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Original Research

Effects of Sepsis Serum on the Fate of Adipose Tissue-Derived Stem Cells

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Abstract

Background: Adipose tissue-derived stem cells (ADSCs), a type of mesenchymal stem cell, have been used extensively in clinical trials for the treatment of multiple conditions, including sepsis. However, increasing evidence indicates that ADSCs vanish from tissues within days of administration. Consequently, it would be desirable to establish the mechanisms underlying the fate of ADSCs following transplantation. **Methods**: In this study, sepsis serum from mouse models was used to mimic microenvironmental effects. Healthy donor-derived human ADSCs were cultured *in vitro* in the presence of mouse serum from normal or lipopolysaccharide (LPS)-induced sepsis models for the purposes of discriminant analysis. The effects of sepsis serum on ADSC surface markers and cell differentiation were analyzed by flow cytometry, and the proliferation of ADSCs was assessed using a Cell Counting Kit-8 (CCK-8) assay. Quantitative real-time PCR (qRT-PCR) was applied to assess the degree of ADSC differentiation. The effects of sepsis serum on the cytokine release and migration of ADSCs were determined based on ELISA and Transwell assays, respectively, and ADSC senescence was assessed by β-galactosidase staining and western blotting. Furthermore, we performed metabolic profiling to determine the rates of extracellular acidification and oxidative phosphorylation and the production of adenosine triphosphate and reactive oxygen species. **Results**: We found that sepsis serum enhanced the cytokine and growth factor secretion and migratory capacities of ADSCs. Moreover, the metabolic pattern of these cells was reprogrammed to a more activated oxidative phosphorylation stage, leading to an increase in osteoblastic differentiation capacity and reductions in adipogenesis and chondrogenesis. **Conclusions**: Our findings in this study reveal that a septic microenvironment can regulate the fate of ADSCs.

Keywords: adipose tissue-derived stem cell; sepsis; cytokine; migration; differentiation; metabolic pattern

1. Introduction

Sepsis is a frequent complication in hospitalized patients characterized by life-threatening organ dysfunctions in response to microbial infection. Annually, some 50 million people worldwide will experience sepsis, among whom at least 11 million will die [1–3]. Most types of microorganism, including bacteria, fungi, viruses, and parasites, can cause sepsis [4], which is the final common pathway leading to death from most infectious diseases and is becoming more prevalent [5]. This increase in prevalence could be ascribed to multiple factors, among which, the global aging population, evolution of drug-resistant pathogens, and worldwide spread viral infections, such as SARS-CoV-2/COVID-19, are often cited [6–8].

Despite considerable research efforts, there have been no therapies developed that directly modify the pathophysiology and injury mechanisms underlying sepsis [4,9,10]. During the past two decades, most sepsis-related studies have focused on suppressing the early proinflammatory response to sepsis. To date, however, there have been more than 40 unsuccessful clinical trials of agents that have been optimistically proposed to reduce pathogen recognition or block proinflammatory cytokines or inflammation-signaling pathways [11,12]. In spite of these failures, however, a number of key insights have accrued in recent years from studies that have evaluated the therapeutic effects mesenchymal stem/stromal cells (MSCs) in attenuating proinflammatory responses whilst enhancing host immune function and the reparative capacity of tissues [13,14]. The findings of multiple preliminary studies from different groups have demonstrated the potential application of MSC therapy in murine models, albeit with varying degrees of efficacy. Plausible factors that might account for these discrepant findings include the use of non-homogenized MSC populations, different donors, different tissue sources, and different passages during *in vitro* ex-

pansion. MSC engraftment to the site of injury and their retention there, at least for a short period, is required for efficacy in preclinical sepsis models [15–17]. Moreover, the findings of recent studies have also indicated that the cell microenvironment can contribute to regulating the fate of MSCs [18–21], which effectively means that the same or different batches of MSCs may have differing effects when transplanted into patients at different stages of the disease. These complex issues accordingly warrant further in-depth study to provide a basis for more effective personalized stem cell therapy.

Adipose tissue-derived stem cells (ADSCs) are a type of MSCs that have the capacity of self-renewal and can be induced to develop into osteoblasts, adipocytes, and chondrocytes [22,23]. Compared with bone marrow-derived MSCS, ADSCs have multiple unique advantages, including the greater ease of access and harvesting, and their use is less constrained by ethical considerations [24]. Given these advantages, ADSCs have been used extensively in clinical trials to evaluate their utility in the treatment of conditions such as diabetes mellitus, liver disease, corneal lesions, and sepsis. The secretory profile of ADSCs includes a wide range of cytokines, chemokines, and growth factors [25]. Studies have revealed that on the basis of their paracrine effects, ADSCs can stimulate angiogenesis, exert antiapoptotic effects, and recruit other cells to the sites of injury [23]. However, there are few studies on the role of pathological environment on MSCs' function. In the present study, we exposed human ADSCs to sepsis serum to assess the regulation of the fate of these cells.

