© 2015 International Society of Nephrology

Migration of smooth muscle cells from the arterial anastomosis of arteriovenous fistulas requires Notch activation to form neointima

Ming Liang[1,2], Yun Wang[2], Anlin Liang[2], William E. Mitch[2], Prabir Roy-Chaudhury[3], Guofeng Han[2,4] and Jizhong Cheng[2]

[1]*Department of Nephrology, Guangzhou First People's Hospital, Guangzhou Medical University, China;* [2]*Division of Nephrology, Department of Medicine, Baylor College of Medicine, Houston, Texas, USA;* [3]*Department of Surgery, Pathology and Radiology and Division of Nephrology, Department of Medicine, University of Cincinnati and Cincinnati VAMC, Cincinnati, Ohio, USA and* [4]*The 455th Hospital, Shanghai, China*

A major factor contributing to failure of arteriovenous fistulas (AVFs) is migration of smooth muscle cells into the forming neointima. To identify the source of smooth muscle cells in neointima, we created end-to-end AVFs by anastomosing the common carotid artery to the jugular vein and studied neural crest–derived smooth muscle cells from the carotid artery, which are Wnt1-positive during development. In Wnt1-cre-GFP mice, smooth muscle cells in the carotid artery but not the jugular vein are labeled with GFP. About half of the cells were GFP–positive in the neointima, indicating their migration from the carotid artery to the jugular vein in AVFs created in these mice. As fibroblast-specific protein-1 (FSP-1) regulates smooth muscle cell migration, we examined FSP-1 in failed AVFs and polytetrafluoroethylene grafts from patients with end-stage kidney disease or from AVFs in mice with chronic kidney disease. In smooth muscle cells of AVFs or polytetrafluoroethylene grafts, FSP-1 and activation of Notch1 are present. In smooth muscle cells, Notch1 increased RBP-Jκ transcription factor activity and RBP-Jκ stimulated FSP-1 expression. Conditional knockout of RBP-Jκ in smooth muscle cells or general knockout of FSP-1 suppressed neointima formation in AVFs in mice. Thus, the artery of AVFs is the major source of smooth muscle cells during neointima formation. Knockout of RBP-Jκ or FSP-1 ameliorates neointima formation and might improve AVF patency during long-term follow-up.

Kidney International advance online publication, 18 March 2015; doi:10.1038/ki.2015.73

KEYWORDS: arteriovenous fistula; chronic kidney disease; fibroblast specific protein 1; neointima formation; Notch; vascular smooth muscle cell

Correspondence: *Jizhong Cheng, Division of Nephrology, Department of Medicine, Baylor College of Medicine, Houston, Texas 77030, USA. E-mail: jizhongc@bcm.edu*

Received 27 May 2014; revised 26 January 2015; accepted 29 January 2015

The success of hemodialysis treatment depends on reliable functioning of an arteriovenous fistula (AVF) or polytetrafluoroethylene (PTFE) graft. However, in the 2 years following creation of the AVF or PTFE graft, nearly 50% fail, generally because of the accumulation of vascular smooth muscle cells (SMCs) in the neointima.[1–3] This problem is costly as loss of AVFs amounts to >US$1 billion dollars per year for surgical and radiological interventions.

Multiple types of cells have been implicated in the formation of a neointima, including fibrocytes and bone marrow–derived, circulating progenitor cells and/or endothelial–mesenchymal transition cells.[4–6] Besides circulating cells, neointima could be formed from local events and local cells, including resident SMCs, or from adventitial cells and smooth muscle progenitor cells. The latter cells can transform into SMCs and participate in formation of the neointima.[7–10] To identify the origin of cells contributing to SMCs in the neointima, we used Wnt1-Cre transgenic mice to label and track neointima SMCs arising from the cardiac outflow tract.[11] Our strategy was based on the finding that during cardiac development, neural crest cells contribute to the smooth muscle layer of the cardiac outflow tract, which includes the common carotid artery. As SMCs arising from vessels that are outside of the neural crest (e.g., the jugular vein) are not labeled.[12–14] This strategy allowed us to examine whether SMCs from the arterial anastomoses contribute to neointima formation.

What could regulate SMC functions in AVFs? Several important SMC growth-regulatory pathways and molecules can modulate neointima formation. These factors include transforming growth factor-β (TGF-β1) and Notch, as well as fibroblast-specific protein-1 (FSP-1).[15,16] FSP-1 is stimulated by growth factors that influence SMC migration and proliferation.[17] In fact, bone marrow–derived FSP-1$^+$ cells were shown to stimulate neointima formation in a mouse model of a vein graft.[18] Others have demonstrated that activation of Notch can promote the vascular remodeling that follows vessel injury.[15,16]

Recently, we developed a mouse model of an AVF model and used it to show that chronic kidney disease (CKD) accelerates neointima formation.[16] There was evidence for endothelial damage in the AVFs of mice with CKD.[19] Specifically, the endothelium of the AVF became leaky, leading to the infiltration of inflammatory cells causing activation of SMCs. Our aim in the present experiments was to identify the origin of SMCs and how their migration is regulated during neointima formation. Our results identify potential targets that might prevent neointima formation in AVFs, the '*Achilles* heel' of the hemodialysis patient.

