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Original Research Article

Hypoxia-Induced Mesenchymal Stem Cell Secretome Enhances IL-10 via STAT3 Pathway in a Rat PCOS Model

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ABSTRACT

Polycystic ovary syndrome (PCOS) is a condition characterized by chronic inflammation and hyperandrogenism, commonly leading to infertility. This is often triggered by hyperglycemia and hyperlipidemia, which cause hyperandrogenism. The secretome of mesenchymal stem cells (MSCs) can suppress pro-inflammatory cytokine secretion by enhancing IL-10 expression and inhibiting androgen production in a PCOS rat model. This study aims to investigate the effect of the administration of hypoxic mesenchymal stem cell secretome on IL-10 and STAT3 gene expression in the PCOS model. *In vivo* experimental research with a post-test-only control group design. The total sample was 24 Wistar female rats, divided into four groups: healthy, negative control (PCOS rats were injected with 0.9% NaCl), T1 (PCOS rats were given secretome at a dose of 200 µl), and T2 (PCOS rats were given Secretome at 400 µl) and were given treatment for 33 days. QRT-PCR determined IL-10 and STAT3 gene expression, and statistical analysis was performed using the One-Way ANOVA test followed by the Post Hoc LSD test. This study demonstrated that the expression of the IL-10 and STAT3 genes significantly differed in the T2 group compared to the negative control. Additionally, there was a notable difference in IL-10 gene expression between groups T2 and T1 compared to the negative control. Furthermore, differences were also observed in the expression of the STAT3 gene in both the T2 and T1 groups. The administration of hypoxic mesenchymal stem cell secretomes increased IL-10 and STAT3 gene expression in PCOS rat models.

Keywords: Mesenchymal Stem Cells, Hypoxia, IL-10 gene expression, STAT3 gene expression

Introduction

Polycystic Ovary Syndrome (PCOS) is a prevalent endocrine disorder affecting women of reproductive age, characterized by hyperandrogenism, anovulation, and polycystic ovaries.[1] The syndrome encompasses a range of metabolic and hormonal disturbances, often leading to irregular menstrual cycles, hirsutism, insulin resistance, and an increased risk of metabolic comorbidities such as obesity and type 2 diabetes mellitus. [2]

Recent advances in regenerative medicine have highlighted the potential of stem cell-based therapies in addressing the complex pathophysiology of PCOS. Mesenchymal stem cells (MSCs), with their self-renewal and differentiation capabilities, have shown promise in restoring ovarian function and ameliorating metabolic imbalances associated with PCOS.[3,4]

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In particular, the hypoxic preconditioning of MSCs has emerged as a strategic approach to enhancing their therapeutic potential.[5] Hypoxic conditions mimic the microenvironment of injured tissues, promoting the secretion of a distinct set of paracrine factors known as the secretome. This bioactive secretome, rich in cytokines, growth factors, and anti-inflammatory molecules, exhibits heightened regenerative and immunomodulatory properties compared to conventionally cultured MSCs.[6]

The interplay between pro-inflammatory and anti-inflammatory factors plays a pivotal role in developing and resolving PCOS-related symptoms. Interleukin-10 (IL-10), an anti-inflammatory cytokine, exerts regulatory effects on the immune response and has been implicated in attenuating hyperandrogenism and insulin resistance in PCOS.[7] Similarly, the signal transducer and activator of transcription 3 (STAT3) is a critical transcription factor in regulating various cellular processes, including inflammation and cell survival, which are perturbed in PCOS.[8]

Despite these promising insights, the specific impact of mesenchymal hypoxia-induced secretome stem cells (MHSSCs) on the expression of IL-10 and STAT3 in the context of PCOS still needs to be explored. Elucidating the molecular mechanisms underlying the interaction between MHSSCs and these key regulatory elements offers a unique opportunity to understand and potentially mitigate the multifaceted pathogenesis of PCOS comprehensively.

This study seeks to bridge this knowledge gap by investigating the influence of MHSSCs on the expression profiles of IL-10 and STAT3 in a validated PCOS animal model. The findings hold significant potential for advancing our therapeutic approaches towards more

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targeted and effective interventions for individuals affected by this complex and challenging syndrome.

Materials and Methods

Ethical approval
This study received approval from Komisi Bioetik Faculty of Medicine Universitas Islam Sultan Agung Semarang under No. 14/II/2024/Komisi Bioetik.

