Original Article

Monitoring IL-13 expression in relation to miRNA-155 and miRNA-133 changes following intra-tracheal administration of mesenchymal stem cells and conditioned media in ovalbumin-sensitized rats

Mahdi Ahmadi[1,2] Reza Rahbarghazi[3,4] Amir-Ali Shahbazfar[5] Rana keyhanmanesh[6*]

Abstract

The overall goal of the current study was to show possibly mechanisms by which mesenchymal stem cells (MSCs) or conditioned media (CM) exert immune-modulatory properties for amelioration of asthmatic changes. Male rats were classified into healthy (C) and ovalbumin-sensitized groups (S), which further were divided into three subgroups (n=6); rats given intratracheally PBS (CPT and SPT groups), CM (CST and SST groups) and MSCs (CCT and SCT groups). Two weeks post treatment, IL-13, miRNA- 133 and miRNA-155 transcripts, pathological injuries and the homing of MSCs into the lung parenchyma were evaluated. The local administration of CM and notably MSCs blunted the expression levels of miRNA-133, miRNA-155, IL-13 and all pathological changes in pulmonary specimens of the sensitized rats compared to the SPT group ($p<0.001$ to $p<0.05$). Successful mesenchymal stem cells transmigration to lung tissue in cell-administrated rats was also shown, although the intensity of asthmatic changes profoundly affected the amount of recruited cells. In summary, our results indicate the potency of CM and especially MSCs in ameliorating pathological changes in rats presumably by regulating miRNAs expression in lung tissue.

Keywords: interleukin-13, mesenchymal stem cells, miRNA-133, miRNA-155

[1]*Tuberculosis and Lung Diseases Research Center, Tabriz University of Medical Sciences, Tabriz, Iran*
[2]*Student Research Committee, Tabriz University of Medical Sciences, Tabriz, Iran*
[3]*Stem Cell Research Center, Tabriz University of Medical Sciences, Tabriz, Iran*
[4]*Applied Cell Sciences, Faculty of Advanced Medical Sciences, Tabriz University of Medical Sciences, Tabriz, Iran*
[5]*Department of Pathology, Faculty of Veterinary Medicine, University of Tabriz, Tabriz, Iran*
[6]*Drug Applied Research Center, Tabriz University of Medical Sciences, Tabriz, Iran*
***Correspondence**: rKeyhanmanesh@gmail.com*

Introduction

Asthma is a life-threatening chronic respiratory disease with very high care costs (Ahmadi et al., 2017). In spite of novel advances in medical science, asthma cannot be cured efficiently by any current protocol guidelines (Ahmadi et al., 2017; Keyhanmanesh et al., 2013). New therapeutic manipulations for asthma must be considered, not only to manage asthma-related manifestations, but also to accelerate the regeneration rate of chronic structural changes focusing on the reversal of Th2/Th1 imbalance and the control of inflammation rate in the lung airway structures (Ahmadi et al., 2017). Interleukin (IL)-13, produced by activated Th2 cells and/or innate lymphoid cells (ILC) especially ILC2, is an essential mediator for the induction of histopathological changes in asthmatic subjects such as tissue fibrosis, mucus production and airway hyper responsiveness (Ghosh, 2013; Kim et al., 2016). On the other hand, it is well established that potential therapeutic effects of IL-10, as an anti-inflammatory cytokine, in allergic asthma are performed via down-regulate Th2-mediated inflammatory response (Fu et al., 2006). Therefore, the modulation of both pro- and anti-inflammatory cytokines is crucial for a functional resolution of inflammatory response in asthma. In line with this hypothesis, mesenchymal stem cells (MSCs) possess immune-regulatory capacity, which is driven by trans-differentiation behavior and paracrine activity. Therefore, it makes them unique therapeutic tools for inflammatory disorders, peculiarly asthma. In response to inflammatory mediators, MSCs can be mobilized from bone marrow and engrafted successfully into injured sites (Kyurkchiev et al., 2014). Due to the low survival and differentiation rate of MSCs at the inflamed niche, it is proposed that the paracrine effects are the principal mechanism for immune-regulatory effects of MSc (Linero and Chaparro, 2014).

It is well-known that bone marrow-derived mesenchymal stem cells (BMMSCs) secrete various growth factors responsible for some of the therapeutic effects, so it seems logical to advise that the paracrine effects of MSCs could be monitored in their conditioned media (CM) (Timmers et al., 2011). An in vitro analysis confirmed that other soluble factors such as miRNAs, exosomes and microparticles were extruded to the supernatant. Any change in the culture condition such as hypoxia and inflammation could affect the entity and composition of secretome in these cells (Madrigal et al., 2014). *Selecting CM* instead of stem cells could circumvent some of the present confounding issues associated with MSCs such as tumorigenic risk, immune compatibility, xenozootic contaminations, total costs, and prolonged period of cellular expansion (Timmers et al., 2011). A great body of studies uncovered that systemic or local administration of stem cells and CM could suppress Th2 cytokine levels and alleviate inflammation in lung injury (Abreu et al., 2013; Ahmadi et al., 2017). Although the immunomodulatory properties of MSCs are very promising, underlying mechanisms dictated by MSCs in regulation of immune responses remain to be elucidated.

