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Characterization of Pdgfrb-Cre Transgenic Mice Reveals Reduction of ROSA26 Reporter Activity in Remodeling Arteries

Anne S. Cuttler, Renee J. LeClair¹, J. Patrizia Stohn, Qiaozeng Wang, Christine M. Sorenson², Lucy Liaw, and Volkhard Lindner

Center for Molecular Medicine, Maine Medical Center Research Institute, Scarborough, ME 04074

¹Department of Biochemistry, University of New England, Biddeford, ME 04005

²Department of Pediatrics, University of Wisconsin-Madison, Madison, WI 53792

Abstract

With the intention to modulate gene expression in vascular mural cells of remodeling vessels, we generated and characterized transgenic mouse lines with Cre recombinase under the control of the platelet-derived growth factor receptor- β promoter, referred to as Tg(Pdgfrb-Cre)^{35Vli}. Transgenic mice were crossed with the Gt(ROSA)26Sor^{tm1Sor} strain and examined for Cre activation by β -galactosidase activity, which was compared with endogenous Pdgfrb expression. In addition, Pdgfrb-Cre mice were used to drive expression of a conditional myc-tagged Cthrc1 transgene. There was good overlap of β -galactosidase activity with endogenous Pdgfrb immunoreactivity. However, dedifferentiation of vascular mural cells induced by carotid artery ligation revealed a dramatic discrepancy between ROSA26 reporter activity and Pdgfrb promoter driven Cre dependent myc-tagged Cthrc1 transgene expression. Our studies demonstrate the capability of the Pdgfrb-Cre mouse to drive conditional transgene expression as a result of prior Cre mediated recombination in tissues known to express endogenous Pdgfrb. In addition, the study shows that ROSA26 promoter driven reporter mice are not suitable for lineage marking of smooth muscle in remodeling blood vessels.

Keywords

vascular smooth muscle; dedifferentiation; Cthrc1

Introduction

Blood vessel development is guided by receptor ligand interactions that allow for crosstalk between the endothelial cells and the surrounding mesenchyme. The role of platelet-derived growth factor (Pdgf) in recruiting mural cells during blood vessel formation has been demonstrated through gene targeting of the ligand (Pdgfb) (Hellstrom *et al.*, 1999; Leveen *et al.*, 1994) and the platelet-derived growth factor receptor beta (Pdgfrb) (Soriano, 1994); however, major arteries and the heart appeared to develop normally in Pdgfrb deficient embryos (Soriano, 1994). The dependence of pericyte/smooth muscle cell (SMC) recruitment on Pdgfb/Pdgfrb signaling was shown to be variable between different tissues, with vascular mural cells expressing Pdgfrb in most tissues except liver, as determined by in situ hybridization (Hellstrom *et al.*, 1999).

The expression of Pdgf receptors has been well characterized and the expression of the Pdgfrb has been demonstrated in fibroblasts, kidney mesangium (Alpers *et al.*, 1993; Seifert *et al.*, 1998), myoblasts and muscle lineages (Crosby *et al.*, 1998), smooth muscle (Majesky *et al.*, 1990), pericytes (Lindahl *et al.*, 1999), and neurons (Smits *et al.*, 1991). In the developing embryo, Pdgfrb expression was prominent in the developing mesenchyme and in the developing gastrointestinal tract (Shinbrot *et al.*, 1994).

In mice and rats, endothelial cells are typically the only cell type that is found in the tunica intima of arteries. Various models have been developed to induce intimal thickening in carotid arteries and our laboratory developed and characterized the mouse carotid artery wire denudation (Lindner *et al.*, 1993) as well as the carotid artery ligation model (Kumar and Lindner, 1997). Intimal lesion formation in these models is preceded by dedifferentiation and proliferation of SMC in the media with subsequent migration of proliferating SMC into the intima where they give rise to the neointima (Kumar and Lindner, 1997; Lindner *et al.*, 1993; Reidy *et al.*, 1992). The dedifferentiation of SMC in this process is characterized by a reduction in SMC specific markers such as smooth muscle alpha actin, SM22-alpha, and smooth muscle myosin heavy chain (SM-MHC) (Lindner *et al.*, 1993; Regan *et al.*, 2000).

