

Bone marrow mesenchymal stem cells modified pathological changes and immunological responses in ovalbumin-induced asthmatic rats possibly by the modulation of *miRNA155* and *miRNA133*

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Abstract. In the current experiment, we investigated the immune-modulatory potential of mesenchymal stem cells (MSCs) and conditioned media (CM) in attenuating of chronic asthmatic changes in a rat model. Male rats were divided into control (C) and ovalbumin-sensitized (S) groups, which further allocated into three subgroups; rats received systemically 50 μ l volume of PBS (C and S groups), CM (CSV and SSV groups) and rats received intravenous infusion of 2×10^6 bone marrow-derived mesenchymal stem cells (rBMMSCs) (CCV and SCV groups). Two weeks later, the expression of interleukin (IL)-4, IL-13, and IL-10, *miRNA133*, and *miRNA155* was measured by real-time PCR. Pathological changes and the recruitment of rBMMSCs into pulmonary parenchyma were evaluated by histopathological and immunofluorescence analyses, respectively. The systemic injection of rBMMSCs, but not CM, decreased the levels of IL-4, IL-13, IL-10, *miRNA133*, *miRNA155* and reduced pathological changes in sensitized rats as compared with other sensitized groups ($p < 0.001$ to $p < 0.05$). rBMMSCs transmigrated to lung tissue in cell-administrated rats, albeit intensity of asthmatic changes, in turn, affected the amount of recruited cells. Collectively, our data suggest the potential role of MSCs, but not CM, in reducing pathological changes possibly *via* the modulation of *miRNA133* and *miRNA155* during asthmatic changes.

Key words: Asthma — Conditioned media — Mesenchymal stem cells — *miRNA133* — *miRNA155*

Introduction

Asthma is commonly regarded as a debilitating chronic respiratory disorder caused by a plethora of factors with profound impacts on socioeconomic costs. Based on statistics, asthma involves approximately 300 million people of all ages worldwide (Murray and Lopez 1997; D’Cruz et al. 1999;

Bharadwaj and Agrawal 2004; Martinez 2007). Despite substantial advances in human medicine, asthma is not curable permanently. The current conventional pharmacotherapy approaches only suppress and alleviate asthma-related attacks and have different side effects. The asthma manifestations are most likely to be returned following treatment discontinuation (Guilbert et al. 2006; Douglas et al. 2008; Keyhanmanesh et al. 2014).

Histopathological changes in the lung tissues of asthmatic subjects are mainly induced by the chronic inflammatory responses due to the up-regulation of cytokines produced by type 2 T helper cells (Th2) (Huang et al. 2001). Therefore,

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emerging therapeutic strategies for asthma management should be focused on the reversal of Th2/Th1 imbalance and the control of inflammation rate (Gholamnezhad et al. 2014; Aslani et al. 2016; Fallahi et al. 2016). Th2-derived cytokines such as interleukin IL-4 and IL-13 orchestrated asthma pathophysiology by the interaction with lung-resident fibroblasts, epithelial cells and smooth muscle cells (Gholamnezhad et al. 2014; Fallahi et al. 2016). On the other hand, IL-10, an anti-inflammatory cytokine is secreted by T regulatory cells could down-regulate Th2-mediated inflammatory response in allergic asthma. Hence, the modulation of pro- and anti-inflammatory cytokines is thought to be essential for a successful resolution of inflammation in asthma (Todorova 2007). In line with this statement, the immune-modulatory characteristics of mesenchymal stem cells (MSCs), are orchestrated by trans-differentiation capacity and paracrine activity, makes them very powerful therapeutic tools for a wide spectrum of inflammatory diseases such as asthma (Kyurkchiev et al. 2014; Ma et al. 2014). The survival and differentiation of the MSCs at the injured sites are confined possibly because of ischemic microenvironment. Therefore, it is proposed that MSCs paracrine effect is essential to exert therapeutic effects (Gallina et al. 2015). Since stem cell-secretome yields some of the therapeutic effects, it is logical that the paracrine effects of MSCs could be observed in their condition media (CM) (Horie et al. 2012; Gallina et al. 2015). The administration of CM could circumvent some of the current problems associated with MSCs such as immunological reactions, tumorigenicity, infectious agents, total costs, and long waiting time for cellular expansion (Sze et al. 2007). A great body of studies unveiled BMMSCs suppressed Th2 cell responses and mitigated inflammation in ovalbumin-induced asthmatic mice (Goodwin et al. 2011; Sun et al. 2012). However, underlying mechanisms driven by MSCs during asthma have still not been fully documented and must be addressed in animal models that are applicable to human, especially rat.

MicroRNAs (miRNAs) are conceived as a novel class of biomarkers for numerous inflammatory diseases (Mi et al. 2013). They possess a single-stranded, small, non-coding RNAs, suppressing gene expression at the post-transcriptional level by the subversion or inhibition of target mRNAs. miRNAs were found to participate in airway inflammatory regulation *via* the modulation of Th2 cell function (Kishore et al. 2014; Rebane and Akdis 2014; Simpson et al. 2014).