Mesenchymal Stem Cells (MSCs) Isolation from Umbilical Cord Blood
Blood was collected from rats that were 21 days pregnant, and the mononuclear cell fraction was isolated using density gradient centrifugation with Ficoll-Paque™ Plus (GE Healthcare, USA). The obtained cells were cultured in T-75 flasks with low-glucose Dulbecco's Modified Eagle Medium (DMEM, Gibco, USA) supplemented with 10% fetal bovine serum (FBS, Gibco, USA) and 1% penicillin-streptomycin (Gibco, USA). Cultures were maintained in a humidified atmosphere at 37°C with 5% CO_2. Cells were passaged at 80-90% confluence using TrypLE™ Express (Gibco, USA).[9]

Validation of MSCs by Flow Cytometry Surface Marker Analysis
The MSCs were cultured for 3-7 days, and passage 3 of the MSCs were harvested, counted, and resuspended in phosphate-buffered saline (PBS) supplemented with 2% FBS. The cells were incubated with anti-human CD73, CD90, CD105, CD34, CD45, and HLA-DR antibodies (BD Biosciences, USA) for 30 minutes at 4°C. After washing, cells were analyzed using a flow cytometer (BD Accuri™ C6, BD Biosciences, USA).

Validation of MSCs by Adipogenic and Osteogenic Staining
For adipogenic differentiation, passage 4 MSCs were cultured in an adipogenic induction medium (Gibco, USA) for 21 days, followed by Oil Red O staining to visualize lipid droplets. For osteogenic differentiation, cells were cultured in an osteogenic induction medium (Gibco, USA) for 21 days, followed by Alizarin Red staining to detect calcium deposits.

Induction of Hypoxia in MSCs
Passage 3 of MSCs were subjected to hypoxia (5% O_2) using a hypoxic chamber (STEMCELL Technologies, Canada) for 24 hours. Control cells were maintained under normoxic conditions (21% O_2).[10]

PCOS Animal Model Induction
Adult female Wistar rats were used. PCOS was induced by subcutaneous injection of dehydroepiandrosterone (DHEA, Sigma-Aldrich, USA) dissolved in sesame oil (6 mg/ 100g body weight) daily for 30 days.[11]

Testosterone Quantification by ELISA
Serum testosterone levels were measured using a commercially available mouse testosterone ELISA kit (Enzo Life Sciences, USA) following the manufacturer's instructions. Standards, controls, and serum samples were briefly added to a microplate pre-coated with a testosterone-specific antibody. After incubation and washing, a testosterone-horseradish peroxidase (HRP) conjugate was added, followed by a substrate solution. The reaction was stopped, and absorbance was read at 450 nm using a microplate reader (BioTek, USA). Testosterone concentrations were determined based on a standard curve.

Validation of PCOS by Estrus Cycle Analysis in HE Staining
After blood collection on day 23, rats were sacrificed, and ovaries were carefully excised, fixed in 10% formalin, and embedded in paraffin for histological analysis. Paraffin-embedded ovarian sections (5 µm thick) were deparaffinized, rehydrated, and stained with hematoxylin for 5 minutes. After rinsing, sections were counterstained with eosin for 1 minute. Slides were dehydrated and coverslipped. A blinded observer examined HE-stained sections under a light microscope (Olympus BX53, Japan). Estrus cycle phases (proestrus, estrus, metestrus, and diestrus) were determined based on morphological characteristics of ovarian structures, vaginal epithelium, and leukocyte infiltration.

MHSSCs administration on PCOS animal model
Dehydroepiandrosterone (DHEA) injections were continued subcutaneously until day 30 to prevent ovarian self-recovery. Simultaneously, MHSSCs were administered from day 23 to day 30, with 200 µL/kg (T1) and 400 µL/kg (T2) given intraperitoneally on days 23, 25, 28, and 30. The negative control group received NaCl treatment. From days 27 to 32, cytological examinations were performed to determine the estrus cycle. On day 33, testosterone levels were assessed, PCR analysis was conducted, and ovarian samples from all groups were collected for histological preparations using paraffin embedding and Hematoxylin-Eosin (HE) staining.

Analysis of IL-10 and STAT3 Gene Expression by qRT-PCR
Ovarian tissues were harvested and homogenized, and total RNA was extracted using TRIzol™ Reagent (Invitrogen, USA). cDNA synthesis was performed using SuperScript™ IV Reverse Transcriptase (Invitrogen, USA). qRT-PCR was conducted using PowerUp™ SYBR™ Green Master Mix (Applied Biosystems, USA) on a QuantStudio™ 3 Real-Time PCR System (Applied Biosystems, USA). Primers for IL-10, STAT3, and housekeeping genes (β-actin) were designed and validated (sequences available upon request). Relative gene expression was calculated using the ΔΔCt method.[12,13]

Statistical Analysis
The normality of the data distributions was assessed using the Shapiro-Wilk test, while the homogeneity of variances was evaluated using Levene's test. A p-value greater than 0.05 will suggest that the data is usually distributed and that the variances are homogeneous. Analysis of Variance (ANOVA) will be used to compare across multiple groups, with a p-value of less than 0.05 indicating significant differences among the treatment groups. The statistical analysis was conducted using SPSS version 26.

