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Molecular Effects of Curcumin Nanomicelles on Wharton's Jelly-Derived Mesenchymal Stem Cells: Integrated Analysis of Inflammatory Genes, Regulatory MicroRNAs, and DNA Methylation

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Abstract

Background: Mesenchymal stem/stromal cells (MSCs) exert therapeutic effects largely through immunomodulatory and paracrine mechanisms. Pharmacological preconditioning strategies have been proposed to enhance MSC functionality and therapeutic efficacy. Curcumin possesses anti-inflammatory and epigenetic regulatory properties; however, the effects of curcumin nanomicelles on inflammation-associated genes, regulatory microRNAs, and DNA methylation patterns in Wharton's jelly-derived MSCs (WJ-MSCs) remain incompletely understood.

Objectives: This study investigated the molecular effects of curcumin nanomicelles on inflammatory regulatory pathways in WJ-MSCs.

Methods: WJ-MSCs were isolated from human umbilical cords and characterized by flow cytometry and multilineage differentiation assays. Cell viability following exposure to curcumin nanomicelles was assessed using the MTT assay. Cells were treated with 20 µM and 70 µM curcumin nanomicelles for 24 h. Expression levels of IκBα, TGF-β1, miR-21, and miR-146a were evaluated by quantitative real-time PCR. Promoter methylation status of IκBα and TGF-β1 was analyzed using methylation-sensitive high-resolution melting (MS-HRM).

Results: Curcumin nanomicelles induced dose-dependent molecular alterations in WJ-MSCs. Treatment with 70 µM significantly increased IκBα expression and reduced miR-21 expression compared with untreated controls ($P < 0.05$). TGF-β1 expression was significantly increased following treatment with 20 µM curcumin nanomicelles, whereas no significant change was observed at 70 µM. miR-146a expression was significantly decreased at both tested concentrations. Despite these transcriptional and post-transcriptional changes, no significant alterations in promoter methylation patterns of either IκBα or TGF-β1 were detected after 24 h of treatment.

Conclusion: Curcumin nanomicelles modulated inflammation-associated gene expression and regulatory microRNAs in WJ-MSCs without inducing detectable promoter methylation changes in IκBα or TGF-β1. These findings suggest that the early molecular effects of curcumin nanomicelles are predominantly mediated by transcriptional and post-transcriptional mechanisms. The observed modulation of inflammatory regulatory pathways supports further investigation of curcumin nanomicelles as a potential MSC preconditioning strategy; however, additional protein-level, functional, and disease-model studies are required to determine their translational relevance.

Keywords: Curcumin nanomicelles; Wharton's jelly-derived mesenchymal stem cells; IκBα; TGF-β1; miR-21; miR-146a; DNA methylation; MSC preconditioning

1. Introduction

Mesenchymal stem/stromal cells (MSCs) have emerged as promising candidates for regenerative medicine because of their immunomodulatory properties, paracrine activity, and capacity to promote tissue repair (1, 2). Increasing evidence indicates that the therapeutic effects of MSCs are predominantly mediated by the secretion of bioactive molecules and modulation of the immune microenvironment rather than by direct differentiation into damaged tissues (3, 4). The therapeutic efficacy of MSC-based approaches has been demonstrated across diverse disease settings in both preclinical and translational studies (5-9). Consequently, substantial efforts have been directed toward developing strategies that enhance MSC functionality and optimize their therapeutic efficacy in inflammatory and immune-mediated disorders.

Among available MSC sources, Wharton's jelly–derived mesenchymal stem cells (WJ-MSCs) have attracted considerable attention due to their high proliferative capacity, low immunogenicity, ease of isolation, and favorable ethical profile. These characteristics make WJ-MSCs an attractive cell source for clinical applications (10). However, the biological activity of MSCs is highly influenced by their microenvironment, and their therapeutic performance can vary substantially depending on culture conditions and inflammatory status. Therefore, pharmacological preconditioning approaches aimed at improving MSC survival and immunomodulatory function have become an important area of investigation (11).

Inflammatory signaling pathways play a central role in regulating MSC behavior. Among these pathways, Nuclear Factor kappa B (NF-κB) is a key transcription factor that regulates cytokine production, cell survival, migration, and interactions with immune cells (12). Under resting conditions, NF-κB remains sequestered in the cytoplasm through its association with inhibitor of kappa B alpha (IκBα). Following inflammatory stimulation, degradation of IκBα permits NF-κB nuclear translocation and activation of pro-inflammatory gene expression programs. Because excessive NF-κB activation can impair MSC function and contribute to chronic inflammation, modulation of the NF-κB/IκBα signaling axis has emerged as a promising strategy to enhance MSC therapeutic potential (2, 13, 14).

