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Original Research Article

Hypoxia-Mesenchymal Stem Cell Exosomes As A Novel Therapy Targeting Socs3/Stat3 Signaling In Androgenic Alopecia

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Abstract

Background: Androgenetic alopecia (AGA) is the most common form of hair loss and is characterized by progressive follicular miniaturization. This condition is associated with dysregulation of suppressor of cytokine signaling 3 (SOCS3) and signal transducer and activator of transcription 3 (STAT3). Current therapies, such as minoxidil, provide limited efficacy and are often accompanied by side effects. Exosomes derived from hypoxia-conditioned mesenchymal stem cells (EH-MSCs) exhibit regenerative, immunomodulatory, and anti-inflammatory properties, which may represent a novel therapeutic approach.

Objective: To evaluate the therapeutic potential of EH-MSC-derived exosomes in regulating SOCS3 and STAT3 signaling in a dihydrotestosterone (DHT)-induced AGA mouse model.

Methods: This in vivo randomized post-test-only control group study was conducted using thirty-four male C57BL/6 mice divided into five groups: healthy control (G1), DHT-induced AGA + saline (G2), DHT-induced AGA + 5% minoxidil (G3), DHT-induced AGA + EH-MSC exosomes 100 µg/kg (G4), and DHT-induced AGA + EH-MSC exosomes 200 µg/kg (G5). Exosomes were isolated from hypoxia-preconditioned umbilical cord-derived MSCs using tangential flow filtration and were validated by flow cytometry using CD63 and CD9 markers. SOCS3 and STAT3 expression levels were quantified using qRT-PCR. Statistical analyses included the Shapiro-Wilk test, Levene's test, one-way ANOVA, and post hoc comparisons.

Results: EH-MSC exosomes significantly modulated SOCS3 and STAT3 expression in a dose-dependent manner. The high-dose EH-MSC group (G5) restored SOCS3 expression (1.25 ± 0.18 pg/mL) to levels comparable with the healthy control group (1.00 ± 0.00 pg/mL) and reduced STAT3 expression (0.89 ± 0.15 pg/mL) toward normal values. In contrast, minoxidil only partially improved SOCS3 (0.49 ± 0.16 pg/mL) and STAT3 (2.32 ± 0.19 pg/mL) compared with untreated AGA controls.

Conclusion: Exosomes derived from hypoxia-preconditioned MSCs demonstrated superior immunomodulatory effects compared with minoxidil by upregulating SOCS3 and downregulating STAT3 expression, thereby restoring follicular homeostasis in AGA. These findings suggest that EH-MSC exosomes may serve as a promising exosome-based therapeutic strategy for androgenetic alopecia, with potential implications for clinical practice in regenerative dermatology.

Keywords: Androgenic Alopecia; Mesenchymal Stem Cells; Exosomes; Cell Hypoxia, Signal Transduction

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INTRODUCTION

Alopecia is a globally prevalent condition with multifactorial causes, including genetic predisposition, hormonal imbalance, immune-mediated inflammation, nutritional deficiencies, environmental exposures, psychological stress, and aging.^{1,2} Among its subtypes, androgenetic alopecia (AGA) is the most common and is characterized by the progressive miniaturization of thick, pigmented terminal hairs into finer vellus hairs due to follicular sensitivity to androgens.³ The prevalence of AGA is higher in men than in women and is particularly common among Caucasian males, with reports indicating that approximately 50% of Caucasian men are affected by the age of 50.^{1,4-7} Studies in Singapore have shown that up to 63% of men experience AGA, with nearly 60% reporting a positive family history.⁵ Beyond its clinical presentation, AGA significantly impacts patients' quality of life, contributing to psychological distress, reduced self-confidence, and impaired social functioning.^{8,9}

The pathophysiology of AGA involves dihydrotestosterone (DHT), which induces chronic scalp tension and inflammatory responses in susceptible tissues, ultimately leading to follicular remodeling and hair loss.^{1,4} At the molecular level, suppressor of cytokine signaling 3 (SOCS3) and signal transducer and activator of transcription 3 (STAT3) are critical regulators of hair follicle biology. SOCS3 modulates immune and inflammatory responses, whereas STAT3 regulates both pro- and anti-inflammatory processes and plays an essential role in hair follicle morphogenesis and anagen initiation.¹⁰⁻¹² Dysregulation of these pathways has been associated with follicular miniaturization and disruption of the hair growth cycle.^{13,14}

Current therapies for AGA include topical corticosteroids, immunomodulators, and the widely used agent minoxidil. Minoxidil has been reported to stimulate dermal papilla cell proliferation and enhance local growth factor activity; however, its clinical effectiveness is limited by adverse effects such as contact dermatitis, scalp irritation, and unwanted hypertrichosis.^{7,15} Therefore, safer and more effective therapeutic alternatives are urgently needed.

