Effects of MRI on Stemness Properties of Wharton's Jelly Derived Mesenchymal Stem Cells

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Research

Keywords: MRI, MSCs, Stemness Properties, Viability, Clonogenicity, Apoptosis

Posted Date: November 22nd, 2021

DOI: https://doi.org/10.21203/rs.3.rs-1040769/v1

Abstract

Background

Mesenchymal stem cells (MSCs), derived from various tissues, have served as a promising source of cells in clinic and regenerative medicine. Umbilical cord-Wharton's jelly (WJ-MSCs)-derived MSCs exhibit advantages over those from adult tissues, such as no ethical concerns, shorter population doubling time, broad differentiation potential, readily available non-invasive source, prolonged maintenance of stemness properties.

Material and methods

The aim of this study was to evaluate the effect of MRI (1.5 T, 10 min) on stemness gene expression patterns (OCT-4, SOX-2, NANOG) of WJ-MSCs. In addition, we assessed cell viability, growth kinetics and apoptosis of WJ-MSCs after MRI treatment.

Results

The quantitative real-time reverse transcription polymerase chain reaction (qRT-PCR) data showed that transcript levels of SOX-2, NANOG in MRI-treated WJ-MSCs were increased 32- and 213-fold, respectively. MTT assay was performed at 24, 48, and 72 hours post-treatment and the viability was not significantly difference between two groups. The doubling time of MRI group was markedly higher than control group. In addition, the colony formation ability of WJ-MSCs after MRI treatment significantly increased. Furthermore, no change in apoptosis was seen before or after MRI treatment.

Conclusions

Our results suggest the use of MRI can improve quality of MSCs and may enhance the efficacy of mesenchymal stem cell-based therapies.

Introduction

Magnetic Resonance Imaging (MRI) as a powerful non-invasive technique is frequently used in the in medical diagnostics. It was indicated that MRI is a safe imaging technology compared to diagnostic tools utilizing ionizing radiation and there are no known side effects and problems for individuals upon examination with the MRI machine (Hsieh et al., 2008). However, several studies demonstrated that strong electromagnetic fields and high static MFs had implications for the occurrence of different type of cancers such as lung cancers, leukemia, and brain cancers(Barregård et al., 1985, Hsieh, Lee, 2008). Nonetheless the safety issue of MRI on human health is still controversial and not well understood.

Mesenchymal stem cells (MSCs) are multipotent cells that have gained significant attention in the field of regenerative medicine and tissue engineering. MSCs can play an influential role in tissue regeneration, repair, and homeostasis via self-renewing capacity with the potential of differentiating into various

tissues including bone, muscle, cartilage, fat, and nerve(Han et al., 2019). MSCs can be isolated from a variety of tissues such as bone marrow, adipose tissue, umbilical cord, and placenta(Kang et al., 2016). Umbilical cord-derived-MSCs (UC-MSCs) have several advantages in comparison with MSCs derived from the other origins and are paid an increasing attention as a source for cell therapy. These advantages include a noninvasive collection procedure for autologous or allogeneic use, more efficient proliferation, low immunogenicity with a good immunosuppressive ability, and minimal societal, ethical and legal constraints(Nagamura-Inoue and He, 2014).

Specific regulatory genes called Oct-4, Sox-2 and Nanog have been shown to play a critical role in maintaining the pluripotency, self-renewality and undifferentiated state of embryonic stem cells (ESCs) (Tsai et al., 2012).Several studies have demonstrated that these pluripotency genes (Oct-4, Sox-2 and Nanog) are essential for the major properties of MSCs and the genes knockdown reduced cell proliferation rate and differentiation potential(Malvicini et al., 2019, Matic et al., 2016, Pitrone et al., 2017, Tsai, Su, 2012).

The aim of this study was to investigate the expression of stemness genes Oct-4, Sox-2 and Nanog in Wharton’s jelly-derived mesenchymal stem cells (WJ-MSCs) after MRI treatment.

Materials And Methods

Wharton's jelly MSCs isolation

The study protocol was approved by the Ethics Committee of Rafsanjan University of Medical Sciences, Rafsanjan, Iran (IR.RUMS.REC.1397.231). Informed consent was obtained from each participant. Umbilical cords were collected immediately after birth and placed in DMEM/F12 medium supplemented with 3% penicillin, streptomycin and amphotericin B and transported to the laboratory at room temperature. The cords were rinsed in sterile phosphate buffered saline (PBS) supplemented with 3% penicillin, streptomycin and amphotericin B and cut longitudinally to expose and remove the two umbilical arteries and the umbilical vein. The remaining umbilical cord tissue including the Wharton's jelly was chopped into 2–5 mm3 explants using single edge razor blades, transferred to 6-well tissue culture plates (Corning Inc., Corning, NY) containing 2 ml of DMEM/F12 (Gibco-Invitrogen Corporation, Carlsbad, CA), 15% fetal bovine serum (Gibco-Invitrogen Corporation, Carlsbad, CA), 1% penicillin/ streptomycin; 1% amphotericin B and maintained in a 37°C incubator with a 5% CO2 atmosphere and saturated humidity. Adherent fibroblast-like cells developed in 3 weeks were isolated by 0.05% trypsin and 0.02% EDTA (Sigma, St. Louis, MO, USA) for further growth and characterization.

MSCs characterization

Mesodermal multilineage differentiation

The differentiation potential of MSC into mesodermal lineages, adipogenic and osteogenic, was tested. For adipogenic and osteogenic differentiation, cells were cultured for 21 days in specific media bought

from Bonyakhte Stem Cell Research Center (Tehran, Iran). After 21 days cells were fixed in 4% paraphormaldeide and stained with Alizarin Red (Sigma) to visualize calcium deposition and Oil Red O (Sigma) to detect the presence of lipid droplets.