Single-stranded microRNAs (miRNAs) have recently been discovered, the evolutionarily conserved non-coding RNA species with less than 25 nucleotides, which suppress gene expression by degradation or repression of target mRNAs and participate in the regulation of airway inflammation via modulating Th2 cells activity. The miRNA expression patterns in the respiratory system can be basically controlled by the array of inflammatory agents (Grimson et al., 2007; Simpson et al., 2014). In close relation to this statement, it has been shown that numerous miRNAs expressed differently in lung tissue of OVA-challenged mice. For instance, different patterns of expression of miRNA-155 and miRNA-133 were certified in asthmatic animals compared to normal subjects. Indeed, miRNAs targeting in the treatment of asthma will inhibit inflammation by returning it to near normal values (Kai et al., 2015). Lists of target genes are under control of both above-mentioned miRNAs such as c-Fos, C-Maf, INF-γ receptor for miRNA-155 and RhoA for miRNA-133 (Kai et al., 2015; Banerjee et al., 2010). To our knowledge, there are few documents on the modulatory effects of MSCs and CM on the expression of miRNAs in asthmatic rats. The illumination of the underlying mechanisms under control of MSCs in asthmatic rat can lead to clinical application *of* stem cell in human medicine. Therefore, the present study proposed that the immune-modulatory effects of MSCs would be mainly performed via the regulation of specific miRNAs expression profile in lung parenchyma of asthmatic rats.

Materials and Methods

Animal ethics: All phases of the current study were conducted in strict accordance with the criteria published by the National Institutes of Health for Laboratory Animal Care (NIH Publication No. 85-23, revised 1996) and accepted by the Animal Care Committee of Tabriz University of Medical Sciences (No: TBZMED.REC.1394.386).

Sensitization protocol and animal groups: Forty adult male Wistar rats, weighing between 200-250 g, were enlisted in this study. The animals were maintained in metal rodent cages under 12:12 light/dark cycle at 18-22°C with free access to water and food. After adaptation to the new environmental condition, four healthy rats were blindly selected for extraction of rBMMSCs. The rest were randomly classified into six groups (6 rats in each group) as follows:

1. Healthy rats receiving only 50 µl PBS (CPT)
2. Healthy rats receiving 50 µl CM (CST)
3. Healthy rats receiving 50 µl PBS containing 2×10^6 rBMMSCs (CCT)
4. Sensitized rats receiving only 50 µl PBS (SPT)
5. Sensitized rats receiving 50 µl CM (SST)
6. Sensitized rats receiving 50 µl PBS containing 2×10^6 rBMMSCs (SCT)

In the sensitized groups, the rats were exposed to ovalbumin (OVA) for a period of 32 ± 1 days based on previously reported guidelines (Ahmadi et al., 2016). Shortly, each animal received 1

mg ovalbumin intra-peritoneally (Sigma-Aldrich, USA) and 200 mg aluminum hydroxide (as adjuvant) which was pre-dissolved in 1 ml sodium chloride solution (0.9% w/v) on the first and 8th days. On day 14, the sensitized rats were challenged daily with aerosolized condition of 4% OVA formed by a nebulizer *for* 5 min (CX3, Omron Co., Netherlands), for 18 ± 1 days without any interruption. This exposure was induced in a special sealed box with dimensions of 30 × 20 × 20 cm^3. In the healthy subjects, sodium chloride solution was injected instead of ovalbumin with the same manner. One day post sensitization, stem cells or relevant CM were directed slowly into the trachea via a ventral neck dissection (Abreu et al., 2014). All measurement was performed two weeks after treatment.

MSC isolation and expansion: rBMMSCs were isolated as published previously by different reliable sources (Rahbarghazi et al., 2012). In short, the animals were euthanized by high doses of ketamine combined with xylazine. Following dissection and cutting ends of each femur, bone marrow cells were extracted by flushing the components of medullary cavity with PBS solution containing 2% fetal bovine serum (FBS; Gibco, USA). Then, fresh bone marrow mononuclear cells were harvested by Ficoll (Gravity density: 1.073; Sigma, USA), centrifuged at 400 g for 20 min, washed twice by PBS and plated in Dulbecco's modified Eagle's medium low glucose (DMEM/LG; Gibco, USA) containing 20% FBS, 100 U/ml penicillin and 100 mg/ml streptomycin (Biosera, UK). Finally, a density of 1 × 10^5 purified cells was seeded in 6-well culture flasks (SPL). The medium was changed regularly every 4 days. The cells were passaged at 70-80% confluency. After passage 1, the cells were immediately transferred to 75 cm^2 T flask (SPL) and at passage 3 were used in subsequent experiment.

Characterization of isolated rBMMSCs: To further validate the multipotential properties of cultured cells, rBMMSCs were evaluated immune-phenotypically using a FACS Calibur apparatus (BD, USA) (Rahbarghazi et al., 2014). A panel of monoclonal anti-rat antibodies was directed against the cell surface antigens. PE-conjugated anti-CD31 was used as well as FITC-conjugated anti-CD133, CD34 and CD44 (all from ebioscience, USA). In addition, appropriate isotype control antibodies were used to determine the level of background staining. Briefly, the cells were detached by 0.025% Trypsin-EDTA solution (Gibco, USA). Afterward, 5 × 10^5 cells were incubated with recommended concentration for each antibody for 30 min at RT and washed twice with PBS. Ultimately, the cells were subjected to flow cytometric system and raw data analyzed by Flow Jo software ver.7.6.1.

MSCs labeling with Cell Tracker: In order to track migrated cells into lung parenchyma, rBMMSCs were labeled with 2 µM Cell Tracker™ CM-Dil at 37°C for 20 min (Catalog No. C-7000; Molecular Probes, Invitrogen, USA). A final volume of 50 µl PBS containing 2 × 10^6 CM-Dil pre-labeled cells was gently administrated intratracheally to each animal one day after sensitization.

