Osteoblast-derived Matrix Vesicles Differ in Characteristics and Role from Exosomes

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DISCLOSURES: Anne M. Skelton (N), D. Joshua Cohen (3B – Pascal Medical Corporation), Barbara D. Boyan (3B – Medtronic; 4 – Pascal Medical Corporation; 5 – Medtronic, Curiteva, Spine Wave, Institut Straumann AG), Zvi Schwartz (5 – A.B. Dental)

INTRODUCTION: Heterogenous, nano-sized, membrane-bound extracellular vesicles can deliver physiologically relevant signaling molecules, such as lipids, proteins, and nucleic acids, without an immunogenic response. There are several classes of extracellular vesicles with classification based on biogenesis pathway and size among other characteristics. In mineralizing tissues, two populations of vesicles include those found in biological fluid, termed exosomes (EX), and those anchored to the extracellular matrix, termed matrix vesicles (MV). Characterization of these two types of vesicles indicates that they differ in phospholipid composition, matrix processing enzymes, and microRNA content. However, additional comparison is needed to further understand how their function within bone is dictated by their physical location and characteristics. Therefore, the goal of the current study was to directly compare exosomes from the conditioned media to matrix vesicles from the cell monolayer with the hypothesis that they are different classes of vesicles and they exert different effects on target cells.

METHODS: MVs and EXs were isolated from human MG-63 osteoblast-like cells cultured in growth media for 24 hours after reaching confluence. MVs were isolated from MG-63 cell monolayers via ultracentrifugation of the ECM trypsin digest at 100,000xg. EXs were similarly isolated via ultracentrifugation of the conditioned media. Total cell lysates (CL) and plasma membrane (PM) fractions from parent cells were used for comparison. Alkaline phosphatase specific activity was measured for all four fractions. Protein expression patterns were evaluated by western blots. MV and EX size was determined by nanoparticle tracking analysis (NTA). Morphology was assessed via transmission electron microscopy (TEM); uptake was examined using vesicles labeled with a fluorescent membrane dye. Finally, isolated MVs and EXs were added to culture media of osteoblast-like cells for 24 and 48 hours followed by assessment of osteoblastic differentiation markers alkaline phosphatase and osteocalcin.

RESULTS: MV alkaline phosphatase specific activity was enriched >4-fold over the PM fraction, constituting a significant difference and confirming isolation purity. EX also exhibited enrichment of alkaline phosphatase activity versus the PM, but to a significantly lesser extent than the MVs. Western blots demonstrated the presence of extracellular vesicle markers CD9 and CD81 within the MV and EX fractions. ALIX, a marker of multivesicular body biogenesis, was also present. However, TSG101, a protein more associated with the formation of the early endosome, showed greater enrichment in the MV fraction than the EX fraction. Na^+/K^+-ATPase and integrins αV and β3 were present in MVs but not in EXs (Figure 1). NTA showed that EXs were smaller than MVs, averaging 57 nm versus 115 nm in diameter. Both MV and EX were incorporated into the MG-63 cells, but had different effects. Alkaline phosphatase and osteocalcin both significantly decreased following 48 hours of MV treatment without any significant differences noted with EX treatment or changes in DNA content (Figure 2).

DISCUSSION: These results indicate that MVs and EXs are distinct extracellular organelles. They have different enrichment of alkaline phosphatase specific activity and size. CD9 and CD81 support their characterization as extracellular vesicles, but only MVs possess integrins αV and β3. This is consistent with the anchorage of MVs in the ECM whereas EXs, as free-floating vesicles, do not need to express these anchoring proteins. However, it is less clear why TSG101 content was lower in the EXs, which are classically linked to the endosomal biogenesis pathway. Na^+/K^+-ATPase enrichment in MVs versus EXs may be related to the role of MVs in mineral formation. These differences in physical and chemical characteristics of the two extracellular vesicle populations suggest different roles. Indeed, MVs decreased osteoblastic differentiation while EXs did not demonstrate such an effect. Others have shown that EXs can stimulate expression of osteoblast differentiation markers. Taken together with our findings, MVs may function as a brake on too rapid mineralization, by controlling the activity of alkaline phosphatase, initial hydroxyapatite formation and, ultimately, the rate of calcification of the ECM.

SIGNIFICANCE/CLINICAL RELEVANCE: This study provides a comparison between two classes of extracellular vesicles based on enzyme activity, protein composition, size, and effect on osteoblast-like cells. It suggests that exosomes and matrix vesicles are similar but separate types of vesicles that are not only located in different areas of bone, but play different roles in osteoblast differentiation and potentially matrix mineralization. Further research is needed to determine their viability as a therapeutic option for different bone pathologies.

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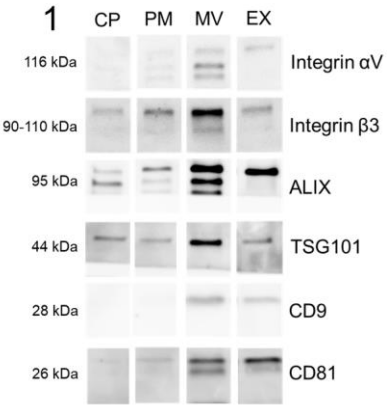

FIGURE 1: Western blot analysis of cell pellet lysate (CP), plasma membrane (PM), matrix vesicle (MV), and exosome (EX) fractions.

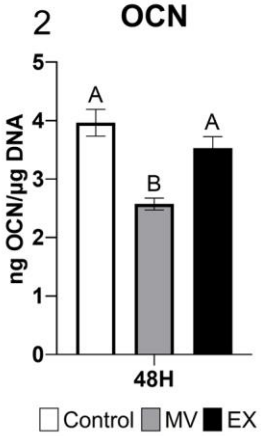

FIGURE 2: Osteocalcin (OCN) analysis after 48 hours of treatment with matrix vesicles (MV), exosome (EX), or control 0.9% NaCl.