Results and Discussion

MSCs isolation and validation
The MSCs were isolated from the umbilical cords of 21-day-old pregnant rats. The isolated cells were then cultured in plastic flasks with the specialized medium. Observation under a microscope after passage 5 revealed adherent cells with spindle-like morphology attached to the flask's surface (Figure 1A-B). Validation of mesenchymal stem cell isolation was performed using flow cytometry to demonstrate the ability of MHSSCs to express various specific surface markers. This study showed that MHSSCs were capable of expressing CD90 (97.60%) and CD29 (97.70%), with low expression of CD45 (1.50%) and CD31 (3.20%) (Figure 1C).
Furthermore, the study assessed the differentiation potential of MHSSCs into various mature cell types. MHSSCs were induced to differentiate into osteocytes and adipocytes by providing a specific medium. The results indicated successful differentiation, as evidenced by calcium deposits and red-stained lipid droplets using Alizarin Red and Oil Red dye staining in respective osteogenic and adipogenic cultures (Figure 1D-E).
MSCs were then incubated under hypoxic conditions with 5% O2 concentration for 24 hours using a hypoxic chamber. Hypoxia-exposed MSCs exhibited denser growth compared to their appearance before culturing under hypoxic conditions (Figure 1B).

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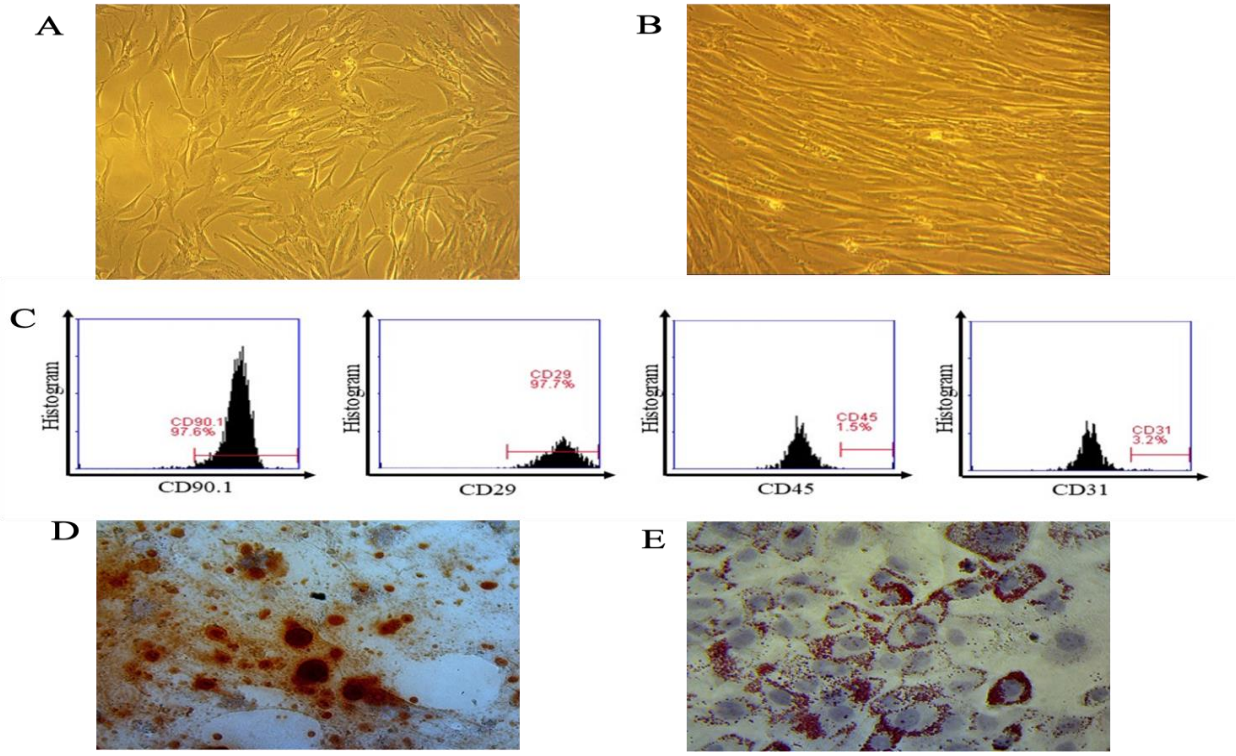

Figure 1: Characterization of Isolated MSCs. (A) Microscopic observation of MSCs after passage 5. (B) Morphology of MSCs under hypoxia condition. (C) Flow Cytometry Analysis of Surface Markers. (D) Osteogenic differentiation Potential of MHSSCs, and (E) Adipogenic differentiation Potential of MHSSCs.

