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The Effect of Hypoxic Mesenchymal Stem Cells (S-HypMSCs) Secretome Creamon AP1S3 Gene Expression (In Vivo Study on Psoriasis-like Rats Model Induced by Imiquimod)

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ABSTRACT: Psoriasis is known as a complex disease with interactions between genes, the body's immune system and environmental factors, which is a chronic inflammation characterized by scaly red plaques. Secretome Hypoxic Mesenchymal Stem Cells (S-HypMSCs) contain various components released by MSCs consisting of chemokines, growth factors and anti-inflammatory cytokines, making it possible as a therapeutic option. This study aims to investigate the effect of S-HypMSCs on psoriasis improvement. MSCs were isolated from the umbilical cord of rats, Secretome which could be mixed with cream and applied to the wounds of psoriasis-like animal models at a dose of 100 µL/kgBW in 100 mg cream for group P1 and 200 µL/kgBW in 100 mg cream for group P2. The control group was treated with a cream base. The mRNA expression of AP1S3 was analyzed using quantitative Reverse Transcription-Polymerase Chain Reaction (qRT-PCR). All examinations were assessed on day 14. There was a significant difference in the healthy group against the psoriasis-like group, psoriasis-like treatment 1 and treatment 2 groups. administration of S-HypMSCs at a dose of 100 µL/kgBW in 100 mg cream and a dose of 200 µL/kgBW in 100 mg cream significantly decreased AP1S3 expression in Psoriasis-like male white rats of the Wistar strain. The administration of S-HypMSCs cream at a dose of 100 µL/kgBW in 100 mg cream and a dose of 200 µL/kgBW in 100 mg cream had an effect on decreasing AP1S3 gene expression between treatment groups compared to controls in psoriasis-like male Wistar rats. imiquimod induced.

KEYWORDS: psoriasis, MSCs, Secretome, AP1S3, IL36

I. INTRODUCTION

Psoriasis is known as a complex disease with interactions between genes, the immune system and environmental factors involved in the onset and development of the disease.[1] Psoriasis is a chronic, immune-mediated inflammation of the skin, characterized by scaly red plaques that most commonly occur on the elbows, knees, scalp, and lower back, but any surface of the skin can be involved.[2] Current psoriasis therapy methods such as topical steroids, phototherapy, conventional systemic agents such as cyclosporine, methotrexate, acitretin and other small molecules remain the mainstay of psoriasis therapy. However, long-term administration of these agents may cause some side effects, such as skin irritation, drug resistance and intolerance.[3] Research on the subcutaneous injection of Secretome Hypoxic Mesenchymal Stem Cells (S-HypMSCs) shows an improvement in psoriasis, thus enabling therapy in the form of a cream as a therapeutic option due to its easier application for patients.[4,5]

Many of the genes associated with increased susceptibility to psoriasis were identified. A Genome Wide Association Studies (GWAS) genetic study identified 15 psoriasis susceptibility, named Psoriasis Susceptibility 1-15 (PSORS 1-15), which are thought to be major contributors to the genetic pathogenesis of psoriasis.[6] The PSORS15 loci is located on chromosome 2q36.[7] in the Sigma 3 (AP1S3) of Adapter Protein-1 gene.[1,8] Several research studies also mention that mutations in the AP1S3 gene are closely related to the occurrence of psoriasis. The impact of mutations affecting AP1S3 is that AP1S3 expression is typically increased in keratinocytes because AP1S3 encodes a protein involved in autophagosome formation and it was found that knockout of AP1S3 impairs keratinocyte autophagy, leading to abnormal accumulation of p62 (protein62), an adapter protein that mediates NF-κB

(nuclear factor kappa-light-chain-enhancer of activated B cells, nuclear factor-kappaB) activation, as a result, cells lacking AP1S3 regulate IL-1 (Interleukin-1) signaling and overexpress an IL-36, a cytokine that has emerged as an important mediator of skin inflammation.[1] This abnormal immune profile was recapitulated by pharmacological inhibition of autophagy and verified in keratinocytes, where they were reversed by IL-36 blockade, these findings suggest that keratinocytes play a key role in skin autoinflammation and identify autophagy modulation of IL-36 signaling as a therapeutic target.[8]

