Original Article

Combined Resveratrol and Curcumin Treatment Suppresses MCF-7 Breast Cancer Cell Growth via TNF-α/NF-κB Pathway Regulation

Xiaodan Fan, Jue Zhu，Yuhui Sun，Huan Chen，Liang Chen，Jing Zhang，Yichen Chen

DOI: 10.34172/PS.026.43448

To appear in: Pharmaceutical Science (https://pstbzmed.com/)

Received date: 3 Nov 2025

Revised date: 23 Feb 2026

Accepted date: 23 Feb 2026

Please cite this article as: Bagheri N, Abolhassani Y, Rassouli FB, Jamialahmadi K. Combined resveratrol and curcumin treatment suppresses MCF-7 breast cancer cell growth via TNF-α/NF-κB pathway regulation. Pharm Sci. 2026. doi: 10.34172/PS.026.43448

This is a PDF file of a manuscript that have been accepted for publication. It is assigned to an issue after technical editing, formatting for publication and author proofing.

Combined Resveratrol and Curcumin Treatment Suppresses MCF-7 Breast Cancer Cell Growth via TNF-α/NF-κB Pathway Regulation

Xiaodan Fan[1 2], Jue Zhu[1 2], Yuhui Sun[1], Huan Chen[2 3], Liang Chen[1 2], Jing Zhang[2*], Yichen Chen[1*]

1. Central Laboratory, Women and Children's Hospital of Ningbo University, Ningbo, China.
2. Department of Gynecology, Women and Children's Hospital of Ningbo University, Ningbo, China.
3. Department of Medicine, Ningbo University, Ningbo, China

* Corresponding authors: https://orcid.org/0000-0002-8954-9870, zhangjyy1978@163.com, E-mail: https://orcid.org/0000-0001-5726-9383, cyc_0605@hotmail.com

Abstract

Background: Breast cancer remains a leading malignancy in women, with limited therapeutic options. Natural compounds such as resveratrol and curcumin exhibit anticancer properties, but their combined mechanisms are not fully understood. This study investigated the synergistic effects of resveratrol and curcumin in MCF-7 breast cancer cells.

Methods: Optimal drug concentrations were determined by CCK-8 assay, followed by flow cytometry to assess apoptosis in the four treatment groups (control, curcumin, resveratrol, and combination). Transcriptome sequencing and Western blotting were performed to identify molecular mechanisms. In parallel, a network pharmacology approach was applied by integrating TNBC-related DEGs from GSE38959 with predicted drug targets, followed by KEGG and GO enrichment analysis.

Results: The combination of 200 μM resveratrol and 20 μM curcumin significantly inhibited cell growth (73.60 ± 6.91% inhibition, $P < 0.001$ vs. control) and increased apoptosis (52.80% apoptotic cells, $P < 0.001$ vs. single agents). RNA-seq and enrichment analysis revealed activation of the TNF signaling pathway and modulation

of the NF-κB signaling pathway in the combination treatment group. Network pharmacology further confirmed the convergence of curcumin and resveratrol targets on the TNF/NF-κB axis, which supported the transcriptomic findings. Western blot validation showed down-regulation of NF-κB, TNF-α, and c-Myc, along with up-regulation of Cleaved caspase-3($P < 0.05$ for all), reinforcing the observed effects from RNA-seq and network pharmacology analysis.

Conclusions: Combined resveratrol and curcumin treatment synergistically induces apoptosis in MCF-7 cells via the TNF-α/NF-κB/c-Myc axis, supporting their potential as a complementary therapy for breast cancer.

Keywords: apoptosis, breast cancer, curcumin, MCF-7, NF-κB signaling pathway, resveratrol

1. Introduction

Breast cancer is one of the most common malignant tumors among Chinese women, characterized by strong invasiveness, high malignancy, and poor prognosis[1]. In recent years, the incidence rate of breast cancer has gradually increased, and its incidence rate and death rate rank first among malignant tumors in Chinese women[2]. Treatment options for breast cancer include surgery, chemotherapy, radiation, and targeted therapy; nevertheless, these modalities often entail several adverse effects throughout treatment, including bone marrow suppression, drug resistance, and metastatic recurrence[3]. Consequently, there is a need for further therapeutic interventions or pharmaceuticals aimed at breast cancer.

In recent years, natural plant-derived compounds have shown significant results in the treatment of cancer, such as luteolin[4-6]. For the treatment of breast cancer, these two natural compounds have obvious advantages in the research of anti-breast cancer: resveratrol and curcumin[7-8]. Resveratrol is a stilbene analog that exists in large quantities in grapes, berries, and other plants. It has been found that resveratrol can

intervene in the occurrence and development of breast cancer through a variety of pathways, including the induction of apoptosis, blocking the cell cycle, and the modulation of autophagy and glycolysis, as well as the reversal of breast cancer's resistance to chemotherapy and radiotherapy[9-10]. Curcumin, as a polyphenol extracted from the rhizome of the traditional Chinese medicine turmeric, has demonstrated significant anti-inflammatory, anti-oxidant, and anti-tumor effects in vitro and vivo studies[11-13]. And it has the advantages of being widely available, inexpensive, safe, and having fewer adverse effects than radiotherapy[14-15]. The outcomes clearly indicate that resveratrol and curcumin may independently influence the prevention and progression of breast cancer. While both substances have been documented for independent use in breast cancer, many elements of traditional Chinese medicine are often used in conjunction throughout therapy. The impact of these two components on breast cancer therapy remains ambiguous, whether synergistic or antagonistic.