The global pattern of miRNA profiles in respiratory niche can be regulated by inflammatory agents (Wang et al. 2011). Commensurate with this statement, a plethora of experiments showed multiple miRNAs expressed differentially in murine lung tissues following sensitization with ovalbumin (Garbacki et al. 2011). For instance, in an animal model of asthma, the up-regulation of *miRNA155* was recorded while *miRNA133* was down-regulated as compared with the

control group (Kai et al. 2015). Actually, the manipulation of these miRNAs in numerous allergic asthmatic models attenuated the inflammatory status and closed it to near-normal ranges (Wang et al. 2011; Kai et al. 2015).

However, no enough data exist on the modulatory effects of MSCs related to the expression of miRNAs in asthmatic rats. The elucidation of the underlying mechanisms governed by MSCs in asthma will help to achieve the beneficial clinical application for stem cell therapy in human medicine. Therefore, we here hypothesized that MSC-mediated immunomodulatory properties would be directed *via* the regulation of miRNA expression in asthmatic lung tissues.

Materials and Methods

Ethical issues

All phases of our study were implemented in a guideline with of "The Care and Use of Laboratory Animals published by the National Institutes of Health" (NIH Publication No. 85-23, revised 1996) and agreed by the local ethical committee of Tabriz University of Medical Sciences (No: TBZMED.REC.1394.386).

Cell isolation and expansion procedure

Rat bone marrow-derived mesenchymal stem cells (rBMMSCs) were extracted in accordance with previously published protocols (Rahbarghazi et al. 2012). In brief, the animals were killed humanely by an overdose combination of xylazine and ketamine solution. After complete dissection and the removal of femurs and cutting the epiphysis with a scissor, the medullary components were aspirated under aseptic conditions immediately by phosphate-buffered solution (PBS) containing 2% fetal bovine serum (FBS; Gibco, USA). Then, fresh bone marrow-derived mononuclear cells were harvested by gradient centrifugation using Ficoll solution (Sigma, USA) at $400 \times g$ for 20 min and washed next twice by PBS. Thereafter, isolated cells were resuspended in Dulbecco's modified Eagle's medium-low glucose (DMEM/LG; Gibco, USA) supplemented with 20% FBS, 100 U/ml penicillin and 100 mg/ml streptomycin (Biosera, UK). Finally, 1×10^5 cells were seeded in 6-well culture plates (SPL). The medium was replenished every 3–4 days. For cell subculture, rBMMSCs were trypsinized at 70–80% confluency and subjected to subsequent analyses, at passage three.

Immunophenotypic characterization of isolated rBMMSCs

rBMMSCs were immunophenotyped by flow cytometry analysis (Rahbarghazi et al. 2014). We used a panel of dif-

ferent monoclonal antibodies directed against the stem cell surface markers, including FITC-conjugated anti-CD133, CD44, and CD34 as well as PE-conjugated anti-CD31 (all purchased from ebioscience, USA). Isotype control antibodies were also used to normalize background staining. Cells were trypsinized by using 0.25% Trypsin-EDTA solution (Gibco, USA), washed by PBS, and blocked by 1% bovine serum albumin (BSA; Sigma, USA) for 15 min. Antibodies were added into 100 μ l of PBS containing 5×10^5 cells and incubated for the next 30 min at RT according to manufacturer's recommendation. Finally, cells were analyzed with BD FACSCalibur Flow Cytometer system (USA) and raw data processed by Flow Jo software version 7.6.1.

Vital staining of rBMSCs via Cell Tracker

We used a Cell Tracker dye to follow recruitment and homing of rBMSCs into the pulmonary parenchyma in sensitized rats. After trypsinization, cells were labeled with 2 μ M Cell Tracker™ CM-Dil at 37°C for 20 min (Catalog No. C-7000; Molecular Probes, Invitrogen, USA). A total number of 2×10^6 CM-Dil pre-labeled cells in 50 μ l of PBS was administered gently to each animal's femoral vein.

Conditioned media (CM) harvesting

Possible paracrine effects of rBMSCs in the alleviation of ovalbumin-induced asthma intensity were investigated by rBMSCs-derived CM. By 70–80% confluency, the exhausted DMEM/LG was discarded and cells were washed three times with PBS. Subsequently, DMEM/LG-free FBS was added to flasks (Mao et al. 2013a). The supernatant was collected after 72 h, centrifuged at $400 \times g$ for 5 min, filtered by 0.20- μ m-pore syringe filter. An approximately 50-fold concentrated solution was prepared by using centrifuge filter tubes with molecular weight cut-offs at 4 kDa (Catalog No. 003099.125, Eppendorf, Germany). The protein content of concentrated CM was measured by using bicinchoninic acid assay (BCA assay, iNtRon, Korea). Our preliminary analysis showed that total protein content of concentrated CM reached to 500 μ g/ml. An equal volume of CM, containing similar protein concentration was used in this experiment. We injected 50 μ l of concentrated CM *per case* intravenously (Abreu et al. 2013a).