Pdgf signaling also plays important roles in arteries and in the response to arterial injury by promoting migration of SMC from the media into the developing neointima (Buetow *et al.*, 2003; Ferns *et al.*, 1991; Jackson *et al.*, 1993; Jawien *et al.*, 1992; Majesky *et al.*, 1990). Using the rat balloon injury model we previously demonstrated that the activated and dedifferentiated SMC of the neointima expressed elevated levels of Pdgfrb (Lindner *et al.*, 1995; Lindner and Reidy, 1995). In the present study we describe the generation of transgenic mice using a Pdgfrb promoter fragment to drive Cre expression. Pdgfrb-Cre mice were crossed with Gt(ROSA)26Sor^{tm1Sor} reporter mice as well as mice carrying a Cre inducible transgene in order to characterize the sites of prior Cre recombinase activity in embryonic tissues, postnatal tissues and in remodeling arteries.

Results

We generated transgenic mice with Cre recombinase under the control of the Pdgfrb promoter and crossed them to the Cre reporter strain B6;129S4-Gt(ROSA)26Sor^{tm1Sor} (Soriano, 1999). In this reporter strain *LacZ* expression is silent until the transcriptional stop, flanked by LoxP sites, is excised by Cre recombinase (Fig. 1). Tg(Pdgfrb-Cre)^{35Vli} mice were also bred with transgenic mice expressing Cre inducible collagen triple helix repeat containing 1 (Cthrc1) with a myc epitope tag fused to its C-terminus, designated strain name Tg(CagGfp-Cthrc1myc)^{11Vli}. In the absence of Cre recombination this transgenic mouse line expresses GFP under the control of the CAG promoter. Upon Cre recombination, the GFP cassette, flanked by LoxP sites, is removed and Cthrc1-myc is expressed (Fig. 1). This allowed for comparison of β -galactosidase staining and myc tag immunoreactivity localization.

The distribution pattern of β -galactosidase activity in various postnatal tissues seen in the Tg(Pdgfrb-Cre)^{35Vli}/Gt(ROSA)26Sor^{tm1Sor} mice was similar to the immunoreactivity of the Cre inducible transgene driven by the Pdgfrb promoter in Tg(Pdgfrb-Cre)^{35Vli}/Tg(CagGfp-Cthrc1myc)^{11Vli} mice (Fig. 2). Consistent with endogenous Pdgfrb expression, β -galactosidase activity and transgene immunoreactivity were seen in vascular and alimentary tract smooth muscle, kidney mesangium, skeletal muscle, adult myocardium and dermal fibroblasts (Fig. 2). The blood vessels of the liver showed no β -galactosidase activity, transgene expression or Pdgfrb immunoreactivity (not shown). The recombination efficiency in esophageal smooth muscle and skeletal muscle of four adult Tg(Pdgfrb-Cre)^{35Vli}/

Gt(ROSA)26Sor^{tm1Sor} mice was determined to be 99.5±0.6% and 99±2.0%, respectively (Table 1). In embryonic tissues, β-galactosidase activity was present in the developing dermis, skeletal muscle, mesenchymal condensation/perichondrium, lung tissue, spinal cord/ectoderm, kidney mesangial cells and the myocardium. Serial sections displaying Pdgfrb immunoreactivity showed an analogous staining pattern (Fig. 3).

We analyzed β-galactosidase activity staining on sections of remodeling and normal carotid arteries from Tg(Pdgfrb-Cre)^{35Vli}/Gt(ROSA)26Sor^{tm1Sor} mice to assess recombination efficiency in vascular SMC. Activity of β-galactosidase was restricted to medial SMC with no detectable staining observed in the adventitia. The percentage of carotid artery SMC exhibiting β-galactosidase activity in uninjured common carotid arteries was similar for the left and right side (not shown), with a range from 35.5% to 79.0% and an average of 61.9±17.9%. Surprisingly, remodeling carotid arteries responding to ligation revealed very few β-galactosidase positive cells in the neointima (Fig. 4). In addition, the percentage of SMC in the media exhibiting β-galactosidase activity also declined. Quantification of β-galactosidase staining in the ligated (left) and corresponding contralateral (non-injured) carotid artery is shown in Table 2 for five adult mice. The medial SMC and dedifferentiated SMC of the neointima did express endogenous Pdgfrb as well as the Pdgfrb-Cre driven myc-tagged transgene (Fig. 4).