2. Materials and Methods

2.1 Human Adipose Tissue-Derived Stem Cell Isolation and Culture

Samples of human adipose tissue were collected from the abdominal wall of healthy donors (20–35 years old healthy females with standard body mass index, no history of diabetes or other metabolic diseases) undergoing cosmetic surgery, using a protocol approved by the Internal Review Board of Zhejiang Provincial People's Hospital. The samples were washed with pre-cooled phosphate-buffered saline (PBS) containing a 1% antibiotic–antimycotic solution (Beyotime, Shanghai, China), diced into small pieces using surgical blades, and then digested with 0.2% Type I collagenase (Sigma, St Louis, MO, USA) in low-glucose Dulbecco's modified Eagle's medium (DMEM-LG; Cellgro, Herndon, VA, USA) at 37 °C shaking at 100 rpm. Cell suspensions were harvested after incubating for 30 min, filtered through an 80-μm mesh, and centrifuged for 10 min at 250 $\times$ g. The cell pellets thus obtained were washed twice with pre-cooled PBS, resuspended with DMEM-LG supplemented with 10% fetal bovine serum (FBS: Gibco), and seeded in 10-cm tissue culture-treated Petri dishes (Sigma-Aldrich, St Louis, MO, USA) at a density of 1×10^3 cells/cm^2. The cultures were incubated at 37 °C in a 5% CO_2 atmosphere at 95% humidity. Non-adherent cells were removed by renewing the medium after 24 h, and the adherent cells were thereafter continuously cultured.

Sterile cloning cylinders and 0.25% trypsin were subsequently used to obtain single colony-derived ADSC populations. Care was taken to select well-separated colonies, thereby ensuring that each cloning cylinder contained only a single colony. The isolated ADSC colonies were then cultured in the aforementioned growth medium and passaged via 0.25% trypsin-EDTA digestion at a 1:5 ratio. Cells of passage five were used in subsequent experiments.

2.2 Preparation of Sepsis Serum

Eight-week-old Balb/c mice were purchased from Shanghai SLAC Laboratory Animal Co., Ltd. and maintained in the specific pathogen-free laboratory of a local animal facility. The experimental protocol was approved by the local medical animal experiment ethics committee (ZJCLA, No. ZJCLA-IACUC-20110043). Lipopolysaccharide-induced sepsis was stimulated as described by other groups [26]. Briefly, lipopolysaccharide (5 mg/kg dosage) was intraperitoneally injected into mice to induce experimental conditions, and survival was recorded at 2 and 20 h post-administration. Thereafter, surviving mice were anesthetized, and blood was collected from the retro-orbital venous plexus. Serum samples were harvested, mixed, and stored at –80 °C. Prior to use for cell culture, the mouse sepsis serum was thawed, added to the DMEM-LG, and passed through a 0.22-μm filter.

2.3 Characterization of ADSCs

The phenotype of ADSCs was examined based on flow cytometric analysis. Briefly, cells were trypsinized, washed, and incubated for 1 h at room temperature with appropriate dilutions of the following fluorescently conjugated antibodies (BD Bioscience, San Jose, CA, USA): anti-CD73 (550257), anti-CD90 (555596), anti-CD105 (560839), anti-CD14 (555397), anti-CD19 (555413), anti-CD34 (555822), anti-CD45 (555483), anti-HLA-DR (555811). Corresponding isotype-matched antibodies were used as the negative controls. Cells were washed twice with PBS and analyzed using a Becton Dickinson Calibur flow cytometer (BD, East Rutherford, NJ, USA).

To evaluate the multilineage differentiation capacity of ADSCs, osteogenic, adipogenic, and chondrogenic inductions were assessed *in vitro* as previously described [27]. Briefly, ADSCs derived from Stromal Vascular Fraction (SVF) were cultured in α-MEM (Invitrogen, Carlsbad, CA, USA) medium containing 10% FBS, 10^{-7} M dexamethasone, 10 mM β-glycerol phosphate, and 50 μM ascorbate-2-phosphate for osteogenic differentiation and stained with Alizarin Red S. ADSCs were incubated for 2 weeks in an adipogenic induction medium (10^{-6} M dexamethasone, 0.5 μM isobutylmethylxanthine, and 10 ng/mL insulin) for adipogenic differentiation, fixed, and stained with Oil Red O

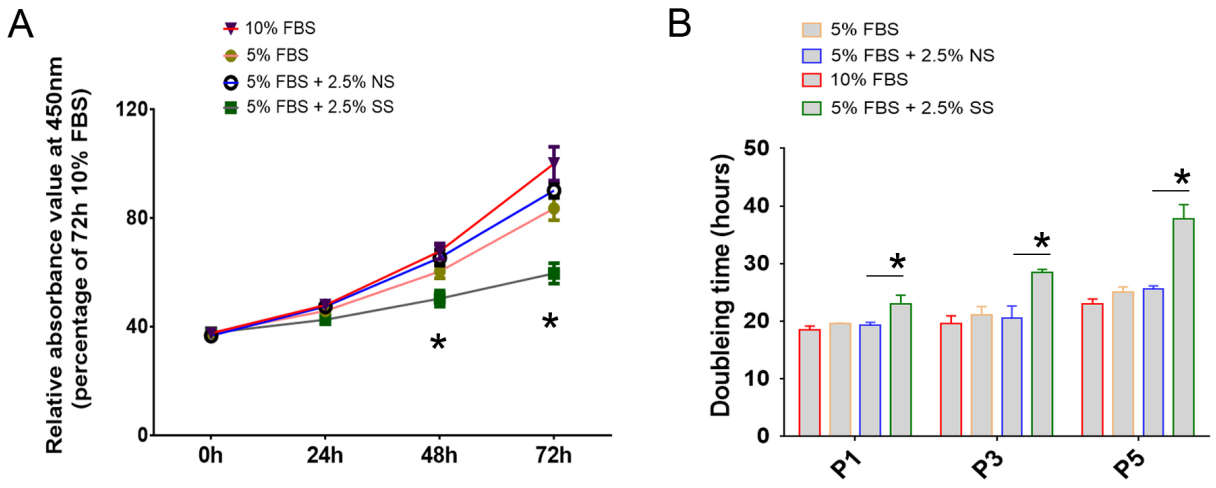

Fig. 1. Sepsis serum treatment inhibit cell proliferation of ADSCs. (A) Cell proliferation was assessed using CCK-8 assays. (B) The population doubling time of various passage ADSCs with different culture mediums. Data are presented as the mean of four experiments. * $p < 0.05$.