Animal sensitization and animal groups

In this study, 40 healthy mature male Wistar rats with body weight at range of 200–250 g were used. The animals were accommodated in standard cages at $20 \pm 2^\circ\text{C}$ under 12:12 light/dark cycle with free access to food and water. Then, four healthy rats were randomly allocated for extraction of rBMSCs prior to sensitization process. The remaining rats

were classified into two main groups; healthy (C) and sensitized (S) rats which further were divided into 3 subgroups, each included six animals. The control (C) and sensitized (S) rats were treated as follows:

1. Rats only received 50 μ l PBS (C and S)
2. Rats received 50 μ l CM (CSV and SSV)
3. Rats received 50 μ l PBS containing 2×10^6 rBMSCs (CCV and SCV)

In the sensitized groups, rats were exposed with ovalbumin over a course of 32 ± 1 days according to previously published methods (Schuster et al. 2000). Each animal was *intraperitoneally (i.p.)* co-injected with the combination of 1 mg of ovalbumin (Sigma-Aldrich, USA) and 200 mg aluminum hydroxide, as adjuvant, pre-dissolved in 1 ml saline on first and 8th days. By 14th day, the sensitized rats were accommodated to aerosolized environment of 4% ovalbumin generating by a nebulizer (CX3, Omron Co., Netherland), for 18 ± 1 consecutive days. Our protocol last for an approximate period of 5 min daily. The experiment was tailored in a special locked box with dimensions $30 \times 20 \times 20 \text{ cm}^3$. In control rats without any sensitization, saline solution was used instead of ovalbumin using the same protocol (Neamati et al. 2013). One-day post-sensitization, the rats were enrolled to the experimental procedure of cell administration or CM treatment through the left femoral vein (Ahmadi et al. 2016). Calling attention, some authorities declared that the respiratory responses related to ovalbumin-induced sensitization continued over a course of two weeks (Khazdair et al. 2013; Gholamnezhad et al. 2014).

Tracking of migrated cell by immunofluorescence assay

Snap-frozen and sectioned at 5 μ m thickness were prepared using a Cryostat apparatus to ascertain the presence of injected cells in respiratory tissues (Rahbarghazi et al. 2014). The prepared sections were thawed at room temperature, washed twice with PBS (5 min each) and stained with 4', 6-diamidino-2-phenylindole (DAPI, 1 μ g/ml, Catalog# D9542, Sigma-Aldrich, USA) for background counterstaining.

Total RNA extraction and real-time PCR

Two weeks after injection of cell suspension or CM, rats were anesthetized with the combination of ketamine and xylazine solution (75 mg/kg and 3 mg/kg *i.p.*). Left lung was instantly excised, washed with saline solution and snap-frozen in liquid nitrogen. Then, total RNA from lung homogenate was extracted using Total RNA extraction mini kit (YTA, Taiwan) according to manufacturer's instruction (Biyashev et al. 2012; Lässer et al. 2012). The purity of isolated RNAs were confirmed by a NanoDrop 1000 Spectrophotometer (Thermo Scientific, Wilmington DE 19810 USA). The ex-

pression of *IL-4*, *IL-13*, *IL-10*, *miRNA133* and *miRNA155* genes were quantitatively assessed by real-time PCR. Primer sequences for each mRNAs were chosen using Gene-Runner Software, version 3.05 (Table 1). Reverse transcription was performed by cDNA Synthesis Kit (YTA, Taiwan) to determine mRNA expression levels. In addition, the expression profile of miRNAs was performed on total RNA extracts by miR-amp kit (Parsgenome Co, Iran). Each cDNA was used as a template for separate assay for miRNAs and mRNAs quantitative real-time PCR by using SYBR Green master mix (YTA, Taiwan). Real-time PCR reactions were carried out on a Rotor-Gene 6000 instrument (Corbett Life Science, Australia). Housekeeping *GAPDH* (glyceraldehyde-3-phosphate dehydrogenase) gene was used to normalize the amount of PCR products for mRNA samples and miRNA191 for *miRNA133* and *miRNA155*. We used $2^{-\Delta\Delta CT}$ formula to ascertain relative-quantitative expression levels of individual miRNAs and mRNAs. The results were expressed as fold change versus the relevant controls.

Pathological evaluation

The right lung of each animal was removed and fixed in buffer formalin 10% (37%, Merck, Germany). After one week, tissue specimens were processed through Passage method by increasing ethanol concentrations (up to 100%), cleared by xylol, and embedded in paraffin. Serial 4-micron thick

sections were cut by a microtome. Finally, all sections were carefully mounted on glass slides stained by hematoxylin-eosin (H&E) solution and assessed by a light microscope. The pathological changes were observed in the lungs of all experimental groups including hyperemia, leukocyte infiltration, epithelialization, emphysema, and atelectasis varied greatly from none to scattered changes. We defined grades according to the following manner: 0, absence of pathologic changes; 1, patchy changes; 2, local changes; 3, scattered changes.

Statistical analysis

All quantitative data were analyzed by one-way analysis of variance (ANOVA) with the Tukey-Kramer post-hoc test and expressed as mean \pm SEM. Histopathological scores between the groups were analyzed using Kruskal-Wallis test with the post-hoc Mann-Whitney test. Significance was accepted at $p < 0.05$.

