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ORIGINAL ARTICLE

# TAZ is Associated with Poor Osteoblast Differentiation of Mesenchymal Stem Cells Under Simulated Microgravity

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**Background:** Exposure to microgravity (MG) leads to many varieties of physiological alterations, including bone loss. Most studies concur that the impaired osteoblast differentiation of mesenchymal stem cells (MSCs) plays an important role in this bone loss. However, the detailed signaling mechanisms underlying the MG-induced bone loss remain to be further clarified. **Materials and Methods:** We utilized a rotary cell culture system (RCCS) to study the role of transcriptional coactivator with PDZ-binding motif (TAZ) in simulated MG. Cells were obtained from the calvarial bone of 5-d old Balb/c mice littermates. The phenotype of the MSCs was confirmed by positive expression of Sca-1 and CD29, and negative for CD45. MSCs were cultured in osteo-induction medium in order to promote differentiation towards osteoblasts (OSTs). **Results:** Upon exposure to MG for 7 days, the abundance of Runx2 was significantly reduced to 0.33 and 0.2-fold in MSCs and OSTs, respectively. In contrast, PPARγ2 was significantly enhanced to 3.8 and 3.0-fold in response to MG in MSCs and OSTs, respectively. TAZ mRNA is decreased to 0.22- and 0.08-fold as compared to normal gravity (NG) in both MSCs and OSTs, respectively. Similarly, the TAZ protein level was also significantly decreased to 0.4-fold in MSCs and to 0.2-fold in OSTs. Moreover, we showed that MG indeed disrupted the interaction of TAZ and Runx2, which disturbed osteoblast-related gene expression. **Conclusions:** We show for the first time that the TAZ is associated with MG-induced impairment of osteoblast differentiation. Our results also suggest that TAZ plays an important role in MG-induced bone loss.

Key words: TAZ, simulated microgravity, mesenchymal stem cells, osteoblast differentiation

## INTRODUCTION

Spaceflight is associated with a low gravitational, cold, and hypoxic environment, which is hazardous for humans adapted to earth conditions. Thus, individuals may face many new physiological challenges when exposed to a space environment. Among them, microgravity (MG)-induced osteopenia is an important health problem after long-term human space travel.[1] It was reported that astronauts suffered from a decrease in bone mineral density of about 1-2% per month, as well as reduced bone strength during spaceflight. These effects are far severer than menopausal osteopenia and consequently might increase the risk of developing disuse osteoporosis. Furthermore, many altered bone biomarkers and bone strength data also supported the facts of dramatic bone loss, even lasting for a period of time after landing,[2] which increases the risks of bone fracture and disability.

Bone homeostasis is principally regulated by the interaction of osteoclastic bone resorption and osteoblastic bone formation. Osteoclasts are bone-resorbing multinucleated giant cells derived from monocytes. MG was reported to trigger proliferation and enhance osteoclast activity by upregulating the release of crucial bone turnover enzymes such as cathepsin K.[3,4] On the other hand; the bone formation is processed by osteoblasts (OSTs), which are differentiated from multipotent precursors, mesenchymal stem cells (MSCs). MSCs obtained from rat bone marrow were decreased to differentiate and maturate toward OSTs under simulated MG.[5] Thus, it is commonly alleged that MG-induced osteopenia resulted from decreased bone formation and increased bone resorption,[6] especially the insufficient OST differentiation from MSCs.[7]

Varieties of tissues, including cartilage, muscle, bone, tendon, marrow, and fat originate from MSCs. These cell fates are determined by complicated interactions of many

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different biochemical and biomechanical factors. Among them, gravity is one of the most important factors.[8] Lack of gravity was reported to direct increased adipogenesis and decreased osteogenesis of human MSCs.[2] In addition, similar results were also observed in the bone marrow stroma of a skeletal-unloading rodent model.[9] These findings affirmatively indicated that the lack of gravity drove MSCs into adipocyte lineage rather than OST differentiation. Until now, though there is an increasing number of studies that focus on the mechanisms of MG suppressing OST differentiation. The detailed mechanisms remain unclear.

Runt-related transcription factor 2 (Runx2) has been identified as "a master gene" for OST differentiation at multiple stages.[10] It was confirmed by *in vivo* evidence that development and maturation of OSTs and ossification are disturbed in Runx2 knockout mice,[11] indicating the essential role of Runx2 in the differentiation of OSTs. On the other hand, peroxisome proliferator-activated receptor γ (PPARγ) plays a critical role in adipocyte differentiation, as deficiency of PPARγ in cells and rodents displayed poor differentiation of adipocyte with increased OST differentiation and bone formation.[12] Taken together, Runx2 and PPARγ2 are two representative markers and essential transcription factors to regulate differentiation of MSCs to either OSTs or adipocytes. The transcriptional coactivator with PDZ-binding motif (TAZ), a transcriptional modulator of MSCs differentiation, can reinforce a Runx2-dependent gene transcription while retarding PPARγ2-dependent gene transcription.[13] This implies that TAZ is an important target for MG-impaired OST differentiation.[14]