Conditioned media preparation: To pinpoint possible paracrine roles of isolated cells in the alleviation of ovalbumin-induced asthma, the CM of rBMMSCs were prepared. Following 70-80% confluency, the DMEM/LG containing 10% FBS was removed and the cells were washed three times with PBS and then incubated with DMEM/LG-free FBS for 72 h (Ahmadi et al., 2016). After this time, the supernatant was collected. The collected media were centrifuged at 400 g for 5 min and filtered by 0.20-µm-pore syringe filter to remove any cellular debris. We further concentrated harvested CM approximately 50-fold by centrifuge filter tubes with molecular weight cut-offs at 4 kDa (Catalog No. 003099.125, Eppendorf, Germany). The protein level of concentrated CM was detected by bicinchoninic acid assay (BCA assay, iNtRon, Korea). Corroborating to initial results, it was demonstrated that total protein content of the concentrated CM reached 500 µg/ml. An equal volume of CM containing similar protein concentration was used in the current experiment. Finally, 50 µl/per case of concentrated CM was administrated intratracheally one day post sensitization (Abreu et al., 2013).

Tracking of migrated cell by immunofluorescence assay: To ensure the existence of transplanted cells in pulmonary parenchyma at the final stage of experimental plan, the isolated lung tissues were embedded in Tissue Freezing Medium, snap-frozen and sectioned at 5 µm thickness by a Cryostat apparatus (Rahbarghazi et al., 2014). Following cryo-sectioning, slides were rinsed twice with PBS for 5 min and stained with 4', 6-diamidino-2-phenylindol (DAPI, 1 µg/mL, Catalog# D9542, Sigma-Aldrich, USA) for nuclear counterstaining.

Total RNA extraction and real-time PCR: Two weeks following the administration of cell or CM, the animals were anesthetized with a mixture of Ketamine and Xylazine intraperitoneally (75 mg/kg and 3 mg/kg, respectively), their left lung was quickly removed post-tracheostomy and stored in liquid nitrogen. Total RNA from each lung tissue was extracted using Total RNA extraction mini kit (YTA, Taiwan) according to the manufacturer's recommendations (Biyashev et al., 2012). The purity of isolated RNAs was inspected and confirmed by a NanoDrop 1000 Spectrophotometer (Thermo Scientific, Wilmington, DE 19810 USA). The IL-13, miRNA-133 and miRNA-155 gene expressions were quantitatively assessed by real-time PCR. Primer sequences for IL-13 were designed using Gene-Runner Software, version 3.05 (Table 1). The expression profiles of miRNA-133 and miRNA-155 were assessed on total RNA extracts by MiR-Amp kit (Parsgenome Co, Iran). In addition, reverse transcription was carried out by cDNA Synthesis Kit (YTA, Taiwan) to determine expression level of IL-13. Each cDNA was used as a template for separate assay for miRNAs and mRNA quantitative real-time PCR using a SYBR Green master mix (YTA, Taiwan). Real-time PCR reactions were accomplished on a Rotor-Gene 6000 instrument (Corbett Life Science, Australia). The amount of PCR products was normalized with GAPDH (glyceraldehyde-3-phosphate dehydrogenase) gene for mRNA sample and miRNA-191 for miRNAs. The 2^-

$^{\Delta\Delta Ct}$ method was employed to ascertain relative-quantitative expression levels of miRNAs and mRNA. Results were expressed as the fold-change of expression to the internal control gene.

Pathological evaluation: The right lung *of* each rat was excised, washed with PBS and kept in 10% buffered formaldehyde (37%, Merck, Germany). On day 7, the tissues were dried through Passage method by a series of increasing alcohol concentrations (70-100%), cleared by xylol, immersed in paraffin and put into blocks. Serial sections were prepared *at* 4 micron-width using microtome (Leica). Finally, the specimens were stained by hematoxylin-eosin (H&E) solution and imaged by a light microscope. Pathological changes were observed in the lung of all sensitized and non-sensitized groups in terms of vascular changes, mucus plugs and respiratory epithelial hyperplasia by an expert pathologist. Injuries were valued and scored according to the following manner: absence of pathologic changes=0; patchy changes=1; local changes=2; scattered changes=3.

Table 1 Design of primers for mRNAs

Gene name	Accession number	Primer sequence	Product length
IL-13	NM_053828.1	Forward: 5'-CGC TTG CCT TGG TGG TCT TG-3' Reverse: 5'-TTC TGG TCT TGT GTG ATG TTG CTC-3'	110 bp
GAPDH	NM_017008.4	Forward: 5'-CAA GTT CAA CGG CAC AGT CAA G-3' Reverse: 5'-ATA CTC AGC ACC AGC ATC ACC -3'	121 bp

Statistical analysis: All quantitative data were analyzed using one-way analysis of variance (ANOVA) followed by Tukey-Kramer test and presented as mean ± SEM. The data of pathological changes were analyzed using nonparametric Kruskal-Wallis test with the post- hoc Mann-Whitney test. Statistical significance was considered at $p<0.05$.

Results

Phenotypic verification of MSCs by Flow Cytometry: The study confirmed that the isolated cells clearly expressed multipotential markers such as CD44 and CD133, while the negative markers, including CD34 and CD31, were not evident (Fig. 1).

IL-13 mRNA transcript were reduced in asthmatic lungs of CM and especially MSC-treated rats: The expression of IL-13 in all sensitized groups were significantly higher than the CPT group ($p<0.001$ to $p<0.05$). Compared with the SPT group, the expression of IL-13 in the asthmatic rats receiving MSCs or CM was diminished significantly ($p<0.01$ to $p<0.05$). A profound decrease in the level of IL-13 was detected in the asthmatic lungs treated by MSCs rather than the CM counterpart ($p<0.05$) (Fig. 2).

