PLANT SCIENCE TODAY
ISSN 2348-1900 (online)
Vol 12(1): 1-17
https://doi.org/10.14719/pst.3566

HORIZON
e-Publishing Group
HePG

RESEARCH ARTICLE

A systems biology-based study to assess the effects of TNF-α ± apigenin in triple-negative breast cancer cell line

Elham Amjad[1,2,¥], Babak Sokouti[3,¥*], Solmaz Asnaashari[3*]& Siavoush Dastmalchi[3,4*]

[1]Student Research Committee, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran
[2]Department of Medical Genetics, School of Medicine, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran
[3]Biotechnology Research Center, Tabriz University of Medical Sciences, Tabriz, 5165665813, Iran
[4]School of Pharmacy, Tabriz University of Medical Sciences, Tabriz, 51664-14766, Iran

*Email: b.sokouti@gmail.com; asnaasharisolmaz@gmail.com; dastmalchi.s@tbzmed.ac.ir
¥The first two authors (EA and BS) contributed equally to this research and should be regarded as joint first authors

OPEN ACCESS

ARTICLE HISTORY
Received: 15 July 2024
Accepted: 10 September 2024
Available online
Version 1.0 : 23 December 2024
Version 2.0 : 01 January 2025

Check for updates

Additional information

Peer review: Publisher thanks Sectional Editor and the other anonymous reviewers for their contribution to the peer review of this work.

Reprints & permissions information is available at https://horizonepublishing.com/journals/index.php/PST/open_access_policy

Publisher's Note: Horizon e-Publishing Group remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Indexing: Plant Science Today, published by Horizon e-Publishing Group, is covered by Scopus, Web of Science, BIOSIS Previews, Clarivate Analytics, NAAS, UGC Care, etc
See https://horizonepublishing.com/journals/index.php/PST/indexing_abstracting

CITE THIS ARTICLE

Amjad E, Sokouti B, Asnaashari S, Dastmalchi S. A systems biology-based study to assess the effects of TNF-α ± apigenin in triple-negative breast cancer cell line. Plant Science Today. 2025; 12(1): 1-17. https://doi.org/10.14719/pst.3566

Abstract

Triple-negative breast cancer (TNBC) is a type of breast cancer that lacks estrogen, progesterone, and HER2 receptors. Various treatment methods are available for breast cancer, but therapies with minimal toxic side effects are particularly important. This study computationally investigates the impact of apigenin, a compound used in traditional Chinese medicine, on the TNBC cell line. The GSE120550 dataset was retrieved from the NCBI-GEO database. BRB-ArrayTools were used for pre- and post-processing to identify significantly differentially expressed genes. Additionally, the DAVID web server was utilized to analyze three main components:"biological process," "cellular component," and "molecular function," along with the KEGG signaling pathway. Finally, a Venn diagram was employed to thoroughly investigate the number of shared genes among 15 groups derived from 6 compared sample sets. The primary analysis of 6 pairs of samples revealed significant differentially expressed genes (DEGs), which were prioritized using the TOPPgene web server. These identified genes, playing key roles in inhibiting the progression of BC, are involved in various signaling pathways. Protein-protein interaction network analysis highlighted the biomarkers associated with the inhibitory effects of apigenin across the 15 sets derived from the 6 sample pairs. The findings of this study confirm the inhibitory effects of apigenin, with no toxic side effects, on patients with TNBC. This natural compound holds promise for future therapeutics and novel drug designs.

Keywords

apigenin; TNF-α; systems biology; pharmacology; triple-negative breast cancer; anti-cancer agent

Introduction

Triple-negative breast cancer (TNBC) arises from the absence of 3 key receptors: Estrogen Receptor (ER), Progesterone Receptor (PR), and human epidermal growth factor receptor 2 (HER2). It accounts for approximately 10-15% of invasive breast cancer cases, with a high risk of relapse even after treatment (1, 2). Diagnosis TNBC can be challenging due to it's histologically similarity to basal-like BC, which is associated with BRCA1 dysfunction in the relevant signaling pathways (3). Common treatments approaches for TNBC include surgery followed by chemotherapy and radiation therapy , or the reverse for more manageable cases (4). As a focus of global cancer research, therapeutic agents are needed to target receptors and signalling

pathways. Tumor necrosis factor-alpha (TNF-α) is a critical target, playing a dual role (a "double-edged sword") in both promoting and inhibiting cancer growth (5). Although no clinical trials have reported effective TNF-α inhibition due to its high toxicity (5), its role in inflammation and cancer progression can be significant in the host cells of cancerous tissues (5, 6). Traditional Chinese medicine, such as Triptolide and Tubeimu, has been used since ancient times as a source for developing anti-metastatic agents to treat TNBC (7, 8).

TNF-α is a crucial pro-inflammatory cytokine that plays a significant role in the tumor microenvironment, particularly in TNBC. Its importance stems from its influence on tumor progression. TBNC lacks estrogen and progesterone receptors and has low HER2 expression, making it one of the most difficult subtypes of breast cancer to treat due to its aggressive nature and high propensity for metastasize (9, 10). The MDA-MB-231 cell line is commonly used in TBNC research due to its high invasiveness and resistance to conventional therapies(9).

TNF-α promotes cell migration, increases invasion, and facilitates metastasis in MDA-MB-231 cells by upregulating signaling pathways such as NF-κB and MAPK (11). Increasing evidence suggests that TNF-α plays a crucial role in cancer progression and may also have therapeutic potential. Studies on MDA-MB-231 cells have shown that TNF-α significantly alters IL-1α expression (12). Additionally, TNF-α secretes chemokines that worsen the tumor microenvironment, further promoting tumor cell migration and invasion (13, 14).

Apigenin, phytonutrient found in fruits and vegetable, has demonstrated promising anti-cancer properties. It inhibits the proliferation of cancer cells, including MDA-MB -231 cell lines (15, 16). Apigenin suppresses pro-tumor factors such as TNF-α, IL-1α, and IL-6, thereby reducing their tumor-promoting effects (14, 16). For instance, apigenin was found to inhibit the release of CCL2, which is induced by TNF-α, thereby preventing tumor migration and metastasis (14). Additionally, apigenin induces apoptosis and cell cycle arrest in MDA-MB-231 cells, highlighting its potential as a therapeutic agent against TNBC, particularly in its early stages (15, 16).

In the future, understanding the interaction between TNF-α and apigenin in TNBC cell lines, such as MDA-MB-231, could help address the complex therapeutic landscape of TNBC treatment. Apigenin not only inhibits inflammatory pathways but also induces cell death, counteracting the tumor-promoting effects of TNF-α. Due to this dual action, a growing body of research suggests that apigenin can enhance the efficacy of chemotherapy in TNBC, particularly when combined with other treatments targeting the TNF-α signaling pathway (12, 17).

Among natural compounds with low toxicity, apigenin derived from the *Apium* genus, is one of the most extensively studied molecules for its anti-cancer properties due to its flavonoid structure (18, 19). Numerous studies have also explored apigenin's antioxidant, anti-allergic, antimicrobial, and anti-inflammatory activities, along with its derivatives (20-22). Apigenin's remarkable role lies in its ability to induce cell cycle arrest and apoptosis by targeting various signaling pathways, including Wnt/β-catenin, PI3K/Akt/mTOR and STAT3 (23-25).

Recent attention has focused on the effects of apigenin treatment, particularly in combination with TNF-α, on gene expression in TNBC cells, including the MDA-MB-231 cell lines. TNF-α, a pro-inflammatory cytokine in the tumor microenvironment, often promotes cancer progression through signalling pathways such as MAPK and NF-κB (26, 27). Apigenin, a flavonoid known for its anti-inflammatory and anti-cancer properties, regulates process such as apoptosis, inflammation, and cell survival (28).

TNF-α stimulates the production of inflammatory cytokines such as IL-6 and IL-1β in MDA-MB-231 cells, leading to increased proliferation and survival (26). These cytokines activate downstream signalling pathways that promote tumor growth and metastasis through various mechanisms. Research has shown that apigenin can effectively counteract these effects by inhibiting the production of IL-6 and TNF-α, both of which contribute to TNF-α-induced inflammation (26). This anti-inflammatory action may significantly reduce the expression of pro-tumorigenic genes in TNBC cells, potentially decreasing the aggressiveness of the tumors.

Apigenin has been shown to induce apoptosis in MDA-MB-231 cells by modulating various apoptotic pathways involved in cell death. Studies indicate that apigenin upregulates pro-apoptotic genes and downregulates anti-apoptotic genes, leading to a significant increase in cell death when TNF-α is present (28). For instance, apigenin induces the expression of BAX, a pro-apoptotic gene, while suppressing BCL-2, an anti-apoptotic gene. This shift in gene expression favors apoptosis, impacting cell survival. To survive, cancer cells must alter their gene expression to overcome the apoptotic signals induced by TNF-α.

Apigenin modulates the NF-κB signaling pathway in a manner that is highly relevant for analysing its effects on TNF-α induced gene expression. TNF-α enhances NF-κB activity, which promotes the transcription of genes involved in inflammation, survival and proliferation when activated (29). Apigenin inhibits the phosphorylation of IκBα, a key regulator in the NF-κB pathway, preventing NF-κB from translocating to the nucleus and thereby inhibiting gene transcription (29). This reduction in NF-κB activity can lead to the downregulation of various NF-κB target genes, including those involved in cell invasion and migration, such as matrix metalloproteinase-9 (MMP-9) (30).

Apigenin has also demonstrated the potential to modulate chemokine expression, an important areas of application. Research indicates that TNF-α promotes the production of chemokines, such as CXCL1, which are associated with tumor cell migration and dissemination

throughout the body (27). When MDA-MB-231 cells are treated with apigenin, their metastatic capabilities may be reduced by downregulating these chemokines (27). Given that TNBC is particular prone to metastasis, which adversely affects prognosis, this effect of apigenin is significant.

The current study focuses on a systems biology approach to analyse significant differentially expressed genes in the TNBC cell line, considering the role of TNF-α as a tumor-promoting agent, both with and without apigenin treatment. Additionally, the study will investigate biological processes, molecular functions, and cellular components to identify the signalling pathways involved, which could inform future novel drug design and discovery.

Materials and Methods

Fig. 1 provides a detailed flowchart of the procedure, serving as a comprehensive diagram for biomarker analyses (31, 32).

