



Differential Gene Expression Induced by Carvacrol in MCF-7 and MDA-MB-231 Breast Cancer Cells

Karvakrolün MCF-7 ve MDA-MB-231 Meme Kanseri Hücrelerinde İndüklediği Diferansiyel Gen Ekspresyon Değişiklikleri

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ABSTRACT

Objective: Breast cancer (BC) is a heterogeneous malignancy driven by dysregulated molecular pathways that promote proliferation, invasion, and therapeutic resistance. Carvacrol, a natural monoterpenoid phenol, has demonstrated anticancer potential; however, its subtype-specific molecular mechanisms in BC remain unclear.

Materials and Methods: This study combined bioinformatic and experimental approaches to evaluate the functional relationships and prognostic relevance of *MKI67*, *E2F4*, *FOSL1*, *CD44*, *CDKN1A (P21)*, *CASP9*, *ABCB1*, *ABCC1*, and *ABCC5*. The STRING database was used to evaluate protein-protein interaction (PPI) networks. Overall survival (OS) and disease-free survival (DFS) analyses were conducted using GEPIA2 based on TCGA-BRCA data. MCF-7 and MDA-MB-231 BC cells were cultured and exposed to 400 μ M carvacrol for 48 hours. Gene expression levels were evaluated by qPCR. Relative mRNA expression was calculated using the $2^{-\Delta\Delta C_t}$ method.

Results: PPI analysis revealed significant functional connectivity among the studied genes ($p=2.15 \times 10^{-9}$). Survival analyses did not demonstrate consistent independent prognostic significance. Carvacrol induced subtype-dependent molecular alterations. In MCF-7 cells, *CD44*, *FOSL1*, and *MKI67* were significantly downregulated ($p<0.05$), indicating suppression of proliferative and invasive pathways. In MDA-MB-231 cells, *P21* expression was significantly upregulated ($p=0.006$), suggesting the induction of cell cycle arrest in this aggressive phenotype. No significant changes were observed in *E2F4*, *CASP9*, *ABCB1*, *ABCC1*, or *ABCC5* expression.

Conclusion: Carvacrol induces subtype-specific alterations in the expression of the studied genes (*CD44*, *FOSL1*, *MKI67*, and *P21*) in BC cells. These findings provide preliminary molecular evidence of its biological activity and warrant further protein-based analyses, functional in vitro experiments, and in vivo studies to elucidate its potential therapeutic role in BC.

Keywords: Breast Cancer, Carvacrol, Gene Expression Modulation, Treatment, Survival.

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ÖZ

Amaç: Meme kanseri, proliferasyon, invazyon ve tedavi direncini artıran düzensiz moleküler yollar tarafından yönlendirilen heterojen bir malignitedir. Doğal bir monoterpenoid fenol olan karvakrol, antikanser potansiyel göstermiştir; ancak meme kanserindeki alt tip-spesifik moleküler mekanizmaları henüz tam olarak aydınlatılamamıştır.

Gereç ve Yöntem: Bu çalışmada, *MKI67*, *E2F4*, *FOSL1*, *CD44*, *CDKN1A (P21)*, *CASP9*, *ABCB1*, *ABCC1* ve *ABCC5* genlerinin fonksiyonel ilişkileri ve prognostik önemi biyoinformatik ve deneysel yaklaşımlar birlikte kullanılarak değerlendirilmiştir. Protein-protein etkileşim (PPI) ağları STRING veritabanı kullanılarak analiz edilmiştir. Genel sağkalım ve hastalıksız sağkalım analizleri, TCGA-BRCA verilerine dayalı olarak GEPIA2 aracılığıyla gerçekleştirilmiştir. MCF-7 ve MDA-MB-231 meme kanseri hücreleri kültüre edilerek 48 saat boyunca 400 µM karvakrol ile muamele edilmiştir. Gen ekspresyon düzeyleri qPCR ile değerlendirilmiştir. Göreceli mRNA ekspresyonu $2^{-\Delta\Delta Ct}$ yöntemi kullanılarak hesaplanmıştır.

