Annexin V disruption impairs mechanically induced calcium signaling in osteoblastic cells

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Abstract
The mechanical environment of the skeleton plays an important role in the establishment and maintenance of structurally competent bone. Biophysical signals induced by mechanical loading elicit a variety of cellular responses in bone cells, however, little is known about the underlying mechanotransduction mechanism. We hypothesized that bone cells detect and transducer biophysical signals into biological responses via a mechanism requiring annexin V (AnxV). AnxV, a calcium-dependent phospholipid binding protein, has several attributes, which suggest it is ideally suited for a role as a mechanosensor, possibly a mechanosensitive ion channel. These include the ability to function as a Ca2+ selective ion channel, and the ability to interact with both extracellular matrix proteins and cytoskeletal elements. To test the hypothesis that AnxV has a role in mechanosensing, we studied the response of osteoblastic cells to oscillating fluid flow, a physiologically relevant physical signal in bone, in the presence and absence of AnxV inhibitors. In addition, we investigated the effects of oscillating flow on the cellular location of AnxV. Oscillating fluid flow increased both [Ca2+]i levels and c-fos protein levels in osteoblasts. Disruption of AnxV with blocking antibodies or a pharmacological inhibitor, K201 (JTV-519), significantly inhibited both responses. Additionally, our data show that the cellular location of AnxV was modulated by oscillating fluid flow. Exposure to oscillating fluid flow resulted in a significant increase in AnxV at both the cell and nuclear membranes. In summary, our data suggest that AnxV mediates flow-induced Ca2+ signaling in osteoblastic cells. These data support the idea of AnxV as a Ca2+ channel, or a component of the signaling pathway, in the mechanism by which mechanical signals are transduced into cellular responses in the osteoblast. Furthermore, the presence of a highly mobile pool of AnxV may provide cells with a powerful mechanism by which cellular responses to mechanical loading might be amplified and regulated.