THE ROLE OF SIGNALING PATHWAYS IN OSTEOBLAST GRAVITY PERCEPTION

Millie Hughes-Fulford
Lab of Cell Growth, Department of Veteran's Affairs Medical Center
and University of California San Francisco, San Francisco, CA 94121
milliehf@aol.com lab site: www.spacedu.com

ABSTRACT

Bone loss is one of the major problems in long term spaceflight. This physiological consequence of microgravity is the rapid loss of weightbearing bone that is associated with skeletal unloading. Moreover, we have previously noted that sera deprived osteoblasts do not have a normal response to sera in microgravity. Where exercise (mechanical loading) has been shown to increase bone formation and stimulate osteoblastic function, the mechanisms underlying signal transduction of mechano-perception is yet to be fully understood. Osteoblasts have been shown to respond to mechanical stress such as fluid shear, bending, flexing and compression. The type of stress and amount of stress determine the osteoblast response Recently we have discovered that the isolated osteoblast responds to a very short pulse of g-force compression. The possible regulatory sensors include mechano-sensitive calcium channels, autrocrine responses to stress, response to FAK/integrin, alterations in the cytoskeleton as well as other known growth factor and cytokine receptors. The secondary signal may include growth factor related kinases such as ERK, p38 and JNK map kinase (MAPK) pathways. Experimental evidence suggests that normal osteoblast response to stress and sera requires normal earth gravity.

1. INTRODUCTION

One principal concern in extended spaceflight is the rapid and continous loss of bone mass during flight. Reduction in bone formation (1-3), mineral content (4, 5) and loss of bone density (6,7) characterize the physiological response of human weight bearing skeleton to microgravity. For the 12 crew members of Gemini 4, 5, and 7, and Apollo 7 and 8, the average post-flight loss from the os calcis (heel) was 3.2 percent over an average of 8.5 days (8). Analysis of in-flight urine, fecal, and plasma samples from Skylab missions revealed changes in urinary output of hydroxy-proline indicating degradation of the collagenous matrix substance of weight bearing bones. Elevated concentrations of urinary calcium were noted in the early studies of Skylab astronauts starting during the first days of flight. In many of the astronauts urinary calcium concentrations remained at elevated levels throughout the mission. In Earth based studies, similar catabolic effects on bone are observed in human and animals subjected to immobilization (9-13). At the cellular level, weightlessness and skeletal unloading seem to modulate bone by changes in the osteoblasts Osteoclasts increase in short duration flights (14), however they return to normal during long duration missions (15). Studies in rats have shown a marked reduction in the periosteal bone suggesting alteration in the osteoblasts metabolism (1). Finally, Roberts group demonstrated that microgravity inhibited differentiation of osteoblasts in microgravity (16, 17). From animal and human studies investigators found that loss of weight bearing bone is as high as 1% per month and this loss is primarily due to lack of new osteoblast growth in spaceflight. It is likely that studies of the osteoblast in microgravity will give us new target molecules for development of pharmalogical agents that will stimulate bone growth. A projected bone loss of 20-30% in astronauts is one of the major physiological 'show stoppers' in the proposed 30-month manned mission to Mars.

Studies have noted that in some astronauts bone is not recovered even after 6 months return to earth (6). Exercise has long been utilized as a countermeasure to weightlessness and to increase bone mass here on Earth (18, 19). Vico and colleagues measured bone mineral density (BMD) at the distal radius and tibia in 15 cosmonauts who spent 1, 2 or 6 months on the Russian MIR space station. They found that neither the cancellous nor cortical bone of the radius was significantly changed at any of the time points. In contrast, at the weight-bearing tibial site, cancellous BMD loss was seen after 2 months of microgravity. After 6-months, loss of cancellous bone was more pronounced than cortical bone. In some individuals, the tibial deterioration was great and BMD loss did not seem to depend on previous exposure to microgravity. Moreover, tibial bone loss persisted after return to earth and was not recovered during the post flight study period, suggesting the recovery time is greater than the time spent in microgravity (6). Since microgravity-induced bone loss is not fully recovered after return to gravity this is a significant complication of long-term missions and must be addressed before a Mars Mission.

