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Global analysis of the extracellular matrix vesicle proteome from differentiating murine osteoblasts

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Introduction:

Bone formation is a complex process involving recruitment, replication and differentiation of osteoblasts and ultimately the accumulation and mineralization of the extracellular matrix. The matrix vesicles (MVs) produced by osteoblasts during this process play pivotal roles in mineralization. MVs are released from osteoblast cell surface via polarized budding into extracellular space where they are thought to function as nucleation centers that initiate mineralization and sustain the accumulation of extracellular matrix. Various osteoblast proteins and minerals, including some MV components were found to participate in this process. A detailed analysis of MV proteins is needed to better understand their contribution to bone formation. Thus the goal of this study is to obtain a comprehensive profile of MV proteins using mass spectrometry.

Methods:

Mouse calvaria-derived osteoblast line MC3T3-E1 was used as a model to isolate and analyze MV proteome. Ascorbic acid and □-glycerophosphate were added to culture to induce MV biogenesis. MVs from the extracellular matrix and media were isolated with two different methods. In one method, a short exposure of osteoblasts to collagenase and dispase released MVs from extracellular matrix. Alternatively, MVs were isolated from culture media by ultracentrifugation. The MV enrichment was monitored by measuring alkaline phosphatase activity. Both MV samples were separated and stained on SDS-PAGE. Twenty four gel bands were excised from each sample and subjected to in-gel digestion. The tryptic peptides were analyzed using nanoflow reversed-phase liquid chromatography (nanoRPLC) coupled online with tandem mass spectrometry (MS/MS).

Abstract:

Extracellular matrix vesicles were isolated from osteoblasts either via collagenase/dispase treatment or ultracentrifugation. Both isolation methods yielded a 2 to 3-fold enrichment of MVs as determined by alkaline phosphatase activity. A total of 51 proteins were identified by at least two unique peptides using the two different preparation methods. These 51 proteins represented the most abundant protein components associated with MVs. As expected, a number of annexins (I, II, IV, V and VI), which can function as calcium channels and have previously been demonstrated integral to MV function were detected. The results also identified a number of proteins previously not known to be associated with matrix vesicles including, collagen (I, VI and XII), 14-3-3/protein kinase C inhibitor proteins and a variety of peptidases. Further identified were two osteoblast specific factors and signal transduction molecules such as calmodulin and Rho GDP-dissociation inhibitor. Other identifications such as three different voltage-dependent anion-selective channel proteins (mitochondrial outer membrane proteins), endoplasmin (endoplasmic reticulum) and lysosome-associated membrane glycoprotein, were suggestive of the potential origins of MVs. Further analysis of these MV protein components is under way to elucidate important factors involved in bone formation and connective tissue related diseases.