Resveratrol reverses myogenic induction supression caused by high glucose through activating SIRT1/AKT/FOXO1 pathway

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Research article

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Abstract

Background: Long-term high glucose environment can cause muscle tissue atrophy, and then lead to musculoskeletal depression or even disability. Regenerative medicine is an extremely attractive select to solve this problem. Resveratrol is a compound which has various clinical therapeutic effects including regulating the myogenic differentiation of bone marrow mesenchymal stem cells (BMSCs). So, the objective of this study is to observe if resveratrol affect myogenic induction of rat BMSCs under high glucose environment and explore the possible mechanism. Methods: Rat BMSCs were isolated and cultured. The phenotypes were identified when cultured to the third passage cells (P3 cells). Then the P3 cells were used to induce to differentiate into myogenic cells by using the conditioned medium. After grouping, glucose, resveratrol and EX527 (inhibitor of SIRT1) were added. The cell viability was measured by MTT assay. The myogenesis related protein was detected by immunofluorescence. The level of reactive oxygen species (ROS) and superoxide dismutase (SOD) activity were detected by use of assay kits. The cell cycle was assayed with flow cytometry. The expression of FOXO1, AKT, p-AKT, MyoD1 and Myogenin were measured by WB. All above indicators in different groups were quantified and compared. Results: During myogenic induction, after 72h treatment, high glucose (35 mmol/L) reduced cell viability and proliferation of rat BMSCs significantly, increased intracellular ROS levels clearly, decreased SOD activity obviously, and restrained AKT/FOXO1 pathway apparently. Resveratrol (15μmol/L) could regulate the process positively and reverse the suppression caused by high glucose partly through restoring cell proliferation and viability, reducing peroxidative damage and activating AKT/FOXO1 pathway. After pretreated the cells with EX527 (20 μmol/L), this reverse effect of resveratrol was eliminated. Conclusion: Resveratrol not only promoted myogenic induction of rat BMSCs, but also partially reversed myogenic induction supression of rat BMSCs caused by high glucose through activating SIRT1/AKT/FOXO1 pathway. [Key words] resveratrol, bone marrow mesenchymal stem cells, myogenic induction, glucose, SIRT1, AKT, FOXO1

Background

Musculoskeletal depression is a main reason of disability. Diabetes is a chronic disease and older diabetic patients are prone to suffer from sarcopenia. Researchers already confirmed that the body's long-term high glucose environment can cause muscle tissue atrophy [1]. At the same time, sarcopenia are closely related to frailty [2], and this status of sarcopenia and/or frailty would increase the risk of negative events such as disability, paralysis and tumble [3]. Regenerative medicine is an extremely attractive select to solve this problem [4, 5].

Bone marrow mesenchymal stem cells (BMSCs) are a subgroup of cells with multi-differentiating potential, and their directed differentiation is a complex process involving multiple cell pathways. The multi- differentiating properties of BMSCs are currently used to treat a variety of diseases such as type 1 diabetes, inflammatory bowel disease and angiogenesis [6–8]. Several studies demonstrated that BMSCs could be induced to differentiate into muscle cells under specific induction conditions [9, 10], and this differentiation might be used to cure the sarcopenia caused by high glucose.

Resveratrol is a non-flavonoid polyphenolic compound containing astragalus structure and mainly derived from peanuts, grapes and mulberry, etc. Resveratrol has various clinical therapeutic effects, including anti-oxidation, anti-inflammation, anti-tumor, anti-diabetes, anti-aging and myocardial protection [11]. Recent study indicated that resvetartrol regulated downstream transcriptional molecules by activated silent information regulator 1 (SIRT1) and increased their activity. Previous experiments of our group have demonstrated that resveratrol can promote angiogenesis in bone marrow mononuclear cells of diabetic mice by activating SIRT1 [12]. SIRT1/FOXO1 played an important role in the process of osteogenic differentiation of BMSCs promoted by resveratrol [13]. Furthermore, FOXO1 also occupied an essential place among signaling pathways which regulated the myogenic differentiation [14].

As far as we know, the role and mechanism of resveratrol in the myogenic induction of BMSCs under the high glucose environment have not been reported. So, the main aims of this study were to observe the effects of high glucose and resveratrol on the myogenic induction of BMSCs. At the same time, the possible mechanisms of resveratrol affected myogenic induction were explored.

Materials And Methods

Materials and reagents

Male and healthy SD rats in SPF grade were provided by Animal Experimental Center of the Second Affiliated Hospital of Harbin Medical University. DMEM/F12 was purchased from Hyclone Laboratories (Logan, UT, USA). Fetal bovine serum was from Biological Industries (Beit Haemek, Israel), bFGF was from PeproTech (Rocky Hill, New Jersey, USA). Dexamethasone was from Solarbio Science & Technology (shanghai, China). Superoxide dismutase (SOD) detection kit was from Nanjing Institute of Bioengineering (Nanjing, China). BCA assay kit, Reactive Oxygen Detection Kit and SDS-PAGE gel preparation kit were purchased from Beyotime Institute of Biotechnology (Shanghai, China). Cell Cycle Detection Kit and Supersensitive ECL luminescent liquid were brought from HaiGene (Harbin, China). Anti-CD44, CD90, CD31, CD34 and goat anti-rabbit IgG-Cy3 were purchased from Absin Bioscience Inc. (Shanghai, China). MyoD1 and Myogenin primary antibodies were purchased from abcam (Cambridge, UK). Anti-FOXO1, anti-AKT and anti-P-AKT were purchased from Cell Signaling Technology, Inc. (Danvers, Massachusetts, USA). Anti-GAPDH antibody was purchased from Zhongshan Golden Bridge Biotechnology (Beijing, China). PBS and other reagents were from Solarbio (Beijing, China).

