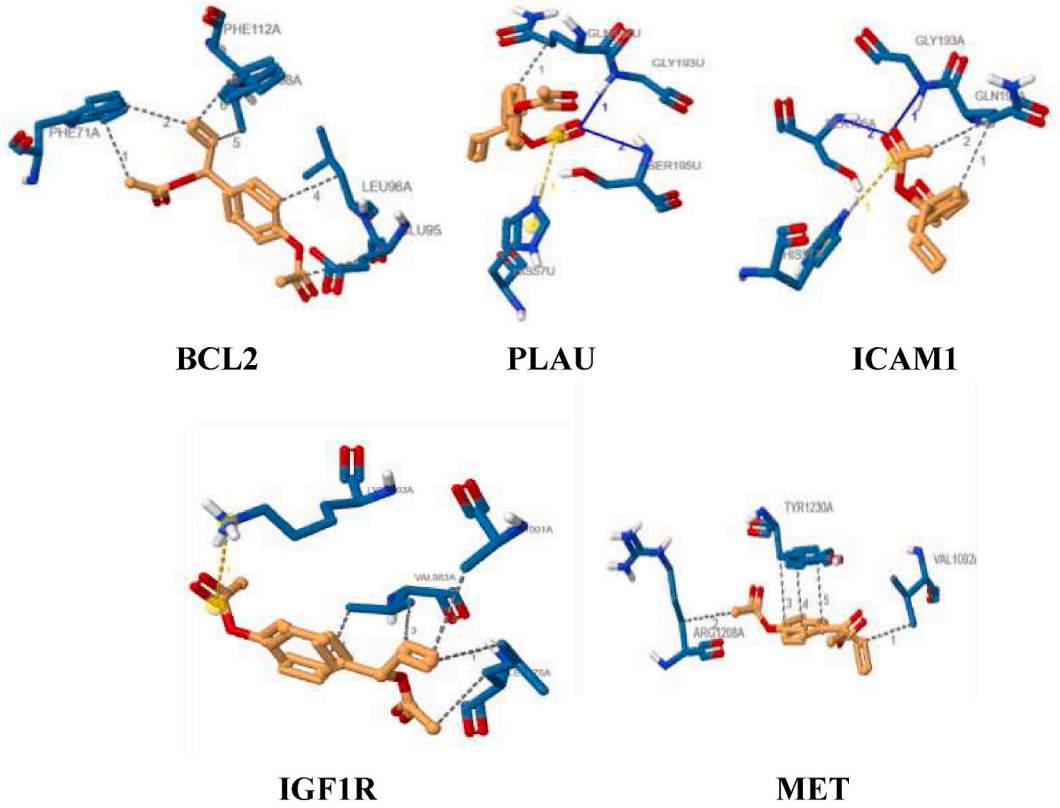


Fig. 4. Molecular docking interactions between the BCL2, PLAU, ICAM1, IGF1R, and MET, their native ligands, and ACA.

4. Discussion

Bioinformatics was applied to explore ACA targets and pathways for lapatinib-resistant breast cancer. GO analysis showed that plasma membrane DEGs are involved in GPCR signaling pathway and signal transduction biological responses. Furthermore, DEGs are involved in the molecular process of protein binding. KEGG pathway analysis revealed the presence of neuroactive ligand-receptor interactions and pathways associated with cancer [27]. The cancer pathways include the cAMP, cGMP-PKG signaling pathways, and calcium [28]. The receptors and ligands on the plasma membrane that link to signaling pathways both within and outside of cells comprise the neuroactive ligand-receptor interaction signaling pathway. Therefore, disrupting the connection between the ligand and the receptor could be an effective strategy to treat lapatinib-resistant cancer. In breast cancer, ACA affects proliferation, migration, and metastasis, disrupting the progression of carcinogenesis [9–13,16,29]. The role of ACA in lapatinib-resistant breast cancer cells is not yet fully understood.