The purpose of this study is to further investigate the detailed mechanisms of MG on MSCs differentiation. In this study, the primary cultured murine calvarial cells were isolated, and the surface markers CD29 (+), CD45 (−), and Sca-1 (+) were identified to confirm the phenotype of MSCs.[15] Simulated MG conditions were carried out with a Rotary Cell Culture System (RCCS). The effect of MG on the expression levels of the TAZ, Runx2, and PPARγ2 were analyzed in both MSCs and OSTs. Our findings indicate that MG suppresses the differentiation of MSCs toward OSTs. The results confirm that MG severely downregulates the osteogenesis-related gene expression and that this effect is associated with the TAZ signal pathway.

## MATERIALS AND METHODS

### Animal

We used 5-day-old newborn BALB/c mice, which were purchased from BioLASCO Taiwan Co., Ltd. The experimental protocol was approved by the Institutional Animal Care and Use Committee of the National Defense Medical Center (IACUC-06-007). The mice were fed with regular food and water ad libitum and maintained with half and half light and dark cycles at 25 ± 0.5°C in the National Defense Medical Center's Laboratory Animal Center.

### Mesenchymal stem cells isolation and the induction of osteogenesis

MSCs isolation was performed as in the previous study with minor modifications.[2] Briefly, mice were euthanized by the use of cervical dislocation. The frontal and parietal bones were dissected and cut into small pieces with scissors (around 1 mm diameter) in phosphate-buffered saline (PBS). After being centrifuged at 1500 rpm for 2 min at 4°C, the supernatant (blood cells) was removed and then resuspended by 0.2% collagenase type I in 5% $CO_2$ at 37°C for 30 min. Minimum essential medium alpha containing 100 IU/ml penicillin, 100 μg/ml streptomycin, and 10% fetal calf serum (Gibco, Gaithersburg, MD, USA) were added to stop the reaction. Subsequently, the homogenates were centrifuged at 1200 rpm for 10 min. Supernatant was removed, and cells were plated in T150 flasks (BD Biosciences). The medium was changed every 2 or 3 days, and the cells were subcultured when grown to approximately 80% confluence. Osteo-induction medium contains regular medium supplemented with $10^{-7}$ M dexamethasone, 50 μM L-ascorbic acid, and 10 mM beta-glycerophosphate disodium salt hydrate (Sigma-Aldrich, MO, USA). MSCs were incubated in an osteo-induction medium for 72 h for osteoblastic differentiation as OSTs preceding the beginning of MG exposure. All the experiments were under osteo-induction medium and carried out using cells with 2-5 passages.

### Flow cytometry

Specific cell surface markers of MSCs were identified by using flow cytometry, as described previously.[15] Briefly, MSCs were stripped from beads, and incubated in PBS solution containing fluorescein isothiocyanate (FITC)-conjugated antibodies against CD29 or CD45 (Santa Cruz, CA, USA), or Sca-1 (BD Biosciences, CA, USA) for 30 min at room temperature, respectively. Subsequently, samples were washed with PBS and centrifuged at 175 ×*g* for 2 min to remove nonspecific binding, finally analyzed by flow cytometry. All data were analyzed with CellQuest software V3.3 (Becton Dickinson, San Jose, CA).

### Rotary Cell Culture System

RCCS (Synthecon, Inc., Houston, TX, USA) [Figure 1] contains rotary wall vessels (RWVs), a cylindrical vessel which consists of a flat silicone rubber and a gas transfer membrane, and a rotator base. RCCS, designed by NASA, is

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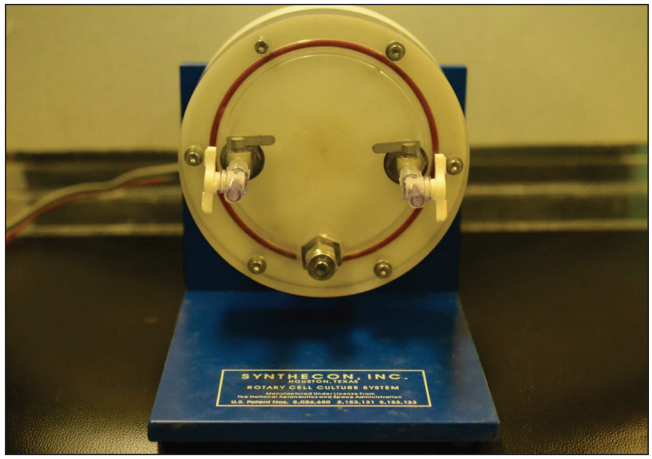


**Figure 1:** Rotary Cell Culture System (RCCS). Rotary Cell Culture System generates a simulated microgravity effects of cell culture environment via rotating a vessel around a horizontal axis to randomize the gravitational directions, whose features include low shear force (low turbulence), high mass transfer of nutrients and gas, and avoiding existing bubbles

a well-established cellular model of MG for studying cellular physiology.[6] The method was as described previously.[16] Briefly, 0.2 g microcarrier beads (Biosilicon, Nunc Labs, UK) are added per $10^7$ cells, then mixed and injected into the RWV. To simulate MG conditions, RWVs rotates along a horizontal axis at a fixed speed (10 rpm), resulting in fluid dynamics that allow a cell suspension environment with low fluid shear turbulence and sufficient oxygenation. The medium was replaced every 2 days.