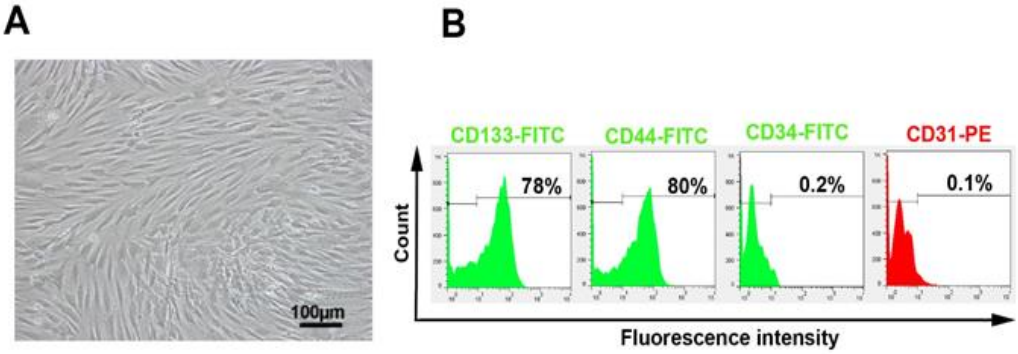

Figure 1 Photomicrographs of rBMMSCs at the third passage (A). Flow cytometric analysis of stem cell surface markers (B). The expression of both positive and negative cell surface markers of cultured cells was assessed by flow cytometry analysis.

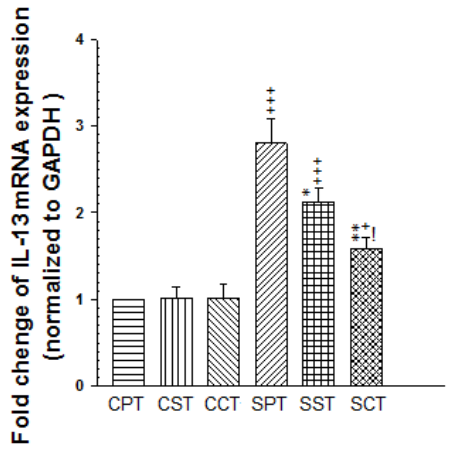

Figure 2 Expression level of IL-13 mRNA in the lung tissues of control group receiving PBS (CPT), control animals receiving CM (CST), control animals receiving rBMMSCs (CCT), sensitized animals receiving PBS (SPT), sensitized animals receiving CM (SST) and sensitized animals receiving rBMMSCs (SCT) (for each group, n = 6). The bars represent mean±SEM. Statistical differences between the control and different groups: +, p<0.01 and +++, p<0.001. Statistical differences between the SST and SCT groups *vs* the SPT group: *, p<0.05 and **, p<0.01. Statistical differences between the SST and SCT groups: !, p<0.05.

CM and noticeably MSCs normalized the expression levels of miR-133 and miR-155 in sensitized rats: The expression level of miRNA-133 in all sensitized groups was significantly lower than that of the CPT group (p<0.001 to p<0.01). A significant increase was noticed in the level of miRNA-133 in the SST and SCT groups compared to the SPT group (p<0.01 and p<0.001, respectively). Interestingly, MSCs had more positive effects in returning miRNA-133 transcriptional level to near-normal values compared with CM (p<0.01) (Fig. 3). In contrast to miRNA-133, the expression level of miRNA-155 in all sensitized groups was significantly higher than the CPT group (p<0.001 to p<0.01). There was an evident decrease in the SST and SCT groups compared to the SPT group (p<0.05 and p<0.01, respectively). Similar to the contributory effect of MSCs on miRNA-133 expression, the potent role of these cells on the expression of miRNA-155 was significantly more than the asthmatic animals treated with CM (p<0.05) (Fig. 4).

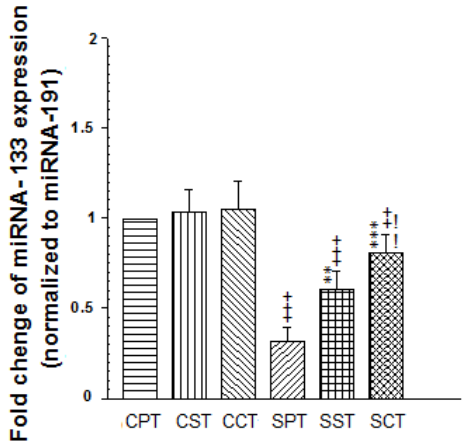

Figure 3 Real-time quantitative RT-PCR analysis of miRNA-133 expression level in the lungs of control group receiving PBS (CPT), control animals receiving CM (CST), control animals receiving rBMMSCs (CCT), sensitized animals receiving PBS (SPT), sensitized animals receiving CM (SST) and sensitized animals receiving rBMMSCs (SCT) (for each group, n = 6). The bars represent mean±SEM. Statistical differences between the control and different groups: ++, p<0.01 and +++, p<0.001. Statistical differences between the SST and SCT groups *vs* the SPT group: **, p<0.01 and ***, p<0.001. Statistical differences between the SST and SCT groups: !!, p<0.01.