The muscle differentiation induction (OM) medium including DMEM/Ham's F12, horse serum (2%), glutamine (1%), bFGF (1 ng/mL) and dexamethasone (0.4 ug/mL) was prepared according to the reference [15].

Methods

Cell culture and identification

This study was conducted in accordance with the Helsinki Declaration II and was approved by the Human Ethics Committee of Second Affiliated Hospital of Harbin Medical University, People's Republic of China.

Primary cultures of rat BMSCs were prepared from SD rats aged 3–4 weeks. They were housed in an air-conditioned room with a humidity level of 45–65% and a temperature of 20 ± 2 °C. They were sacrificed by cervical dislocation and soaked in 75% ethanol for 10 min. The bilateral femur and tibia of rats were removed under aseptic conditions. The periosteum and muscle tissue were dissected and then washed with PBS three times. Next, the medullary cavity was cut and washed repeatedly with DMEM/F12 medium containing 10% fetal bovine serum. The fluid was centrifuged and the sediment was inoculated into cell bottles. The medium was changed after 48 hours. When the cells were covered with 80% of the cell bottle, then passaged, and the third generation was used to conduct the subsequent experiment [8]. Well-grown P3 cells were selected for identification. The cells were fixed with paraformaldehyde for 15 min at room temperature, after that, added with blocking solution and shaken gently for 30 min on a horizontal shaker. Anti-CD44, anti-CD90, anti-CD31, anti-CD34 were added and incubated overnight at 4 °C. Afterwards, the fluorescent secondary antibody was added and incubated for 1 hour at room temperature. After the cells were stained with DAPI for 5 min, the fluorescent expression was observed under fluorescence microscope.

Treatment and grouping

The P3 cells were treat with OM medium, different concentrate glucose (25 mmol/L and 35 mmol/L), resveratrol (15 μmol/L) [16] with or without EX527 (SIRT1 inhibitor, 20 μmol/L) [16] according to the group design. After the MTT assay, the suitable concentrate of glucose (35 mmol/L) was confirmed and six experimental groups were set randomly and parallelly ($n \geq 3$), they were control group, myogenic induction group (OM group), myogenic induction + glucose group (OM + G group), myogenic induction + resveratrol group (OM + R group), myogenic induction + glucose + resveratrol group (OM + G + R group), myogenic induction + glucose + resveratrol + EX527 group (OM + G + R + EX527 group). The cells in the OM + G + R + EX527 group were pretreatment with EX527 for 24 h [9]. Except for EX527, both glucose and resveratrol were added together with OM medium.

MTT assay

After different treatment of 72 h, 20 μl MTT solution (5 g/L) was added to each well of the plates and cells were incubated for 4 h in the incubator (37 °C and 5%CO_2). Then, the medium in each well was aspirated and 100 μl of DMSO was added. The plate was shaked at room temperature for 10 min. The optical density values at a wavelength of 490 nm were measured on an imaging reader. Viability results were expressed as percentages. The absorbance measured from non-treated cells was taken to be 100%.

Measurement of intracellular reactive oxygen species (ROS) levels

Cells were treated according to the experimental grouping, DFCH-DA (20 μmol/L) which diluted in serum-free medium was added in each well of the plate and the plate was, incubated at 37 °C for 30 min in the

dark. Finally, the fluorescence intensity of each well was measured by fluorescence micro-plate reader.

SOD activity

The total protein was extracted from the treated cells and the total protein concentration was determined with a BCA assay kit. The SOD activity was determined by use of SOD detection kit. Both assays were performed and the results calculated according to the manuals provided with the kits.

The changes of cell cycle

The cell pellet of treated cells in different groups was collected and washed, then pre-cooled ethanol (75%) was added and the cells were fixed at 4 °C overnight. Afterwards, the cells were stained and detected with flow cytometry (FACSCalibur, BD Biosciences; Franklin Lakes, NJ, USA). In general, 2–3 million cells were counted. The results were analyzed by using the cell cycle fitting software (ModFit).

Immunofluorescence detection

The MyoD1 and Myogenin expressions in different groups were identified with immunofluorescence after 14 days of myogenic induction. The cells were incubated with primary antibody at 4 °C overnight, After incubation for 1 h at room temperature in the dark with the fluorescent secondary antibody, the fluorescence expression was observed under a fluorescence microscope.

Western Blot

The total protein was extracted from the treated cells and the total protein concentration was determined with a BCA assay kit. An equal amount of protein for each sample in loading buffer was heated at 100 °C for 5 min and loaded onto 12% polyacrylamide gels. After electrophoresis, proteins were transferred onto polyvinylidene difluoride membranes. Next, the membrane was blocked, was incubated with the primary antibodies overnight a 4 °C and the secondary antibody for 1 h in turn. Signals were detected by Protein Gel Imaging System (Clinx; Shanghai, China) and data was expressed as normalized ratios to GAPDH.