Results

Stemness characterization

We confirmed that expanded MSCs evidently expressed stemness-related markers CD133 and CD44 while the

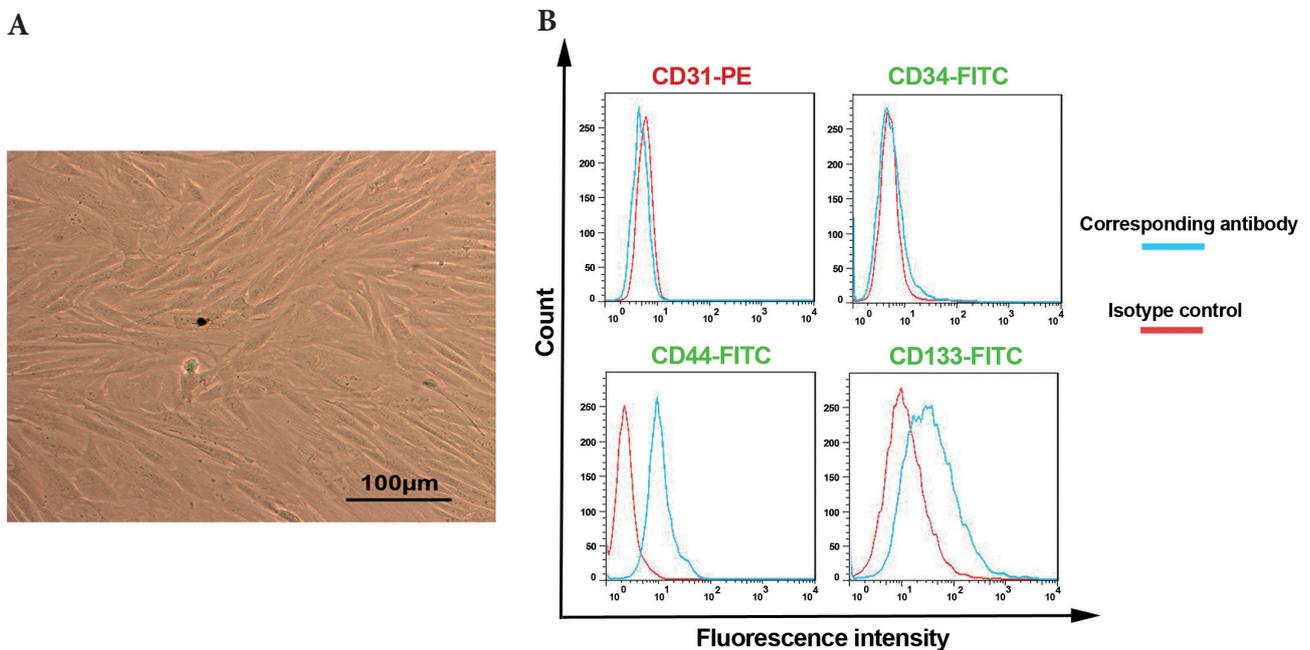


Figure 1. A. Phase contrast micrographs of the confluent monolayer of rBMSCs at third passage. B. Cultured rBMSCs phenotypic characterization. The expression of both positive and negative cell surface markers of cultured cells was evaluated by flow cytometry analysis. Histograms demonstrate cells positively stained with CD133 and CD44. (See online version for color figure.)

negative markers, CD31 and CD34 were not identified (Figure 1).

MSCs efficiently modulated the IL-4, IL-13 and IL-10 mRNA levels in asthmatic lungs

Quantitative transcript analysis of the interleukins in all sensitized groups showed a marked expression in the levels of IL-4 and IL-13 with decrease of IL-10 in comparison with C group ($p < 0.001$ to $p < 0.01$). Compared with other sensitized groups, the expression of interleukins in asthmatic rats received MSCs showed drastic difference. In detail, the expression levels of IL-4 and IL-13 diminished significantly ($p < 0.05$), whereas IL-10 increased simultaneously ($p < 0.01$). Based to our results, no significant modulatory effects of CM injection were achieved in terms of IL-4, IL-13 and IL-10 levels in asthmatic rats (Figure 2).

MSCs normalized the expression of miRNA133 and miRNA155 in asthmatic rats

We detected that *miRNA133* gene expression was decreased significantly in all sensitized groups in comparison with the control rats ($p < 0.001$). There was a significant increase in SCV group as compared to other sensitized groups ($p < 0.01$). However, there were no significant differences between S and SSV groups. *miRNA133* gene expression in CCV and CSV groups were more similar to control group (Figure 3A). On the other hand, *miRNA155* gene expression was increased significantly in all sensitized groups as compared with C group ($p < 0.001$ to $p < 0.01$). There was a significant decrease in SCV group compared to other sensitized groups ($p < 0.05$). Similar to *miRNA133*, there were no significant differences in the expression of *miRNA155* between S and SSV groups. In addition, the pattern of *miRNA155* expression in CCV and CSV groups were more similar to group C (Figure 3B).

Pre-labeled cells appropriately recruited into pulmonary parenchyma

The presence of pre-labeled rBMSCs was determined either in non-sensitized and peculiarly sensitized animals received cells *via* systemic injection. Our data were also shown that injected MSCs could recruit into lungs of non-sensitized control rats, regarding the homing proficiency of MSCs (Figure 4).

