Original Research Article

Expression profiling of matrix metalloproteinase in adipose-derived stem cells under simulated microgravity condition

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ABSTRACT

The extracellular matrix (ECM) provides the cellular environment required during development and morphogenesis. The mechanical forces could influence the expression of ECM components. Matrix metalloproteinase (MMP) is a family of proteolytic enzymes participated in ECM remodeling and degradation. We investigated the effect of simulated microgravity on gene expression of some MMPs including *MMP1*, *MMP2*, *MMP3*, *MMP14*, *TIMP1* and *TIMP2* in adipose-derived stem cells (ADSCs) using real-time PCR technique. Our results showed that the expression of selected genes was differentially regulated following microgravity simulation. Therefore, with a better understanding of this mechanical force and its effect on cells, it is likely to use microgravity alone or besides the biochemical inducer for cell manipulation.

***Keywords*:** Simulated microgravity, matrix metalloproteinase, adipose derived stem cell

INTRODUCTION

The extracellular matrix (ECM) is a complex network of non-cellular molecules that creates structural, adhesive and biochemical signaling support to the cells. Normal condition of ECM is essential during development of multi-cellular organisms,

morphogenesis, tissue homeostasis, tissue repairing and remodeling. However, dysregulation of ECM has been reported in many disorders including arthritis, nephritis, cancer, encephalomyelitis, chronic ulcers, and fibrosis [1].

Matrix metalloproteinases (MMPs), called matrixins, are a family of zinc-dependent proteolytic enzymes that participated in ECM remodeling and degradation. Now, 24 types of MMPs are discovered in verteberates, which 23 of them are existed in humans. In healthy tissues, the activity of most MMPs is generally low or absent. However, during physiological process, diseases, or inflammatory events, matrixin levels are often elevated. Further regulation of matrixins activity occurs by activation of the precursor zymogens and expression of tissue inhibitors of metalloproteinases (TIMPs). TIMPs are the main inhibitors of MMPs that containing a 4-membered family of inhibitors: TIMP1, TIMP2, TIMP3 and TIMP4 [2]. The balance between matrixins and TIMPs are essential for the normal physiological function of ECM.

Furthermore, the expression of ECM components can be influenced by mechanical stress [3]. One of the most important mechanical factors that affect all types of life on earth is gravity. Microgravity has been confirmed to affect growth and physiology of cell through impacting on intracellular signaling mechanisms, cell secretions and gene expression [4]. It has been shown that components of cytoskeleton such as actin polymer are gravity sensitive and reorganized in microgravity condition. This could lead to change in cell morphology and fate. With regards to ECM roles and the communication between ECM and cytoskeleton, it is expected that changes in mechanical forces have a significant effect on the ECM structure.

Here we investigated the effect of simulated microgravity condition on gene expression of some MMPs and TIMPs in adipose-derived stem cells (ADSCs). ADSCs are propounded as a great source of mesenchymal stem cells (MSCs) that are easily achievable from adipose tissue via liposuction [5]. Matrixins have an important role in the differentiation of MSCs into different cell types. Studies have shown that MMPs are involved in the differentiation, angiogenesis, proliferation, and migration of MSCs [6]. Therefore, overexpression of MMPs or TIMPs in MSCs may promote differentiation into specific cell types that can be used for regeneration medicine.

MATERIALS AND METHODS

All experiments were performed according to the Institutional Ethics Committee (IEC-SCTIMST). Tissue samples were acquired from donors in the age group from 34 to 48 years through cosmetic liposuction. Cell isolation was done by enzymatic digestion method as described by Zhk in 2001 and as described previously [7,8]. Flow cytometric analysis was done as described previously [8] using PE and FITC-conjugated antibodies (BD Biosciences PharMingen, USA) against CD90-PE, CD105-FITC, CD73-PE as positive markers, and CD34-PE and CD45-FITC as negative markers. Flow cytometric analysis was carrying out using a Cyflow Space (Partec) flow cytometer. To examine multipotent potential of the cells, adipogenic and osteogenic differentiation were performed using Human Mesenchymal Stem Cell Functional Identification Kit (R&D systems, USA) according to the manufacturer recommendations. Lipid vacuoles and accumulation of calcium were confirmed by Oil Red O and Alizarin Red S (Sigma, USA) staining, respectively [8].

Clinostat was used for simulating microgravity. This device prevents cells feeling gravity by rotating. Clinostat was sterilized by UV and ethanol (70 %) and put in 37 °C CO_2 incubator. ADSCs were seeded on 25 cm^2 culture flask. Flasks filled completely by medium and fixed at the center of the clinostat. To maintain the pH balance, the medium was supplemented with 15 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES). The clinostat rotation speed was 30 rpm. The rotation times were 1, 2 and 3 days.