Statistical processing

All experiments were repeated for at least 3 times independently. The statistical analysis was performed using software SPSS 24.0 software package (International Business Machines Corporation, Armonk, New York, United States). The experimental results were presented as mean ± standard deviation (Mean ± SE) and evaluated with t test between two groups or one-way analysis of variance (One-Way-ANOVA) among all the experimental groups. Dunnett's T3 and the least significant difference tests were used for two-group comparisons followed inter-group comparison. All tests were two-tailed, and P values less than 0.05 were considered statistically significant.

Results

Cell culture and identification

The primary rat BMSCs were isolated and purified for 72 hours, then fibroblast-like adherent cells (Fig. 1A) were observed. Unlike the P0 cells, the morphology of P3 cells was more uniform and orderly. The cells appear in a spiral and fence-like arrangement (Fig. 1B). The results of fluorescence identification showed that the P3 cells were positive for CD44 (Fig. 1C) and CD90 (Figur1D), negative for CD34 (Fig. 1E) and CD31 (Fig. 1F), and they were BMSCs with high purity. So, the P3 cells with good growth status were selected for subsequent experiments.

Modeling of the myogenic induction of BMSCs

After 14 days' myogenic differentiation, MyoD1 and Myogenin expressions were positive and increased sharply (Fig. 2A and 2B). At the same time, the expression of FOXO1 and p-AKT/AKT in the OM group were statistically higher than those in the control group (t_{Foxo1}=-14.84, P_{Foxo1}<0.001, $t_{p-AKT/AKT}$=-6.94, $P_{p-AKT/AKT}$=0.002, Fig. 2C).

The effect of different glucose concentration and time points on the cell viability during myogenic induction

No significant difference was observed in the cell viability of the 25 mmol/L glucose group from 24 h to 72 h (F = 3.95, P = 0.053). But the cell viability in the 35 mmol/L glucose group lowered at 48 h, and decreased obviously at 72 h. The cell viability at 72 h was significantly lower than those in other three time points ($P_{72h\ vs\ 48h}$=0.014, $P_{72h\ vs\ 24h}$=0.001, $P_{72h\ vs\ 48h}$=0.001, Fig. 3). According to above results, the concentration of glucose at 35 mmol/L was selected for subsequent experiments, and the cells in all groups were treated for 72 h or 14 days.

The decrease effects of high glucose and the increase effects of resveratrol on cell viability and proliferation during myogenic induction of BMSCs

After the treatment of 72 h, there were significant differences in cell viabilities and proliferations (the percentage of G2 + S) among five groups ($F_{viability}$=20.46, $P_{viability}$ <0.001, $F_{proliferation}$=124.73, $P_{proliferation}$<0.001, Fig. 4). Cell viability and proliferation in the OM + R group were highest, and significantly than those in all other four groups ($P_{viability\ OM+R\ vs\ OM}$=0.005, $P_{proliferation\ OM+R\ vs\ OM}$<0.001, $P_{viability\ OM+R\ vs\ OM+G}$<0.001, $P_{proliferation\ OM+R\ vs\ OM+G}$<0.001, $P_{viability\ OM+R\ vs\ OM+G+R}$=0.001, $P_{proliferation\ OM+R\ vs\ OM+G+R}$<0.001, $P_{viability\ OM+R\ vs\ OM+G+R+EX527}$<0.001, $P_{proliferation\ OM+R\ vs\ OM+G+R+EX527}$<0.001).

Cell viabilities and proliferations in the OM and OM + G + R groups were significantly higher than those in OM + G and OM + G + R + EX527 groups ($P_{viability\ OM+G\ vs\ OM}$=0.002, $P_{proliferation\ OM+G\ vs\ OM}$<0.001, $P_{viability\ OM+G+R+EX527\ vs\ OM}$=0.003, $P_{proliferation\ OM+G+R+EX527\ vs\ OM}$<0.001, $P_{viability\ OM+G+R\ vs\ OM+G}$=0.006, $P_{proliferation\ OM+G+R\ vs\ OM+G}$=0.003, $P_{viability\ OM+G+R+EX527\ vs\ OM+G+R}$=0.011, $P_{proliferation\ OM+G+R+EX527\ vs\ OM+G+R}$=0.004).

Compared to the OM group, the cell viability in the OM + R + G group changed barely (P = 0.446). Differently, the cell proliferation in the OM + R + G group decreased significantly (P < 0.001). Unlike above

two groups, there were no significant differences in cell viabilities and proliferations between the OM + G group and the OM + G + R + EX527 group ($P_{viability}$ =0.725, $P_{proliferation}$=0.881).

The effect of high glucose and resveratrol on the expression of MyoD1 and Myogenin

After 14 days' treatment, there were significant differences in MyoD1 and Myogenin expressions among all five groups (F_{MyoD1}=70.93, P_{MyoD1}<0.001, $F_{Myogenin}$=77.45, $P_{Myogenin}$<0.001, Fig. 5). The expressions of MyoD1 and Myogeni in the OM + R group were highest and obviously higher than those in all other groups ($P_{MyoD1\ OM+R\ vs\ OM}$<0.001, $P_{Myogenin\ OM+R\ vs\ OM}$ =0.006, $P_{MyoD1\ OM+R\ vs\ OM+G}$<0.001, $P_{Myogenin\ OM+R\ vs\ OM+G}$<0.001, $P_{MyoD1\ OM+G+R\ vs\ OM+R}$<0.001, $P_{MyoD1\ OM+G+R+EX527\ vs\ OM+R}$<0.001, $P_{Myogenin\ OM+G+R\ vs\ OM+R}$=0.001, $P_{Myogenin\ OM+G+R+EX527\ vs\ OM+R}$<0.001).