Exosomes derived from hypoxia-conditioned mesenchymal stem cells (EH-MSCs) represent a promising therapeutic approach for AGA treatment. EH-MSCs exhibit anti-inflammatory, regenerative, and immunomodulatory properties mediated through the paracrine release of growth factors, cytokines, and genetic materials, including microRNAs.^{12,16,17} Exosomes can deliver bioactive molecules, including regulatory microRNAs and proteins involved in intracellular signaling, to recipient cells, thereby modulating pathways essential for tissue regeneration.^{3,18} Previous studies have demonstrated the therapeutic potential of MSC-derived exosomes in skin regeneration and hair growth, including in AGA models.^{3,6,19} However, studies specifically investigating the role of EH-MSC-derived exosomes in modulating SOCS3 and STAT3 expression in AGA remain limited.

Given these gaps, this study aims to explore the therapeutic potential of EH-MSC exosomes in regulating SOCS3 and STAT3 signaling in a DHT-induced AGA model, thereby providing insights into novel exosome-based interventions for hair loss.

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MATERIALS AND METHODS

This study employed an in vivo experimental design using a randomized post-test-only control group approach with five parallel arms. Thirty-four male C57BL/6 mice aged 6–8 weeks and weighing 20–25 g were obtained from Stem Cell and Cancer Research (SCCR) Indonesia and acclimatized for seven days under controlled laboratory conditions (20–28 °C; ventilated housing; food and water ad libitum). The animals were randomly divided into five groups: G1 (healthy control, no treatment), G2 (dihydrotestosterone [DHT]-induced androgenetic alopecia + 0.9% NaCl), G3 (DHT-induced + 5% topical minoxidil), G4 (DHT-induced + exosomes derived from hypoxia-preconditioned mesenchymal stem cells [EH-MSCs] 100 µg/kg body weight), and G5 (DHT-induced + EH-MSC exosomes 200 µg/kg body weight). Group allocation and sample size were estimated using the Federer formula, resulting in a minimum of five animals per group; to account for potential mortality, six animals were assigned to each group, and an additional four were used for validation, producing a total of thirty-four animals. Inclusion criteria consisted of male C57BL/6 mice with a healthy phenotype and no anatomical abnormalities, while exclusion criteria included failure to develop the alopecia phenotype after induction, prior use in other experiments, or illness during the study. Ethical clearance was granted by the Ethics Committee of the Faculty of Medicine, Universitas Islam Sultan Agung Semarang.

Mesenchymal stem cells were isolated from human umbilical cord tissue obtained from healthy full-term donors with appropriate informed consent and institutional approval through SCCR Indonesia. The use of human umbilical cord-derived MSC exosomes in murine models has been widely reported in preclinical studies and is supported by the relatively low immunogenicity and favorable safety profile of MSC-derived extracellular vesicles in cross-species experimental settings.²⁰

Human umbilical cord MSCs were isolated using an explant method under aseptic conditions in a Class II biosafety cabinet. Tissues were washed with phosphate-buffered saline (PBS), vessels were removed, and the remaining tissue was minced and cultured in T-25 flasks containing Dulbecco's Modified Eagle Medium (DMEM) supplemented with fetal bovine serum, antibiotics, and antifungal agents. Cells were maintained in a CO₂ incubator at 37 °C with 5% CO₂, and the medium was changed every three days until reaching 80% confluence. Hypoxic preconditioning was performed by placing culture

flasks in a hypoxia chamber with oxygen reduced to 5% for 24 hours. Hypoxic culture conditions have been reported to preserve the typical fibroblast-like MSC morphology while enhancing MSC regenerative potency and modulating secretome/extracellular vesicle profiles.²¹

Conditioned medium was harvested after hypoxia exposure and subjected to exosome isolation using tangential flow filtration (TFF) with sequential filtration (100 kDa and 500 kDa), followed by concentration and purification. Exosomes were validated using flow cytometry for exosomal surface markers CD81, CD63, and CD9. In accordance with current minimal information standards for extracellular vesicle research (MISEV), comprehensive exosome characterization ideally includes additional assessments such as Transmission Electron Microscopy (TEM) for morphology and Nanoparticle Tracking Analysis (NTA) for particle size distribution and concentration.²² Because TEM and NTA were not performed in the present study, this limitation was acknowledged in the discussion as a potential constraint on the completeness of extracellular vesicle characterization. Purified exosome preparations were stored at 2–8 °C until administration.

