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Quercetin-Mediated Modulation of Tumor Suppressor miR-15a, miR-34a, and p53 Signaling in MCF-7 Breast Cancer Cells

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Abstract: Breast cancer remains one of the most prevalent types of cancer among women globally, contributing significantly to cancer-related mortality. Despite advancements in treatment, many cases continue to exhibit resistance to chemotherapy, radiotherapy, and hormonal therapies, often resulting in drug resistance, high recurrence rates, and severe side effects. Consequently, the role of the natural food components in cancer prevention and treatment is gaining increasing attention in modern medicine. This study focuses on quercetin, a phytochemical compound, and its effects on the breast cancer cell line MCF-7. Specifically, the study has investigated changes in the expression of miR-15a and miR-34a—microRNAs (miRNAs) known to regulate gene expression at the post-transcriptional level—and the *P53* gene, which is critically involved in apoptosis. The analysis was performed using quantitative polymerase chain reaction (qPCR) and Western blot techniques. The results demonstrated that quercetin treatment at concentrations of 40 µM and 80 µM led to a 1.34-fold and 2.73-fold increase in *P53* gene expression, respectively. Additionally, the tumor suppressor miRNA miR-15a showed expression changes of 1.48-fold and 1.69-fold at the same quercetin concentrations. Similarly, miR-34a expression levels increased by 1.23-fold and 1.39-fold at 40 µM and 80 µM, respectively. These findings suggest that dietary phytochemicals, such as quercetin, may have therapeutic potential by modulating miRNA expression and targeting the p53 pathway. In conclusion, quercetin emerges as a promising natural therapeutic agent for breast cancer, warranting further in vivo studies and clinical trials to confirm its efficacy and explore its potential as a part of combination therapies.

Keywords: Flavonoids, cancer, non-coding RNAs, P53

MCF-7 Meme Kanseri Hücrelerinde Tümör Baskılayıcı miR-15a, miR-34a ve p53 Sinyalizasyonunun Kuersetin Aracılı Modülasyonu

Öz: Meme kanseri, küresel olarak kadınlarda en yaygın kanser türlerinden biri olmaya devam etmekte ve kanserle ilişkili ölümlere önemli ölçüde sebep olmaktadır. Tedavideki gelişmelere rağmen, birçok vaka kemoterapi, radyoterapi ve hormonal tedavilere direnç göstermeye devam etmekte ve bu da sıklıkla ilaç direnci, yüksek tekrarlama oranları ve ciddi yan etkilerle sonuçlanmaktadır. Bu nedenle, doğal gıda bileşenlerinin kanser önleme ve tedavisindeki rolü modern tıpta giderek daha fazla ilgi görmektedir. Bu çalışma, bir fitokimyasal bileşik olan kuersetin ve meme kanseri hücre hattı MCF-7 üzerindeki etkilerine odaklanmaktadır. Çalışmada, özellikle, gen ifadesini transkripsiyon sonrası düzeyde düzenlediği bilinen mikroRNA'lar (miRNA) olan miR-15a ve miR-34a'nın ve apoptozda kritik rol oynayan *P53* geninin ifadesindeki değişiklikler araştırılmıştır. Analiz, kantitatif polimeraz zincir reaksiyonu (qPCR) ve Western blot teknikleri kullanılarak gerçekleştirilmiştir. Sonuçlar, 40 µM ve 80 µM konsantrasyonlarında kuersetin tedavisinin *P53* gen ekspresyonunda sırasıyla 1,34 kat ve 2,73 kat artışa yol açtığını göstermiştir. Ek olarak, tümör baskılayıcı miRNA miR-15a aynı kuersetin konsantrasyonlarında 1,48 kat ve 1,69 kat ekspresyon değişiklikleri olduğu saptanmıştır. Benzer şekilde, miR-34a ekspresyon seviyeleri sırasıyla 40 µM ve 80 µM'de 1,23 kat ve 1,39 kat artmıştır. Bu bulgular, kuersetin gibi diyet fitokimyasallarının miRNA ekspresyonunu düzenleyerek ve p53 yolunu hedefleyerek terapötik potansiyele sahip olabileceğini düşündürmektedir. Sonuç olarak, kuersetin meme kanseri için umut verici bir doğal terapötik ajan olarak ortaya çıkmakta ve etkinliğini doğrulamak ve kombinasyon terapilerinin bir parçası olarak potansiyelini keşfetmek için daha fazla in vivo çalışma ve klinik denemeyi gerektirmektedir.