Validation of PCOS Animal Model through Macroscopic and Microscopic Observation

Polycystic Ovary Syndrome (PCOS) is a condition characterized by prolonged anovulation and often accompanied by hyperandrogenism, which frequently leads to fertility issues.[14] PCOS is closely associated with chronic inflammation triggered by hyperglycemia and hyperlipidemia, ultimately resulting in hyperandrogenism. This inflammatory state increases reactive oxygen species (ROS) levels, activating the nuclear transcription factor kappa beta (NF-κB) and enhancing the expression of pro-inflammatory cytokines such as IL-6 and TNF-alpha.[15] This study used DHEA, a testosterone and dihydrotestosterone (DHT) synthesis precursor, to induce inflammation and oxidative stress in ovarian tissues and activate the TGF-β1/Smads and CTGF pathways, leading to PCOS development.[16] Based on macroscopic observations, the induction of DHEA in rats revealed an increased number of palpable nodules compared to healthy rats, suspected to be follicular cysts, a characteristic sign of PCOS (Figure 2A-B). Microscopic examination following DHEA induction displayed the presence of cystic follicles, further confirming a distinctive sign of PCOS (Figure 3A-B). Furthermore, administration of DHEA induction in rats led to an elevation in testosterone levels, a key characteristic associated with the development of PCOS (Figure 2).

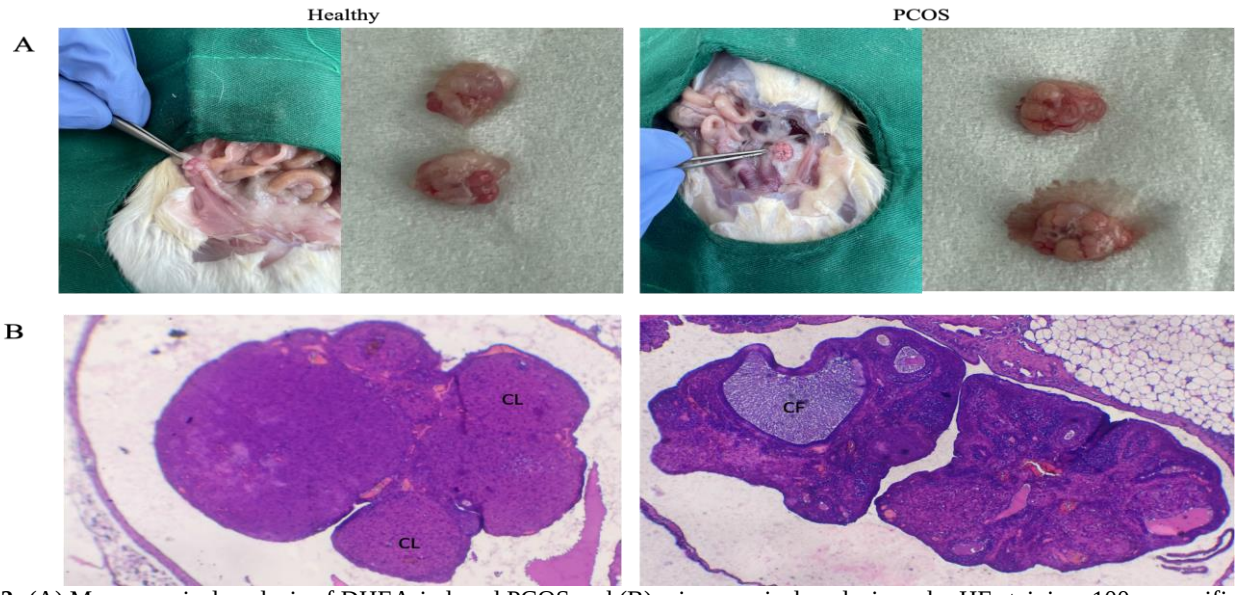

Figure 2: (A) Macroscopical analysis of DHEA-induced PCOS and (B) microscopical analysis under HE staining. 100x magnification, CL: corpus luteum, CF: cystic follicle