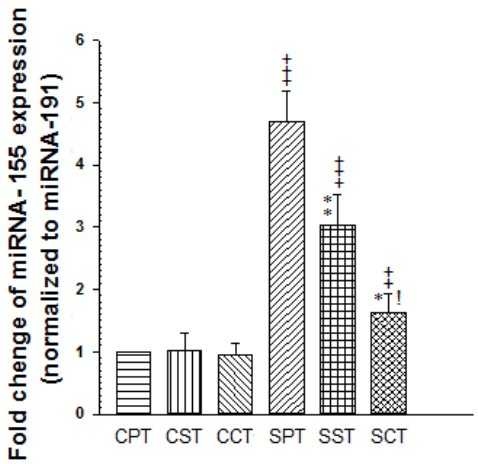

Figure 4 Real-time analysis of miR-155 expression in the lungs of control group receiving PBS (CPT), control animals receiving CM (CST), control animals receiving rBMMSCs (CCT), sensitized animals receiving PBS (SPT), sensitized animals receiving CM (SST) and sensitized animals receiving rBMMSCs (SCT) (for each group, n = 6). The bars represent mean±SEM. Statistical differences between the control and different groups: ++, p<0.01 and +++, p<0.001. Statistical differences between the SST and SCT groups *vs* the SPT group: *, p<0.05 and **; p<0.01. Statistical differences between the SST and SCT groups: !, p<0.05.

Successful homing of pre-labeled MSCs into pulmonary parenchyma: MSCs homing success was confirmed by examining their presence via immunofluorescence assay either in the normal or

especially in the sensitized rats receiving cells intratracheally. Notably, the existence of MSCs in the non-sensitized rats was also confirmed (Fig. 5).

Ameliorating effects of CM and especially MSCs on pathological injuries: Pathological changes observed in the pulmonary system of all sensitized groups were significantly higher than those of the CPT group, except vascular changes for SCT group, ($p<0.001$ to $p<0.05$). There was a significant decrease in all pathological changes of the SCT and SST groups compared with the SPT group ($p<0.001$ to $p<0.05$). Also, MSCs could profoundly diminish the asthmatic associate pathological changes compared with CM ($p<0.05$) (Table 2).

Discussion

Based on literature, local and systemic injection of stem cell and CM alleviated Th2 cytokine levels and airway inflammation in lung injury, but little data exist on the therapeutic effects of MSCs on the expression values of miRNAs studied here (Ahmadi et al., 2017; Goodwin et al., 2011; Sutsko et al., 2012). Our goal, in applying a cellular fraction or relevant CM, was to decipher the fundamental mechanisms by which MSCs fulfill their immune-modulatory properties for the alleviation of structural changes in the asthmatic airways.

Our pathological findings indicate prominent lesions in the lung tissue of the ovalbumin-sensitized groups, confirming that a rat model of asthma was constructed successfully (Ahmadi et al., 2017). Similar to previous experiments, the significant reduction in transcript level of miRNA-133 coincided with the remarkable augmentation in the expression of miRNA-155 and IL-13 in the lung tissues of all sensitized groups compared to control rats (Chiba et al., 2009; Malmhäll et al., 2014).

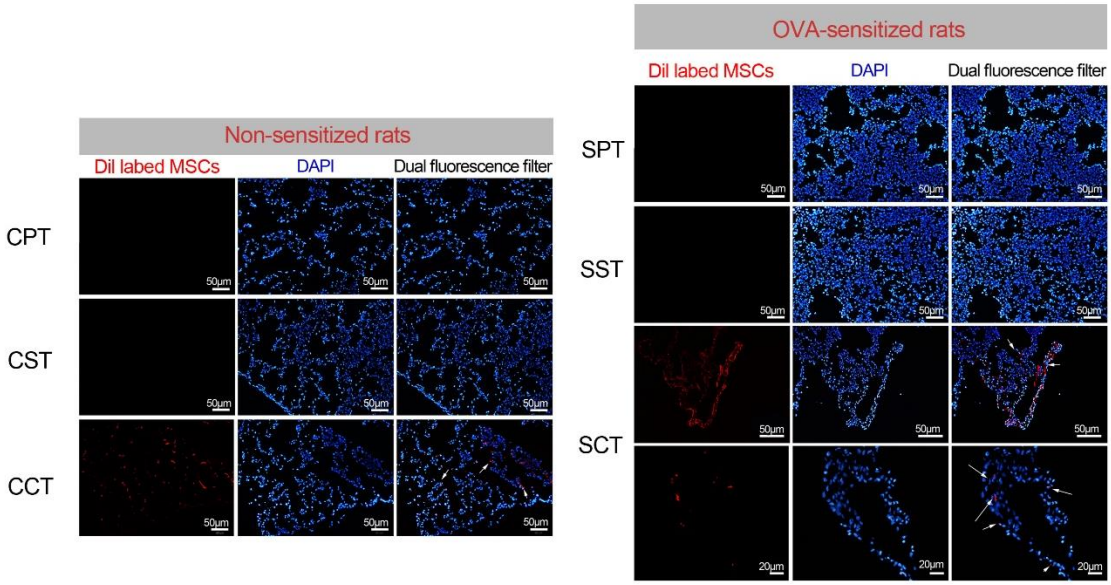

Figure 5 Representative images of the lung tissues from control and ovalbumin-sensitized rats receiving rBMMSC-CM or pre-labeled rBMMSCs administrated intratracheally. The presence of stem cells was demonstrated in all rats receiving rBMMSCs. Interestingly, the number of recruited cells was more prominent in the sensitized groups compared with the non-sensitized rats. For nuclear counterstaining, directed cells were stained with DAPI solution. The control group received PBS (CPT), control animals received CM (CST), control animals received rBMMSCs (CCT), sensitized animals received PBS (SPT), sensitized animals received CM (SST) and sensitized animals received rBMMSCs (SCT).

Table 2 Pathological finding scores in lung of animals

Pathological findings	Scores in groups (for each group, n = 6) (Minimum-Maximum)					
	CPT	CST	CCT	SPT	SST	SCT
Vascular changes	(0-0)	(0-0)	(0-0)	(1-3) +++	(1-2) ++ *	(0-1) ** !
Mucus plugs	(0-0)	(0-0)	(0-0)	(2-3) +++	(0-2) +++*	(0-1) + ***!
Respiratory epithelial hyperplasia	(0-0)	(0-0)	(0-0)	(2-3) +++	(1-2) +++**	(0-1) +***!