The Secretome Hypoxic Mesenchymal Stem Cells (S-HypMSCs) are the result of conditioned MSCs metabolites, which contain more large extracellular vesicles, microvesicles, and exosomes.[9] S-HypMSCs contain various components released by MSCs consisting of chemokines, growth factors and anti-inflammatory cytokines such as Interleukin-10 (IL-10) and growth factors consisting of Vascular Endothelial Growth Factor (VEGF), PDGF (Platelet-Derived Growth Factor), and Transforming Growth Factor beta (TGF-β).[4,7] Data collected shows that the paracrine factors are secreted by stem cells to facilitate cellular survival and regeneration. In addition, the paracrine factors can mediate anti-inflammatory and immunomodulatory functions.[8] Mechanistic studies reveal that IL10 inhibits NF-κB activation. S-HypMSCs containing IL-10 are expected to inhibit the expression of the AP1S3 gene which modulates NF-κB, this is because IL-10 controls the inflammatory process by suppressing the production of IL-36 which is known to be controlled transcriptionally by NF-κB.[10] Therefore, it is possible to have the potential of S-HypMSCs as an alternative agent for psoriasis therapy. In this study we would like to investigate the effect of S-HypMSCs on the expression of the associated AP1S3 gene the imiquimod-induced psoriasis rat model.

II. MATERIAL AND METHOD

MSC Isolation and Culture

MSC isolated from the umbilical cord of pregnant Wistar rats. Samples were collected in sterile culture dishes with 0.9% NaCl. After washing with phosphate-buffered saline (PBS), the umbilical cord is separated from its attachments. The umbilical cord is mechanically chopped and the vessels removed. Each sample was cultured in a 25T flask containing Dulbecco's Modified Eagle's Medium (DMEM), fungizon, penicillin/streptomycin, and 10% Fetal Bovine Serum (FBS) for 3 minutes. The flasks were incubated at 37 °C and 5% C and the medium was replaced with fresh complete medium every three days. MSC will appear in approximately 14 days. After reaching 80% confluence, cells were separated using the BDTM accutase cell releasing solution (cat No. 561527).[11] MSCs were then incubated under conditions hypoxia with O concentration 2.5% for 24 hours using chamber *hypoxia.*[9]

Modeling Animal

A 24 male Wistar rats between 250 and 300 g of weight were kept in polypropylene cages in a standard room with a temperature of 23–35 °C with 12 light-dark cycles and 40–70% humidity. Animals have free access to AIN 76A standard food and water. Experiments were carried out after 3 days of acclimatization.

All of experimental subject were anesthetized with a mixture of ketamine (60 mg/kgBW) and xylazine (20 mg/kgBW). The hair on the back of the mouse is cut clean. The backs of the rats were smeared with imiquimod cream 10 times, from 1st to 10th day).[6] Psoriasis is treated by applying daily topical cream containing Secretome. Sham rats were not given any therapy, while control rats were given cream base treatment.

Administration of S-HypMSCs

All of experimental subject were randomly divided into four groups and each group consisting of 6 rats. The intervention were divided into 4 treatments: Sham treatment (no treatment), positive control (only exposed to 120 mg of imiquimod), P1 (exposed to 120 mg of imiquimod and treated with 100 µL/kgBW cream in 100 mg of cream), and P2 (exposed to imiquimod 120 mg and treated with S-HypMSCs cream at a dose of 200 µL/kgBW in 100 mg cream).

Animal Termination

The termination of subject using a lethal dose of cocktail before organ harvesting. The preparation of 10 mL cocktail used Ketamine 50 mg/kgBW, Xylazine 10 mg/kgBW and Acepromazine 2 mg/kgBW which were injected intramuscularly. After the subject died, the skin was collected and then stored in cryotubes free of RNAse at -80°C in RNA later.

Data analysis

Data are shown as mean ±standard deviation. Data analysis were performed using SPSS 25.0 (IBM Corp., Armonk, NY, USA). All of experimental variable were tested using normality and homogeneity test. The significance test of independent quantitative variables using ANOVA test and followed by Kruskal Wallis test. A p value of <0.01 was considered as significant.

III. RESULT

Mesenchymal Stem Cell Differentiation and Characteristics

The MSCs isolated from rat umbilical cord were morphologically identified as fibroblast cells (Figure 1). It is induced to differentiate in terms of its phenotype and its ability to give rise to both osteogenic and adipogenic lineages.

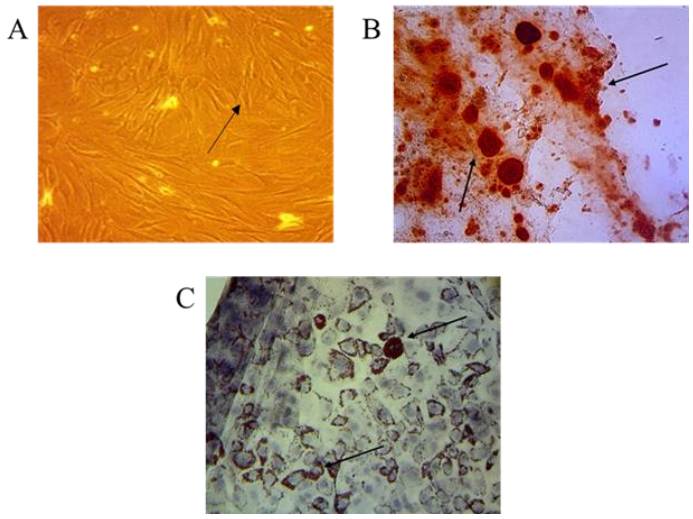

Figure 1. (A) Isolation of MSCs with 80% confluency showed spindle-like cells (pointed by arrows) at 100x magnification. (B) Osteogenic differentiation using Alizarin Red staining appears in the MSC population at 100x magnification. (C) Adipogenic differentiation using Oil Red O appears in the MSC population at 100x magnification.