To establish the androgenetic alopecia model, mice in groups G2–G5 were anesthetized with intraperitoneal ketamine (60 mg/kg) and xylazine (20 mg/kg). The dorsal fur was shaved, and DHT was administered intraperitoneally at 1 mg/day for 17 consecutive days. On day 25, macroscopic validation of alopecia was conducted by observing the absence of hair regrowth, and microscopic validation was performed by histological examination of hematoxylin and eosin (H&E)-stained skin sections, confirming the lack of hair follicles in the anagen phase.

Treatments were initiated on day 25 as follows: G2 received intraperitoneal 0.9% NaCl, G3 received 5% topical minoxidil daily for 14 days, G4 received intraperitoneal EH-MSC exosomes at 100 µg/kg body weight on days 25 and 32, and G5 received EH-MSC exosomes at 200 µg/kg body weight on the same schedule. The intraperitoneal route was selected as a systemic delivery approach to achieve broader biodistribution and immunomodulatory effects, as systemic extracellular vesicle administration has been shown to distribute predominantly to organs such as liver and spleen and may reach peripheral tissues through circulation and lymphatic trafficking. This rationale was included to justify the systemic route over localized delivery for a dermatological target. On day 39, animals were euthanized using a lethal combination of ketamine and xylazine. Dorsal skin samples were excised and preserved in RNA later at –80 °C until molecular analysis.

Total RNA was extracted from approximately 50 mg of frozen skin tissue using TRIzol reagent following standard protocols. Chloroform was added to separate phases by centrifugation, and RNA was precipitated with isopropanol, washed with 75% ethanol, and resuspended in nuclease-free water. RNA concentration and purity were quantified using a microplate spectrophotometer. Complementary DNA (cDNA) synthesis was performed by reverse

transcription of 0.1 µg total RNA using a master mix protocol with sequential incubation at 37 °C, 50 °C, and 98 °C. Quantitative real-time PCR (qRT-PCR) was then carried out using SYBR Green chemistry on an Eco Study platform, with primers specific for SOCS3 and STAT3. Each reaction contained 1 µg cDNA, 10 µg 2× SYBR Green master mix, 0.8 µg forward and reverse primers, and nuclease-free water in a 20 µg reaction volume. Amplification conditions consisted of an initial denaturation at 95 °C for 2 minutes, followed by 40 cycles of 95 °C for 5 seconds and 56 °C for 20 seconds. Fluorescence was detected at approximately 520 nm, and data were analyzed using EcoStudy software.

For statistical analysis, all results were expressed as mean ± standard deviation. The Shapiro–Wilk test was used to evaluate normality, and Levene’s test was employed to assess homogeneity of variances. When data were normally distributed and homogeneous, comparisons among groups were performed using one-way analysis of variance (ANOVA), followed by least significant difference (LSD) post hoc tests for pairwise comparisons. If normal but non-homogeneous, ANOVA with Tamhane post hoc correction was applied. For non-normal distributions, the Kruskal–Wallis test was used, followed by Mann–Whitney U tests for intergroup comparisons. A p-value less than 0.05 was considered statistically significant.

The entire study was conducted at the Animal Model Research Center, SCCR Indonesia, between May and July 2025. The workflow consisted of acclimatization, induction of androgenetic alopecia by DHT (days 8–24), validation of alopecia (day 25), preparation and administration of EH-MSCs, treatment application, termination of animals on day 39, and subsequent molecular analysis of SOCS3 and STAT3 expression.

RESULTS

The mesenchymal stem cells (MSCs) isolated from rat umbilical cords exhibited spindle-shaped, fibroblast-like morphology and adhered firmly to culture flasks, consistent with standard MSC characteristics (Figure 1A). Flow cytometry analysis confirmed the MSC phenotype, showing high expression of CD90 (98.7%) and CD29 (98.8%), with low expression of hematopoietic/endothelial markers CD45 (0.36%) and CD31 (4.81%) (Figure 1B). These findings are consistent with previous reports defining MSCs as CD90⁺/CD29⁺ cells with minimal expression of hematopoietic markers. The multipotency of MSCs was validated by successful differentiation into osteogenic and adipogenic lineages, demonstrated by positive Alizarin Red staining for mineralized osteocytes and Oil Red O staining for lipid-laden adipocytes (Figure 2). Following hypoxic incubation at 5% O₂ for 24 hours, exosomes were isolated using tangential flow filtration (100–500 kDa). Flow cytometry characterization confirmed the presence of exosomal surface markers CD63 and CD9, and the final exosome yield was quantified at 80 µg/mL (Figure 3).