To address the utility of the Gt(ROSA)26Sor^{tm1Sor} reporter line for analyzing Cre recombinase activity in remodeling arteries we applied the carotid artery ligation model to B6;129S-Gt(ROSA)26Sor mice with constitutive expression of *LacZ* under ROSA26 promoter control (Friedrich and Soriano, 1991). β-galactosidase activity localization in these mice also exhibited a significant decrease in the number of β-galactosidase positive SMC in the media as well as in the developing neointima of the remodeling artery (Fig. 4). Quantitative real time reverse transcription PCR (qRT-PCR) data revealed a 21.98 ± 1.56 fold decrease (p=0.00003) in *LacZ* expression in remodeling carotid arteries when compared to expression in normal carotid arteries (Fig. 5). These findings indicate that the decrease in ROSA26 reporter activity is independent of Cre mediated recombination.

Discussion

One major reason for developing Pdgfrb-Cre transgenic mice was the high activity of the Pdgfrb promoter in dedifferentiated vascular smooth muscle of remodeling and injured arteries (Lindner and Reidy, 1995; Majesky *et al.*, 1990). While a Pdgfrb-Cre transgenic mouse line has been previously developed by others (Abraham *et al.*, 2008; Foo *et al.*, 2006), a detailed analysis of this mouse line has not been published. The purpose of the study conducted here was to characterize a Pdgfrb-Cre transgenic mouse line with emphasis on vascular smooth muscle and mural cells. The Tg(Pdgfrb-Cre)^{35Vli}/Gt(ROSA)26Sor^{tm1Sor} mice displayed β-galactosidase activity in most medial SMC of the carotid arteries, indicating that these cells were indelibly tagged by Cre recombination. With β-galactosidase now under the control of ROSA26 regulatory elements and the ROSA26 promoter thought to be active in most cells and tissues, we anticipated that SMC forming the neointima would display β-galactosidase activity because they originated from cells in the media (Reidy *et al.*, 1992). The low β-galactosidase activity seen in dedifferentiated SMC was therefore surprising and raised the question whether the ROSA26 reporter is suppressed in dedifferentiated SMC.

Using Gt(ROSA)26Sor mice that have constitutive β-galactosidase activity (in the absence of any prior Cre mediated recombination), we found low β-galactosidase activity levels (Fig. 3) and a significant reduction in *LacZ* expression (Fig. 5) in dedifferentiated SMC. These data indicated a relative reduction of ROSA26 reporter activity in dedifferentiated SMC. We

ruled out the possibility that only *LacZ* negative cells from the media contributed to neointima formation because SMC undergoing dedifferentiation in the media also lost expression of *LacZ* (Table 2). We could also rule out a potential discrepancy between *LacZ* expression and β -galactosidase enzyme activity because remodeling and control tissue specimens were meticulously treated in an identical fashion and because β -galactosidase activity was still detectable in some SMC that had not undergone dedifferentiation within the remodeling artery.

We previously discovered *Cthrc1* as a gene expressed in remodeling arteries and we generated a transgenic mouse expressing myc-tagged *Cthrc1*, Tg(CagGfp-Cthrc1myc)^{11Vli}, in the presence of Cre recombinase (Fig. 1). Using myc-tagged *Cthrc1* transgene expression as an alternative readout for prior Cre recombinase activity, we clearly demonstrated that Cre recombination had occurred in the SMC that dedifferentiated in response to carotid artery ligation in Tg(Pdgfrb-Cre)^{35Vli}/Tg(CagGfp-Cthrc1myc)^{11Vli} double mutant mice. We were not able to directly demonstrate Cre protein expression in remodeling arteries by immunohistochemistry due to the lack of suitable antibodies.

We previously generated transgenic mouse lines that constitutively overexpress *Cthrc1* under the control of the CMV promoter (Pyagay *et al.*, 2005). When we crossed two different transgenic mouse lines, we observed brittle bones with reduced collagen matrix deposition (Pyagay *et al.*, 2005). Yamamoto *et al.* (Yamamoto *et al.*, 2008) as well as Kimura *et al.* (Kimura *et al.*, 2008) reported that targeted disruption of the *Cthrc1* in mice did not result in any developmental abnormalities. Here we used a mutant mouse strain with inducible expression of *Cthrc1* following Pdgfrb-Cre mediated recombination. We deliberately chose expression of the myc-tagged *Cthrc1* transgene as a readout for prior Cre activity for this study because we anticipated no major developmental abnormalities that would have complicated the interpretation of the transgene expression results. Although not investigated in any detail here, we did not observe any obvious developmental abnormalities in Tg(Pdgfrb-Cre)^{35Vli}/Tg(CagGfp-Cthrc1myc)^{11Vli} double mutant mice. Because *Cthrc1* is an efficiently secreted protein and Pdgfrb is a cell surface receptor, the distribution of the immunoreactivity for these two proteins is different at the cellular level.