Despite advances in therapy, breast cancer management remains challenged by treatment toxicity, drug resistance, and disease recurrence, necessitating safer adjunctive approaches. Natural compounds such as resveratrol and curcumin exhibit individual anticancer properties; however, the mechanistic nature of their combined effects—whether synergistic, additive, or antagonistic—is not well defined. We hypothesize that co-treatment with resveratrol and curcumin may provide a synergistic advantage by concurrently targeting interconnected pro-survival signaling pathways, notably the TNF-α/NF-κB axis. To systematically decipher this interaction, we integrate in vitro validation in MCF-7 breast cancer cells with multi-omics analysis and network pharmacology. This study aims to elucidate the synergistic mechanism of resveratrol and curcumin combination therapy, offering a rationale for developing novel natural compound-based strategies against breast cancer.

2. Materials and methods

2.1 Integration Analysis of Network Pharmacology and Metabolomics

By comparing the potential pathways enriched by the network pharmacological targets with the metabolic pathways enriched by differential metabolites, the common metabolic pathways that were regarded as the key pathways were screened out. The KEGG database was used to obtain the proteins contained in the key metabolic pathways to screen the key targets from the potential targets of network pharmacology. Through the literature research to reveal the possible mechanism of resveratrol and curcumin in the treatment of breast cancer.

2.2. Cell Culture and Reagents

The MCF-7 cell line in this study was obtained from the Chinese Academy of Sciences (CAS) Cell Bank and cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1 % penicillin/streptomycin (PS) at 37°C, 5% CO2. Resveratrol and curcumin were purchased from Shanghai Yuanye Bio-Technology Company, dissolved in dimethyl sulfoxide (DMSO), and used at multiple indicated concentrations.

2.3. Cell Proliferation-toxicity Test

MCF-7 cells were seeded in 96-well plates with about 1×10^4 cells per well. 200 microliters of DMEM with 10% FBS and 1% PS were added to each well. After overnight incubation, cells were treated with different individual and combined drug concentrations under cell culture conditions as described above for 24h. Using the Cell Counting Kit-8 (CCK8) assay(1:10, Biosharp, China) measured the dose-dependent effect of resveratrol and curcumin on cell viability in MCF-7 cells. We first identified the IC_{50} values for resveratrol (~200 μM) and curcumin (~20 μM) in MCF-7 cells. To test for synergy, we chose: Sub-optimal doses (100 μM Res + 20 μM Cur) to assess whether lower resveratrol could still enhance curcumin's effect; Dose-reversal combinations (200 μM Res + 10 μM Cur) to examine whether curcumin could be reduced while maintaining efficacy; IC_{50}-based combination (200 μM Res + 20 μM Cur) as the maximum tolerated dose without excessive cytotoxicity to normal cells.

2.4. Flow Cytometry

MCF cells were cultured and divided equally into four groups, which were incubated with DMSO (Con group), 200um resveratrol (Res group), 20um curcumin (Cur group), 200um resveratrol and 20um curcumin (Dual group) for 24h, then digested and collected all the cells. Then count the cells and adjust to a desired concentration (10 million cells per mL). 10 µL of Annexin V-FITC and 5 µL of Propidium iodide (PI) (Multi Sciences, China) were added to each tube. Finally, use flow cytometry software to analyze the results and identify populations of live, early apoptotic, late apoptotic, and necrotic cells based on the staining patterns.

2.5. Network Pharmacology Analysis

The transcriptomic dataset GSE38959, comprising triple-negative breast cancer (TNBC) and normal breast tissue samples, was downloaded from the Gene Expression Omnibus (GEO) database. Differential expression analysis was performed using the limma package (version 3.54.2) in R (version 4.2.2). Genes with an adjusted p value < 0.05 and $|\log2 \text{ fold change}| > 1$ were defined as differentially expressed genes (DEGs). A volcano plot was generated to visualize the overall distribution of DEGs.

Potential molecular targets of resveratrol and curcumin were retrieved from multiple pharmacological databases, including the Traditional Chinese Medicine Systems Pharmacology Database (TCMSP), SwissTargetPrediction, and DrugBank. The intersecting genes between compound-related targets and TNBC-associated DEGs were identified as candidate therapeutic targets.

Functional enrichment analyses of overlapping genes were conducted using the clusterProfiler package (version 4.6.2). Gene Ontology (GO) enrichment covered biological processes (BP), cellular components (CC), and molecular functions (MF), while Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis was applied to reveal the signaling pathways potentially regulated by resveratrol and curcumin. Results were visualized using R packages including ggplot2 and enrichplot.

2.6. RNA Sequencing (RNA-seq) and Differential Gene Bioinformatics Analysis

Following 24 h of selected doses of resveratrol and curcumin treatment in MCF-7 cells. Total RNA was isolated using Trizol(Takara, Japan), then the first-strand cDNA was transcribed by using iScript cDNA Synthesis Kit(Takara, Japan). Normalized RNA-seq expression data were analyzed by using reference genome comparison, KEGG/GO enrichment analysis, GSEA analysis, and so on.

