

NEW PLAYERS IN CKD-MBD

SaO024

CALCIPROTEIN PARTICLE RIPENING INDUCES RUNX2-INDEPENDENT MINERALISATION OF HUMAN AORTIC VASCULAR SMOOTH MUSCLE CELLS

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Introduction and Aims: Vascular calcification is widely regarded as an active cell-mediated phenomenon, triggered by chronic exposure of the vasculature to inappropriate environmental cues like high phosphate concentrations, which drive the transdifferentiation of vascular cells toward a mineralising phenotype. Recent work has focused on the involvement of phosphate-induced mineral nanocrystals as direct mediators of this process, but their role in the pathogenesis of arterial calcification remains unclear.

Methods: Here we investigated the effect of native serum-derived calciprotein particles (CPP), on mineral deposition, calcification inhibitory pathways and changes in mineralisation- and inflammation-related gene expression in primary human aortic vascular smooth muscle cells (HAVSMC) *in vitro*. We assessed the dependency of changes in HAVSMC mineralisation on phenotypic reprogramming directed by

Runx-related factor-2 (Runx2), a key osteogenic transcription factor, and defined the temporal inter-relationship of spontaneous CPP formation, HAVSMC mineralisation and phenotypic adaptations in response to high-phosphate culture. Since the ripening of CPP from small amorphous calcium phosphate-containing particles (CPP-I) to larger particles with a crystalline mineral core (CPP-II) appears to be a critical determinant of biological effect and predictive of patient outcome, we also investigated the effect of CPP maturation on these mineralisation phenomena.

Results: Exposure of HAVSMC to CPP-II, but not CPP-I, accelerated the passive mineralisation of HAVSMC in normophosphataemic media (1 mmol/L), but only induced limited expression of bone-related markers (osteopontin, matrix Gla protein) and without changes in Runx2 transcriptional activation or the expression of downstream effectors (alkaline phosphatase). Although culture of HAVSMC in high-phosphate containing media (2.5 mmol/L) resulted in modest Runx2 transcriptional activation, these cellular changes were preceded by de novo CPP-I formation, ripening to CPP-II and passive deposition of mineral on the cell monolayer. Critically, while inhibition of CPP formation with pyrophosphate or phosphonofunctional acid (crystal poisons) completely abrogated mineralisation in HAVSMC, it had minimal effect on high-phosphate induced Runx2 up-regulation. Moreover, stable knockdown of Runx2 in HAVSMC using a shRNA lentiviral expression system had no effect on either CPP or high-phosphate induced mineral deposition.

Conclusions: High-phosphate-induced mineralisation of HAVSMC *in vitro* is strongly dependent on CPP formation, whereas Runx2 upregulation may not be an absolute prerequisite but may in contrast be induced by phosphate independent of CPP generation. Therapies aimed at minimising the transformation of CPP-I to CPP-II, or enhancing particle clearance may help limit CPP-related vascular calcification and cardiovascular sequelae.