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Renal tubule-derived EVs carrying complement 3 aggravate CKD vascular calcification by downregulating autophagy in vascular smooth muscle cells

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Objectives : Chronic kidney disease (CKD) often leads to vascular calcification (VC), a serious complication recognized as a major cardiovascular risk. The mechanisms of VC in CKD remain unclear. This study investigates how renal-origin EVs carrying complement mediate CKD-associated VC.
Methods : Techniques including WB, PCR, and immunofluorescence detected renal tubular complement activation and localization in the mouse model of CKD-associated VC. EVs were isolated from kidney tissue and plasma. WB examined C3 and tubular markers of EVs. Simultaneously, the Elisa method quantified C3 in plasma, plasma EV, and EV-depleted plasma. Immunofluorescence detected tubular-derived EV uptake in calcified arteries. In vitro experiments intervened with tubular-derived EVs, C3aR inhibitors (SB290157), and autophagy inhibitors (3-MA) in high-phosphate induced vascular smooth muscle cells (VSMCs). PCR and WB assessed calcification indicators, C3aR, and autophagy-related markers. Alizarin Red staining visualized calcification nodules. Transcriptome sequencing was performed on VSMCs. An in vivo model was infused renal tubular-derived EVs and injected SB290157 and 3-MA. Additionally, plasma EVs from 30 CKD patients with VC and healthy controls were collected for C3 detection.
Results : The CKD-associated VC mice model revealed complement C3 activation in renal tubules, secreted into the blood through EVs. The C3 proportion in plasma EVs of the CKD group significantly exceeded the control group. Immunofluorescence indicated tubular-derived EV uptake by the calcified vascular wall. In vitro, tubular-derived EVs enhanced VSMC osteogenic differentiation, upregulated C3aR, mimicking C3a intervention effects. C3aR inhibitors produced opposing effects. Transcriptome sequencing suggested renal tubular-derived EVs downregulated VSMC autophagy-related pathways, autophagy inhibition reversed osteogenic differentiation. In vivo, reinfusing renal tubular-derived EVs exacerbated complement activation and VC, while injecting SB290157 and 3-MA alleviated VC. In CKD patients with VC, the C3 level of plasma EVs significantly increased, correlating with the thoracic aorta calcium score.
Conclusions : This study confirms that tubular-derived EVs carrying C3 downregulate autophagy in VSMCs, mediating CKD-associated VC.