Cell surface protein profile

Flow cytometry analysis was used to investigation of the cell surface marker profile of MSC. Cells at passage 3 to 6 were incubated with following anti-human monoclonal antibodies conjugated with Fluorescence isothyocyanate or Phycoerythrin: CD34, CD45, CD73, CD90, CD105 (eBioScience, San Diego, CA), and subjected to flow cytometric analysis using a Beckman Colter flow cytometer and FACScan program (eBioScience).

MRI

For magnetic field treatment, the MSCs in the logarithmic growth phase were examined under a 1.5 Tesla MAGNETOM ESSENZA Siemens MRI scanner (Ali-ebn Abitaleb Hospital, Rafsanjan, Iran). Protocols of MRI can be a little different at different MRI centers, however in this study MRI protocol included T2 – STIR (Short-Tau Inversion-Recovery) and T1 – TSE (Turbo Spin Echo) in coronal plane, T2 – TSE in coronal, sagittal and Axial plane, T1 – TSE and T1 – TSE with spectral Fat Saturation (FS) in Axial plane. These 7 pulse sequences were performed in total scan time of 10 minutes for samples. Table 1 shows the sequences and physical parameters in details.

The effect of MRI on mesenchymal stem cells doubling time

To compare the growth and proliferative characteristics of the cells, the population doubling time were measured. Doubling time was calculated using the formula DT = (t2 − t1) log(2)/ log(n2 − n1) where n1 and n2 are initial and final cell numbers and, t2 and t1 are the number of days in culture.

The effect of MRI on mesenchymal stem cells colony forming potential

The capacity of the cells for self-renewal can be evaluated by colony forming assay. The MSCs were seeded at a density of 1.6-2 cells/ cm culture flask for colony development. After 14 days, the colonies were stained with 0.5% crystal violet (Sigma-Aldrich, St. Louis, MO, USA) for 5 min at room temperature. The colonies on the plates were photographed and counted for comparison of plating efficiency (PE) and surviving fraction (SF) (Clonogenic Assay: Adherent Cells):

PE (The ratio of the number of colonies to the number of cells) was calculated by the following formula: PE = number of colonies counted/ number of cells plated. SF parameter as the number of colonies that arise after treatment of cells is calculated using the formula: SF = number of colonies counted/ number of cells plated × PE.

Cytotoxicity (Viability) assay

Spectrophotometric 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assays was used to determine possible toxic effect of MRI on MSCs viability. After incubation of MRI-treated and untreated MSCs for 24 h and 48 h at 37 °C, cells were incubated with MTT solution for 4 h and then the medium was substituted by DMSO followed by measuring optical density at 570 nm using a ELISA reader (BioTek ELX800, Winooski,Vermont, USA).

Apoptosis assay

To assess the cell death of MRI-treated and untreated MSCs by Annexin V/PI double staining methodology, 1 × 106 cells/mL were labelled with Annexin V-FITC and PI, and then cells were analyzed using a flow cytometer (Becton-Dickinson, San Jose, CA). FloMax software was used to determine the fraction of cells in each quadrant.

Real Time -PCR analysis for gene expression

Total RNA from MRI-treated and untreated MSCs was extracted using TRIzol reagent (Invitrogen, Carlsbad, CA, USA) and converted to cDNA using the SuperScript TMII enzyme (Inc., CA, USA) according to the manufacturer's instructions, and cDNA was amplified with gene specific primers. qRT-PCR was performed using Fast SYBR Green Master Mix (Applied Biosystems, Foster City, CA) applying the primers detailed in Table 2. As an internal control, levels of GAPDH were quantified in parallel with target genes.

Cryopreservation-induced cell death (CICD)

MRI -treated and -untreated MSCs were cryopreserved for at least 4 month prior to further evaluation. To evaluate MSCs after thawing, cryovials were retrieved from liquid nitrogen storage and and rapidly thawed in a 37°C water bathwater.

Statistical analysis

All the values are expressed as mean ± standard deviation. Statistical analysis was performed using SPSS 18 (SPSS, Munich, Germany). Differences between treatment group and control were determined using independent sample Student t-test. A p-value less than 0.05 was considered statistical significant.

Results

Characterization of MSCs

Umbilical cord derived-MSCs were isolated and expanded based on their plastic adherence. Phenotypic characterization showed that isolated MSCs were positive for the MSC-specific markers CD90 and CD105 but negative for the CD34 and CD45 (hematopoietic markers). Multi-lineage differentiation potential of MSCs cultured in adipogenic and osteogenic differentiation media showed positive staining with Oil Red O and Alizarin Red, respectively (Figure1).

Toxicity assay

Viability assay showed that MRI is safe in that dose (1.5 T). Analysis of changes in cell viability after MRI treatment was done using the MTT assay. An increase in cell viability was shown in MRI-treated group compared to untreated-control group at 24 h, 48 h and 72 h, but the differences were not statistically significant (Figure2).

Doubling time

The proliferation potential of MSCs in MRI-treated group and control group was assessed by calculating the population doubling time. The DT of MRI-treated MSCs was significantly higher than untreated control MSCs. The data indicating that MSCs in MRI group had slower growth than MSCs in control group (Figure3).

Colony Forming Assay

The colony forming assay was applied to examine the self-renewal potential of the cells. The present study showed that MRI led to a dramatically increase in the clonogenicity of mesenchymal stem cells with PE of 3.03±0.743 and SF of 28.9±13.6 compared to MSCs without treating (Figure4 and Table3).

Apoptosis assay

In order to quantify and analyze the percentage of apoptotic cells after MRI treatment, MSCs were stained with Annexin-V FITC/PI and analyzed by flow cytometry. Living, apoptotic, and necrotic cells were defined as Annexin-V^{-}/PI^{-}, Annexin-V^{+}/ PI$^{-/+}$, and Annexin-V^{-}/PI^{+}, respectively. The apoptosis of mesenchymal stem cells after the MRI treating showed no differences between the MRI group in comparison with the untreated-control group (Figure5).

Gene expression analysis by real time PCR

- **Bax and Bcl-2**

Results showed that the mRNA level of Bax, as a proapoptotic marker, significantly increased, whereas the mRNA level of Bcl-2, as an antiapoptotic marker, was not significantly changed in the MRI-treated MSCs (Figure6).