The expressions of MyoD1 and Myogeni in the OM and OM + G + R groups were significantly higher than those in OM + G and OM + G + R + EX527 groups (all P ≤ 0.001). At last, no clear changes were seen in MyoD1 and Myogenin expressions between the OM + G + R group and the OM group (P_{MyoD1} =0.727, $P_{Myogenin}$ =0.196). Compared to the OM + G group, the expression of MyoD1 in the OM + R + G + E527 group changed a little (P = 0.924), differently, the expression of Myogeni in the OM + R + G group decreased significantly (P < 0.001).

The effect of high glucose and resveratrol on the ROS level and SOD activity

During the process of myogenic differentiation of BMSCs, significant differences were seen in the ROS levels and SOD activities among all five groups (F_{ROS}=150.09, P_{ROS}<0.001, F_{SOD}=30.42, P_{SOD}<0.001, Fig. 6). The ROS levels in OM and OM + R groups were significantly lower than those in the OM + G, OM + G + R and OM + G + R + EX527 groups (all P < 0.001), while SOD activities in these three groups were obviously lowered than those in OM and OM + R groups (all P < 0.001).

No significant differences were seen in ROS level and SOD activity between the OM + R group and the OM group (P_{ROS}=0.171, P_{SOD}=0.797). The ROS levels in OM + G + R and OM + G + R + EX527 groups were significantly lower than that in the OM + G group ($P_{OM+G+R\ vs\ OM+G}$<0.001, $P_{OM+G+R+EX527\ vs\ OM+G}$=0.019).

On the contrary, SOD activity in the OM + G + R group were significantly higher than that in the OM + G group ($P_{OM+G+R\ vs\ OM+G}$=0.03), but there was no significant difference in SOD activity between the OM + G + R + EX527 group and the OM + G group (P = 0.801).Finally, compared to the OM + G + R group, the ROS level in the OM + G + R + EX527 group elevated significantly (P = 0.001) and the SOD activity lowered significantly(P = 0.019).

The expressions of FOXO1 and the ratios of p-AKT/AKT in different groups

No FOXO1 were detected in the OM + G + R + EX527 group. There were significant differences both in the expressions of FOXO1 and p-AKT/AKT among other four groups (F = 179.78, P < 0.001, F = 208.45, P <

0.001, Fig. 7). The expression of FOXO1 and p-AKT/AKT in the OM + R group were highest, and significantly higher than those in other groups (all $P < 0.001$).

Except the OM + R group, the expression of FOXO1 in the OM + G was lowest, and obviously lower than those in OM and OM + G + R groups ($P_{OM\ vs\ OM+G}<0.001$, $P_{OM+G+R\ vs\ OM+G}=0.023$). Moreover, significant difference was seen between the OM group and the OM + G + R group ($P = 0.003$, Fig. 7A).

Besides the OM + R group, p-AKT/AKT in the OM group was apparently higher than those in OM + G, OM + G + R and OM + G + R + EX527 groups (all $P < 0.001$, Fig. 7B). No significant difference was seen in p-AKT/AKT among OM + G, OM + G + R and OM + G + R + EX527 groups ($P_{OM+G+R\ vs\ OM+G}=0.063$, $P_{OM+G+R+EX527\ vs\ OM+G}=0.547$), but significant difference was seen between the OM + G + R group and the OM + G + R + EX527 group ($P = 0.022$).

Discussion

In BMSCs, the expression of CD44 and CD90 are positive, while the expression of CD31 and CD34 are negative [17, 18]. In this study, rat BMSCs were cultured and identified to meet the surface antigen characteristics of BMSCs. MyoD1 and Myogenin are specific genes that determine myogenic differentiation, and the expression product is an important marker in the early stage of myogenic differentiation [19, 20]. In this experiment, MyoD1 and Myogenin expressions increased sharply after 14 days' myogenic differentiation. These results indicated that the myogenic differentiation of rat BMSCs was induced successfully.

In vitro, high glucose environment inhibited the proliferation of BMSCs and regulated the osteogenic and myogenic differentiation negatively [21, 22]. In our experiments, high glucose affected myogenic differentiation negatively, decreased cell viability, cell proliferation index and the expressions of MyoD1 and Myogenin significantly. These results were in agreement with the conclusions of above references. In this study, resveratrol was the opposite of the high glucose, and could partially reverse above changes caused by high glucose partly. However, this reversed effect would be blocked by the EX527 (an inhibitor of SIRT1). The results preliminarily suggested that SIRT1 played a role in the process of resveratrol promoted myogenic differentiation.

Diabetes or high glucose can cause oxidative damage in a variety of cells [23, 24]. The above conclusions were all got corroborate mutually in our study. High glucose lowered the SOD activity and elevated the ROS level, and this effect could reverse partly by resveratrol. In addition, the reversed effect of resveratrol could be interfered by the EX527. The changed characteristics of oxidative stress in each group were consistant with changes of cell viability, cell proliferation index and the expressions of MyoD1 and Myogenin of different groups.