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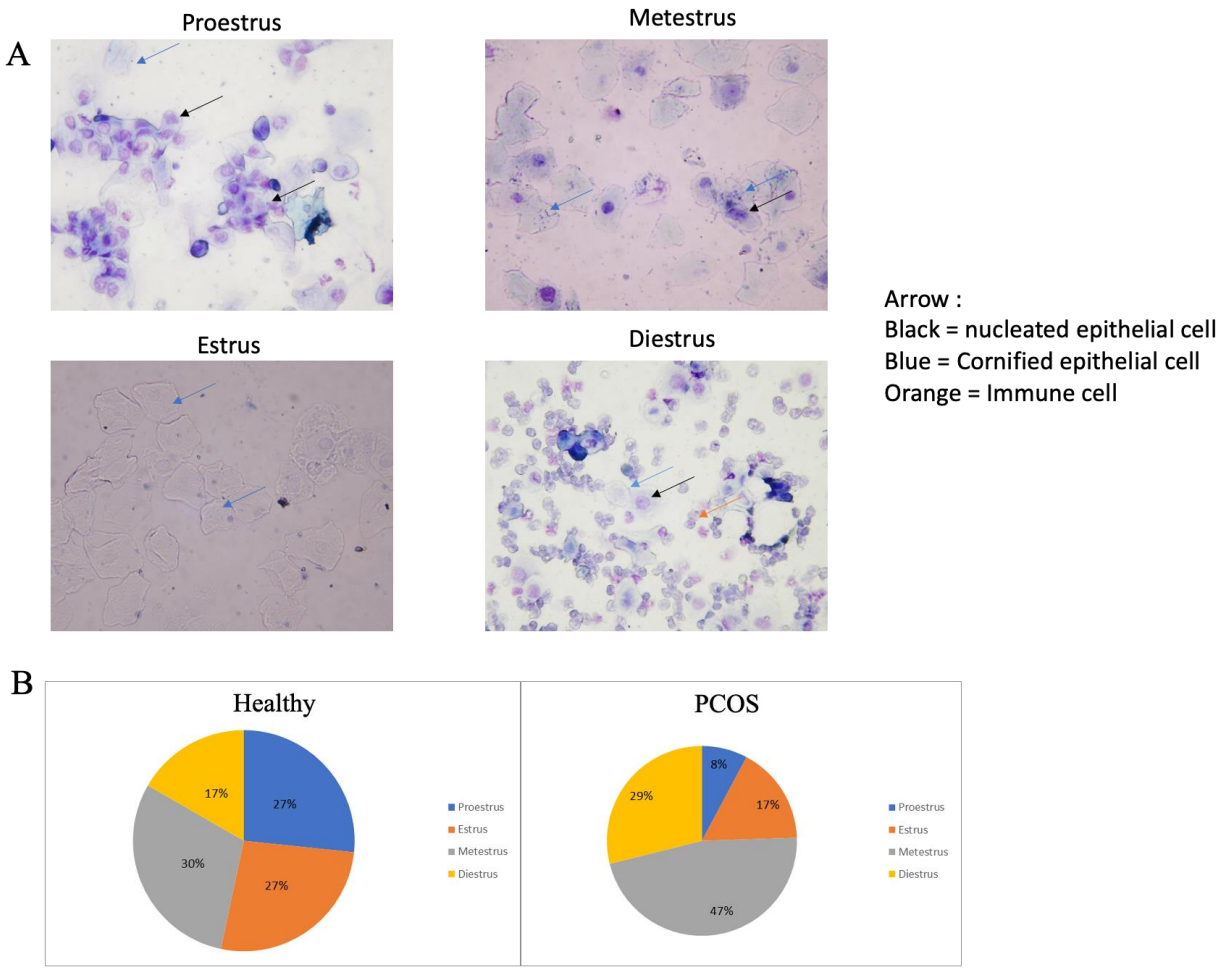

Figure 3: (A) Representative histological section of proestrus, metestrus, estrus, and diestrus under HE staining. (B) the percentage of total PCOS stages.

MHSSCs induced IL-10 gene expression in PCOS animal model

Chronic inflammation occurs in the ovaries and surrounding tissues, marked by an abundance of type 1 macrophages (M1), which produce pro-inflammatory cytokines like IL-6, TNF-α, and IL-1β, thus perpetuating chronic inflammation.[17] Previous studies have shown that in some cases of PCOS, there is an imbalance between M1 activity and IL-10 levels.[18] As shown in Figure 4A, the group treated with a higher dose of MHSSCs (400 µL) exhibited a significant upregulation in IL-10 gene expression compared to the control group ($p < 0.05$). The administration of MHSSCs can transform M1 into anti-inflammatory M2 macrophages.[19] Differentiation of M1 into M2 balances the macrophage population within the ovaries, halting the inflammatory process and reducing folliculogenesis, ultimately preventing PCOS [6]. IL-10 can stop inflammation by inhibiting NF-KB activation through several mechanisms, one of which is inhibiting IkappaB kinase (IKK) activation, an initial step in NF-κB activation.[20] Additionally, IL-10 can inhibit the TLR pathway by suppressing the phosphorylation of mitogen-activated protein kinase (MAPK) and TGF-beta-activated kinase 1 (TAK1), leading to reduced NF-KB activation induced by TLR receptor signaling. IL-10 also inhibits the activity of tumor necrosis factor receptor-associated factor 6 (TRAF6), a critical mediator in NF-KB activation leading to healing.[21,22]

MHSSCs induced STAT3 gene expression in PCOS animal model

Similarly, in Figure 4B, the same group showed a significant increase in STAT3 gene expression compared to the control group ($p<0.05$). Furthermore, a dose-dependent relationship was observed in the administration of MHSSCs. Specifically, the group receiving a higher dose demonstrated a more pronounced upregulation of both IL-10 and STAT3 gene expression levels than the group receiving a lower dose (200 µL) of MHSSCs. These findings highlight the potential of MHSSCs in modulating the expression of key genes involved in immune regulation and inflammation, particularly IL-10 and STAT3, in the PCOS animal model. The dose-dependent response further suggests optimizing the dosage for potential therapeutic applications.