Transforming growth factor-beta 1 (TGF-β1) is another critical regulator of MSC biology. TGF-β1 participates in multiple cellular processes, including immunoregulation, extracellular matrix remodeling, differentiation, and tissue repair. Although physiological TGF-β1 signaling contributes to regenerative responses, dysregulated activation may promote fibrosis and adverse tissue remodeling. Therefore, understanding how potential MSC-modulating agents influence TGF-β1 expression is important for evaluating their therapeutic applicability (15, 16).

In addition to transcriptional regulation, epigenetic mechanisms play an essential role in determining MSC phenotype and function. MicroRNAs (miRNAs) are small non-coding RNAs that regulate gene expression post-transcriptionally and contribute to the fine-tuning of inflammatory signaling networks (17). Among them, miR-21 and miR-146a have been widely implicated in regulating immune and inflammatory responses. miR-21 is frequently associated with cell survival, proliferation, and inflammatory signaling, whereas miR-146a primarily acts as a negative feedback regulator of NF-κB activity (18). Alterations in the expression of these miRNAs may significantly influence

MSC immunomodulatory properties and therapeutic performance (19). Furthermore, DNA methylation is a stable epigenetic mechanism that regulates gene transcription and long-term cellular behavior. Changes in promoter methylation of inflammation-related genes may therefore contribute to sustained alterations in MSC function (20, 21).

Curcumin, a naturally occurring polyphenolic compound derived from Curcuma longa, has received considerable attention for its anti-inflammatory, antioxidant, and epigenetic regulatory properties (22). Previous studies have demonstrated that curcumin can modulate NF-κB signaling, influence TGF-β-related pathways, and alter miRNA expression in various cellular systems. However, its broader therapeutic application has been limited by poor aqueous solubility and low bioavailability. To overcome these limitations, nanomicellar formulations have been developed to improve curcumin stability, solubility, and intracellular delivery, thereby enhancing its biological activity (23, 24).

Although the anti-inflammatory effects of curcumin have been extensively investigated in different cell types, its integrated effects on inflammatory gene expression, inflammation-associated miRNAs, and epigenetic modifications in WJ-MSCs remain incompletely understood. In particular, limited information is available regarding whether curcumin nanomicelles can simultaneously influence NF-κB-related signaling components, regulatory microRNAs, and promoter methylation patterns in WJ-MSCs. Addressing this knowledge gap may provide valuable insights into the development of pharmacological preconditioning strategies to improve MSC-based therapies (25).

Therefore, the present study aimed to investigate the effects of curcumin nanomicelles on the expression of IκBα, TGF-β1, miR-21, and miR-146a, as well as the promoter methylation status of IκBα and TGF-β1 in WJ-MSCs. By integrating transcriptional, post-transcriptional, and epigenetic analyses, we sought to characterize better the molecular responses of WJ-MSCs to curcumin nanomicelle treatment and to evaluate its potential as an MSC preconditioning approach.

2. Materials and Methods

2.1. Study Design

This study was designed as an in vitro experimental investigation to evaluate the effects of curcumin nanomicelles on microRNA expression and genomic DNA methylation in human WJ-MSCs. Ethical approval was obtained from the Ethics Committee of Mashhad University of Medical Sciences (Approval code: IR.MUMS.REC.1402.189). Written informed consent was obtained from all donors prior to sample collection in accordance with the Declaration of Helsinki.

2.2. Isolation and Culture of Wharton's Jelly–Derived Mesenchymal Stem Cells

Human umbilical cords were obtained following full-term deliveries with written informed consent and in accordance with institutional ethical guidelines. Under sterile conditions, umbilical blood vessels were removed, and Wharton's jelly tissue was dissected into small fragments. The explants were cultured in Dulbecco's Modified Eagle Medium–Low Glucose (DMEM-LG) supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin at 37 °C in a humidified atmosphere containing 5% CO_2. Medium was replaced every 3–4 days. Adherent fibroblast-like cells were expanded, and cells at passages 3–5 were used for all experiments.

2.3. Phenotypic Characterization of WJ-MSCs

Immunophenotypic characterization of WJ-MSCs was performed by flow cytometry according to the minimal criteria defined by the International Society for Cell and Gene Therapy. Cells were harvested, washed with PBS, and incubated with fluorochrome-conjugated antibodies against CD73, CD90, and CD105 (positive markers) and CD34, CD45, CD14, CD19, and HLA-DR (negative markers). Data acquisition was performed using a flow cytometer, and analysis was conducted with appropriate software. Only cell populations fulfilling MSC surface marker criteria were used for downstream experiments.

2.4. Curcumin Nanomicelle Treatment

Curcumin nanomicelles were prepared according to the manufacturer's instructions and stored in the dark. WJ-MSCs were seeded and allowed to adhere overnight, then treated with curcumin nanomicelles at final concentrations of 20 µM and 70 µM for 24 hours. Untreated cells served as controls.