Our results indicate that the Pdgfrb -4.7/+0.1kb regulatory sequence of the Pdgfrb gene used here drives Cre mediated recombination and subsequent transgene expression in most cell types that express endogenous Pdgfrb. The Tg(Pdgfrb-Cre)^{35Vli} strain should be a useful tool for researchers in various fields including vascular and kidney biology. We also show that the Gt(ROSA)26Sor^{tm1Sor} reporter mice are not suitable for cell lineage tracking in blood vessels undergoing remodeling because *LacZ* expression as well β -galactosidase activity under direct control of the ROSA26 locus are decreased in dedifferentiated SMC. These results may be limited to reporter mice driven by the endogenous ROSA26 promoter and may not apply to more recent ROSA26 reporter strains such as those with the CAG promoter knocked into the ROSA26 locus (Yamamoto *et al.*, 2009). The most likely explanation for these results is that the activity of the ROSA26 promoter is decreased in remodeling vasculature. However, in the absence of any in-depth characterization of the ROSA26 promoter we cannot rule out decreased stability of the *LacZ* transcript as an alternative explanation.

Methods

Construct generation

A -4.7/+0.1 kb fragment of the Pdgfrb promoter including the first exon was amplified from mouse genomic DNA using the forward primer (CGTTGCTTCCTTGATGTTTGCTTGTGA) and the reverse primer

(GCTGTCTTCTGTTATTTTGCTGGACCCA). The amplified sequence was fused to a nuclear targeted Cre recombinase sequence.

Animals

All studies involving mice were approved by the Institutional Animal Care and Use Committee of the Maine Medical Center Research Institute, which adheres to all applicable federal laws and rules, and the guidelines and recommendations spelled out in the "Guide for Care and Use of Laboratory Animals."

Oocytes from an FVB female mated with a homozygous male of the B6;129S4-GT(ROSA)26Sor^{tm1Sor1} strain (JAX #003309) (Soriano, 1999) were injected with the construct using standard methodologies. Founder animals were identified and crossed to Gt(ROSA)26Sor^{tm1Sor} mice to analyze Cre recombinase activity by examining β -galactosidase activity in embryonic tissue (E10.5, E13.5 and E17.5), adult organs and after vascular injury. Three month old animals (n=5) were used in the carotid artery flow cessation model as described (Kumar and Lindner, 1997). The carotid arteries, aortae and organs were harvested 4 weeks later for analysis of β -galactosidase activity.

Cre-inducible transgenic mice expressing myc-tagged Cthrc1, Tg(CagGfp-Cthrc1myc)^{11Vli}, were generated (Fig. 1) and bred with Tg(Pdgfrb-Cre)^{35Vli} mice to obtain double transgenic mice. The myc tag allowed for specific detection of the transgene by immunohistochemistry. Three month old animals (n=5) were used in the carotid artery flow cessation model as described (Kumar and Lindner, 1997). The carotid arteries, aortae and organs were harvested 4 weeks later for anti-myc and Pdgfrb immunohistochemistry.

To assess the utility of the ROSA26 promoter driven reporter mice in remodeling arteries more directly, B6; 129S-Gt(ROSA)26Sor mice (JAX # 002073) (Friedrich and Soriano, 1991) with constitutive expression of *LacZ* under ROSA26 promoter control were subjected to carotid artery ligation to induce arterial remodeling. The carotid arteries, aortae and hearts were harvested 4 weeks later for histological analysis of β -galactosidase activity or one week later for analysis of *LacZ* expression.

Histological analysis, immunohistochemistry and morphometry

Frozen sections of carotid arteries, aortae and embryonic tissues (20 μ m thick) were prepared for β -galactosidase activity localization with nuclear Fast Red counterstain as described (Weiler *et al.*, 2001). Immunohistochemistry for Pdgfrb (Epitomics, Inc., #1469-1, 1:250 dilution) and the myc epitope tag were performed with rabbit monoclonal antibodies on serial sections of paraformaldehyde fixed tissues embedded in paraffin following antigen retrieval with heat in citrate buffer pH=6.0 (LeClair *et al.*, 2007). The myc epitope tag specific rabbit monoclonal antibody (clone Vli-1, 10ng/ml dilution) was raised against a synthetic peptide derived from the human c-myc proto-oncogene (EQKLISEEDL). Adjacent sections were also stained without a primary antibody to serve as negative immunohistochemistry controls.