AGA induction was confirmed by visual observation of reduced hair density in

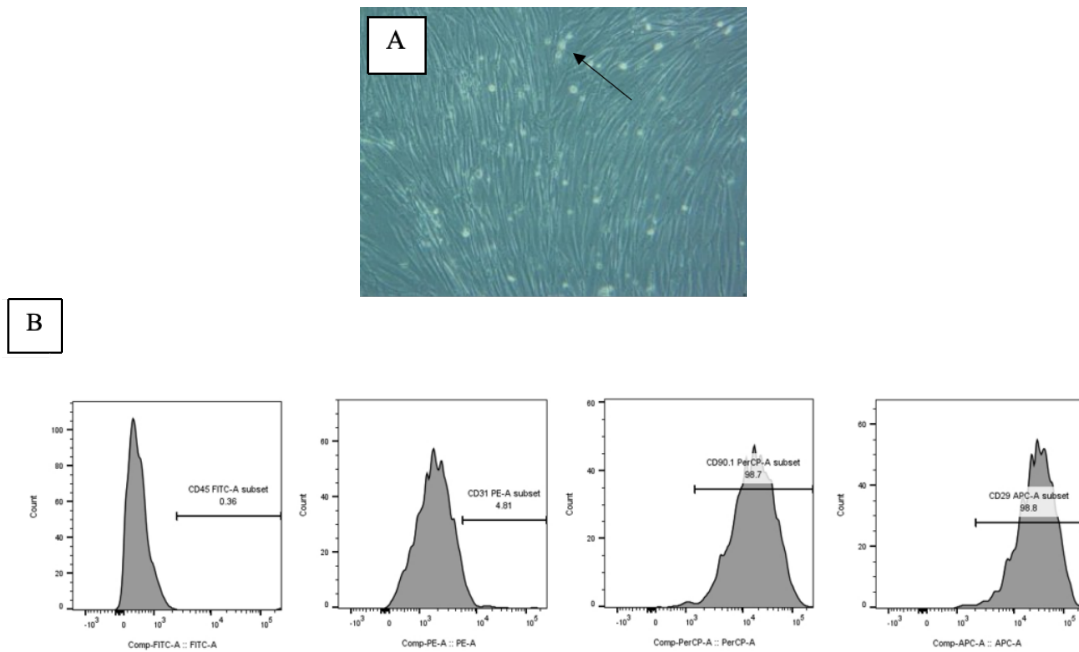


Figure 1. MSC morphology. (A) MSC morphology is fibroblast-like (indicated by arrows) at 100x magnification. (B) Flow cytometry analysis of CD45, CD31, CD90, and CD29 expression.

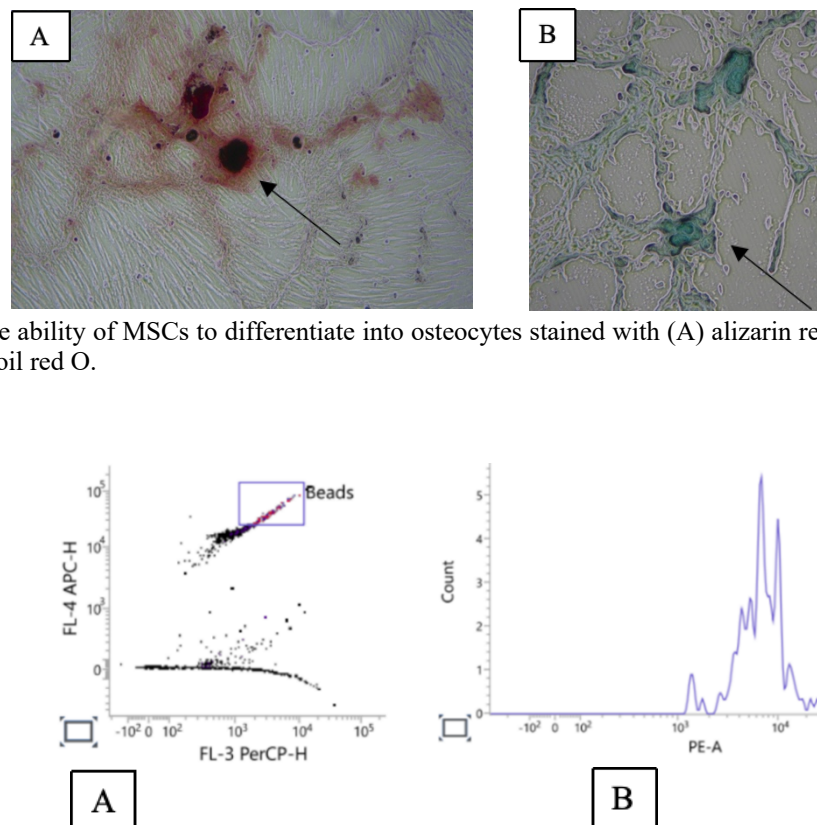


Figure 2. The ability of MSCs to differentiate into osteocytes stained with (A) alizarin red and (B) adipocytes stained with oil red O.