2.5. Cell Viability Assay

Cell viability and cytotoxicity of curcumin nanomicelles were assessed using the MTT assay. Briefly, WJ-MSCs were seeded into 96-well plates and treated with increasing concentrations of curcumin nanomicelles for 24 hours.

Following treatment, MTT solution (5 mg/mL) was added to each well and incubated for 4 hours at 37 °C. The resulting formazan crystals were dissolved using dimethyl sulfoxide (DMSO), and absorbance was measured at 570 nm using a microplate reader. Cell viability was calculated relative to untreated controls, and the IC_{50} value was determined.

2.6. RNA Extraction, cDNA Synthesis, and Quantitative Real-Time PCR

Total RNA, including small RNAs, was extracted from treated and control WJ-MSCs using a commercial RNA extraction kit according to the manufacturer's protocol. RNA concentration and purity were evaluated spectrophotometrically by measuring absorbance at 260 and 280 nm, and only samples with A260/A280 ratios between 1.8 and 2.0 were used for downstream analyses. For mRNA expression analysis, complementary DNA (cDNA) was synthesized from 1 µg of total RNA using a reverse transcription kit following the manufacturer's instructions.

Quantitative real-time PCR (qRT-PCR) was performed using SYBR Green master mix on a real-time PCR system to assess the expression levels of IκBα and TGF-β1. The primer sequences used were IκBα forward 5′-CAGCAGACTTTCAGGGACTG-3′ and reverse 5′-TGGGTCAGAGAGGTCACTGT-3′; TGF-β1 forward 5′-TGAGTCACCACCACTTCAGT-3′ and reverse 5′-CACGATCATGTTGGACAGCT-3 ′. GAPDH was used as the internal reference gene (forward 5′-GAAGGTGAAGGTCGGAGT-3′ and reverse 5′-GAAGATGGTGATGGGATTTC-3′). Relative gene expression levels were calculated using the $2^{-}\Delta\Delta Ct$ method.

For microRNA expression analysis, cDNA was synthesized using stem-loop primers specific to each microRNA. Real-time PCR was conducted to determine the expression levels of miR-21 and miR-146a using the following primers: miR-21 forward 5′-TAGCTTATCAGACTGATGTTGA-3′ and miR-146a forward 5′-TGAGAACTGAATTCCATGGGT-3′. U6 small nuclear RNA was used as the endogenous control with forward primer 5′-CTCGCTTCGGCAGCACA-3′ in combination with a universal reverse primer (5′-AACGCTTCACGAATTTGCGT-3′). All reactions were performed in triplicate, and amplification specificity was confirmed by melting curve analysis.

2.7. DNA Extraction, Bisulfite Conversion, and Methylation Analysis

Genomic DNA was extracted using a commercial DNA extraction kit according to the manufacturer's instructions. DNA concentration and purity were assessed spectrophotometrically. Bisulfite conversion of genomic DNA was performed using the EZ DNA Methylation™ Kit. The methylation status of the IκBα and TGF-β1 promoter regions was analyzed using methylation-specific high-resolution melting (MS-HRM). Melting profiles were compared with fully methylated and unmethylated control DNA samples.

2.8. Statistical Analysis

Statistical analyses were performed using GraphPad Prism version 10 (GraphPad Software, San Diego, CA, USA). Data normality was assessed using the Shapiro–Wilk test. Comparisons among groups were conducted using a one-way ANOVA followed by Tukey's post hoc test. All data are presented as mean ± SD from at least three independent biological replicates. P values < 0.05 were considered statistically significant.

3. Result

3.1. Isolation and Morphology of WJ-MSCs

Primary cultures derived from human Wharton's jelly exhibited typical mesenchymal stem cell morphology. Cells migrated from tissue explants and adhered to plastic surfaces, displaying a spindle-shaped, fibroblast-like appearance. Upon successive passages, the cells maintained a homogeneous morphology and reached 70–80% confluence without noticeable morphological abnormalities (Figure 1).