Microarray Database

The GSE120550 dataset, from platform GPL17692 [HuGene-2_1-st] Affymetrix Human Gene 2.1 ST Array [transcript (gene) version], was obtained from the publicly available NCBI-GEO database of the National Center (i.e., https://www.ncbi.nlm.nih.gov/gds/). The dataset includes 12 samples of MDA-MB-231 cells treated with TNF-α [40 ng/ml] ± apigenin [40 μM] for (n TNF-α =3: GSM3402894, GSM3402895 and GSM3402896; n apigenin = 3: GSM3402897, GSM3402898 and GSM3402899; n TNF-α+apigenin = 3: GSM3402900, GSM3402901 and GSM3402902) and untreated control (n=3; GSM3402891, GSM3402892 and GSM3402893).

Identification of significant genes between four types of samples and gene prioritization

Dr. Richard Simon and his team developed BRB-ArrayTools (v4.6.0) (https://brb.nci.nih.gov/BRB-ArrayTools/) to identify significant differentially expressed genes (DEGs) in treated and untreated MDA-MB-231 cell lines.

The pre-treatment procedure included quantile normalization and gene annotation using the R package,"pd.hugene.2.1.st" (33). Next, the "gcrma" R package

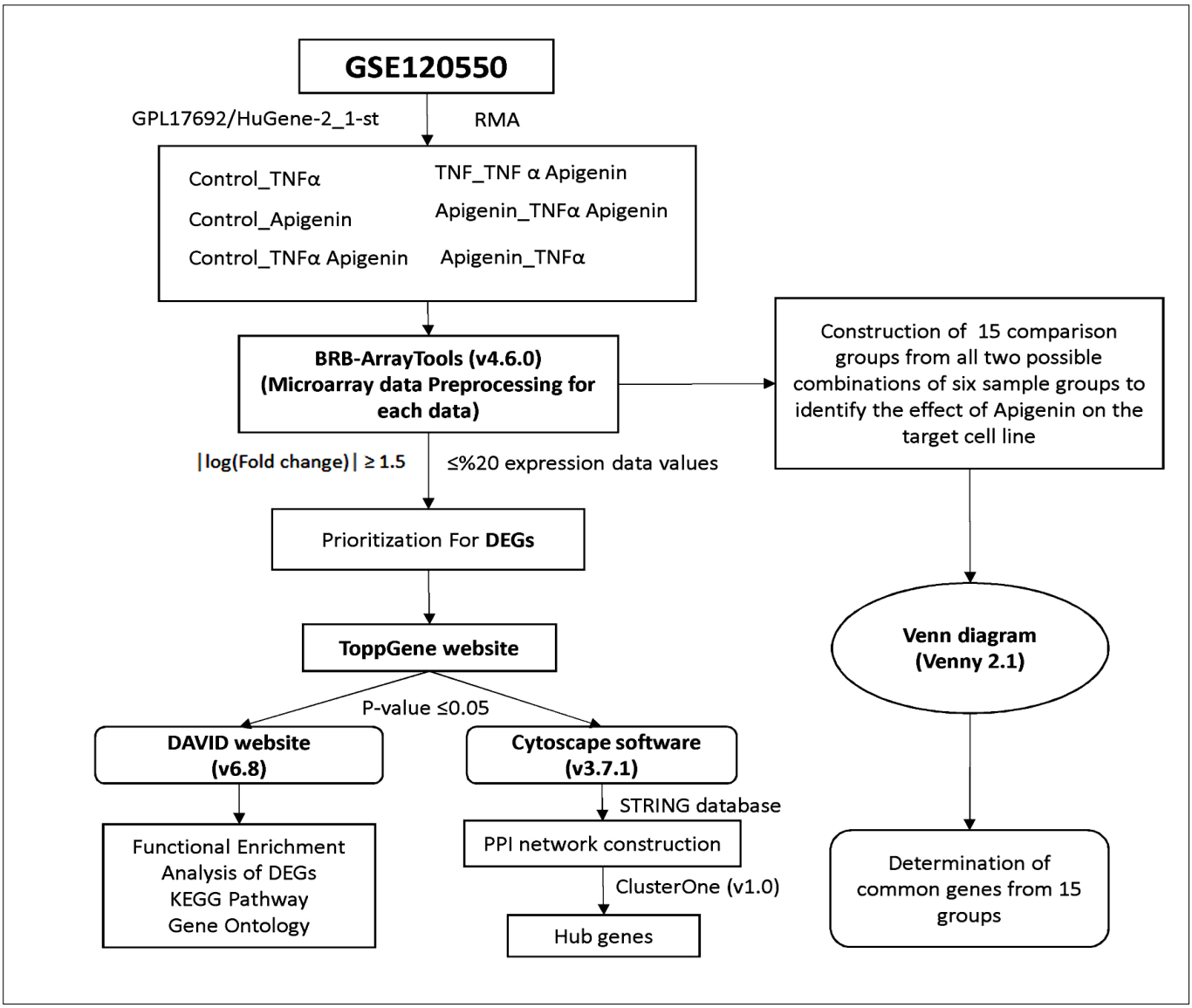


Fig. 1. An step by step flowchart to identify the conclusion on effect of TNF-α ± Apigenin on TNBC cell line cancer. (BRB-ArrayTools (v4.6.0) (i.e., https://brb.nci.nih.gov/BRB-ArrayTools/), ToppGene website ((i.e., https://toppgene.cchmc.org), DAVID website (v6.8) (https://david.ncifcrf.gov/), Cytoscape software (v3.7.1) (https://cytoscape.org/), Venn diagram (Venny 2.1) (https://bioinfogp.cnb.csic.es/tools/venny/).

was used to assess probe intensities from the raw microarray data. Differentially expressed genes were then classified between the 2 sample groups using univariate permutation tests and fold change threshold values of 10,000 and 1.5.

To prioritize the identified DEGs, two websites were utilized: GeneCards (17) and ToPPGene (https://toppgene.cchmc.org). The keyword “breast cancer" was used to search GeneCards to extract gene symbol evidence for the training group. The ToPPGene websites then ranked the significant DEGs (test group) from the BRB-ArrayTools output based on a p-value less than or equal to 0.05, using the training genes evidence obtained from GeneCards.

GEO and KEGG enrichment analyses

DAVID v. 6.8 (Database for Annotation, Visualization, and Integrated Discovery) was used to identify various Gene Ontology (GO) terms and enriched functional gene groups, including biological processes, cellular components, and molecular functions, as well as to perform KEGG (Kyoto Encyclopedia of Genes and Genomes) signalling pathway analysis (34, 35).

Construction of protein-protein interaction, gene-disease, and gene-drug networks

To create the protein-protein interaction (PPI) network, the STRING database was used with a confidence score cutoff value of 0.4, and the network was visualized with Cytoscape v.3.7.1 using ClusterOne v.1.0 (36, 37). Associations between target genes within statistically significant modules (p-value of ≤ 0.05) were examined to identify potential biomarkers with the highest connectivity degree.

Venn diagram

Venny2.1.0 (38) was used to map the genes from any two of the six constructed groups (i.e., 15 sets) to illustrate the number and rate of shared genes between them.

Results

Statistical analyses revealed several upregulated and downregulated differentially expressed genes (DEGs) (Table 1). However, there were no significant DEGs identified when comparing the apigenin and TNF-α + apigenin treatments in the MDA-MB-231 cancer cell line. Additionally, the ToPPGene web server ranked DEGs for further pro-

Table 1. List of top 10 significant DEGs determined by BRB-ArrayTools.

	Gene symbol	FC	P-value
(A) Control_ TNF-α	**Upregulated**		
	VIPR1	2.47	1.48E-05
	CDK15	2.26	0.0003859
	OLFML2A	2.16	3.81E-05
	PLPP4	1.99	0.0002055
	ITGB4	1.98	7.30E-05
	RHOD	1.96	0.0004039
	HTRA1	1.89	0.0008368
	IGFBP4	1.85	0.0003397
	CPA4	1.85	0.0003467
	BMP4	1.73	8.99E-05
	Downregulated		
	IER3	0.67	0.0004881
	OPTN	0.66	0.0006317
	IER3	0.65	0.0005174
	SERPINB8	0.64	0.0007988
	PKIA	0.63	0.0005714
	ARHGAP42	0.62	0.0003596
	4-Mar	0.62	0.0004407
	ACO1	0.62	0.0009106
	ETS1	0.61	0.0001548
	DOCK9	0.61	0.0003044

	Gene symbol	FC	P-value
(B) Control_Apigenin	**Upregulated**		
	LOC284344	10.37	< 1e-07
	MIR100	10.14	3.00E-07
	ARC	6.32	2.29E-05
	ARRDC4	6.08	5.08E-05
	MIR3143	5.91	8.00E-07
	IDI2-AS1	5.37	2.10E-06
	RASD1	5.27	2.70E-06
	EGR1	5.13	1.30E-05
	SLC30A1	5.05	6.00E-07
	LOC284344	10.37	< 1e-07
	Downregulated		
	NUB1	0.67	0.00061
	SASS6	0.66	0.000471
	EHD4	0.66	0.000579
	ACTR3	0.66	0.00071
	FYN	0.66	0.00088
	DUSP6	0.66	0.000924
	STC1	0.66	0.000927
	CCDC77	0.66	0.00094
	ITSN2	0.66	0.00095
	HPS3	0.66	0.000958

	Gene symbol	FC	P value
(C) Cotrol_TNF-α Apigenin	**Upregulated**		
	CYP1B1	6.31	0.000123
	CAVIN2	6.28	9.50E-05
	PDE7B	5.73	0.000153
	PLK1	5.2	0.00013

	Gene symbol	FC	P value
(D) TNF-α _TNF-α Apigenin	**Upregulated**		
	IL1A	9.19	5.00E-07
	CEMIP	5.94	1.20E-06
	CYP1B1	5.79	1.90E-06
	IL24	5.76	2.30E-06

	ADAMTS15	4.84	0.000671
	SPTLC3	4.81	2.29E-05
	IGFBP1	4.78	6.43E-05
	KIF20A	4.51	0.000148
	KLHL4	3.8	0.000504
	CYP1B1	6.31	0.000123
	Downregulated		
(C) Cotrol_TNF-α Apigenin	MICB	0.66	0.000141
	ARPC5L	0.66	0.00037
	PLK3	0.66	0.000828
	RNPS1	0.65	0.00021
	SUPT16H	0.65	0.000498
	BAIAP2	0.65	0.000541
	SMIM15	0.65	0.00071
	TSR1	0.64	8.90E-06
	ZNF850	0.64	0.000151
	SLC35F2	0.64	0.000343