solution (Cyagen, Santa Clara, CA, USA). ADSCs were centrifuged to form a pelleted micromass and maintained in high-glucose DMEM containing 10^{-7} M dexamethasone, 1% insulin–transferrin–sodium selenite, 50 μM ascorbate-2-phosphate, 1 mM sodium pyruvate, 50 μg/mL proline, and 20 ng/mL TGF-β3 for chondrogenic differentiation and stained with Alcian blue.

2.4 Cell Growth Analysis

Cell proliferation assays were conducted using a Cell Counting Kit-8 (CCK-8) cell viability kit (Yeasen) according to the manufacturer's protocol. Briefly, ADSCs at the log-phase of growth were trypsinized, resuspended, and seeded at a density of 2×10^3 cells/well in 96-well plates, to which 10 μL of CCK-8 reagent was added at the indicated time points. Following incubation for 1 h at 37 °C, the absorbance the well contents was measured at 450 nm using a Synergy H1 microplate reader (BioTek, Winooski, VT, USA). For population doubling time analysis, 1×10^5 cells were seeded in 35-mm dishes (Corning Incorporated, Corning, NY, USA) and cultured for 120 h. The medium was changed at 2-day intervals, and the number of viable cells in each plate was estimated based on hemocytometer counts. The population doubling time after 120 h was determined using the software (http://www.doubling-time.com/ Doubling Time, Mathieu, France).

2.5 Quantitative Real-Time Polymerase Chain Reaction

Total RNA was extracted using TRIzol (Sigma, St Louis, MO,USA) using a standard protocol and quantified using a NanoDrop™ 2000 spectrophotometer. Reverse transcription reactions were performed using a high-capacity cDNA reverse transcription kit (Applied Biosystems, Waltham, MA, USA) according to the manufacturer's instructions. Quantitative real-time PCR (qRT-PCR) reactions were conducted using gene-specific primers (**Supplementary Table 1**) in an Applied Biosystems 7900 thermocycler (Waltham, MA, USA).

2.6 Cell Migration Assay

For an analysis of cell migration, we used Boyden's chambers (Transwell Assay) containing Transwell inserts of pore size 4.5 μm. Briefly, ADSCs at the log-phase of growth were harvested, resuspended in DMEM-LG supplemented with 1% FBS, and seeded at 2×10^4 cells in the upper chamber of the Transwell apparatus. To the lower chamber, we added 2 mL of DMEM-LG containing 10% FBS to create a chemotactic gradient. The quantification of migrated cells was performed after incubating for 18 h. The transwell insert from the plate was removed and a cotton-tipped applicator was used carefully to remove the media and remaining cells on the top of the membrane without damaging it. Then, the transwell insert was placed in methanol for 10 minutes to allow cell fixation and stain with 0.2% crystal violet for 5 minutes. Images were captured under an inverted light microscope. The number of migrating cells in different fields of view was counted and the mean value was calculated.

2.7 Quantification of Cytokine Release

ADSCs were exposed to either 5% FBS + 2.5% normal mouse serum (NS) or 5% FBS + 2.5% sepsis mouse serum (SS) media for 72 h. Then, the cells were harvested and seeded on 96-well plates at a density of 6700 cells/well. Cells were cultured for 48 h in 200 μL DMEM-LG 5% FBS/well before the culture medium was collected. To determine the levels of cytokines released into culture supernatants, we used a Quantikine® QuicKitTM Enzyme-linked immunosorbent assay (ELISA) kit (R&D Systems) according to the manufacturer's instructions, with