The PI3K-AKT-FOXO1 pathway has been reported that it played an important role in several systems, especially in bones and muscles [25–27]. The activation of AKT can enhance the activity of MyoD in myogenesis, then induce the differentiation of myocytes and make them integrate into the regenerated

muscle fibers [28]. In the process of modeling of this study, the expression of FOXO1 and p-AKT/AKT statistically increased with the increased expressions of MyoD1 and Myogenin. Namely the AKT/FOXO1 pathway was activated and had a role during myogenic differentiation. It was found that p-AKT/AKT and FOXO1 always showed a synchronous change trend after treatment of high glucose, resveratrol and EX527. High glucose decreased them and resveratrol increased them. Encouragingly, the increased effect of resveratrol on them again interfered while inhibited SIRT1 with EX527. These tendencies were essentially uniform with the changes of oxidative stress, cell viability, cell proliferation index, expressions of MyoD1 and Myogenin in different groups.

Conclusions

Together, our results indicated that high glucose could regulate myogenic induction of rat BMSCs negatively through inhibiting cell proliferation, reducing cell viability, oxidative damage and inactivating AKT/FOXO1 pathway. And fortunately, resveratrol could regulate the process positively through restoring cell proliferation and viability, reducing peroxidative damage and activating SIRT1/AKT/FOXO1 pathway. In other word, resveratrol not only promoted myogenic induction of rat BMSCs, but also reversed myogenic induction supression of rat BMSCs caused by high glucose through activating SIRT1/AKT/FOXO1 pathway.

Abbreviations

P3 cells: The third passage cells

OM : Muscle differentiation induction

G: Glucose

R: Resveratrol

Declarations

Availability of data and materials

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

Ethics approval and consent to participate

This study was conducted in accordance with the Helsinki Declaration II and was approved by the Human Ethics Committee of Second Affiliated Hospital of Harbin Medical University, People’s Republic of China.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

L designed the study; ML and LY carried out the experiment; XL and HF analyzed the data; HZ searched literature; ML, LY and MF wrote and revised the manuscript; All authors read and approved the final manuscript.

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Figures

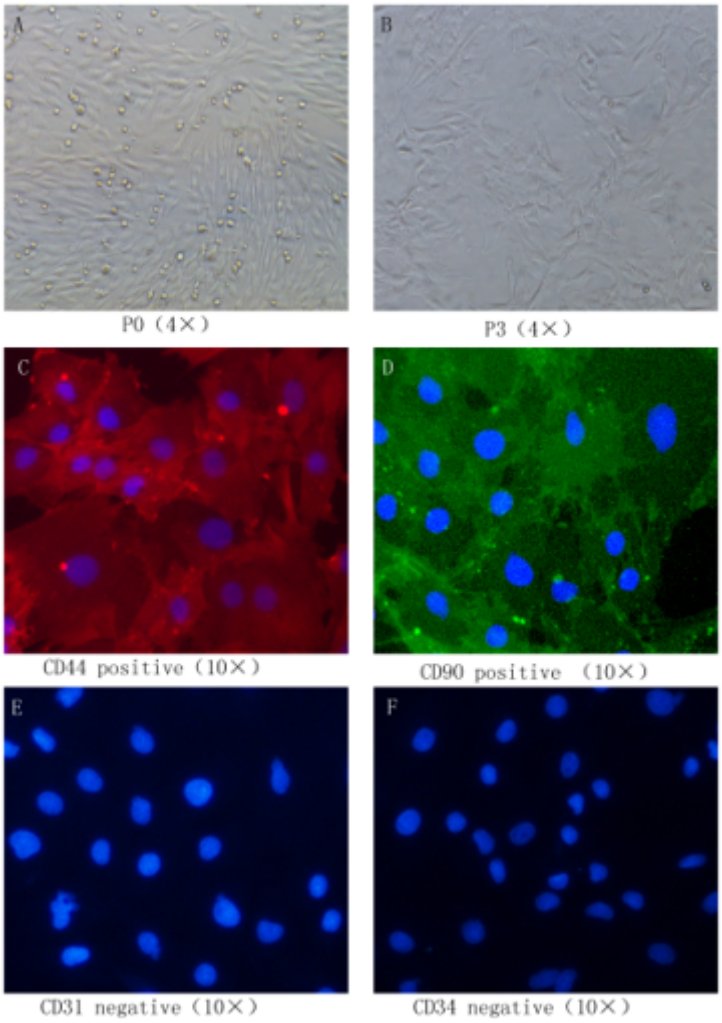

Figure 1

Culture and identification of BMSCs derived from rat bone marrow. A: P0 BMSCs cultured for 72h (4×); B: P3 BMSCs (4×); C: The express of CD44 in P3 cells is positive (10×); D: The express of CD90 in P3 cells is positive (10×); E: The express of CD34 in P3 cells is negative (10×); F: The express of CD31 in P3 cells is negative (10×).