	IKBKE	5.01	2.00E-07
	PLK1	4.78	2.00E-07
	CAVIN2	4.67	6.10E-06
	MIR4435-2HG	4.32	5.21E-05
	SHISA2	4.16	1.50E-06
	KIF20A	3.73	7.20E-06
	Downregulated		
(D) TNF-α _TNF-α Apigenin	NCOA4	0.67	0.000627
	NEB	0.67	0.000638
	GLYR1	0.66	0.000331
	MAK16	0.66	0.000449
	INF2	0.66	0.000583
	LUZP1	0.65	0.000226
	IPO7	0.65	0.000294
	SPECC1	0.65	0.000377
	ARL5B	0.65	0.000383
	COPS2	0.65	0.000538

Gene symbol	**FC**	**P value**
Upregulated		
SNORA2B	2.02	0.000758
-	-	-
-	-	-
-	-	-
-	-	-
-	-	-
-	-	-
-	-	-
-	-	-
-	-	-
Downregulated		
ATP2B1	0.67	0.000708
FAM98A	0.67	0.000943
CFLAR	0.65	0.000264
DDX58	0.65	0.000686
CDV3	0.64	0.000374
POMK	0.64	0.00039
PAGE5	0.64	0.000707
R3HCC1L	0.63	0.000169
SLC25A22	0.63	0.000303
HIVEP1	0.62	0.000574

(E) Apigenin_ TNF-α Apigenin

Gene symbol	**FC**	**P value**
Upregulated		
MIR100	12.25	0.000356
LOC284344	9.15	7.86E-05
MIR3143	6.86	0.000159
RASD1	6.36	0.000535
SLC30A1	5.33	2.71E-05
ARC	5.07	0.00056
IDI2-AS1	4.83	0.000546
GADD45B	4.74	6.00E-07
HIF1A-AS2	4.49	0.000364
VN1R108P	4.32	0.000624
Downregulated		
CCL28	0.66	0.000283
BCKDK	0.65	0.000119
PIK3CA	0.65	0.00021
NCEH1	0.65	0.000212
PDE6D	0.65	0.000341
CKAP5	0.65	0.000823
MCM8	0.64	6.59E-05
VAMP7	0.64	0.000237
ACTR3	0.64	0.000633
MYO1B	0.64	0.000757

(F) Apigenin_TNF-α

tein-protein interaction analysis (Table 2). The PPI network analysis identified hub genes affected by various treatments, including apigenin and TNF-α (Table 3).

The GO analysis, encompassing biological processes, cellular components, molecular functions, and KEGG pathway mapping, was performed using DAVID v6.8, the Database for Annotation, Visualization, and Integrated Discovery. The results revealed significant terms for groups 2 and 6 (Control vs. Apigenin, Apigenin vs. TNF-α+Apigenin, and Control vs. TNF-α+Apigenin):

- Biological Processes (BP): GO:0043981~histone H4-K5 acetylation, GO:0043982~histone H4-K8 acetylation, GO:0043984~histone H4-K16 acetylation; GO:0043547~positive regulation of the GTPase ac-

Table 2. The list of 10 top DEGs ranked by ToPPGene web server (i.e., https://toppgene.cchmc.org)

	Rank	Gene symbol	Overall p Value
(A) Control_TNF-α	1	OPTN	0.003685
	2	IRAK2	0.007693
	3	PANX1	0.012981
	4	TNIP1	0.017472
	5	GPRC5B	0.024466
	6	SERPINB8	0.025938
	7	MFAP2	0.027366
	8	ACO1	0.028311
	9	ROBO4	0.035488
	10	NUAK2	0.042567
	Rank	**Gene symbol**	**Overall p Value**
(B) Control_Apigenin	1	ANKRD1	0.005784
	2	DYNC2H1	0.005877
	3	MUL1	0.00624
	4	RASA3	0.006318
	5	IL1RAPL1	0.006765
	6	TAF2	0.006776
	7	NDC1	0.007182
	8	LAMTOR3	0.00807
	9	NFIA	0.008719
	10	IL31RA	0.008975
	Rank	**Gene symbol**	**Overall p Value**
(C) Cotrol_TNF-α Apigenin	1	ANKRD1	0.005366
	2	MUL1	0.005921
	3	TAF13	0.009175
	4	TAF9B	0.010446
	5	TFDP2	0.010772
	6	PANX1	0.011572
	7	SEC24B	0.014874
	8	WIPI1	0.014885
	9	TNIP1	0.015007
	10	PPP1R15B	0.015423
	Rank	**Gene symbol**	**Overall p Value**
(D)TNF-α _TNF-α Apigenin	1	TAF7	0.0051
	2	RASA3	0.006503
	3	ANKRD1	0.006634
	4	MUL1	0.00727
	5	NDC1	0.007911
	6	CREB3L2	0.009494
	7	MBD5	0.010524
	8	NUP42	0.010755
	9	IL31RA	0.010787
	10	CRISPLD2	0.012033
	Rank	**Gene symbol**	**Overall p Value**
(E) Apigenin_ TNF-α Apigenin	1	IRAK2	0.005829
	2	POMK	0.013584
	3	NAV2	0.013962
	4	TNIP1	0.014482
	5	DOCK10	0.023973
	6	MLLT6	0.07229
	7	REXO4	0.074127
	8	ARHGAP42	0.082052
	9	PARP12	0.092027
	10	IRAK2	0.203029
	Rank	**Gene symbol**	**Overall p Value**
(F) Apigenin_TNF-α	1	CDK5RAP2	0.003076
	2	ANKRD1	0.005095
	3	RASA3	0.006446
	4	IL1RAPL1	0.006578
	5	IRAK2	0.007118
	6	NUP42	0.009236
	7	RAB3GAP1	0.009582
	8	ABR	0.010544
	9	GYS1	0.011166
	10	ARAP1	0.012808

tivity, GO:0007264~small GTPase mediated signal transduction, GO:0031023~microtubule organizing center organization; and GO:0016236 ~ macroautophagy, O:0006368~transcription elongation from RNA polymerase II promoter, GO:0000290~deadenylation-dependent decapping of nuclear-transcribed mRNA).

- Cellular component (CC): GO:0005654 ~ nucleoplasm, GO:0005829~cytosol, GO:0000123~histone; acetyltransferase complex; GO:0005829~cytosol, GO:0005794~Golgi apparatus, GO:0030659 ~ cytoplasmic vesicle membrane; and GO:0005669 ~ transcription factor TFIID complex, GO:0005654 ~ nucleoplasm, GO:0005829~cytosol).

- Molecular Function (MF): GO:0008270~zinc ion binding, GO:0061630~ubiquitin protein ligase activity, GO:0005085~guanyl-nucleotide exchange factor activity; GO:0005096~GTPase activator activity, GO:0005515~protein binding, GO:0005085~guanyl-nucleotide exchange factor activity; and GO:0044822~poly(A) RNA binding, GO:0005515 ~ pro -tein binding, GO:0008270~zinc ion binding).

- KEGG pathways: hsa00562~Inositol phosphate metabolism, hsa03013~RNA transport, hsa04070~ Phosphatidylinositol signalling system; hsa00500~ Starch and sucrose metabolism; and hsa03022 ~ Ba -sal transcription factors, hsa03040~Spliceosome, hsa05168~Herpes simplex infection.

Table 3. Potential significant hub genes identified using ClusterOne v1.0 Cytoscape plugin

Groups	Item	Gene Symbol	Connectivity Degree	Up/Downregulated
(B)	1	DDX46	5	Downregulated
	2	RNPS1	5	Upregulated
	3	TFIP11	5	Upregulated
	4	UBA3	5	Downregulated
	5	SPC25	4	Downregulated
	6	ATP8A1	3	Downregulated
	7	LAMTOR3	3	Upregulated
	8	SLCO4C1	3	Downregulated
	9	XPOT	3	Downregulated
	Item	**Gene Symbol**	**Connectivity Degree**	**Up/Downregulated**
(C)	1	CHERP	4	Downregulated
	2	SYF2	4	Downregulated
	3	TAF3	3	Downregulated
	4	SPC25	3	Upregulated
	5	DIEXF (UTP25)	2	Downregulated
	6	FKBP15	2	Downregulated
	7	MAK16	2	Downregulated
	Item	**Gene Symbol**	**Connectivity Degree**	**Up/Downregulated**
(D)	1	DDX46	3	Upregulated
	2	SF3A2	3	Downregulated
	3	SYF2	3	Downregulated
	4	TFIP11	3	Downregulated
	5	MGAM	3	Downregulated
	6	CENPI	2	Upregulated
	7	CENPN	2	Downregulated
	8	MIS12	2	Downregulated
	9	ACO1	2	Upregulated
	10	UBE2J1	3	Upregulated

Additionally significant terms for BP included GO: 0060325~face morphogenesis, GO: 0010976 ~ positive regulation of neuron projection development, GO: 0016567~protein ubiquitination; GO: 0002755~MyD88-dependent toll-like receptor signalling pathway.

