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Hypergravity-induced enrichment of β1 integrin on the cell membranes of osteoblast-like cells via caveolae-dependent endocytosis

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ABSTRACT

In bone cells, integrins on the cellular surface are the primary sensors of their mechanical environment. Although gravitational changes are known to affect the adhesion and functions of bone cells, whether integrins respond to hypergravity in osteoblasts remains unclear. In this work, we demonstrate that exposure to a hypergravitational environment ($20 \times g$ via centrifugation) resulted in the concentration of β1, but not β3, integrin on the cell membrane of osteoblast-like (MC3T3-E1) cells. Notably, the total expression of both integrins was unaffected by the hypergravitational environment. In addition, caveolin-dependent endocytosis was discovered to be involved in the regulation of the enrichment of β1 integrin on the cell surface after stimulation by hypergravity. These findings could aid in the improvement of our understanding of the mechanisms underlying the effects of different gravitational forces on the human body.

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1. Introduction

Not only the chemical but also the mechanical environment can regulate cellular properties including the cell cycle, shape, differentiation, and/or motility. The transduction of mechanical stimuli in bone cells is crucial to bone generation, resorption, and regeneration [1–6]. Hypergravity, accomplished with centrifugation, is a convenient approach to forge mechanical stress on cultured cells and has been found to impact osteogenesis. In addition, as an inevitable consequence of acceleration in space flight, the effects of hypergravity on bone cells have been investigated [7,8]. These recent findings suggest that hypergravity triggers certain mechanotransduction pathways and results in altered cell adhesion and the post-translational modification of extracellular matrix (ECM) proteins in bone cells [7,8]. However, the mechanisms underlying the effects of gravity on osteoblasts have yet to be clearly defined.

Of the various mediators involved in mechanotransduction, integrins play a crucial role in the response of osteoblasts to mechanical forces, and are positioned at the start of most mechanical sensing pathways. By assembling into obligate αβ heterodimers, integrins couple the ECM outside a cell to the actin bundles inside the cell. Upon activation by mechanical stimuli, integrins undergo tension-dependent conformational changes that affect kinase activity, phosphorylation site availability, intracellular localization, and/or ligand affinity [9,10]. Osteoblasts express a variety of integrin protein subunits, including β1, β3, α1, α2, α3, α4, α5, α6, and αv [2,11]. Different types of integrins recognize and bind diverse ECM proteins and mediate mechanotransduction independently or cooperatively.

Osteoblasts primarily express the integrin β subunits β1 and β3. The interaction of β1 integrin with fibronectin is essential for the survival and proliferation of osteoblasts [12,13], while the interaction of β1 integrin with collagen is important for the activity of the Runx2 transcription factor [14,15]. However, by binding to vitronectin and fibronectin, β3 integrin negatively modulates bone mineralization and osteoblast differentiation [16]. Therefore, these two subunits were examined in the present work to evaluate their role in hypergravity-sensing processes.

By employing centrifugation to apply a hypergravitational condition of $20 \times g$ for 24 h to a well-established osteoblast-like cell line (MC3T3-E1), we determined the total expression of both β1 and β3 integrin and their individual contribution to the cell membrane protein content during hypergravity. In addition, we probed the possible mechanisms involved in the resulting β1