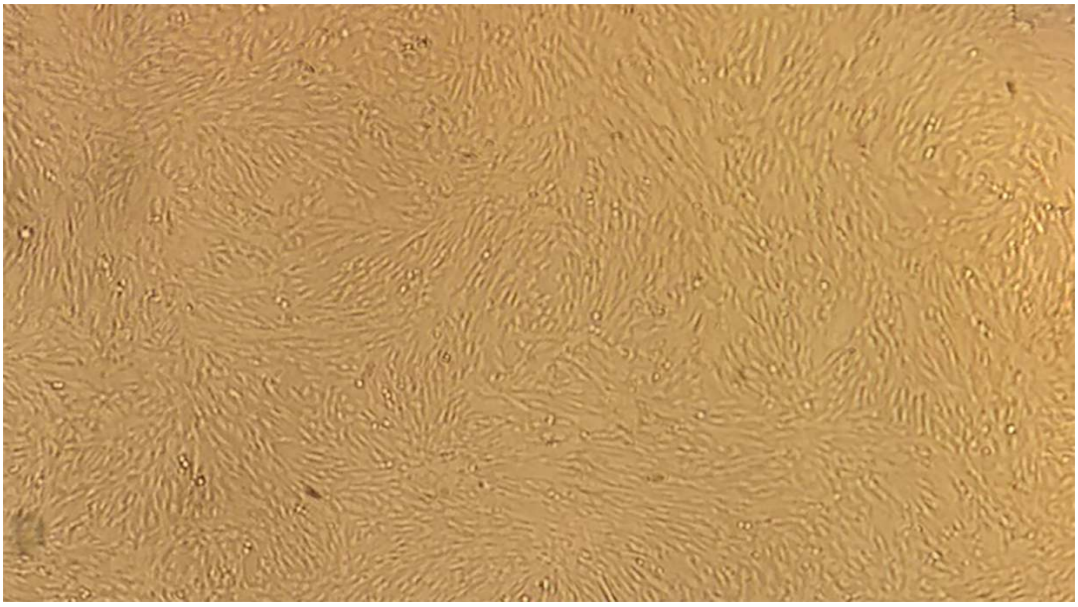


Figure 1: Morphology of Wharton's jelly–derived mesenchymal stem cells (WJ-MSCs)

3.2. Cytotoxicity Assessment of Curcumin Nanomicelles

The cytotoxic effects of curcumin nanomicelles on WJ-MSCs were evaluated using the MTT assay following 24 hours of treatment. Cell viability decreased in a dose-dependent manner with increasing concentrations of curcumin nanomicelles. The half-maximal inhibitory concentration (IC_{50}) was approximately 70 µM. Based on these results, 20 µM (sub-cytotoxic) and 70 µM (near-IC_{50}) concentrations were selected for subsequent experiments. The higher concentration may partially induce cellular stress responses, which should be considered when interpreting transcriptional changes (Figure 2).

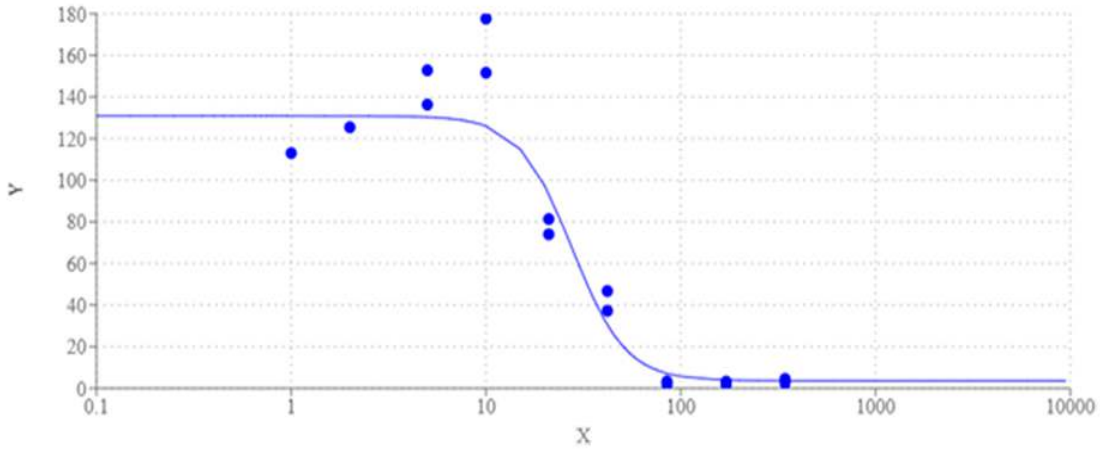


Figure 2: Cell viability of WJ-MSCs following 24-hour treatment with increasing concentrations of curcumin nanomicelles was assessed using the MTT assay. Results are presented as a percentage of viable cells relative to untreated controls. The half-maximal inhibitory concentration (IC_{50}) was approximately 70 µM. Based on these results, 20 µM (sub-cytotoxic) and 70 µM (near-IC_{50} range) concentrations were selected for subsequent experiments.

3.3. Effects of curcumin on IκBα and TGF-β1 expression

Curcumin nanomicelles induced a concentration-dependent increase in IκBα expression. Treatment with 20 µM curcumin did not significantly alter IκBα expression compared with untreated controls (P = 0.269). In contrast, exposure to 70 µM significantly upregulated IκBα levels (P = 0.010). Furthermore, IκBα expression was significantly higher in the 70 µM group than in the 20 µM group (P = 0.021), indicating a dose-dependent regulatory effect (Figure 3-A).