Bulgular: PPI analizi, incelenen genler arasında anlamlı fonksiyonel bağlantılar bulunduğunu ortaya koymuştur ($p=2.15e-09$). Sağkalım analizleri, bu genlerin bağımsız prognostik önemini tutarlı bir şekilde göstermemiştir. Karvakrol, meme kanseri alt tiplerine bağlı olarak gen ekspresyonunda farklı değişikliklere neden olmuştur. MCF-7 hücrelerinde *CD44*, *FOSL1* ve *MKI67* genlerinin ekspresyon düzeyleri anlamlı olarak azalmıştır ($p<0.05$). MDA-MB-231 hücrelerinde ise *P21* gen ekspresyonu anlamlı düzeyde artmıştır ($p=0.006$). Buna karşın *E2F4*, *CASP9*, *ABCB1*, *ABCC1* ve *ABCC5* genlerinin ekspresyon düzeylerinde istatistiksel olarak anlamlı bir değişiklik saptanmamıştır.

Sonuç: Karvakrol, meme kanseri hücrelerinde incelenen genlerin (*CD44*, *FOSL1*, *MKI67* ve *P21*) ekspresyonunda alt tipe özgü değişiklikler oluşturmuştur. Bu bulgular, karvakrolün biyolojik etkilerine ilişkin ön moleküler kanıtlar sağlamakta olup, meme kanserindeki terapötik potansiyelinin aydınlatılabilmesi için protein düzeyinde doğrulama çalışmaları, fonksiyonel in vitro deneyler ve in vivo araştırmalarla desteklenmesi gerekmektedir.

Anahtar Sözcükler: Gen Ekspresyonu Modülasyonu, Karvakrol, Meme Kanseri, Sağkalım, Tedavi.

Introduction

Worldwide, breast cancer (BC) is the most frequently diagnosed cancer in women and a leading cause of cancer-related morbidity and mortality (1). The disease is characterized by marked biological and clinical heterogeneity, which significantly influences tumor progression, treatment response, and overall prognosis (2). Furthermore, distinct demographic disparities exist in both incidence and survival; for example, despite having similar incidence rates, Black women have a mortality rate nearly 40% higher than that of White women (3). Abnormal cellular signaling and disruption of molecular pathways are primary drivers of BC progression and therapeutic resistance. This underscores the need for a more comprehensive understanding of these mechanisms to design precise, targeted therapies (4, 5). Although modern screening and therapeutic strategies have markedly improved patient survival, the long-term use of conventional chemotherapeutic agents is often limited by systemic toxicity, adverse effects, and the development of multidrug resistance. Thus, identifying safer and more effective therapeutic alternatives remains a major focus of current oncology research (6-9).

Carvacrol ($C_{10}H_{14}O$, 150.22 g/mol) is a naturally occurring monoterpenoid phenol with both hydrophilic and hydrophobic properties and is predominantly isolated from aromatic herbs such as *Thymus vulgaris* and *Origanum vulgare* (10)(11). This compound has broad therapeutic potential, including antioxidant, anti-inflammatory, antimicrobial, and anticancer properties (12-16). Its ability to neutralize free radicals and mitigate oxidative stress may help reduce the risk of chronic diseases, including malignancies (17). Moreover, carvacrol has been shown to suppress cancer cell proliferation and migration while inducing apoptosis by interfering with critical survival and metastatic signaling pathways (18). Given its favorable safety profile (recognized as a safe food additive by the FDA and the European Council), carvacrol is an attractive therapeutic candidate with fewer adverse effects than synthetic alternatives (19).

Malignant transformation and tumor progression are regulated by various genes. Specifically, genes such as *MKI67*, *E2F4*, *FOSL1*, *CD44*, *CDKN1A* (*p21*), *CASP9*, *ABCB1* (*MDR1*), *ABCC1*, and *ABCC5* govern critical cellular functions ranging from proliferation,

adhesion, and cell cycle regulation to apoptosis and drug efflux mechanisms (20, 21). In our previous investigations, we demonstrated the antitumor activity of carvacrol in MCF-7 and MDA-MB-231 BC cell lines. Following exposure to 400 μ M carvacrol (based on established IC₅₀ values), both cell lines exhibited significantly reduced viability and migration, lower reactive oxygen species (ROS) production, and a reduction in the subpopulation of CD44⁺ cancer stem-like cells. Additionally, treatment increased the BAX/BCL2 ratio, promoting apoptosis, and induced subtype-specific transcriptomic changes, such as differential *ABCG2* regulation and uniform *NF- κ B1* upregulation (8).

Building on these findings, the present study aimed to further characterize the molecular effects of treatment with 400 μ M carvacrol for 48 hours on the expression profiles of these key genes in BC cell lines. Furthermore, the prognostic value of these genes for overall and disease-free survival in patients with BC was evaluated using Kaplan-Meier analyses via GEPIA2, and their functional interactions were mapped using the STRING protein-protein interaction (PPI) database.

