Research Paper

Investigating the Impact of *Astragalus* Polysaccharide on Angiogenesis in Human Adipose-Derived Stem Cells

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This study aims to investigate the influence of *Astragalus* polysaccharides on angiogenesis in human adipose-derived stem cells. Three experimental groups were established; control group (group A), a low-dose *Astragalus* polysaccharides intervention group (group B), and a high-dose *Astragalus* polysaccharides intervention group (group C). Group A comprised untreated human adipose-derived stem cells cultured under standard conditions. Group B received an *Astragalus* polysaccharides concentration of 80 µg/ml, while group C was treated with 160 µg/ml of *Astragalus* polysaccharides. Cell proliferation, migration, and angiogenic potential were assessed using cell counting kit-8 assay, Western blot analysis, quantitative polymerase chain reaction, and Transwell migration assays. Relative to group A, group B exhibited enhanced cell migration and proliferation, with further increases observed in group C. Angiopoietin-1 and vascular endothelial growth factor levels were elevated in group B compared to group A, with an additional increase in group C. Conversely, the expression levels of pro-apoptotic proteins Bcl-2-associated X protein and caspase-3 were decreased in group B relative to group A, and further reduced in group C. Hypoxia-inducible factor 1 and nuclear factor kappa B expression were diminished in group B compared to group A, with a more pronounced reduction in group C. *Astragalus* polysaccharides modulate angiogenesis in human adipose-derived stem cells by downregulating nuclear factor kappa B expression, thereby inhibiting hypoxia-inducible factor 1 and nuclear factor kappa B pathways. This modulation facilitates the proliferation and angiogenic activity of adipose-derived stem cells, suggesting a promising therapeutic potential of *Astragalus* polysaccharides in regenerative medicine and tissue engineering.

Key words: *Astragalus* polysaccharides, human adipose-derived stem cells, angiogenesis, nuclear transcription, hypoxia

In addition to the formation of scar tissue, wound healing is an unusually continuous and complex process that involves proliferation, inflammation, coagulation and hemostasis[1]. The improper management of wounds can lead to complications, such as non-healing and prolonged healing[2]. Although significant progress has been made in traditional treatments, including wound dressings, hyperbaric oxygen therapy, the use of sensitive antibiotics and wound debridement, patients often experience persistent wound healing[3]. Applying stem cells has the potential to accelerate tissue regeneration and wound repair. Adipose-Derived Stem Cells (ADSCs) are adult stem cells with high regeneration ability identified in adipose tissue[4]. Previous studies have shown that ADSCs can regulate neovascularization and angiogenesis. ADSCs can release a variety of bioactive factors, including Hepatocyte Growth Factor (HGF), Epidermal Growth Factor (EGF) and Vascular Endothelial Growth Factor (VEGF), which can promote angiogenesis and enhance endothelial cell function[5]. *Astragalus* Polysaccharides (APS) is one of the main active components of radix Astragali, which is obtained by high-tech extraction and isolation. It can enhance immune function and promote the development and proliferation of stem cells. It has been proved that it can promote the

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Accepted 12 April 2024
Revised 05 August 2023
Received 04 November 2022
Indian J Pharm Sci 2024;86(2):620-624

growth, proliferation and differentiation of many kinds of stem cells[6]. In light of this, this study investigated the impact of APS on angiogenesis of human ADSCs, in order to provide reference for the choice of clinical treatment.

MATERIALS AND METHODS

Materials and reagents:

Human ADSCs were from the American Type Culture Collection (ATCC). APS injection was purchased from Tianjin Sainuo Pharmaceutical Co., Ltd.; Hypoxia-Inducible Factor 1 (HIF-1), Nuclear Factor Kappa B (NF-κB) messenger Ribonucleic Acid (mRNA) primers and β-actin primers (Sigma Co., Ltd.,); quantitative Polymerase Chain Reaction (qPCR) detection kit, immunohistochemical sheep anti-rabbit second antibody, Cell Counting Kit-8 (CCK-8) detection kit, Annexin V-Fluorescein Isothiocyanate (FITC)/ Propidium Iodide (PI) apoptosis kit (Shanghai Biyuntian Co., Ltd.,); Transwell chamber (Corning Co., Ltd.,). Angiopoietin-1 (Ang-1), VEGF, Bcl-2-Associated X Protein (BAX), HIF-1 and NF-κB antibody (Abcam Biotechnology Co., Ltd.,).

Method:

Cell culture and treatment: Following resuscitation, the human ADSCs were grown in a constant temperature incubator (37°, 5 % Carbon dioxide (CO_2)) and inoculated in a T25 culture bottle with Roswell Park Memorial Institute (RPMI)-1640 media containing 10 % Fetal Bovine Serum (FBS). APS injection purchased from Tianjin Sainuo Pharmaceutical Co., Ltd., was injected into sterile water at the configuration and final concentration of 80 and 160 µg/ml. APS injection of 80 and 160 µg/ml was added. Set up control group (group A), low-dose APS intervention group (group B) and high-dose APS intervention group (group C). Human ADSCs were the cells in group A; they were routinely grown and received no treatment, the group B was treated with 80 µg/ml APS, and the group C was treated with 160 µg/ml APS. Each experiment was repeated 6 times.

