Changes of expression of regulators of calcification in different stages of atherosclerosis in vasculature and bone.

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Introduction and aim:
Calcification is physiologically present in bone but also pathophysiologically in the vasculature, favoring cardiovascular diseases. Cardiovascular diseases are one of the most common causes of death within patients with chronic kidney disease and crucial for kidney transplantation (RTX) outcomes. The present knowledge about expression of calcification regulators in bone compared to vasculature is fragmentary. Our aim was to investigate these changes in the expression of calcification regulators (CR) during vascular calcification simultaneously in bone and vasculature. Furthermore we tested the effect of systemic regulators of bone calcification in HEK293, HOS, EA.hy926 cells and HUVECs.

Material and methods:
Gene expression levels of OPG, RANKL, OPN, MGP, BSP-II and RUNX2 were determined in bone, aorta and arteria iliaca externa tissue samples of 21 transplant donors. Realtime PCR was performed with pre-designed TaqMan assays (Life Technologies) using the LC480 (Roche). Normalisation of the Ct values of CRs was done by division. Beta actin was used as housekeeping gene. The influence of PTH, 25(OH)-Vitamin-D3 and 1,25(OH)2-Vitamin-D3 on the expression of CR was tested in HEK293, HOS, EA.hy926 cells and HUVECs. In addition, serum measurements of PTH, Fetuin A, 25(OH)-Vitamin-D3 and 1,25(OH)2-Vitamin-D3 were performed.

In RTX donors only atherosclerosis and no media sclerosis was present. The RTX donors were subgrouped using three histological stages of atherosclerosis (0) no changes, (1) intimal thickening or (2) intima calcification. Patients’ other tissue samples were subgrouped accordingly.

![Fig. 1: Changes in the expression of CR in artery ilica (A), aorta (AO) and (B), in 3 stages of atherosclerosis. The expression (normalised Cp values) of MGP, OPG and OPN in stage 1 was significantly lower in bone than in the vessels. In stage 2, the expression of OPG and OPN decreased in the vessels as low as in bone, abolishing the previously seen difference. Stage 0: A1: n=3, A0: n=3, B1: n=2, B0: n=11; stage 2: A1: n=8, A0 and B: n=7.](image1)

![Fig. 2: Changes in the expression of CR (normalised Cp values) in 3 stages of atherosclerosis in bone (A) and artery ilica (B) and in 2 stages of atherosclerosis in bone (C) and artery ilica (D). A: Cp values of BSP-II, MGP, OC, Rankl and RUNX2 increased in bone in stage 1 and were still elevated in stage 2. B: In artery ilica tissue, Cp values of BSP-II (p=0.057), OPN (p=0.096) und Rankl (p=0.19) decreased in stage 1 and increased slightly in stage 2. C and D: RTX donors were subgrouped in 2 stages of atherosclerosis: Stage 0 = no changes in the vessel wall, stage 1 = changes in the vessel wall. C: In bone, Cp values of BSP-II, MGP and Rankl decreased in the presence of changes in the vessel wall. D: In artery ilica tissue, Cp values of the same CRs that were decreased in bone were conversely increased.](image2)

![Fig. 3: Changes of expression of CR in HUVECs following addition of 10 nM PTH: The expression of Rankl and RUNX2 (relative Cp values) increased after addition of PTH. Addition of the vitamin D3 metabolites 25OH- and 1,25(OH)2-Vitamin-D3 did not change the expression of CR. None of the tested metabolites was able to significantly change the expression of CR in HEK293, EA.hy926 and HOS cells.](image3)

Summary:
- The expression of calcification regulator genes (CR) changes in the three histological stages of atherosclerosis.
- In the vessels, the expression of CR increases significantly after the first histological signs of calcification in the vessel wall but does not change significantly in further stages.
- At the same time, the expression of CR decreases in bone, starting in the stage of vessel wall thickening.
- The expression of Rankl and RUNX2 increases after induction with PTH in HUVECs, but not in HEK293, HOS and EA.hy926 cells.
- Serum levels of Fetuin A were significantly lower in stage 2 patients compared to the other stages (p=0.007).

Conclusion:
We were able to demonstrate that changes in the expression of calcification regulators occur in the stage of vessel thickening even prior to vessel wall calcification. Changes in the expression of CRs in the vessels were counterbalanced in bone.

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