Materials and Methods

Bioinformatic Analysis

To establish a theoretical basis for the targeted genes, a comprehensive literature search was conducted using the PubMed database. Search queries included individual gene identifiers alongside terms such as "breast cancer," "BC," "oncogene," "tumor suppressor," "overexpression," "up-regulation", "down-regulation", "drug resistance," "apoptosis," and "cell cycle". Additional genetic annotations were retrieved from the GeneCards and Ensembl repositories (22, 23). To evaluate functional associations among the proteins encoded by these genes, we used the STRING database (version 12.0) to construct PPI networks (24). Prognostic assessments, specifically overall survival (OS) and disease-free survival (DFS), were performed using the GEPIA2 web platform and Kaplan-Meier methods (25, 26).

Cell Culture and Carvacrol Preparation

Two human breast cancer cell models, hormone receptor-positive MCF-7 and triple-negative MDA-MB-

231 (TNBC), were used in this study. Both cell lines were cultured in high-glucose Dulbecco's Modified Eagle Medium (DMEM; EcoTech Biotechnology, Türkiye) supplemented with 10% fetal bovine serum (FBS; Gibco, UK) and 1% antibiotic solution containing penicillin and streptomycin (Gibco, NY, USA). The cultures were maintained in a controlled incubator (Wiggins WCI-180, Wuppertal, Germany) in a humidified atmosphere (>90%) containing 5% CO₂ at 37°C.

A 200 mM carvacrol stock solution (5-isopropyl-2-methylphenol, 99.56% purity; Ambeed, Buffalo Grove, USA) was prepared in dimethyl sulfoxide (DMSO; EcoTech Biotechnology, Türkiye). This stock solution was protected from light and stored at room temperature. For subsequent cellular assays, carvacrol was administered to the cells at a final concentration of 400 µM for 48 hours.

Relative Gene Expression Analyses

RNA Isolation

MCF-7 and MDA-MB-231 cells were seeded into six-well culture plates at a density of 4×10^4 cells per well in 2 mL of complete medium. After 24 hours, the cells in the experimental group were exposed to 400 µM carvacrol, while the untreated cells served as the

negative control. After 48 hours of continuous incubation, the cells were harvested by centrifugation. Total RNA was extracted by lysing the cell pellets in 1 mL of TRIzol Reagent (Invitrogen, USA). The concentration and purity of the isolated RNA were subsequently measured using a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, USA).

Gene Expression Analysis by Quantitative Real-Time PCR

A total of 1000 ng of isolated RNA was used as the template for complementary DNA (cDNA) synthesis with the OneScript® Plus cDNA Synthesis Kit (ABM Inc., Canada). This reverse transcription step was performed in a T100 Thermal Cycler (Bio-Rad, Singapore) at 52°C for 15 minutes.

For the quantitative real-time PCR (qPCR) assays, the BlasTaq™ 2X qPCR Master Mix (ABM Inc., Canada) was used according to the manufacturer's instructions. The specific primer sets used for amplification are detailed in Table I. Each sample was analyzed in triplicate using the Bio-Rad CFX96™ Real-Time PCR System. Target gene expression was normalized to the endogenous reference (housekeeping) gene *GAPDH*, and relative fold changes were calculated using the $2^{-\Delta\Delta C_t}$ method.

Table I: Primer sequences used for quantitative real-time PCR analysis.

Gene Name	Forward primer	Reverse primer	Ref.
<i>GAPDH</i>	5'-GAAGGTGAAGGTCGGAGTCA-3'	5'-TGACAAGCTTCCCGTTCTCA-3'	(16)
<i>MKI67</i>	5'-GAAAGAGTGGCAACCTGCCTTC-3'	5'-GCACCAAGTTTACTACATCTGCC-3'	(47)
<i>E2F4</i>	5'-GGAAGGTATCGGGCTAATCGAG-3'	5'-AGCTCCTCGATCTCTGCCTTGA-3'	(48)
<i>FOSL1</i>	5'-CAGGCGGAGACTGACAACTG-3'	5'-TCCTTCCGGGATTTTGCAGAT-3'	(49)
<i>CD44</i>	5'-CTGCCGCTTTGCAGGTGTA-3'	5'-CATTGTGGCAAGGTGCTATT-3'	(50)
<i>P21/CDKN1A</i>	5'-GACTGTGATGCGCTAATGGC-3'	5'-CCGTGGGAAGGTAGAGCTTG-3'	(51)
<i>CASP9</i>	5'-GTTTGAGGACCTTCGACCAGCT-3'	5'-CAACGTACCAGGAGCCACTCTT-3'	(52)
<i>ABCB1/MDR1</i>	5'-GAAACCAACTGTCAGTGTATTTTC-3'	5'-AGAGGAAGTCCAGCCCC-3'	(53)
<i>ABCC1</i>	5'-TCAGCCAGAAAATCCTCCAC-3'	5'-GGCACCATGAGGACCATC-3'	(53)
<i>ABCC5</i>	5'-TGAGGGAGAGAACCAGCACT-3'	5'-AAGTAGTCCGGATGGGCTTC-3'	(53)