For groups 4, 5, and 1, the following GO terms were identified:

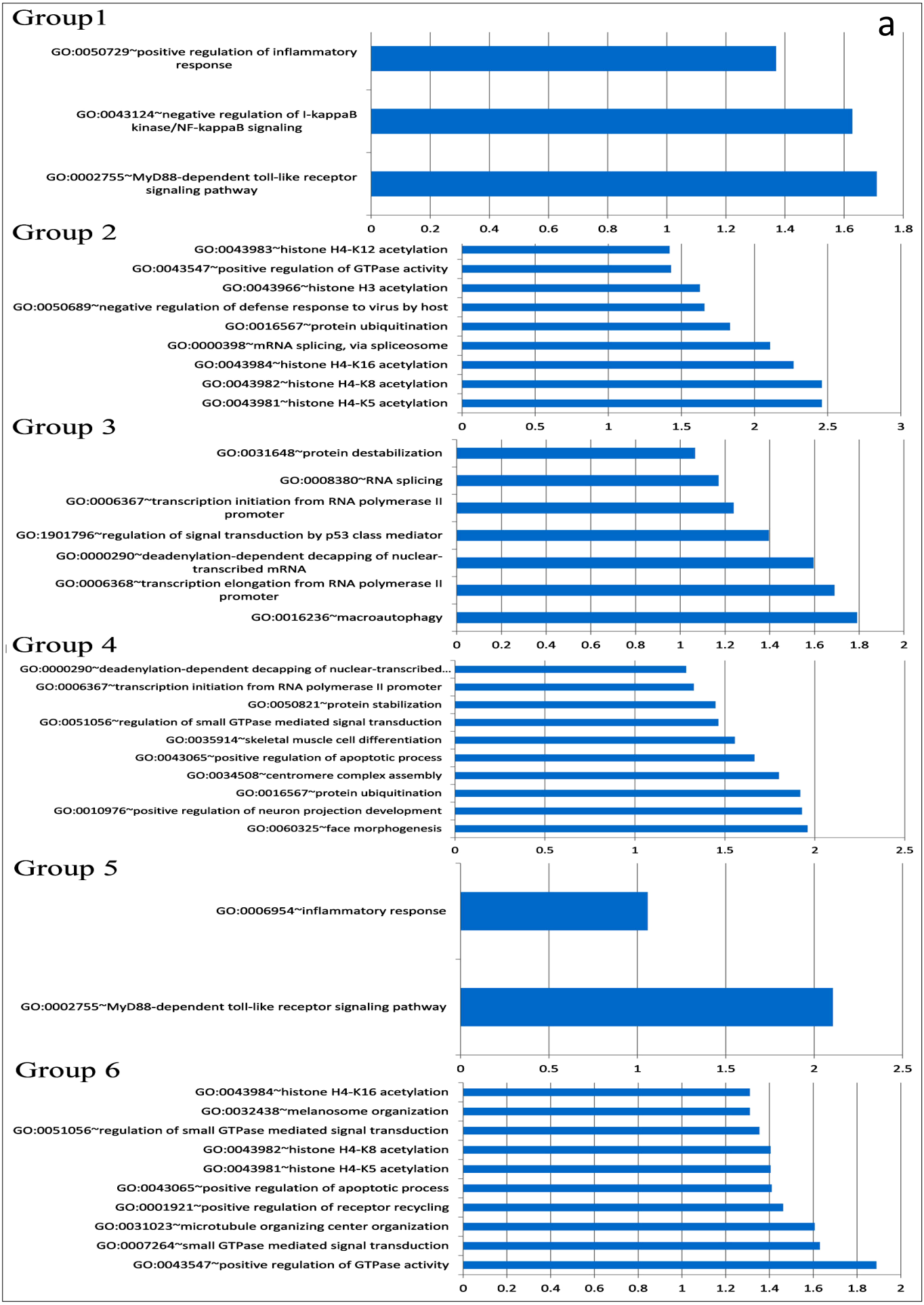
- Biological Process (BP): GO: 0006954~inflammatory response; and GO: 0002755~MyD88-dependent toll-like receptor signaling pathway, GO: 0043124 ~ negative regulation of I-kappaB kinase/NF-kappaB signaling, GO: 0050729~positive regulation of inflammatory response.
- Cellular Component (CC): GO: 0005654 ~ nucleoplasm, GO: 0005829~cytosol, GO: 0005856 ~ cytoskeleton; and GO: 0005829~cytosol.
- Molecular Functions (MF): GO: 0008270~zinc ion binding, GO: 0008134~transcription factor binding, GO: 0005085~guanyl-nucleotide exchange factor activity; GO: 0005524~ATP binding.

No KEGG pathways were identified for these groups. Fig. 2 presents the results of these analyses.

This study investigates the effect of apigenin and TNF-α on MDA-MB-231 cancer cells, as well as the interaction between these 2 molecules. To achieve this, the study analyzed six treated and untreated groups across fifteen combinations. This detailed investigation aims to clarify the role of apigenin in inhibiting breast adenocarcinoma and invasive breast cancer.

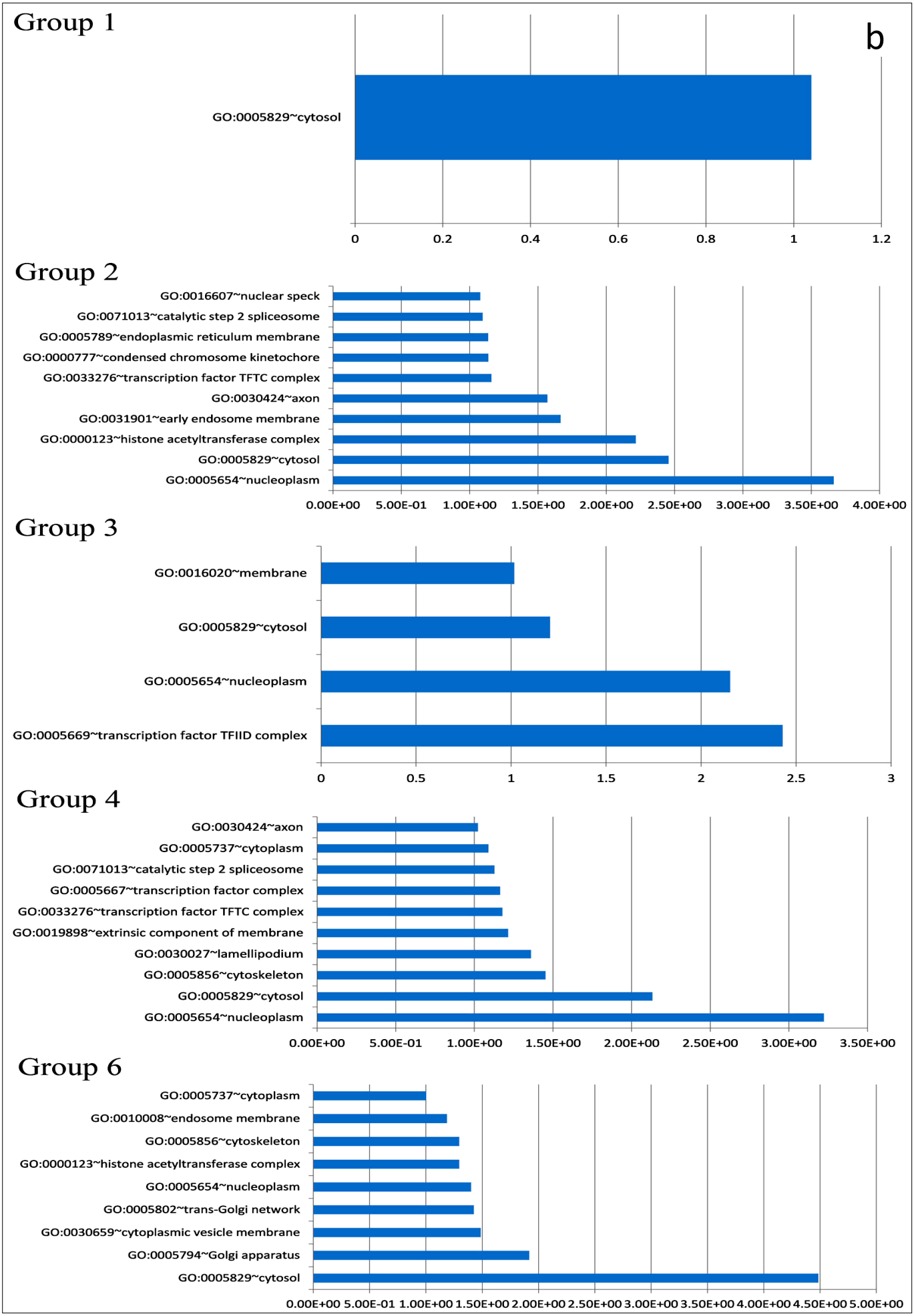
In group 1, although the number of shared genes is limited, the inhibitory effect of apigenin is evident in this comparison. In group 2, apigenin shares only one out of twenty-nine genes, indicating that it could not counteract the effects of TNF-α.

In group 3, the number of genes with altered expression levels suggests that apigenin inhibits the development of TNF-α. This means that apigenin's impact is more similar to its effects on normal cancer cells rather than on TNF-α-aggravated ones. In group 4, apigenin does

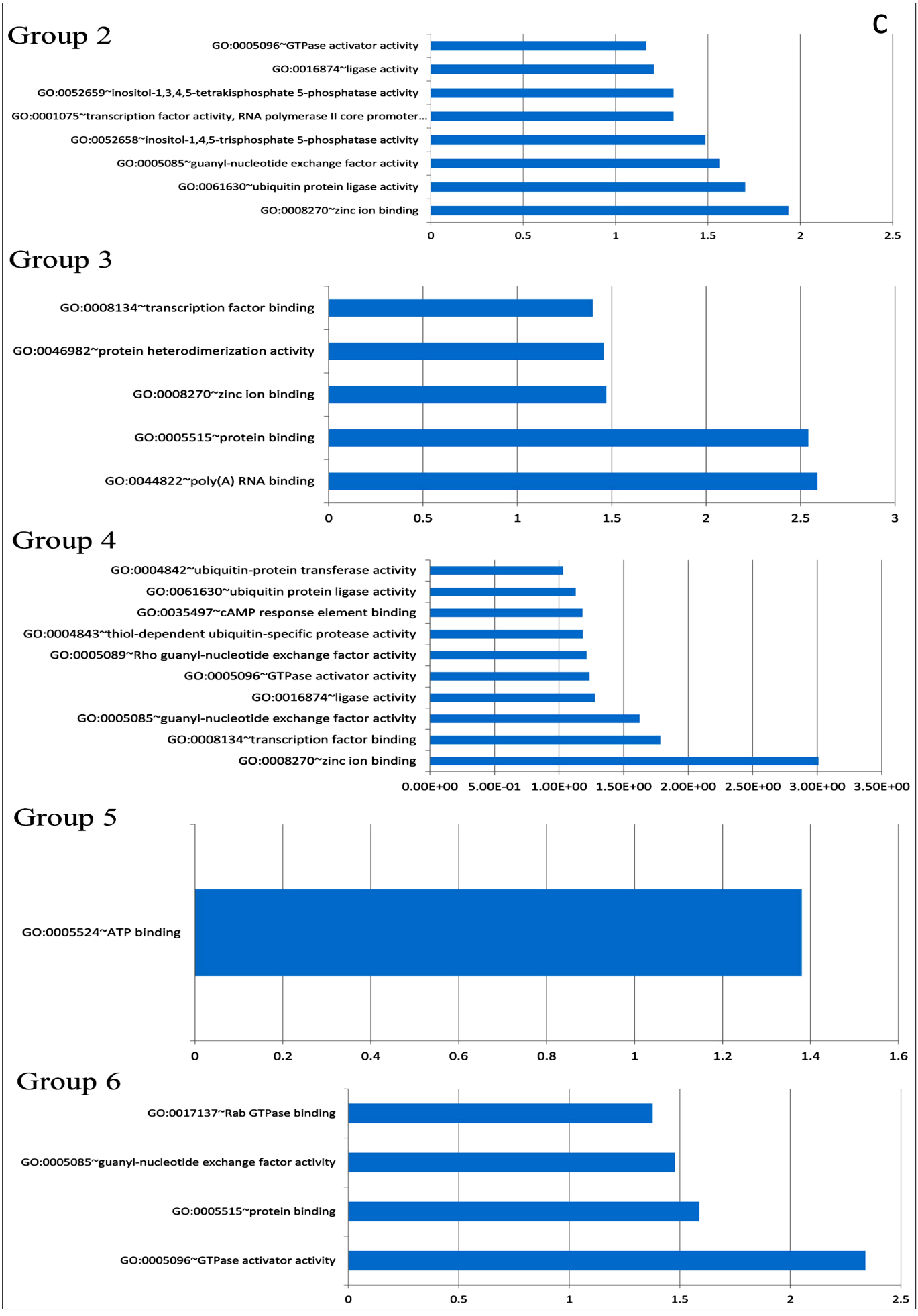


not alter gene expression, implying that it has minimal effect on TNF-α-aggravated cancer cells.