### Isolation of RNAs and quantitative real-time-polymerase chain reaction

Relative gene expression of TAZ in the MSCs and OSTs were examined by quantitative real-time polymerase chain reaction (Q-PCR). Briefly, after MG or normal gravity (NG) exposure, total RNA was extracted by Trizol reagent (Invitrogen, Irvine, CA, USA). RNA (1 μg) was reverse-transcribed to cDNA using the procedures provided in commercial kits (Applied Biosystems, Darmstadt, Germany). The PCR primers and programs were as described previously:[17] TAZ 5' primer, 5'-CGTCCATCA CTTCCACCTCG-3'; 3' primer, 5'-ACTGTAGCACCCTAACCCCAGG-3'; ß-actin 5' primer, 5'-GTC CCTGTA TGC CTC TGG TC-3'; 3' primer, 5'-TCG TAC TCC TGC TTG CTGAT-3'. The PCR programs for TAZ were as follows: 95°C for 1 min, 58°C for 30 s, and 72°C for 7 min for total 30 cycles. The annealing temperature for ß-actin is 60°C. The SYBR Green Q-PCR was assayed on the StepOne real-time PCR System with a commercial kit (Applied Biosystems, Foster City, CA, USA). Data were determined by the StepOne v2.0 software (Applied Biosystems, Foster City, CA, USA).

### Western blot analysis

The expression of specific proteins in MSCs and OSTs was quantified as described previously.[18] Briefly, the cells were harvested in lysis buffer (7 M urea, 2 M thiourea, 1% NP-40, and 60 mM DTT) with protease inhibitors and centrifuged at 14000 rpm for 20 min at 4°C. The supernatant was collected. Equal amounts of protein from each sample were separated using sodium dodecyl sulfate (SDS)-10% polyacrylamide gel electrophoresis (PAGE) and transblotted onto polyvinylidenedifluoride (PVDF) membranes (Millipore, Bedford, MA, USA). Protein concentration was quantified of using the BCA protein assay (Pierce, Rockford, IL, USA). Immunoblotting was performed with antibodies for PPARγ2, Runx2, TAZ (Santa Cruz, CA, USA), and β-actin (Chemicon, Temecula, CA, USA). The signals were visualized with an enhanced chemiluminescence kit (ECL, Amersham, UK), followed by exposure to X-ray films.

### Immunofluorescence

The method was to investigate the subcellular localization of TAZ and Runx2 as described previously.[19] After harvest, cells were fixed in methanol at −20°C for 10 min and then incubated at 37°C with TAZ or Runx2 antibody (Santa Cruz, CA, USA) for 1 h followed by conjugated with donkey anti-goat IgG-FITC (Santa Cruz, CA, USA) for another 1 h, subsequently, supplemented with propidium iodide (PI) staining to stain nuclei. Primary and secondary antibodies were used at 1:10 dilutions in PBS. Emission and excitation wavelengths are 488/530 nm and 530/585 nm for IgG-FITC and PI, respectively. Images were examined with a laser scanning confocal microscope (ZeissAxiovert 100 M, Oberkochen, Germany).

### Statistical analysis

All the data represent the mean ± standard error of the mean. Significant differences among group means were determined by a one-way ANOVA, with repeated measures followed by Newman-Keuls test using SPSS version 14.0 for windows software. $P < 0.05$ was considered as statistically significant.

## RESULTS

### Characterization of cellular surface antigens and morphology of mesenchymal stem cells

We obtained the MSCs from the calvarial bone of a 5-day-old newborn mouse. To characterize the cellular properties of MSCs, three specific markers were analyzed by flow cytometry.[15] As shown in Figure 2a, over 90% of the cells expressed CD29, which is an extracellular matrix receptor. Furthermore, Sca-1, a surface marker of stem cells,

