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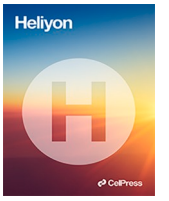

Research article

Preventive effect of human umbilical cord mesenchymal stem cells on skin aging in rats

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ABSTRACT

The irreversibility of aging makes anti-aging become an important research direction in the field of medical research. As the most direct manifestation of human aging, skin aging has been paid more and more attention. Stem cells have been used as a basis for anti-aging studies in skin, of which adipose-derived mesenchymal stem cells are more commonly used. In this study, human umbilical cord mesenchymal stem cells were used, and human umbilical cord mesenchymal stem cells were intervened while making a skin aging model, which was planned to reduce the process of preventing skin aging in the study method. At the end of the experiment, rat skin and serum were taken for relevant data detection. The results showed that the contents of EGF and VEGF in serum and skin tissue of rats increased and the content of MDA decreased after the application of human umbilical cord mesenchymal stem cells. At the same time, hUCMSC intervention increased skin thickness, increased dermal vessels, increased type I collagen type III collagen mRNA expression, and decreased MMP-1 content in rats. The results showed that hUCMSC could prevent skin aging in rats.

1. Introduction

The aging problem of the global population is becoming more and more serious, and clarifying the aging mechanism and strengthening anti-aging therapy have become hot spots in the field of modern medical research. Aging is a process in which the structure and function of the human body gradually decline with age and is a natural dynamic process of life. Studying how to fight aging is equal to helping people achieve the desire to prolong life span and restore body function and vitality. We are always looking for ways to delay this physiological process and provide better treatments for human life span and quality of life. To date, methods to combat and reverse aging have been limited.

The most intuitive manifestation of body aging is skin aging, which is physiological and inevitable, and its specific manifestations are various, including tissue becoming atrophic and fragile, with repair defects, immunodeficiency, and susceptibility to infection [1]. The molecular mechanisms of skin aging include cellular aging theory, decreased cellular DNA repair capacity and telomere loss, extranuclear mitochondrial DNA point mutations, oxidative stress, increased frequency of chromosomal abnormalities, single gene mutations, sugar reduction, and chronic inflammation [2]. Skin aging is influenced by many different factors such as intrinsic and extrinsic factors, while leaving a variety of imprints locally in aging. Intrinsic factors proceed over time and involve all tissues of our

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organism. Exogenous aging, also called photoaging, is caused by the continuous exposure of the skin to environmental factors. Persistent exposure of skin to UV radiation increases DNA damage and mutations and leads to premature aging [3]. Clinically, skin aging often presents with fine lines and wrinkles, loss of elasticity, pigmentary abnormalities, thinning of the epidermis, and increased roughness. Skin aging leads to a series of adverse consequences, making people increasingly need to fight aging.

Because the inevitability of skin aging and skin aging caused by the improvement of people 's living standards have received more attention, it brings a strong market demand and research demand in the field of anti-aging. Stem cells are a newest biological resource that secrete a variety of growth factors that promote tissue regeneration, such as basic fibroblast growth factor, vascular endothelial growth factor, and transforming growth factor β, which are characteristics of stem cells that distinguish them from other cell types [4]. At present, the stem cells studied for the field of anti-aging cosmetic medicine mainly include mesenchymal stem cells, induced pluripotent stem cells and some other novel stem cells [5], of which mesenchymal stem cells are more used, mesenchymal stem cells stimulate angiogenesis, protect other cells from peroxide-mediated damage, and regulate inflammation, pain and immune tolerance [6–8]. Adipose-derived mesenchymal stem cells (ADSCs) are widely used in various types of mesenchymal stem cells. Umbilical cord-derived mesenchymal stem cells not only have the same advantages of mesenchymal stem cells (MSCs) from other sources, but also have more primitive, stronger proliferation ability, are not easy to differentiate after long-term passage, are not easy to produce tumor cells, have a relatively low probability of pathogenic microorganism infection and transmission, and are relatively simple to obtain samples and can be used after ethical approval, which is an ideal method for skin anti-aging. The aim of this study was to observe the aging performance of rats after D-galactose modeling and the appearance of human umbilical cord mesenchymal stem cells (hUCMSC) after subcutaneous injection. To investigate the changes of skin tissue in skin aging and the effect of hUCMSC on preventing skin aging, and to explore the mechanism of hUCMSC in preventing skin aging.

2. Materials and methods

2.1. Isolation, extraction, culture and identification of umbilical cord mesenchymal stem cells

After obtaining approval from the Ethics Committee of the Affiliated Hospital of Guizhou Medical University, informed consent was obtained from pregnant women and their families to collect umbilical cord tissue from healthy fetuses delivered at term, about 15 cm in length, and placed in tissue transfer solution and transferred to the laboratory. The associated containers containing umbilical cord tissue were disinfected and placed in the cell culture room. After disinfection, the umbilical cord tissue was removed and washed repeatedly with PBS to remove the umbilical vessels and umbilical cord adventitia. The umbilical cord tissue was cut into uniform tissue fragments, and the tissue blocks were transferred into 75 cm^2 culture flasks, placed evenly, and 0.5 ml of culture medium was added; put the culture bottle flat in an incubator with 37 °C and 5 % CO2 for continuous culture for 24 h, so that the tissue blocks are laid flat in the culture bottle, and then the culture flasks were placed with the mouth facing upwards. After changing the solution every 3 days, the umbilical cord mesenchymal cells will gradually crawl out of the tissue. When the periphery of the tissue block is fully fused, the tissue block is carefully removed and 0.25 % trypsin solution is added for digestion, so that the cells are detached from the culture flask wall and observed under a microscope. After the digestion is completed, FBS is added to terminate the digestion. Centrifuge at 1500 rpm for 5 min. A 75 cm^2 flask of low-glucose DMEM containing 10 % FBS was added and placed in a 37 °C incubator with 5 % CO_2 to continue the culture. The isolated cells were subcultured to passage 3, and CD29, CD90, CD34, and CD45 were flow identified. Single cell suspension was washed and prepared at a concentration of 2×10^6 cells/ml using PBS.