2. MECHANICAL STRESS IN SPACEFLIGHT

Exercise has been used as a countermeasure for bone loss for decades. However, exercise alone is not a total solution for the countermeasures, since paradoxically, excessive exercise aggravates bone loss. Stein et al. reported a negative energy balance in longer-term missions (20-24). This strongly suggests the need for a programmatic and efficient exercise program that would not result in a catabolic state. The cause of bone loss in response to reduction of mechanical stress is not yet known, but several ground studies have demonstrated that eicosanoids may be involved. Prostaglandins are released with exercise and are key regulators in exercise-induced bone growth *in vivo* (25). The specific cyclooxygenase-2 inhibitor, NS-389, completely blocked mechanical stress induced bone formation *in vivo*. Other studies have

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demonstrated that PGE_2 augments bone growth *in vivo* (26) and *in vitro* (27).

On the cellular level, numberous studies have demonstrated that mechanical force (fluid shear, flexing, bending and compression) can induce osteoblasts proliferation (28-31). The mechanisms by which these various mechanical strains induce growth may involve multiple pathways. The possible mechanism of action and signal transduction of gravity perception by the osteoblasts are discussed. How the mammalian cell perceives gravity is of upmost importance since it will determine the proliferative state of the osteoblasts.

3. CELL PROLIFERATION IS KEY: The problem of cell proliferation in microgravity is a key issue in cell biology that may determine if multiple generations of gravity evolved life can adapt to a novel evolutionary existence in microgravity. Although flight opportunities have been limited and control experiments sometimes inadequate, the volume of data about microgravity effects on cell proliferation has been accumulating. Specifically, it has been demonstrated that cell growth of lymphocytes (32-34) and osteoblasts (35-37) is inhibited or altered in a microgravity environment. Cytoskeletal changes in actin, intermediate filament and microtubule networks have been found in microgravity as well (35, 38, 39). In addition, several investigators have noted that specific gene expression is modulated by the lack of gravity (35, 40-43). These data point to a possible inhibition of anabolic stimuli in the absence of Earth's gravity. There are several anabolic signals that are regulated by mechanical stress and these same signals may be downregulated in microgravity. The transducers are tyrosine kinase and serine/threonine kinase growth factors, receptors and mechanical stress and all depend on the central controlling point, MAPK for signal transduction. In addition, integrins, actin cytoskeleton, and G proteins can act through the MAPK pathway. Inhibition of any one of these steps may inhibit activation of nuclear transcription factors and/or induction of early immediate genes. Taken together, the data suggest that the inhibition of cell proliferation is due to changes in early signal transduction in microgravity, which lead to alterations in downstream events that effect gene expression and cell cycle in spaceflight.

4. MECHANO-SENSING RESPONSE PATHWAYS: Mechanical force is an important regulator of cell morphology and function especially in osteoblasts. Mechanical stress is known to activate *cox-2* both in *vitro* and *in vivo* leading to bone growth (25, 44). Others have shown that mechanical stress causes induction of *cox-2* and *c-fos* in osteoblasts (45). It has recently been shown that mechanical stress (Flexcell™ or 50rpm clinorotation) in ROS 17/2.8 osteoblasts cause increase in erg-1 and NFkB nuclear translocation (31). The promoter regions of both *cox-2* and *c-fos* are driven by both erg-1 and NFkB, it is interesting to note that both of these transcription factors are upregulated by MAPK (31).