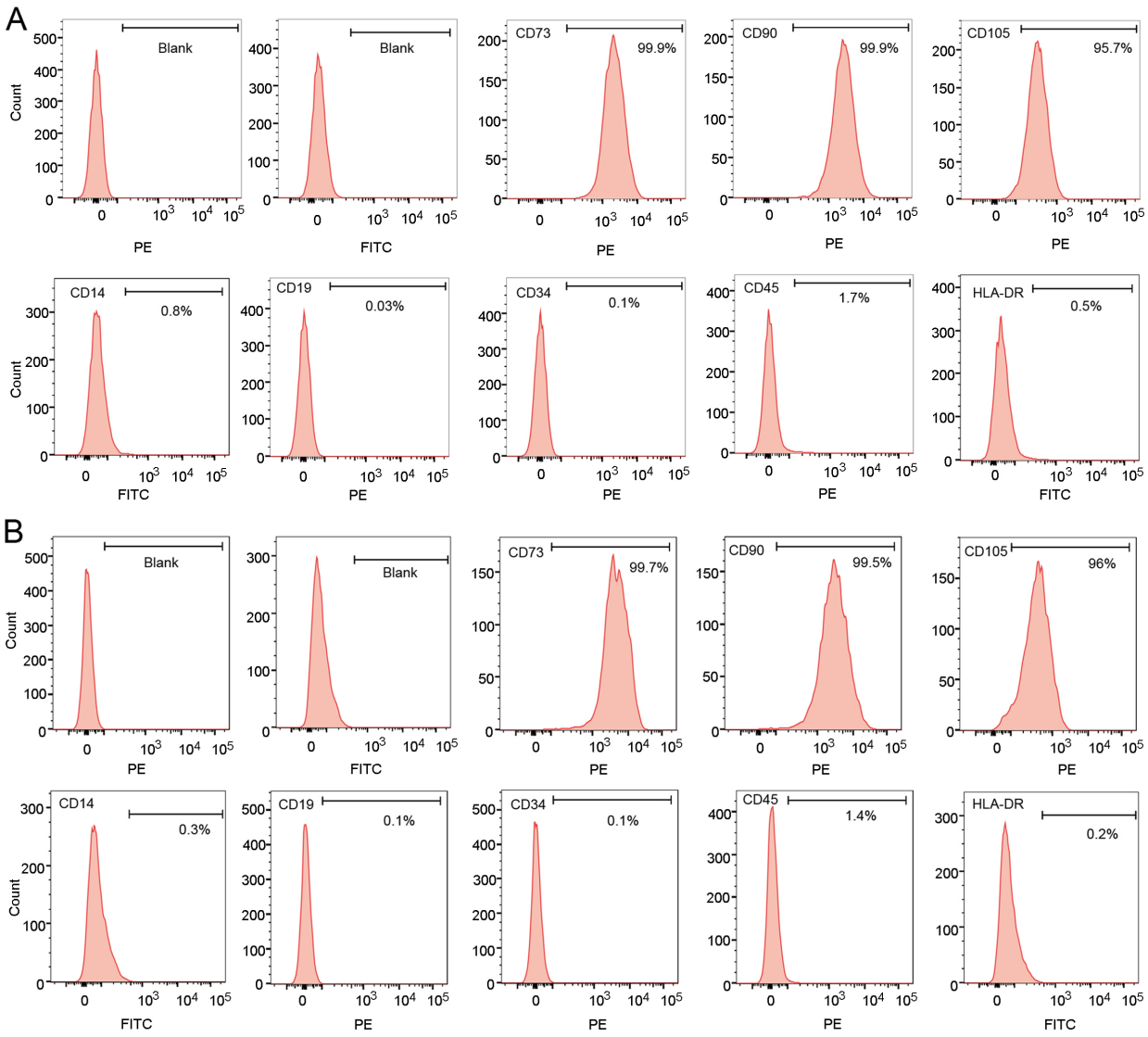

Fig. 2. Representative flow cytometry analysis of cell surface markers in ADSCs. (A) ADSCs cultured in DMEM-LG supplemented with 5% FBS + 2.5% NS. (B) ADSCs cultured in DMEM-LG supplemented with 5% FBS + 2.5% SS.

absorbance being measured at 450 nm using a SpectraMax Plus384 microplate reader (Molecular Devices, Wokingham, UK).

2.8 Western Blot Analysis

Whole-cell lysates were run on 10% polyacrylamide gels and the separated proteins were transferred to Hybond-P membranes. The membranes were blocked with 5% skim milk and immunoblotted overnight at 4 °C with the following primary antibodies: P53 (Beyotime, Shanghai, China; AF1162), P21 (Beyotime, Shanghai, China; AP021) and Lamin B (Proteintech, Rosemont, IL, USA; 12987-1). As a loading control, we used Rabbit anti-human GAPDH (Beyotime, Shanghai, China; AF1186). Following primary antibody incubation, the membranes were incubated with appropriate secondary antibodies for 1 h at room temperature, and were subsequently developed using an ECL Chemiluminescence Kit, with quantification being performed using ImageJ software 2.0 (NIH, Bethesda, MD, USA).

2.9 Measurement of Extracellular Acidification and Oxidative Phosphorylation

Changes in the rates of extracellular acidification (ECAR) and oxygen consumption (OCR) in ADSCs were determined in real-time using a Seahorse XF96 Flux Analyzer (Seahorse, Agilent, MA, USA) in conjunction with a Seahorse XF Glycolytic Rate Assay Kit (Seahorse Bioscience, USA) and a Seahorse XF Mitochondrial Respiration Assay Kit (Seahorse Bioscience, USA), respectively, following the manufacturers' instructions.

2.10 Measurement of the Adenosine Triphosphate and Reactive Oxygen Species

The levels of cellular ATP and reactive oxygen species (ROS) produced by ADSCs were measured using standard