Furthermore, compared with the control group, the expression level of TGF-β1 was notably increased in cells treated with 20 µM of curcumin nanomicelles (p=0.010); however, it was not significantly increased in cells treated with 70 µM of curcumin nanomicelles (p=0.230). The increased TGF-β1 expression in the 20µM treatment group was significant compared to the 70 µM treatment group (p=0.020) (Figure B).

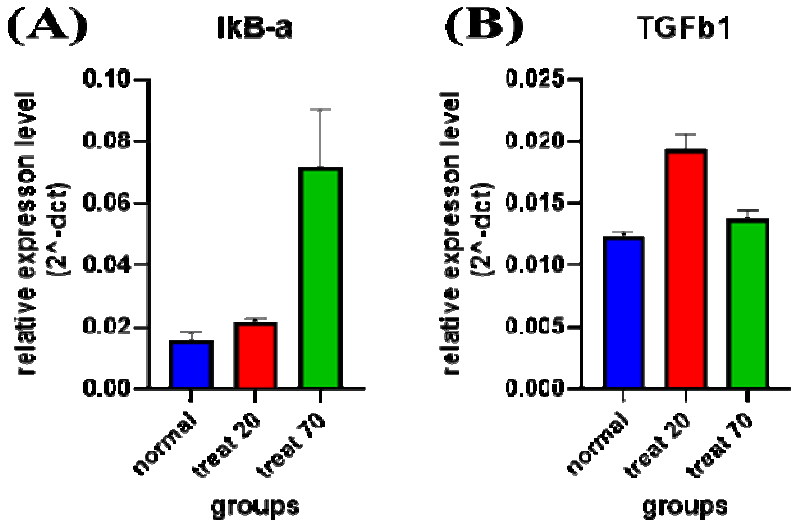


Figure 3. The relative IκBα (A) and TGF-β1 (B) expression of the control group, the group treated with 20 µM of nano-micellar curcumin, and the group treated with 70 µM of nano-micellar curcumin. The error bars represent standard error.

3.4. Effects of curcumin on miR-21 and miR-146a expression

Compared to the control group, miR-21 gene expression was not significantly decreased in cells treated with 20 µM of curcumin nanomicelles (p=0.231); however, it was significantly decreased in cells treated with 70 µM of curcumin nanomicelles (p=0.018). The decreased miR-21 gene expression in the 70 µM treatment group was significant compared to the 20 µM treatment group (p=0.047) (Figure A).

Compared with the control group, miR-146a expression was significantly downregulated in MSCs treated with 20 µM (p=0.011) and 70 µM (p=0.012) of curcumin nanomicelles. Furthermore, the difference between the 20 µM and the 70 µM treatment groups was not significant (p=0.847) (Figure B).

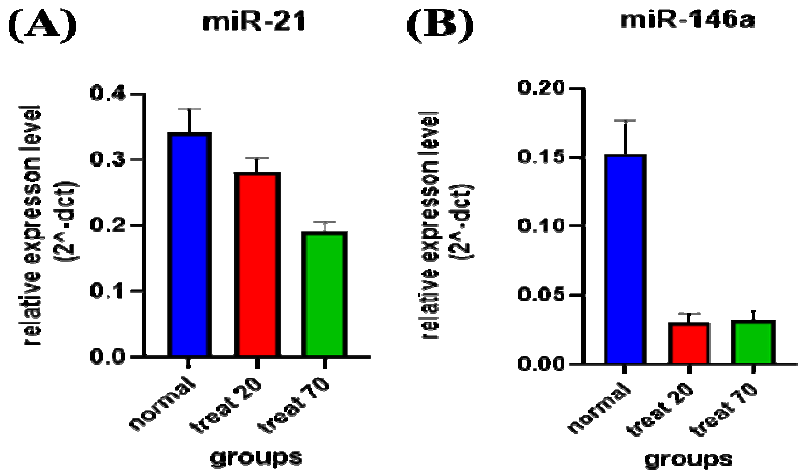
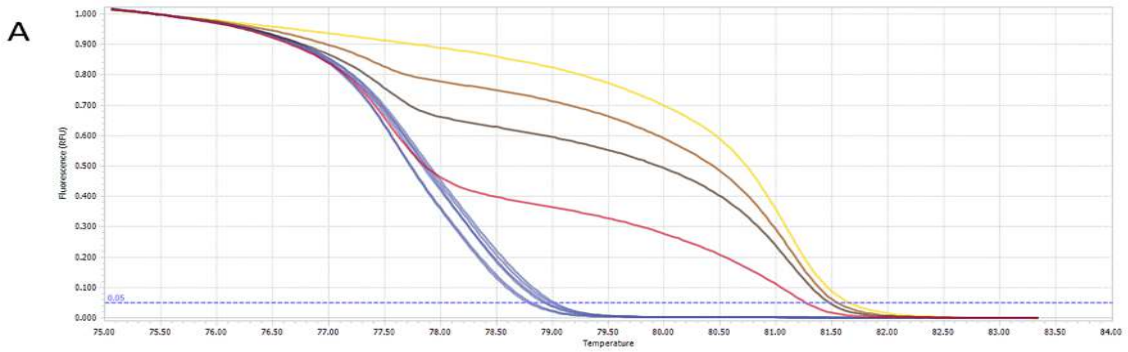


Figure 4. The relative miR-21 (A) and miR-146a (B) expression of the control group, the group treated with 20 µM of nano-micellar curcumin, and the group treated with 70 µM of nano-micellar curcumin. The error bars represent standard error.