Statistical Analysis

All statistical analyses were performed using GraphPad Prism software (version 9) (San Diego, CA, USA). Differences in relative gene expression between the carvacrol-treated and untreated control groups were evaluated using Student's t-test. The data are presented as the mean $\pm$ standard deviation (SD). Statistical significance thresholds were defined as * $p < 0.033$, $p < 0.002$, and *** $p < 0.001$.

Results

Bioinformatic Results

The selected genes are known to be closely associated with BC progression and key oncogenic processes. *MKI67*, *E2F4*, *FOSL1*, and *CD44* are involved in cell proliferation, migration, adhesion, and cell cycle regulation, and are typically upregulated in cancer. The multidrug resistance-related genes *ABCB1/MDR1*, *ABCC1*, and *ABCC5* contribute to chemotherapy resistance by mediating drug efflux mechanisms. In contrast, *CASP9*, an important apoptotic gene, is

commonly downregulated in cancer, leading to impaired programmed cell death. Similarly, *P21* (*CDKN1A*), a critical regulator of cell cycle progression, is often downregulated in malignant cells. Given that all nine genes play significant roles in BC biology, they were considered suitable candidates for *in vitro* investigation.

Analysis of the PPI network using the STRING database revealed a highly significant functional association among these nine proteins (PPI enrichment $p = 2.15\text{e-}09$) (Figure I). Despite their documented roles in tumor biology, Kaplan-Meier analyses using GEPIA2 indicated that the transcript levels of these individual genes were not significantly associated with OS (Figure II) or DFS (Figure III) in BC cohorts. This finding suggests that although these genes are differentially expressed during tumorigenesis, their individual expression levels may not be sufficiently robust to serve as independent prognostic biomarkers.

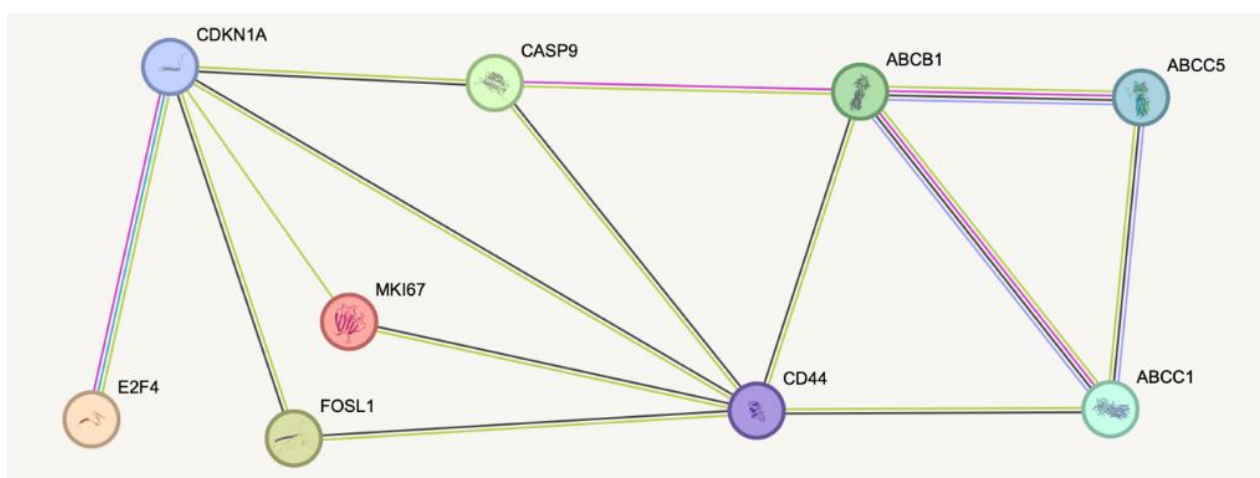


Figure I. Protein-protein interaction network (using the STRING database) of MKI67, E2F4, FOSL1, CD44, P21, CASP9, ABCB1, ABCC1, and ABCC5 (Nodes: 9; Edges: 14; Average node degree: 3.11; Average local clustering coefficient: 0.719; Expected edges: 2; PPI enrichment p -value = $2.15\text{e-}09$).