Anahtar kelimeler: Flavonoidler, kanser, kodlama yapmayan RNAlar, P53

1. Introduction

Breast cancer continues to be a major global health challenge, accounting for approximately 30% of all cancer cases worldwide, as reported by Siegel et al. (2020). This alarming prevalence underscores the pressing need to develop innovative and effective therapeutic strategies. In recent years, flavonoids have emerged as a promising class of natural compounds, gaining significant attention due to their diverse biological activities and potential therapeutic applications. These natural polyphenolic compounds possess antioxidant, anti-inflammatory, anticancer, antidiabetic, antiviral, and antiallergic properties (Kopustinskiene et al., 2020). They have shown remarkable potential in cancer therapy by inhibiting tumor cell proliferation, inducing apoptosis, and suppressing angiogenesis (Tang et al., 2021).

Quercetin, a well-known flavonoid commonly found in fruits, vegetables, and medicinal plants, has been

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extensively studied for its pharmacological effects, including antioxidant, anti-inflammatory, antimicrobial, and antitumor activities (Hashemzaei et al., 2017; Li et al., 2020; Rhman et al., 2022). Its anticancer properties, such as inhibiting cell proliferation, inducing apoptosis, and delaying cancer cell invasion and metastasis, make it an attractive candidate for therapeutic intervention (Wang et al., 2021). Given its favorable safety profile and pharmacological value, quercetin has garnered increasing research interest, with numerous studies exploring its potential applications as a chemopreventive and chemotherapeutic agent (Iqbal et al., 2018; Chen et al., 2022). Moreover, its lower toxicity and side effect profile compared to the synthetic drugs make it an appealing option in cancer treatment (Zhang et al., 2019). Emerging evidence suggests that quercetin can modulate metabolic functions through regulatory effects on transcription factors and critical proteins within cellular signaling pathways (Ezzati et al., 2020). Additionally, flavonoids have been shown to regulate non-coding microRNAs (miRNAs), which are essential in controlling gene expression and various cellular processes (Mansoori et al., 2020; Adinew et al., 2021). In particular, quercetin has demonstrated the ability to modulate the expression of functional genes through miRNA-associated regulatory pathways (Tao et al., 2015).

MicroRNAs (miRNAs) are small, non-coding RNAs that regulate gene expression through post-transcriptional gene silencing, playing crucial roles in development, proliferation, metabolism, and inflammation (Kawaguchi et al., 2017; O'Brien et al., 2018). The expression of miRNAs varies significantly under pathological conditions, including cancer, and is closely linked to patient survival (Misso et al., 2014; Zhang et al., 2019). Aberrant miRNA expression is often associated with treatment resistance in tumors, making miRNAs attractive therapeutic targets (Ferdin et al., 2010; Gungormez & Acar, 2019). Among tumor suppressor miRNAs, miR-15a plays a vital role as a tumor suppressor in various cancers, including breast cancer (Bandi et al., 2009; Erbes et al., 2015). In MCF-7 breast cancer cell lines, miR-15a demonstrated anticancer activity by inhibiting cell proliferation and promoting apoptosis (Calin et al., 2008; Zhang et al., 2021). It interacts with the *P53* tumor suppressor gene, enhancing p53 pathway activation and subsequently arresting the cell cycle and triggering programmed cell death (Muller et al., 2011) Notably, decreased miR-15a expression correlates with tumor progression and metastasis in breast cancer, highlighting its potential as both a diagnostic biomarker and a therapeutic target (Tang et al., 2012). Similarly, miR-34a has garnered considerable attention due to its pivotal role in cancer biology (Agarwal et al., 2015; Imani et al., 2018; Kang et al., 2015). It exerts anticancer effects by inducing apoptosis through p53 signaling pathways and inhibiting the epithelial-mesenchymal transition (EMT) process (Wang et al., 2021). Due to its regulatory functions and impact on apoptosis, miR-34a holds substantial therapeutic potential, particularly in breast cancer (Chen et al., 2022). Its relevance as a tumor suppressor molecule is underscored by its association with p53 dysfunction, epigenetic silencing, and genomic losses (Chang et al., 2007; Zhang et al., 2019).