3.5. DNA Methylation Analysis of IκBα and TGF-β1 Promoter Regions

The methylation status of the IκBα and TGF-β1 promoter regions was assessed using methylation-specific high-resolution melting (MS-HRM). Comparison of melting curves between treated and control groups revealed no detectable differences in DNA methylation patterns for either gene following 24 hours of treatment with curcumin nanomicelles at both tested concentrations. These results indicate that short-term exposure to curcumin nanomicelles did not significantly alter the promoter methylation status of IκBα or TGF-β1 in WJ-MSCs.(Figure 5:A and B).



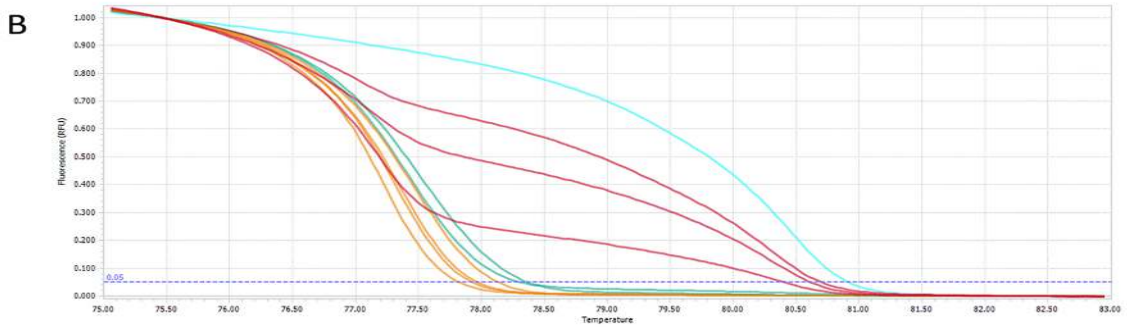


Figure 5: A) MS-HRM analysis of IκBα gene methylation. B) MS-HRM analysis of TGF-β1 gene methylation.

4. Discussion

The present study investigated the effects of curcumin nanomicelles on inflammation-associated gene expression, regulatory microRNAs, and promoter methylation patterns in WJ-MSCs. Our findings demonstrated that curcumin nanomicelles induced dose-dependent molecular alterations characterized by increased IκBα expression, modulation of TGF-β1 expression, and downregulation of miR-21 and miR-146a. In contrast, no detectable changes in the promoter methylation status of IκBα or TGF-β1 were observed after 24 h of treatment. Collectively, these findings suggest that the early effects of curcumin nanomicelles on WJ-MSCs are mediated predominantly through transcriptional and post-transcriptional mechanisms rather than detectable promoter methylation remodeling.

One of the most notable findings of this study was the significant upregulation of IκBα expression following exposure to 70 µM curcumin nanomicelles. IκBα functions as a major negative regulator of NF-κB signaling by sequestering NF-κB complexes in the cytoplasm and limiting transcription of pro-inflammatory genes(26). Numerous studies have demonstrated that curcumin exerts anti-inflammatory effects by suppressing NF-κB activity across a wide variety of cellular systems, including mesenchymal stem cells, macrophages, epithelial cells, and cancer cells (27-29). Previous reports have shown that curcumin can inhibit IKK activation, stabilize IκBα, and reduce NF-κB nuclear translocation, ultimately attenuating inflammatory responses. Therefore, the increased IκBα expression observed in the present study is consistent with the established anti-inflammatory profile of curcumin and may indicate modulation of NF-κB-associated signaling pathways in WJ-MSCs(29, 30).

Importantly, the use of a nanomicellar formulation may have contributed to the observed biological effects. Conventional curcumin exhibits poor aqueous solubility and limited cellular bioavailability, whereas nanomicellar formulations improve stability and intracellular delivery. Enhanced cellular uptake may therefore amplify curcumin's capacity to regulate inflammatory signaling networks, even during relatively short exposure periods (31, 32). Nevertheless, because NF-κB activation, nuclear translocation, and cytokine production were not directly assessed in the present study, the observed increase in IκBα should be interpreted as evidence of potential modulation of NF-κB-related pathways rather than definitive inhibition of NF-κB activity.