2.2. Animal experiment

2.2.1. Preparation of natural aging model in rats

Five-week-old male Sprague-Dawley rats (n = 30) weighing 100–150 g were fed adaptively in the animal room for 7 days and equally divided into 3 groups by random number table, with 10 rats in each group. They were raised by the Laboratory Animal Center of Guizhou Medical University. In this experiment, the aging model was prepared by subcutaneous injection of D-galactose, and the specific procedures were as follows: Five-week-old male SD rats were selected, 20, that is, the two groups, and 10 ml/kg of 5 % D-galactose was subcutaneously injected daily on the back of the neck for 42 days. The unmodeled group received daily injections of the corresponding amount of saline into the neck and back for 42 days.

2.2.2. hUCMSCs transplantation

In this study, hUCMSCs suspension was subcutaneously injected at the same time of skin aging model to simulate the effect of human umbilical cord mesenchymal stem cells on delaying skin aging to a greater extent and achieve the purpose of preventing aging. Each rat was shaved weekly on the back, approximately 3×3 cm^2. In the treatment group, 2×10^6 hUCMSCs were injected into the dorsal skin of rats at multiple points on the first day of each week while preparing the aging model, and the same amount of PBS was injected into the unmodeled group and the modeling group for a total of 6 weeks. Forty-two days after the experiment, blood samples were collected from the heart and immediately centrifuged at 4000 r/min for 15 min in a centrifuge, and the upper serum was aspirated with a pipette gun after completion to avoid aspiration to the lower red blood cells and stored in a −80 °C freezer.

The whole skin layer of the back experimental area was taken as the test sample, and the sample was divided into three parts for preservation: 1. paraformaldehyde soaked, paraffin block embedded and sectioned; 2. the sample was placed in mRNA protective solution and stored in a −80 °C freezer; 3. the sample was cut into small pieces and stored in a −80 °C freezer. The specific experimental flow is shown in Fig. 1.

2.3. Results testing

2.3.1. HE staining

The skin tissues were dehydrated in paraffin and fixed in neutral gum. The slides were immersed in hematoxylin staining solution for 3 min and carefully washed with tap water. Immerse in 1 % hydrochloric acid in alcohol for 5 s and wash carefully with tap water. Soak in light ammonia water for 5 s and wash carefully with tap water. They were placed in eosin staining solution for 2 min and carefully washed with tap water. Removal was performed sequentially by passing 80 % alcohol for 3 min, 95 % alcohol for 1 min, 100 % alcohol for 1 min, and dehydration step by step. After clearing with xylene, the sections were mounted again in neutral gum and observed microscopically.

2.3.2. Masson 's staining

Paraffin sections of skin tissue were dehydrated and washed sequentially with tap water and distilled water. Regaud 's hematoxylin stain was used to stain the nuclei for 8 min and washed again with tap water and distilled water. Masson Ponceau red acid fuchsin solution was used for staining for 7 min, washed with 2 % glacial acetic acid aqueous solution for a moment, fully differentiated with 1 % phosphomolybdic acid aqueous solution for 5 min, stained with aniline blue for 5 min, washed with 0.2 % glacial acetic acid aqueous solution for a moment, and then cleared with 95 % alcohol, absolute alcohol, xylene, and blocked and fixed with neutral gum.

2.3.3. PCR assay

Prior to use, absolute ethanol was added first as instructed, homogenization was performed, and the well-ground homogenate was transferred to a DNA-Cleaning Column for centrifugation at 12,000 rpm for 2 min. The supernatant in the collection tube was retained after removing the DNA-CleaningColumn.Add an appropriate amount of Buffer RL2 to the above supernatant and mix appropriately for 7 times.0.7 ml of the mixture was then transferred to an RNA-only Column (12000 rpm for 1 min) and the waste from the collection tube was discarded. The remaining mixture is added to the purification column and centrifuged for sample addition to remove residual Buffer RW2. After transfer to a new centrifuge tube, add 100 μl of RNase-Free ddH2O preheated at 65 °C to the center of the membrane of the purification column and place at room temperature for 2 min. RNA solution was collected after centrifugation. Genomic DNA removal and reverse transcription reactions were recorded. The complete sequences of the genes used in the experiment were searched from the National Center for Biotechnology Information (NCBI) database, and the corresponding primers for each gene were screened using Primer Premier design. All primers were designed and synthesized from Shanghai Sangon Bioengineering Technical Services Co., Ltd., and purified with ULTRAPAGE. Real-time PCR reactions were recorded. The CT (Threshold cycle) values of each sample tested during PCR were analyzed using Thermo Scientific PikoReal software (Thermo). Relative X mRNA expression levels were calculated by 2-△△CT.

2.3.4. Elisa

In a microtiter plate pre-coated with anti-rat Vascular endothelial growth factor (VEGF), Epidermal growth factor(EGF) and Matrix metalloproteinase 1(MMP-1) antibodies (solid phase antibodies), rat VEGF, EGF and MMP-1 calibrators and samples to be tested were added, while another HRP-labeled anti-rat VEGF, EGF and MMP-1 antibody was added. After incubation and washing, the unbound components were removed and a solid phase antibody-antigen-enzyme-labeled antibody complex was formed on the solid phase