2.7. Western Blot

The total cells were lysed with RIPA buffer supplemented with proteinase inhibitors. The protein concentrations were measured using the BCA protein assay kit(Thermo Fisher Scientific, USA). Protein samples were separated on a 10% SDS-PAGE electrophoresis gel and then transferred onto polyvinylidene fluoride (PVDF) membranes. The membranes were then blocked in skim milk solution for 1 hour at room temperature and then incubated overnight with primary antibodies at 4°C. The following primary antibodies were used in this blotting: anti-NF-κB (ab16502, Lot: GR308937-1, 1:1000, Abcam, Cambridge, UK), anti-TNF-α(AF7041, 1:1000, Affinity Biosciences, USA), anti-Cleaved caspase-3(ab32351, Lot:1093798-85, 1:5000, Abcam, Cambridge, UK), anti-c-Myc(AF6054, Lot:#34b1408, 1:1000, Affinity Biosciences, USA) and anti-β-ACTIN(AF7018, lot:#12w2944, 1:3000, Affinity Biosciences, USA). The next day, after washing the membranes with TBST, the membranes were incubated with appropriate horseradish peroxidase (HRP-) labeled secondary antibodies(Cat No.:RGAR001, Lot: 20001572, 1:5000, Proteintech, USA) at room temperature for 1h. Finally, the membranes were washed before being analyzed using an electrophoresis gel imaging system.

2.8. Statistical Analysis

Statistical analyses were performed using GraphPad Prism version 6.0 (GraphPad Software, USA) for graphical presentation and intergroup comparisons via two-tailed Student’s t-test, and SPSS version 20.0 (IBM Corp., USA) for data management and

descriptive statistics. Since the analyses focused on pairwise comparisons between each treatment group and the control group, two-tailed Student's t-tests were applied. For future multi-group comparisons, one-way ANOVA with appropriate post-hoc tests is recommended. The qualification of results was presented as mean ± standard deviation. $P < 0.05$ was considered statistically significant.

3. Result

3.1. Combination of Resveratrol and Curcumin significantly inhibits MCF-7 cell growth

To determine the optimal concentration of resveratrol and curcumin, cell viability was assessed by CCK-8 assay after 24 h treatment of MCF-7 cells with different concentrations of each compound alone or in combination. Treatment groups included control, resveratrol (100, 200, and 400 µM), curcumin (10, 20, and 40 µM), and three combination regimens (100 µM resveratrol + 20 µM curcumin, 200 µM resveratrol + 10 µM curcumin, and 200 µM resveratrol + 20 µM curcumin). Cell growth inhibition rates were calculated relative to the control group.

Both resveratrol and curcumin inhibited MCF-7 cell proliferation in a dose-dependent manner, with resveratrol showing greater potency. However, the highest concentrations (400 µM resveratrol and 40 µM curcumin) also markedly suppressed the growth of normal breast epithelial cells, and were therefore excluded from further combination studies. Among the three combined treatment groups, the regimen of 200 µM resveratrol with 20 µM curcumin exhibited the strongest effect, reducing cell growth by 73.60% ± 6.91 (N = 3), while the other two combinations achieved inhibition rates of 45.48% ± 10.32 (N = 3) and 45.64% ± 3.32 (N = 3), respectively (Figure 1). Based on these results, the combination of 200 µM resveratrol and 20 µM curcumin was selected for subsequent experiments.

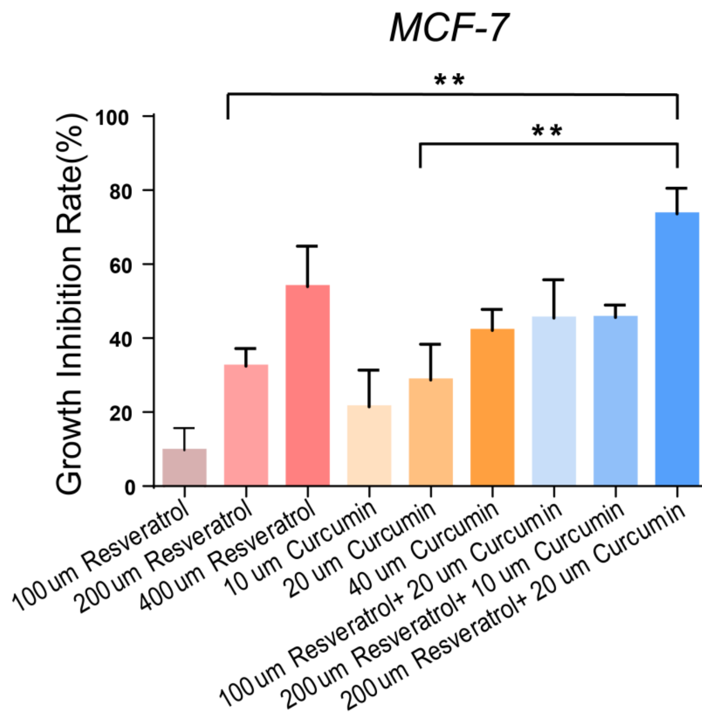

Figure1. Dose-dependent inhibitory effects of resveratrol and curcumin (24h) on MCF7 cells using CCK8 assay based on measurement of optical density (OD450). Growth inhibition rate was calculated based on the control group. The date in the figure was expressed by mean±SD in SPSS. Comparison between the two groups was analyzed by a t-test in GraphPad Prism. n=3, **P < 0.01.

3.2. Combination of resveratrol and curcumin enhances apoptosis in MCF-7 breast cancer cells

To evaluate the pro-apoptotic effects of resveratrol and curcumin, MCF-7 cells were divided into four groups (control, resveratrol, curcumin, and combination) and treated for 24 h. Apoptosis was then quantified by flow cytometry (Figure 2).

Both resveratrol and curcumin alone significantly increased apoptosis compared with the control group, with total (early + late) apoptotic rates of 27.37% and 35.61%, respectively, versus 11.19% in controls. Notably, the combination treatment induced a much stronger apoptotic response, with a total apoptotic rate of 52.80%, which was

significantly higher than control ($p < 0.0005$), resveratrol alone ($p < 0.0005$), and curcumin alone ($p < 0.001$). These results demonstrate that combined resveratrol and curcumin treatment exerts a synergistic pro-apoptotic effect in MCF-7 breast cancer cells, exceeding the efficacy of either agent alone.