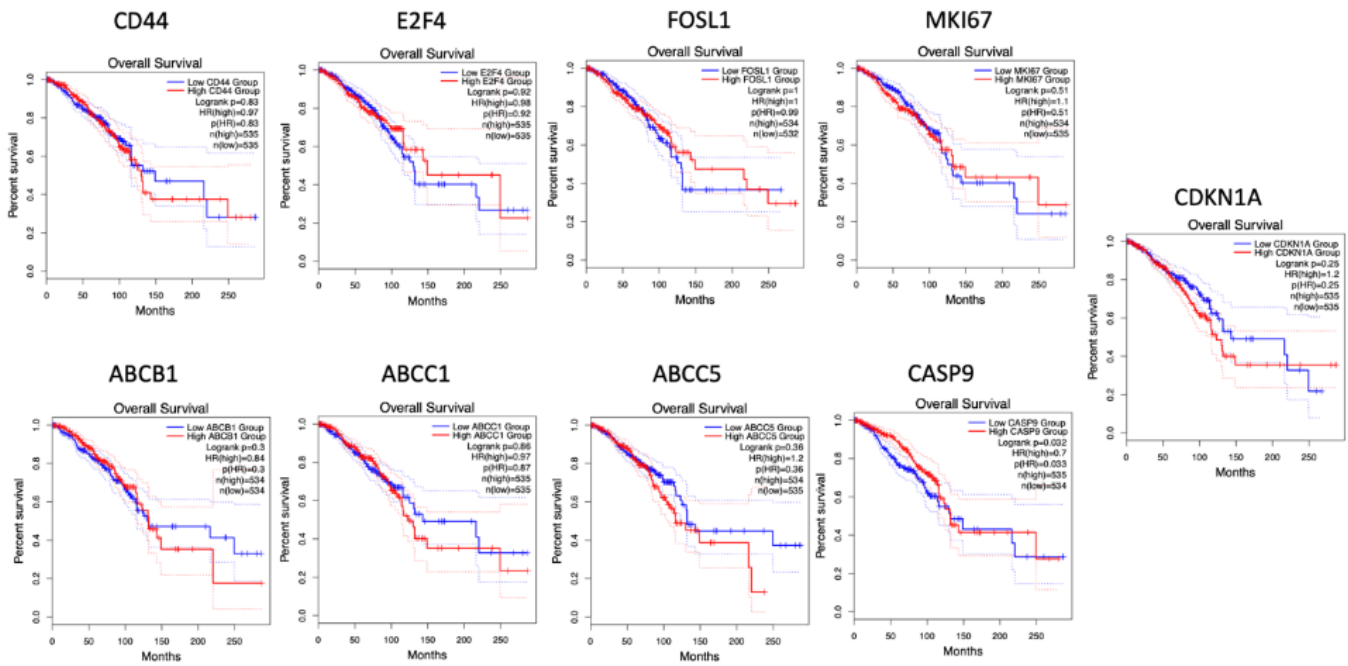


Figure II. Kaplan-Meier overall survival analysis (using GEPIA2) of BC patients using median expression as cutoff: CD44 ($p=0.83$), E2F4 ($p=0.92$), FOSL1 ($p=0.99$), MKI67 ($p=0.51$), ABCB1 ($p=0.3$), ABCC1 ($p=0.87$), ABCC5 ($p=0.36$), CASP9 ($p=0.033$), and P21 ($p=0.25$).