The expression of selected genes was analyzed in all samples by real-time RT-PCR as described previously [8]. PCR program was: initial denaturation at 95 ºC for 2 min; followed by 40 cycles of denaturation at 95 ºC for 5 sec and annealing at 60 ºC for 30 sec. The expression of GAPDH was measured as a normalization control. Specific primers were designed as follows (5′ to 3′): F-MMP1:TCACACCTCTGACATTCACCAAG, R-MMP1:TCCCGATGATCTCCCCTGAC, (79 bp); F-MMP2:TTGACCAGAATACCATCGAGACC, R-MMP2:TGTGTAGCCAATGATCCTGTATGT, (131 bp); F-MMP3:ACAAAGGATACAACAGGGACCAA, R-MMP3:ATCTTGAGACAGGCGGAACC, (153 bp); F-MMP14:CCAGTTCGCCGACTAAGCAG, R-MMP14:CGCTGTGTGTGGGTACGTAG, (395 bp); F-

TIMP1:CCCAGAGAGACACCAGAGAAC, R-TIMP1:GCAACAACAGGATGCCAGAAG, (69 bp); F-TIMP2: GAGCACCACCCAGAAGAAGAG, R-TIMP2:GATGTAGCACGGGATCATGGG, (91 bp); F-GAPDH: TGCACCACCAACTGCTTAGC and R-GAPDH: GGCATGGACTGTGGTCATGAG, (87 bp). Statistical correlation was performed using independent samples t-test and $p<0.05$ was considered statistically significant.

RESULTS

After 48 h of culture of cells, fibroblast-like spindle shape cells were observed (figure 1A). Both the shape and adhesion properties of the cells confirmed that the isolated cells were mesenchymal stem cells. Flow cytometric analysis of ADSCs showed that a higher percentage of ADSCs (>95 %) expressed CD90, CD73 and CD105 (MSCs markers) but not expressed CD34 and CD45 (hematopoitic markers). To demonstrate pluripotent capacity of ADSCs, they were differentiated into adipocyte and osteocyte. Staining results by Oil Red O (figure. 2B) and Alizarin Red S (figure. 2C) confirmed that the isolated cells differentiated well into adipocyte and osteoblast lineages [8].

We employed a real-time PCR method to measure any potential changes in gene expression of MMP1, MMP2, MMP3, MMP14, TIMP1, and TIMP2. As shown in figure 2, simulated microgravity increased the expression of MMP2 (30 %), MMP3 (12 %), MMP14 (20 %), TIMP1 (50 %), and TIMP2 (50 %) one day after treatment. However, the expressions of MMP2, TIMP1 and TIMP2 decreased by 3 days after treatment (40 %, 40 % and 30 %; respectively) compared to the control group. The expression of MMP3 declined by 2 days after microgravity simulation and increased thereafter by 3 days after treatment compared to the control group. The expression of MMP14 came back to the normal levels by 3 days. However, the expression of MMP1 was increased after microgravity simulation, their changes were not significant.

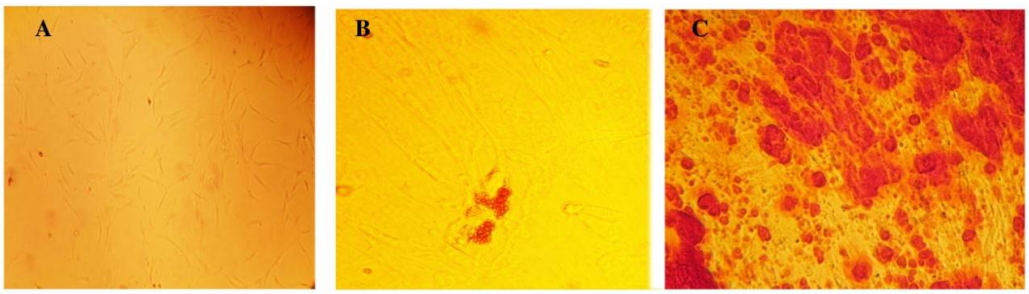

Figure1. Cell isolation and characterization. A) Fibroblast-like spindle shape cells after 48 h culture. B) Adipogenic differentiation of ADSCs. C) Osteogenic differentiation of ADSCs [8].