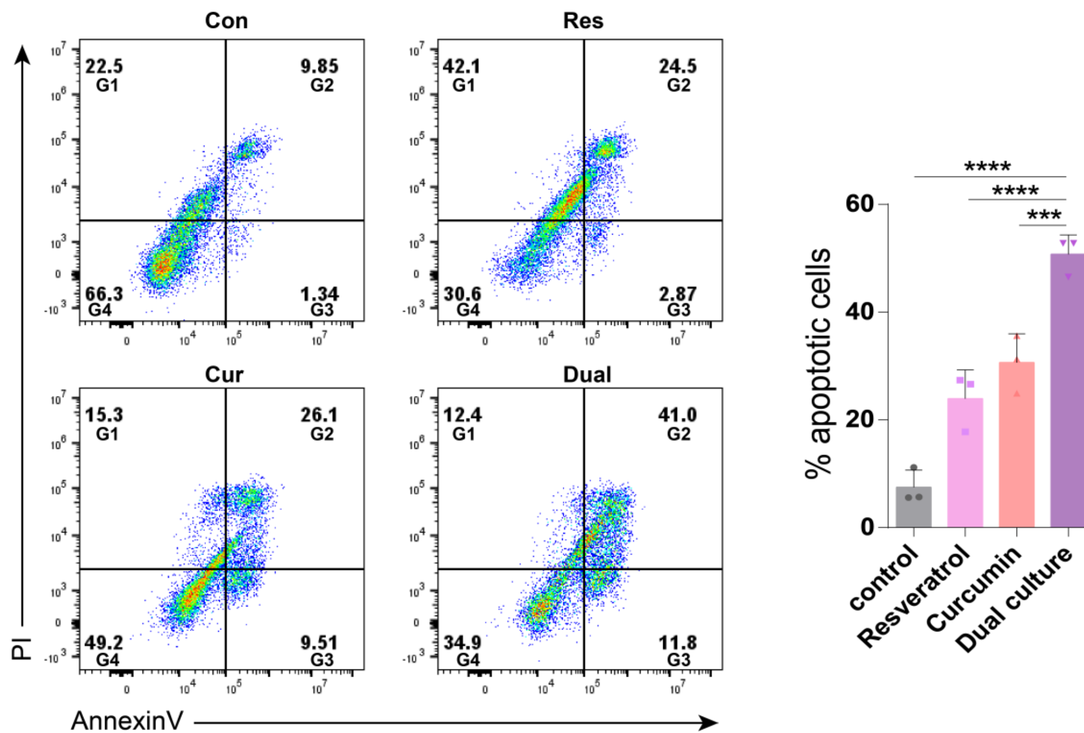

Figure2. Flow cytometry validation of apoptosis in four groups of MCF-7 cells. G2: Late apoptotic and necrotic cells; G3: Early apoptotic cells; G4: live cells. %apoptotic cells: G2+G3. The date in the figure was expressed by mean±SD in SPSS. Comparison between two groups was analyzed by one-way ANOVA in GraphPad Prism. n=3, ***$P < 0.001$, ****$P < 0.0005$. The control group (Con) received the DMSO vehicle only, and all statistical comparisons of drug effects are made relative to this baseline.

3.3. Combination of resveratrol and curcumin up-regulated the TNF signaling pathway in MCF-7 cells

To investigate the mechanism underlying the combined effect of resveratrol and curcumin on breast cancer cells, RNA was extracted from four groups of treated MCF-

7 cells and subjected to transcriptome sequencing. After normalization, differentially expressed genes (DEGs) were identified by comparing each treatment with the control group. PCA and DEG distribution plots revealed that the transcriptomic profiles of the control and curcumin groups, as well as the resveratrol and dual-treatment groups, were relatively similar; in contrast, substantial differences were observed between the control and dual-treatment groups (Fig. 3A–B). This suggested that resveratrol exerted a greater transcriptional effect and that its impact was further amplified under the combined treatment.

Heatmap visualization and KEGG enrichment analysis of DEGs between the control and dual groups revealed the top 20 enriched pathways (Fig. 3C–D). Notably, the TNF signaling pathway and NF-κB signaling pathway were significantly up-regulated in the dual-treatment group, with several representative genes highlighted in the volcano plot (Fig. 3E). Although NF-κB activation via TNF is typically associated with cell survival, this observation contrasted with the flow cytometry results (Fig. 2), which demonstrated increased apoptosis following combined treatment. These findings suggest that curcumin, despite exerting a modest individual transcriptional effect, may counteract NF-κB–mediated pro-survival signaling when combined with resveratrol, thereby promoting apoptosis in MCF-7 cells.