Upon analyzing the protein-protein interaction network, it was found that the genes BCL2, CXCR2, and CDC42 had the highest

Table 4
The molecular docking data of BCL2, PLAU, ICAM1, IGF1R, and MET RET, ErbB4, FGFR2, their respective natural ligands, and ACA.

No	Protein	PDB ID	Docking Score (kcal/mol)	
			Native Ligand	ACA
1	IGF1R	2OJ9	−8.373	−7.775
2	BCL2	2WL3	−1.853	−1.841
3	MET	3DKF	−8.956	−8.358
4	PLAU	3KID	−5.125	−5.06
5	ICAM1	5MZA	−2.277	−3.134

Table 5
The RMSD scores of BCL2, PLAU, ICAM1, IGF1R, and MET RET, ErbB4, FGFR2, their respective natural ligands, and ACA.

No	Protein	PDB ID	RMSD	
			Native Ligand	ACA
1	IGF1R	2OJ9	1.406	1.655
2	BCL2	2WL3	1.466	1.314
3	MET	3DKF	1.407	1.853
4	PLAU	3KID	1.104	1.058
5	ICAM1	5MZA	1.856	1.911

degree scores. BCL2, CXCR2, and CDC42. SSTR3, PLAU, and ICAM1 were the genes that changed the most in breast cancer samples, most of which were over-expressed. Analysis using cBioPortal showed that ICAM1, SSTR3, CXCR2, PLAU, and IGF1R exhibited high co-occurrence in mutual exclusivity. Our pathway enrichment study found a link between RTK-Ras and changes in the genes for CDC42, BCL2, SSTR3, PLAU, ICAM1, IGF1R, and MET. Our GEPIA analysis revealed significantly higher expression levels of PLAU and CDC42 in tumor tissues compared to normal tissues. Patients exhibiting low levels of CXCR2, ICAM2, SSTR3, and MET showed a notably increased overall survival rate. IGF1R expression levels demonstrated significant prognostic power relationships, with AUC values of 0.712. The eight proteins play a vital role in breast cancer carcinogenesis, with IGF1R, MET, and ICAM-1 being particularly significant. The data suggest that those genes have a crucial role in ACA therapy.

The RTK-Ras pathway is essential for breast cancer resistance [30]. It regulate major signaling pathways including MAPK, PI3K/Akt, and JAK/STAT, which drive cancer stemness, angiogenesis, and metastasis [31,32]. In breast cancer, several RTKs were significantly overexpressed, including vascular endothelial growth factor receptors (VEGFRs) [33], epidermal growth factor receptors (EGFRs) [34], insulin-like growth factor receptors (IGFRs) [35], platelet-derived growth factor receptors (PDGFRs) [36], and fibroblast growth factor receptors (FGFRs) [37]. Kinase and Ras/PI3K/Akt pathways are also crucial in controlling proliferation, differentiation, and survival [38]. Ras mutations with oncogenic properties are linked to promoting resistance to cancer drugs, specifically in breast cancer [31]. When the RTK-Ras pathway is activated in breast cancer, therapies targeting the HER2 receptor, such as trastuzumab and lapatinib, may not be as effective. Various mechanisms have been identified to explain why some cells become resistant to RTK inhibitors. These include changes in RTKs and their affected cells, as well as the increased activity of additional RTKs [32,39]. Lapatinib-resistant breast cancer cells have been found to overexpress the IGF1R receptor. This receptor shares downstream signaling pathways with HER2 in breast cancer, such as PI3K/Akt/mTOR and MAPK [35]. Interaction between IGF1R and HER2 may lead to continuous HER2 activation even while trastuzumab is present, causing resistance [35,40]. IGF1R signaling may affect the expression of proteins related to drug resistance, including MDR1, MRP3, and BCRP, which play a role in chemotherapeutic resistance [41,42]. IGF1R overexpression has been reported as an alternative mechanism for HER2-positive breast cancer cells to evade resistance, particularly in the context of targeted therapy [43]. Moreover, resistance in breast cancer has been linked to other RTK members, such as EGFR, AXL, VEGFR, and c- Met [43,44]. c-Met overexpression in HER2+ breast cancer cells is associated with trastuzumab resistance [45]. Studies have shown that small-molecule inhibitors targeting c-Met may improve the efficacy of EGFR inhibitors [46]. MET, which encodes c-Met in HER2-overexpressing breast cancer cells, has been shown to contribute to trastuzumab resistance [47]. The interaction between c-Met and MET may result in prolonged HER2 phosphorylation in the presence of trastuzumab, leading to resistance. Additionally, ICAM-1 acts as an adapter protein that interacts with c-MET, enhancing its activity. Phosphorylation of ICAM-1 by c-Met creates a positive feedback loop that enhances SRC activity and promotes cancer progression. Several studies have reported that targeting ICAM-1 could be beneficially to enhance the efficacy of chemotherapy and improve patient outcomes by reducing metastasis and angiogenesis [48,49]. Utilizing a multi-targeted therapeutic approach that simultaneously targets multiple pathways may be the most effective option for overcoming resistance in breast cancer.

