Extracellular vesicles signal from bones to vessels – an answer to the "calcification paradox"?

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As we age, bone resorption increases, leading to bone-fat imbalance and osteoporosis. Surprisingly, at the same time as bones lose calcium, vascular calcification increases, leading to increased risk of atherosclerotic plaque rupture, heart disease or stroke. The coexistence of bone resorption and vascular calcification has been called the calcification paradox, because bone formation and vascular calcification share common mechanisms[1,2]. A recent study suggests that extracellular vesicles (EVs) may help to resolve this paradox.[4]

EVs are 50-1000nm lipid bilayer vesicles released by virtually all cells, which contain biological information that can be transferred to distal tissues to exert their actions, both in health and disease[3]. In a comprehensive study performed in a mouse model, Wang et al.[4] demonstrated that EVs derived from bone marrow (and more specifically, from osteocytes), are released to regulate bone marrow mesenchymal stem cells (BMSC) differentiation and vascular smooth muscle cells (VSMC) calcification, contributing to the calcification paradox, as summarized in **Figure 1**. In fact, BMSC internalize EV from aged and young bone matrix, and these EVs can be transported to the blood vessels. Interestingly, EVs derived from aged bone matrix promoted the expression of genes related to the adipogenesis pathway and lipid droplet formation, whereas EVs from young bones stimulated the osteogenic pathway and the formation of calcium nodes in BMSCs. Oppositely, in VSMC, aged but not young EVs from bone matrix increased calcium deposition and induced the osteogenic pathway, promoting the switching from VSMC to an osteogenic phenotype. Intravenous injection of aged EVs from bone marrow (BM-EVs) increased calcium deposition lesion areas in abdominal aorta. Moreover, the authors evaluated the capacity of aged BM-EVs to induce bone loss and fat accumulation in bone marrow in young and aged mice. Both young and aged mice treated with aged BM-EVs showed lower bone strength, lower trabecular mass, volume fraction, number and thickness and higher trabecular separation compared to mice treated with young BM-EVs. In addition, mice treated with aged BM-EVs showed increased number of adipocytes and lower number of osteoblasts in bone marrow, without affecting osteoclast formation and functionality. In turn, it has been recently observed in an *in vitro* model that the co-incubation of calcified aortas with osteoblasts inhibited their calcification and upregulated osteopontin and the progressive ankylosis protein homolog[5], both inhibitors of bone mineralization.