- **Stemness markers (Oct-4, Sox-2 and Nanog)**

To investigate the effect of MRI on MSCs, the mRNA expression levels of three major stemness markers, Oct-4, Sox-2and Nanog, were assessed. The quantitative results revealed that the expression of Sox-2and Nanog in MRI group was significantly higher than that in control group (213- and 32-fold increase, respectively, for Nanog and Sox-2) (Figure7)., but there was no significant difference in Oct-4 mRNA level.

Cryopreservation-induced cell death assessment

To identify and characterize the influence of cryopreservation on cell viability and apoptosis of MSCs, cells were cryopreserved with DMSO and analyzed after thawing. Results show that there is no regrowth of MRI-treated MSCs after thawing (Figure8).

Discussion

MSC-based cell therapy as a promising therapeutic strategy is used in various clinical fields, including tissue regeneration and immunomodulatory therapy.

MSCs are considered to be relatively safe in terms of genomic instability compared to induced pluripotential stem (iPS) cells and embryonic stem (ES) cells (Kim et al. , 2017). In the advancement of stem cell therapy, attempts are being made to develop an efficient production system to produce sufficient clinically relevant numbers of MSCs in a timely manner (Han et al. , 2014).

The aim of this study was to investigate of MRI effects on stemness genes (Oct-4, Sox-2 and Nanog) expressions on MSCs.

Our results are in consistent with previous studies that showed ectopic expression of stemness genes, such as Oct-4, Nanog, and Sox-2, promotes the proliferation potential of NIH 3T3 fibroblasts and MSCs while maintaining the lineage differentiation capacity (Go et al. , 2008, Zhang et al. , 2005).

Lengner and colleagues showed that oct-4 has no role in the self-renewal of somatic stem cell (Lengner et al. , 2007). Recent study revealed that Nanog maintains self-renewal capability of MSCs by delaying cellular senescence, increases the endogenous expression of Oct-4 and Sox-2, and preserves stemness (Park et al. , 2019).Many studies have shown that both Oct-4 and Sox-2 bind the Nanog promoter and induce Nanog expression (Park, Jun, 2019).Park et al. reported that secretome from Nanog-overexpressing MSCs accelerated the telogen-to-anagen transition in hair follicles and could be an excellent candidate as a powerful anagen inducer and hair growth stimulator for the treatment of alopecia (Park, Jun, 2019).

In this study, we also showed that long term effect of exposure to 1.5 T MRI for 10 min enhanced colony formation capacity (approximately 25 folds) of WJ-MSCs and increased number of active stem cells. In addition, the findings of the flow cytometry revealed that MRI treatment does not influence percentages of WJ-MSCs in apoptosis. Result showed that short term exposure to magnetic field did not induce apoptosis after 24 and 48 hours.

In the current investigation, we evaluated the cytotoxic effects of MRI treatment on WJ-MSCs using the MTT assay. MTT assay determines cell viability by quantitative assessment of a metabolic product and indicates activity of mitochondria in living cells; which has a direct relationship with cell proliferation and longevity (Tabatabaei et al. , 2015). The results indicated that MRI does not significantly impact on metabolic activity, mitochondria function and viability of WJ-MSCs.

In addition, we utilized doubling time assay to investigate the average duration of cell growth and division. The findings reported that MRI can decrease the average division time of WJ-MSC in short time, however, colony formation assay showed that this change was not permanent and reversed after a short time.

The result of MRI effect on regrowth of the cryopreserved MSCs suggests that the cell death of all MRI-treated MSCs may be due to increased sensitivity of these cells to the physical and chemical stress caused by the freezing process.

There is growing evidence that the increase in ROS content is the most common event for various types of cells after cryopreservation (Savitskaya and Onishchenko, 2016).

It has been shown that cryopreservation procedures can induce both apoptosis and necrosis (Savitskaya and Onishchenko, 2016). It was previously shown that during thawing in mesenchymal stem cells, apoptosis was induced by the activation of apoptosis-related proteins including Ca^{2+}-dependent protease calpain, caspase-8, -9, and -3(Bissoyi and Pramanik, 2014).

Previous investigations have demonstrated that extended exposure to magnetic field decreased the viability percent and/or proliferation rate of stem cell and terminally differentiated somatic cells(Javani Jouni et al. , 2013, Marędziak et al. , 2014, Raylman et al. , 1996, Rosen and Chastney, 2009).

Seven-day exposure to 0.5 t static magnetic fields (SMF) inhibited viability, proliferation, cytokines secretion, surface antigen expression, and adipogenic and osteogenic differentiation of adipose-derived stem cells (ASCs) (Wang et al. , 2016). Several studies have demostrated that different intensities of the magnetic field and durations of exposure did not cause DNA damage in cells, such as ASCs (Wang, Xiang, 2016), THP1 (Amara et al. , 2007), peripheral blood mononuclear cells (Reddig et al. , 2015) and leukocytes(Kubinyi et al. , 2010).

Gruchlik et al. demonstrated that exposure to a SMF (300mT) for 24 hours inhibited the IL-6 secretion in normal human colon myofibroblasts (Gruchlik et al. , 2012).

Vergallo et al. reported that SMF exposure (1.4T, 24 h) had a significant inhibitory effect on the release of IL-6, IL-8, and INF-α from macrophage (Vergallo et al. , 2013).

In another study, exposure to a strong SMF (4.75T) for 24–48 hours significantly decreased the production of IL-2 in human peripheral blood mononuclear cells (Aldinucci et al. , 2003).

According to previous studies, it seems that the exposure to magnetic fields is associated with epigenetic changes but not genetic changes (mutations or DNA damage).

Review of the current literature suggests that Wharton's jelly-derived mesenchymal stem cells (WJ-MSCs) have the best potential to differentiate into adipocytes, chondrocytes, osteocytes, muscle cells, neurons, cardiomyocytes, and hepatocytes (Beeravolu et al. , 2017).