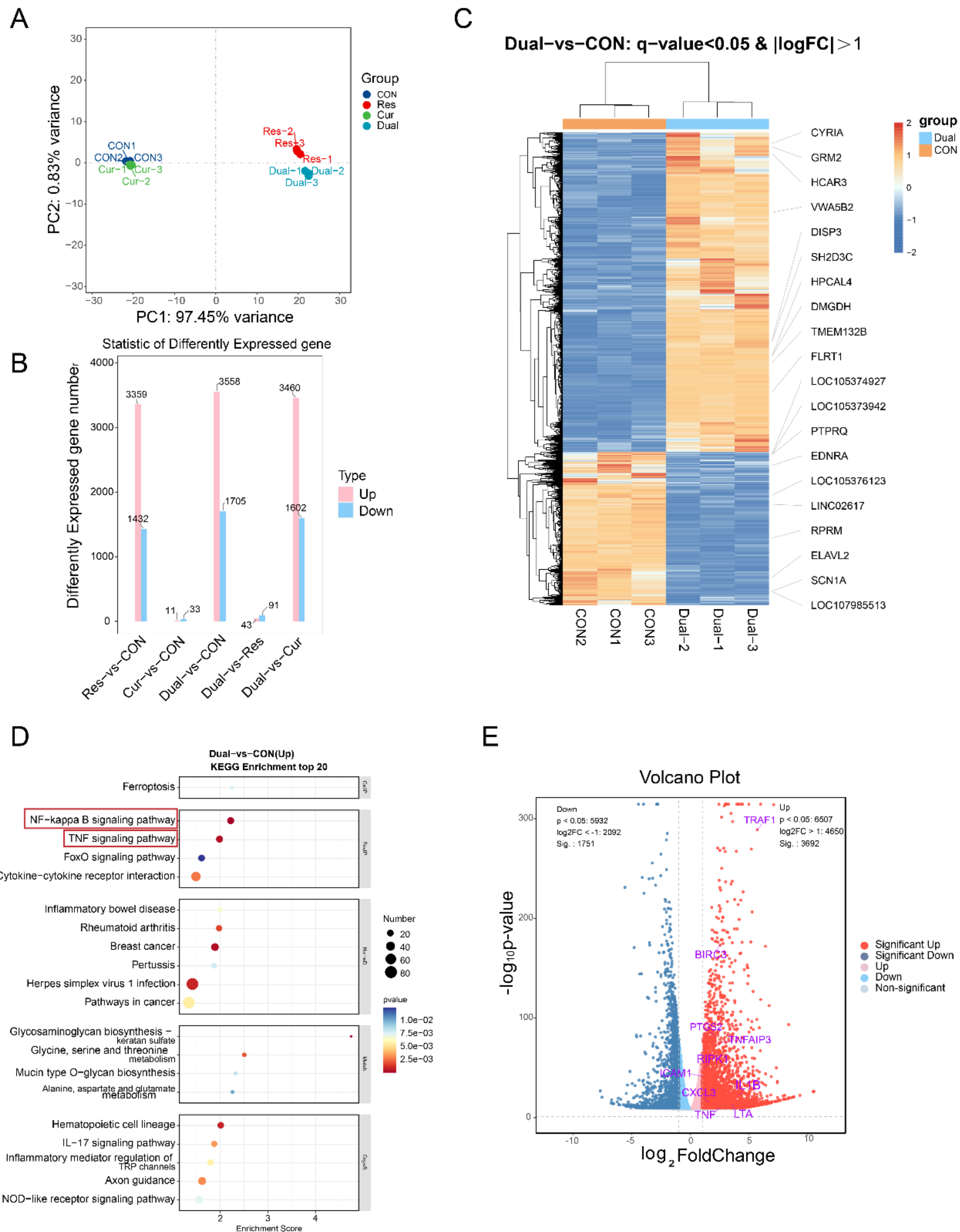

Figure 3. Transcriptomic profiling of MCF-7 cells under combined resveratrol and curcumin treatment. (A) PCA plot showing global transcriptomic differences among control (Con), curcumin (Cur), resveratrol (Res), and dual-treatment (Dual) groups. (B) Histogram of differentially expressed genes (DEGs) across treatment groups. (C) Heatmap of DEGs between control and dual-treatment groups. (D) KEGG enrichment analysis of DEGs in the dual-treatment group, displaying the top 20 enriched pathways.

(E) Volcano plot of DEGs between control and dual-treatment groups, with up-regulated genes in the TNF and NF-κB signaling pathways highlighted.

3.4. Network pharmacology validation of transcriptomic findings

To further clarify the integration of our experimental and computational findings, the connection between the RNA-seq and network pharmacology results can be made more explicit. Differentially expressed genes from the TNBC dataset GSE38959 were first identified and used as a reference set (Fig. 4A). By intersecting these genes with the predicted molecular targets of curcumin and resveratrol, overlapping candidate genes were obtained for each compound (Fig. 4B–C). KEGG enrichment analysis revealed that resveratrol-associated intersecting genes were significantly enriched in the TNF and NF-κB signaling pathways, whereas curcumin-associated genes were primarily enriched in the NF-κB pathway along with the HIF-1α signaling pathway, which is known to be regulated downstream of both TNF and NF-κB signaling [16] (Fig. 4D–E). Complementary GO enrichment analysis further indicated involvement in inflammatory response, apoptosis, and proliferation (Fig. 4F–G). Importantly, these pathways were consistent with the transcriptomic alterations observed in MCF-7 cells under dual treatment, thereby providing cross-validation that both compounds converge on modulation of the TNF-α/NF-κB axis in breast cancer.

Figure 4. Network pharmacology validation of TNBC-related targets for curcumin and resveratrol. (A)Volcano plot of DEGs from the TNBC dataset GSE38959. (B–C) Venn diagrams showing overlaps between TNBC DEGs and predicted targets of curcumin (B) or resveratrol (C). (D–E) KEGG enrichment of overlapping genes for

curcumin (D) and resveratrol (E), highlighting TNF and NF-κB signaling pathways. (F–G) GO enrichment of overlapping genes for curcumin (F) and resveratrol (G), showing enrichment in inflammatory response, apoptosis, and proliferation. Cur: Curcumin; Res: Resveratrol.

