2. Tojo A, Endou H. Intrarenal handling of proteins in rats using fractional micropuncture technique. *Am J Physiol*. 1992;263:F601–F606.
3. Russo LM, Sandoval RM, McKee M, et al. The normal kidney filters nephrotic levels of albumin retrieved by proximal tubule cells: retrieval is disrupted in nephrotic states. *Kidney Int*. 2007;71:504–513.
4. Tenten V, Menzel S, Kunter U, et al. Albumin is recycled from the primary urine by tubular transcytosis. *J Am Soc Nephrol*. 2013;24:1966–1980.
5. Weyer K, Andersen PK, Schmidt K, et al. Abolishment of proximal tubule albumin endocytosis does not affect plasma albumin during nephrotic syndrome in mice. *Kidney Int*. 2018;93:335–342.
6. Nielsen R, Mollet G, Esquivel EL, et al. Increased lysosomal proteolysis counteracts protein accumulation in the proximal tubule during focal segmental glomerulosclerosis. *Kidney Int*. 2013;84:902–910.
7. Eisner C, Faulhaber-Walter R, Wang Y, et al. Major contribution of tubular secretion to creatinine clearance in mice. *Kidney Int*. 2010;77:519–526.
8. Aseem O, Smith BT, Cooley MA, et al. Cubilin maintains blood levels of HDL and albumin. *J Am Soc Nephrol*. 2014;25:1028–1036.
9. Wagner MC, Campos-Bilderback SB, Chowdhury M, et al. Proximal tubules have the capacity to regulate uptake of albumin. *J Am Soc Nephrol*. 2016;27:482–494.

Not all vascular smooth muscle cell exosomes calcify equally in chronic kidney disease

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Prevention of medial calcification in patients with chronic kidney disease requires the maintenance of vascular smooth muscle cell fitness. To preserve viability under chronic kidney disease–induced stress, vascular smooth muscle cells increase exosome formation and release, but the result is aggravated pathological calcification. Now Chen *et al.* report that microvesicles from calcifying vascular smooth muscle cells may propagate procalcifying signals to normal vascular smooth muscle cells. To help design effective strategies to impair procalcifying cell-to-cell communication, this commentary updates the current understanding of the main regulators of microvesicle/exosome biogenesis and secretion.

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Vascular calcification is the pathological deposition of calcium phosphate salts in the vasculature and is a prevalent and serious complication in patients with chronic kidney disease (CKD), where it associates positively with a disproportionately high risk of cardiovascular mortality. In the course of CKD, vascular calcification develops early, affecting 25% of patients at CKD stages 3 to 4 and increasing to 50%–80% in patients starting hemodialysis (reviewed in Paloian and Giachelli[1]).

In patients with CKD, calcification affects both the intimal and medial layers of the arterial wall. However, medial calcification, a process that resembles bone mineralization and is carried out by vascular smooth muscle cells (VSMCs), is more pronounced in CKD and is the exclusive form of vascular calcification in pediatric CKD (reviewed in Paloian and Giachelli[1]).

Important for treatment, despite similar clinical and biochemical risk factors, medial calcification progresses faster in hemodialysis patients with existing calcified lesions compared with patients with noncalcified CKD. In this issue of *Kidney International*, Chen and collaborators (2018) examine the ability of microvesicles generated by calcifying VSMCs cultured from arteries of rats with CKD to extend procalcifying signals to adjacent VSMCs cultured from normal rats.[2] The rationale for the study was the recognized key role of exosomes in cell-to-cell communication and the recent identification of a critical contribution of exosome biosynthesis and release by VSMCs to vascular calcification.[3] The results of the study by Chen *et al.* have revealed a greater complexity of mechanisms regulating intracellular control and compartmentalization of mineralization by VSMCs.

This commentary updates the current understanding and highlights the unanswered questions underlying microvesicle/exosome biogenesis and secretion from VSMCs. Ultimately, this knowledge will be essential to customize therapeutic strategies aimed at attenuating or delaying both the initiation of calcification and potentially the propagation of calcifying signals between VSMCs.

Figure 1 summarizes the mechanisms for pathological calcium deposition. First, VSMCs undergo a process of phenotypic transition that involves the loss of their contractile phenotype, required to maintain vascular tone, together with the upregulation of markers of osteochondrogenesis. Simultaneously, VSMCs release matrix vesicles that colocalize with elastin and collagen fibrils and form the nidus for mineralization.[1]

Recent characterization of the biogenesis of these calcifying matrix vesicles identified at least a subpopulation as exosomes, because the endosomal pathway and inward budding of the membrane of late endosomes or multivesicular bodies (MVB) participate in their formation,[3] as depicted in Figure 1. In addition, the role of