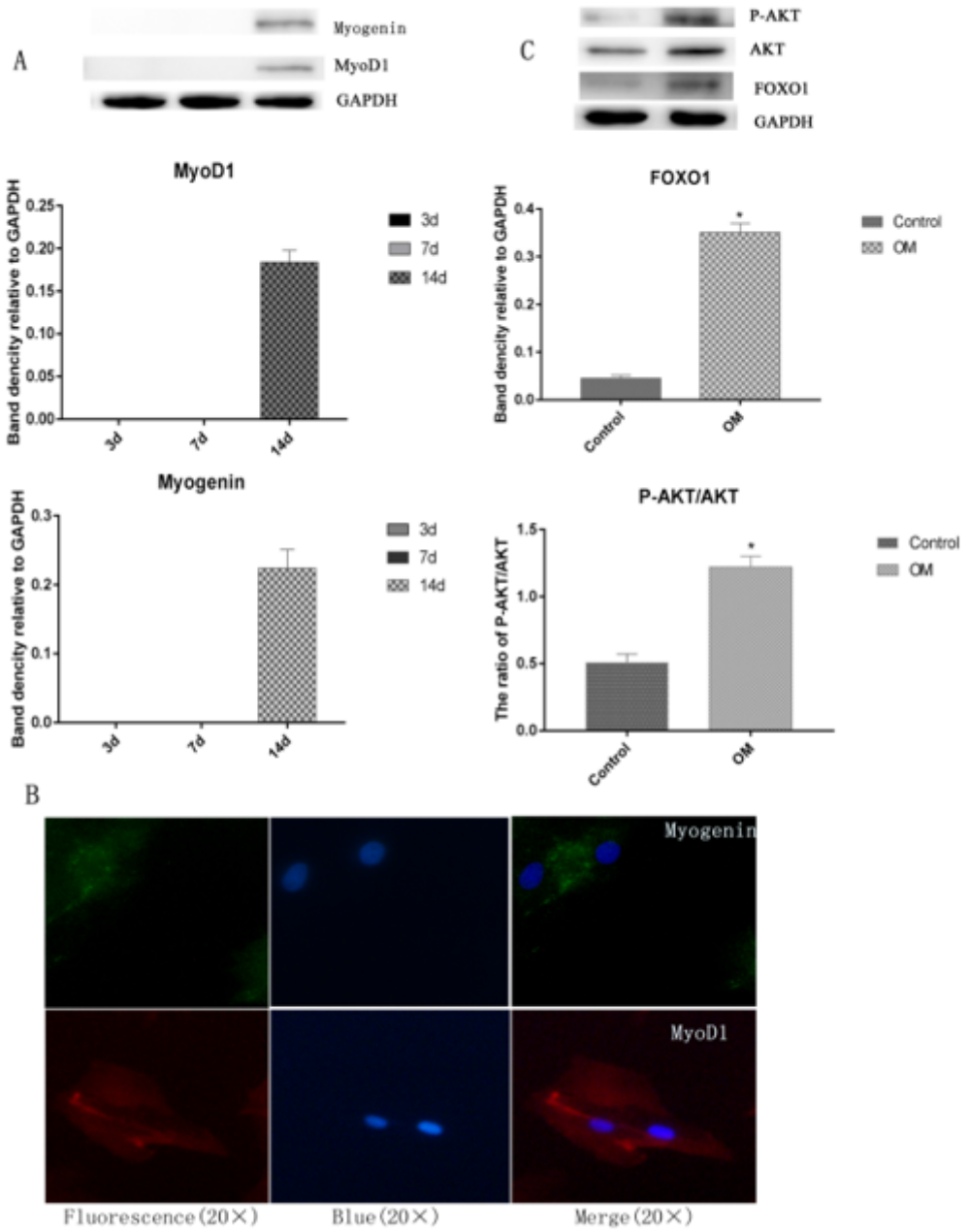

Figure 2

The expressions of MyoD1, Myogenin, FOXO1 and p-AKT/AKT during the myogenic differentiation of BMSCs. A: the expression of MyoD1 and Myogenin at 3d, 7d and 14d; B: The expression of MyoD1 and Myogenin at 14d; C: the expression of FOXO1 and p-AKT/AKT during the myogenic differentiation of BMSCs. *P<0.05 compared with the control group.

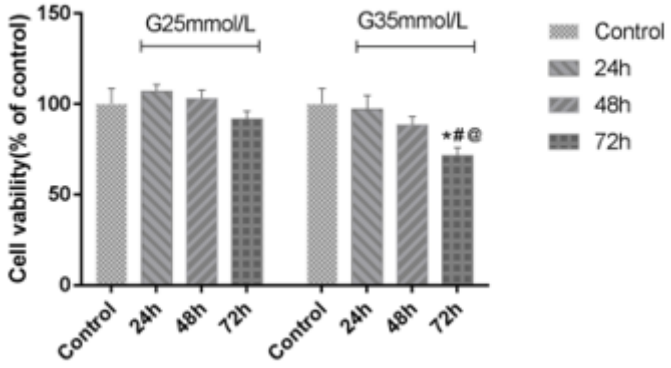

Figure 3

The effect of different glucose concentration and treated time on the cell viability. The cell viability was inhibited significantly when they were cultured at a glucose concentration at 35mmol/L or 72 h. *P<0.05 compared with the control group, #P<0.05 compared with the 24h group, @P<0.05 compared with the 48h group.