3.5. Combined treatment downregulates key proteins in the TNF-α/NF-κB/c-Myc.

To validate the functional outcome of the signaling pathways identified by transcriptomics and network pharmacology, protein expression of key mediators was examined by Western blot after 24 h of treatment. Compared with the control, the combination treatment significantly downregulated the protein levels of NF-κ, TNF-α, and the downstream oncoprotein c-Myc. Conversely, the expression of Cleaved caspase-3 was markedly upregulated, confirming the induction of apoptosis (Figure 5).

Notably, TNF-α protein was elevated in the resveratrol-alone group but reduced in both the curcumin-alone and combination groups, suggesting that curcumin plays a dominant role in suppressing TNF-α protein expression or stability, thereby attenuating the NF-κB signal. The synergistic downregulation of c-Myc, a critical proliferative driver downstream of NF-κB, aligns with the enhanced apoptotic rate observed in the combination group. These protein-level findings collectively demonstrate that the combined treatment ultimately inhibits the functional output of the TNF-α/NF-κB/c-Myc axis, providing a direct mechanistic basis for the synergistic induction of apoptosis.

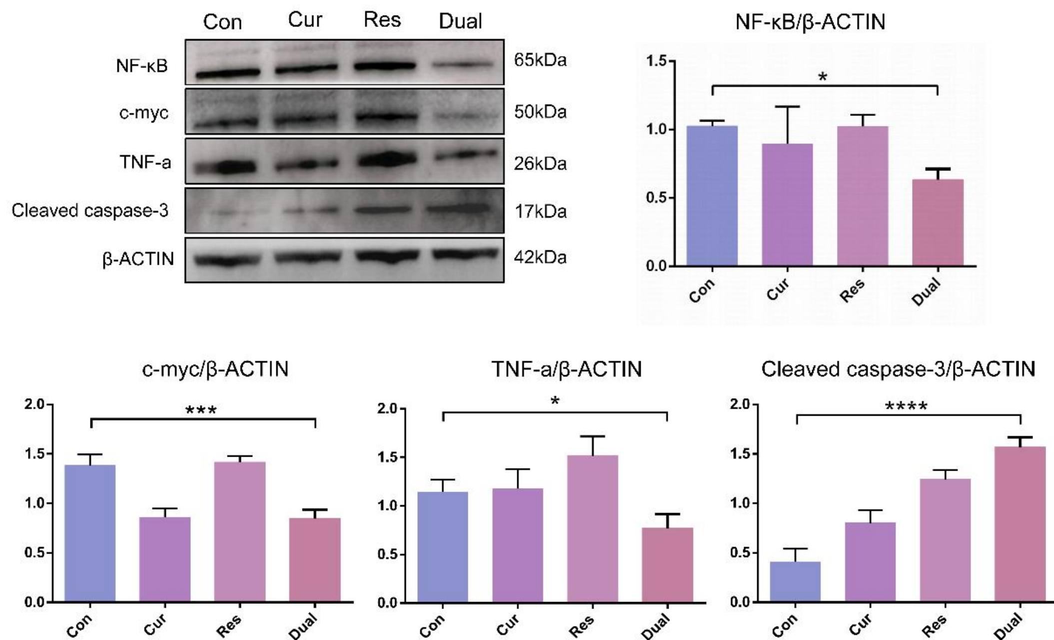

Figure 5. Western blot analysis was carried out to detect the protein expression in the signaling pathway in MCF-7 cells. Protein levels in MCF-7 cells from all four groups were normalized to those of β-ACTIN. The expression of NF-κB, c-myc, and TNF-α was significantly decreased (*P< 0.05, ***P< 0.001), while the expression of Cleaved caspase-3 was markedly upregulated (****P< 0.0005). The date in the figure was expressed by mean ± SD in SPSS, and comparison between two groups was analyzed by one-way ANOVA in GraphPad Prism. The experiment was repeated three times independently.

4. Discussion

In this study, we demonstrated that the combination of resveratrol and curcumin exerted significantly stronger anticancer effects on MCF-7 breast cancer cells than either agent alone, highlighting the therapeutic potential of dual phytochemical strategies. While both compounds individually inhibited proliferation and induced apoptosis in a dose-dependent manner, their combination achieved the greatest reduction in cell viability and markedly increased apoptosis, consistent with a

synergistic rather than an additive effect. Similar synergistic interactions between natural compounds have been increasingly reported, offering the advantage of enhanced efficacy while reducing the effective dose of each compound and thereby minimizing toxicity[17].

Transcriptomic analysis revealed that the dual-treatment group exhibited pronounced enrichment of the TNF signaling pathway and modulation of the NF-κB signaling pathway, both of which are central to inflammation-associated tumor progression. Network pharmacology further validated these findings by integrating TNBC-related DEGs from GSE38959 with predicted drug targets. Resveratrol-associated targets were mainly enriched in TNF and NF-κB pathways, while curcumin-associated targets were enriched in NF-κB together with the HIF-1α signaling pathway, which functions downstream of TNF/NF-κB and mediates hypoxia adaptation, angiogenesis, and metabolic reprogramming [18-19]. This complementary targeting suggests that resveratrol primes stress- and immune-related TNF signaling, whereas curcumin antagonizes NF-κB–mediated pro-survival signaling and its downstream effectors, including HIF-1α and c-Myc, thereby shifting the cellular balance toward apoptosis.