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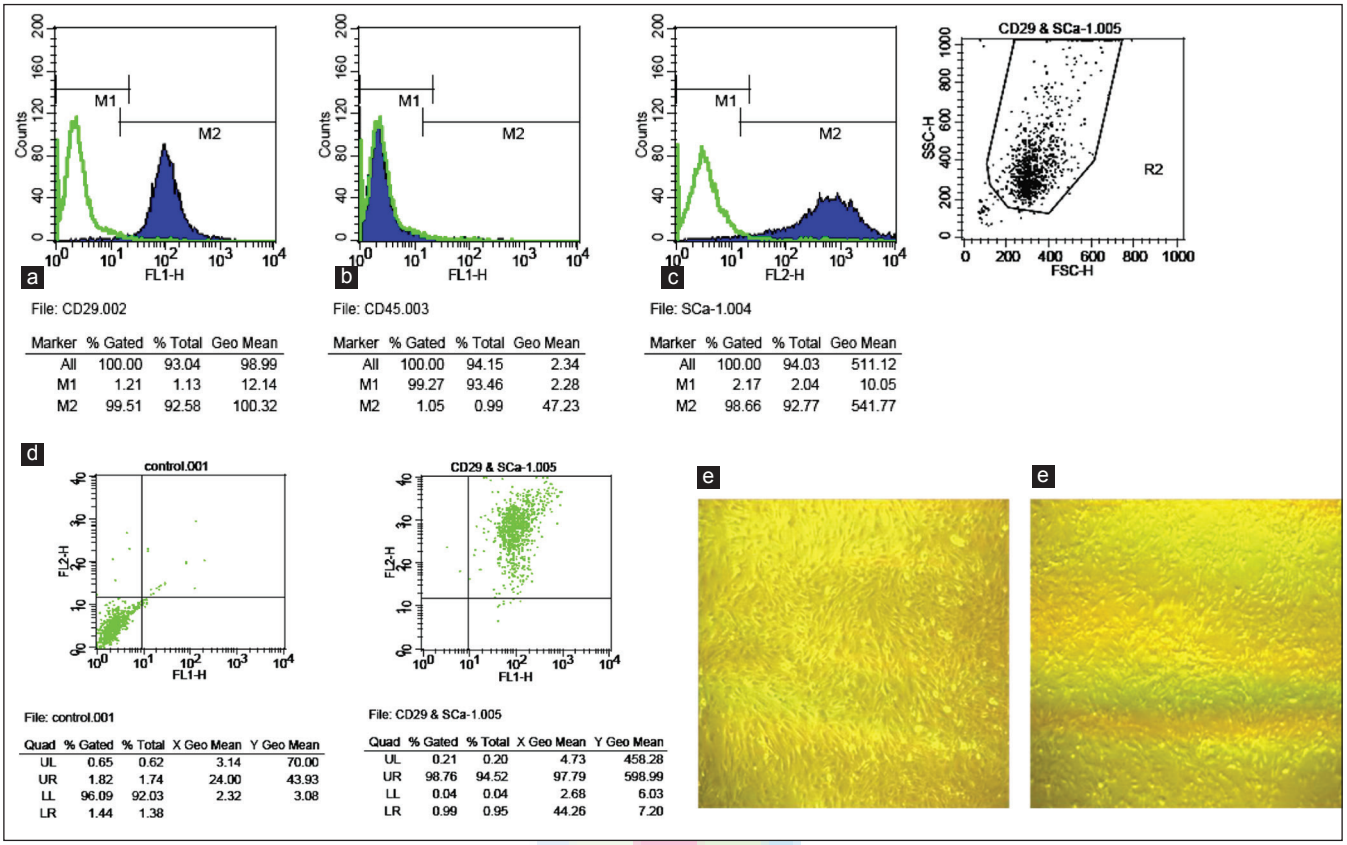


**Figure 2:** Identification and morphology of mesenchymal stem cells. Fluorescent activated cell sorter (FACS, 1 × $10^6$ cells each sample) analysis demonstrated that cells expressed positive for (a) CD29 and (c) Sca-1, negative for (b) CD45. Double stain method further showed the double positive of CD29 and Sca-1 (d) to confirm the properties of mesenchymal stem cells. Morphologic characteristics of mesenchymal stem cells (e) were different from those of osteoblasts (f), which were incubated in special osteoinductive medium for 72 h. Images were performed by photomicrography (original magnification ×400)

is also expressed predominantly on the majority of the cells [Figure 2c]. In contrast, CD45, a hallmark of hematopoietic cells, is almost negative [Figure 2b]. To further confirm the results, the CD29 and Sca-1 double staining method was used. We showed over 94% of double positive cells, representing the characterization of MSCs [Figure 2d]. In addition to MSCs, we also used special osteo-induction medium to induce osteogenic differentiation of MSCs, as previously described.[2] The cellular morphology has obviously changed from the long-tailed fibroblast-like morphology of MSCs to the flat and stellate-like morphology of OSTs after 72 h osteo-induction. It indicates the success in differentiating MSCs into OSTs [Figure 2e and f].

### Microgravity increased peroxisome proliferator-activated receptor γ2 but decreased Runt-related transcription factor 2 expression

To verify the effects of MG on MSCs differentiation, Western blot analysis was performed. As shown in Figures 3a and b, MG significantly increased PPARγ2 levels compared with NG in either MSCs (3.78 ± 0.32-fold, $P < 0.001$) or OSTs (3.02 ± 0.07-fold, $P < 0.001$), respectively. In contrast, the Runx2 protein level was dramatically suppressed in response to MG as compared with NG in both of the MSCs (0.33 ± 0.05-fold, $P < 0.001$, in Figure 3c) and OSTs (0.2 ± 0.02-fold, $P < 0.001$, in Figure 3d).