Pathological results

Pathological changes observed in the pulmonary environments of all sensitized groups were significantly higher than C group ($p < 0.001$ to $p < 0.05$). There was a significant decrease in the all-pathological changes of SCV group, unless

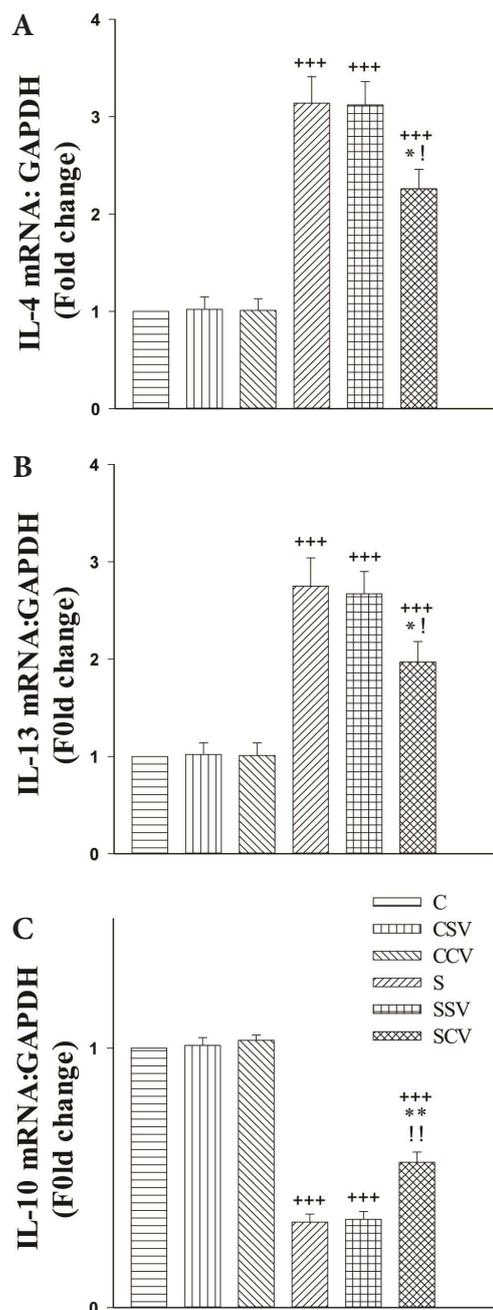


Figure 2. Expression levels of IL-4 (A), IL-13 (B) and IL-10 (C) mRNA in the lung tissue of control group received PBS (C), control group received CM (CSV), control group received rBMSCs (CCV), sensitized animals received PBS (S), sensitized animals received CM (SSV), sensitized animals received rBMSCs (SCV) (for each group, $n = 6$). Bars represent the mean \pm SEM. Statistical differences between control and different groups: $^{++} p < 0.01$ and $^{+++} p < 0.001$. Statistical differences between SSV and SCV vs. S group: $^* p < 0.05$ and $^{**} p < 0.01$. Statistical differences between SSV and SCV groups: $^! p < 0.05$ and $^{!!} p < 0.01$. CM, conditioned media; PBS, phosphate-buffered solution; rBMSCs, bone marrow-derived mesenchymal stem cells.

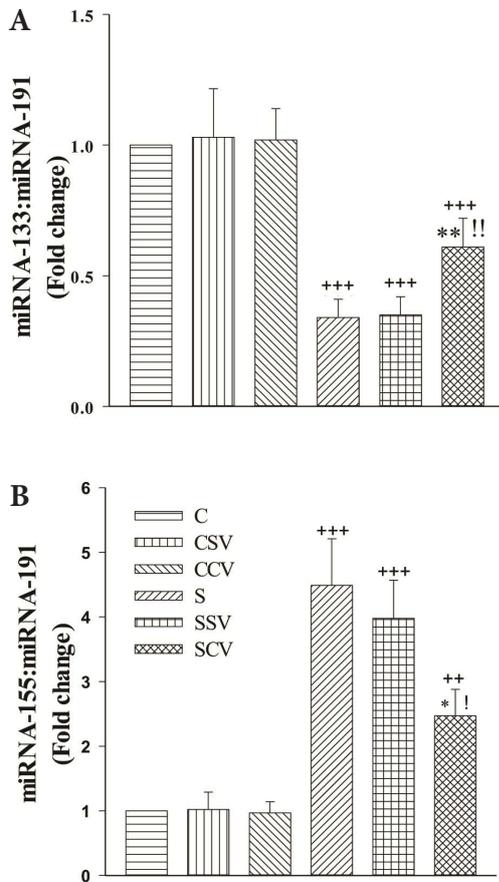


Figure 3. Real-time quantitative RT-PCR analysis of miR-133 (A) and miR-155 (B) expression level in lung of control group received PBS (C), control group received CM (CSV), control group received rBMMSCs (CCV), sensitized animals received PBS (S), sensitized animals received CM (SSV), sensitized animals received rBMMSCs (SCV) (for each group, $n = 6$). Bars represent the mean \pm SEM. Statistical differences between control and different groups: $^{++} p < 0.01$, $^{+++} p < 0.001$. Statistical differences between SSV and SCV vs. S group: $^* p < 0.05$, $^{**} p < 0.01$. Statistical differences between SSV and SCV groups: $^! p < 0.05$, $^{!!} p < 0.01$. For abbreviations, see Fig. 2.

atelectasis, in comparison with S and SSV groups ($p < 0.01$ to $p < 0.05$). Calling attention, the pathological changes in SSV group were more similar to S group (Figure 5, Table 2).

