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CHRONIC KIDNEY DISEASE

Gli1$^+$ adventitial cells have a critical role in vascular calcification in CKD

> Genetic ablation of Gli1$^+$ MSC-like cells … completely abolished vascular calcification

Vascular calcification is a major complication of chronic kidney disease (CKD) that increases cardiovascular risk. New findings from Rafael Kramann and colleagues in Benjamin Humphreys' laboratory show that in mice, Gli1$^+$ adventitial mesenchymal stem cell (MSC)-like cells generate osteoblast-like cells that drive CKD-induced arterial calcification. "It had been hypothesized that adventitial progenitors might contribute to vascular calcification, but our study is the first inducible genetic fate-tracing experiment that actually proves this," says Kramann.

In previous work, Kramann and coworkers identified Gli1 as a specific marker of MSCs. In this study, they used a genetic approach to induce the expression of a red fluorescent protein (tdTomato) in Gli1$^+$ positive cells and trace their fate. "Once Gli1$^+$ cells are tagged by tdTomato, they permanently express this protein and give it to all daughter cell populations," explains Kramann.

Using this tracing technique to isolate Gli1$^+$ cells, the researchers found that Gli1$^+$ adventitial progenitors are multipotent *in vitro;* they can generate adipocytes, chondrocytes and osteoblasts. In a model of femoral artery wire injury, the researchers showed that Gli1$^+$ adventitial cells can migrate into the media and contribute to arterial repair after acute injury. "Approximately 50% of all vascular smooth muscle cells (VSMCs) that were newly formed after wire injury to the femoral artery were derived from Gli1$^+$ progenitors," says Kramann.

To assess the function of Gli1$^+$ progenitors during vascular calcification, Kramann and colleagues induced CKD by performing a two-step subtotal nephrectomy in ApoE deficient mice with tdTomato-tagged Gli1$^+$ cells. In these mice, which developed severe atherosclerosis 16 weeks after CKD induction, the number of Gli1$^+$ cells in the media and the neointima was markedly higher than in litter-mate controls. Adventitial Gli1$^+$ cells first differentiated towards the VSMC lineage and then into osteoblast-like cells, as shown by *Runx2* expression and alkaline phosphatase activity, two characteristic markers of osteogenic differentiation. Genetic ablation of Gli1$^+$ MSC-like cells with diphtheria toxin before the onset of CKD reduced *Runx2* mRNA expression and completely abolished vascular calcification. "These findings suggest that Gli1$^+$ MSCs are an important therapeutic target," says Kramann.

As Gli1 is a read-out of Shh pathway activation, the researchers also investigated the effects of stimulating the SHH coreceptor Smoothened on Gli1$^+$ progenitors. SAG, a Smoothened agonist, stimulated the proliferation of Gli1$^+$ progenitors *in vitro* and enhanced calcification during their osteogenic differentiation.

Finally, the researchers report that GLI1 and SHH were predominantly expressed in the adventitia of healthy human arteries, whereas they were highly upregulated and localized to the media, adventitia and plaques in the calcified arteries of deceased patients with CKD. Kramann comments that whether this increase in *Gli1* expression in calcified arteries is due to a human progenitor population similar to that found in mice is not yet clear.

"It will be critical to study the mechanisms that recruit Gli1$^+$ cells from the adventitia and drive their expansion and differentiation to develop novel targeted therapeutics," says Kramann. The researchers also plan to investigate whether a Gli1$^+$ progenitor population exists in human arteries.

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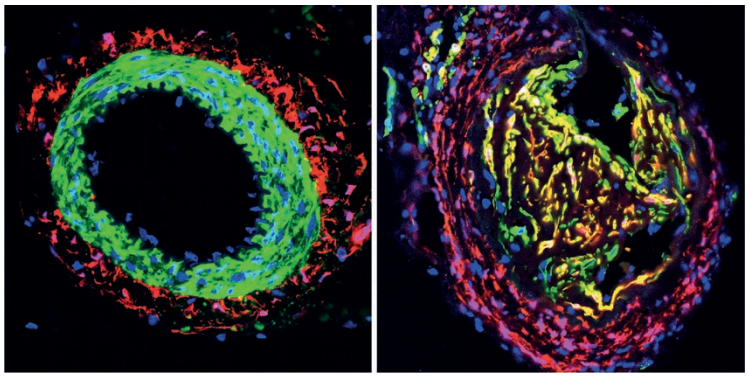

Gli1-expressing cells (red) are localized in the perivascular zone in control animals (left) and generate α-SMA$^+$ vascular smooth muscle cells (green) after wire injury (right). Image courtesy of R. Kramann.

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