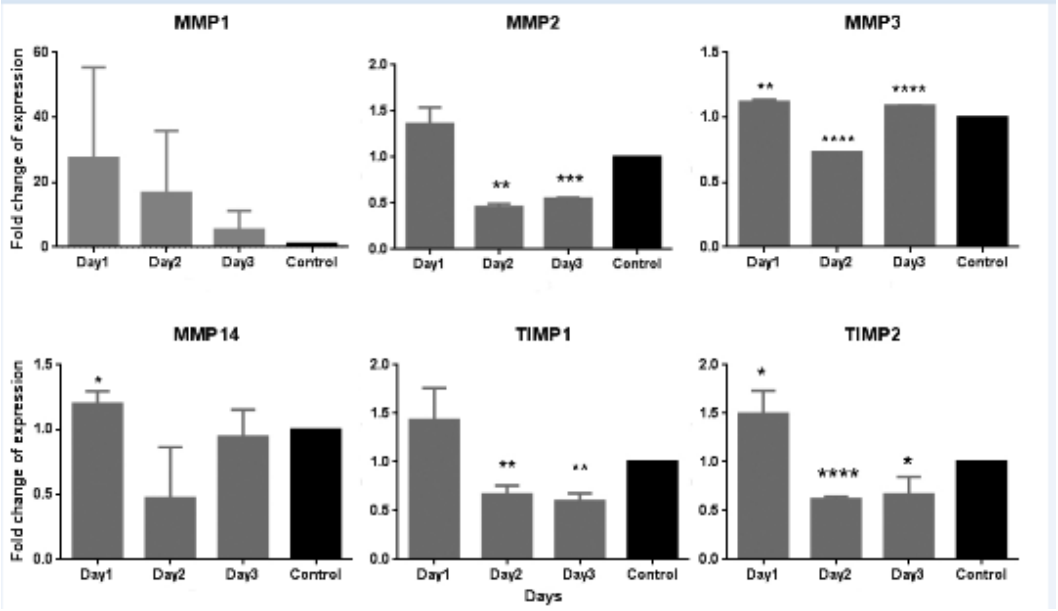

Figure 2. Real-time quantitative PCR analysis of MMPs and TIMPs of ADSCs cultured on simulated microgravity condition.

DISCUSSION

Gravity is one of the most important mechanical factors on the earth that has extensive effect on evolution and physiology of all living organisms. It is believed that changes in value of gravity cause changes in physiology of cells and organisms [9]. Here we were examined the changes in the expression of some MMP and TIMP enzymes that participated in ECM remodeling and degradation in isolated human ADSCs under simulated microgravity condition including *MMP1*, *MMP2*, *MMP3*, *MMP14*, *TIMP1* and *TIMP2*. ECM provides an environment that is necessary during development and morphogenesis of multi-

cellular organisms [1]. Interaction of ECM molecules with cell receptors initiate signal transduction pathways and regulate the cell fate and cell differentiation [10,11]. Our results showed that the expression of selected genes was differentially regulated following microgravity simulation in ADSCs. MMP1 degrades collagen I, II and III. MMP2 digest gelatin and MMP3 responsible for degradation of some noncollagen ECM components. In contrast, MMP14 are intracellularly activated transmembrane molecules.

The MMPs were inhibited by specific TIMPs including TIMP1, TIMP2, TIMP3 and TIMP4 [2]. Here we investigated the effect of simulated microgravity on TIMP1 and TIMP2 expression.

ADSCs are propound as a great source of MSCs that are easily achievable from adipose tissue via liposuction. They have great potential for cell-based therapy and regenerative medicine because of their capacity for self-renewal and differentiation into different cell lineages [12]. Some reports are shown that some matrixins probably affect the fate of MSCs and regulates the differentiation of them into different cell lineages. Also, studies suggest that pericellular localization of matrixin functions could be control through interaction with MSCs. Recent studies suggested the association of MMPs in the differentiation, angiogenesis, proliferation, and migration of MSCs [13]. Therefore, we believed that treatment of ADSCs by simulated microgravity may increase the differentiation capacity of these cells through alterations in expression of MMPs and remodeling of ECM structure. Previous studies suggested that MMPs have a crucial role in differentiation of MSCs into adipocytes. Sillat and his colleagues showed that the expression of TIMP2 significantly increased during adipogenic differentiation of MSCs [14]. Also, upregulation of MMP2 and down regulation of MMP11 were observed during differentiation of mouse embryonic cells into fat tissues [15]. The roles of MMPs during chondrogenic, osteogenic and endothelial differentiation have also been reported [16-19]. Data by Jin and their group [16] revealed that the expression of MMP2 is required for chondrogenic differentiation of MSCs. This is probably the result of downregulation of focal adhesion kinase (FAK)–β1 integrin interaction, which leads to phosphorylation of FAK. Downregulation of MMP-1 and MMP-8 and upregulation of TIMP2 and TIMP4 were observed following osteogenic differentiation of human MSCs [17]. Today we know that ECM signaling is required for angiogenesis induction through digestion of the ECM and exposure of endothelial cells to

collagen type I. Therefore, degradation of ECM by MMPs has an important role in angiogenesis and differentiation of endothelial cells [18].

Therefore, with a better understanding of this mechanical force and its effect on cells, it is likely to use microgravity alone or besides the biochemical inducer for cell manipulation and differentiation. Our findings could provide a new strategy for differentiation of ADSCs which are used in medicine for cell therapy of some disorders. Also, the potential of three dimensional growth of cells under microgravity condition offers opportunity for tissue engineering without using scaffold for regenerative medicine.

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