Discussion

In spite of apparent advances in human medicine, asthma still is not curable. Pathological changes in the lung structure of asthmatic subjects are mainly induced by chronic inflammatory responses. Accordingly, the efficient strategy must be directed to control the intensity and period of aberrant inflammation (Kavanagh and Mahon 2011; Vosoghi et al.

2013; Keyhanmanesh et al. 2014). Concurrently, owning immune-regulatory features, it makes MSCs and CM eligible to be used extensively for ameliorating lung inflammatory disorders such as asthma and COPD (Goodwin et al. 2011; Ionescu et al. 2012; Abreu et al. 2014; Feizpour et al. 2014; Chen et al. 2015; Ahmadi et al. 2017). Because of numerous similarities to human, rats were therefore selected in the current experiment. Previously, Abreu showed that therapeutic effects of BMMCs reduced in ovalbumin-sensitized mice as compared with healthy animals, MSCs were obtained from non-sensitized rats (Pauluhn and Mohr 2005; Abreu et al. 2014). It has been conceived that BMMSCs could significantly suppress Th2 cytokines levels, promoted Treg cell responses, and prevent allergic airway inflammation in ovalbumin-induced asthma. Nevertheless, no enough data exist on the modulatory effects of MSCs on the expression levels of miRNAs studied in rat model of asthma (Bonfield et al. 2010; Kavanagh and Mahon 2011; Zeng et al. 2015). Our aim, in selecting a suitable cell fraction as well as CM was to explore the underlying mechanisms by which MSCs exert immune-modulatory properties for attenuating chronic asthmatic changes.

In the current study, the paracrine effects of intravenously injected rBMMSCs and relevant CM phase were individually investigated in terms of the expression of IL-4, IL-13, IL-10, *miRNA133*, *miRNA155* along with pathological changes in the lungs of sensitized rats. Our pathological findings revealed that lesions observed in the lung tissue of the ovalbumin-sensitized groups were significantly higher than the control rats, indicating the rat model of asthma was constructed successfully (Ebrahimi et al. 2016; Mohammadian et al. 2016b). Similar to previous findings, a significant reduction in the expression of IL-10 and *miRNA133*, coincided with a remarkable increase in the expression of IL-4, IL-13 and *miRNA155* were seen in all sensitized groups as compared with healthy rats (Rankin et al. 1996; Chiba et al. 2009; Dong et al. 2014; Malmhäll et al. 2014; Fallahi et al. 2016).

Based on data, following systemic injection of rBMMSCs, the observed pathological changes were significantly reduced unless atelectasis, showing that rBMMSCs significantly diminished inflammation rate which was supported by some previous experimental findings (Firinci et al. 2011; Mohammadian et al. 2016b). In addition, the systemic injection of rBMMSCs blunted the levels of IL-4, IL-13, IL-10, *miRNA133*, and *miRNA155* in pulmonary specimens of sensitized rats to near normal levels. In contrary, MSCs-CM did not initiate any obvious effects on the levels of measured miRNAs, interleukins and pathological changes in lung tissues of sensitized rats. The application of both MSCs and CM has inert effects on normal healthy tissue, and we did not find any obvious change in the levels of measured miRNAs and interleukins of saline-challenged rats. Unlike to our results, some experi-

ments showed that the intravenous injection of MSC-CM decreased inflammation and improved local tissue damage and therapeutic effects of direct administration of CM were most similar to BMMSCs (Timmers et al. 2011; Ionescu et al.

2012; Gazdhar et al. 2014; Zhu et al. 2014). Failure of systemic administration of CM on the factors measured in this study could be possibly related to animal species studied, sensitization technique, the source, volume, route and time of cell