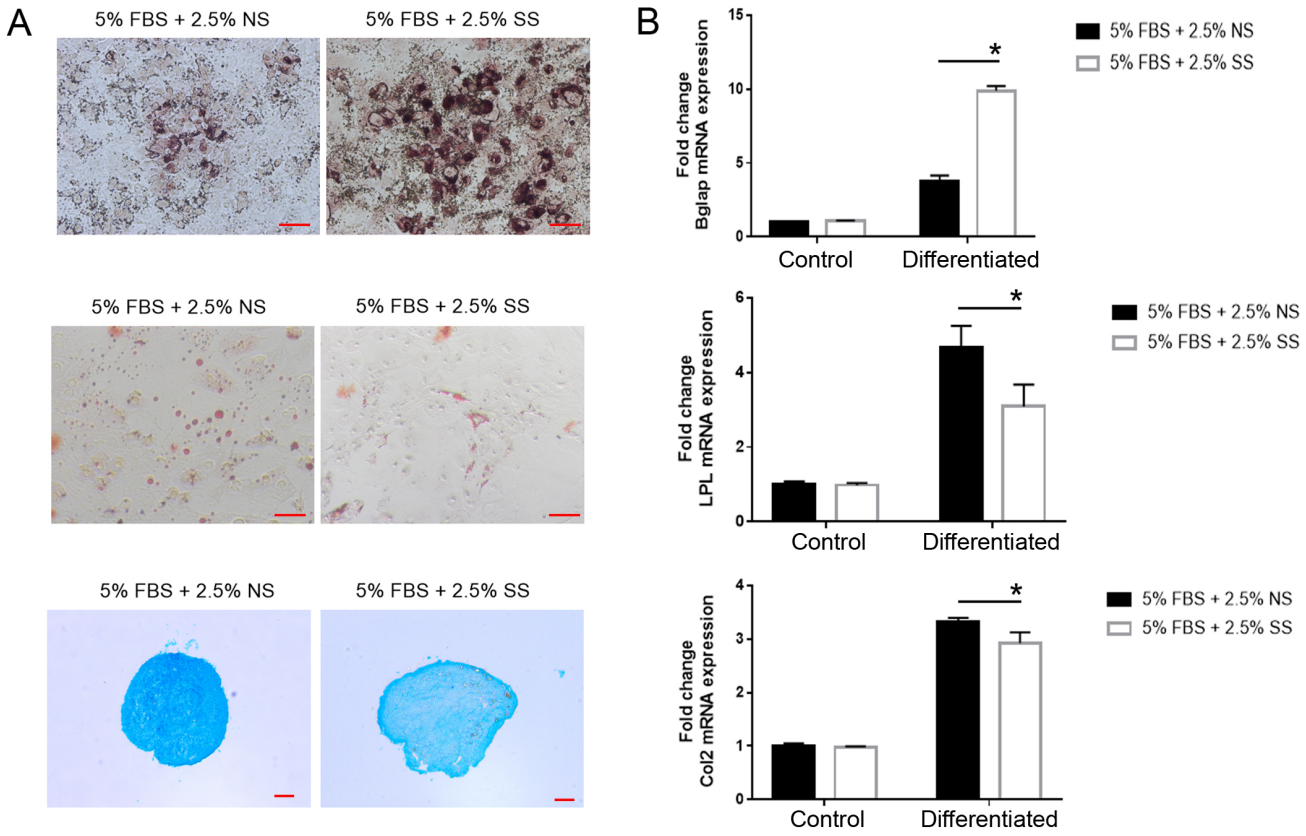

Fig. 3. Sepsis serum treatment influence the differentiation bias of ADSCs. (A) ADSCs cultured in 5% FBS + 2.5% NS and 5% FBS + 2.5% SS were induced to differentiate along adipogenic, osteogenic, and chondrogenic lineages. (B) The differentiation degrees were evaluated by qRT-PCR analysis of lineage marker gene expression. GAPDH was used as a housekeeping gene. All measures were performed in triplicate. * $p < 0.05$.

assay Kits purchased from Beyotime according to the manufacturer's instructions. The data presented are the average of three independent experiments.

2.11 Cell Senescence Assessment

ADSC senescence was determined microscopically based on β-galactosidase staining using a dedicated detection kit (Cell Signal Technology, VIC, AUS).

2.12 Statistical Analysis

Statistical analyses were performed using GraphPad Prism 6.0 (GraphPad software, San Diego, CA, USA). Data are presented as the means $\pm$ standard deviations [28] of the results of at least three independent replicates. Student's *t*-tests were used to identify differences between groups. *p*-values < 0.05 were considered to be statistically significant.

3. Results

3.1 Effect of Sepsis Serum on the Growth of ADSCs

To investigate the effects of sepsis serum on ADSCs, we monitored the growth of these cells. Rapidly growing passage 5 ADSCs were harvested and seeded for one of the following four treatments: standard culture (10% FBS), 5% FBS culture, 5% FBS + 2.5% NS, and 5% FBS + 2.5% SS. CCK-8 analysis indicated that among these culture conditions, the 10% FBS standard culture was the most effective in terms of supporting ADSC growth. Compared with the 5% FBS medium, the addition of normal mouse serum was found to enhance cell growth, whereas the addition of mouse sepsis serum had the opposite effect, with significantly inhibited ADSC growth being detected at 48 and 72 h ($p < 0.05$, Fig. 1A). We subsequently repeated the aforementioned assessments using passage 1 and 3 ADSCs, and calculated the cell doubling times. Cells cultured in the presence of sepsis serum were observed to be characterized by a prolonged time to doubling, and the effects were more pronounced with successive *in vitro* passages (Fig. 1B). To further evaluate the effects of sepsis serum on ADSCs, in subsequent experiments we systematically compare the fates of cells exposed to the 5% FBS + 2.5% NS and 5% FBS + 2.5% SS media.

3.2 Influence of Sepsis Serum on Cell Surface Markers and the Differentiation of ADSCs

As shown in Fig. 2, the results of flow cytometry analysis revealed that ADSCs cultured in both the 5% FBS + 2.5% NS and 5% FBS + 2.5% SS media exhibited a typical