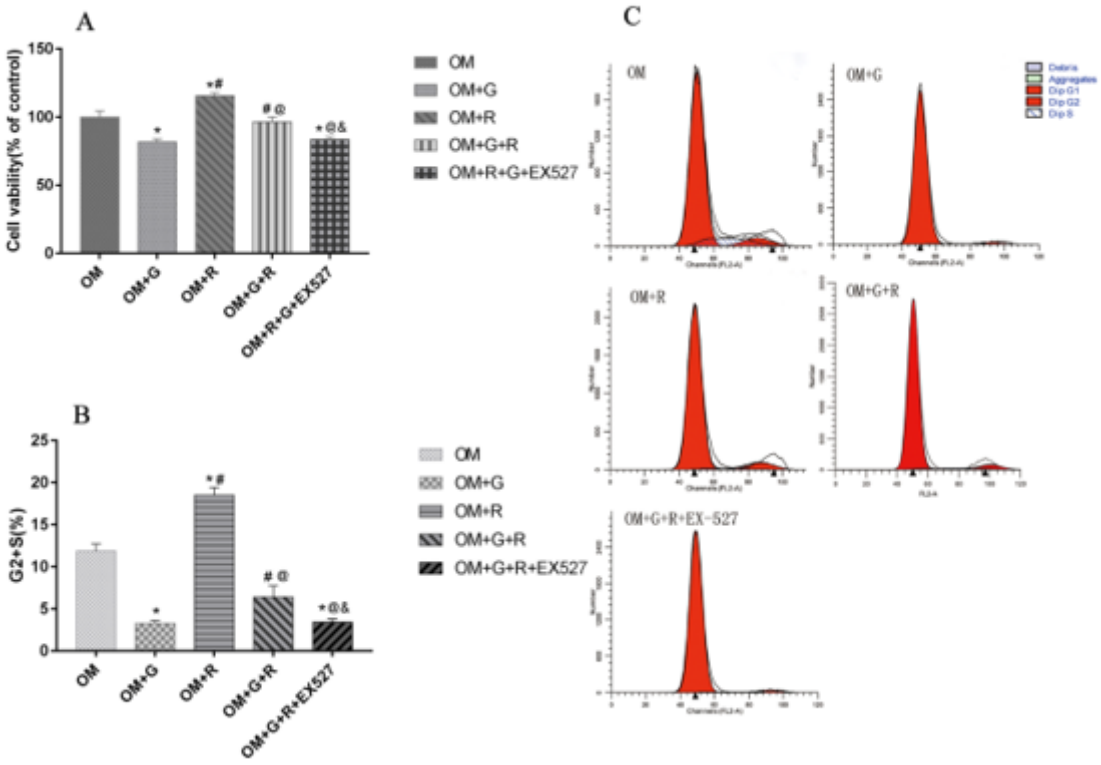

Figure 4

The effects of high glucose and resveratrol on cell viability and proliferation index during myogenic induction of BMSCs. A: the cell viability in different groups. B+C: the proliferation index in different groups. *P<0.05 compared with the OM group, #P<0.05 compared with the OM+G group, @P<0.05 compared with the OM+R group, &P<0.05 compared with the OM+G+R group.

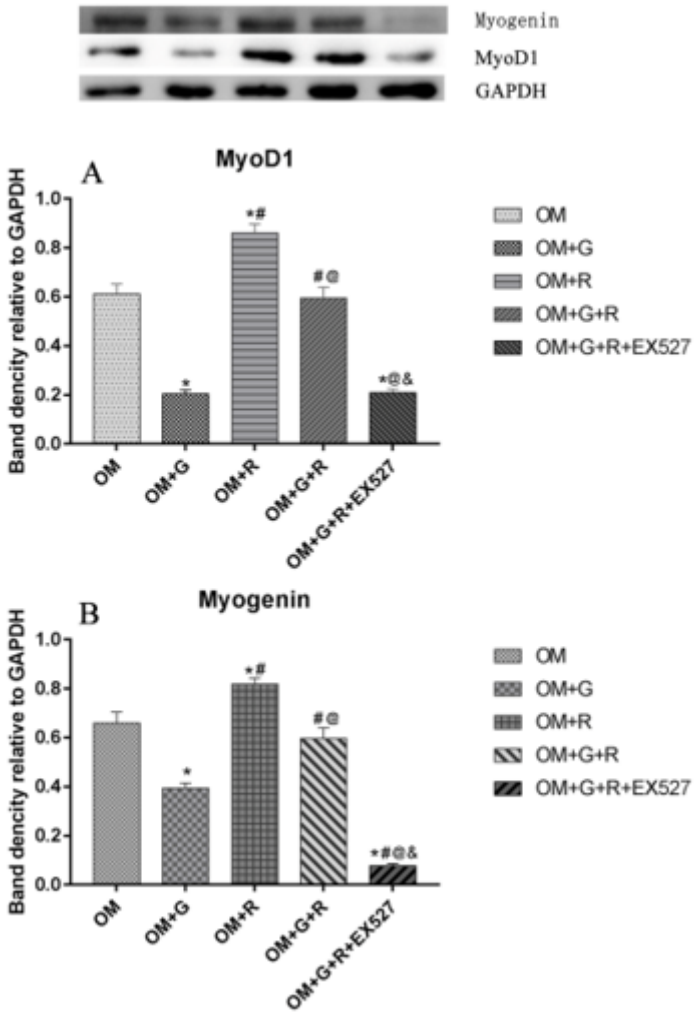

Figure 5

The expressions of MyoD1 and Myogenin in different groups. A: the expression of MyoD1 of each group; B: the expression of Myogenin in all groups. *P<0.05 compared with the OM group, #P<0.05 compared with the OM+G group, @P<0.05 compared with the OM+R group, &P<0.05 compared with the OM+G+R group.