WJ-MSCs have been widely explored for cell-based therapy of immune-mediated, inflammatory, and degenerative diseases, due to their remarkable anti-inflammatory, immunosuppressive, immunomodulatory and regenerative potentials (Liau et al. , 2020, Noronha et al. , 2019).

Overall, our findings suggest that MRI treatment could be a useful approach to produce high-quality MSCs by improving their stemness properties.

This strategy has great potential for developing pluripotency in WJ-MSCs to become more versatile in clinical applications.

To the best of our knowledge, no other study has investigated the MRI effects on the stemness genes expression of MSCs.

However, it is worth noting that cancer stem cells, as a key driver of tumor formation and metastasis, express deregulated stemness-associated genes, such as OCT-4, Nanog, SOX-2 (Müller et al. , 2016). In addition, the personal exposure to MRI has the potential for deregulation of stemness related genes in normal stem cells. Therefore, it seems that precautions should be taken to reduce the possible risks of MRI exposure. Further studies are needed to determine MRI-treated MSCs have no adverse or unwanted effects following cell therapy. Our findings suggest that MRI treatment may be a useful strategy for gaining high-quality MSCs in clinical studies of MSC-based therapies.

Finally, preclinical and clinical studies should be designed to identify whether MRI treatment can be an effective approach to improve the therapeutic function of MSCs.

Conclusion

MSCs exposed to MRI promote colony-forming potential and induce stemness genes (Oct-4, Sox-2 and Nanog) expression. Therefore, it suggests that MRI may enhance the efficacy of mesenchymal stem cell-based cell therapy.

Declarations

Ethics approval and consent to participate

The work was approved by the local ethical review board.

Consent for publication

All authors gave consent for the publication.

Competing interests

None.

Funding

This project was supported by Rafsanjan University of Medical Sciences, Rafsanjan, Iran.

Authors' contributions

Mahnaz Tashakori designed the experiments, supervise the project, writing the manuscript; **Fatemeh Asadi** performed the experiments**,** data analysis**; Faezeh-Sadat Khorram** performed the experiments**; Azita Manshoori** contributed to sample preparation**; Ali Hosseini-Chegeni** wrote the manuscript**; Mahdieh Ahmadi Kamalabadi** designed the experiments**; Aliakbar Yousefi-Ahmadipour** designed and performed the experiments, data analysis, wrote the manuscript, supervised the project

Data Availability Statement

The data that support the findings of this study are available on request to the corresponding author.

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Tables

Due to technical limitations, table 1, 2 and 3 is only available as a download in the Supplemental Files section.