The results of S-MSCs isolation were validated using flow cytometry to show the ability of MSCs to express various specific surface markers. Quantitative results were the percentage of positive expression of CD90.1 (99.50%), CD29 (96.10%), negative expression of CD45 (1.30%), and CD31 (6.60%) (Figure 2).

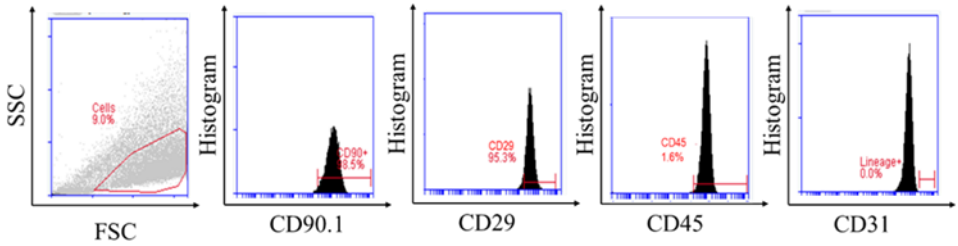

Figure 2. Detection of surface markers CD90.1, CD29, negative expression of CD45 and CD31.

MSCs were then incubated under hypoxic conditions with a concentration of 5% O2 for 24 hours using a hypoxic chamber to obtain a more denses, spindle-like cell appearance (Figure 3).

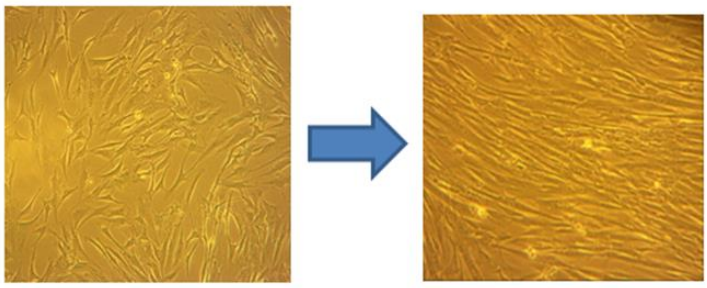

Figure 3. Different visualize of hypoxic mesenchymal stem cells

Validation Test of The Psoriasis-Like Animal Model

Based on the results and validation of the psoriasis-like model mice (Fig. 4) proved that 120 mg of imiquimod induced psoriasis-like which was proven to have significant erythema, scale, and ticknes.