RESULTS

SMCs from the arterial anastomosis contribute to neointima formation

We used a Wnt1-Cre reporter mouse strain in which SMCs from the artery are specifically labeled, whereas venous SMCs were not labeled. Wnt1-Cre transgenic mice with dual-fluorescent, RFP-Stop$^{flox/flox}$-GFP/Wnt1-Cre^{+} mice (Supplemental Figure S1 online) were studied to determine whether SMCs in the neointima are derived from the artery of an AVF. This identification was possible because SMCs of the cardiac outflow tract express green fluorescent protein (GFP) in Wnt1-Cre^{+} reporter mice, but other, non-neuron crest–derived cells (including SMCs of the vein) are positive for RFP. Although SMCs from the common carotid artery in RFP-Stop$^{flox/flox}$-GFP/Wnt1-Cre^{+} mice were GFP^{+}, endothelial cells were RFP^{+} (Figure 1a) and GFP and RFP signals were not colocalized. There was also no nonspecific staining of elastin (Figure 1a). Note that endothelial cells or SMCs arising from the jugular vein were not GFP^{+} signals (Figure 1a). Next, we examined whether GFP^{+}-SMCs were present in AVFs created in the RFP-Stop$^{flox/flox}$-GFP/Wnt1-Cre^{+} mice. GFP^{+} cells were observed in frozen sections of the neointima at the venous side near the anastomosis (Figure 1b). Costaining with an SMC marker showed that around 50% of neointima cells in the AVF were GFP^{+} and most expressed smooth muscle α-actin (SMA-α). There were some GFP^{-} SMCs in the neointima of the AVFs (Figure 1c). Our results indicate that SMCs from anastomosed artery contribute to as much as 50% of SMCs in the neointima.

As expected, Wnt1 was expressed in the vagus nerve (Figure 1d) but was not detected in neointima cells of AVFs (Figure 1e). These results indicate that: (1) Wnt1 is expressed and will activate Cre in the vagus nerve leading to GFP expression; (2) GFP^{+} SMCs present in the forming neointima originated from neural crest–derived SMCs because they were Wnt1^{-} but GFP^{+}. The results raise the question, what stimulates arterial SMCs to migrate into the forming neointima?

CKD stimulates FSP-1 expression and Notch activation in SMCs of the neointima

There were significantly increased mRNA levels of FSP-1 in the AVFs created in mice with CKD (Figure 2a). In fact, there was increased expression of the FSP-1 protein in cells of the neointima in AVFs created in mice with CKD versus results in control mice (Figure 2b and c). Notably, FSP-1^{+} cells in the neointima also stained positive for SMA-α (Figure 2d). We characterized neointima cells by staining for the SMC terminal differentiation marker (smooth muscle myosin heavy chain, SMMHC). These neointima cells costained positively for SMMHC and SMA-α, indicating that SMCs are present in the neointima (Figure 2e). Immunostaining for vimentin was detected in the adventitia and was slightly stained in the neointima (Figure 2f). Notably, these vimentin-positive cells costained positively with FSP-1 (Figure 2g). We conclude that SMCs are the major cellular contributor to neointima formation because they express the terminal differentiation marker of SMCs, SMMHC.

As Notch activation and Notch/RBP-Jκ signaling have been linked to worsening of vascular remodeling in arterial injury models,[20,21] we examined how CKD affects Notch and its signaling pathway. CKD increased the expression of Notch receptors and their target genes, Hes1 and Hey1, in AVFs (Figure 2h). CKD was also associated with activation of Notch1 intracellular domain (N1ICD), which was present in the nuclei of neointima cells coexpressing FSP-1 (Figure 2i). Thus, FSP-1 expression could be regulated by the Notch signaling pathway.

Notch regulates FSP-1 transcription

In AVFs created in CKD mice, Notch activation was increased compared with results in AVFs placed in control mice. A potential mediator is TGF-β1 because it can stimulate Notch activation.[22,23] In fact, we found that adding TGF-β1 to SMCs induced the cells to express the Notch ligand, Jagged1, and FSP-1 in a time-dependent manner (Figure 3a). As we found that the TGF-β1-induced FSP-1 expression is suppressed by pretreatment with the Notch inhibitor, DAPT (*N*-[*N*-(3,5-difluorophenacetyl)-L-alanyl]-*S*-phenylglycine *t*-butyl ester) (Figure 3b), our results suggest that CKD induces FSP-1 expression via a TGF-β1 to the Notch pathway.

We examined the FSP-1 promoter and found potential binding sites of RBP-Jκ upstream of the transcription start site (Figure 3c). To determine whether RBP-Jκ targeting sites in FSP-1 promoter can activate the transcription of FSP-1, we created luciferase-reporter constructs of different fragments of the FSP-1 promoter (left panel of Figure 3d). In the absence of RBP-Jκ sequences, there was no increase in luciferase activity in response to TGF-β1 (Figure 3d, right panel). Moreover, DAPT, the Notch inhibitor, suppressed TGF-β1-induced FSP-1 promoter activity by ~50% in cultured SMCs (Figure 3e). In SMCs, knockout (KO) of RBP-Jκ suppressed TGF-β1-induced FSP-1 promoter activities by ~40% versus results in wild-type SMCs (Figure 3f). Taken together, these results indicate that Notch activation stimulates FSP-1 transcription.

TGF-β1-stimulated DNA-binding activity is regulated by Notch signaling

We designed eight pairs of primers (P1–8) to amplify the DNA fragments that contain the potential RBP-Jκ binding

Download English Version:

https://daneshyari.com/en/article/6162046

Download Persian Version:

https://daneshyari.com/article/6162046

Daneshyari.com