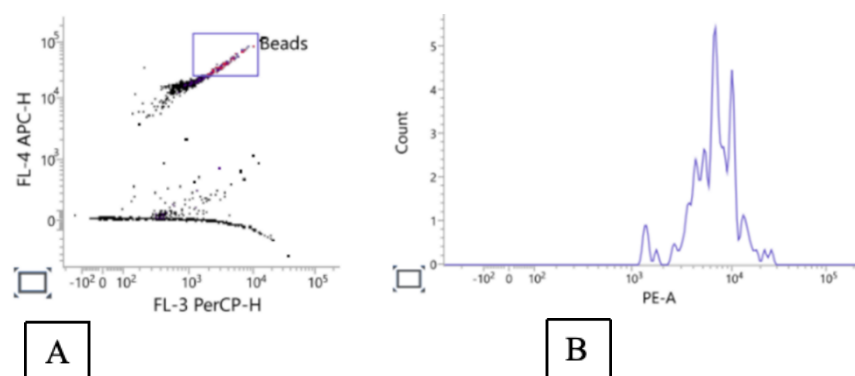


Figure 3. Results of Exosome Level Analysis using (A) CD63 and (B) CD9 markers

dihydrotestosterone-treated mice compared to healthy controls (Figure 4). Control animals displayed dense hair growth, whereas AGA mice showed markedly sparse hair coverage, validating successful induction of the alopecia phenotype. Importantly, the therapeutic intervention produced functional improvements in the

hair follicle phenotype, as reflected by the recovery of hair coverage in treated groups. Compared with minoxidil (G3), EH-MSC exosome therapy (G4 and G5) demonstrated a clearer dose-dependent improvement in hair density, with the high-dose exosome group (G5) showing the most apparent

normalization toward the healthy phenotype. This phenotypic recovery supports the functional relevance of exosome treatment and indicates that modulation of intracellular signaling pathways translated into visible improvement of follicular performance, whereas minoxidil exhibited only partial restoration.

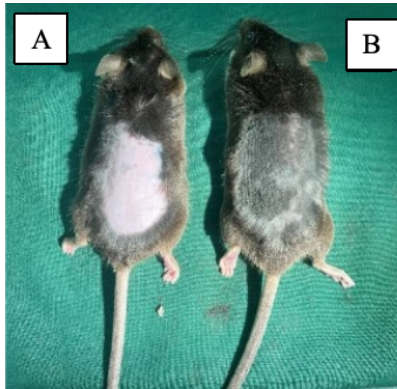


Figure 4. Validation of Androgenic Alopecia in Mice (A) Mice exposed to dihydrotestosterone have lower hair density, while in image (B, Control) mice have higher hair density.

Quantitative PCR revealed significant differences in SOCS3 expression across groups (Table 1, Figure 5). The highest SOCS3 level was observed in the EH-MSC high-dose group (G5: 1.25 ± 0.18 pg/mL), which was comparable to healthy controls (G1: 1.00 ± 0.00 pg/mL). The low-dose EH-MSC group (G4: 0.81 ± 0.23 pg/mL) also showed increased SOCS3

expression compared to the AGA untreated group (G2: 0.18 ± 0.09 pg/mL) and the minoxidil group (G3: 0.49 ± 0.16 pg/mL). Statistical testing confirmed normal data distribution (Shapiro–Wilk, $p > 0.05$); however, Levene’s test indicated heterogeneity of variance ($p = 0.002$, $p < 0.05$). One-way ANOVA demonstrated a significant overall difference among groups ($p = 0.002$). Post hoc Tamhane analysis showed that G5 differed significantly from G2, G3, and G4 ($p < 0.05$), but not from G1 ($p > 0.05$). These results indicate that EH-MSC exosomes, particularly at higher doses, restored SOCS3 expression to levels comparable to healthy controls, whereas minoxidil only partially improved SOCS3 expression. Functionally, this stronger restoration of SOCS3 in the exosome-treated group is consistent with its superior improvement in hair follicle phenotype compared to minoxidil.