### Microgravity decreased TAZ mRNA and protein expression in both mesenchymal stem cells and osteoblasts

TAZ is a crucial co-transcription factor of Runx2, a switch of osteogenesis, and an upstream regulator of Runx2 and PPARγ2. To test whether MG has impacts on TAZ gene transcription, mRNA expression of TAZ was examined in both MSCs and OSTs under NG or MG. As shown in Figure 4a, real-time reverse transcription-PCR results showed that MG downregulated TAZ mRNA levels compared with NG in MSCs after MG exposure for 7 days. Meanwhile, we also observed that TAZ mRNA under MG is also much lower than NG in OSTs (0.08-fold, Figure 4b) . Similar results were also

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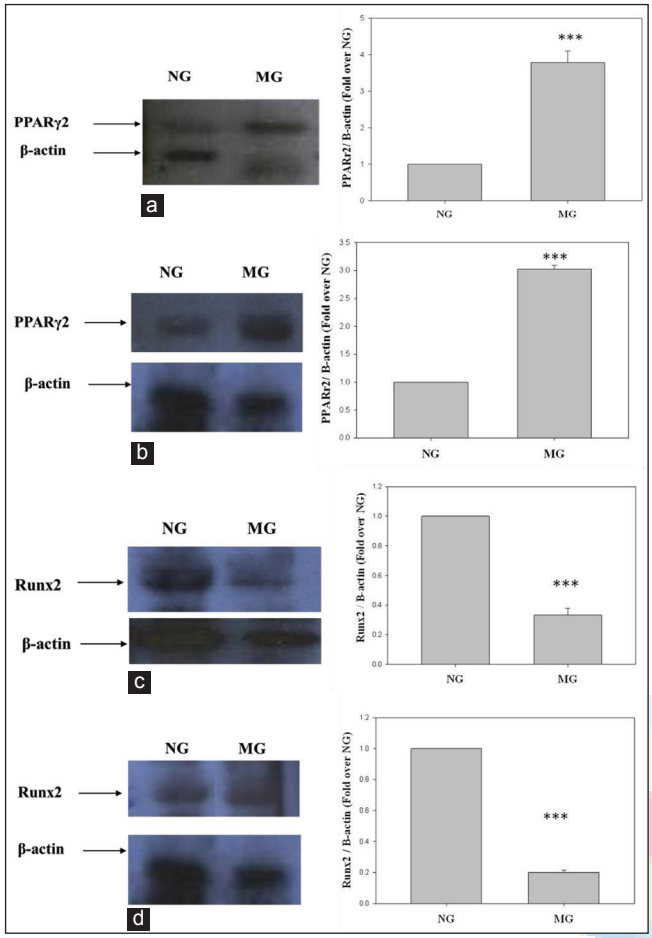


**Figure 3:** Microgravity altered Runt-related transcription factor 2, and peroxisome proliferator-activated receptor γ2 protein level in both mesenchymal stem cells and osteoblasts. After 7 days of microgravity exposure, cells were harvested for Western blot analysis. The relative levels of peroxisome proliferator-activated receptor γ2 and Runt-related transcription factor 2 were analyzed in mesenchymal stem cells (a and c) or osteoblasts (b and d), respectively. The results represent the mean ± standard error of mean of three independent experiments. ***$P < 0.001$ versus normal gravity