Marketed cancer drugs, such as trastuzumab and lapatinib, are essential HER2-targeted breast cancer treatments, . However, some cancer may not respond to or develop resistance to these drugs. In patients with HER2-positive cancer, lapatinib is often less effective because it blocks the HER2 receptor [50], which in turn inhibits the tyrosine kinase domain in the cytoplasm. Researchers have identified BCL2 as relevant to these events because it governs cell viability by blocking apoptosis [51]. Breast cancer resistance to lapatinib is a multifaceted issue that includes changes in apoptotic signaling pathways and the development of novel driver mutations. Changes in BCL2 family members, specifically MCL-1 and BAX, have been seen in cases of lapatinib resistance, suggesting that they may play a role in this resistance [51,52]. Studies have linked CXCR2, a chemokine receptor, to a worse prognosis in breast cancer patients [53]. CXCR2 may contribute to lapatinib resistance by influencing the tumor microenvironment or innate host immunity [54,55]. MET and PLAU are key players in the HGF/MET signaling pathway, and have been linked to lapatinib resistance [56]. MET overexpression is observed in lapatinib-resistant breast cancer cells, suggesting a role in resistance [46]. IGF1R plays a crucial role in cell survival, proliferation, and angiogenesis as a receptor tyrosine kinase. Reports of IGF1R overexpression in breast cancer cells resistant to lapatinib suggest that IGF1R may play a role in lapatinib resistance [43]. ICAM1, a cell adhesion molecule, is associated with resistance to cancer treatments such as lapatinib [48,57]. ICAM1 may contribute to lapatinib resistance by influencing the tumor microenvironment or innate host immunity. SSTR3 is a somatostatin receptor associated with resistance to breast cancer treatments, such as lapatinib. SSTR3 may contribute to lapatinib resistance by influencing the tumor microenvironment or innate host immunity [58]. CDC42 is a small GTPase involved in cell migration, adhesion, and cytoskeleton rearrangement. May contribute to lapatinib resistance by influencing the tumor microenvironment or innate host immunity [59,60]. Our results from molecular docking analysis revealed a comparative affinity of ACA and native ligands of BCL2, CXCR2, MET, PLAU, IGF1R, ICAM2, SSTR3, and CDC42. We

anticipate that ACA may play a role in combating lapatinib resistance through RTK-Ras signaling pathway and alter the ligand interactions of those protein targets. Utilizing a multi-targeted therapeutic approach that concurrently targets multiple pathways may be the most effective strategy for overcoming lapatinib resistance in breast cancer patients.