In Table 2, Statistical testing confirmed normal data distribution (Shapiro–Wilk, $p > 0.05$) but heterogeneity of variance (Levene’s test, $p > 0.05$). One-Way ANOVA showed significant differences among groups ($p = 0.002$). Post hoc Tamhane analysis revealed that G5 was significantly different from G2, G3, and G4 ($p < 0.05$), but not from G1 ($p > 0.05$). These results indicate that EH-MSCs, particularly at higher doses, restored SOCS3 expression to levels comparable to healthy controls, whereas minoxidil only partially improved SOCS3 expression. This contrasts with earlier studies, where minoxidil was reported as the main stimulator of SOCS3 upregulation, suEH-MSC exosomes may provide a stronger modulatory effect.

Table 1. SOCS3 Expression Analysis Results Data

Variable	Group (Mean $\pm$ SD)					p-value
	G1 (pg/mL)	G2 (pg/mL)	G3 (pg/mL)	G4 (pg/mL)	G5 (pg/mL)	
SOCS3	1,00 $\pm$ 0,00	0,18 $\pm$ 0,09	0,49 $\pm$ 0,16	0,81 $\pm$ 0,23	1,25 $\pm$ 0,18	
Shapiro Wilk	0,00	0,34	0,48	0,13	0,20	
Lavene Test						0,002
One Way-Anova						<0,001

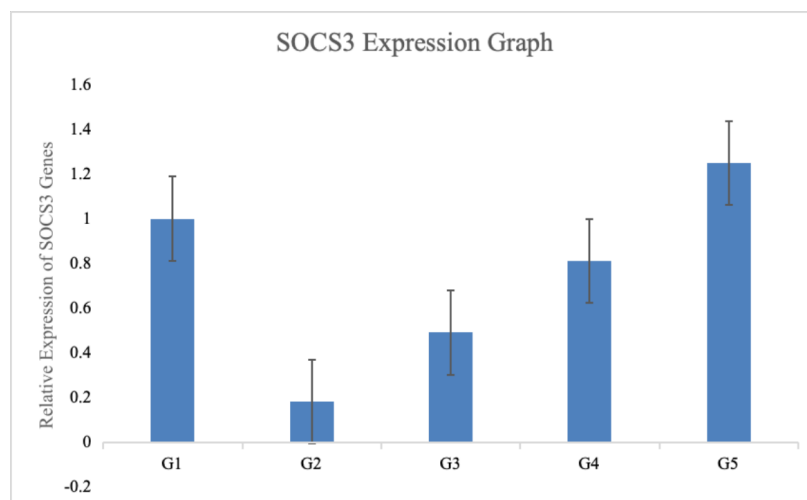


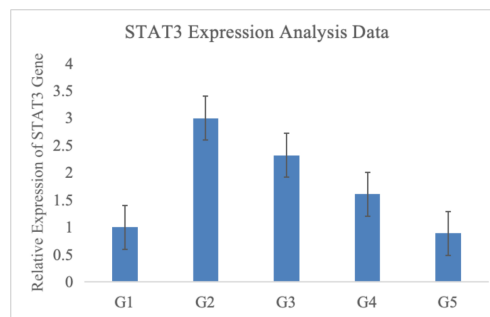
Figure 5. SOCS3 Expression Graph

Table 2. The difference in mean SOCS3 expression between the two groups using the Tamhene Post Hoc Test

Group	Comparison Group	Significant
G1	G2	0,001
	G3	0,005
	G4	0,648
	G5	0,166
G2	G3	0,03
	G4	0,004
	G5	0,001
G3	G4	0,158
	G5	0,001
G4	G5	0,04

Table 3. STAT3 Expression Analysis Results Data

Variable	Group					p-value
	G1	G2	G3	G4	G5	
STAT3	1,00±0,00	3,00±1,28	2,32±0,19	1,61±0,12	0,89±0,15	
Shapiro Wilk	0,00	0,51	0,72	0,10	0,91	
Lavene Test						0,27
One Way-Anova						0,001

Figure 6. STAT3 Expression Analysis Data**Table 4.** Difference in mean STAT3 expression between the two groups using Tamhane's Post Hoc Test

Group	Comparison Group	Significant
G1	G2	0,001
	G3	0,001
	G4	0,001
	G5	0,725
G2	G3	0,001
	G4	0,001
	G5	0,001
G3	G4	0,001
	G5	0,001
G4	G5	0,001