This study aimed to explore the anticancer potential of quercetin on the MCF-7 breast cancer cell line, focusing on the synergistic roles of miR-15a, miR-34a, and *P53*. This research examines their coordinated effects in cancer biology and provides valuable insights into the therapeutic potential of natural compounds in combating breast cancer.

2. Material and Method

2.1. Dataset Selection

In this study, microarray data were obtained from the Gene Expression Omnibus (GEO) database (https://www.ncbi.nlm.nih.gov/gds/). The miRNA profiles were collected from three platforms: GPL570 (6 assays), GPL571 (8 assays), and GPL6244 (25 assays), with additional screening performed using the Affymetrix Human Gene 1.0 ST Array.

Based on the observed changes in the expression levels of miR-15a, miR-34a, and p53 in the sampled datasets, the study focused on analyzing ARR files from the GPL platform through TAC processing. Among the miRNA datasets with the highest overlap in expression levels, miR-15a and miR-34a were identified as the top two miRNAs, with expression values ranging between -1.5 and 2.5 on the scale. These findings provided critical guidance for the design and planning of the study.

2.2. Cell Culture and Treatments

The MCF-7 cell line, kindly provided by Prof. Dr. İlknur Keskin from Medipol University, was cultured in Dulbecco's Modified Eagle Medium/Nutrient Mixture F-12 (DMEM/F12) supplemented with 10% (v/v) fetal bovine serum and 1% (v/v) penicillin-streptomycin. The cells were maintained under standard conditions at 37°C in a humidified incubator with 5% CO_2. The culture medium was refreshed every 24 to 48 hours and all procedures were performed under aseptic conditions following standard cell culture protocols.

Quercetin (CAS number: 117-39-5; Lot ID: SLBM7336V) was purchased from Sigma-Aldrich Pty Ltd. Stock solutions were prepared by dissolving quercetin in dimethyl sulfoxide (DMSO) at a concentration of 10 mM. For experimental treatments, cells were exposed to quercetin at final concentrations of 40 µM and 80 µM, chosen based on their proven efficacy in inhibiting cell proliferation. To minimize potential cytotoxic effects, the final concentration of DMSO in all treatment conditions was kept below 1%.

2.3. Proliferation Analysis

The effect of quercetin on cell proliferation was assessed using the Alamar Blue assay, a method that quantifies metabolic activity by detecting the reduction of resazurin to resorufin in viable cells. MCF-7 cells were seeded at a density of 1×10^4 cells per well in 96-well plates and incubated for 24 hours. Following incubation, the culture medium was replaced with fresh medium containing either 40 µM or 80 µM quercetin. These concentrations were determined by our preliminary experiments. To maintain consistency, the medium was refreshed daily at the same time and the treatment continued for 48 hours. At the end of the treatment period, the culture medium was carefully removed and the fresh medium containing Alamar Blue solution (Invitrogen, USA) at a 1:10 ratio was added to each well. The plates were shielded from light

and incubated for 4 hours under standard culture conditions. Absorbance was then measured at 570 nm and 600 nm using a microplate reader (Thermo MultiSkanIt, USA) to determine cell viability based on the recorded absorbance values (Aktas & Ayan, 2021). To ensure the reliability of the results, all experiments were performed in triplicate.