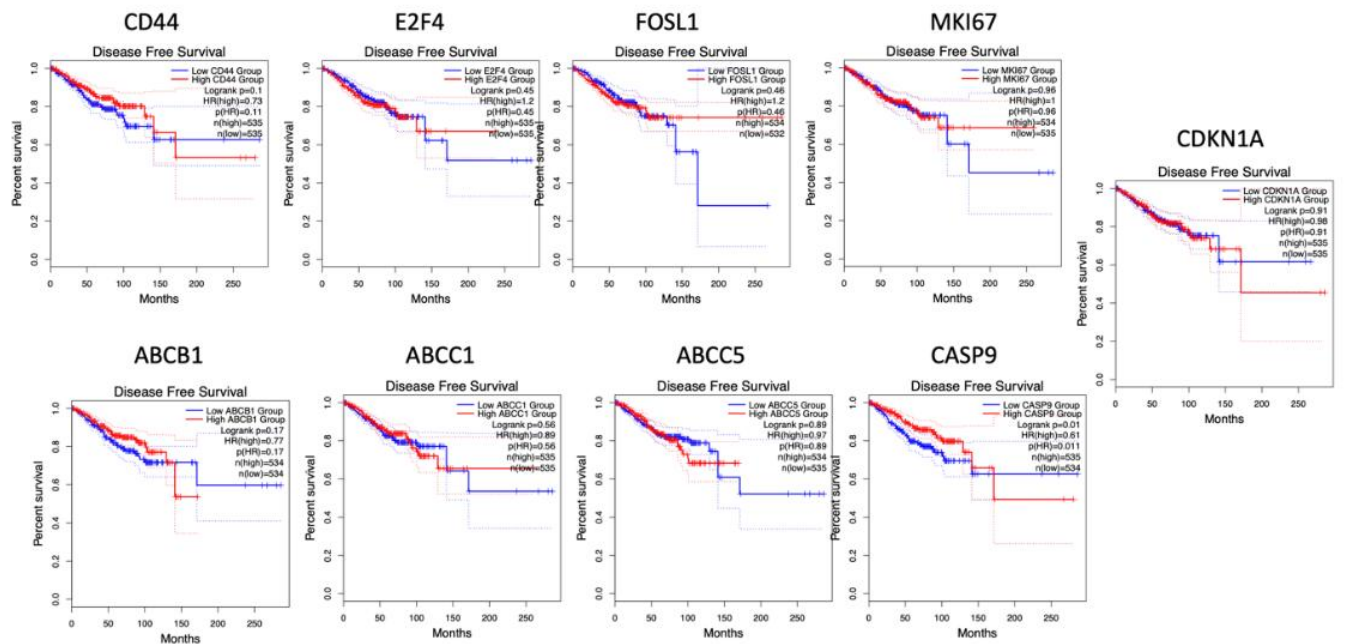


Figure III. Kaplan-Meier disease-free survival analysis (using GEPIA2) of BC patients using median expression as cutoff: CD44 ($p=0.73$), E2F4 ($p=0.45$), FOSL1 ($p=0.46$), MKI67 ($p=0.96$), ABCB1 ($p=0.17$), ABCC1 ($p=0.56$), ABCC5 ($p=0.89$), CASP9 ($p=0.011$), and P21 ($p=0.91$).

Gene Expression Results

In MCF-7 cells, carvacrol treatment resulted in a significant downregulation of *CD44*, *FOSL1*, and *MKI67* expression ($p=0.024$, $p=0.022$, and $p=0.010$, respectively). In contrast, these genes did not exhibit statistically significant changes in MDA-MB-231 cells ($p=0.423$, $p=0.672$, and $p=0.165$, respectively), suggesting a subtype-dependent molecular response. Expression of *E2F4* remained unchanged in both cell lines ($p=0.090$ in MCF-7, and $p=0.623$ in MDA-MB-231), indicating that carvacrol did not significantly influence this cell cycle-associated transcription factor under the tested conditions (Figure IV).

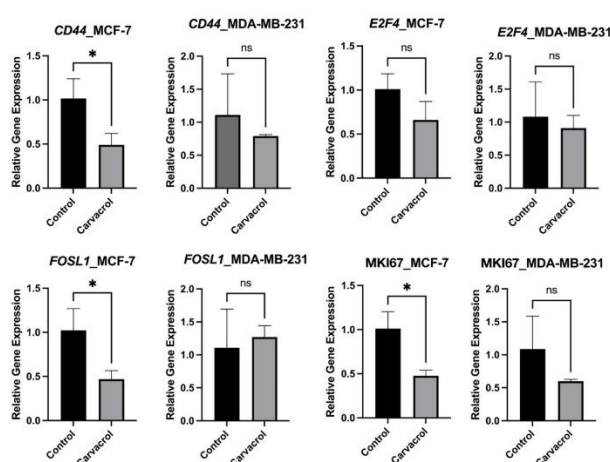


Figure IV. qPCR evaluation of *CD44*, *FOSL1*, *MKI67*, and *E2F4* gene expression in MCF-7 and MDA-MB-231 cell lines after carvacrol treatment. A significant reduction in *CD44*, *FOSL1*, and *MKI67* mRNA expression was observed in MCF-7 cells, while MDA-MB-231 cells exhibited no such changes. *E2F4* levels were unaffected in both models. Transcript abundance was normalized to the GAPDH reference gene using the $2^{-\Delta\Delta Ct}$ method. Values represent the mean

$\pm$ standard deviation (SD). Statistical significance is denoted by an asterisk (* $p<0.033$).