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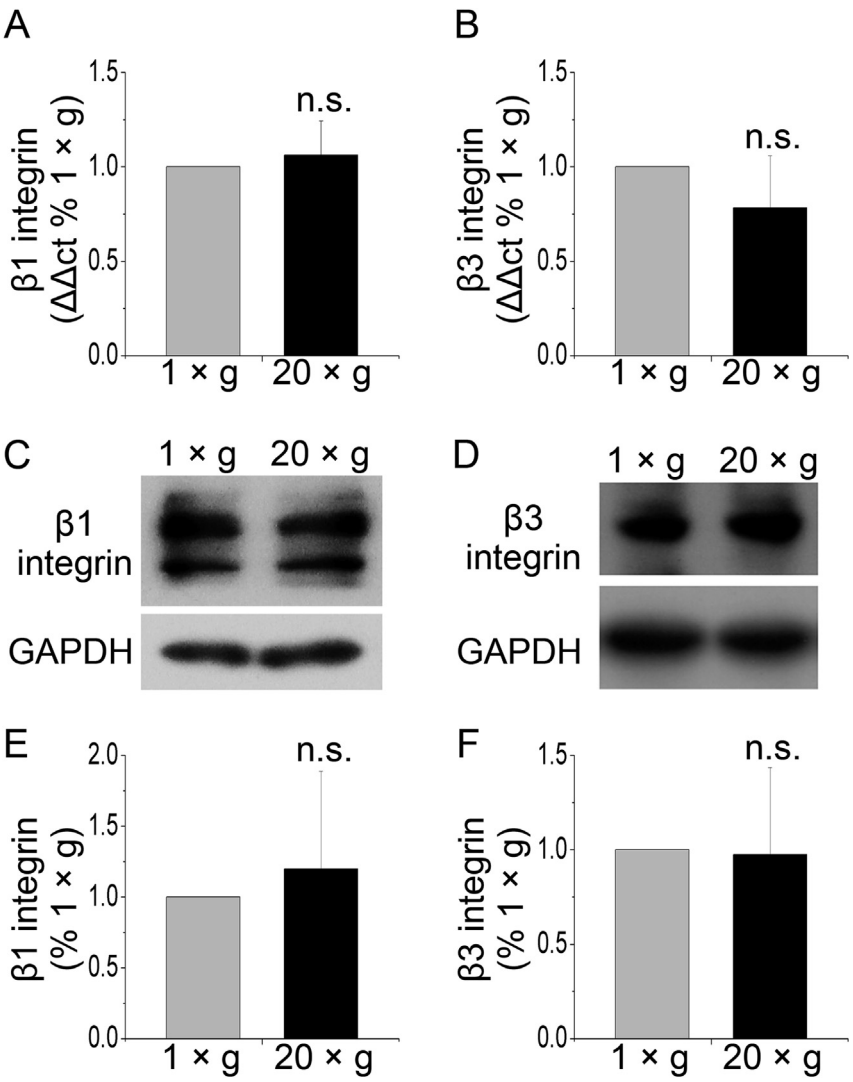

Fig. 1. The effects of gravity on the total expression of β1 and β3 integrins in MC3T3-E1 osteoblast-like cells. The mRNA and protein expression levels of β1 and β3 integrins after 24 h at either 1 × *g* or 20 × *g* are shown. (A) Comparative CT quantitation of real-time PCR results of β1 expression (mean ± SEM; n = 4). (B) Comparative CT quantitation of real-time PCR results of β3 expression (mean ± SEM; n = 4). (C) Western blots probed with anti-β1 integrin antibody, with anti-GAPDH as a loading control, in cells. (D) Western blotting results with anti-β3 integrin antibody, with anti-GAPDH as a loading control, in cells. (E) Statistical analyses of results from C. The means are shown as a percentage of the control values ± SEM from four experiments. (F) Statistical analyses of results from D. Means are represented as a percentage of the 1 × *g* values (mean ± SEM; n = 6). n.s. indicates no statistical difference between 1 × *g* and 20 × *g*.

integrin concentration on the cell membrane of MC3T3-E1 cells due to the hypergravitational environment.

2. Materials and methods

2.1. Reagents

Primaquine (PQ), monodansylcadaverine (MDC), and methyl-β-cyclodextrin (MBCD) were purchased from Sigma–Aldrich.

2.2. Cell culture

Osteoblast-like MC3T3-E1 cells were purchased from the cell center of the School of Basic Medicine of Peking Union Medical College. The cells were cultured in DMEM supplemented with 10% fetal bovine serum (FBS), 2 mmol/L L-glutamine, and 1% penicillin–streptomycin at 37 °C in an atmosphere containing 5% CO_2. After reaching confluence, the cells were trypsinized; 1 × 10^5 cells were seeded for 2 h in flasks, and then exposed to 20 × *g* hypergravity for 24 h in a 37 °C incubator. PH of the culture medium was adjusted by adding 15 mM Hepes. In some experiments, MC3T3-E1 cells were incubated with the specific inhibitors PQ (60 μM), MDC (200 μM), or MBCD (10 mM) during exposure to hypergravity. The control cells were subjected to the same conditions as the experimental cells in terms of timing, incubation media, and other procedures, with the exception of the gravity condition; for this, control cells were incubated at 1 × *g* for 24 h in the 37 °C incubator as that used for the experimental cells.

2.3. RNA isolation and RT-PCR

The total RNA was isolated by TRIzol (Invitrogen, Carlsbad, CA, USA) and converted to cDNA by using a reverse transcription kit (Tiangen Biotech, Beijing, China), according to the manufacturer's instructions. The primers were designed using the Primer3Web software [17]:

β1 integrin, (sense, 5′-GCCAGGGCTGGTTATACAGA-3′; antisense, 5′-TCACAATGGCACACAGGTTT-3′), *β3 integrin*, (sense, 5′- GCTCA TTGGCCTTGCTACTC-3′; antisense, 5′-TAATGGCAGAGAGTCCC ACG-3′), GAPDH, (sense, 5′-TGCACCACCAACTGCTTAG-3′; antisense, 5′-GGATGCAGGGATGATGTTC-3′).

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