Studies on annexins in primary cultures of human osteoblasts and in the human osteosarcoma cell line MG-63

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Calcium plays a central role in the regulation of many physiological processes. Calmodulin is a major calcium-binding protein that regulates cell function. Over the past few years another major class of calcium-binding protein has emerged which binds calcium through an alternative calcium-binding site. These proteins are called "annexins". Various physiological roles have been suggested for annexins including phospholipase A2 inhibition, membrane fusion, anti-coagulation, cell differentiation and interaction with cytoskeletal proteins (1,2). These proteins could also play a role in the regulation of calcium in osteoblasts and in the mineralization of bone through their ability to bind to calcium and phospholipids (3). It has been reported that annexins I, II and V bind to actin and annexin V binds to type I, II and X collagen (4). Hence annexins could contribute to a variety of processes required for mineralization including the regulation of calcium transport into human osteoblasts and interactions between macromolecules in the extracellular matrix.

We have attempted to identify the annexins present in primary cultures of human osteoblasts and in the osteosarcoma cell line MG-63 (5), and to quantitate annexin V. We have also studied the presence of annexin V in the extracellular medium and the effect of cell culture conditions on the expression of annexin V in MG-63 cells.

The cells were grown in 90 mm tissue culture dishes with Dulbecco's modified minimum essential medium containing 10% (v/v) fetal calf serum and then proteins were extracted with 2% SDS. Proteins were analysed by SDS-PAGE (6) on 10% polyacrylamide gels. Separated proteins were transferred from the gel onto nitrocellulose membranes according to Burnette (7) using an electoblotting apparatus operated at constant current 400mA for 3 h or 100mA overnight. Immunopробing was carried out by exposing nitrocellulose paper to antibodies against each annexin and then incubating with peroxidase-conjugated second antibody. Diaminobenzidine (DAB) and ECL methods were used to detect immobilized peroxidase. Finally, we used densitometry to quantitate the amount of annexin V in primary cultures of human osteoblasts and in MG-63 cells.

This study demonstrates that five members of the annexin family: annexin I; annexin II; annexin IV; annexin V; and annexin VI are produced by primary cultures of human osteoblasts and by the human osteosarcoma cell line MG-63 (Fig.1). To our knowledge, this is the first description of the expression of annexins in human bone cells.

From the quantitation studies the amount of annexin V in MG-63 cells is more than 3 times the level found in primary cultures of human osteoblasts (Fig.2).

Fig. 2 Quantitation of annexin V in primary cultures of human osteoblast and in MG-63 cells.

Results were expressed as a percentage of total cell protein and are presented as the mean ± S.E. of 9 determinations.

Some annexin V was detected in the culture medium but was sedimentable on centrifuging at 100000 rpm for 2 hours. This indicates that a small amount of annexin V is released from MG-63 cells, possibly in the form of vesicles shed from the plasma membrane.

The level of expression of annexin V in MG-63 cells is affected by the final time of cell feeding and FCS concentration. In particular, the amount of annexin V was increased 72 and 48 hours after final cell feeding compared with 24 hours after final cell feeding and appeared to be highest when the concentration of FCS was 5%(v/v). The observed changes appear to be correlated with some aspect of MG-63 growth or division.

In summary, we report the expression of annexins I, III, IV, V and VI by human osteoblasts in primary culture and by MG-63 cells. Because of their biochemical properties and their specific regulation, these proteins are likely to play a significant role in the function of osteoblasts and in bone metabolism.

References