Figures

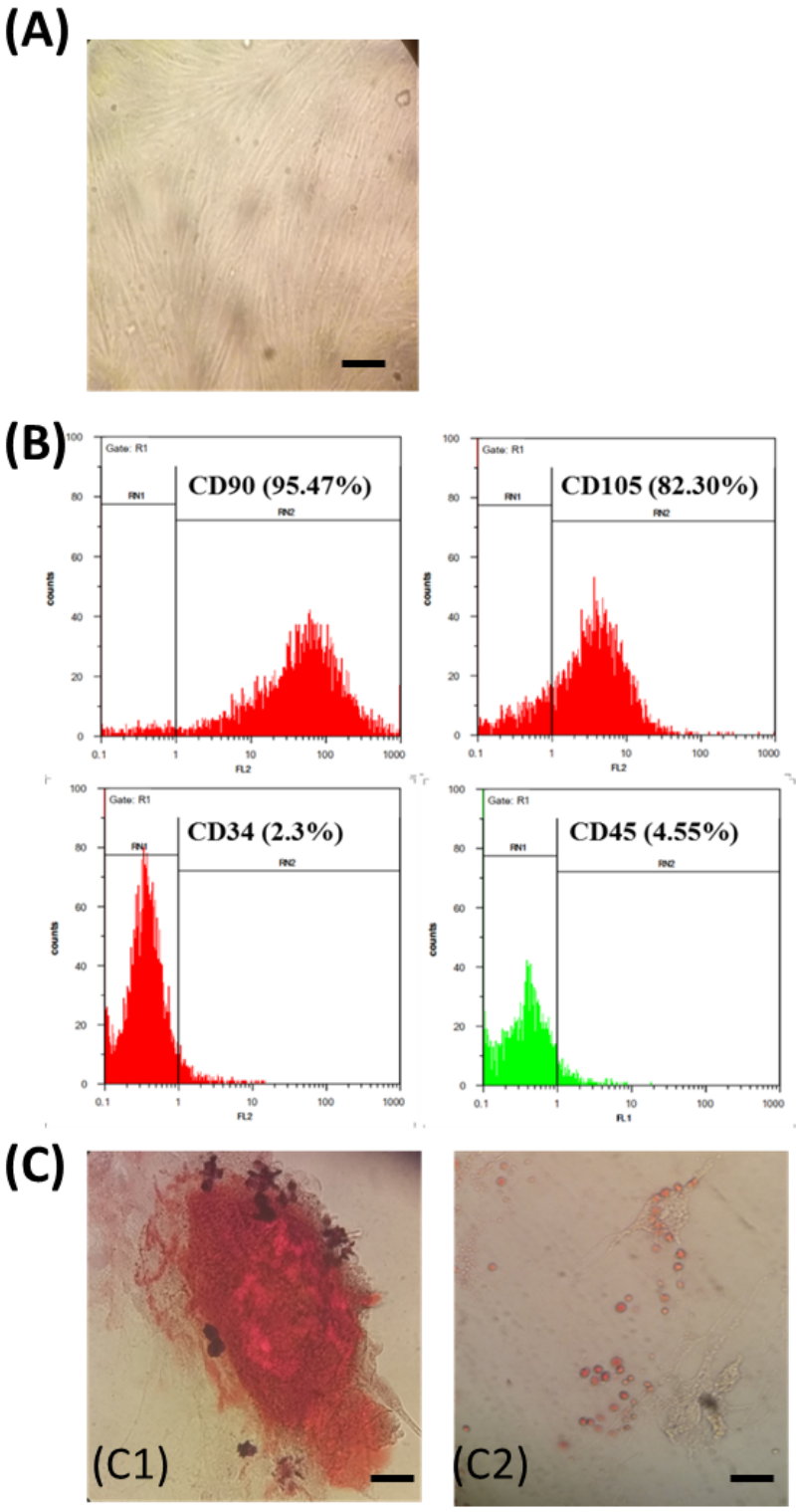

Figure 1

Isolation, characterization and differentiation capacity of umbilical cord mesenchymal stem cells. (A) Morphology of the adherent-mesenchymal stem cells (magnifications: ×100). (B) Immunophenotype of MSCs. Immunophenotype of MSCs analyzed by flowcytometry. Positive markers (CD90, and CD105) were expressed whereas negative markers (CD34 and CD45) were not. (C) The representative images of

osteogenic (C1, ×100) and adipogenic (C2, ×100) differentiation of MSC in vitro. Scale bar indicates 50 μm.

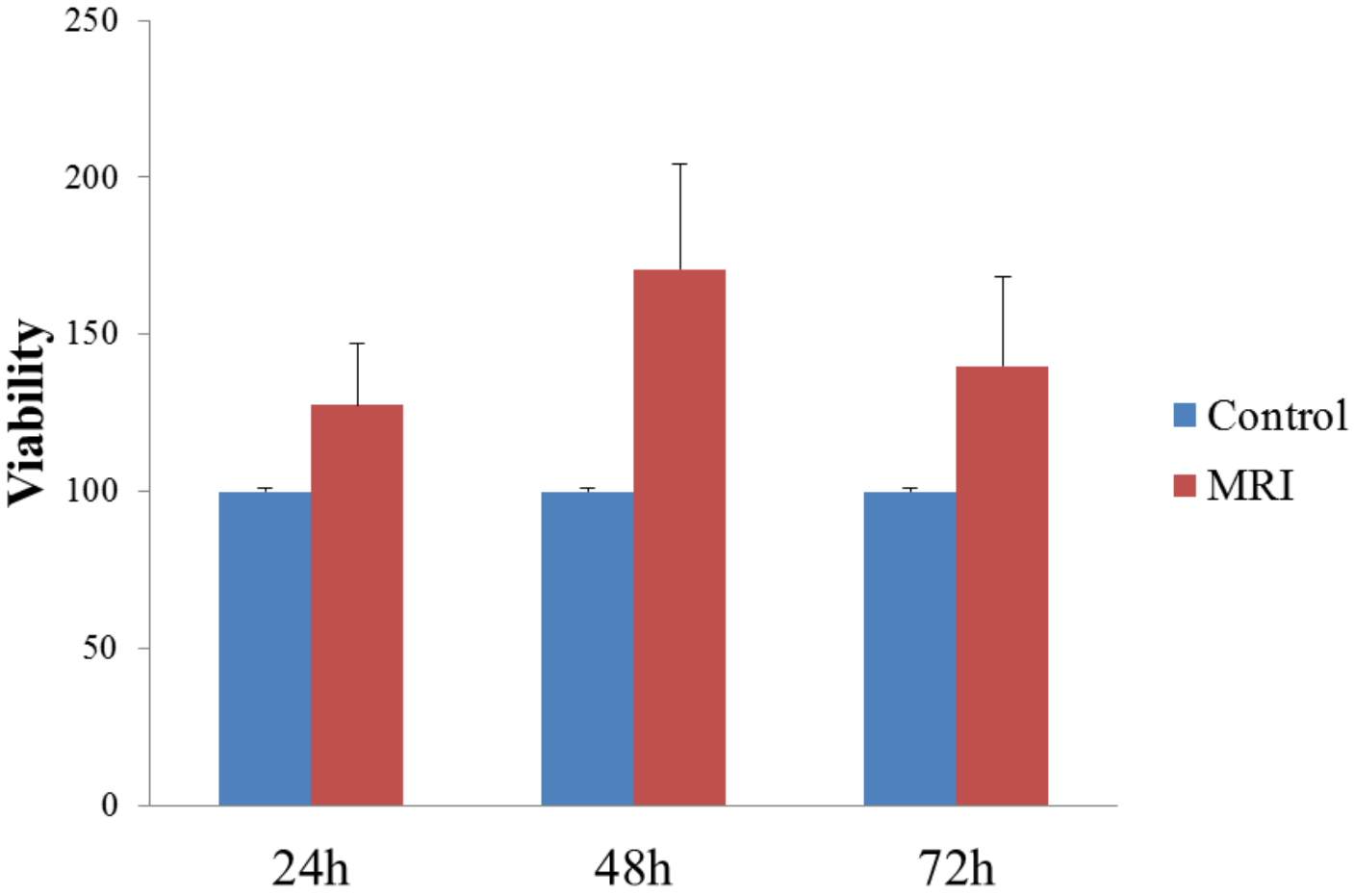

Figure 2

Effects of MRI on cell viability. There was no significant difference in MSCs viability in two treatment groups after 24 h, 48 h and 72 h.