An apparent yet interpretable discrepancy emerged when comparing transcriptional and protein data. While RNA-seq indicated an upregulation of gene expression in the TNF/NF-κB pathway (Section 3.3), Western blot revealed a reduction in the corresponding proteins at the same 24-hour endpoint. This pattern is not contradictory but reflects a dynamic, time-resolved biological response. The initial transcriptional upregulation detected by RNA-seq likely represents a cellular stress response triggered by the potent combination treatment. Subsequently, this activation may engage strong negative feedback mechanisms and/or promote targeted protein degradation—processes in which curcumin is known to participate, for instance, by inhibiting IκB kinase and promoting proteasomal degradation of NF-κB. Consequently, by 24 hours, despite elevated pathway-related mRNA, the net protein levels and functional activity of the pro-survival axis are suppressed, leading

to the observed downregulation of NF-κB, TNF-α, and c-Myc, and the activation of Cleaved caspase-3.

Our findings align with and extend the growing paradigm that targeting interconnected nodes within a robust signaling network (like TNF-α/NF-κB/c-Myc/HIF-1α) is more effective than single-target inhibition. The network pharmacology prediction linking curcumin's targets to the HIF-1α pathway is particularly relevant, as HIF-1α is stabilized by both NF-κB and inflammatory signals, and is a key mediator of therapy resistance[20]. The dual suppression of NF-κB and its downstream partner HIF-1α represents a powerful, complementary anti-tumor strategy that impairs survival, proliferation, and adaptive responses[21-22].

Despite these promising findings, several limitations should be acknowledged. First, the present work was conducted in vitro using a single luminal breast cancer cell line (MCF-7). Given the heterogeneity of breast cancer, validation in other subtypes—particularly triple-negative breast cancer—is essential. Second, both resveratrol and curcumin suffer from poor solubility, rapid metabolism, and consequently low bioavailability in vivo, which may restrict their clinical application[23]. Nanoparticle-based formulations and structural analogs have shown promise in enhancing stability and systemic delivery of these compounds, but further optimization and in vivo studies are required[24-25]. Finally, pharmacokinetics, dose–response relationships, and systemic safety of the combined regimen remain to be systematically evaluated.

5. Conclusion

In summary, we provide compelling mechanistic evidence that the combination of resveratrol and curcumin synergistically induces apoptosis in breast cancer cells through a coordinated attack on the TNF-α/NF-κB/c-Myc axis. This work underscores the therapeutic potential of rationally combining natural compounds with complementary mechanisms of action to disrupt oncogenic signaling networks more completely, offering a promising strategy for adjuvant breast cancer therapy.

6. Author Contributions

Conceptualization: Liang Chen, Jing Zhang, Yichen Chen

Formal analysis: Jue Zhu, Yuhui Sun

Software: Huan Chen

Resources: Liang Chen, Jing Zhang

Funding acquisition: Liang Chen, Jing Zhang, Yichen Chen

Investigation: Xiaodan Fan, Jue Zhu, Yuhui Sun, Huan Chen

Data Curation: Xiaodan Fan, Huan Chen, Yichen Chen

Methodology: Xiaodan Fan, Yuhui Sun, Huan Chen，

Project administration: Jue Zhu, Liang Chen, Jing Zhang

Visualization: Yichen Chen

Supervision: Liang Chen, Jing Zhang, Yichen Chen

Validation: Xiaodan Fan, Jue Zhu, Huan Chen，

Writing-original drat: Xiaodan Fan

Writing-Review & editing: Xiaodan Fan, Yichen Chen

7. Acknowledgments

None.

8. Competing Interests

The authors have no conflict of interest to declare.

9. Funding

This work was supported by Ningbo Clinical Research Center for Gynecological Diseases (Grant no: 2024L002); Ningbo Top Medical and Health Research Program (Grant no: 2024021020); Ningbo Key Laboratory for Reproductive Health in Major Gynecological Diseases; Zhejiang Province Major Health and Wellness Science and Technology Project Fund (Grant no: WKJ-ZJ-2447 and LQN26H040009); Natural Science Foundation of Zhejiang Province (Grant no: LY24H040002 and Grant no. LMS25H040004).

10. References

[1] Dvir K, Giordano S, Leone JP. Immunotherapy in Breast Cancer. Int J Mol Sci. 2024;25(14):7517. doi: 10.3390/ijms25147517.

[2] Heater NK, Warrior S, Lu J. Current and future immunotherapy for breast cancer. J Hematol Oncol. 2024;17(1):131. doi: 10.1186/s13045-024-01649-z.

[3] Sakach E, O'Regan R, Meisel J, Li X. Molecular Classification of Triple Negative Breast Cancer and the Emergence of Targeted Therapies. Clin Breast Cancer. 2021;21(6):509-520. doi: 10.1016/j.clbc.2021.09.003.

[4] Wang X, Zhang L, Dai Q, Si H, Zhang L, Eltom SE, et al. Combined Luteolin and Indole-3-Carbinol Synergistically Constrains ERα-Positive Breast Cancer by Dual Inhibiting Estrogen Receptor Alpha and Cyclin-Dependent Kinase 4/6 Pathway in Cultured Cells and Xenograft Mice. Cancers(Basel). 2021;13(9):2116. doi: 10.3390/cancers13092116.

[5] Baena Ruiz R, Salinas Hernández P. Cancer chemoprevention by dietary phytochemicals: Epidemiological evidence. Maturitas. 2016;94:13-19. doi: 10.1016/j.maturitas.2016.08.004.

[6] Siddiqui JA, Singh A, Chagtoo M, Singh N, Godbole MM, Chakravarti B. Phytochemicals for breast cancer therapy: current status and future implications. Curr Cancer Drug Targets. 2015;15(2):116-35. doi: 10.2174/1568009615666141229152256.

