INTEGRIN SIGNALING AND THE RESPONSE OF OSTEOCYTES TO OSCILLATORY FLUID FLOW

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INTRODUCTION
Osteocytes are mechanosensitive cells in bone and are believed to be responsible for initiating and coordinating osteogenic and osteoclastic processes in vivo. Dynamic fluid flow has been shown to be a potent regulator of bone cell metabolism, but the molecular mechanism through which osteocytes sense mechanical stimuli is unknown [1,2]. Integrins, heterodimeric cell adhesion proteins, are ideal candidates for mechanosensitive molecules in bone due to their structural and catalytic capabilities. The aim of this study is to investigate the role of integrins in mechanotransduction in osteocytes.

In response to mechanical stimuli, osteocytes coordinate a cellular response to load by directing effector cells to deposit or resorb bone [3,4]. Intracellular calcium ([Ca$^{2+}$]) mobilization and MAPK signaling in response to fluid shear stress are associated the deposition of bone matrix proteins in vitro [2]. Independent of calcium signaling, an increase in cyclooxygenase-2 transcription results in prostaglandin release from osteocytic cells and in bone formation in vivo [5–7]. It has also been shown that osteocytes have a loading-induced inhibitory effect on osteoclastogenesis, as measured by a decrease in the ratio of RANKL to OPG [8]. We hypothesize that inhibiting integrin signaling in osteocytic cells will decrease the production of molecular signals that drive osteogenic and osteoclastic processes in bone.

MATERIALS AND METHODS

β1DN Construct: Stably transfected cell lines of MLO-Y4 osteocytic cells were generated by transfecting parental cells either with an expression plasmid encoding a dominant negative form of the β1 integrin monomer (β1DN) or with the empty vector (vector controls). Oscillatory Fluid Flow (OFF): For 48 hours prior to OFF exposure, cells were subcultured on glass slides coated with type I bovine collagen and were measured in triplicate.

Statistical Analysis: A student t test was used to compare samples, and a p<0.05 was considered significant (*). Data is reported as mean ±SD.

RESULTS
Over 44 percent of the vector control cells responded to OFF with a release of [Ca$^{2+}$], compared to 12 percent of the β1DN cells (p=0.031, N=6) (Figure 1). Application of OFF resulted in a significant increase in COX-2 mRNA levels in vector control cells (p=0.0007, N=4), but no significant change in β1DN cells (p=0.406, N=4) (Figure 2).

REFERENCES

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Figure 1. Intracellular calcium mobilization in β1DN and control cells exposed to oscillatory fluid flow. * p<0.05

Figure 2. COX-2 gene expression in cells with the application of oscillatory fluid flow (OFF) compared to controls (NF). ** p<0.005

Figure 3. RANKL/OPG gene expression with the application of oscillatory fluid flow (OFF) compared to controls (NF). ** p<0.005

RANKL/OPG mRNA levels decreased significantly after exposure to OFF in vector control cells (p=0.0007, N=7) but not in β1DN cells (p=0.247, N=7) (Figure 3).