In the current experiment, pathological changes were significantly diminished following the direct injection of rBMMSCs and CM in asthmatic rats, except atelectasis for the SST group, indicating that cell mass and cell secretome modulate host inflammation responses in lung tissue (Abreu et al., 2013; Ahmadi et al., 2017). In addition, it was determined that the local presentation of both MSCs and CM blunted the levels of measured miRNAs and IL-13 in the pulmonary specimens of sensitized rats to near normal levels. The

effects of rBMMSCs on the pathological changes, levels of IL-13 and measured miRNAs in the sensitized rats were more substantial than those of CM.

Up to the present, there have been conflicting results regarding the anti-inflammatory effects of MSCs and CM (Ahmadi et al., 2016; Sutsko et al., 2012; Ahmadi et al., 2017). The logic explanation for this dissimilarity could be presumably originated from animal species and age, sensitization technique, the source, dosage, volume, route and time of cell or CM injection and in vitro culture conditions (Ahmadi et al., 2016; Madrigal et al., 2014). Furthermore, MSCs could release soluble factors "spontaneously" or in response to different stimuli. It seems that one of the possible reasons for the higher effects of rBMMSCs compared with CM on the parameters evaluated here is the variety in type and amount of secreted agents (Kyurkchiev et al., 2014; Madrigal et al., 2014). Another possible reason for the higher effective outcome of BMSCs in comparison to CM is due to the fact that limited content of the factors existing in CM is adsorbed rapidly by the adjacent damaged or normal tissues, whereas stem cells provide a sustained release source of soluble factors. Therefore, it is noteworthy to mention that CM injection with high volume and/or repeated doses could be more therapeutic (Sutsko et al., 2012). Some authorities also acclaimed that distinct post-transplantation period must be assumed to trace injected MSCs in specific tissues. Similarly, Ghorbani et al. (2014) confirmed the presence of pre-labeled MSCs in injured lungs after 2 weeks. Of note, in the present study, an appropriate number of pre-labeled MSCs were observed in the 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., 2013; Ghorbani et al., 2014). It is hypothesized that many factors could alter the homing capacity of MSCs in terms of animal strain and species, source, dosage, volume, route and recovery time after cell administration, grade and type of tissue injury and volume and type of cell tracker used in different experiments (Abreu et al., 2013; Trzil et al., 2014).

Up to now, numerous possible mechanisms regarding asthma immunomodulatory properties have been published. Based on some studies, multiple miRNAs showed different patterns and seems to play a key role in the induction of OVA-challenged mice (Garbacki et al., 2011). For instance, down-regulation of miRNA-133 and up-regulation of miRNA-155 and miRNA-126 were shown in asthmatic animals compared to normal mice. Actually, miRNAs targeting in treatment of allergic asthma altered the inflammatory response and returned Th2 cytokines to near-normal levels (Chiba and Misawa, 2010; Kai et al., 2015; Solberg et al., 2012). Of 296 miRNAs studied via miRNA PCR and mRNA PCR arrays in samples collected from lung tissues, Tang et al. (2016) presented that miR-21/Acvr2a axis played actively critical role in the induction of inflammatory process in the murine ovalbumin asthma model. They further revealed that Th2 cytokines expression levels and miRNA expression profile remarkably were affected directly after MSCs therapy (Tang et al., 2016). In addition, in one study directed by Kuo and co-workers, it was observed that increase in IL-8 levels was due to an elevated miRNA-155 expression and human BM-MSCs down-regulate miRNA-155 expression and IL-8 levels in cultured human bronchial epithelial cells (Kuo et al., 2013). Therefore, dynamics of miRNAs and IL-13 expression in lung niche during pre- and post-MSC and CM transplantation, in SCT and SST rats, could infer that the therapeutic effects of MSCs and CM on pathological changes and Th2 cytokines occurred most likely by the modulation of miRNAs expression levels. There are also certain limitations regarding the current experiment. The most prominent limitation related to the present study is that the miRNA target genes and related signaling pathways were not investigated. The CM entity and existence of modulatory factors must be addressed. Moreover, long-term monitoring would be helpful to mimic real changes during asthmatic changes. In conclusion, the results of this study showed that the local administration of rBMMSCs and CM could be effective in the amelioration of ovalbumin-sensitized changes in rats presumably by regulating miRNAs expression in lung tissue. However, the therapeutic outcomes observed post-intratracheal administrations of rBMMSCs were higher than CM.

Acknowledgements

This study was supported by a grant from the Student Research Center of Tabriz University of Medical Sciences.

References

Abreu SC, Antunes MA, Maron-Gutierrez T, Cruz FF, Ornellas DS, Silva AL, Diaz BL, Ab'Saber AM, Capelozzi VL, Xisto DG and Morales MM 2013. Bone marrow mononuclear cell therapy in experimental allergic asthma: Intratracheal versus intravenous administration. Respir Physiol Neurobiol. 185(3): 615-624.

Abreu SC, Antunes MA, Mendonça L, Branco VC, de Melo EB, Olsen PC, Diaz BL, Weiss DJ, Paredes BD, Xisto DG and Morales MM 2014. Effects of bone marrow mononuclear cells from healthy or ovalbumin-induced lung inflammation donors on recipient allergic asthma mice. Stem Cell Res Ther. 5(5): 108.