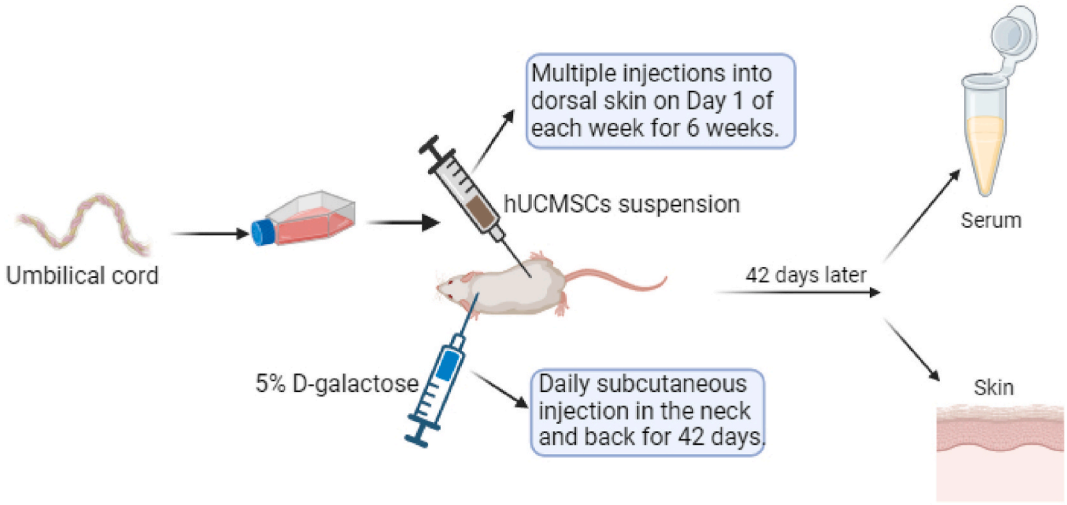

Fig. 1. Rats in the stem cell intervention group were given 5 % D-galactose 10 ml/kg subcutaneously on the back of the neck daily for 42 days to create a skin aging model. At the same time of model establishment, 2×10^6 hUCMSCs suspension was injected subcutaneously into the back at multiple points on the first day of each week for the same period of 6 weeks.

surface of the microplate. With the substrate added, the substrate presents a blue color catalyzed by HRP, which converts to a yellow color after exposure to 2 M sulfuric acid, and the absorbance (OD value) is measured at a wavelength of 450 nm on a microplate reader, which is positively correlated with the concentrations of rat VEGF, EGF, and MMP-1 in the sample to be tested. Calibrator curves were fitted to calculate the concentrations of rat VEGF, EGF, and MMP-1 in the samples, respectively.

2.3.5. MDA activity assay

Approximately 0.1 g of tissue was homogenized in 1 ml of extract at 4 °C. Centrifuge at 12,000 rpm for 10 min, and take the supernatant as the test solution. Allow the reader to warm up for more than 30 min and adjust the wavelength to 450 nm. Prior to determination, place reagents in 25 °C water bath for more than 5 min. Each reagent and sample were added sequentially to a 96-well plate. Mix thoroughly, allow to stand at room temperature (25 °C) for 30 min (accurate time) in the dark, and determine the absorbance of each tube at 450 nm. Malondialdehyde (MDA) activity was calculated.

2.3.6. Immunohistochemistry

The sections were successively placed in xylene I for 15 min, xylene II for 15 min, xylene III for 15 min, absolute ethanol I for 5 min; absolute ethanol II for 5 min, 85 % alcohol for 5 min, 75 % alcohol for 5 min, and distilled water for washing; the sections were immersed in citrate buffer solution (PH 6.0), heated at high fire in a microwave oven for 10 min, ceasefire for 8 min, and reheated at medium and high fire for 10 min; after cooling, PBS was washed three times for 5 min each; endogenous peroxidase was blocked and blocked with serum, the blocking solution was shaken off, PBS was dropped on the sections to prepare the primary antibody in a certain proportion, and the sections were incubated at 4 °C overnight in a wet box; PBS was washed three times for 5 min each time; secondary antibody was dropped, 37 °C for 30 min; PBS was washed three times for 5 min each time; fresh DAB chromogenic solution was prepared, and the color was developed on the tissue, and the color was controlled under a microscope, and the color development time was positive brownish-yellow, and the sections were washed with distilled water to terminate the color; hematoxylin was washed with tap water for 3 min, and finally washed with clean water; Scanning and browsing software was used to collect images of the sections. Each section was observed at low magnification before 200× microscopic images were collected, and a total of 3 sections were collected. Hematoxylin-stained nuclei were blue, and CD31 showed positive expression brownish-yellow.

3. Results

3.1. hUCMSC identification results

After subculturing hUCMSC, cell morphology was observed microscopically to be long spindle-shaped and tightly arranged, as shown in Fig. 2. Identification of hUCMSC surface markers by flow cytometry, as shown in Fig. 3. The experimental results met the identification criteria for hUCMSC.

3.2. Modeling results

The model group and stem cell intervention group were subcutaneously injected with 5 % D-galactose 10 ml/kg daily on the back of the neck for 42 consecutive days, and 42 days later, it was observed that the model group rats showed significant aging manifestations such as skin wrinkles, hair loss and dim coat color compared with the non-model group.

3.3. Skin appearance changes in rats

After D-galactose application, the skin of rats showed wrinkles, dim coat color, and hair loss, compared with the modeling group,

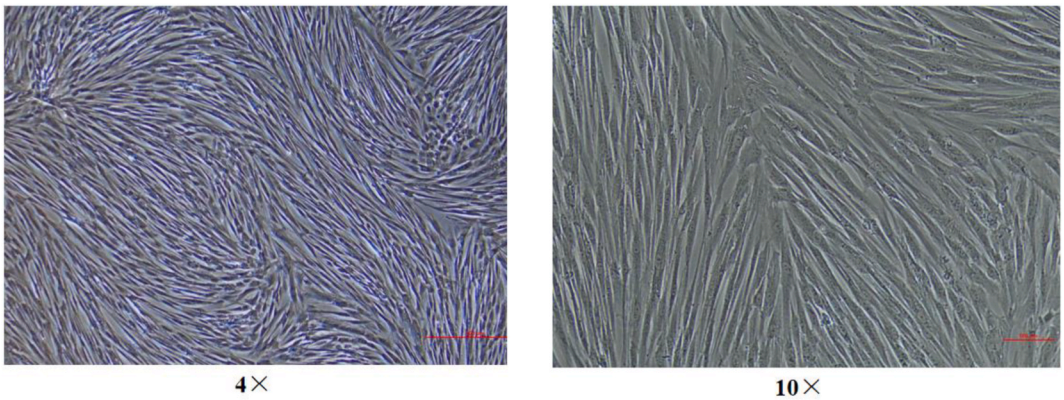

Fig. 2. Fifth generation human umbilical cord mesenchymal stem cells observed under a microscope at different magnifications.