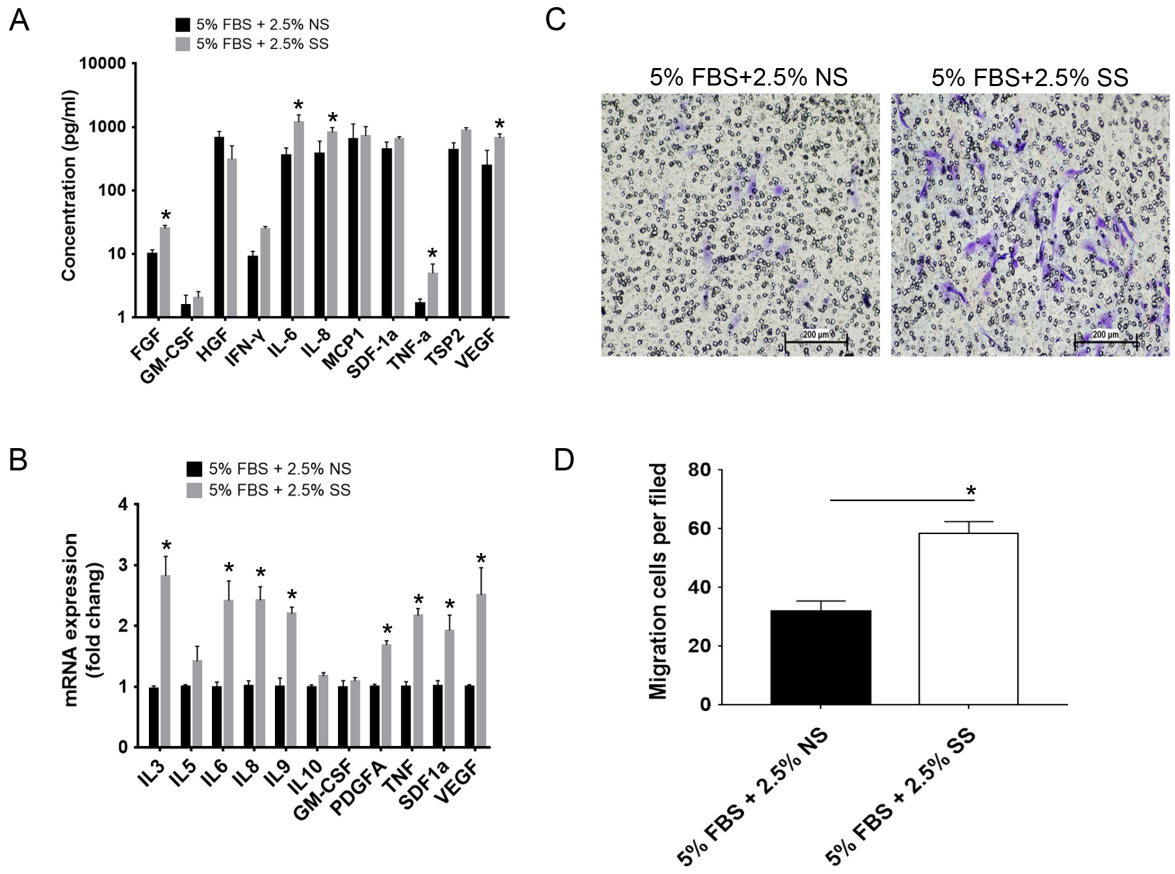

Fig. 4. Cell behavior. (A) Cytokine and growth factor secretion of ADSCs exposed to normal mouse serum and sepsis serum were determined. (B) qRT-PCR verification of mRNA expression of representative cytokines and growth factors in two groups of ADSCs. (C) Transwell assay evaluated ADSCs migration after 24 h of incubation. (D) Data are presented as means ± SEM of triplicate experiments. Three independent experiments were performed in each group. * $p < 0.05$.

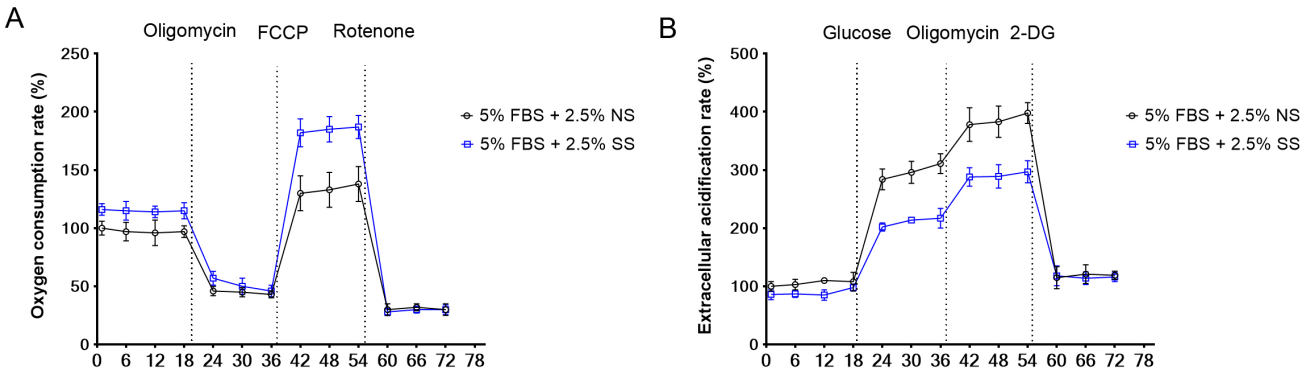

Fig. 5. Respiration assay using the Agilent Seahorse XF technology. (A) An analysis of O_2 consumption in two groups of cells using different inhibitors. (B) Representative graph of the ECAR determination curves reveals the differences in glycolysis.

cell surface phenotype characteristic of multipotent mesenchymal stem cells, as defined by the International Society for Cellular Therapy guidelines published in 2006 [29]. These cells were established to be positive for the markers CD73, CD90, and CD105, and negative for CD14, CD19, CD34, CD45, and HLA-DR. In addition, we found that the cells can successfully differentiate into classical mesenchymal derivatives, including osteoblasts, adipocytes, and chondrocytes, albeit with differing differentiation efficiency. Histochemical staining results and qRT-PCR data for lineage-specific markers indicated that ADSCs exposed to sepsis serum were characterized by an increased capacity to differentiate into osteoblasts but reduced capacity to undergo adipogenic and chondrogenic differentiation (Fig. 3). These findings would thus tend to indicate that sepsis serum may regulate factors determining the fate of ADSCs.