Western blot: First, we lysed cell samples in Radio-Immunoprecipitation Assay (RIPA) lysis buffer. Then we separated protein lysates on 10 % Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis (SDS-PAGE), followed by transferring to the Polyvinylidene Difluoride (PVDF) membranes. Later, the 5 % nonfat milk powder was utilized to blockage and the membranes were cultured with the primary antibodies at 4° for one night. After that, the Horseradish Peroxidase (HRP)-conjugated secondary antibodies were used to cultivation for 2 h at 37°. Finally, samples were exposed by Enhanced Chemiluminescence (ECL) luminous liquid (Pierce, Illinois, United States of America (USA)). There were more than three independent repetitions in this experiment.

qPCR: The cell was inoculated and cultured, After being cultured in the incubator for 48 h, each group of cells used RNA extraction kit to extract RNA, One Step Prime Script microRNA (miRNA) complementary Deoxyribonucleic Acid (cDNA) synthesis kit was used to reverse transcription miRNA into cDNA, miRNA fluorescence qPCR detection kit was used for quantitative real-time PCR and then performed the amplification reactions as per the instructions provided with the TransStart® Top Green qPCR Super mix. Using $2^{-\Delta\Delta Ct}$ method to analysis and the outcomes were standardized to Glyceraldehyde-3-Phosphate Dehydrogenase (GAPDH) or U6.

CCK-8: The cell density of each group was adjusted to 1×103/well and inoculated. 10 µl to every well, CCK-8 solution was added. The capacity of each group's cells to proliferate was measured using an enzyme labeling device (Optical Density (OD) 450 nm) in an incubator maintained at a constant temperature of 37° and 5 % Carbon dioxide (CO_2).

Transwell method: We collected 2×10^4 cells of A549 and H1299 after transfection was finished and then re-suspended cells in serum-free medium. The transwell upper chamber was utilized to seed cells, and 100 % culture media was applied to the bottom chamber. 24 h later, we used 4 % paraformaldehyde to fix the migrating cells and then stained them with crystal violet. Cell migration was analyzed in 5 random filed by use of optical microscope (Olympus, Tokyo, Japan). There were more than three independent repetitions in this experiment.

Statistical method:

The whole experiments in our research contained more than three independent repeats. The data was displayed as the mean±Standard Deviation (SD). Group difference was determined by the application of Student's t-test or one-way Analysis of Variance (ANOVA) *via* Graph Pad PRISM 6. $p<0.05$ was regarded as significant.

RESULTS AND DISCUSSION

Compared to group A, group B had higher cell migration rates and a higher rate of cell proliferation. Group C exhibited higher levels of cell migration and proliferation activity compared to group B (Table 1).

The Ang-1 and VEGF in the group B were raised than those in the group A, and these in the group C were increased than the group B (Table 2).

The protein levels of BAX and caspase-3 in the group B were reduced than those in the group A, and these in the group C were decreased than the group B (Table 3).

The relative expressions of HIF-1 and NF-κB, mRNA in the group B were reduced than the group A, while these in the group C were decreased than the group B (Table 4).

The protein levels of HIF-1 and NF-κB in the group B were reduced than the group A, while these in the group C were decreased than the group B (Table 5).

TABLE 1: EFFECTS OF APS ON PROLIFERATION AND MIGRATION OF ADSCs

Group	n	Cell proliferative activity	Number of cell migration
A	6	0.10±0.01	73.25±15.70
B	6	0.18±0.03[a]	106.48±17.44[a]
C	6	0.22±0.04[ab]	142.74±24.34[ab]
F		25.846	19.022
p		0.000	0.000

Note: [ab]p<0.05

TABLE 2: EFFECT OF APS OF ANGIOGENESIS-RELATED PROTEINS IN ADSCs

Group	n	Ang-1	VEGF
A	6	0.41±0.13	0.30±0.11
B	6	0.63±0.14[a]	0.59±0.13[a]
C	6	0.94±0.25[ab]	0.71±0.12[ab]
F		97.823	53.632
p		0.000	0.000

Note: [ab]p<0.05

TABLE 3: EFFECT OF APS ON APOPTOTIC PROTEIN EXPRESSION OF ADSCs

Group	n	BAX	Caspase-3
A	6	0.36±0.06	0.38±0.02
B	6	0.23±0.04[a]	0.24±0.04[a]
C	6	0.13±0.03[ab]	0.11±0.04[ab]
F		45.413	47.276
p		0.000	0.000

Note: [ab]p<0.05

TABLE 4: EFFECTS OF APS ON THE HIF-1 AND NF-κB mRNA IN ADSCs

Group	n	HIF-1 mRNA	NF-κB mRNA
A	6	2.47±0.54	2.05±0.46
B	6	1.65±0.40[a]	1.32±0.35[a]
C	6	1.24±0.32[ab]	0.77±0.09[ab]
F		212.744	321.687
p		0.000	0.000