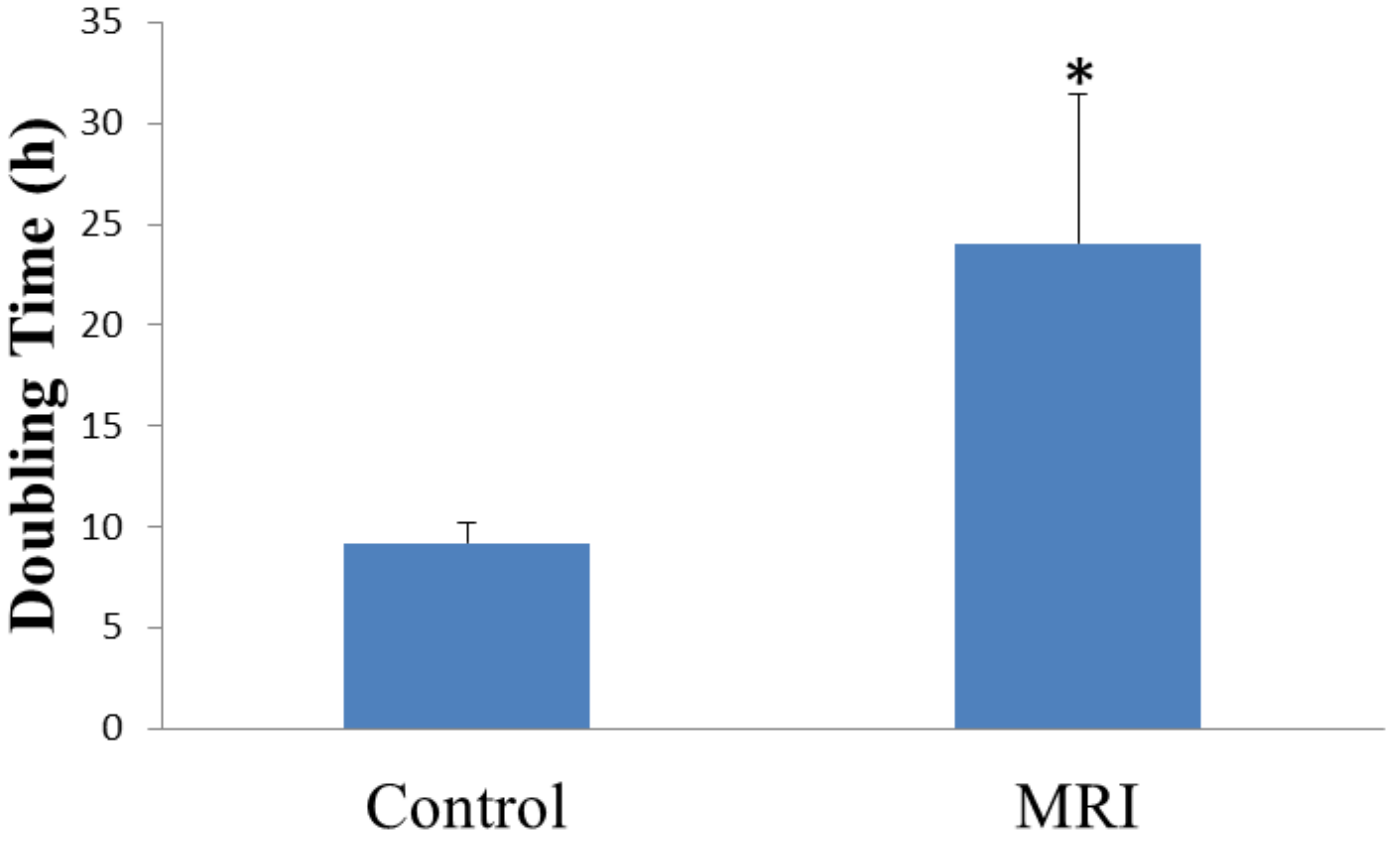

Figure 3

The doubling time of MRI-treated mesenchymal stem cells compared with untreated mesenchymal stem cells was significantly increased. *p < 0.05 versus control group

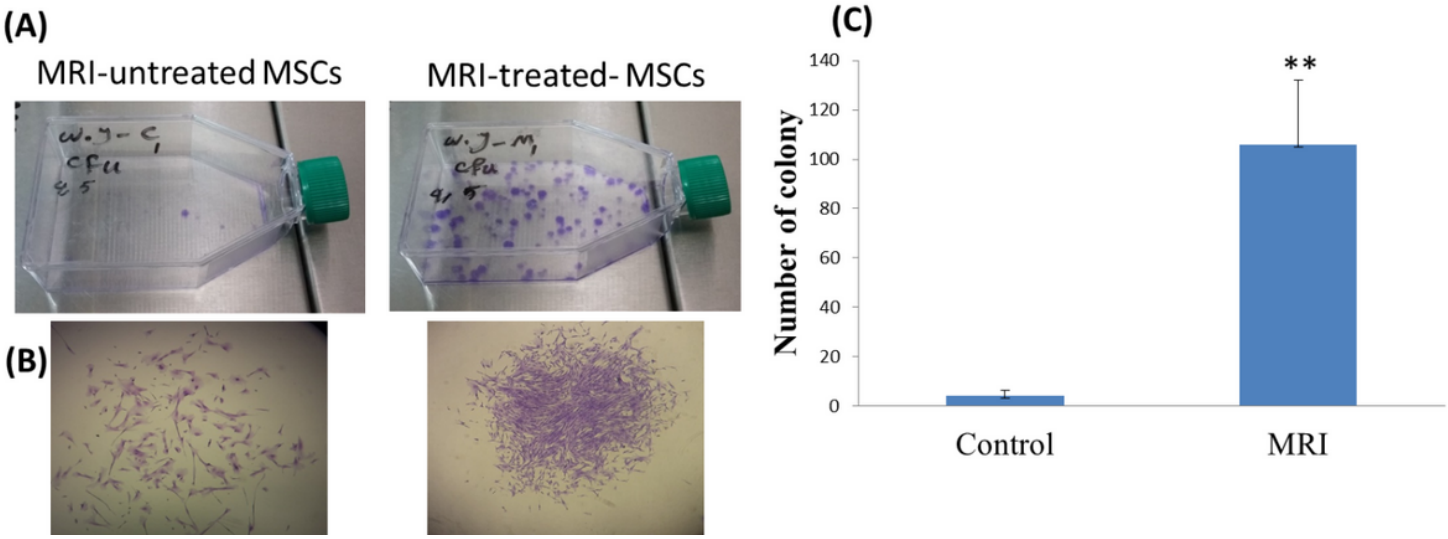

Figure 4

MRI enhanced colony formation ability of mesenchymal stem cells. A colony formation assay was performed on MRI-treated MSCs for 14 days. (A) Macroscopic and (B) microscopic appearance of MSCs

in different groups. (C) Colony number of MSCs in two treatment groups. **p < 0.01 versus control group

