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P343 -Uraemic sera-induced in-vitro calcification of human aortic smooth muscle cells involves regulation of Klotho and RUNX2

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Introduction
Vascular calcification (VC) is common in subjects with chronic kidney disease (CKD) and is associated with increased cardiovascular risk. It is an active process involving trans-differentiation of arterial smooth muscle cells into osteogenic phenotype. Previous work has demonstrated the uraemic sera can induce calcification of rat aortic smooth muscle cells (RASMC). The mechanisms are unknown. Whether this translates to human aortic smooth muscle cells (HASMC) is also unknown. Both these factors may be important in designing potential interventions
Methods
We investigated the ability of serum from CKD subjects to induce calcification in HASMCs in vitro (calcific potential of sera: CP), and associated changes in expression of RUNX2, SM22 and Klotho. Sera from subjects with CKD (18 stage 3, 17 stage 4/5, and 29 stage 5D) and 20 controls were added to cultured human SMCs and CP quantified.
Results
CP of CKD sera in the model HAMSC was greater ($p<0.01$) than that of controls, though, unlike in RASMC, was not influinduce enced by CKD stage. MDRD-4 eGFR ($p<0.001$), serum phosphate ($p= 0.042$), RANKL ($p= 0.001$), PTH ($p= 0.014$) and HDL/Cholesterol ratio ($p= 0.026$) were independent predictors of CP accounting for 45% of variation. Adding calcification buffer (CB: calcium chloride [7mM] and β glycerophosphate [7mM]) increased the CP of control sera to approximate that of CKD sera. CP of CKD sera was unchanged. CKD sera increased RUNX2 expression ($p<0.01$) in human SMCs and decreased SM22 expression ($p<0.05$). Co-incubating control but not CKD serum with CB further increased RUNX2 expression ($p<0.01$). Both SM22 and Klotho expression decreased significantly ($p<0.01$) in the presence of CKD serum, and were virtually abolished with Stage 5D sera.
Conclusions
These findings demonstrate that CKD sera can induce calcification of HASMC in vitro, and highlight potentially important species differences. They also support active regulation by CKD serum of in vitro VC by induction of RUNX2 and suppression of SM22 and Klotho. Further work is necessary to relate in vitro CP to in vivo VC at different stages of CKD – both cross-sectionally and over time.