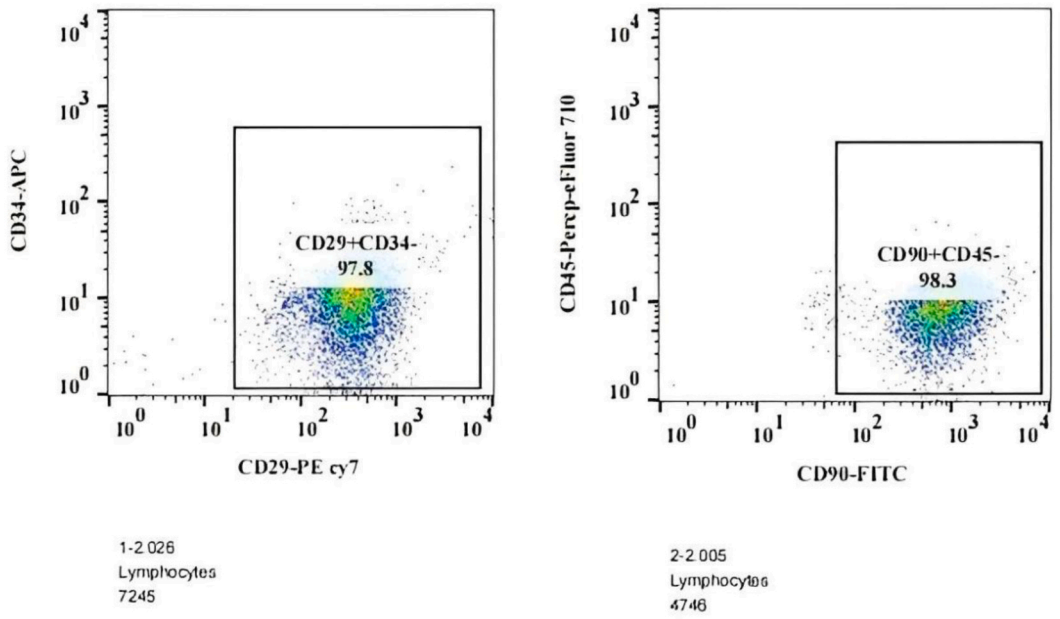

Fig. 3. Cells were identified to express the hUCMSC surface markers CD29 and CD90, but not the hematopoietic stem cell surface marker CD34 and the leukocyte surface marker CD45.

the rats in the hUCMSC intervention group had no obvious wrinkles, and hair loss was rare Fig. 4.

3.4. Effect of hUCMSC on oxidation levels in serum and skin of rats

The MDA levels in the skin and serum tissues of rats in the modeling group were significantly higher than those in the hUCMSC intervention group and the non-modeling group, as shown in Fig. 5.

3.5. Effect of hUCMSC application on rat epidermis

HE staining of the skin of rats in each group showed that the epidermis of rats in the modeling group became thinner significantly, and the epidermis of rats in the hUCMSC intervention group thickened significantly and epidermal proliferation was active, as shown in Fig. 6. The content of EGF in serum skin tissue of rats in each group was significantly higher in the intervention group than in the modeling group as shown in Fig. 7.

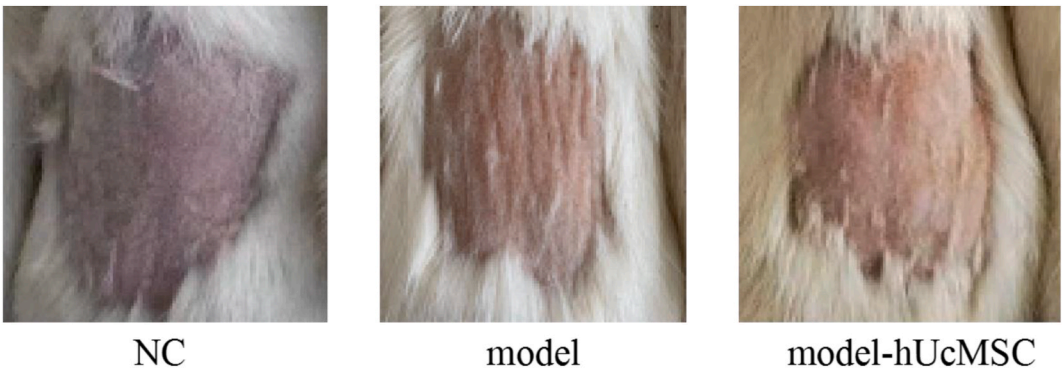

Fig. 4. Rats were shaved on the back every week with a shaved area of about 3 × 3 cm^2. During the experiment, it was observed that the skin of rats showed wrinkles, dim coat color, and hair loss after D-galactose application, and compared with the control group, the rats in the stem cell intervention group had no obvious wrinkles and less hair loss. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

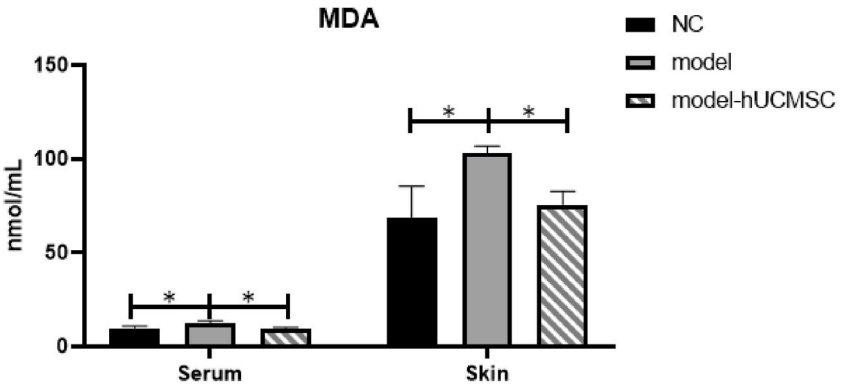

Fig. 5. The MDA levels in the skin and clear tissue of rats in the model group were significantly higher than those in the hUCMSCs intervention group and the non-model group. (n = 3 p < 0.05) .

