IDENTIFICATION OF CAVEOLIN 1 IN NORMAL HUMAN OSTEOBLASTS

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INTRODUCTION
Nitric oxide (NO) has been studied extensively over the past decade, and has been identified as an important biological mediator in a broad range of systems. More recently, NO has been shown to be a signaling molecule involved in normal bone function, playing a role in bone formation and resorption in response to different types of stimuli. Studies have shown that osteoblasts produce NO in response to mechanical stimulation, though it is still unclear whether this is due to stretch or fluid shear. NO is produced as a co-product in a reaction catalyzed by the enzyme nitric oxide synthase (NOS), in which arginine is converted to citrulline. NOS can exist as one of three isozymes; nNOS, iNOS and eNOS. In osteoblasts that are under the effects of mechanical stimulation, the enzyme responsible for this NO production is the calcium-dependent eNOS isom.

The enzyme eNOS was originally identified in endothelial cells, and much of the work into its location and mode of action has been carried out on this cell type. More recently, eNOS has been localized to specialized plasma membrane invaginations, termed caveolae, located on the cell surface. Caveolae are characterized by the presence of the 21 kDa transmembrane protein caveolin and are thought to serve as sites for the colocalisation of NOS-coupled signaling molecules. It is possible that the localization of eNOS to the caveole, and in particular, its interaction with the protein caveolin, may act as another important regulator of NO production.

In other biological systems, studies have identified caveolin in tissues such as endothelium and muscle. Further, several different forms of caveolin exist and are specific to certain tissues; endothelial cells express caveolin 1 on their surface, while muscle cells express only caveolin 3. These proteins and the cell surface structures, caveolae, in which they are found, are currently the subject of intense study. However, at the time of writing, caveolin has not been identified in osteoblasts. The purpose of this study was to identify caveolin 1 in human osteoblasts for the first time.

MATERIALS AND METHODS
Normal human osteoblasts were isolated from explants of cortical bone by collagenase digestion. They were isolated by outgrowth in 10cm Petri dishes, and cultured in HY medium. They were confirmed as being osteoblasts by their production of alkaline phosphatase and collagen I, as demonstrated by immunocytochemical staining. Alkaline phosphatase activity was quantified by a P-nitrophenylphosphate conversion assay (Sigma Diagnostics). In addition, they were shown to produce the osteoblast-specific protein, osteocalcin, by immunoradioactive assay of the supernatant (Immunotopics Inc.). The cells were passaged and seeded onto T75 culture dishes, and cultured in HY medium. They were confirmed as being osteoblasts.

After reaching confluence, approximately 10 million cells were harvested from five T75 flasks. They were dissolved in lysis buffer, and the protein concentration was measured using a modified Bradford method (Biorad, Hercules, CA). 100μg of protein was loaded into each well of a ten-

RESULTS
Western blot using anti-caveolin 1 antibody revealed a single positive band corresponding to a 21 kDa protein in the osteoblast lanes. The positive control lane (BAE cells), also showed a strongly positive band at this level. There was no detectable band in the negative control lane (myocytes). In contrast, when anti-caveolin 3 antibody was used, there were no visible bands in the human osteoblast lanes. There was also no visible band in the negative control lane (BAE cells). However, there was a strongly positive band in the positive control lane (myocytes).

CONCLUSION
We have shown for the first time that normal human osteoblasts contain the protein caveolin 1. This makes it highly probable that, as has been demonstrated in other tissues such as endothelium and muscle, osteoblasts exhibit cell-surface plasmalemmal caveolae. The isolation from osteoblasts of this protein, which is currently under investigation in other tissues, raises exciting possibilities to further study cell signaling. In particular, it may provide a clue to further understanding the role of eNOS function in the response of osteoblasts to mechanical stimulation.

Figure. Western blot identifying caveolin 1 in normal human osteoblasts

References

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