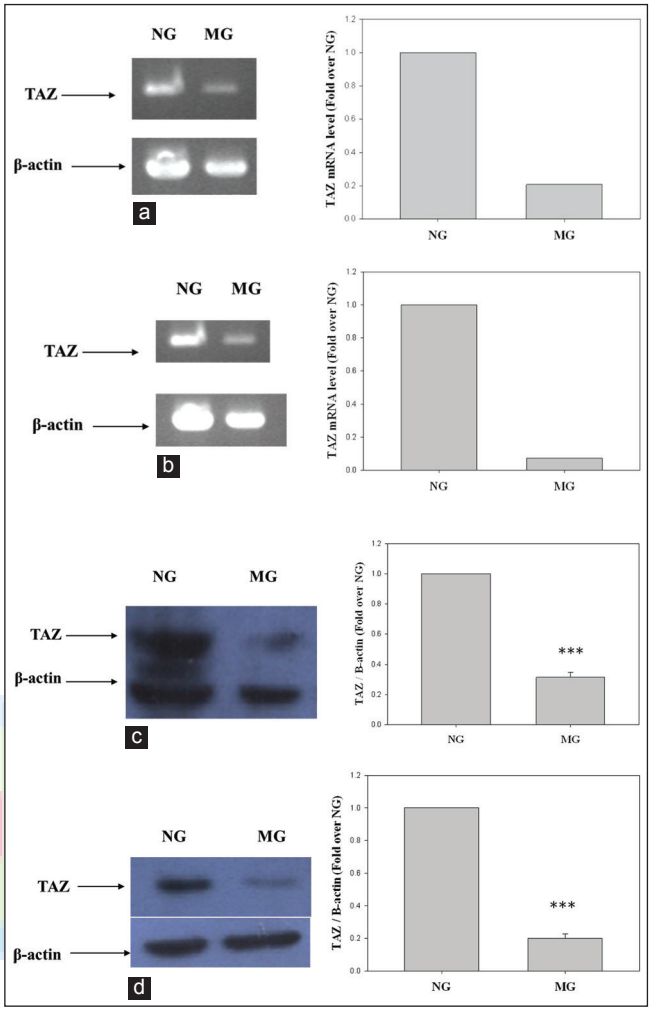


**Figure 4:** Microgravity significantly decreased TAZ mRNA and protein expression in both mesenchymal stem cells and osteoblasts. After 7 days of microgravity exposure, RNA was extracted from cells to examine the relative TAZ mRNA levels by quantitative real-time polymerase chain reaction. The result was normalized by β-actin level. The relative mRNA and protein expression of TAZ in mesenchymal stem cells (a and c) or osteoblasts (b and d). Results represent mean ± standard error of mean of three independent experiments. ***$P < 0.001$ versus normal gravity

found in the protein level of TAZ in either MSCs (0.31 ± 0.03-fold, $P < 0.001$, in Figure 4c) or OSTs (0.2 ± 0.03-fold, $P < 0.001$, in Figure 4d).

### Microgravity decreased the nuclear localization of TAZ and Runt-related transcription factor 2

Nonviable cells were assessed by propidium iodide staining assay. Results showed similar viability in response to either MG or NG, indicating that MG conferred no apparent cytotoxicity to the cells under our experimental conditions [Figure 5a and b]. To investigate the physical interaction of TAZ and Runx2 in response to MG, OSTs were examined by using laser scanning confocal microscope assay in either NG or MG. The localization of TAZ or Runx2 was expressed as a red or green fluorescence signal in Figures 5c and d, respectively, showing that TAZ and Runx2 were predominantly located in the nuclei under NG. Notably, merging the two images showed a strong yellow light, indicating the colocalization of TAZ and Runx2 in the nuclei [Figure 5e]. Next, we tested the cellular distribution of TAZ and Runx2 after 18 h of exposure to MG. Neither TAZ nor Runx2 was present in nuclei; this result was totally opposite to that in NG [Figure 5f-h).

## DISCUSSION

It is known that gravity is an important factor for the maintenance of bone mass because of the essential role of

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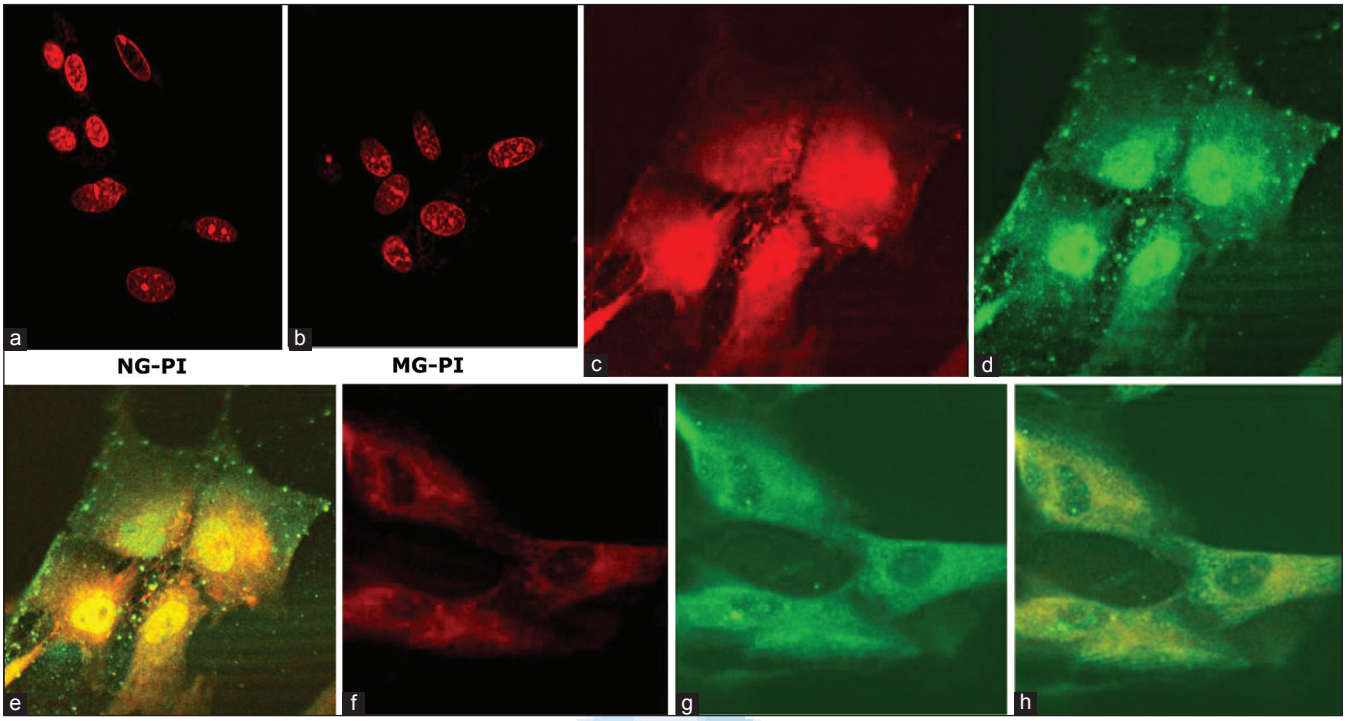