Ahmadi M, Rahbarghazi R, Aslani MR, Shahbazfar AA, Kazemi M and Keyhanmanesh R 2017. Bone marrow mesenchymal stem cells and their conditioned media could potentially ameliorate ovalbumin-induced asthmatic changes. Biomed Pharmacother.85: 28-40.

Ahmadi M, Rahbarghazi R, Soltani S, Aslani MR and Keyhanmanesh R 2016. Contributory Anti-Inflammatory Effects of Mesenchymal Stem Cells, Not Conditioned Media, On Ovalbumin-Induced Asthmatic Changes in Male Rats. Inflammation. 39(6): 1960-1971.

Banerjee A, Schambach F, DeJong CS, Hammond SM and Reiner SL 2010. Micro-RNA-155 inhibits IFN-γ signaling in CD4+ T cells. Eur. J. Immunol .40(1): 225-231.

Biyashev D, Veliceasa D, Topczewski J, Topczewska JM, Mizgirev I, Vinokour E, Reddi AL, Licht JD,

Revskoy SY and Volpert OV 2012. miR-27b controls venous specification and tip cell fate. Blood. 119(11): 2679-2687.

Chiba Y and Misawa M 2010. MicroRNAs and their therapeutic potential for human diseases: MiR-133a and bronchial smooth muscle hyperresponsiveness in asthma. J Pharmacol Sci. 114(3): 264-268.

Chiba Y, Tanabe M, Goto K, Sakai H and Misawa M 2009 .Down-regulation of miR-133a contributes to up-regulation of Rhoa in bronchial smooth muscle cells. Respir Crit Care Med. 180(8): 713-719.

Fu CL, Ye YL, Lee YL and Chiang BL 2006. Effects of overexpression of IL-10, IL-12, TGF-β and IL 4-on allergen induced change in bronchial responsiveness. Respir Res. 7(1): 72.

Garbacki N, Di Valentin E, Geurts P, Irrthum A, Crahay C, Arnould T, Deroanne C, Piette J, Cataldo D and Colige A 2011. MicroRNAs profiling in murine models of acute and chronic asthma: a relationship with mRNAs targets. PloS one. 6(1): e16509.

Ghorbani A, Feizpour A, Hashemzahi M, Gholami L, Hosseini M, Soukhtanloo M, Bagheri FV, Khodaei E, Roshan NM and Boskabady MH 2014. The effect of adipose derived stromal cells on oxidative stress level, lung emphysema and white blood cells of guinea pigs model of chronic obstructive pulmonary disease. Daru.22(1): 22-26.

Ghosh B 2013. Involvement of microRNA in Asthma: New perspective in respiratory biology. Indian J Allergy Asthma Immunol. 27(1): 3-8.

Goodwin M, Sueblinvong V, Eisenhauer P, Ziats NP, LeClair L, Poynter ME, Steele C, Rincon M and Weiss DJ 2011. Bone marrow-derived mesenchymal stromal cells inhibit Th2-mediated allergic airways inflammation in mice. Stem cells. 29(7): 1137-1148.

Grimson A, Farh KK, Johnston WK, Garrett-Engele P, Lim LP and Bartel DP 2007. MicroRNA targeting specificity in mammals: determinants beyond seed pairing. Mol Cell. 27(1): 91-105.

Kai W, Xu Q and Wu ZQ 2015. MicroRNAs and Asthma Regulation. Iran J Allergy Asthma Immunol.14(2): 120-125.

Keyhanmanesh R, Bagban H, Nazemiyeh H, Bavil FM, Alipour MR and Ahmady M 2013. The relaxant effects of different methanolic fractions of Nigella sativa on guinea pig tracheal chains. Iran J Basic Med Sci. 16(2): 123-128.

Kim HY, Umetsu DT and Dekruyff RH 2016. Innate lymphoid cells in asthma: will they take your breath away? Eur. J. Immunol. 46(4): 795-806.

Kuo YC, Li YS, Zhou J, Shih YR, Miller M, Broide D, Lee OK and Chien S 2013. Human Mesenchymal Stem Cells Suppress the Stretch–Induced Inflammatory miR-155 and Cytokines in Bronchial Epithelial Cells. PloS one.8 (8): e71342.

Kyurkchiev D, Bochev I, Ivanova-Todorova E, Mourdjeva M, Oreshkova T, Belemezova K and Kyurkchiev S 2014. Secretion of immunoregulatory cytokines by mesenchymal stem cells. World J Stem Cells. 6(5): 552-570.

Linero I and Chaparro O 2014. Paracrine effect of mesenchymal stem cells derived from human adipose tissue in bone regeneration. PloS One. 9(9): e107001.

Madrigal M, Rao KS and Riordan NH 2014. A review of therapeutic effects of mesenchymal stem cell secretions and induction of secretory modification by different culture methods. J Transl Med. 12(1): 1.

Malmhäll C, Alawieh S, Lu Y, Sjöstrand M, Bossios A, Eldh M and Rådinger M 2014. MicroRNA-155 is essential for T H 2-mediated allergen-induced eosinophilic inflammation in the lung. Journal of J Allergy Clin Immunol.133(5): 1429-1438. e1427.

Rahbarghazi R, Nassiri SM, Ahmadi SH, Mohammadi E, Rabbani S and Araghi A 2014. Dynamic induction of pro-angiogenic milieu after transplantation of marrow-derived mesenchymal stem cells in experimental myocardial infarction. Int j cardiol. 173(3): 453-466.

Rahbarghazi R, Nassiri SM, Khazraiinia P, Kajbafzadeh AM, Ahmadi SH, Mohammadi E, Molazem M and Zamani-Ahmadmahmudi M 2012. Juxtacrine and paracrine interactions of rat marrow-derived mesenchymal stem cells, muscle-derived satellite cells, and neonatal cardiomyocytes with endothelial cells in angiogenesis dynamics. Stem cells Dev.22(6): 855-865.