As an anti-inflammatory agent, IL-10's presence in MHSSCs activates STAT3, significantly increasing group T2. The transcription factor STAT3 binds to the promoter region of genes regulating pro-inflammatory cytokine expression, inhibiting their transcription and reducing pro-inflammatory cytokine production in chronic PCOS inflammation.[23,24] STAT3 plays a crucial role in regulating regulatory T cell (Treg) function, a key player in alleviating inflammation and maintaining immunological tolerance.[15] Activation of STAT3 in Treg enhances their ability to inhibit excessive immune responses and alleviate inflammation.[25] Moreover, STAT3 can direct macrophage differentiation towards the M2 phenotype, resulting in fewer pro-inflammatory cytokines and more anti-inflammatory cytokines, reducing inflammation.[15]

Other studies have shown a decrease in inflammation through activating the IL-10 STAT3 pathways. IL-10 binds to IL-10R, resulting in JAK 1 activation, inducing STAT3 phosphorylation.[15] The STAT3 protein translocates to the nucleus, activating mRNA SOSC3 sequences, which are then intracellularly expressed and can suppress pro-inflammatory signaling pathways, namely NF-κB.[26] Suppression of the NF-κB pathway leads to a decrease in pro-inflammatory cytokine secretion, including IL-6.[27,28] Additional research utilizing Fibrin Facilitates MSCs has demonstrated that MSCs regulate estradiol and progesterone, decrease gonadotropin (LH/FSH), testosterone (T), and TGF-β1,

maintain regular estrus cycles, increase granulosa cell counts, and decrease immature cystic follicles.[29]

IL-10 from MHSSCs activates the STAT3 pathway through JAK-1 translocation from the intracellular membrane to the cytoplasm. This results in STAT3 phosphorylation, culminating in its translocation to the nucleus. Nuclear translocation of STAT3 activates the SOCS3 gene, which is subsequently synthesized intracellularly and can inhibit intracellular NF-kB signaling, preventing NF-κB translocation to the nucleus and the consequent expression of pro-inflammatory genes. The significant increase in IL-10 and STAT3 expression in group T4 (MHSSCs 400µl) compared to group T2 rats indicates that MHSSCs have the potential to act as an anti-inflammatory.

Overall, the results demonstrate the promising immunomodulatory effects of MHSSCs and underscore their potential as a targeted intervention in managing PCOS-related inflammatory processes. However, this study did not analyze the expression of pro-inflammatory cytokines, representing a limitation of this research. Further studies are warranted to elucidate the underlying mechanisms and validate the clinical relevance of these findings.

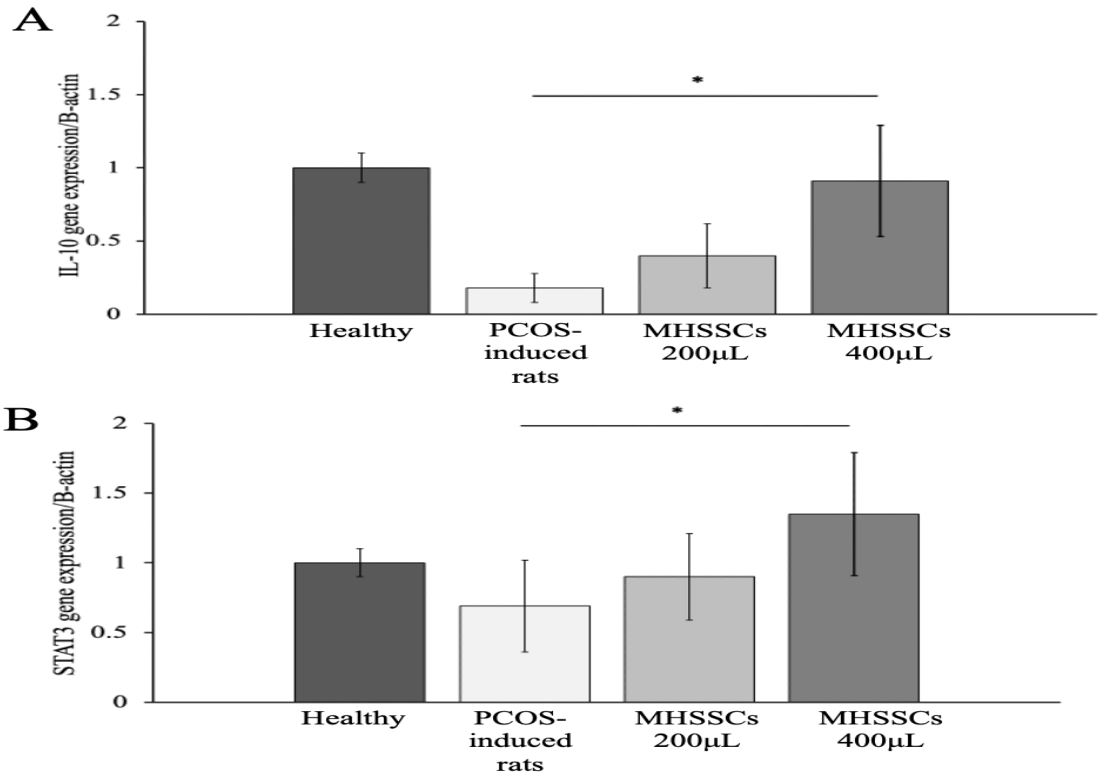

Figure 4. Gene expression ratio of (A) IL-10 and (B) STAT-3 after MHSSCs treatment on PCOS model under qRT-PCR analysis. The data represent the average and SD from 3 different independent experiments. *p<0.05= significant different.