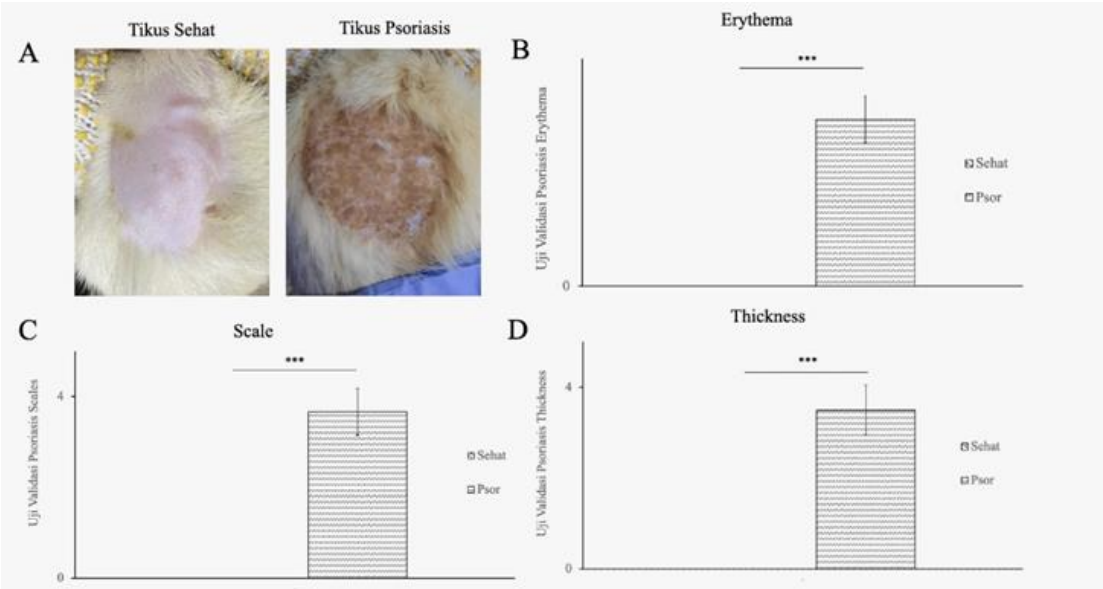

Figure 4. (A) dorsal view of healthy rats and mice induced with 100 mg immoquinot, (B) Graph of psoriasis erythema validation of healthy group and psoriasis group, (C) Graph of validation of psoriasis scale of healthy group and control group, and (D) Graph validation of the psoriasis thickness of the healthy group and the control group. Data represents an average of 3 replicates +/- SD. ** * p < 0.05 indicates a significant difference.

AP1S3 Expression

The relative expression of mRNA in rat skin tissue samples was measured by the qRT-PCR method and calculated by the Livak method to determine the relative quantification (qRT) value. In this study, the researchers found that S-HypMSCs were able to significantly reduce AP1S3 levels in male rats of the psoriasis-like model, depending on the dose (Figure 5).

Based on Figure 5. The mean AP1S3 gene expression in the Psoriasis negative control group was the highest (5.24 ± 1.91), followed by the average AP1S3 level in the P1 group (2.58 ± 1.10). Furthermore, the mean AP1S3 gene expression in the P2 group (1.75 ± 0.84) and the mean AP1S3 levels in the Sham group (1.00 ± 0.00).

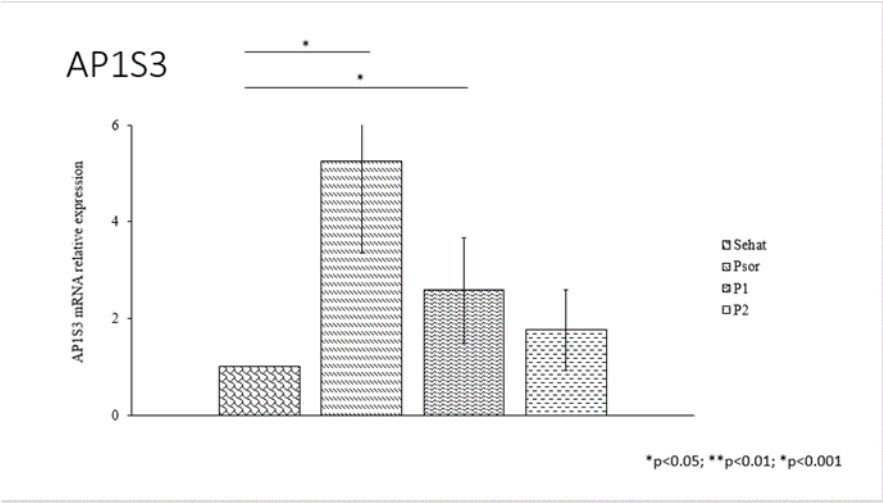

Figure 5. Graph of AP1S3 Gene Expression Levels in All Treatment Groups.

IV. DISCUSSION

A MSCs secrete a secretome that contains mediators and growth factors that support skin regeneration, including anti-inflammatory cytokines (IL-10), VEGF, PDGF, TNF-α, fibroblast growth factor (FGF), TGF β1 angiopoietin, IL-1, IL-6, and IFN γ which will stimulate collagen formation and accelerate skin regeneration.[12,13] Hypoxic conditions will further trigger MSCs to secrete soluble molecule secretomes that contain more anti-inflammatory mediators and growth factors.[14]

The AP1S3 mutation causes AP1S3 gene expression to increase resulting in abnormal IL-1 signaling and increased IL-36 expression.[3,15] Available evidence suggests that the cytokine IL-36 is an important driver of autoinflammatory response. 20 21 22 In this study it can be seen that the mutation of the AP1S3 gene increased resulting in increased.[16]

The secretome containing IL-10 can inhibit the expression of the AP1S3 gene which modulates NF-κB, this is because IL-10 controls the inflammatory process by suppressing the production of pro-inflammatory cytokines which are known to be controlled transcriptionally by NF-κB.[17] 49 IL-10 inhibits NF-κB activation at least two different ways: by inhibiting activation of IkB kinase-similar to salicylates and by inhibiting DNA-binding NF-κB activity (the latter mechanism is not understood).[18]

V. CONCLUSION

The administration of S-HypMSCs can increase the psoriasis healing by decreasing the AP1S3 expression.

VI. ACKNOWLEDGEMENT

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