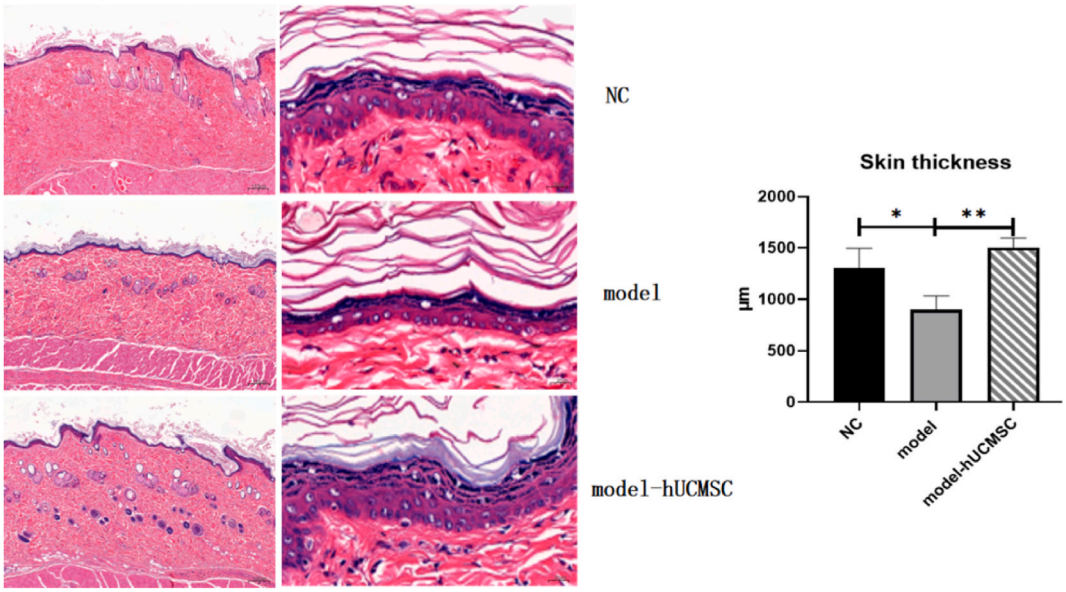

Fig. 6. HE staining of rat skin at different magnifications (10 × ,63 ×) . It could be observed that the epidermis of rats in the stem cell intervention group was thicker than that in the modeling group. At the same time, the epidermal thickness of rats in each group was statistically analyzed, and the results supported that the epidermis of rats in the stem cell intervention group was thicker than that in the modeling group. (n = 3 p < 0.05) .

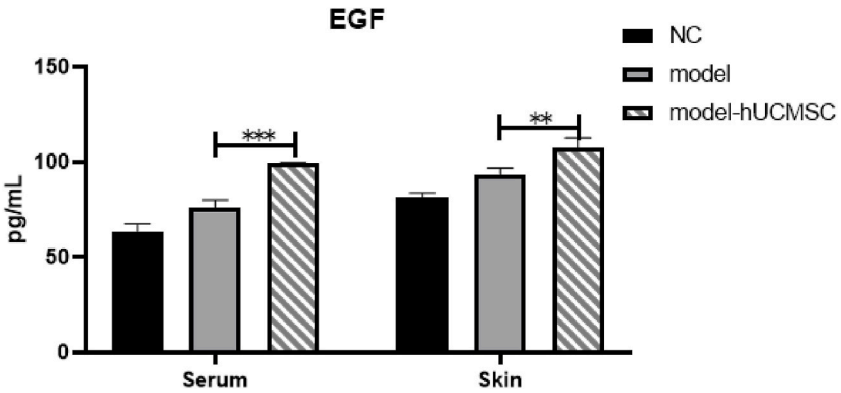

Fig. 7. The content of EGF in serum and skin tissue of rats in each group was significantly higher in the intervention group than in the modeling group.(n = 3 p < 0.05).

3.6. Application of hUCMSC increases collagen synthesis and decreases degradation in rat skin

Masson staining of the skin tissue of rats in each group showed that the dermal collagen of rats in the modeling group was sparse and disorganized, and the collagen of rats in the hUCMSC intervention group was increased compared with the modeling group, as shown in Fig. 8. The results of detecting the expression of type I collagen I mRNA and type III collagen III mRNA in rat skin tissue by PCR showed that the mRNA contents of type I and type III collagen in the intervention group were higher than those in the modeling group, as shown in Fig. 8. The levels of MMP-1 in skin and serum of rats in each group were significantly lower in the hUCMSC intervention group than in the modeling group, as shown in Fig. 8.

3.7. Application of hUCMSC promotes dermal angiogenesis

Masson staining of the skin tissue of rats in each group showed that the thickness of the dermal tissue layer became thinner and the blood vessels decreased in the model group, and the dermis was thicker and the number of blood vessels increased significantly in the intervention group, as shown in Fig. 9. CD31 immunohistochemical staining and microvascular count (MVC) analysis were performed in rats of each group. The counting results indicate that MVC in intervention group is significantly higher than that in model group, as shown in Fig. 10. The VEGF content in the corresponding intervention group was significantly increased compared with the modeling group, as shown in Fig. 11.

4. Discussion

The most intuitive manifestation of body aging is skin aging, which is the largest organ of the human body, the outermost barrier, and the organ with the largest body surface area. It protects the body from physical and chemical damage, invasion by pathogenic microorganisms, and other environmental insults. It also plays an important role in maintaining body temperature and preventing water loss. Skin aging is physiological and inevitable. The manifestations of skin aging are various, mainly manifested as dryness and roughness, laxity, and deepening of wrinkles. Histopathologically, it is mainly characterized by decreased collagen, loss of skin water, diminished epidermal stem cell activity, and dermis disorganization. Aging can lead to skin barrier dysfunction, lead to dry skin, increase the risk of skin diseases, but also increase the risk of skin cancer, delaying skin aging is important for maintaining people 's mental health and normal skin physiological function. Therefore, preventing and delaying skin aging has become one of the hotspots in life science research. However, there is no effective way to delay the process of skin aging from the root cause. In order to meet people 's increasing medical needs and improve the quality of life, how to root cause anti-skin aging is the current research focus, and it is also an important issue in the field of medical cosmetology.