Image analysis was used to quantify β -galactosidase activity on cross-sections of aortae and ligated as well as contralateral carotid arteries as previously described (LeClair *et al.*, 2007). These measurements were also used to assess recombination efficiency. In all cases, recombination efficiency was similar in left and right common carotid arteries from unmanipulated mice. The percentage of medial and neointimal area exhibiting blue β -galactosidase staining in the remodeling artery was determined and expressed as a percentage of the blue area determined for the non-ligated carotid artery. Recombination efficiency for esophageal smooth muscle and skeletal muscle was determined by quantifying

the percentage of muscle tissue showing β -galactosidase activity in tissue sections from four adult mice.

Quantitative real time reverse transcription PCR (qRT-PCR)

Levels of *LacZ* transcript in remodeling and normal carotid arteries from Gt(ROSA)26Sor mice with constitutive expression of *LacZ* under ROSA26 promoter control were compared by isolating RNA from remodeling (left) and normal (right) carotid arteries (n=7). cDNA was prepared by reverse transcription and transcript levels were determined using qRT-PCR with glyceraldehyde-3-phosphate dehydrogenase (*Gapdh*) as a reference gene. qRT-PCR was performed on the Bio-Rad iQ5 cyclor using the iQ-SYBR Green Supermix (Bio-Rad). Primers amplifying *LacZ* (GGTAAACTGGCTCGGATTAGGG and GCTGATGTTGAAGTGAAGTCGC) and *mGAPDH* (ATCACTGCCACCCAGAAGACTG and ATCCACGACGGACACATTGG) were used. A non-template control was included for all qRT-PCR reactions. All samples were run in triplicate. The thermal profile was 95°C for 10 min, 40 cycles of 95°C for 15 sec and 58°C for 1 min. Samples were normalized with respect to *Gapdh* and expression levels in the remodeling carotid arteries were expressed as a fold change relative to the normal carotid arteries using the comparative C_t method ($\Delta\Delta C_t = \Delta C_{t \text{ sample}} - \Delta C_{t \text{ reference}}$).

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Figure 1.

Crossing the Tg(Pdgfrb-Cre)^{35Vli} mice (b) with the Gt(ROSA)26Sor^{tm1Sor} reporter mice (a) allows Cre recombinase to excise the stop sequence between the loxP sites resulting in *LacZ* expression and β-galactosidase activity under the control of the ROSA26 promoter in cells where the Pdgfrb promoter is also active (d). Crossing the Tg(Pdgfrb-Cre)^{35Vli} mice (b) with Tg(CagGfp-Cthrc1-myc)^{11Vli} mice (c) elicits the Cre recombinase protein to remove the GFP cassette flanked by loxP sites. Subsequently, the Cthrc1-myc transgene is expressed under the control of the CAG promoter in cells where the Pdgfrb promoter is active (e). In the absence of Cre recombinase, the Tg(CagGfp-Cthrc1myc)^{11Vli} transgenic mouse line expresses GFP under the control of the CAG promoter. Generation of a myc epitope tag rabbit monoclonal antibody allowed for specific detection of the transgene.

Figure 2. Characterization of the $Tg(Pdgfrb-Cre)^{35Vli}$ strain crossed to the ROSA26 reporter strain, $Gt(ROSA)26Sor^{tm1Sor}$, was performed by detection of β -galactosidase activity (left column). The ability to activate a Cre-inducible transgene was determined by crossing the $Tg(Pdgfrb-Cre)^{35Vli}$ strain with the $Tg(CagGfp-Cthrc1myc)^{11Vli}$ strain and transgene expression was analyzed by anti-myc immunohistochemistry (left center column, immunoreactivity in brown). Endogenous expression of $Pdgfrb$ in serial sections was assessed by anti- $Pdgfrb$ immunohistochemistry (right center column). Negative controls for the antibody stains are also shown on serial sections (right column). In both the ROSA26 and myc-tagged $Cthrc1$ reporter crosses, prior Cre mediated recombination was detected in the areas where endogenous $Pdgfrb$ expression is present: the myocardium (MC), esophageal smooth muscle (ESM), skeletal muscle, fibroblasts of the dermis (DF), mesangial cells of kidney glomeruli (M), and vascular smooth muscle cells (vSMC) of the lung and the brain. All images were taken at the same magnification, scale bar = 50 μ m.

Figure 3.