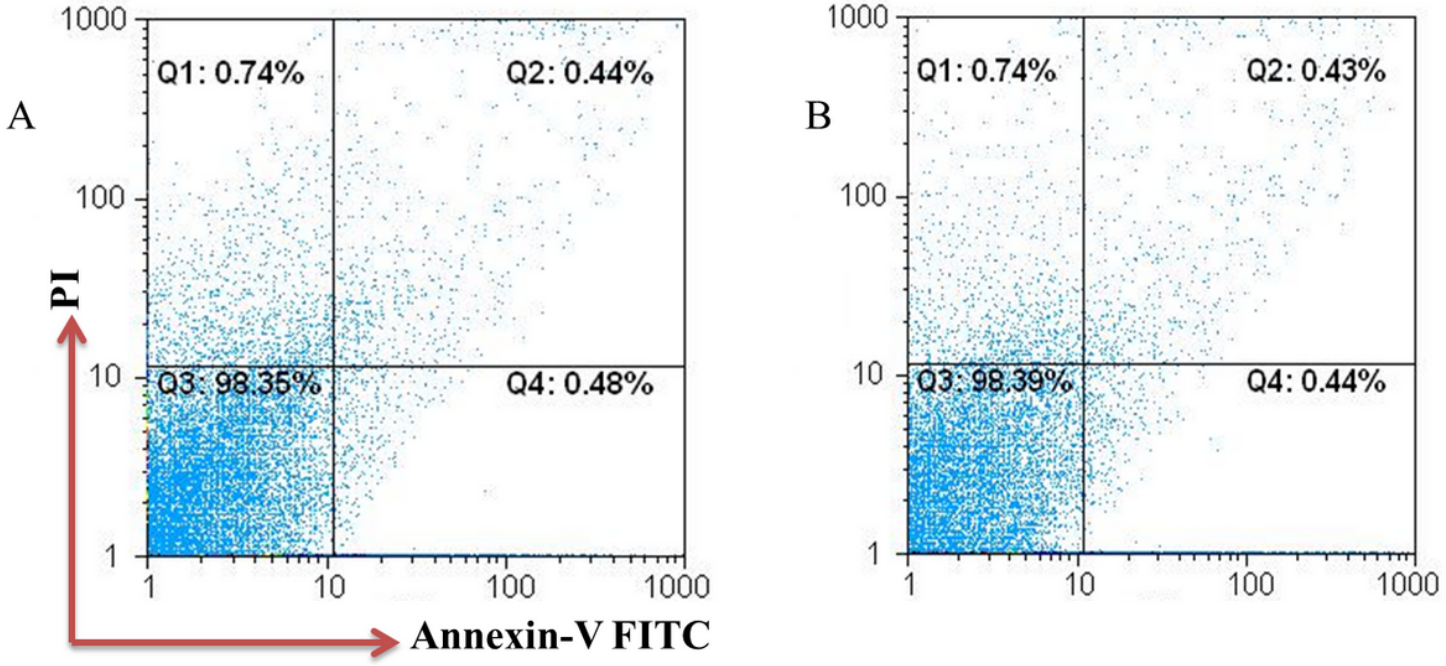

Figure 5

Representative dot plot of untreated MSCs (A) and MRI treated MSCs (B). Flow cytometry data indicate that apoptosis was not significantly different between the two groups.