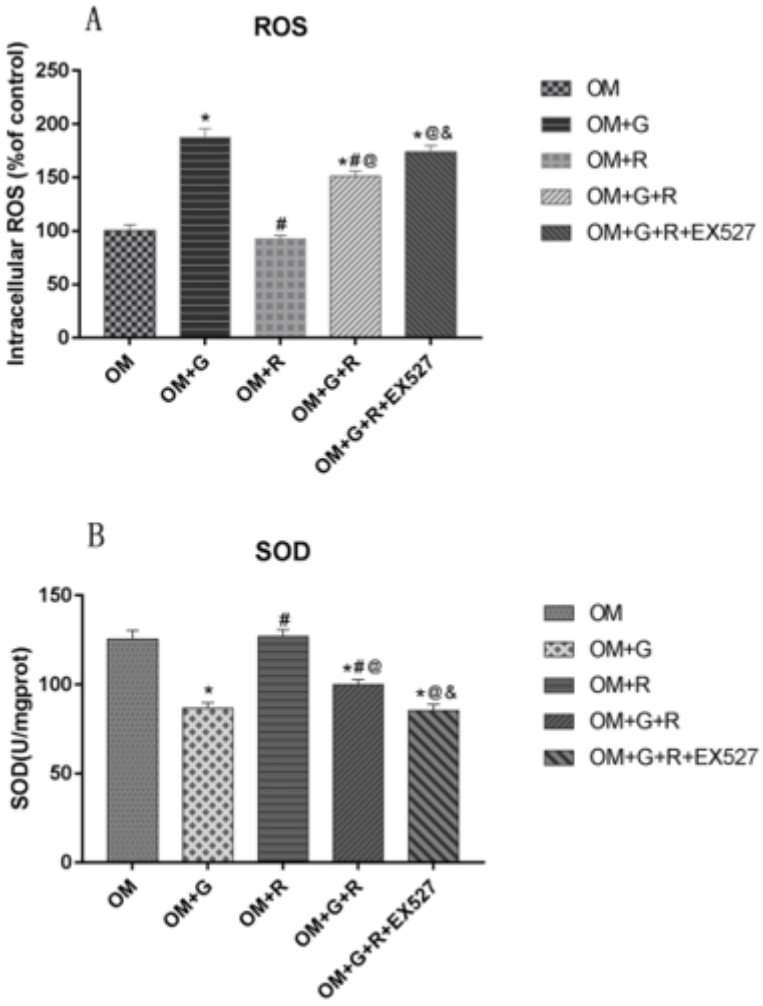

Figure 6

The ROS levels and SOD activities in all groups during myogenic induction of BMSCs. A: the ROS levels of different groups; B: SOD activity of each group. *P<0.05 compared with the OM group, #P<0.05 compared with the OM+G group, @P<0.05 compared with the OM+R group, &P<0.05 compared with the OM+G+R group.

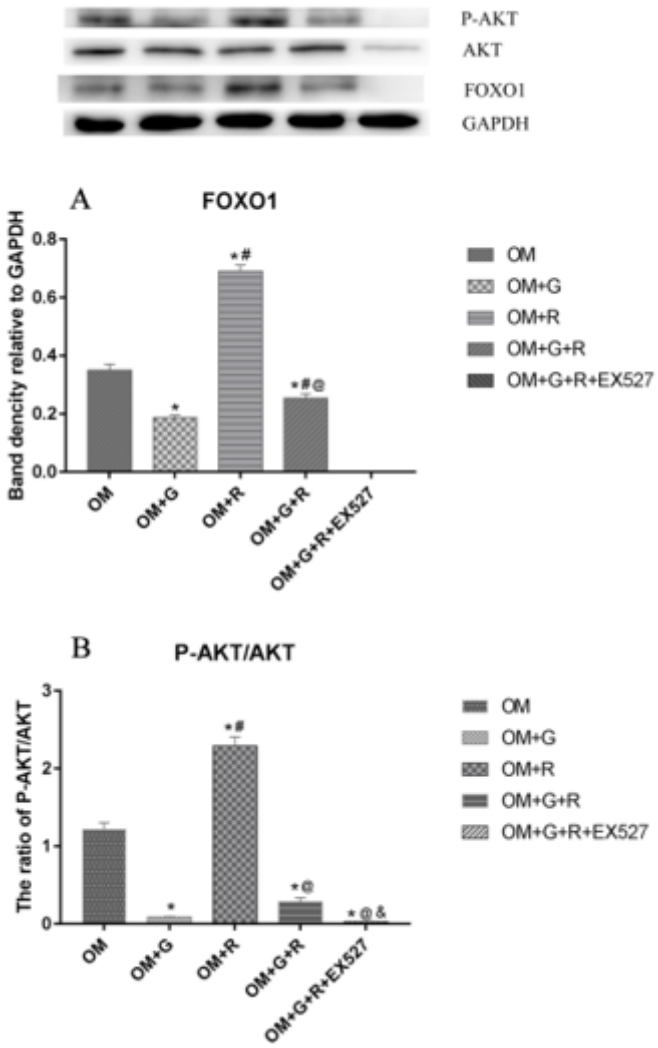

Figure 7

The expression of FOXO1 and p-AKT/AKT during myogenic induction. A+B: The expression of FOXO1 in each group; A+C: p-AKT/AKT in different groups. *P<0.05 compared with the OM group, #P<0.05 compared with the OM+G group, @P<0.05 compared with the OM+R group, &P<0.05 compared with the OM+G+R group.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- NC3RsARRIVEGuidelinesChecklist.docx