In group 5, despite several shared genes, only 1 gene shows a different expression, suggesting the antici-



pated impact of apigenin. In group 6, the increasing num- ber of shared genes with altered expression levels demon-



strates apigenin's positive effect on normal cancer cells.

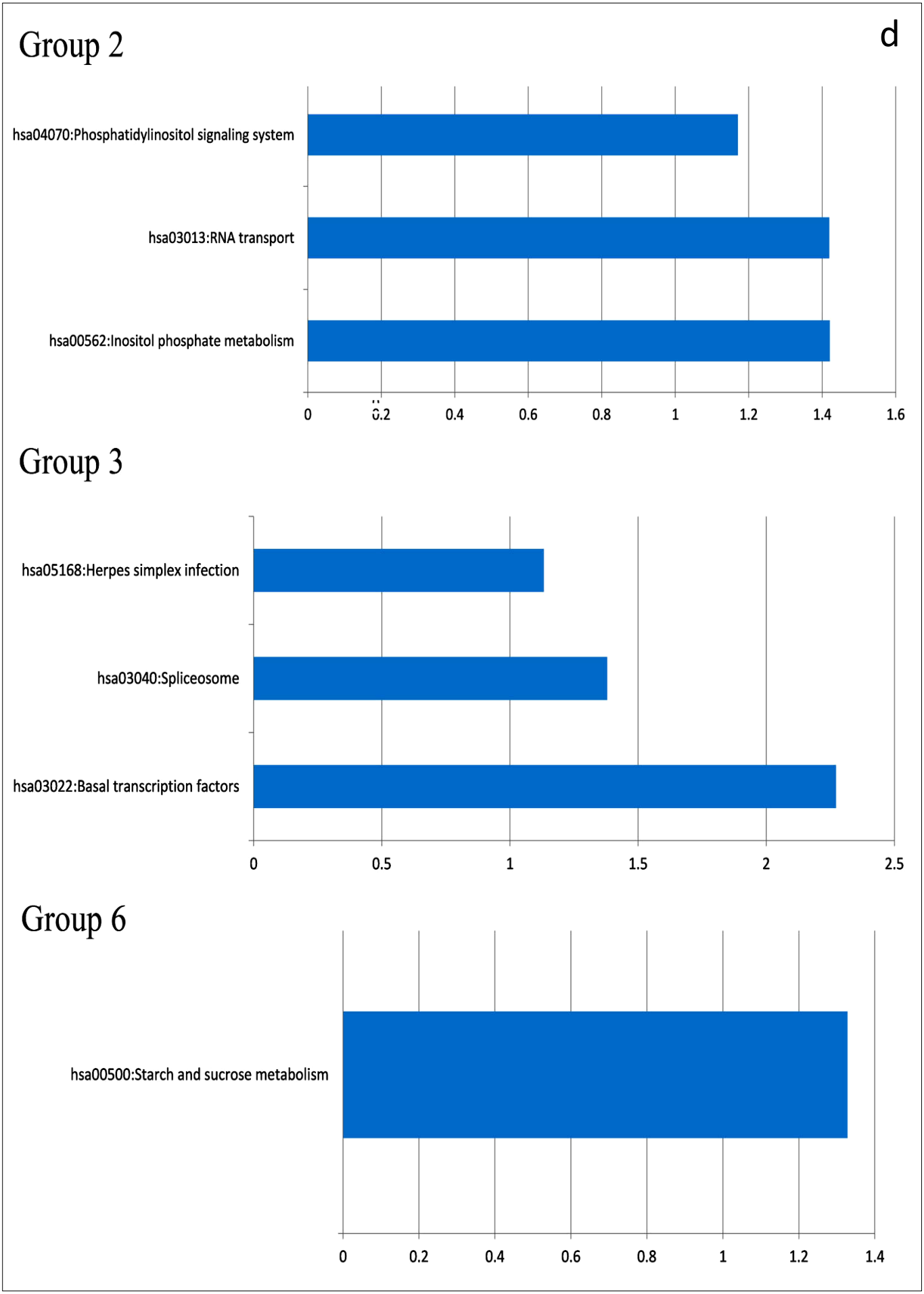


Fig. 2. DAVID's webserver results demonstrated for six groups on (**a**) biological processes (BP), (**b**) cellular components (CC), (**c**) molecular functions (MF), and (**d**) the KEGG signaling pathway analysis.

In group 7, numerous shared genes and changes in expression reveal both the inhibitory and cancer-inhibiting effects of apigenin on non-exacerbated cells. In group 8, the dominance of TNF-α over apigenin is observed, indicating that apigenin has a minimal impact on aggravated cancer cells. In group 9, despite the presence of shared genes, apigenin did not alter gene expression levels. In groups 10 and 11, apigenin's low impact on TNF-α-aggravated cancer cells is observed. In groups 12 and 13, apigenin's effect remains the same as in intensified cancer cells. In group 14, the expression changes in all shared genes demonstrate apigenin's positive impact on cancer cells not aggravated by TNF-α. In group 15, apigenin did not affect TNF-α-induced cancer cells.

Table 4 and Fig. 3 summarizes these findings, with the Venn diagrams providing further detail.

In summary, the aim of this article, as illustrated by the results in Table 4 and Fig. 3, is to compare the effects of TNF-α and apigenin on breast cancer and evaluate the potential of apigenin as a therapeutic drug. The table was prepared by analysing 15 comparative groups and conducting various analyses. Table 4 highlights which combination has a dominant effect in each group and its impact on gene expression. Additionally, the goal is to assess the influence of apigenin at both high and low concentrations of TNF-α on cancer cells and to determine its effectiveness in regulating the expression of genes affected by TNF-α.

Discussion

The potential anti-cancer effects of apigenin, a flavonoids found in many fruits and vegetables, have been extensively studied, particularly in breast cancer cells such as MDA-MB-231. This cell line is commonly used as a model for TNBC research. Apigenin inhibits the cell proliferation, migration, and invasion of MDA-MB-231 through various mechanisms. One of the key ways apigenin combat cancer is by inducing apoptosis in these cells. This cell death is mediated by caspases, and apigenin has been shown to trigger apoptosis by increasing the expression of p21WAF1/CIP1 and enhancing histone H3 acetylation, among other mechanisms (16, 39). Additionally, apigenin induces oxidative stress and DNA damage, both of which are critical factors in initiating apoptosis in cancer cells (16, 40).

MDA-MB-231 cells are known for their ability to migrate and invade surrounding tissues, which is crucial for cancer progression and metastasis. Apigenin not only promotes apoptosis but also blocks the migration and invasion of these cells. Studies have shown that apigenin suppresses the expression of matrix metalloproteinases (MMP-2 and MMP-9), enzymes critical for breaking down the extracellular matrix, a key process in tumor invasion (41). By downregulating these proteins, apigenin effectively reduces the invasive potential of MDA-MB-231 cells.

Moreover, apigenin inhibits tumor growth by exhibiting strong anti-inflammatory properties. Studies have shown that apigenin reduces the expression of pro-inflammatory cytokines, such as IL-6 and TNF-α, in MDA-MB-231 cells (12, 14). In addition to decreasing inflammation, which can contribute to tumor growth, apigenin disrupts signalling pathways involved in cancer cell proliferation and survival. By suppressing IL-6 signalling, apigenin may reduce the activation of STAT3, a key factor in the progression of aggressive breast cancers (14).

TNF-α, an inflammatory cytokine, plays a crucial role in the progression of various cancers, including breast

Table 4. Summary of effects for Apigenin treatment using fifteen groups' comparison

Group	Description	Gene ratio of DEGs in common	Effect	
			Apigenin	TNFα
1	Control-Apigenin *vs.* Control-TNFα	8/16		
2	Control-TNFα-Apigenin *vs.* Control-TNFα	1/29		
3	TNFα-Apigenin *vs.* Control-TNFα	52/55		
4	TNFα-TNFα-Apigenin *vs.* Control-TNFα	0/33		
5	Apigenin-TNFα-Apigenin *vs.* Control-TNFα	1/50		
6	Control-TNFα-Apigenin *vs.* Control-Apigenin	258/258		
7	TNFα-Apigenin *vs.* Control-Apigenin	430/430		
8	TNFα-TNFα-Apigenin *vs.* Control-Apigenin	1/7		
9	Apigenin- TNFα-Apigenin *vs.* Control-Apigenin	0/209		
10	TNFα-Apigenin *vs.* Control-TNFα-Apigenin	4/221		
11	TNFα-TNFα-Apigenin *vs.* Control-TNFα-Apigenin	1/12		
12	Apigenin-TNFα-Apigenin *vs.* Control-TNFα-Apigenin	84/91		
13	TNFα-TNFα-Apigenin *vs.* TNFα-Apigenin	18/19		
14	Apigenin-TNFα-Apigenin *vs.* TNFα-Apigenin	245/245		
15	Apigenin-TNFα-Apigenin *vs.* TNFα-TNFα-Apigenin	0/24		