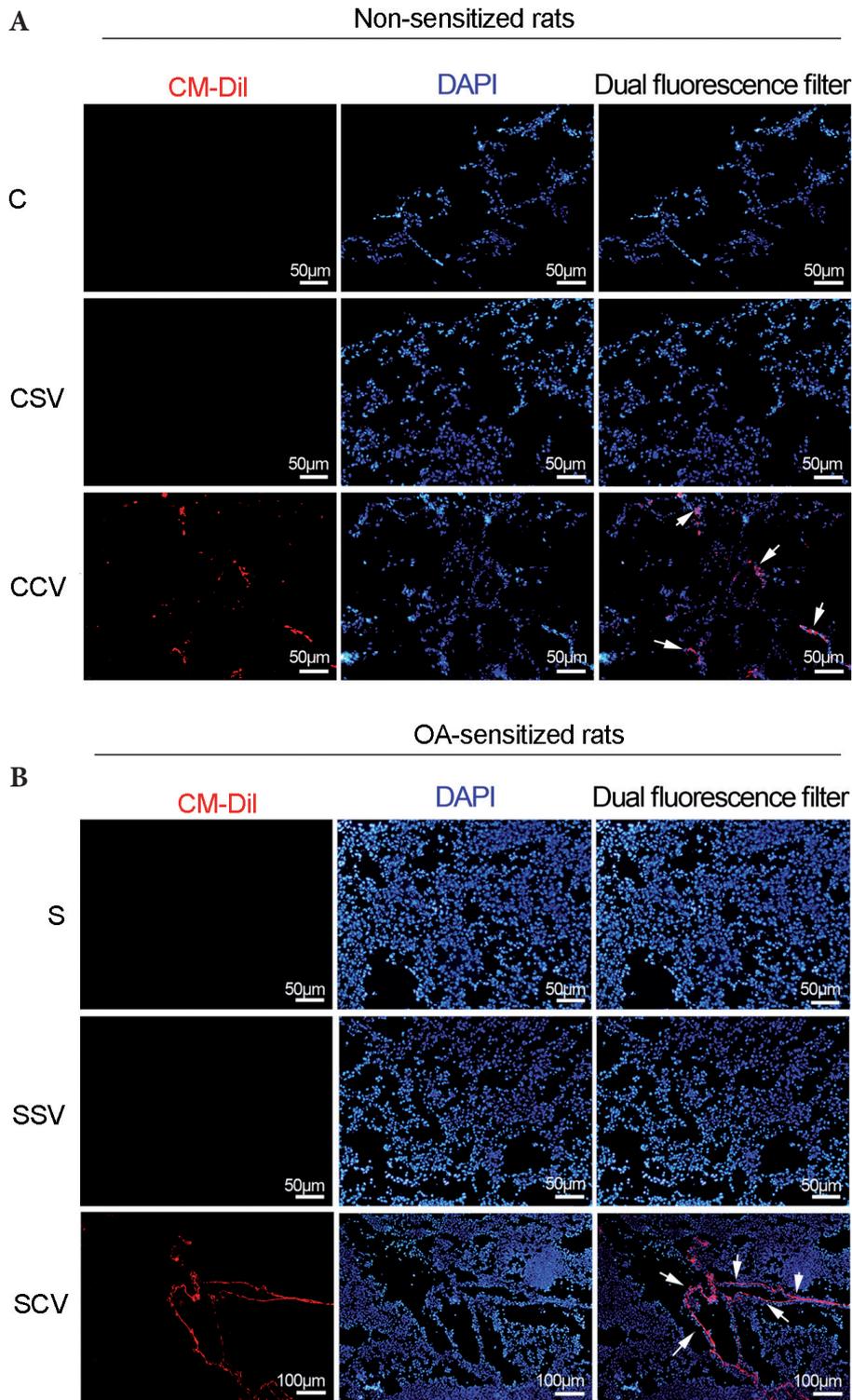


Figure 4. Representative images of fluorescently-tagged rBMMSCs in lungs parenchyma of non-sensitized (A) and sensitized rats (B). The existence of rBMMSCs was evident in all rats given cells by systemic approach. Interestingly, the number of recruited cells was more profound in the sensitized rats as compared with non-sensitized control. For background staining, DAPI solution was used. Control group received PBS (C), control group received CM (CSV), control group received rBMMSCs (CCV), sensitized animals received PBS(S), sensitized animals received CM (SSV), sensitized animals received rBMMSCs (SCV). (See online version for color figure.)

and CM administration (Bonios et al. 2011; Ma et al. 2014). Moreover, MSCs release their active factors “spontaneously” or after induction by the local situations, it seems that one of the most possible reasons for effects of rBMMSCs compared with CM is the variation in quality and quantity of secreted agents. On the other hand, the secretome could be easily dispersed in the peripheral tissues and not reach efficiently and completely into the injured sites. Another possible for this statement is due to the fact that the factors within the CM are consumed by the surrounding injured tissue, because the CM contains only a limited amount of factors whereas the presence of the stem cells provides an available source for the chronic secretion of soluble agents (Sutsko et al. 2012; Kyurkchiev et al. 2014). Injection with high volume, recurrent doses, and intra-tracheal injection may be effective.

To date, several possible mechanisms regarding asthma pathophysiology have been reported. Given that numerous

miRNAs expressed differentially in asthmatic subjects as compared with healthy subjects, miRNAs were described to play a key role in the induction of asthma (Tang et al. 2016). A plethora of experiments showed, for example, the up-regulation of *miRNA155* and down-regulation of *miRNA133* in the mouse model of OVA-induced asthma and miRNAs dynamic targeting could add invaluable data in allergic asthma therapy regarding on asthma Th2 and Treg cytokines levels (Chiba et al. 2009; Wang et al. 2011; Kuo et al. 2013; Malmhäll et al. 2014; Kai et al. 2015). Of 296 miRNAs studied *via* miRNA PCR in samples collected from lung tissues in mice model, Tang et al. (2016) introduced miR-21/Acyr2a axis play actively key role on the induction of asthmatic inflammation. For example, they also showed that miRNA21a and Th2 cytokines levels remarkably was affected immediately after administration of MSCs. The dynamic changes of both miRNAs expression along with