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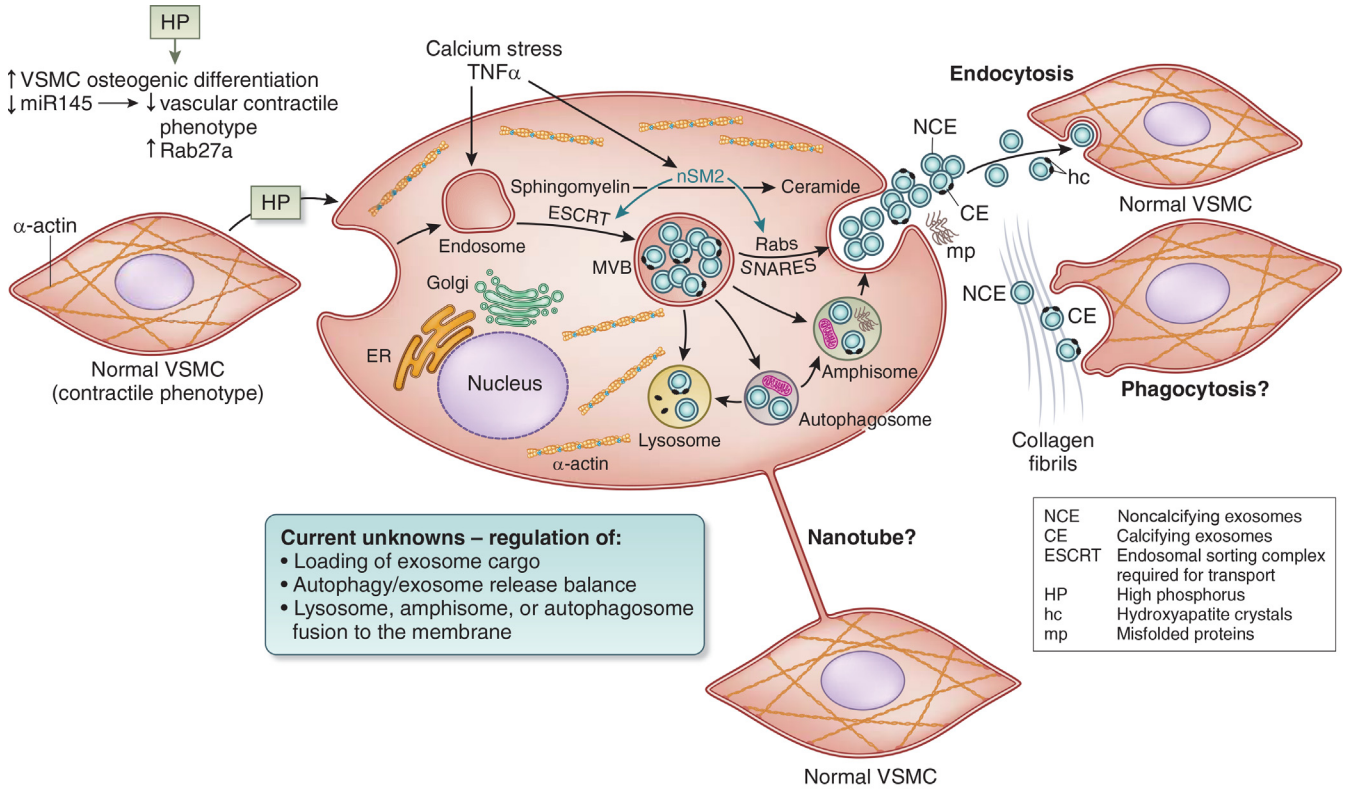

Figure 1 | Vascular smooth muscle cell (VSMC) exosome biogenesis and release. ER, endoplasmic reticulum; miR145, micro RNA 145; MVB, multivesicular bodies; nSM2, neutral sphingomyelinase 2; SNARES, Soluble NSF Attachment protein REceptor superfamily; TNF-α, tumor necrosis factor alpha.

sphingomyelin phosphodiesterase 3, also known as neutral sphingomyelinase 2 (nSM2), in the regulation of exosome release was shown. Furthermore, although elevated extracellular calcium and tumor necrosis factor α, common features in CKD, increased the expression of nSM2 and the secretion of calcifying exosomes, chemical inhibition of nSM2 prevented VSMC calcification.[3] These findings render the inhibition of nSM2 a novel strategy to attenuate calcification initiation. The severe bone mineralization defects in the nSM2 null mice strongly support the contribution of this mechanism to bone and vascular calcification.

Intriguingly, Chen and coworkers[2] demonstrate multiple procalcifying signals induced exclusively by the so called "cellular-derived exosome-like vesicles" obtained from collagenase digestion of VSMCs cultured from arteries of CKD rats upon their endocytosis by cultured VSMCs from normal rats exposed to high phosphorus. In contrast, there were no calcifying signals from exosome-like vesicles freely released into the incubation media by identical cells in culture. The calcifying signals from these matrix-trapped cellular-derived exosomes included increased intracellular calcium and oxidative stress in recipient VSMCs. However, exposure to high phosphate had no effect on the calcifying potency of the cellular exosome-like vesicles in increasing intracellular calcium and oxidative stress. They also showed that activation of transient calcium rises by these exosome-like vesicles was dependent on mitogen-activated protein kinase signaling; however, this was independent of the activation of oxidative stress and osteogenic gene expression. Thus, the authors show a potential contribution for intercellular signaling in propagating calcifying signals, but the mechanisms driving this calcification potential remain unclear. In addition, a critical unanswered question is how these procalcifying signals from matrix-trapped cellular-derived exosome-like vesicles reach a neighboring normal VSMC. One mechanism could be uptake from the extracellular matrix by phagocytotic processes. Alternatively, nanotubes, bridging CKD-derived VSMCs and normal VSMCs, could contribute, although the authors indicated that nanotube formation was not observed in these studies.

It is important to note that in the work by Chen *et al.* the vesicles were not fully characterized and therefore their composition as well as release and uptake pathways are difficult to determine. Both matrix-trapped and free-floating exosome-like vesicles were positive for CD63, but this is not necessarily indicative of an endosomal origin for both. Indeed, the main difference between these 2 vesicle populations was that the matrix-trapped population lacked fetuin-A, whereas the media-derived exosomes had a high fetuin content. These findings raise 2 important questions regarding the biogenesis of the 2 vesicle populations: (i) What is the origin of the fetuin in media-derived exosomes because VSMCs do not express fetuin? and (ii)