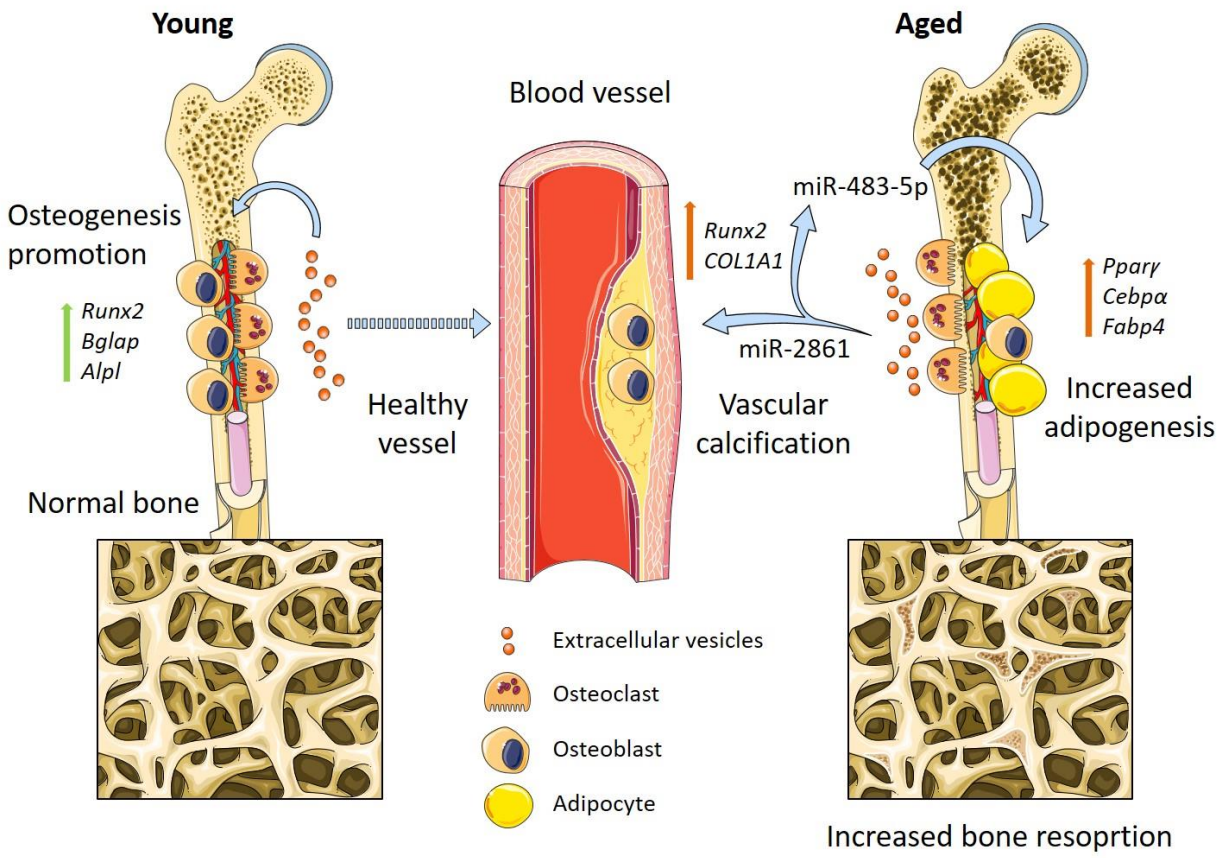

Figure 1. Differential effects of young and aged extracellular vesicles released from bone marrow in bones and blood vessels. This figure was created using Smart-Servier Medical Art (https://smart.servier.com).

The authors also explored the potential involvement of miRNAs contained in aged BM-EVs in bone-fat imbalance. MiR-483-5p and miR-2861 were the most abundant miRNAs in aged BM-EVs compared with young BM-EVs. Moreover, the content of these miRNAs in BM-EVs increased with the age of mice, as well as the calcium content in these EVs. MiR-483-5p has been shown to stimulate adipogenesis[6] and miR-2861 promotes osteogenic differentiation[7]. Consequently, miR-483-5p and miR-2861 expression was increased in bones, vessels and liver of aged mice compared with young mice, but in other tissues their expression remained constant. The inhibition of miR483-5p resulted in an impaired ability of aged BM-EVs to induce trabecular bone loss, and the inhibition of miR-2861 reduced the calcium deposition areas and the vascular calcium content induced by the aged BM-EVs.

Until the "ossification" of blood vessels was discovered in 1993[8], vascular calcification had been assumed to be a passive consequence of aging[9]. This was an important finding which further unveiled the role of the bone-vascular axis in several pathological contexts such as chronic kidney disease, diabetes or osteoporosis, among others. In this setting the work from Wang et al.[4] reveals a physiological role of BM-EVs in bone and vascular health maintenance. This is of importance as the levels and role of EVs in health maintenance, not only derived from bone marrow, are not yet fully elucidated.

The findings of Wang et al.[4], help to explaining the calcification paradox, and might have important cardiovascular implications, as vascular calcification –and more specifically coronary artery calcium score- is an independent risk factor for cardiovascular disease. In this setting, it has been recently demonstrated that bone marrow and vascular activation (measured as ^{18}F- fluorodeoxyglucose uptake), is associated with higher plaque burden and number of plaques in humans[10]. Therefore, the contribution of activated BM- EVs in plaque progression might be further studied.

Overall these findings might have potential clinical translation, once the associated limitations of the study of EVs in clinical trials have been overcome. On one hand, the inhibition of bone marrow-derived EVs shedding in aged subjects may delay vascular calcification and osteoporosis progression. In this context, the inhibition of the packaging of miR-483-5p and miR-2861 in BM-EVs might serve as a therapeutic target for cardiovascular disease. On the other hand, the reparative effects of young BM- EVs on vascular calcification or their contribution to the maintenance of vascular functionality in aged subjects might also be investigated as a potential treatment for the prevention of a major cardiovascular event. At the same time, the effects of young BM-EVs in maintaining bone density or repairing osteoporosis in aged people might also be of clinical relevance.

In conclusion, aging induces the packaging of miR-483-5p and miR-2861 into BM-EVs, thereby promoting adipogenic differentiation of BMSCs. These EVs are also released to the circulation and incorporated in blood vessels, inducing vascular calcification. These findings open a new window to make possible for the calcification paradox not to be a paradox anymore, through the inhibition of vascular calcification and bone resorption.

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Biography: Prof. Sean M Davidson leads a research group at the Hatter Cardiovascular Institute (University College London), investigating novel methods to protect the heart from ischaemia and reperfusion injury. He currently leads a programme of research to investigate the cellular response to extracellular vesicles, and their potential role in cardioprotection, funded by grants from the Medical Research Council and British Heart Foundation. He chairs the ESC working group on Cellular Biology of the Heart, is council member of the International Society of Heart Research (ISHR) - European Section, and associate editor for Cardiovascular Research, Basic Research in Cardiology and Cardiovascular Drugs and Therapy.