Similarly, the multidrug resistance-associated genes *ABCB1/MDR1*, *ABCC1*, and *ABCC5* showed no significant transcriptional changes in either cell line ($p=0.718$, $p=0.675$, and $p=0.754$ in MCF-7; $p=0.593$, $p=0.394$, and $p=0.359$ in MDA-MB-231, respectively), suggesting that carvacrol does not directly modulate drug efflux transporter expression under these experimental conditions (Figure V).

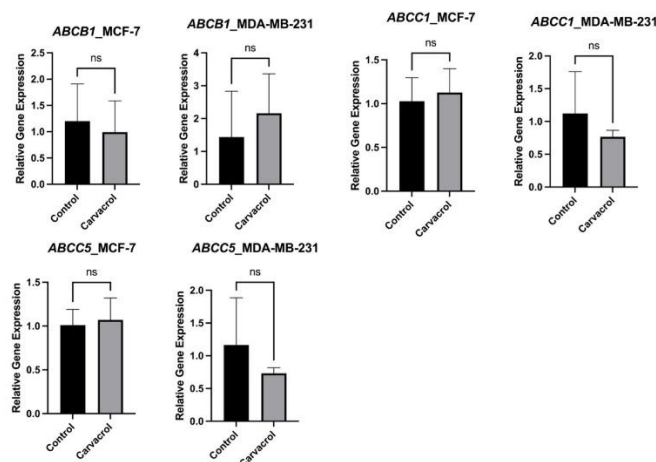


Figure V. Gene expression levels of the multidrug resistance transporters *ABCB1/MDR1*, *ABCC1*, and *ABCC5* following carvacrol treatment, as assessed by qPCR. No significant changes were detected in either cell line relative to the untreated controls. Expression data were normalized to *GAPDH* using the $2^{-\Delta\Delta Ct}$ method. Results are presented as the mean $\pm$ SD.

Furthermore, *CASP9* expression was not significantly altered in either cell line ($p=0.952$ in MCF-7, and $p=0.185$ in MDA-MB-231), suggesting that apoptotic signaling at the transcriptional level may not be primarily mediated through *CASP9* regulation. Regarding *P21*, no significant change was observed in MCF-7 cells ($p=0.072$); however, its expression was significantly altered in MDA-MB-231 cells ($p=0.006$), highlighting differential regulation of cell cycle control mechanisms between luminal and TNBC phenotypes (Figure VI).

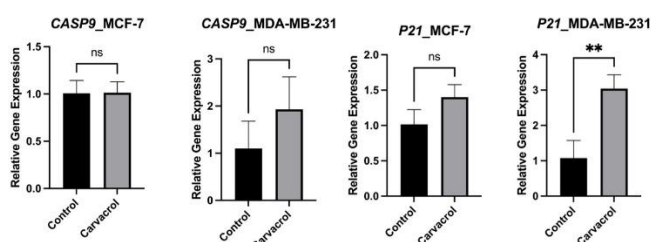


Figure VI. Assessment of *CASP9* and *P21* mRNA expression profiles in MCF-7 and MDA-MB-231 cells after carvacrol treatment. Although *CASP9* levels remained statistically unchanged in both cell lines, *P21* was significantly upregulated only in MDA-MB-231 cells. Relative gene expression was calculated using the $2^{-\Delta\Delta Ct}$ method, with *GAPDH* as the endogenous control. Data are expressed as the mean $\pm$ SD ($p<0.002$).

Discussion

BC remains the most prevalent malignancy among women worldwide and is primarily driven by complex genetic and epigenetic alterations that disrupt multiple cellular signaling pathways. Tumor heterogeneity and various microenvironmental factors often contribute to therapeutic resistance and a wide range of adverse effects in patients (27, 28). In recent years, natural bioactive compounds have attracted considerable interest in oncology research. Among these, carvacrol is a monoterpenoid predominantly derived from *Thymus vulgaris* and has been extensively studied for its antioxidant and antitumor properties (13, 16). In our previous study, we established that carvacrol reduced the viability and migratory capacity of both estrogen receptor-positive MCF-7 and TNBC MDA-MB-231 cells in a dose-dependent manner, with IC₅₀ values of 397.2 μ M and 401.7 μ M, respectively (8). The current study expands on these findings by examining the effect of carvacrol on the expression of a multigene panel comprising *MKI67*, *E2F4*, *FOSL1*, *CD44*, *P21/CDKN1A*, *CASP9*, *ABCB1/MDR1*, *ABCC1*, and *ABCC5*.