Note: [ab]p<0.05

TABLE 5: EFFECTS OF APS ON THE EXPRESSION OF HIF-1 AND NF-κB PROTEIN IN ADSCs

Group	n	HIF-1	NF-κB
A	6	0.86±0.28	0.78±0.12
B	6	0.57±0.08[a]	0.44±0.14[a]
C	6	0.28±0.04[b]	0.23±0.14[b]
F		115.521	218.481
p		0.000	0.000

Note: [ab]p<0.05

Incisions, thermal burns, accidental injuries, and chronic ulcers can all result in acute or refractory wounds[7]. Wounds that go untreated can result in hyperplastic scarring and even disabilities, as well as serious mental and economic hardship for patients and their families. Hemostasis, inflammation, proliferation, and tissue remodeling are the four stages of skin wound healing. The development of new strategies, treatments, and techniques for wound healing is urgently needed[8]. ADSCs are a kind of mesenchymal stem cells, which have a wide range of sources, easy to isolate and expand, and poor immunogenicity. ADSCs are increasingly being shown to promote wound healing and tissue regeneration[9]. Therefore, the investigation and study of protein and angiogenesis secreted by ADSCs is very important to understand the molecular mechanism of ADSCs regulating wound healing.

Compared to group A, group B cells migration and proliferation activities were higher, while these in the group C were increased than in the group B. The BAX and caspase-3 in the group B were reduced than those in the group A, while these in the group C were decreased than the group B. It is proposed that APS can promote the proliferation of human ADSCs and inhibit their apoptosis. *Astragalus membranaceus* is extracted from the roots of plants and belongs to Leguminosae. It is widely used in traditional Chinese medicine. Because of its well-established safety and effectiveness, it is a highly esteemed element in herbal preparations[10]. A natural herbal substance derived from the root of the plant, *Astragalus membranaceus* contains important components that contribute to its pharmacological effect. APS has a chemical structure similar to saponins. It possesses several pharmacological properties, including as immunomodulatory, antiviral, anti-inflammatory, and antioxidant properties[11]. In addition, many studies *in vitro* and *in vivo* have shown that astragaloside can inhibit the growth of various types of stem cells and promote cell differentiation, and its mechanism may be related to its induction of mesenchymal stem cells to secrete cytokines[12].

Studies have shown that stem cells promote wound healing through enhanced vascularization. Angiogenesis is an important process of wound healing[13]. In order to heal wounds and repair tissues, new capillaries must be formed. In addition to increasing blood flow to the wound, neovascularization provides adequate oxygen and nutrition to aid wound healing, removes local metabolites, and accelerates tissue repair[14]. After healing, angiogenesis occurs in the wound, including endothelial cell proliferation, migration and tube formation. ADSCs have strong differentiation potential and the ability to promote angiogenesis, so they are proposed as important seed cells to promote tissue repair in regenerative medicine stem cell therapy[15]. Angiogenesis is stimulated by growth factors such as VEGF, which is an important process of wound healing. ADSCs secrete VEGF, which partly explains why ADSCs can promote wound healing[16]. The Ang-1 and VEGF in the group B were raised than the group A, while these in the group C were increased than the group B. It is proposed that APS can promote the angiogenesis of human ADSCs. Angiogenesis is a multi-molecule process that occurs in a range of cells. In the theory of traditional Chinese medicine, Huang Miao can replenish qi, invigorate blood, promote blood circulation, and support toxin and muscle. The researchers speculate that the traditional Chinese medicine for tonifying qi and blood should have the function of promoting angiogenesis. Modern medical research shows that Huang Miao polysaccharide, as the main active ingredient, has been proved to have the effects of promoting the secretion of cytokines, promoting neovascularization and protecting vascular endothelial cells in many studies[17]. HIF-1α exists widely in humans and mammals. During intracellular oxygen metabolism, it regulates dimer transcription factors. The genes regulated

by HIF-1α mainly act on some key links in the occurrence and development of tumor, such as cell invasion and migration, tumor metabolism, apoptosis and so on[18]. Additionally, studies on the molecular etiology of endocrine-related illnesses, such as cancers, center on the signal route. Nuclear transcription factor NF-κB is a multifunctional protein found in cells across all bodily systems. It controls the transcription of adhesion molecules, complements, cytokines, chemokines, and other cytokines[19]. In inflammatory cells including lymphocytes, neutrophils, macrophages, and chondrocytes, it is extensively disseminated, although it has no biological activity at rest, existing in the cytoplasm as an inactive trimer. Furthermore, it aggravates the body's inflammatory response and impairs wound healing by starting the transcription of relevant target genes and taking part in pathological processes including immune response, inflammatory reaction, and apoptosis[20]. The HIF-1, NF-κB, mRNA and protein in the group B was reduced than the group A, while these in the group C was decreased than the group B, suggesting that APS inhibit HIF-1 and NF-κB in ADSCs, thus promoting wound healing.

To sum up, APS can promote the proliferation and angiogenesis of human adipose stem cells by inhibiting HIF-1 and NF-κB of ADSCs.

Conflict of interests:

The authors declared no conflict of interests.

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