Simpson LJ, Patel S, Bhakta NR, Choy DF, Brightbill HD, Ren X, Wang Y, Pua HH, Baumjohann D, Montoya MM and Panduro M 2014 .A microRNA upregulated in asthma airway T cells promotes TH2 cytokine production. Nat Immunol. 15(12): 1162-1170.

Solberg OD, Ostrin EJ, Love MI, Peng JC, Bhakta NR, Hou L, Nguyen C, Solon M, Nguyen C, Barczak AJ and Zlock LT 2012. Airway epithelial miRNA expression is altered in asthma. Am J Respir Crit Care Med. 186(10): 965-974.

Sutsko RP, Young KC, Ribeiro A, Torres E, Rodriguez M, Hehre D, Devia C, McNiece I and Suguihara C 2012. Long-term reparative effects of mesenchymal stem cell therapy following neonatal hyperoxia-induced lung injury. Pediatr Res. 73(1): 46-53.

Tang GN, Li CL, Yao Y, Xu ZB, Deng MX, Wang SY, Sun YQ, Shi JB and Fu QL 2016. MicroRNAs Involved in Asthma After Mesenchymal Stem Cells Treatment. Treatment. Stem Cells Dev. 25(12): 883-896.

Timmers L, Lim SK, Hoefer IE, Arslan F, Lai RC, van Oorschot AA, Goumans MJ, Strijder C, Sze SK, Choo A and Piek JJ 2011.Human mesenchymal stem cell-conditioned medium improves cardiac function following myocardial infarction. Stem Cell Res. 6(3): 206-214.

Trzil JE, Masseau I, Webb TL, Chang CH, Dodam JR, Cohn LA, Liu H, Quimby JM, Dow SW and Reinero CR 2014. Long-term evaluation of mesenchymal stem cell therapy in a feline model of chronic allergic asthma. Clin Exp Allergy. 44(12): 1546-1557.

บทคัดย่อ

การตรวจติดตามการแสดงออกของ IL-13 ที่สัมพันธ์กับการเปลี่ยนแปลงของ miRNA-155 และ miRNA-133 ภายหลังการได้รับเซลล์ต้นกำเนิดเนื้อเยื่อเกี่ยวพันผ่านหลอดลม ภายใต้สภาวะ Ovalbumin-sensitized ในหนูขาว

มัดดิ อาห์มาดี[1,2] เรซา ราห์บาร์กาซี[3,4] อามิร์-อาลี ซาห์บาซ์ฟาร์[5] รานา คีย์ฮันมาเนสห์[6*]

วัตถุประสงค์ของการศึกษาในภาพรวม คือ ศึกษาความเป็นไปได้ของกลไกของเซลล์ต้นกำเนิดของเนื่อเยื่อเกี่ยวพันหรือสภาวะของน้ำยาเลี้ยงเซลล์ ในการกระตุ้นระบบภูมิคุ้มกันต่อภาวะโรคภูมิแพ้ หนูขาวเพศผู้ ถูกแบ่งเป็น 2 กลุ่มคือ สุขภาพดี **(**C**)** และกลุ่ม Ovalbumin-sensitized (S) หนูในกลุ่มนี้ถูกแบ่งเป็น **3** กลุ่มย่อย คือ หนูที่ได้รับ PBS ทางหลอดลม **(**CPT **และ** SPT**)**, CM **(**CST **และ** SST**)** และ MSCs **(**CCT **และ** SCT**)** หลังจากนั้น **2** สัปดาห์ ตรวจการแสดงออกของยีน IL-13, miRNA- 133 and miRNA-155 transcripts พยาธิวิทยาระดับเซลล์ และการเปลี่ยนแปลงของเซลล์ต้นกำเนิดของเนื่อเยื่อเกี่ยวพันที่ปอด ในหนุกลุ่ม CM และกลุ่ม MSCs สามารถลดการแสดงออกของยีนดังกล่าว เมื่อเทียบกับกลุ่ม SPT อย่างมีนัยสำคัญทางสถิติ **(**$p < 0.001$ - $p < 0.05$**)** ตรวจพบการเปลี่ยนแปลงเซลล์ต้นกำเนิดของเนื่อเยื่อเกี่ยวพันที่ปอด ซึ่งแสดงถึงการเปลี่ยนแปลงของเนื้อเยื่อปอด บทสรุป ผลการทดลองพบว่า ความสามารถของ CM และ MSCs ในการควบคุมการแสดงออกของยีนในเนื้อเยื่อปอดของหนูขาว

คำสำคัญ: อินเตอลิวคิน-13 เมเซนไคมอล สเต็ม เซลล์ ไมโครอาร์เอ็นเอ-133 ไมโครอาร์เอ็นเอ-155

[1]Tuberculosis and Lung Diseases Research Center, Tabriz University of Medical Sciences, Tabriz, Iran

[2]Student Research Committee, Tabriz University of Medical Sciences, Tabriz, Iran

[3]Stem Cell Research Center, Tabriz University of Medical Sciences, Tabriz, Iran

[4]Applied Cell Sciences, Faculty of Advanced Medical Sciences, Tabriz University of Medical Sciences, Tabriz, Iran

[5]Department of Pathology, Faculty of Veterinary Medicine, University of Tabriz, Tabriz, Iran

[6]Drug Applied Research Center, Tabriz University of Medical Sciences, Tabriz, Iran

****ผู้รับผิดชอบบทความ** E-mail: rKeyhanmanesh@gmail.com*