(Table 3, Table 4, Figure 6). The AGA untreated group (G2: 3.00 ± 1.28 pg/mL) exhibited the highest STAT3 expression, followed by minoxidil (G3: 2.32 ± 0.19 pg/mL) and low-dose EH-MSC exosomes (G4: 1.61 ± 0.12 pg/mL). Healthy controls were set at 1.00 ± 0.00 pg/mL, while the high-dose EH-MSC exosome group (G5: 0.89 ± 0.15 pg/mL) demonstrated STAT3 levels closest to normal. Shapiro–Wilk testing confirmed normal distribution ($p > 0.05$), and Levene’s test showed homogeneity of variance ($p = 0.27$, $p > 0.05$). One-way ANOVA revealed significant differences between groups ($p = 0.001$). Post hoc analysis demonstrated that G5 differed significantly from G2 and G3 ($p < 0.05$), but not from G1 or G4 ($p > 0.05$). Notably, only G5 showed STAT3 levels statistically equivalent to healthy controls, aligning with the most prominent recovery of hair density in this group. In contrast, minoxidil reduced STAT3 only partially and was associated with less complete phenotypic restoration than EH-MSC exosome treatment.

DISCUSSION

This study demonstrates that hypoxia-conditioned mesenchymal stem cell exosomes (EH-MSCs) exert significant immunomodulatory and regenerative effects in an androgenic alopecia (AGA) model by enhancing SOCS3 expression while suppressing STAT3 signaling. At the gene-expression level, the disease model showed a disrupted SOCS3/STAT3 equilibrium, consistent with a microenvironment dominated by persistent cytokine-driven stress. Importantly, EH-MSCs at 200 μ g/kg most effectively returned SOCS3 and STAT3 expression patterns toward those of healthy controls, whereas minoxidil produced only partial normalization. This molecular recovery correlated with functional improvement in follicular regeneration (e.g., the emergence of newly growing follicles and improved follicular architecture), supporting the interpretation that rebalancing SOCS3/STAT3 is not merely a biomarker change but a mechanistic axis linked to hair follicle restoration.

A deeper interpretation of the SOCS3 gene-expression shift provides a critical mechanistic explanation for the superior performance of high-dose EH-MSCs. SOCS3 is a central negative-feedback regulator of cytokine signaling, functioning as an endogenous “brake” on inflammatory JAK/STAT activation, thereby limiting prolonged immune stimulation and tissue injury. In inflammatory alopecia contexts, SOCS3 has been implicated in preventing immune-mediated follicular destruction by counteracting cytokine-dependent immune privilege collapse and excessive antigen presentation^{10,13,23}. In our dataset, the substantial SOCS3 upregulation observed after EH-MSCs 200 μ g/kg indicates reconstitution of this regulatory checkpoint, which plausibly reduces cytokine amplification loops that sustain perifollicular inflammation. Mechanistically, this may reflect exosomal delivery of immunoregulatory cargo (including miRNAs) that reprograms immune–epithelial interactions toward tolerance and homeostasis. Supporting this, adipose MSC-derived exosomes enriched in miR-122-5p have been shown to

counteract dihydrotestosterone (DHT)-mediated suppression of hair follicles, highlighting that exosomal miRNA cargo can directly influence growth-inhibitory signaling in androgen-driven alopecia states²⁴. Previous work has shown that SOCS3 dysregulation contributes to autoimmune-driven hair follicle damage^{14,25}, and our results extend this by demonstrating that EH-MSC exosomes can reverse such deficits *in vivo*. Thus, the SOCS3 rescue observed here likely represents restoration of anti-inflammatory feedback and stabilization of the follicular niche rather than a nonspecific growth-factor effect.

Conversely, STAT3 suppression observed in the high-dose EH-MSC group highlights another therapeutic dimension. STAT3 is known to mediate chronic inflammatory signaling and promote fibrosis in hair follicle microenvironments^{11,12}. While physiological STAT3 signaling is required for epidermal and follicular homeostasis, sustained STAT3 activation under inflammatory cues can become pathologic, amplifying cytokine-driven stress responses²⁶. In AGA-related settings, elevated STAT3 expression has been associated with inflammatory imbalance and microenvironmental impairment, which can converge on miniaturization and compromised follicular support. Therefore, the “normalizing” reduction of STAT3 toward healthy levels by EH-MSCs 200 μ g/kg may alleviate prolonged inflammatory transcriptional programs while preserving baseline homeostatic signaling. Functionally, this STAT3 normalization plausibly improves the conditions needed for follicular cycling recovery and structural regeneration, aligning with the stronger follicle-growth outcomes in the high-dose EH-MSC group compared with minoxidil¹². By lowering STAT3 activity towards normal levels, EH-MSCs appear to both reduce chronic inflammatory stress and facilitate vascular remodeling essential for follicle regeneration. This dual regulation of SOCS3 and STAT3 underscores a dose-dependent mechanism unique to hypoxia-conditioned exosomes.