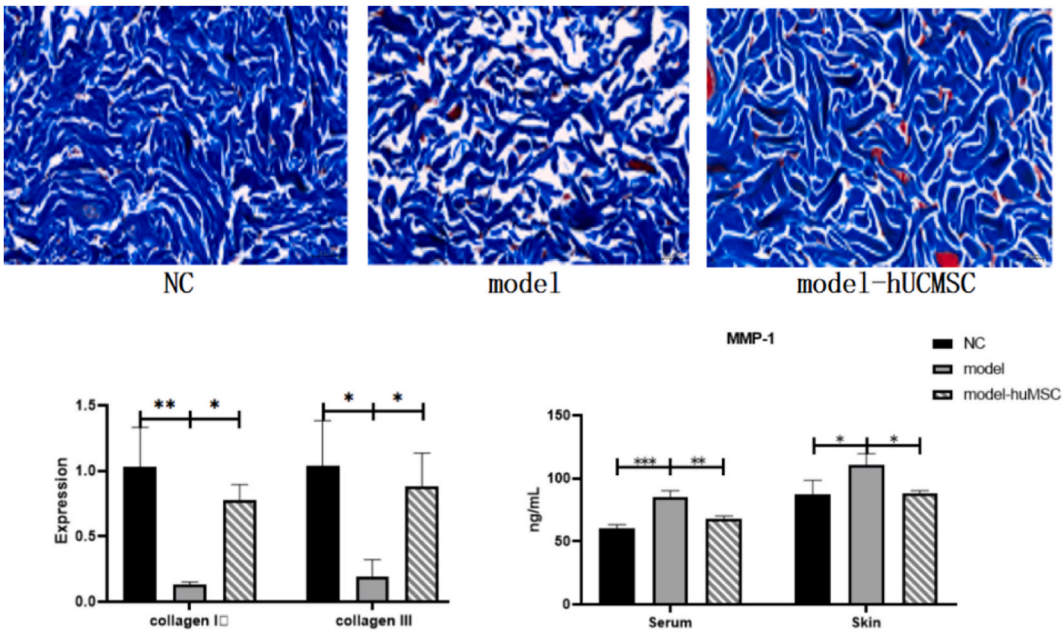

Fig. 8. Masson staining of the skin tissue of rats in each group showed that the collagen of rats in the hUCMSC intervention group was denser than that in the modeling group(20 ×). The expression of type I collagen I mRNA and type III collagen III mRNA in rat skin tissue was detected by PCR. The results showed that the mRNA contents of type I and type III collagen in the intervention group were higher than those in the modeling group. The levels of MMP-1 in skin and serum of rats in each group were significantly lower in the hUCMSC intervention group than in the modeling group. ($n = 3$ $p < 0.05$).

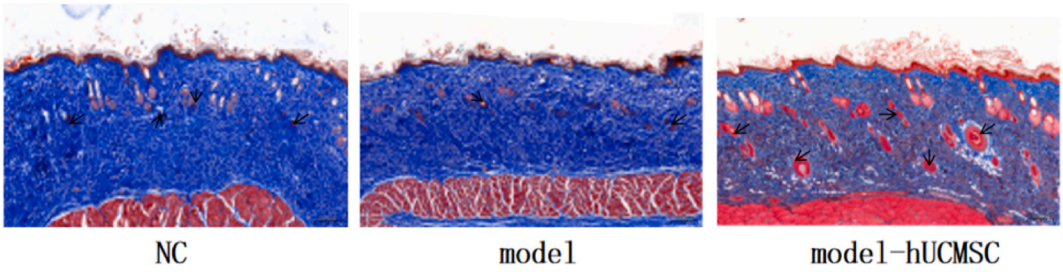

Fig. 9. Masson staining of skin tissue of rats in each group showed that the dermal vessels of rats in the stem cell intervention group were densely distributed compared with those in the modeling group. (5 ×).