2.4. Total RNA Extraction and qPCR

Total RNA was extracted from the cells using the miRNeasy Mini Kit (Qiagen, Germany), following the manufacturer's instructions. The RNA concentration was determined by using a NanoDrop spectrophotometer and RNA integrity was assessed by electrophoresis on a 1.2% agarose gel. After RNA isolation, complementary DNA (cDNA) synthesis was carried out using the High Capacity cDNA Reverse Transcription Kit (Applied Biosystems™) according to the manufacturer's protocol. The synthesized cDNA was either utilized immediately for real-time PCR or stored at -80°C for future experiments. Quantitative real-time PCR (qRT-PCR) was performed using the BlasTaq 2X qPCR MasterMix (ABM, Canada), following the guidelines provided by the manufacturer Gene expression levels were analyzed using the delta-delta Ct (ΔΔCt) method, with normalization performed against suitable reference genes (RNU6 for miRNAs, GAPDH for p53) to ensure accurate quantification.

2.5. Western Blot Analysis

Cell disruption was performed using a RIPA lysis buffer system (Santa Cruz Biotechnology, USA). The lysates were then centrifuged at 14,000 rpm to separate the supernatant and the protein concentration was measured using the Bradford assay. Protein samples (40 µg) were resolved by 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and subsequently transferred onto polyvinylidene difluoride (PVDF) membranes. For immunoblotting, membranes were blocked with 1% bovine serum albumin (BSA) to prevent non-specific binding and incubated overnight at 4°C with primary antibodies targeting p53 (1:1000; Boster Bio, China). After thorough washing, the membranes were incubated for one hour with horseradish peroxidase (HRP)-conjugated secondary antibodies at a 1:5000 dilution ratio. Protein bands were visualized using enhanced chemiluminescence (ECL) reagents (Santa Cruz Biotechnology, USA) and detected using an imaging system (Bio-Rad ChemiDoc Imaging System, USA).

2.6. Bioinformatics and Statistical Analysis

FunRich (Functional Enrichment Analysis Tool) is a standalone software application specifically designed for functional enrichment and interaction network analyses of genes, miRNAs, and proteins. In this study, FunRich version 3.1.3 was employed to perform functional enrichment analyses, enabling the identification of biologically relevant pathways, molecular functions, and cellular components that were significantly enriched within the datasets (Gungormez, 2024). At least three petri dishes were used for an experiment and each experiment was repeated at least three times. The results were presented as mean ± standard deviation (x ± SD). Statistical analysis was conducted using SPSS software. Differences in gene expression levels between control and treated cells were evaluated through one-way analysis of variance (ANOVA), followed by Dunnett's post hoc test for multiple comparisons. A p-value<0.05 was deemed statistically significant unless otherwise adjusted for FDR in enrichment analyses.

3. Results and Discussion

3.1. Quercetin induces miR-15a and miR-34a expression

The expression levels of miR-15a and miR-34a were assessed in MCF-7 cells following treatment with 40 µM and 80 µM quercetin. Relative expression analysis using the ΔΔCt method demonstrated that miR-15a expression increased by 1.48-fold at 40 µM and 1.69-fold at 80 µM quercetin, both statistically significant ($p<0.05$) (Fig. 1A). Similarly, miR-34a expression levels increased by 1.23-fold and 1.39-fold at the respective quercetin concentrations (Fig. 1B). No statistically significant difference was observed between DMSO-treated cells (used as a solvent control) and untreated cells ($p>0.05$). However, the differences in expression levels between quercetin-treated cells and the control group were statistically significant ($p<0.05$).