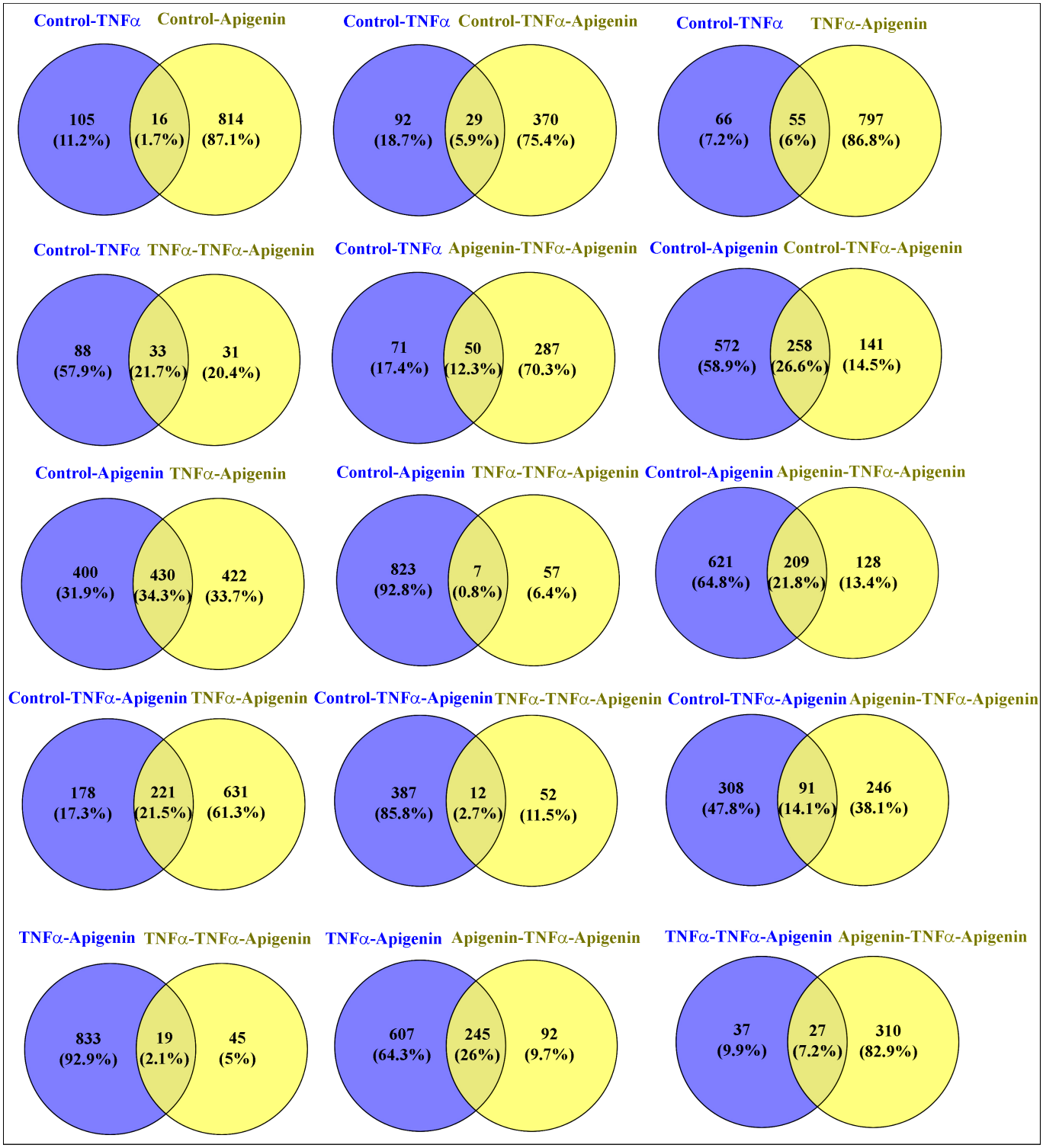


Fig. 3. The presentation of fifteen Venn Diagrams for identification of the dissimilar and shared genes in TNBC cell line dataset which are ordered per sets listed in Table 4, respectively (left to right).

cancer. The MDA-MB-231 cell line, known for its triple-negative breast cancer phenotype, is ideal for studying TNF-α's effects on cancer cells. TNF-α promotes tumor growth and invasion in these cells by activating signalling pathways and upregulating adhesion molecules. One of the key mechanisms by which TNF-α influences MDA-MB-231 cells is through the NF-κB signalling pathway, which drives the expression of genes associated with inflammation and cancer progression. Upon exposure to TNF-α, MDA-MB-231 cells show increased levels of adhesion molecules like VCAM-1 and ICAM-1, which enhance adhesion to endothelial cells and promote metastasis (42, 43). Additionally, TNF-α stimulates the release of matrix metalloproteinases (MMPs), particularly MMP-9, which degrade extracellular matrix components, facilitating tumor cell invasion (44, 45).

TNF-α induces epithelial-mesenchymal transition (EMT) in MDA-MB-231 cells, leading to the loss of epithelial markers and the retention of mesenchymal characteristics, a critical step in cancer progression (46). Additionally, cancer cells expressing vimentin, a protein linked to increased motility, exhibit greater responsiveness to TNF-α treatment, further supporting its role in promoting EMT (47). TNF-α significantly influences MDA-MB-231 cells and

the tumor microenvironment by fostering an inflammatory setting that accelerates tumor growth and enhances treatment resistance. TNF-α facilitates tumor progression and metastasis by increasing the secretion of pro-inflammatory cytokines, such as IL-6 (48, 49).The interaction of TNF-α with other inflammatory mediators complicates the breast cancer microenvironment, making it a key factor in promoting inflammation and enhancing cancer cell invasiveness. For instance, studies on MDA-MB-231 cells show that TNF-α activates the P2Y2 receptor, which in turn stimulates inflammasomes that contribute to tumor progression and radiation resistance (30). Furthermore, TNF-α has been found to induce the production of granulocyte-macrophage colony-stimulating factor (GM-CSF) in MDA-MB-231 cells, indicating its involvement in shaping a pro-tumorigenic environment while limiting cancer cell proliferation (50).

Several studies indicate that breast cancer is the most common malignancy in both developing and developed countries (51, 52). Researchers have extensively explored the inhibitory effects of flavonoid-containing plants, such as Quercetin, Cuminum and Tangeretin, using various *in vitro* and *in situ* methodologies (53-56). Six studies have specifically highlighted the anti-cancer properties of apigenin in the MDA-MB-231 cell line through experimental approaches (12, 16, 39, 57-59). Since early diagnosis of breast cancer is critical in effectively inhibiting the disease, investigating the effects of apigenin is particularly important.This study was designed to explore the inhibitory impact of apigenin on the TNBC cell line. A comprehensive systems biology approach was employed to examine various biological processes, molecular functions, cellular components and signaling pathways affected by the presence or absence of TNF-α with or without apigenin. An extensive assessment of 15 treatment variations demonstrated the anti-cancer effect of apigenin during the early and potentially intermediate stages of breast cancer, as confirmed by literature (12). However, apigenin's inhibitory effect may also extend to the expression of certain genes during advanced stages of breast cancer, as suggested by *in vivo* studies (58).The significant influence of TNF-α on the MDA-MB-231 cell line, which served as a control group for comparisons, was evident (shown in Table 4 and Fig. 3). Moreover, recent studies have emphasized the importance of early breast cancer diagnosis through a comprehensive systems biology-based approach, identifying key biomarkers at each stage of the disease (31).

Furthermore, the biomarkers identified in this study provided valuable insights into predicting the stage of breast cancer, as highlighted in another computational-based study (32). A notable advantage of the current research is its comprehensive analysis of the entire genome, unlike other studies that focused on the expression of some specific genes, as seen in the six previously mentioned studies. Our results indicated that Histone H4 acetylation plays a central role in development or inhibition of breast cancer by influencing the expression of p53 or p21 proteins. This finding aligns with previous studies that observed overexpression of Histone H4 (60, 61). Additionally, the positive regulation of GTPase pathways is of particular interest for breast cancer treatment, as supported by earlier research (62, 63). Among the various signalling pathways examined, the inositol phosphate metabolism pathway emerged as a significant factor associated with breast cancer and was the first major pathway identified through KEGG signaling in the study (64). The Phosphatidylinositol signaling system (i.e., PI3K signaling pathway) is well-recognized in the research community for its critical role in breast cancer, and computational approaches have demonstrated the potential positive effects of apigenin for breast cancer patients (65-67). Furthermore, since basal transcription factors are crucial in tumor growth and breast cancer development, targeting this pathway with apigenin is a significant aspect of its therapeutic potential (68).

Moreover, both experimental and computation research have demonstrated that apigenin has an inhibitory effect on Herpes simplex infection (69, 70). Additionally, the activation of nuclear factor-kappaB (NF-κB) has been observed in patients with breast cancer. The computational analysis in this study highlighted the significant inhibitory role of apigenin in this pro inflammatory transcription factor (71, 72). In conclusion, the results of this extensive investigation into inhibiting TNBC development and progression suggests that apigenin, a naturally occurring flavonoid, holds promise for future novel drug design and discovery.

Conclusion

In the current study, the dataset for triple-negative breast cancer (TNBC) using the MDA-MB-231 cell line was analyzed to evaluate the inhibitory effects of apigenin on cancerous samples. Six sample pairs from the dataset were pre- and post-processed to identify significant DEGs. These DEG's were then ranked and prioritized for further analysis of their enrichment in BP, CC, MF and KEGG signaling pathways. Essential and statistically significant hub genes among the 6 groups were determined. Additionally, 15 combinations from the possible paired sets of these 6 groups were examined to confirm the inhibitory effect of apigenin on breast cancer. Overall, apigenin demonstrated effective inhibition of cancer cell growth, which could aid in the development of novel therapies.

Acknowledgements

The authors would like to thank the Research Office of Tabriz University of Medical Sciences and acknowledge the Biotechnology Research Center for providing financial support under grant no. 66410.