Conclusion

The findings demonstrate that MHSSCs, particularly at a dose of 400µl, effectively increase IL-10 and STAT3 expression in PCOS, suggesting its potential as a therapeutic agent through multiple mechanisms: the transformation of pro-inflammatory M1 to anti-inflammatory M2 macrophages, inhibition of NF-κB activation through IL-10-mediated pathways, and activation of the STAT3 signaling cascade.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

References

1. Deswal R, Narwal V, Dang A, Pundir CS. The Prevalence of Polycystic Ovary Syndrome: A Brief Systematic Review. J Hum Reprod Sci. 2020; 13(4):261-271.
2. Chaudhuri A. Polycystic ovary syndrome: Causes, symptoms, pathophysiology, and remedies. Obes Med. 2023; 39:100480-100489.
3. Hadidi M, Karimabadi K, Ghanbari E, Rezakhani L, Khazaei M. Stem cells and exosomes: as biological agents in the diagnosis and treatment of polycystic ovary syndrome (PCOS). Front Endocrinol. 2023; 14:1269266-1269276.
4. Prayitno GD, Lestari K, Sartika CR, Djuwantono T, Widjaya A, Muharam R, Hidayat YM, Wulandari D, Haifa R, Naura NF, Marbun KT, Zahrah A. Potential of Mesenchymal Stem Cells and Their Secretomes in Decreasing Inflammation Markers in Polycystic Ovary Syndrome Treatment: A Systematic Review. Medicines. 2022; 10(1):1-11.
5. Putra A, Widyatmoko A, Ibrahim S, Amansyah F, Amansyah F, Berlian MA, Retnaningsih R, Pasongka Z, Sari FE, Rachmad B. Case series of the first three severe COVID-19 patients treated with the secretome of hypoxia-mesenchymal stem cells in Indonesia. F1000Res. 2021; 10:1-19.
6. Chugh RM, Park H soo, El Andaloussi A, Elsharoud A, Esfandyari S, Ulin M, Bakir L, Aboalsoud A, Ali M, Ashour D, Igboeli P, Ismail N, McAllister J, Al-Hendy A. Mesenchymal stem cell therapy ameliorates metabolic dysfunction and restores fertility in a PCOS mouse model through interleukin-10. Stem Cell Res Ther. 2021;12 (1):1-19.
7. You M, Wang Z, Kim HJ, Lee YH, Kim HA. Pear pomace alleviated atopic dermatitis in NC/Nga mice and inhibited LPS-induced inflammation in RAW 264.7 macrophages. Nutr Res Pract. 2022; 16(5):577-588.