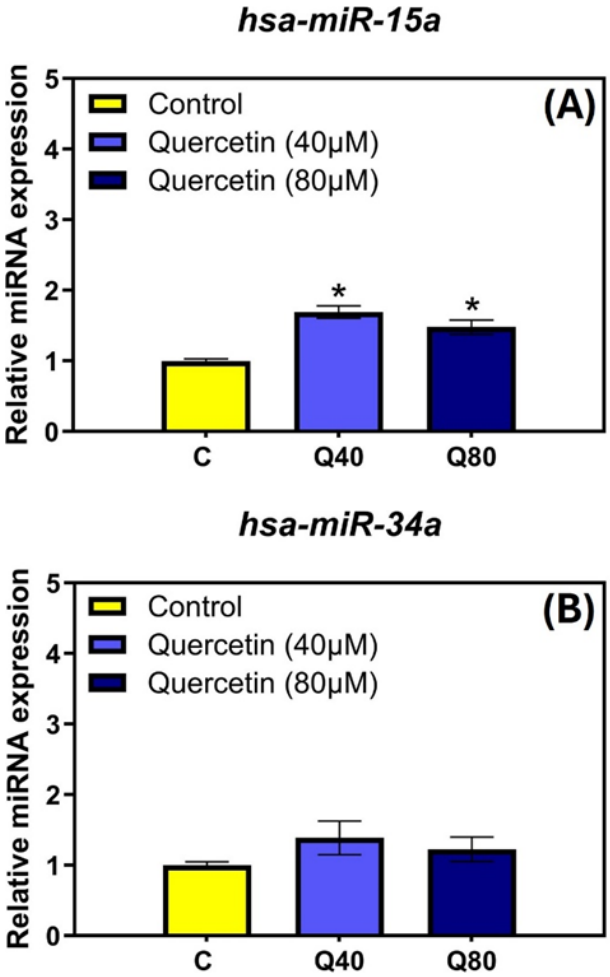

Figure 1. Relative epression level of (A) hsa-miR-34a and (B) hsa-miR-15a. * Statistically significant difference between control and quercetin treatment groups $p<0.05$.

miRNAs, particularly miR-34a, play critical roles in tumor suppression through mechanisms such as apoptosis induction and inhibition of the epithelial-mesenchymal transition (EMT) process via p53 pathways (Imani et al., 2016; Rodríguez-García et al., 2019; Gilbert & Liu, 2010). In breast cancer, metastasis remains the leading cause of

mortality and flavonoids such as quercetin have been identified as bioactive compounds capable of modulating these processes. Previous studies have reported that flavonoids can inhibit metastasis through miRNA activation, particularly miR-34a, which is a well-known tumor suppressor (Imani et al., 2018). Our results suggest that quercetin-mediated upregulation of miR-15a and miR-34a may contribute to tumor-suppressive pathways, aligning with findings that dietary phytochemicals can exert therapeutic effects by modulating miRNA expression.

3.2. Quercetin upregulates P53 expression at both mRNA and protein level

To further elucidate the anticancer effects of quercetin, we examined its impact on p53 expression. The results demonstrated that quercetin treatment significantly upregulated p53 expression in a dose-dependent manner. Specifically, p53 mRNA levels increased by 1.34-fold at 40 µM and 2.73-fold at 80 µM compared to the control ($p<0.05$). Western blot analysis also revealed a concentration-dependent increase in p53 protein levels. These results strongly suggest that quercetin enhances p53-mediated tumor suppression, corroborating previous findings that link flavonoids to p53 activation in cancer cells (Ezzati et al., 2020; Hashemzaei et al., 2017).