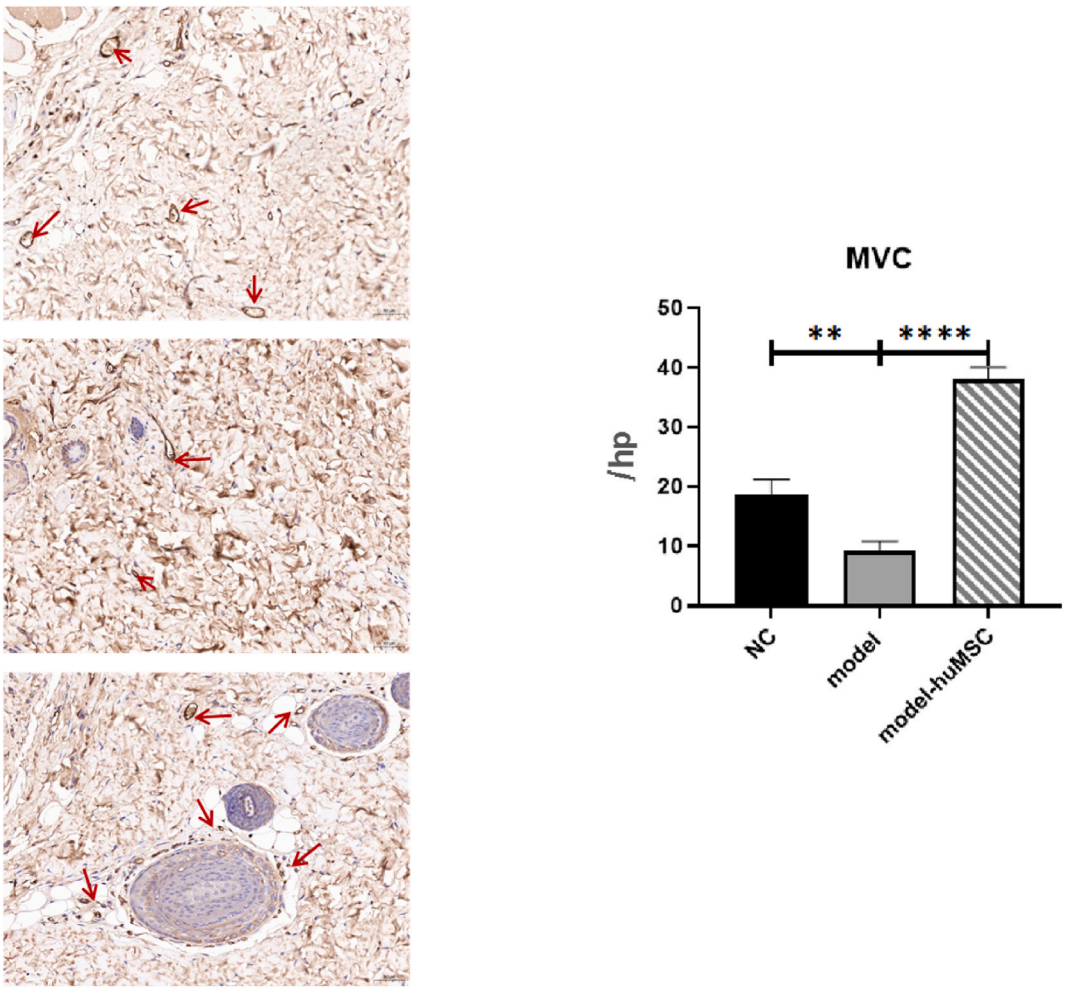

Fig. 10. Immuno-organization of CD31 in skin tissue, CD31 showed positive expression brownish-yellow. The number of microvessels per image was counted. The results showed that the number of microvessels in the stem cell intervention group was significantly higher than that in the modeling group.(9n = 3 $p < 0.05$). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

4.1. Analysis of experimental results

D-galactose-induced subacute aging rat model, as a simple, easy and economical animal model preparation method, has been widely used for organ aging research and drug testing [9]. Prolonged subcutaneous injection of low doses of D-galactose can lead to increased intracellular galactose concentrations in the body. It is reduced to galactinol under the catalysis of aldose reductase, which

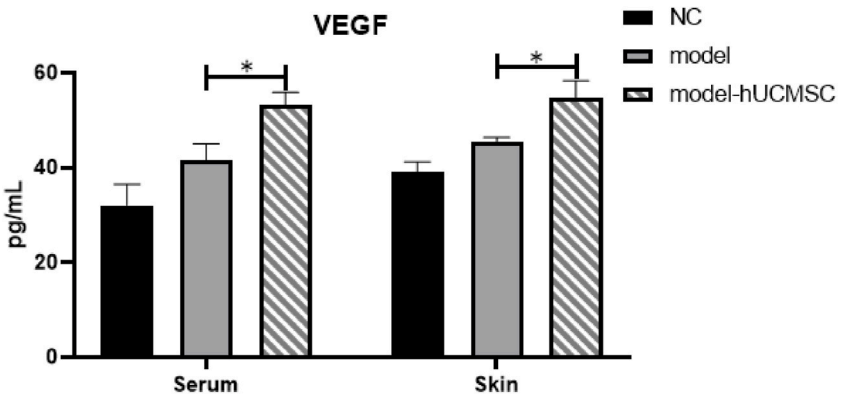

Fig. 11. The VEGF content in the corresponding intervention group was significantly higher than that in the modeling group($n = 3$ $p < 0.05$).

cannot be further metabolized by cells and accumulates in cells, which leads to cell swelling, dysfunction, metabolic disorders, and the formation of excessive superoxide anion radicals. Free radical overproduction is the increase of reactive oxygen species synthesis, the addition of reactive oxygen species to the double bonds of bases destroys bases to generate pyrimidine and purine free radicals, and the alkali free radicals bind to each other or are peroxidized, causing base loss or even main chain breakage and producing genetic mutations [10]. Therefore, in this study, we used subcutaneous injection of D-galactose to create a skin aging model. And after modeling, the related symptoms of skin aging were observed, such as skin wrinkles, hair loss, skin thinning, collagen reduction and so on. It further suggests that it is feasible to make skin aging model by injecting D-hemisugar.

Reactive oxygen species (ROS) are important causes of aging [11]. Aging induces a large number of cellular disturbances within cells, such as elevated ROS, including free radical and non-free radical oxygen-containing molecules, which are thought to play a key role in extracellular matrix alterations in skin with intrinsic aging and photo-aging. Reactive oxygen species can be produced from different sources, including mitochondrial electron transport chain, peroxisomal and endoplasmic reticulum localized proteins, and Fenton reaction [11]. Excess ROS affects cellular metabolic regulation which in turn leads to cellular damage or dysfunction. During aerobic metabolism, reactive oxygen species are continuously produced in the electron transport chain of mitochondria as by-products, and the massive production of superoxide anion may impair cellular function and lead to cellular senescence [12]. At higher concentrations, ROS can modify macromolecules such as proteins, lipids, and nucleic acids, making them toxic to cells and even inducing DNA damage [13]). Our study observed that MDA levels were significantly lower in the stem cell intervention group than in the control group, which suggests that subcutaneous injection of hUCMSC can improve skin antioxidant capacity. Reduce oxidative damage due to skin aging.

Epidermal stem cells (EpdSCs) are located in the innermost layer (basal layer) of the skin epidermis. Basement membranes rich in extracellular matrix (ECM) proteins and growth factors are located at the epidermal-dermal boundary and are mainly produced and secreted by EpdSCs.In adult skin, EpdSCs divide parallel to the basement membrane. When their proliferating progeny committed to terminal differentiation, they left the basal layer and moved outwards, undergoing three morphologically and biochemically distinct stages: spinous cells, granular cells, and stratum corneum cells. Keratinocytes are barriers between the body and the external environment. These cells are continuously detached from the skin surface and replaced by outwardly moving inner cells. In coordination with EpdSCs, the balance between proliferation and differentiation must be finely controlled to maintain balance and maintain skin barrier regeneration. The ultimate goal is to allow EpdSCs to fine-tune their proliferation in the basal layer while producing a constant flow of terminally differentiated protective cells that maintain homeostasis while creating a brand-new skin barrier every few weeks. Therefore, the epidermis is in a state of continuous proliferation, differentiation, and apoptosis.

With aging, ESC are excessively depleted and no longer maintain epidermal tissue homeostasis and repair damaged tissue [14,15]. In the skin, the proliferation capacity of stem cells is also reduced, which can be demonstrated by thinning of the epidermis and reduced hair regeneration capacity during homeostasis [16]. In our study, HE staining observed that the epidermis of aging model rats became significantly thinner, while the thickness of epidermis of subcutaneous hUCMSC rats was close to that of blank group, and epidermal cell proliferation was active. Epidermal growth factor was also detected to be significantly increased in the intervention group compared with the control group.