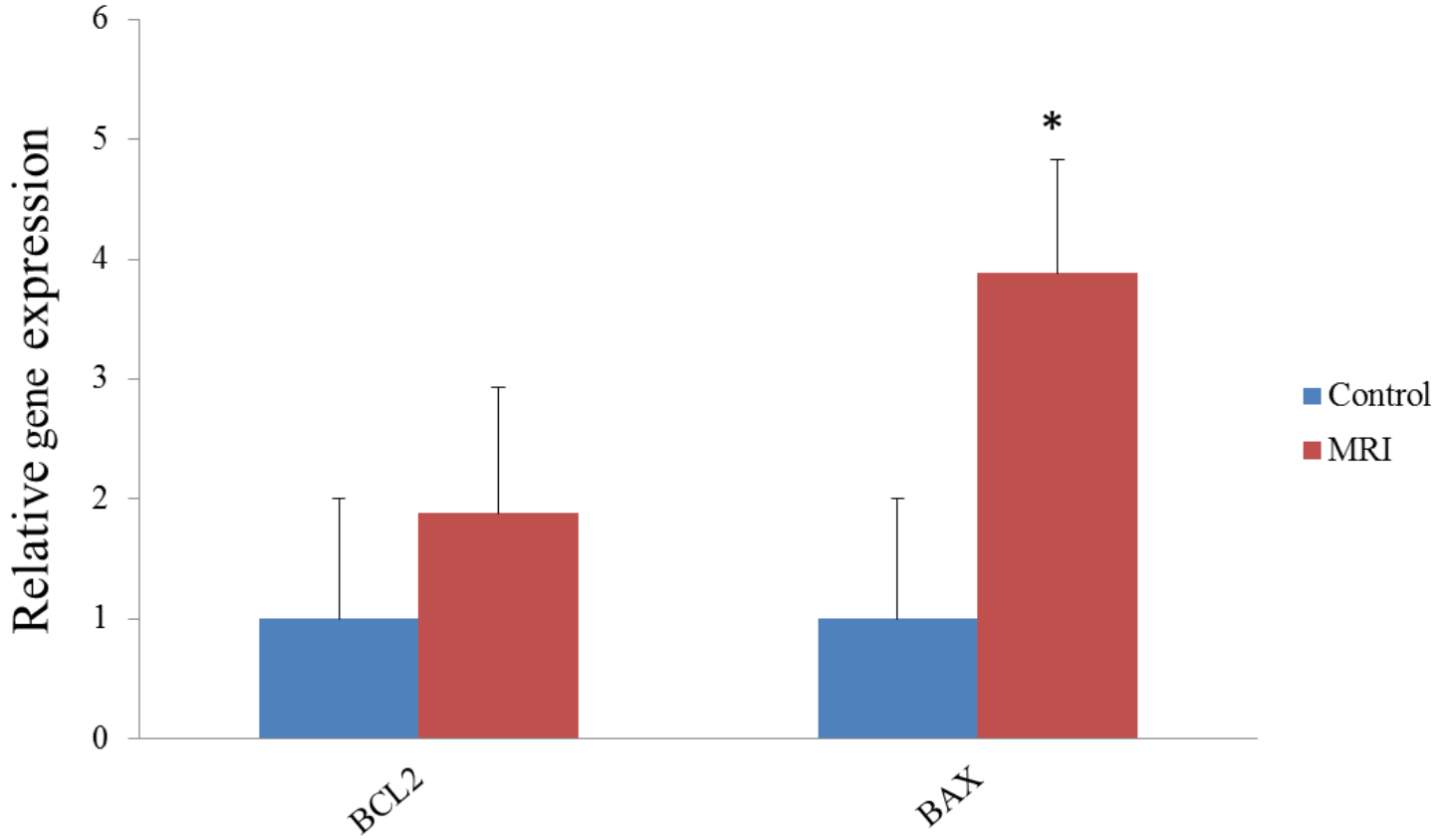

Figure 6

Relative mRNA expression levels of apoptosis-associated genes, Bax and Bcl-2, by real-time PCR. The results are expressed as mean ± SD of three independent experiments performed in triplicate. *p < 0.05 versus control group

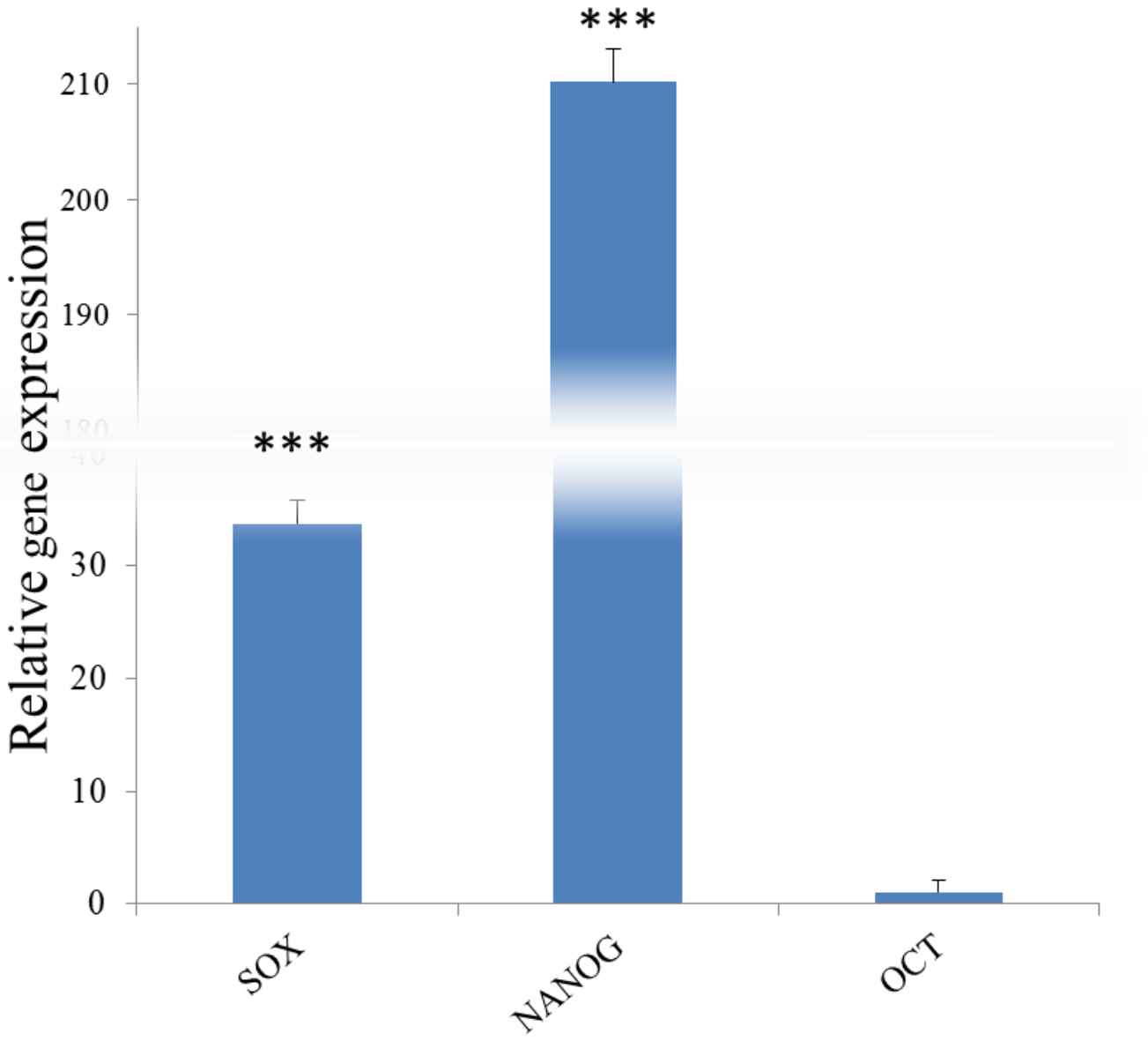

Figure 7

Relative mRNA expression levels of stemness genes, Oct-4, Sox-2 and Nanog, in MSCs after MRI treatment by real-time PCR. The results are expressed as mean ± SD of three independent experiments performed in triplicate. ***p < 0.001 versus control group

Supplementary Files

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

- Table1.docx

- Table2.docx
- Table3.docx