**Figure 5:** Microgravity decreased nuclear colocalization of TAZ and Runt-related transcription factor 2. Mesenchymal stem cells were incubated with osteo-inductive medium for three passages for osteoblast differentiation as osteoblasts preceding the experiment. After 7 days, PI staining was shown as normal gravity (a) and microgravity (b). The cellular location of Runt-related transcription factor 2, TAZ, and merge section were analyzed by immunocytochemistry under normal gravity (c-e) or microgravity (f-h) for 18 h, respectively

mechanical stress in the OST differentiation.[20] MG has been known to have negative impacts on bone formation and the mechanisms still remain unclear. However, it is uncommon and can be very expensive for most researchers to investigate the effects of MG via the approaches of parabolic flight or space flight. Until now, several ground-based analog methods were developed, including the 6° head-down tilt bed rest for human study and hindlimb unloading rodent model. Among them, RCCS is a small-scale, simple, NASA-recommended, and well-established system used to simulate MG on the ground by mimicking free-fall orbit. Free-fall orbit provides a suspension cell culture environment by rotating vessels at a constant speed.[21] In this study, we used the RCCS in order to simulate MG conditions to test the effect of MG on OST differentiation of MSCs, which was from primary cultured mouse bone of calvaria. To clarify the effect of MG on the different stages in OST development, MSCs under osteo-induction medium and OSTs differentiated from MSCs were used, respectively. Our results all demonstrated the comparable trends on the primary transcription factors involved in osteoblastic differentiation in both of the MSCs and OSTs, including protein levels, mRNA expression, and intracellular localizations. These results suggest that MG have similar impacts at different stages of OST lineage.

Lack of mechanical stress predominantly drove MSCs to be differentiated into lipocytes rather than OSTs, which is difficulty reversed to OST lineage, and vice-versa as described previously.[2,5,22,23] Consistently, more lipid droplets were found in the spongy bone tissue of Japanese quails after long-term hypodynamy than in the same tissue of the control animals.[24] These findings indicated that a decrease in bone strength under MG is due to altered constitutions of bone structure, especially in fat formation. These processes may also explain why osteoporosis often exists reciprocally with obesity.[25] In the present study, we showed that MG significantly reduced the OST development as it lowered Runx2 protein expression and contrarily increased PPARγ2 levels in both MSCs and OSTs [Figure 3]. Furthermore, similar results were also observed in different sources of MSCs in previous studies, including human MSCs from surgical waste tissues (31) and rat MSCs from bone marrow (8). Combined with our results, these findings confirmed that MSCs, under MG conditions, tend to differentiate toward adipogenesis instead of osteogenesis. Except for the impacts on MSCs differentiation, some recent studies focused on the benefits of MG on MSCs. It was reported that three-dimensional simulated MG reinforced human MSCs the abilities of proliferation and differentiation for hyaline cartilage after transplantation,[26] suggesting a potential method

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for use in regenerative medicine. It is consistent with a recent study in which simulated MG significantly promoted MSCs toward chondrogenic differentiation.[27] However, all these findings were about graft developments of chondrogenic lineage. Other tissue-engineered aspects, such as the potentials and methods for construction of muscle, bone, and tendon *in vitro* still need to be further explored.