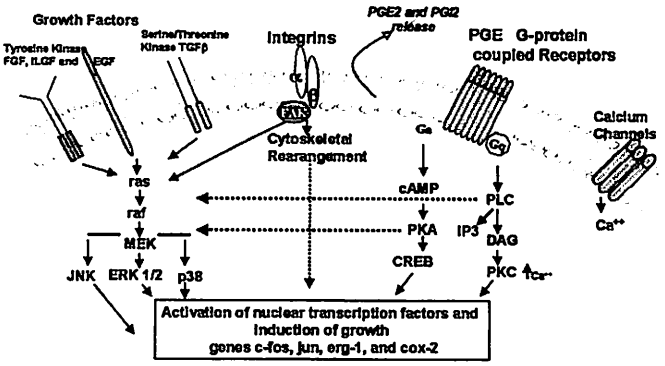

Fig. 1: Pathways stimulated by mechanical stress that may be downregulated in microgravity

We have discovered that mechanical loading of gravity increased induction of *c-fos* and *cox-2*. We have also found that gravity induces MAPK activity and upregulates *c-fos* within 30 minutes of stress. This activation is inhibited by addition of U0126 (MEK kinase inhibitor) but not SB203580 (p38 inhibitor). ERK 1/2 phosphorlation most probably causes translocation of activated ERK to the nucleus in the osteoblast within minutes of sera activation. Translocation of ERK 1/2 to the nucleus is critical since activation of erg-1 and NFkB by intra-nuclear ERK causes induction of immediate early gene *c-fos* (as well other genes regulated by NFkβ and erg-1) since addition of MAPK inhibitors inhibits *c-fos* induction. Taken together, these data suggest that anabolic signal transduction is regulated, at least in part, by the MAPK phosphorylation pathway. It is not yet known if MAPK plays a role in the downregulation of signal transduction in spaceflight.

5. INTRA-NUCLEAR ACTION OF MAPK SIGNALING PATHWAY: TRANSLOCATION OF ERK TO NUCLEUS IS CRITICAL TO COMPLETE SIGNAL TRANSDUCTION.

Many of the signaling processes leading to induction of gene expression by fetal calf serum are mediated through MAPK pathway. Signal transduction generated by stress, growth factors or sera have the same approximate timed response, with ERK1/2 activation occurring within minutes of the stimulus, reaching a maximum signal at about 30 minutes. Phosphorylation of ERK1/2 occurs within minutes and these data show that translocation to the nucleus occurs during this time period. As seen below, translocation of activated ERK1/2 to the nucleus (Fig D) occurs within 30 minutes of sera activation. Phosphorylation of ERK and translocation to the nucleus occurs in gravity stimulation over the same timecourse (46). This translocation is mandatory for proper signal transduction in the osteoblast. As seen below, addition of sera to quiescent osteoblasts can cause phosphorylation of ERK and translocation to the nucleus.

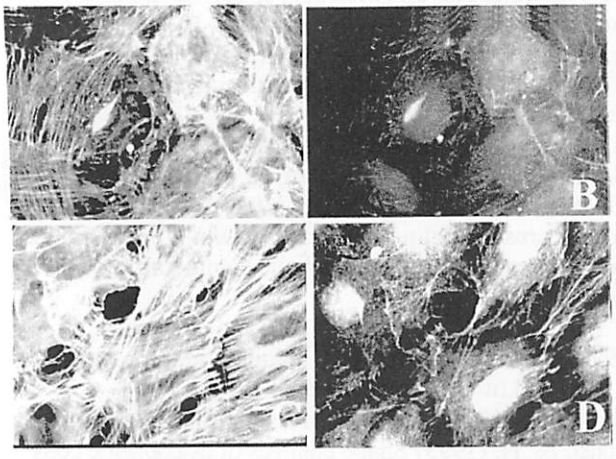

Fig. 2: F-Actin (A and C) and phospho ERK (B and D) in cell activated with sera (C and D)

6. FINAL OVERVIEW Mechanical stress can affect several signaling pathways of the osteoblasts. Reports from the Duncan lab (47) have demonstrated that blocking the mechano sensitive Ca channel or the L-channel did not block mechanically induced gene expression. Moreover, results from our laboratory (46) suggests that compression force induced phosphorylation of ERK, not p38, FAK or JNK kinase pathways. Therefore, candidate pathways for deactivation in microgravity include the growth factor receptors that activate signaling kinases, the cytoskeleton, the seven-domain transmembrane receptors and their associated GTPases, and signaling enzymes PKA, PK-3 and PKC.

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