A primary challenge in cancer therapy is the emergence of drug resistance, which is frequently mediated by the *ABCB1* gene and its role in the efflux of chemotherapeutic agents from cells. This gene is typically overexpressed in BC tissues (29). We initially hypothesized that carvacrol might reverse this resistance by downregulating *ABCB1* expression; however, our data revealed no statistically significant changes in *ABCB1* levels in either cell line. Similarly, *ABCC1* and *ABCC5*, other key transporters associated with multidrug resistance and poor clinical prognosis, remained transcriptionally stable following carvacrol treatment (30)(31). These results suggest that the antitumor effects of carvacrol in these specific BC models are likely mediated through pathways independent of these classical drug efflux transporters.

One of the most notable findings of this study involves the *CD44* gene, a well-known cancer stem cell marker that facilitates tumor progression and metastasis. Higher CD44 expression is often associated with increased aggressiveness in BC (32). Our previous work indicated that carvacrol has multitarget activity partly through the modulation of CD44 (8). In the present study, carvacrol treatment led

to a significant reduction in CD44 mRNA levels in MCF-7 cells, although no significant change was observed in MDA-MB-231 cells. This selective downregulation suggests that carvacrol may inhibit tumor recurrence and "stemness" properties in certain BC subtypes. This trend was further supported by significant decreases in *FOSL1* and *MKI67* expression in MCF-7 cells. *FOSL1* is a known driver of proliferation and invasion (33), while *MKI67* is a gold standard marker of cellular proliferation that is directly associated with tumor growth rates (34). The simultaneous suppression of these three genes indicates that carvacrol attenuates the proliferative and invasive potential of the luminal subtype. The lack of a similar response in MDA-MB-231 cells highlights the subtype-dependent nature of carvacrol's action, which is likely influenced by intrinsic biological differences between luminal and TNBC phenotypes, such as receptor status and distinct transcriptional profiles.

Although *E2F4*, a transcriptional repressor often dysregulated in BC (35), remained unchanged in our study, we observed a significant effect on *P21* expression. Although MCF-7 cells showed no significant change, carvacrol treatment induced a statistically significant upregulation of *P21* in the aggressive MDA-MB-231 cell line. This finding is consistent with recent literature describing the ability of carvacrol to modulate cell cycle checkpoints in other cancers, such as oral squamous cell carcinoma and cervical cancer (44)(45). Furthermore, reports that carvacrol nanoemulsions overcome resistance in lung cancer by upregulating *P21* support our observation (46). The marked increase in *P21* expression in the TNBC phenotype suggests that carvacrol may reactivate tumor suppressor pathways that inhibit the progression of highly metastatic cell lines.

Finally, we evaluated the apoptotic initiator *CASP9*, which is frequently downregulated in BC (36). Although several studies suggest that carvacrol or its isomer thymol can upregulate *CASP9* and trigger mitochondrial apoptosis in various cancer models (37)(38)(39)(40)(41), our results showed no significant transcriptional changes in either cell line. This discrepancy indicates that under our specific experimental conditions, the cytotoxic effects of carvacrol may be mediated by other caspases,

caspase-independent pathways, or alternative programmed cell death mechanisms.

In summary, this study integrates bioinformatic analyses with gene expression profiling to demonstrate that carvacrol modulates key oncogenic drivers in a subtype-dependent manner. Assessing changes only at the mRNA level is a limitation; nevertheless, these findings provide a basis for future protein-based and functional pathway analyses. Another limitation is the absence of a separate DMSO vehicle control in the gene expression experiments. Further preclinical and clinical investigations are needed to fully determine the safety and therapeutic potential of carvacrol in patients with BC.

Conclusion

Carvacrol demonstrated subtype-dependent activity, significantly downregulating *CD44*, *FOSL1*, and *MKI67* in MCF-7 cells while upregulating *P21* in MDA-MB-231 cells. These findings suggest that carvacrol may exert its anticancer effects by modulating pathways involved in cell proliferation and invasion. No significant modulation was observed in the multidrug resistance genes studied or in *CASP9*, suggesting that carvacrol may act independently of classical chemoresistance mechanisms and the *CASP9-mediated* intrinsic apoptotic pathway. Further protein-level validation and preclinical and clinical studies are warranted to establish the therapeutic relevance of carvacrol in BC.

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