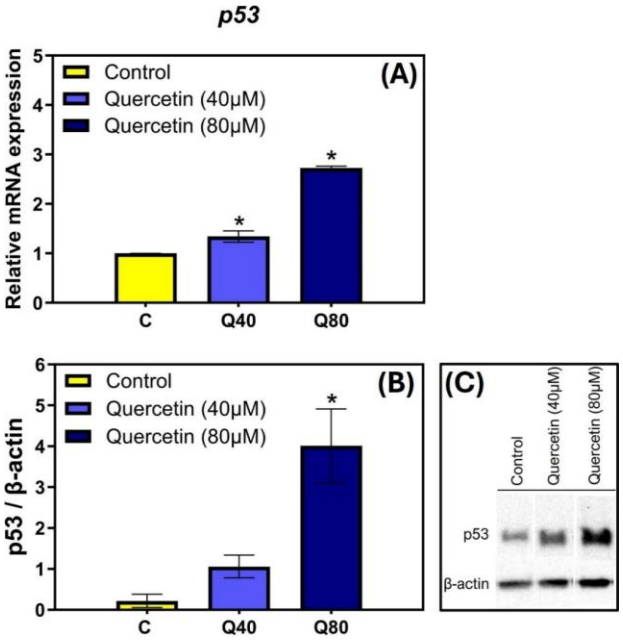

Figure 2. Relative (A) mRNA (B), (C) protein expression levels of p53 after quercetin treatment. *Statistically significant difference between control and quercetin treatment groups $p<0.05$.

The tumor suppressor p53 plays a critical role in regulating cell cycle arrest and apoptosis in response to DNA damage and oncogenic stress. Studies have shown that quercetin enhances p53-mediated tumor suppression mechanisms through multiple pathways, including transcriptional regulation of key apoptotic genes (Murakami et al., 2008). The observed upregulation of p53 protein expression in our study further corroborates quercetin's role in activating p53-dependent apoptotic mechanisms, which has been previously reported in multiple cancer models (Tang et al., 2021; Amit et al., 2020; Kim et al., 2014; Rauf et al., 2018). These results suggest that quercetin may be a promising natural compound for enhancing tumor suppression via p53 activation.

3.3. Bioinformatic analyses of miR-15a and miR-34a functions

The regulatory roles of miR-15a and miR-34a in tumor-suppressive pathways were assessed by bioinformatic analyses. Functional enrichment analysis performed using FunRich (version 3.1.3) demonstrated that miR-15a and miR-34a target genes were significantly enriched in the following molecular functions: transcription factor activity (6.5%), cell adhesion molecule activity (3.4%), and protein serine/threonine kinase activity (2.8%) ($p < 0.05$) (Fig. 3A). These findings suggest that miR-15a and miR-34a play essential roles in gene regulation and signal transduction, potentially through the p53 signaling pathway. Cellular component analysis indicated that these target genes are predominantly localized in the cytoplasm (46.6%), plasma membrane (29%), cytosol (11.1%), endosome (3.8%), and microtubule-associated complex (0.9%) ($p < 0.05$) (Fig. 3B). These results provide valuable information on the spatial distribution of miRNA-regulated proteins, emphasizing their functional relevance in various cellular compartments.