Another important observation was the concentration-dependent effect of curcumin nanomicelles on TGF-β1 expression. Treatment with 20 µM curcumin significantly increased TGF-β1 expression, whereas no

significant alteration was detected at 70 µM. TGF-β1 is a multifunctional cytokine that plays essential roles in tissue repair, immune regulation, extracellular matrix remodeling, and stem cell differentiation(33). Previous studies examining the interaction between curcumin and TGF-β signaling have produced apparently conflicting results(34). In fibrotic disorders and in activated fibroblasts, curcumin frequently suppresses TGF-β signaling and reduces fibrosis-related gene expression (35, 36). Conversely, transient enhancement of TGF-β1 expression has been reported in certain wound-healing and regenerative contexts, suggesting that curcumin may exert context-dependent regulatory effects(37, 38).

The increase observed at the lower concentration in the present study may therefore reflect an adaptive response of WJ-MSCs rather than sustained activation of a profibrotic program(39). Because downstream mediators of TGF-β signaling, extracellular matrix markers, and differentiation-associated genes were not evaluated, the biological significance of this response remains uncertain. Furthermore, the absence of TGF-β1 upregulation at 70 µM suggests that concentration-dependent regulatory mechanisms may be at work. Since this concentration approached the IC50 value identified in the viability assay, it is also possible that cellular stress responses contributed to the altered transcriptional profile observed at higher doses.

In addition to altering gene expression, curcumin nanomicelles significantly altered microRNA expression. Among the analyzed microRNAs, miR-21 showed marked downregulation following treatment with 70 µM curcumin. miR-21 is widely recognized as a critical regulator of inflammation, fibrosis, proliferation, and cellular survival. It has been implicated in the pathogenesis of numerous inflammatory and fibrotic disorders. Elevated miR-21 expression has been associated with NF-κB signaling activation, PTEN suppression, and the promotion of profibrotic responses. Consistent with our findings, several studies have demonstrated that curcumin suppresses miR-21 expression in inflammatory, malignant, and fibrotic cellular models(40). Curcumin-mediated downregulation of miR-21 has been linked to attenuation of inflammatory signaling pathways and restoration of cellular homeostasis. Therefore, the reduction in miR-21 observed in WJ-MSCs may represent an additional mechanism through which curcumin modulates inflammatory regulatory networks (41).

However, interpretation of miR-21 suppression in MSCs requires caution. While excessive miR-21 expression is frequently associated with pathological inflammation and fibrosis, physiological levels of miR-21 have also been implicated in MSC survival, migration, and paracrine activity. Consequently, the long-term functional implications of reduced miR-21 expression in WJ-MSCs remain unclear and require further investigation through functional assays and disease-relevant experimental models(42, 43).

A particularly interesting finding was the significant reduction of miR-146a expression at both tested concentrations. miR-146a is generally regarded as an anti-inflammatory microRNA that functions as a negative feedback regulator of NF-κB signaling by targeting IRAK1 and TRAF6 (44). Several studies have reported increased miR-146a expression following curcumin treatment, particularly in inflammatory disease models, where its induction was associated with attenuation of inflammatory responses. Furthermore, enhanced expression of miR-146a has been linked to improved immunomodulatory activity of MSCs and increased therapeutic efficacy in experimental models of inflammation(45).

At first glance, the downregulation of miR-146a observed in the present study appears inconsistent with these reports. However, several factors may explain this discrepancy. First, the experiments were conducted under basal culture conditions without inflammatory stimulation. Because miR-146a is frequently induced as part of a negative feedback response to inflammatory activation, its regulation under non-inflammatory conditions may differ substantially from that observed in activated cells(46). Second, differences in cell type, treatment duration, curcumin formulation, and cellular microenvironment may contribute to variable responses across studies. Therefore, the observed reduction in miR-146a should not necessarily be interpreted as a pro-inflammatory event. Instead, it may reflect context-dependent modulation of feedback regulatory mechanisms in unstimulated WJ-MSCs. Future studies employing inflammatory challenge models will be essential to determine whether curcumin nanomicelles alter the inducible miR-146a response under disease-relevant conditions(47).

Despite observed transcriptional and post-transcriptional changes, no significant alterations in promoter methylation were detected for either IκBα or TGF-β1. These findings suggest that short-term exposure to curcumin nanomicelles is insufficient to induce measurable methylation remodeling at these loci. DNA methylation is generally considered a relatively stable epigenetic modification that often requires prolonged environmental stimulation before detectable changes become apparent(21). Although curcumin has been reported to influence DNA methyltransferase activity and epigenetic regulation in several cancer and non-cancer models, such effects are frequently observed after longer treatment durations or under specific pathological conditions(48). Consequently, the absence of methylation changes in the present study does not exclude potential epigenetic effects of curcumin. However, it indicates that promoter methylation is unlikely to account for the early molecular responses observed in WJ-MSCs.