Authors' contributions

EA carried out the gathering and analzying of studies and drafted the manuscript. SA participated in the design of the study and performed the statistical analysis. BS and SD conceived of the study and participated in its design and coordination as well as the analyses of data. All authors

read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

Ethical issues: None

References

1. Pérez-García J, Soberino J, Racca F, Gion M, Stradella A, Cortés J. Atezolizumab in the treatment of metastatic triple-negative breast cancer. Expert Opin Biol Ther. 2020;1-9. https://doi.org/10.1080/14712598.2020.1769063
2. He J, Peng T, Peng Y, Ai L, Deng Z, Wang X-Q, et al. Molecularly engineering triptolide with aptamers for high specificity and cytotoxicity for triple-negative breast cancer. J Am Chem Soc. 2020;142(6):2699-703. https://doi.org/10.1021/jacs.9b10510
3. Cleator S, Heller W, Coombes RC. Triple-negative breast cancer: therapeutic options. The Lancet Oncology. 2007;8(3):235-44. https://doi.org/10.1016/S1470-2045(07)70074-8
4. Hulamani S, Durgannavar NA. Triple negative breast cancer (TNBC). In: Rajenderan R, Mishra Sakun, Vhanalakar A, editors. Advances in and Humanities Research; 2020: p. 8-16.
5. Cruceriu D, Baldasici O, Balacescu O, Berindan-Neagoe I. The dual role of tumor necrosis factor-alpha (TNF-α) in breast cancer: molecular insights and therapeutic approaches. Cell Oncol. 2020;43(1):1-18. https://doi.org/10.1007/s13402-019-00489-1
6. Mahdavi Sharif P, Jabbari P, Razi S, Keshavarz-Fathi M, Rezaei N. Importance of TNF-alpha and its alterations in the development of cancers. Cytokine. 2020;130:155066. https://doi.org/10.1016/j.cyto.2020.155066
7. Tao X, Lipsky PE. The chinese anti-inflammatory and immunosuppressive herbal remedy *Tripterygium wilfordii* Hook *f*. Rheumatic Disease Clinics of North America. 2000;26(1):29-50. https://doi.org/10.1016/s0889-857x(05)70118-6
8. Wang J, Yang X, Han H, Wang L, Bao W, Wang S, et al. Inhibition of growth and metastasis of triple-negative breast cancer targeted by traditional chinese medicine tubeimu in orthotopic mice models. Chinese Journal of Cancer Research = Chung-Kuo Yen Cheng Yen Chiu. 2018;30(1):112-21. https://doi.org/10.21147/j.issn.1000-9604.2018.01.12
9. Marshall ML, Fung KY, Jans DA, Wagstaff KM. Tumour-specific phosphorylation of serine 419 drives alpha-enolase (ENO1) nuclear export in triple negative breast cancer progression. Cell Biosci.2024;14(1):74. https://doi.org/10.1186/s13578-024-01249-x
10. Won KA, Spruck C. Triple⊠negative breast cancer therapy: Current and future perspectives (Review). Int J Oncol. 2020;57(6):1245-61. https://doi.org/10.3892/ijo.2020.5135
11. Su YY, Liu YL, Huang HC, Lin CC. Ensemble learning model for identifying the hallmark genes of NFkappaB/TNF signaling pathway in cancers. J Transl Med. 2023;21(1):485. https://doi.org/10.1186/s12967-023-04355-5
12. Bauer D, Mazzio E, Soliman KFA. Whole transcriptomic analysis of apigenin on TNFα immuno-activated MDA-MB-231 breast cancer cells. Cancer Genomics and Proteomics. 2019;16(6):421-32. https://doi.org/10.21873/cgp.20146
13. Bauer D, Mazzio E, Hilliard A, Oriaku ET, Soliman KFA. Effect of apigenin on whole transcriptome profile of TNFalpha-activated MDA-MB-468 triple negative breast cancer cells. Oncol Lett. 2020;19(3):2123-32. https://doi.org/10.3892/ol.2020.11327
14. Lee HH, Jung J, Moon A, Kang H, Cho H. Antitumor and anti-invasive effect of apigenin on human breast carcinoma through suppression of IL-6 expression. Int J Mol Sci. 2019;20(13):3143. https://doi.org/10.3390%2Fijms20133143
15. Kabała-Dzik A, Rzepecka-Stojko A, Kubina R, Iriti M, Wojtyczka RD, Buszman E, et al. Flavonoids, bioactive components of propolis, exhibit cytotoxic activity and induce cell cycle arrest and apoptosis in human breast cancer cells MDA-MB-231 and MCF-7 – A comparative study. Cellular and Molecular Biology. 2018;64(8):1-10. https://dx.doi.org/10.14715/cmb/2018.64.8.1
16. Lee HH, Cho H. Anti-cancer effect of apigenin on human breast carcinoma MDA-MB-231 through cell cycle arrest and apoptosis. Microbiology and Biotechnology Letters. 2019;47(1):34-42. https://doi.org/10.3390/ijms25105569
17. Golonko A, Olichwier AJ, Szklaruk A, Paszko A, Swislocka R, Szczerbinski L, et al. Apigenin's modulation of doxorubicin efficacy in breast cancer. Molecules. 2024;29(11):2603. https://doi.org/10.3390/molecules29112603
18. Madunić J, Madunić IV, Gajski G, Popić J, Garaj-Vrhovac V. Apigenin: A dietary flavonoid with diverse anti-cancer properties. Cancer Lett. 2018;413:11-22. https://doi.org/10.1016/j.canlet.2017.10.041
19. Sung B, Chung HY, Kim ND. Role of apigenin in cancer prevention via the induction of apoptosis and autophagy. Journal of Cancer Prevention. 2016;21(4):216. https://doi.org/10.15430/jcp.2016.21.4.216
20. Park C-H, Min S-Y, Yu H-W, Kim K, Kim S, Lee H-J, et al. Effects of apigenin on RBL-2H3, RAW264.7 and HaCaT cells: anti-allergic, anti-inflammatory and skin-protective activities. Int J Mol Sci. 2020;21(13):4620. https://doi.org/10.3390/ijms21134620
21. Wang M, Firrman J, Liu L, Yam K. A review on flavonoid apigenin: Dietary intake, ADME, antimicrobial effects and interactions with human gut microbiota. BioMed Research International. 2019;2019. https://doi.org/10.1155/2019/7010467
22. Wang W, Yue RF, Jin Z, He LM, Shen R, Du D, et al. Efficiency comparison of apigenin-7-O-glucoside and trolox in antioxidative stress and anti-inflammatory properties. J Pharm Pharmacol. 2020. https://doi.org/10.1111/jphp.13347
23. Tong X, Pelling JC. Targeting the PI3K/Akt/mTOR axis by apigenin for cancer prevention. Anti-cancer Agents Med Chem. 2013;13(7):971-78. https://doi.org/10.2174/18715206113139990119
24. Xu M, Wang S, Song Y, Yao J, Huang K, Zhu X. Apigenin suppresses colorectal cancer cell proliferation, migration and invasion via inhibition of the Wnt/β-catenin signaling pathway. Oncol Lett. 2016;11(5):3075-80. https://doi.org/10.3892/ol.2016.4331
25. Cao H-H, Chu J-H, Kwan H-Y, Su T, Yu H, Cheng C-Y, et al. Inhibition of the STAT3 signaling pathway contributes to apigenin-mediated anti-metastatic effect in melanoma. Sci Rep. 2016;6(1):1-12. https://doi.org/10.1038/srep21731
26. Melaibari M, Alkreathy HM, Esmat A, Rajeh NA, Shaik RA, Alghamdi AA, et al. Anti-fibrotic efficacy of apigenin in a mice model of carbon tetrachloride-induced hepatic fibrosis by modulation of oxidative stress, inflammation and fibrogenesis: A preclinical study. Biomedicines. 2023;11(5):1342. https://doi.org/10.3390/biomedicines11051342
27. Mendonca P, Alghamdi S, Messeha S, Soliman KFA. Pentagalloyl glucose inhibits TNF-alpha-activated CXCL1/GRO-alpha expression and induces apoptosis-related genes in triple-negative breast cancer cells. Sci Rep. 2021;11(1):5649. https://doi.org/10.1038/s41598-021-85090-z
28. Ahmed SA, Mendonca P, Messeha SS, Oriaku ET, Soliman KFA. The anti-cancer effects of marine carotenoid fucoxanthin through phosphatidylinositol 3-kinase (PI3K)-AKT signaling on triple-negative breast cancer cells. Molecules. 2023;29(1):61. https://doi.org/10.3390/molecules29010061

29. Truong VN, Nguyen YT, Cho SK. Ampelopsin suppresses stem cell properties accompanied by attenuation of oxidative phosphorylation in chemo- and radio-resistant MDA-MB-231 breast cancer cells. Pharmaceuticals (Basel). 2021;14(8):794. https://doi.org/10.3390/ph14080794

30. Jin H, Ko YS, Kim HJ. P2Y2R-mediated inflammasome activation is involved in tumor progression in breast cancer cells and in radiotherapy-resistant breast cancer. Int J Oncol. 2018;53 (5):1953-66. https://doi.org/10.3892/ijo.2018.4552

31. Amjad E, Asnaashari S, Sokouti B, Dastmalchi S. Systems biology comprehensive analysis on breast cancer for identification of key gene modules and genes associated with TNM-based clinical stages. Sci Rep. 2020;10(1):10816. https://doi.org/10.1038/s41598-020-67643-w

32. Amjad E, Asnaashari S, Sokouti B, Dastmalchi S. Impact of gene biomarker discovery tools based on protein–protein interaction and machine learning on performance of artificial intelligence models in predicting clinical stages of breast cancer. Interdisciplinary Sciences: Computational Life Sciences. 2020. https://doi.org/10.1007/s12539-020-00390-8

33. Carvalho B. pd.hugene.1.0.st.v1: Platform Design Info for Affymetrix HuGene-1_0-st-v1. R package. 2015. https://www.bioconductor.org/packages/release/data/annotation/html/pd.hugene.1.0.st.v1.html

34. Huang da W, Sherman BT, Lempicki RA. Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. Nat Protoc. 2009;4(1):44-57. https://doi.org/10.1038/nprot.2008.211

35. Huang da W, Sherman BT, Lempicki RA. Bioinformatics enrichment tools: paths toward the comprehensive functional analysis of large gene lists. Nucleic Acids Res. 2009;37(1):1-13. https://doi.org/10.1093/nar/gkn923

36. Zhou G, Soufan O, Ewald J, Hancock REW, Basu N, Xia J. NetworkAnalyst 3.0: a visual analytics platform for comprehensive gene expression profiling and meta-analysis. NAR. 2019;47 (W1):W234-W41. https://doi.org/10.1093/nar/gkz240