These findings align with recent studies reporting the regenerative potential of MSC-derived exosomes in dermatological applications^{17,27}. For example, adipose-derived MSC exosomes enriched in miR-122-5p have been shown to antagonize the inhibitory effect of dihydrotestosterone on follicular growth²⁴. Similarly, miR-221, another key exosomal cargo, has been implicated in activating angiogenic pathways via STAT3 signaling, thereby improving follicular blood supply^{11,28,29}. Our study expands these insights by showing that EH-MSC exosomes not only deliver such miRNAs but also rebalance downstream SOCS3/STAT3 interactions in a more physiologically restorative manner.

In contrast, previous literature indicates that minoxidil primarily promotes follicular growth through angiogenic and anagen-supportive signaling rather than dominant immunoregulatory control. At the molecular level, minoxidil has been shown to upregulate VEGF expression in human dermal papilla cells, supporting enhanced perifollicular vascularization and nutrient delivery³⁰. Beyond VEGF, minoxidil can activate the Wnt/ β -catenin pathway in dermal papilla cells, a

mechanism that helps extend the anagen phase and sustain active hair growth³¹. Minoxidil has also been linked to increased prostaglandin (PGE2) production via activation of prostaglandin synthase-1, suggesting a cytoprotective component that may support follicular survival in stress conditions³². Collectively, these pathways explain why minoxidil often yields measurable functional improvement (new growth induction and anagen prolongation), yet in our study it did not fully restore the SOCS3/STAT3 axis: its molecular action is weighted toward vascular and growth-cycle stimulation rather than robust cytokine-feedback reprogramming. This biological “scope difference” provides a coherent mechanistic rationale for the partial SOCS3 restoration and incomplete STAT3 normalization in the minoxidil group, despite improvements in follicular outcomes relative to untreated AGA controls.

Compared with minoxidil, which primarily stimulates follicular vascularization through potassium channel opening^{7,15}, EH-MSCs provide a broader biological effect by simultaneously modulating immune responses, reducing chronic inflammation, and enhancing angiogenesis. This may explain why SOCS3 restoration was only partial with minoxidil but complete with EH-MSCs. Interestingly, our results differ from earlier clinical data suggesting that exosome therapy primarily acts through paracrine growth factors^{19,33}. Instead, we observed a clear immunomodulatory effect mediated via SOCS3/STAT3 pathways, suggesting that the therapeutic impact of exosomes extends beyond growth factor delivery to precise signaling control.

The immunological implications are noteworthy. The increase in regulatory T cell activity inferred from SOCS3 induction indicates that EH-MSCs may mitigate autoimmunity against hair follicles, a mechanism consistent with earlier reports in alopecia areata models^{13,18,28}. Moreover, by dampening STAT3-driven proinflammatory cascades, EH-MSCs may protect follicular stem cell niches from oxidative stress and premature apoptosis^{8,14,19}. This combined immunoregulatory and regenerative effect places EH-MSCs in a unique therapeutic category distinct from pharmacologic agents currently used in AGA.

Nevertheless, several limitations must be acknowledged. The study was conducted in an animal model, which may not fully recapitulate the chronic and multifactorial nature of human AGA. Additionally, the exosomal miRNA cargo was not profiled in detail; thus, while miR-122 and miR-221 are strong candidates, the precise molecular drivers of SOCS3/STAT3 modulation remain to be elucidated. Future studies should include transcriptomic or proteomic profiling of EH-MSC exosomes to map specific mediators of follicular regeneration^{6,27}. Moreover, dose-response relationships in humans may differ from those in rodents, and clinical translation will require careful evaluation of safety, scalability, and delivery methods.

CONCLUSION

The findings of this study demonstrate that intraperitoneal administration of exosomes derived from hypoxia-preconditioned mesenchymal stem cells

(EH-MSCs) effectively increased SOCS3 and decreased STAT3 expression in a dihydrotestosterone-induced mouse model of androgenetic alopecia. These outcomes suggest that EH-MSCs hold considerable promise as a therapeutic strategy for androgenetic alopecia yielding stronger effects. These outcomes suggest that EH-MSCs hold considerable promise as a therapeutic strategy for androgenetic alopecia by simultaneously modulating immune responses and promoting hair follicle regeneration.

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