

MODULATED EXOSOME SECRETION BY VASCULAR SMOOTH MUSCLE CELLS IS A NOVEL REGULATORY MECHANISM OF VASCULAR CALCIFICATION

A Kapustin,¹ M Chatrou,² I Drozdov,¹ D Soong,¹ M Furmanik,¹ D Alvarez-Hernandez,¹ P Sanchis,¹ R Shroff,³ X Yin,¹ J Skepper,⁴ M Mayr,¹ C Reutelingsperger,² L Schurgers,² C Shanahan¹ ¹King's College London; ²Maastricht University; ³Great Ormond Street Hospital; ⁴Cambridge University

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Vascular calcification is a regulated pathological process similar to bone formation which is mediated by vascular smooth muscle cells (VSMCs) undergoing osteogenic transdifferentiation. Initiation of vascular calcification occurs in small membrane-bound matrix vesicles (MVs), secreted by VSMCs into the extracellular matrix however the mechanisms regulating MV biogenesis and secretion are unclear.

Fetuin-A, a circulating protein abundant in MVs was used to trace the origin of VSMC-derived MVs. Alexa488-labelled fetuin-A was rapidly uptaken by human VSMCs and appeared in the late endosomal system and multivesicular bodies (MVBs) indicating that MVs are secreted from the endosomal compartment similar to exosomes; extracellular vesicles originating from MVBs and secreted by the range of cells. Biochemical analysis of MVs showed that they were enriched with exosomal markers, CD9 and CD63. Furthermore, inhibition of sphingomyelin phosphodiesterase 3 (SMPD3), which is involved in exosome biogenesis abrogated MV secretion by VSMCs confirming that MVs represent exosomes. Calcifying conditions induced exosome secretion by VSMCs and this was accompanied by elevated SMPD3 expression. Importantly, an inhibition of SMPD3 prevented VSMC calcification. Phenotypic modulation of VSMCs and loss of the contractile phenotype resulted in elevated exosome secretion while contractile VSMCs secreted significantly less exosomes. Notably, elevated extracellular calcium rapidly induced calcification of synthetic VSMCs whilst contractile VSMCs did not calcify. In agreement with this data, abundant immunohistochemical staining for CD63 was observed only in atherosclerotic human aorta in close association with calcified areas with little staining detected in the healthy vessel wall.

Taken together this study demonstrates that MVs originate from MVBs and are secreted via the exosomal pathway. Loss of the

contractile phenotype and mineral imbalance promote VSMC calcification by enhanced exosome secretion. Targeting the mechanisms of VSMC exosome secretion and/or their loading with calcification inhibitors may provide novel therapeutic interventions aimed for the prevention of vascular calcification.