37. Xia J, Gill EE, Hancock REW. NetworkAnalyst for statistical, visual and network-based meta-analysis of gene expression data. Nat Protoc. 2015;10(6):823-44. https://doi.org/10.1038/nprot.2015.052

38. Oliveros J. 2007–2015. Venny. An interactive tool for comparing lists with Venn's diagrams. 2017. https://bioinfogp.cnb.csic.es/tools/venny/

39. Tseng TH, Chien MH, Lin WL, Wen YC, Chow JM, Chen CK, et al. Inhibition of MDA-MB-231 breast cancer cell proliferation and tumor growth by apigenin through induction of G2/M arrest and histone H3 acetylation-mediated p21(WAF1/CIP1) expression. Environmental Toxicology. 2017;32(2):434-44. https://doi.org/10.1002/tox.22247

40. Nabavi SM, Habtemariam S, Daglia M, Nabavi SF. Apigenin and breast cancers: From chemistry to medicine. Anti-Cancer Agents in Medicinal Chemistry. 2015;15(6):728-35. https://doi.org/10.2174/1871520615666150304120643

41. Huang C, Wei YX, Shen MC, Tu YH, Wang CC, Huang HC. Chrysin, abundant in *Morinda citrifolia* fruit water-EtOAc extracts, combined with apigenin synergistically induced apoptosis and inhibited migration in human breast and liver cancer cells. Journal of Agricultural and Food Chemistry. 2016;64(21):4235-45. https://doi.org/10.1021/acs.jafc.6b00766

42. Evani SJ, Prabhu RG, Gnanaruban V, Finol EA, Ramasubramanian AK. Monocytes mediate metastatic breast tumor cell adhesion to endothelium under flow. FASEB Journal : Official Publication of the Federation of American Societies for Experimental Biology. 2013;27(8):3017-29. https://doi.org/10.1096%2Ffj.12-224824

43. Montemagno C, Dumas L, Cavailles P, Ahmadi M, Bacot S, Debiossat M, et al. *In vivo* assessment of VCAM-1 expression by SPECT/CT imaging in mice models of human triple negative breast cancer. Cancers (Basel). 2019;11(7):1039. https://doi.org/10.3390/cancers11071039

44. Hong OY, Jang HY, Lee YR, Jung SH, Youn HJ, Kim JS. Inhibition of cell invasion and migration by targeting matrix metalloproteinase-9 expression via sirtuin 6 silencing in human breast cancer cells. Sci Rep. 2022;12(1):12125. https://doi.org/10.1038/s41598-022-16405-x

45. Zhao X, Sun X, Gao F, Luo J, Sun Z. Effects of ulinastatin and docataxel on breast tumor growth and expression of IL-6, IL-8, and TNF-α. Journal of Experimental and Clinical Cancer Research. 2011;30(1):22. https://doi.org/10.1186/1756-9966-30-22

46. Choi YK, Cho SG, Woo SM, Yun YJ, Jo J, Kim W, et al. *Saussurea lappa* Clarke-derived costunolide prevents TNF α- induced breast cancer cell migration and invasion by inhibiting NF- κB activity. Evidence-Based Complementary and Alternative Medicine:eCAM.2013;2013:936257. https://doi.org/10.1155/2013/936257

47. Lee J, Hahm ER, Marcus AI, Singh SV. Withaferin A inhibits experimental epithelial-mesenchymal transition in MCF-10A cells and suppresses vimentin protein level *in vivo* in breast tumors. Molecular Carcinogenesis. 2015;54(6):417-29. https://doi.org/10.1002%2Fmc.22110

48. El Hasasna H, Saleh A, Al Samri H, Athamneh K, Attoub S, Arafat K, et al. *Rhus coriaria* suppresses angiogenesis, metastasis and tumor growth of breast cancer through inhibition of STAT3, NFκB and nitric oxide pathways. Sci Rep. 2016;6:21144. https://doi.org/10.1038/srep21144

49. Geng Y, Chandrasekaran S, Hsu JW, Gidwani M, Hughes AD, King MR. Phenotypic switch in blood: effects of pro-inflammatory cytokines on breast cancer cell aggregation and adhesion. PloS One. 2013;8(1):e54959. https://doi.org/10.1371%2Fjournal.pone.0054959

50. Thomas R, Al-Rashed F, Akhter N, Al-Mulla F, Ahmad R. ACSL1 regulates TNFalpha-induced GM-CSF production by breast cancer MDA-MB-231 cells. Biomolecules. 2019;9(10):555. https://doi.org/10.3390/biom9100555

51. Mungrue K, Ramdath J, Ali S, Cuffie WA, Dodough N, Gangar M, et al. Challenges to the control of breast cancer in a small developing country. Breast Cancer: Basic and Clinical Research. 2014;8(1):7-13. https://doi.org/10.4137/bcbcr.s12780

52. Feng XY, Lu S, Hao XS, Liu H. Breast cancer screening: Review of history and current status in western developed countries. Tumor. 2015;35(4):453-60 and 66. http://dx.doi.org/10.3781/j.issn.1000-7431.2015.55.746

53. Song YK, Yoon JH, Woo JK, Kang JH, Lee KR, Oh SH, et al. Quercetin is a flavonoid breast cancer resistance protein inhibitor with an impact on the oral pharmacokinetics of sulfasalazine in rats. Pharmaceutics. 2020;12(5). https://doi.org/10.3390/pharmaceutics12050397

54. Goodarzi S, Tabatabaei MJ, Mohammad Jafari R, Shemirani F, Tavakoli S, Mofasseri M, et al. *Cuminum cyminum* fruits as source of luteolin- 7-O-glucoside, potent cytotoxic flavonoid against breast cancer cell lines. Nat Prod Res. 2020;34(11):1602-06. https://doi.org/10.1080/14786419.2018.1519824

55. Ko YC, Choi HS, Liu R, Kim JH, Kim SL, Yun BS, et al. Inhibitory effects of tangeretin, a citrus peel-derived flavonoid, on breast cancer stem cell formation through suppression of Stat3 signaling. Molecules (Basel, Switzerland). 2020;25(11). https://doi.org/10.3390/molecules25112599

56. Abd Razik BA, Ezzat MO, Al-Shohani ADH. Molecular docking and design study for anti-cancer activity of flavonoid derivatives

against breast cancer. Indian Drugs. 2020;57(4):7-14. http://doi.org/10.53879/id.57.04.12202

57. Hong J, Fristiohady A, Nguyen CH, Milovanovic D, Huttary N, Krieger S, et al. Apigenin and luteolin attenuate the breaching of MDA-MB231 breast cancer spheroids through the lymph endothelial barrier *in vitro*. Front Pharmacol. 2018;9(MAR). https://doi.org/10.3389/fphar.2018.00220

58. Bauer D, Redmon N, Mazzio E, Soliman KF. Apigenin inhibits TNFα/IL-1α-induced CCL2 release through IKBK-epsilon signaling in MDA-MB-231 human breast cancer cells. PLoS One. 2017;12(4). https://doi.org/10.1371/journal.pone.0175558

59. Lee WJ, Chen WK, Wang CJ, Lin WL, Tseng TH. Apigenin inhibits HGF-promoted invasive growth and metastasis involving blocking PI3K/Akt pathway and β4 integrin function in MDA-MB-231 breast cancer cells. Toxicol Appl Pharmacol. 2008;226(2):178-91. https://doi.org/10.1016/j.taap.2007.09.013

60. Nandy D, Rajam SM, Dutta D. A three layered histone epigenetics in breast cancer metastasis. Cell Biosci. 2020;10:1-23. https://doi.org/10.1186/s13578-020-00415-1

61. Long M, Sun X, Shi W, Yanru A, Leung ST, Ding D, et al. A novel histone H4 variant H4G regulates rDNA transcription in breast cancer. NAR. 2019;47(16):8399-409. https://doi.org/10.1093/nar/gkz547

62. Prieto-Dominguez N, Parnell C, Teng Y. Drugging the small GTPase pathways in cancer treatment: promises and challenges. Cells. 2019;8(3):255. https://doi.org/10.3390/cells8030255

63. Haga RB, Ridley AJ. Rho GTPases: Regulation and roles in cancer cell biology. Small GTPases. 2016;7(4):207-21. https://doi.org/10.1080%2F21541248.2016.1232583

64. Tan J, Yu C-Y, Wang Z-H, Chen H-Y, Guan J, Chen Y-X, et al. Genetic variants in the inositol phosphate metabolism pathway and risk of different types of cancer. Sci Rep. 2015;5:8473. https://doi.org/10.1038/srep08473

65. Hernandez-Aya LF, Gonzalez-Angulo AM. Targeting the phosphatidylinositol 3-kinase signaling pathway in breast cancer. The Oncologist. 2011;16(4):404-14. https://doi.org/10.1634/theoncologist.2010-0402

66. Miller TW, Rexer BN, Garrett JT, Arteaga CL. Mutations in the phosphatidylinositol 3-kinase pathway: role in tumor progression and therapeutic implications in breast cancer. Breast Cancer Research: BCR. 2011;13(6):224. https://doi.org/10.1186/bcr3039

67. Fry MJ. Phosphoinositide 3-kinase signalling in breast cancer: how big a role might it play? Breast Cancer Res. 2001;3(5):304. https://doi.org/10.1186/bcr312

68. Farman FU, Haq F, Muhammad N, Ali N, Rahman H, Saeed M. Aberrant promoter methylation status is associated with upregulation of the E2F4 gene in breast cancer. Oncol Lett. 2018;15(6):8461-69. https://doi.org/10.3892/ol.2018.8382

69. Simon JW. The association of Herpes simplex virus and cervical cancer: A review. Gynecol Oncol. 1976;4(1):108-16. https://doi.org/10.1016/0090-8258(76)90011-1

70. Li M, Guo Y, Feng Y-M, Zhang N. Identification of triple-negative breast cancer genes and a novel high-risk breast cancer prediction model development based on PPI data and support vector machines. Frontiers in Genetics. 2019;10(180). https://doi.org/10.3389/fgene.2019.00180

71. Wang W, Nag SA, Zhang R. Targeting the NFκB signaling pathways for breast cancer prevention and therapy. Curr Med Chem. 2015;22(2):264-89. https://doi.org/ 10.2174/0929867321666141106124315

72. Smith SM, Lyu YL, Cai L. NF-κB affects proliferation and invasiveness of breast cancer cells by regulating CD44 expression. PLoS One. 2014;9(9):e106966. https://doi.org/10.1371/journal.pone.0106966