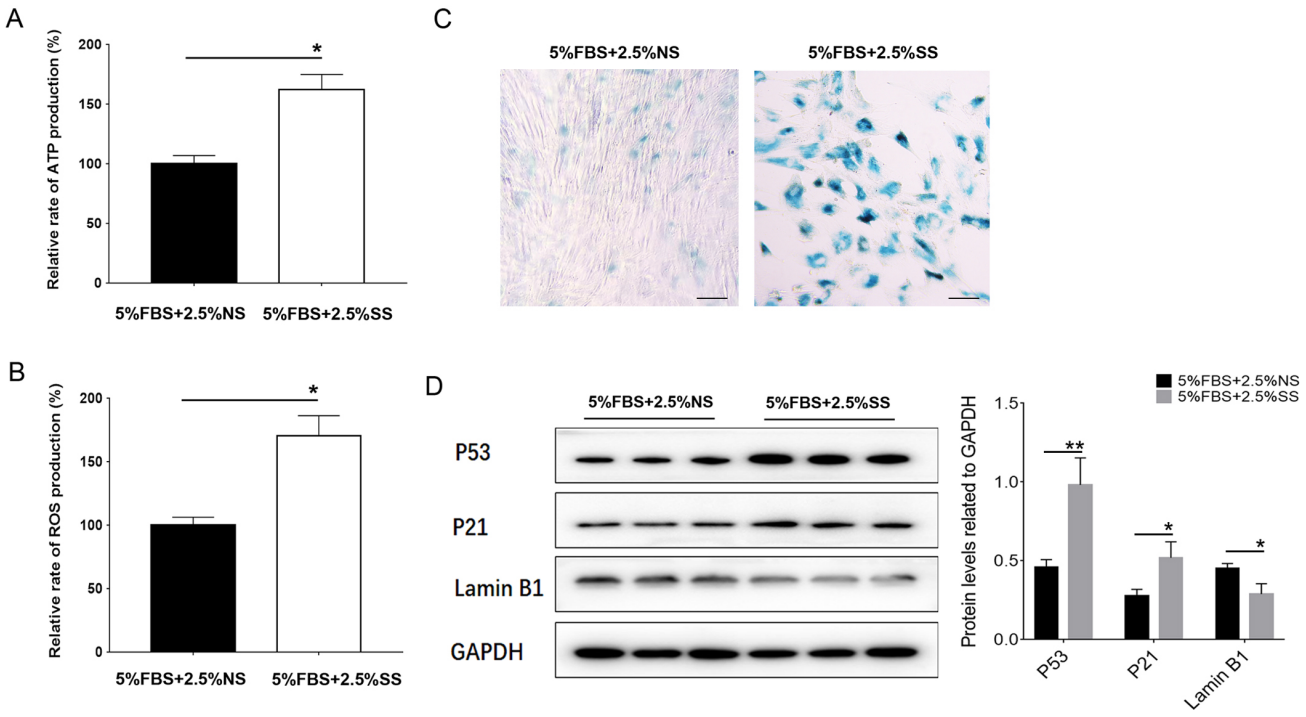

Fig. 6. ADSCs exposed to sepsis serum are more senescent than controls. (A) Average rates of ATP level in mitochondria. (B) Relative levels of ROS production. (C) Representative microscopies of β-galactosidase staining of the two groups of ADSCs. Scale bars = 50 μm. (D) Expression levels of the P53, P21 and Lamin B1 were determined using western blot and normalized to GAPDH. * $p < 0.05$, ** $p < 0.01$.

3.3 Effect of Sepsis Serum on the Cytokine Secretion and Migration of ADSCs

To further to assess the impact of sepsis serum on ADSC behavior, we determined the cytokine secretion profiles of these cells. We accordingly found that the expression of a majority of these factors were increased in ADSCs cultured in the 5% FBS + 2.5% SS medium. Among which, we detected approximately 2.3-, 3.3-, 1.6-, 3.2-, 2.1-fold significant increases in the secretion of IFN-γ, IL6, SDF1a, TNF-a, and VEGF, respectively (Fig. 4A). These responses were subsequently verified by qRT-PCR analysis of the mRNA expression of these cytokines in ADSCs (Fig. 4B). Furthermore, Transwell migration analysis revealed a significant increase the proportion of migratory ADSCs among those exposed to 5% FBS + 2.5% SS compared with cells pre-cultured in DMEM-LG supplemented with 5% FBS + 2.5% NS (Fig. 4C,D).

3.4 Sepsis Serum Treatment Significantly Down-Regulates the Levels of Oxygen Consumption and Extracellular Acidification in ADSCs

To evaluate whether sepsis serum treatment alters the cellular bioenergetics of ADSCs, we examined the rates of oxygen consumption and extracellular acidification in these cells. OCR was measured following sequential addition of the inhibitors oligomycin, FCCP, rotenone, and antimycin (Fig. 5A). The OCR curves of the two groups of cells showed minimal overlap, thereby tending to indicate a significant change in oxidative phosphorylation. ADSCs exposed to sepsis serum were characterized by significant increases in basal OCR, maximal OCR, and reserve capacity, whereas in contrast, we detected no significant difference with respect to non-mitochondrial OCR. ECAR analysis revealed that in response to sepsis serum treatment, the glycolysis and glycolytic capacity of ADSCs were reduced relative to the mean values measured in control cells.