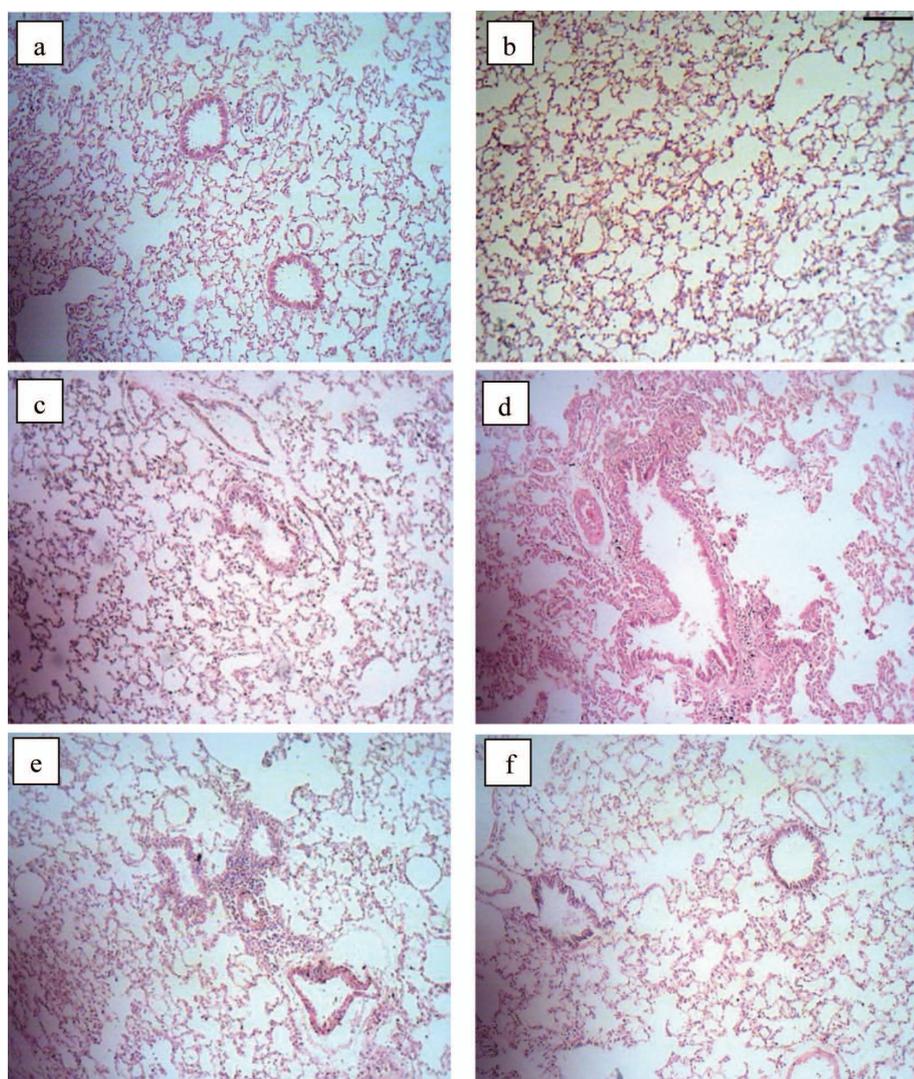


Figure 5. Photographs of a lung specimen in different groups (magnification for each group; 8×10). Normal lung tissue in control group received PBS (C, a), control group received CM (CSV, b) and control group received rBMMSCs (CCV, c). Cell infiltration and emphysema in sensitized group received PBS (S, d), cell infiltration in sensitized group received CM (SSV, e) and sensitized group received rBMMSCs (SCV, f). (See online version for color figure.)

pathological changes and measured cytokines pre- and post-MSCT transplantation, mainly in SCV rats, could denote that the therapeutic effects of MSCs occurred likely by the modulation of our targeted miRNAs.

Based on the crucial effect of both *miR155* and *miR133a* in ongoing pathological changes of asthma, it was elucidated that over-expression of *miR155* seen during T lymphocyte differentiation, recruitment and the production of inflammatory cytokines (Kai et al. 2015). It was well-established that MSC-derived micro-particles, notably exosomes, contained miRNA array that could act as RNA interference (RNAi) in the recipient cells and tissues (Pegtel et al. 2010). By producing and the release of numerous anti-immunogenic factors, such as TGF- β , PGE2 and NO via MSCs exosome, it was shown the dynamic changes of miRNAs could be regulated by affecting the function of T and B lymphocytes as well as natural killer cells in target tissues containing progenitor cells (Di Trapani et al. 2016). In the current study, an appropriate number of pre-labeled MSCs were proved in pulmonary tissues of non-asthmatic and peculiarly asthmatic rats. In contrast to our result, some authorities revealed MSCs homing, exclusively in injured lung tissue (Abreu et al. 2013b; Ghorbani et al. 2014; Mohammadian et al. 2016a). It is hypothesized that many factors could alter the homing capacity of MSCs. According to animal strain and species, stem cells type, injection routes and volumes, recovery time after cell therapy, severity and type of tissue injury and volume and type of cell tracker used in different experiments, a controversial data has obtained (Abe et al. 2004; Herzog et al. 2006; Lee et al. 2009; Abreu et al. 2013b; Trzil et al. 2014). There are also certain limitations regarding current experiment. For example, we did not investigate the *miRNA* target genes and related signaling pathways. Also, long-term monitoring would be helpful to mimic the real changes during asthmatic changes. In conclusion, the outcomes of current investigation showed that intravenous administration of rBMMSCs, but not CM, could be effective in alleviation of asthma pathophysiology, presumably by the modulation of essential miRNAs in ovalbumin-sensitized rats.

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Conflict of Interest. None declared.

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