To investigate the detailed action mechanisms of MG on MSCs differentiation, upstream signaling pathways of Runx2 and PPARγ2 were examined. TAZ also called the WW domain-containing transcriptional coregulator, has been shown to be in charge of cell differentiation, proliferation, and development.[28] It was reported that TAZ is able to coordinate the OST differentiation cascade by cooperating with Runx2, which is translocated to the nucleus, thereby promoting OST-related gene expression while decreasing adipogenesis via blockage of PPARγ2 pathway in MSCs.[17,29] These findings indicate that nuclear retention of TAZ and Runx2 is essential for the regulation of OST function and differentiation. Evidence *in vitro* also showed that TAZ-depleted zebra fish is deficient in skeletal ossification and bone development.[13] In contrast, overexpressed TAZ in MSCs enhanced Runx2-associated osteoblastogenesis and inhibited adipogenesis by suppressing the level of PPARγ expression,[13] thus confirming that TAZ is a key switch between osteogenesis and adipogenesis from MSCs. Although TAZ has been shown as a crucial modulator in the differentiation of MSCs *in vivo* and *in vitro*, the role of TAZ on MSCs' differentiation under MG conditions remains unclear. Recent studies imply that TAZ is the putative signal transduction pathway linking MG and MSCs differentiation.[14] In this study, we showed evidence that MG significantly decreased TAZ protein and mRNA expression in both of the MSCs and OSTs [Figure 4]. Furthermore, results from the confocal study further recognized the facts that MG indeed altered the distributions of TAZ from nuclear retention to cytosolic sequestration [Figure 5f]. We had the direct evidence to demonstrate clearly the negative regulation of MG in Runx2-related gene expression by showing a decreased localization in the nucleus of TAZ and Runx2 upon applying MG [Figure 5h]. Taken together, these findings indicated that MG disturbed the expression and activity of TAZ, thereby suppressing the interactions with Runx2. This interaction consequently suppressed OST-related gene transcriptions that contributed to impaired osteoblastic differentiation of MSCs [Figure 6]. These results also suggest that TAZ may not only play a central role in MSCs differentiation, but it may also be an important factor in MG-induced bone loss. It was reported that disuse condition altered osteogenic and adipogenic potentials of MSCs in a rodent model, which led to decreased bone formation.[30] Thus, the results obtained from this study provide better understanding of the mechanism of MG on osteoblastic differentiation and form the basis for *in vivo* animal or human studies in the future.

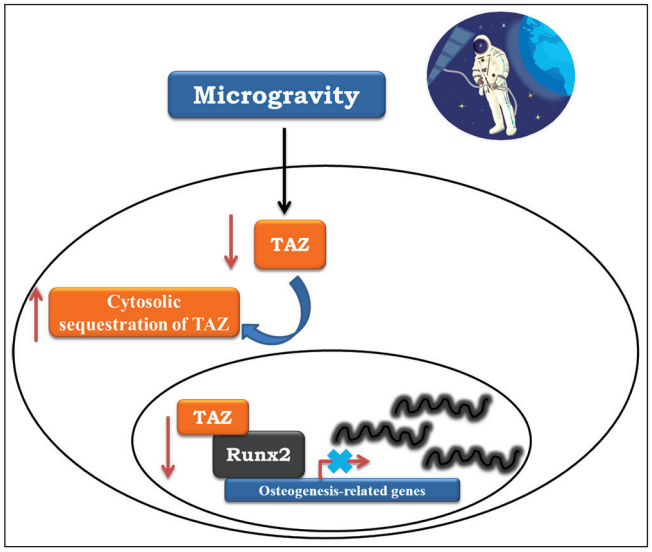


**Figure 6:** The proposed mechanism of microgravity on osteoblast differentiation. Microgravity suppresses the osteoblast differentiation of mesenchymal stem cells by inhibiting the expression and activity of TAZ

Up to now, the regulation of signal transduction pathway by MG on TAZ has not yet been characterized. 14-3-3 proteins, which are involved in many important cellular functions of eukaryotes, were reported to inhibit TAZ activity by directly conjugating with TAZ. These proteins are regarded as an important upstream regulator of TAZ.[31] In addition, increased 14-3-3 expression was also reported in SH-SY5Y cells under simulated MG conditions.[32] . Thus, it is interesting to investigate whether MG promotes 14-3-3 expression, thereby inhibiting TAZ. Apart from this, it was demonstrated that the phosphorylation status of TAZ, which is mainly regulated by two kinases, macrophage stimulating 1/2 and large tumor suppressor homolog 1/2,[33] is a crucial signal for nuclear localization or cytosolic sequestration. Thus, the phosphorylated TAZ should be examined to clarify how MG will regulate the function of TAZ in the future. Furthermore, TAZ has recently been reported as a principal downstream factor of Wnt signaling, which is important for proliferation, differentiation, and maturation of OSTs. Combined with a recent *in vivo* study which found that mechanical unloading led to retardation of Wnt signaling, resulting in a significant decrease in the activity and viability of OSTs,[23] these studies imply that MG suppress osteoblastic differentiation through suppression of Wnt-TAZ signaling. Thus, the detailed relationships among MG, OST differentiation, and Wnt-TAZ signaling require further elucidations.

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## CONCLUSIONS

We demonstrated that the effect of simulated MG on mouse MSCs differentiated into fat development instead of osteoblastogenesis was via suppressing the expression and activity of TAZ. Our study is the first to offer evidence to support the idea of TAZ being associated with MG-induced impairment of OST function, which suggests that TAZ should be an important factor involved in MG-induced bone loss. The findings may also imply that TAZ is a novel potential therapeutic target for disuse-related osteoporosis, such as astronauts, disabled, and bedridden patients.

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