8. Tan W, Zou JL, Yoshida S, Jiang B, Zhou Y di. Increased vitreal levels of interleukin-10 in diabetic retinopathy: A Meta-analysis. Int J Ophthalmol. 2020; 13(9):1477-1483.
9. Prajoko YW, Putra A, Dirja BT, Muhar AM, Amalina ND. The Ameliorating Effects of MSCs in Controlling Treg-mediated B-Cell Depletion by Indoleamine 2, 3-dioxygenase Induction in PBMC of SLE Patients. Open Access Maced J Med Sci. 2022; 10:6-11.
10. Sunarto H, Trisnadi S, Putra A, Sa'dyah NAC, Tjipta A, Chodidjah C. The Role of Hypoxic Mesenchymal Stem Cells Conditioned Medium in Increasing Vascular Endothelial Growth Factors (VEGF) Levels and Collagen Synthesis to Accelerate Wound Healing. Ind J Can Chemoprev. 2020; 11(3): 134-141.
11. Xie Q, Xiong X, Xiao N, He K, Chen M, Peng J, Su X, Mei H, Dai Y, Wei D, Lin G, Cheng L. Mesenchymal Stem Cells Alleviate DHEA-Induced Polycystic Ovary Syndrome (PCOS) by Inhibiting Inflammation in Mice. Stem Cells Int. 2019; 2019(1):1-12.
12. Widyaningsih W, Putra A, Priyantini S, Muhar AM, Sumarawati T, Trisnadi S, Amalina ND, Alif I, Prasetio A, Irawan RC. Secretome of Hypoxia-Preconditioned Mesenchymal Stem Cells Ameliorates Hyperglycemia in Type 2 Diabetes Mellitus Rats. Trends in Sci. 2024; 21(5):7278-7285.
13. Restimulia L, Ilyas S, Munir D, Putra A, Madiadipoera T, Farhat F, Sembiring RJ, Ichwan M, Amalina D, Alif I. The CD4+CD25+FoxP3+ Regulatory T Cells Regulated by MSCs Suppress Plasma Cells in a Mouse Model of Allergic Rhinitis. Med Arch. 2021;4(75):256-261.
14. Dong J, Rees DA. Polycystic ovary syndrome: pathophysiology and therapeutic opportunities. BMJ Med. 2023; 2(1): 548-554.
15. Wang H, Feng X, Wang T, Pan J, Zheng Z, Su Y, Weng H, Zhang L, Chen L, Zhou L, Zheng L. Role and mechanism of the p-JAK2/p-STAT3 signaling pathway in follicular development in PCOS rats. Gen Comp Endocrinol. 2023; 330:114-138.
16. Li Y, Zheng Q, Sun D, Cui X, Chen S, Bulbul A, Liu S, Yan Q. Dehydroepiandrosterone stimulates inflammation and impairs ovarian functions of polycystic ovary syndrome. J Cell Physiol. 2019; 234(5):7435-7447.
17. Miki S, Suzuki J, Takashima M, Ishida M, Kokubo H, Yoshizumi M. S-1-Propenylcysteine promotes IL-10-induced M2c macrophage polarization through prolonged activation of IL-10R/STAT3 signaling. Sci Rep. 2021; 11(1):1-14.
18. Chugh RM, Park HS, Esfandyari S, Elsharoud A, Ulin M, Al-Hendy A. Mesenchymal stem cell-conditioned media regulate steroidogenesis and inhibit androgen secretion in a PCOS cell model via BMP-2. Int J Mol Sci. 2021; 22(17):1-12.
19. Afriani N, Darwin E, Ali H, Syafrita Y. The Effects of Mesenchymal Stem Cell on Calpains, Glycogen Synthase Kinase-3β, and Ryanodine Receptor 3 in Animal Models of Alzheimer's Disease: A Systematic Review. Trop J Nat Prod Res. 2024; 8(8):7941-7946.
20. Baumann D, Drebant J, Hägele T, Burger L, Serger C, Lauenstein C, Dudys P, Erdmann G, Offringa R. P38 MAPK signaling in M1 macrophages results in selective elimination of M2 macrophages by MEK inhibition. J Immunother Cancer. 2021; 9(7):1-9.
21. Cho KA, Lee JK, Kim YH, Park M, Woo SY, Ryu KH. Mesenchymal stem cells ameliorate B-cell-mediated immune responses and increase IL-10-expressing regulatory B cells in an EBI3-dependent manner. Cell Mol Immunol. 2017; 14(11):895-908.
22. Fakhrurrazi F, Hakim RF, Cahyani D, Henny H, Mardhiah S. Effect of Andong (*Cordyline fruticosa*) Leaf Extract on the Acceleration of Incised Wound Healing of Oral Mucosa in Wistar Rats (*Rattus norvegicus*). Trop J Nat Prod Res. 2022; 6(1): 20-23.
23. Li M, Chi X, Wang Y, Setrerrahmane S, Xie W, Xu H. Trends in insulin resistance: insights into mechanisms and therapeutic strategy. Signal Transduct Target Ther. 2022; 7(1):216-224.
24. Wang K, Li Y. Signaling pathways and targeted therapeutic strategies for polycystic ovary syndrome. Front Endocrinol. 2023; 14:1-28.
25. Oparaugo NC, Ouyang K, Nguyen NPN, Nelson AM, Agak GW. Human Regulatory T Cells: Understanding the Role of Tregs in Select Autoimmune Skin Diseases and Post-Transplant Nonmelanoma Skin Cancers. Int J Mol Sci. 2023; 24(2):1527-1537.
26. Xu Y, Tong Y, Ying J, Lei Z, Wan L, Zhu X, Ye F, Mao P, Wu X, Pan R, Peng B, Liu Y, Zhu J. Chrysin induces cell growth arrest, apoptosis, and ER stress and inhibits the activation of STAT3 through the generation of ROS in bladder cancer cells. Oncol Lett. 2018; 15 (6):9117-9125.
27. Guo Q, Jin Y, Chen X, Ye X, Shen X, Lin M, Zeng C, Zhou T, Zhang J. NF-κB in biology and targeted therapy: new insights and translational implications. Signal Transduct Target Ther. 2024; 9(1):53-67.
28. Yang JL, Lin WL, Tai SB, Ciou YS, Chung CL, Chen JJ, Liu PF, Lin MW, Chen CL. Suppression of TGFβ-Induced Interleukin-6 Secretion by Sinulariolide from Soft Corals through Attenuation of the p38–NF-kB Pathway in Carcinoma Cells. Int J Mol Sci. 2023; 24(14):1-18.
29. Masyithah Darlan D, Munir D, Karmila Jusuf N, Putra A, Ikhsan R, Alif I. In vitro regulation of IL-6 and TGF-ß by mesenchymal stem cells in systemic lupus erythematosus patients. Med Glas. 2020; 17(2):408-413.