Taken together, the present findings demonstrate that curcumin nanomicelles can modulate key components of inflammatory regulatory pathways in WJ-MSCs. The simultaneous upregulation of IκBα and suppression of miR-21 are broadly consistent with the anti-inflammatory properties previously attributed to curcumin. In contrast, the concentration-dependent regulation of TGF-β1 and the reduction of miR-146a highlight the complexity of curcumin-mediated signaling in stem cells. Importantly, these molecular alterations occurred in the absence of detectable promoter methylation changes, supporting the notion that transcriptional and post-transcriptional mechanisms predominantly mediate early curcumin responses.

Several limitations should be acknowledged. First, only a single treatment duration was evaluated, potentially underestimating delayed transcriptional and epigenetic responses. Second, functional analyses, including cytokine secretion profiling, macrophage polarization assays, differentiation studies, secretome characterization, extracellular vesicle analysis, and direct assessment of NF-κB protein activity, were not performed. Third, the physicochemical behavior and intracellular kinetics of curcumin nanomicelles were not investigated; finally, the absence of inflammatory stimulation limits the interpretation of curcumin-mediated responses under disease-relevant conditions. Future studies integrating protein-level analyses, functional immunomodulatory assays, inflammatory challenge models, and comprehensive epigenetic profiling will be required to determine whether the molecular alterations

observed in this study translate into enhanced therapeutic efficacy of WJ-MSCs.

5. *Conclusion*

In conclusion, curcumin nanomicelles induced dose-dependent alterations in inflammatory regulatory pathways in WJ-MSCs, characterized by increased IκBα expression and reduced miR-21 expression, together with concentration-dependent modulation of TGF-β1 and miR-146a. These molecular changes occurred in the absence of detectable promoter methylation alterations in IκBα and TGF-β1, suggesting that the early effects of curcumin nanomicelles are predominantly mediated by transcriptional and post-transcriptional mechanisms. Collectively, the findings support the potential of curcumin nanomicelles as a pharmacological preconditioning strategy for WJ-MSCs; however, further protein-level, functional, and disease-model studies are required to determine whether these molecular alterations translate into enhanced therapeutic efficacy.

List of Abbreviations

MSCs: Mesenchymal Stem/Stromal Cells

WJ-MSCs: Wharton's Jelly-Derived Mesenchymal Stem/Stromal Cells

NF-κB: Nuclear Factor Kappa B

IκBα: Inhibitor of Nuclear Factor Kappa B Alpha

TGF-β1: Transforming Growth Factor Beta 1

miRNA: MicroRNA

miR-21: MicroRNA-21

miR-146a: MicroRNA-146a

MS-HRM: Methylation-Sensitive High-Resolution Melting

qRT-PCR: Quantitative Real-Time Polymerase Chain Reaction

cDNA: Complementary DNA

DMEM-LG: Dulbecco's Modified Eagle Medium–Low Glucose

FBS: Fetal Bovine Serum

PBS: Phosphate-Buffered Saline

MTT: 3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide

DMSO: Dimethyl Sulfoxide

GAPDH: Glyceraldehyde-3-Phosphate Dehydrogenase

IC50: Half-Maximal Inhibitory Concentration

DNMTs: DNA Methyltransferases

TNF-α: Tumor Necrosis Factor Alpha

IL-1β: Interleukin-1 Beta

TRAF6: TNF Receptor-Associated Factor 6

IRAK1: Interleukin-1 Receptor-Associated Kinase 1

TLR: Toll-Like Receptor

PTEN: Phosphatase and Tensin Homolog

PI3K: Phosphatidylinositol 3-Kinase

Akt: Protein Kinase B

AP-1: Activator Protein 1

ROS: Reactive Oxygen Species

IKK: IκB Kinase

IKKβ: IκB Kinase Beta

PPARγ: Peroxisome Proliferator-Activated Receptor Gamma

α-SMA: Alpha-Smooth Muscle Actin

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Ethical Approval and Consent to Participate: The study was approved by the Ethics Committee of Mashhad University of Medical Sciences (IR.MUMS.REC.1402.189). Written

informed consent was obtained from all donors prior to sample collection. he study was conducted in accordance with the ethical principles outlined in the Declaration of Helsinki.

Consent for publication: All authors consent to the publication of the article.

Conflicts of Interest: None.

Author contributions:H.M. and R.R.: Writing – Original Draft, Writing – Review & Editing. G.A.: Formal Analysis, Methodology, Conceptualization. A.R.B., R.M., and A.Y.K.: Data Curation. H.R.R.: Writing – Review & Editing, Investigation, Supervision, Project Administration. H.M. and R.R. contributed equally to this work. All authors read and approved the final manuscript.

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