3.5 Sepsis Serum Treatment Results in the Earlier Senescence of ADSCs

We subsequently examined whether changes in ADSC metabolism were associated with altered mitochondrial function. In line with expectations, cells exposed to sepsis serum were found to have significantly higher levels of ATP ($p < 0.05$, Fig. 6A). Furthermore, we detected significant increases in the generation of ROS in cells exposed to sepsis serum ($p < 0.05$, Fig. 6B). Similarly, ADSCs exposed to sepsis serum exhibited significantly higher β-galactosidase activity and flattened morphology in (Fig. 6C). In addition, western blotting analysis indicated that the expression of P53 and P21 were increased, while Lamin B was reduced in the sepsis serum-treated ADSCs (Fig. 6D). Collectively, these findings tend to indicate that sepsis serum promote the senescence of ADSCs, which may partially responsible for the altered differentiation bias.

4. Discussion

Mesenchymal stem cells are a rarely occurring type of undifferentiated cell that can be isolated from a large range of adult tissues, including bone marrow, adipose tissue, cord blood, amniotic fluid, dental pulp, and skeletal muscle [30–33]. Several factors indicate the therapeutic potential of MSCs for the treatment of sepsis. For example, it has been established that MSCs can differentiate into a diverse range of cell lineages both *in vitro* and *in vivo*, thereby contributing to the healing of damaged tissue or organs. MSCs also produce large quantities of cytokines and extracellular matrix that are conducive to reconstruction of the microenvironment of injured tissues, and these cells have strong immunosuppressive effects by contributing to the reduction of inflammatory cell numbers and inflammatory cytokine production. Nevertheless, despite the considerable amount of research conducted on these cells over the past decade, further studies are needed to develop optimized and stable treatment regimens for the management of sepsis. Therefore, we explored the influence of sepsis microenvironment on ADSCs in present study. We demonstrated that sepsis serum promotes the cytokine and growth factor secretion and migratory capacity of ADSCs. However, given that these findings were based on *in vitro* studies, more detailed studies of ADSCs in septic animals are required. In the future, we will continue conduct *in vitro* and *in vivo* experiments to gain further clarification of the efficacy and safety of ADSCs in the treatment of sepsis.

In recent years, concerns regarding the potential long-term effects of stem cell therapy have to a larger extent been allayed by the discovery that these cells tend to disappear from tissues within days of administration. Regulation of stem cell self-renewal and differentiation depends on the provision of an appropriate niche, which is the microenvironment comprising the extracellular matrix, growth factors, morphogenic factors, small molecules, and cytokines secreted by stem cells and the surrounding cells. Accumulating evidence indicates that an inflammatory microenvironment can modulate MSCs, and consequently, there is a pressing need to elucidate the mechanisms underlying the fate of ADSCs after having been transplanted into the body. In this study, we exposed ADSCs to simulated microenvironments comprising normal mouse serum or sepsis serum, and subsequent cell proliferation assays revealed an inhibitory effect of mouse sepsis serum, along with altered cell differentiation capacities. Mesenchymal stem cells have high osteogenesis and adipogenesis potential, and the switch between osteogenetic and adipogenic commitment and differentiation is mediated through numerous transcription factors and signaling pathways, as well as metabolic patterns. Here, we showed that the cell differentiation capabilities were altered in sepsis serum-treated ADSCs. Furthermore, the metabolic analysis revealed metabolism reprogramming in ADSCs after sepsis serum administration, along with enhanced OCR and reduced ECAR. ROS accumulation in MSCs may contribute to the loss of homeostasis, leading to senescence. Indeed, our findings suggested that ADSCs administrated with mouse sepsis serum exhibited senescence markers, including delayed proliferation, increased expression of senescence-associated β-galactosidase (SA-β-gal), and senescence-associated protein alterations.

The significance of this experiment for clinical research is that it reveals the variabilities of ADSC preparations. ADSCs treated with sepsis serum may exhibit properties that are higher than the therapeutic efficacy of normal ADSCs due to fate reprogramming.

5. Conclusions

In conclusion, our findings show that mouse sepsis serum seriously affects the growth and cell fate determination of *in vitro* cultured ADSCs, which hint that the microenvironmental factors of sepsis recipients may regulate the cell fate of ADSCs, leading to different outcomes in the clinical trials under sepsis conditions. This calls for further investigation of the mechanisms of sepsis serum-mediated ADSCs fate alteration and the development of efficient means to reverse it.

Availability of Data and Materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Author Contributions

JF, RL, YL, HWang and HWei designed and performed the experiments; JW, QL, HL analyzed and interpreted the data; ZZ, LZ collect literatures and drafted manuscript; RP, YC proposed the concept, revise and review the manuscript. All authors contributed to author contributed to editorial changes in the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

The animal experimental protocol was approved by the local medical animal experiment ethics committee (ZJCLA, No. ZJCLA- IACUC-20110043). Samples of human adipose tissue were collected using a protocol approved by the Internal Review Board of Zhejiang Provincial People's Hospital (2018KY977).

Acknowledgment

Not applicable.

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Conflict of Interest

Authors LZ and RP were employed by S-Evans Biosciences. The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Supplementary Material

Supplementary material associated with this article can be found, in the online version, at https://doi.org/10.31083/j.fbl2804072.

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