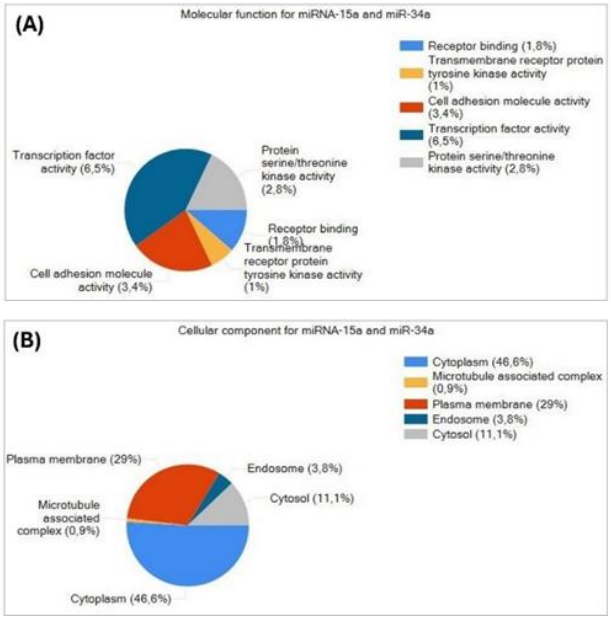

Figure 3. Functional Enrichment Analysis of miR-15a and miR-34a

The regulation of miRNA expression is crucial in breast cancer progression. miR-34a is known to function as a key tumor suppressor by targeting genes involved in cell proliferation, apoptosis, and EMT (Raver-Shapira et al., 2007; Misso et al., 2014). Additionally, miR-15a has been implicated in multiple cancer types, including breast, lung, and colon cancers (Hermeking, 2010; Pang et al., 2010). Given that quercetin significantly upregulated both miRNAs, our findings suggest that quercetin may exert its anticancer effects by modulating these key regulatory molecules.

3.4. Implications of quercetin-mediated miRNA regulation

Recent studies suggest that quercetin modulates multiple signaling pathways, including NF-κB, STAT3, and PI3K/AKT, all of which contribute to cancer progression (Hämäläinen et al., 2007; Kim et al., 2014; Rauf et al., 2018; Tang et al., 2021). Additionally, its regulatory effects on

miRNAs have gained attention due to their critical roles in post-transcriptional gene silencing (Mansoori et al., 2020). In this study, we demonstrated that quercetin enhances the expression of miR-34a and miR-15a, further supporting the hypothesis that dietary flavonoids can exert anticancer effects via miRNA-mediated gene regulation (Chen et al., 2022).

The observed interaction between miR-34a, miR-15a, and p53 in this study aligns with prior research indicating that p53 activation leads to the upregulation of these tumor suppressor miRNAs, facilitating apoptosis and inhibiting tumor growth (Misso et al., 2014; Raver-Shapira et al., 2007). Moreover, the potential of miR-34a as a non-invasive biomarker for breast cancer has been previously reported, highlighting its diagnostic and therapeutic value (Li et al., 2019). Given these findings, the modulation of miRNA expression by quercetin may provide new therapeutic avenues for breast cancer treatment.

Therapeutic strategies targeting miR-15a and miR-34a have been proposed as promising approaches for cancer treatment. Restoring or supplementing the expression of miR-15a has been shown to inhibit tumor growth and enhance chemotherapy sensitivity (Calin et al., 2008). Similarly, strategies that restore p53 function, such as MDM2 inhibitors, have demonstrated efficacy in various cancer models (Vousden & Lane, 2007). Given the observed increase in miR-15a, miR-34a, and p53 expression following quercetin treatment, our findings suggest that quercetin may serve as a potential adjunctive therapeutic agent for breast cancer treatment.

4. Conclusion

This study demonstrates the significant anticancer potential of quercetin through its regulatory effects on tumor suppressor miRNAs (miR-15a and miR-34a) and the p53 signaling pathway in MCF-7 breast cancer cells. Quercetin treatment resulted in a dose-dependent upregulation of miR-15a, miR-34a, and p53 at both the mRNA and protein levels, suggesting its ability to activate tumor suppressive mechanisms. Additionally, bioinformatic analyses revealed that these miRNAs are involved in critical cellular functions, including transcription factor activity and cell adhesion, emphasizing their role in cancer progression and metastasis inhibition.

Our findings align with previous reports on the therapeutic potential of quercetin as a natural compound that modulates key molecular pathways in cancer biology. Quercetin may promote apoptosis or inhibit cell proliferation and metastasis by activating p53 through increasing the expression of miR-15a and miR-34a. This multifaceted action highlights quercetin as a promising candidate for adjunctive cancer therapy, offering a natural and potentially less toxic alternative to conventional treatments.

Given the promising outcomes of this study, further in vivo investigations and clinical trials are warranted to validate the efficacy of quercetin and fully elucidate its therapeutic potential in breast cancer treatment. Additionally, exploring quercetin's role in combination therapies may provide new avenues for enhancing the effectiveness of existing cancer treatments.

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