The connective tissue of the skin is mainly composed of collagen and elastin. Collagen accounts for 70 %–80 % of dry skin weight and confers mechanical strength and structural integrity. Collagen is involved in the strength, elasticity, and stability of the skin [17]. Degradation of collagen and elastin results in loss of strength and elasticity of the skin and is clinically characterized by wrinkles and ptosis [18]. In naturally aging skin, the amount of dermal collagen decreases and elastin accumulates structurally abnormally [19]. In this study, Masson staining of rat epidermis showed that the collagen content in the dermis of aging model rats was reduced, and showed structural abnormalities. However, the collagen content in the dermis of rats injected with hUCMSC increased significantly compared with the model group. Corresponding to the Masson staining results, the PCR results suggested that the expression of type I and type II collagen mRNA in the skin tissue of rats treated with hUCMSC was significantly increased compared with the aging model group. Furthermore, subcutaneous injection of hUCMSC decreased levels of matrix metalloproteinases.Matrix metalloproteinases, a class of proteolytic enzymes, are considered to be the main physiological cause of extracellular matrix protein breakdown in skin. In

aged skin and aging fibroblasts, expression and activity of matrix metalloproteinases are increased, whereas expression of tissue inhibitors of metalloproteinases is decreased [20]. It can be concluded that hUCMSC can not only increase collagen production, but also reduce collagen degradation, and play a role in preventing skin aging by increasing collagen content in the dermis and improving skin mechanical strength and structural integrity.

Age-related changes, as well as those associated with disease progression, usually first appear in the cutaneous circulation. In addition, impaired vascular signaling and consequent endothelial dysfunction are the earliest indicators of cardiovascular pathogenesis and occur in multiple tissue beds throughout the body, including the skin, in a similar manner. Because microvascular dysfunction predicts long-term outcomes and adverse cardiovascular events better than macrovascular disease, it is important to understand age-related changes in cutaneous microcirculatory control in humans [21]. Because the number of dermal vessels decreases, the cutaneous blood supply decreases significantly. This is caused by endothelial dysfunction, including reduced angiogenic capacity, abnormal expression of adhesion molecules, and impaired vasodilation [22]. In this study, it was observed that subcutaneous injection of hUCMSC could increase the number of dermal microvessels, and the corresponding CD31 immunohistochemical results suggested that CD31 expression in the skin of rats in the hUCMSC intervention group was significantly higher than that in the modeling group. The counting results indicate that MVC in intervention group is significantly higher than that in model group. At the same time, Elisa detection of skin tissue and blood showed that hUCMSC could significantly increase the content of vascular endothelial growth factor. Therefore, we believe that hUCMSC can promote angiogenesis in the dermis and increase the number of blood vessels, which will bring better blood circulation to the skin and promote local metabolism, thus playing a role in preventing aging.

4.2. Insufficient for this study

Aging factors include endogenous and exogenous factors. Intrinsic factors proceed over time and involve all tissues of our organism. Intrinsic aging is characterized primarily by non-exposed skin features such as wrinkles, skin drooping, and dryness, which may be caused by hormonal changes, estrogen, and androgens. Exogenous aging, also called photoaging, is caused by the continuous exposure of the skin to environmental factors. In this study, D-galactose subcutaneous injection was used to create skin aging effect, which could not completely simulate natural skin aging, especially skin photoaging. This study demonstrated that hUCMSC can delay skin aging by increasing epidermal thickness, collagen content, and dermal vascular mass. However, there are few explanations for how hUCMSC causes these changes, which still need to be further explored.

4.3. Future prospects for skin anti-aging

Skin is the carrier of direct contact between the human body and the external environment. As the first line of defense against external harmful factors, the human body has to face the challenges of various physical and chemical and biological damage factors from endogenous and exogenous sources throughout its life. Skin aging, on the one hand, will make it decline in the body protection ability for individuals, and ultimately cause disease; on the other hand, more and more people are now concerned about the impact of skin aging on personal appearance, including skin laxity, wrinkles, pigmentation, etc; these factors bring about a research boom in the field of skin anti-aging. At present, there are various skin anti-aging treatment methods used in clinical practice, such as: injection, filling, physical suspension and so on. But more of it is to live in the appearance to alleviate the symptoms of aging. And there is a lack of anti-aging treatment from the mechanism of aging, and this study will be an important research process for us to break through anti-aging treatment. This study demonstrated that hUCMSC had a significant effect on delaying skin aging. However, there is still a huge exploration space for its specific anti-aging mechanism of action. And there is increasing evidence that MSC exosomes have anti-inflammatory and immunomodulatory properties similar to MSC. Its safety, effectiveness and operability have more advantages. Further in-depth study of aging and anti-aging mechanisms will take us to further achieve anti-aging results. The advantages of hUCMSC in skin anti-aging make them have a very strong research and application prospect in the field of anti-aging 。Both stem cells themselves and their derivatives will have a very considerable clinical application prospect.

5. Conclusion

5.1 hUCMSC has the effect of preventing skin aging, which is reflected in the fact that hUCMSC can reduce skin wrinkles, hair loss, increase epidermal thickness and collagen content, and increase dermal vascular mass in rats.

5.2 The mechanisms of hUCMSC in preventing skin aging include promoting the proliferation and differentiation of epidermal stem cells, promoting collagen synthesis and inhibiting collagen breakdown, reducing oxidative stress, and secreting growth factors.

Data availability statement

The [DATA TYPE] data used to support the findings of this study are included within the supplementary information file(s).

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CRediT authorship contribution statement

Juan Xiong: Writing – original draft, Software, Project administration, Methodology, Formal analysis, Data curation, Conceptualization. **Fan Wang:** Visualization, Project administration, Methodology, Formal analysis. **Yutong Yang:** Visualization, Methodology, Data curation. **Yuxi Yang:** Methodology, Data curation. **Zhongshan Liu:** Writing – review & editing, Validation, Supervision, Methodology, Funding acquisition, Formal analysis, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e24342.

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