[7] Schmidt B, Ferreira C, Alves Passos CL, Silva JL, Fialho E. Resveratrol, Curcumin and Piperine Alter Human Glyoxalase 1 in MCF-7 Breast Cancer Cells. Int J Mol Sci. 2020;21(15):5244. doi: 10.3390/ijms21155244.

[8] Shindikar A, Singh A, Nobre M, Kirolikar S. Curcumin and Resveratrol as Promising Natural Remedies with Nanomedicine Approach for the Effective Treatment of Triple Negative Breast Cancer. J Oncol. 2016;2016:9750785. doi: 10.1155/2016/9750785.

[9] Kurzava Kendall L, Ma Y, Yang T, Lubecka K, Stefanska B. Epigenetic Effects of Resveratrol on Oncogenic Signaling in Breast Cancer. Nutrients. 2024;16(5):699. doi: 10.3390/nu16050699.

[10] Zhang E, Wang Y, Zhang H, Li X, Su Y, Cui J, et al. Resveratrol induces ferroptosis in triple-negative breast cancer through NEDD4L-mediated GPX4 ubiquitination and

degradation. Free Radic Biol Med. 2025;235:231-247. doi: 10.1016/j.freeradbiomed.2025.04.052.

[11] Hasanzadeh S, Read MI, Bland AR, Majeed M, Jamialahmadi T, Sahebkar A. Curcumin: an inflammasome silencer. Pharmacol Res. 2020;159:104921. doi: 10.1016/j.phrs.2020.104921.

[12] El-Saadony MT, Saad AM, Mohammed DM, Alkafaas SS, Ghosh S, Negm SH, et al. Curcumin, an active component of turmeric: biological activities, nutritional aspects, immunological, bioavailability, and human health benefits - a comprehensive review. Front Immunol. 2025;16:1603018. doi: 10.3389/fimmu.2025.1603018.

[13] Huang M, Zhai BT, Fan Y, Sun J, Shi YJ, Zhang XF, et al. Targeted Drug Delivery Systems for Curcumin in Breast Cancer Therapy. Int J Nanomedicine. 2023;18:4275-4311. doi: 10.2147/IJN.S410688.

[14] Pan-On S, Dilokthornsakul P, Tiyaboonchai W. Trends in advanced oral drug delivery systems for curcumin: A systematic review. J Control Release. 2022;348:335-345. doi: 10.1016/j.jconrel.2022.05.048.

[15] Zhou Y, Gong J, Deng X, Shen L, Wu S, Fan H, et al. Curcumin and nanodelivery systems: New directions for targeted therapy and diagnosis of breast cancer. Biomed Pharmacother. 2024;180:117404. doi: 10.1016/j.biopha.2024.117404.

[16] Kim KW, Lee SJ, Kim JC. TNF-α upregulates HIF-1α expression in pterygium fibroblasts and enhances their susceptibility to VEGF independent of hypoxia. Exp Eye Res. 2017;164:74-81. doi: 10.1016/j.exer.2017.08.008.

[17] Castañeda AM, Meléndez CM, Uribe D, Pedroza-Díaz J. Synergistic effects of natural compounds and conventional chemotherapeutic agents: recent insights for the development of cancer treatment strategies. Heliyon. 2022;8(6):e09519. doi: 10.1016/j.heliyon.2022.e09519.

[18] Luo S, Jiang Y, Anfu Zheng, Zhao Y, Wu X, Li M, et al. Targeting hypoxia-inducible factors for breast cancer therapy: A narrative review. Front Pharmacol. 2022;13:1064661. doi: 10.3389/fphar.2022.1064661.

[19] Guo Q, Jin Y, Chen X, Ye X, Shen X, Lin M, et al. NF-κB in biology and targeted therapy: new insights and translational implications. Signal Transduct Target Ther.

2024;9(1):53. doi: 10.1038/s41392-024-01757-9.
[20] Sarighieh MA, Montazeri V, Shadboorestan A, Ghahremani MH, Ostad SN. The Inhibitory Effect of Curcumin on Hypoxia Inducer Factors (Hifs) as a Regulatory Factor in the Growth of Tumor Cells in Breast Cancer Stem-Like Cells. Drug Res. 2020;70(11):512-518. doi: 10.1055/a-1201-2602.
[21] Szlosarek PW, Balkwill FR. Tumour necrosis factor alpha: a potential target for the therapy of solid tumours. Lancet Oncol. 2003;4(9):565-73. doi: 10.1016/s1470-2045(03)01196-3.
[22] Liang M, Zhang P, Fu J. Up-regulation of LOX-1 expression by TNF-αlpha promotes trans-endothelial migration of MDA-MB-231 breast cancer cells. Cancer Lett. 2007;258(1):31-7. doi: 10.1016/j.canlet.2007.08.003.
[23] Wang W, Li M, Wang L, Chen L, Goh BC. Curcumin in cancer therapy: Exploring molecular mechanisms and overcoming clinical challenges. Cancer Lett. 2023;570:216332. doi: 10.1016/j.canlet.2023.216332.
[24] Hashim PK, Poonkuzhi NP, Cheruthu NM, Kuruniyan MS. Drug Delivery Systems for Natural Medicines in Cancer Therapy. Curr Top Med Chem. 2025;25(17):2072-2085. doi: 10.2174/0115680266315985240710063754.
[25] Patra S, Pradhan B, Nayak R, Behera C, Rout L, Jena M, et al. Chemotherapeutic efficacy of curcumin and resveratrol against cancer: Chemoprevention, chemoprotection, drug synergism and clinical pharmacokinetics. Semin Cancer Biol. 2021;73:310-320. doi: 10.1016/j.semcancer.2020.10.010.