Several previous preclinical studies on ACA have revealed that this compound exhibits mechanisms of action and potential therapeutic applications against various cancer types, but fewer studies reported on its selectivity and optimum concentration [16]. ACA induces apoptosis in cancer cells by inhibiting the NF-κB signaling pathway, which is crucial for cell survival and proliferation. The compound also activates caspases, leading to DNA fragmentation and cell death. In the term of cell cycle, ACA has been shown to arrest at the G2/M phase, preventing their division and proliferating. ACA also inhibits angiogenesis, which is the formation of new blood vessels that supply nutrients to growing tumors [9–12,29]. The compound has also been found to enhance the efficacy of other anticancer drugs when used in combination therapy, including transtuzumab, cisplatin, and doxorubicin [11,16]. While ACA has demonstrated synergistic effects when combined with other anticancer agents, such as cisplatin or recombinant human alpha fetoprotein, these combinations are still under investigation [61–63]. Other studies on acute and sub-acute toxicity in rats have shown that ACA is non-toxic at low doses, with no lethality or behavioral changes observed. Researchers have studied ACA as a regulator of RTK/ERK MAPK signaling. ACA has been also used in conjunction with other anticancer drugs. In MDA-MB-231, 4T1, and MCF7 breast cancer cells, ACA enhanced the cytotoxic effects of cisplatin and doxorubicin. Several studies have reported that the clinical development of ACA has been hindered by its poor in vivo solubility, degradation of biological activity upon exposure to an aqueous environment, and non-specific targeting of tumor cells. To improve the solubility and bioavailability of ACA, researchers have developed nanostructured lipid carriers (NLCs) for targeted delivery [13]. These NLCs enhance the specificity and efficacy of ACA, reducing systemic side effects and improving therapeutic outcome. Several studies on ACA have been conducted and this study provided a preliminary data to further explore in laboratorium.

One drawback of this work is that the researchers indirectly conducted data mining on lapatinib-resistant BT474-J4 cells. Although the data mining approach effectively demonstrated the resistance phenomenon of lapatinib, the fact that the microarray data originated from a single cell line is an additional limitation. As a result, it is critical to assess microarray data from various HER2+ breast cancer cell types and further investigate at the protein level. The researchers employed a bioinformatics strategy to determine the most likely ACA target genes. An in vitro study is urgently needed to corroborate the bioinformatics findings based on mRNA-level measurements. This approach could accelerate the search for molecular targets and methods by which ACA might be used to treat breast cancer cells that do not respond to lapatinib. Future studies should validate these results using both in vitro and in vivo protein levels assessments and clinical trials.

5. Conclusions

Gene expression, survival prediction, and genetic modifications siggest that ACA may target BCL2, PLAU, ICAM1, IGF1R, and MET to overcome lapatinib resistance in breast cancer. Furthermore, molecular docking analysis showed that ACA counteracts lapatinib resistance by blocking ICAM1 signaling in breast cancer cells. BCL2, SSTR3, PLAU, IGF1R, and MET docked similarly to ACA and their natural ligands. This research requires further in vitro, in vivo, and clinical trials to confirm its findings.

CRediT authorship contribution statement

Febri Wulandari: Writing – review & editing, Writing – original draft, Supervision, Data curation, Conceptualization. **Ahmad Fauzi:** Visualization, Software, Methodology, Data curation. **Muhammad Da'i:** Writing – original draft, Visualization, Validation, Supervision, Funding acquisition, Conceptualization. **Mahmoud Mirzaei:** Supervision, Funding acquisition, Data curation, Conceptualization. **Maryati:** Writing – review & editing, Visualization, Supervision, Data curation, Writing – review & editing, Visualization, Supervision, Data curation, Conceptualization. **Kun Harismah:** Writing – review & editing, Funding acquisition, Data curation, Conceptualization.

Data availability

GSE16179 was used as primary data in this study. The data collected or analyzed during this research can be found in the article, accompanying supplemental materials, available from the corresponding author upon request.

Ethic declarations

This study does not include living organisms as subject experiments, is conducted using computational methods, and was written without the help of artificial intelligence (AI).

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Muhammad Da'i reports financial support was provided by Muhammadiyah University of Surakarta. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e40769.

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