Embryos were harvested from $Tg(Pdgfrb-Cre)^{35Vli}/Gt(ROSA)26Sor^{tm1Sor}$ crosses at E10.5 (a–d), E13.5 (e–j) and E17.5 (k–n). β -galactosidase activity is depicted in blue (right column) and *Pdgfrb* immunoreactivity is shown in brown (left column). In adjacent sections of E10.5 embryos, β -galactosidase activity and endogenous *Pdgfrb* expression were detected in the developing dermis (a, b) and developing skeletal muscle of the limb (c, d). In adjacent sections of E13.5 embryos, the mesenchymal condensation/perichondrium (MC/P) (e, f), lung tissue (g, h) but not the airway epithelium (A), and the spinal cord/ectoderm (i, j) showed β -galactosidase activity and endogenous *Pdgfrb* expression. Mesangial cells of kidney glomeruli (M) (k, l) and the myocardium (MC) (m, n) showed β -galactosidase activity and endogenous *Pdgfrb* expression in adjacent sections of E17.5 embryos. All images were taken at the same magnification, scale bar = 50 μ m.

Figure 4.

Remodeling carotid arteries of $Tg(Pdgfrb-Cre)^{35Vli}$ mice crossed to the $Gt(ROSA)26Sor^{tm1Sor}$ reporter strain reveal reduction of β -galactosidase activity in dedifferentiated SMC and medial smooth muscle cells (mSMC) (b) when compared to the high β -galactosidase activity of the mSMC in the contralateral artery control (a). Remodeling arteries of $Gt(ROSA)26Sor$ mice with constitutive expression of *LacZ* under ROSA26 promoter control show a similar reduction of β -galactosidase activity (d) compared to the contralateral carotid artery (c) indicating that the reduction of ROSA26 reporter activity in dedifferentiated SMC is independent of Cre recombinase activity. Serial sections of remodeling carotid arteries of $Tg(Pdgfrb-Cre)^{35Vli}$ mice crossed with the $Tg(CagGfp-Cthrc1-myc)^{11Vli}$ Cre-inducible transgenic line reveal transgene activity in the neointimal SMC (h) comparable to endogenous *Pdgfrb* expression (f). The transgene expression in mSMC of the contralateral artery control (g) is also analogous to the endogenous *Pdgfrb* staining of mSMC (e) in serial sections. All images were taken at the same magnification, scale bar = 50 μ m. Medial smooth muscle cells, mSMC; inner elastic lamellae, IEL, indicating the boundary between the mSMC and neointimal lesion; $Tg(Pdgfrb-Cre)^{35Vli} \times Gt(ROSA)26Sor^{tm1Sor}$, Cre35 $\times$ R26R; $Gt(ROSA)26Sor$, ROSALacZ; $Tg(Pdgfrb-Cre)^{35Vli} \times Tg(CagGfp-Cthrc1-myc)^{11Vli}$, Cre35 $\times$ Cag13.

Figure 5.

LacZ transcript levels in remodeling and normal carotid arteries from Gt(ROSA)26Sor mice with constitutive expression of *LacZ* under ROSA26 promoter control were analyzed using qRT-PCR. *LacZ* expression in the remodeling carotid arteries was quantified as a fold-change relative to expression in normal carotid arteries (-21.98 ± 1.56 fold change, $p=0.00003$).

Table 1

Recombination efficiency in skeletal and smooth muscle

	Esophageal Smooth Muscle (% β -gal positive muscle area)	Skeletal Muscle (% β -gal positive muscle area)
Pdgfrb-Cre/R26R #1	100.0	100.0
Pdgfrb-Cre/R26R #2	100.0	100.0
Pdgfrb-Cre/R26R #3	99.0	96.0
Pdgfrb-Cre/R26R #4	99.0	100.0
Average$\pm$SD	99.5 $\pm$ 0.6	99.0 $\pm$ 2.0

To assess recombination efficiency in esophageal smooth muscle and skeletal muscle, the percentage of muscle displaying β -galactosidase staining was quantified in 4 three month old Tg(Pdgfrb-Cre)^{35Vli}/Gt(ROSA)26Sor^{tm1Sor} mice.

Table 2

Quantification of β -galactosidase activity in normal and remodeling carotid arteries.

	Control Carotid Artery (% β -gal positive medial area)	Ligated Carotid Artery (% β -gal positive medial area)	Ligated Carotid Artery (% β -gal positive intimal area)
Pdgfrb-Cre/R26R #1	79.0	3.1	6.9
Pdgfrb-Cre/R26R #2	51.6	23.7	11.0
Pdgfrb-Cre/R26R #3	35.5	1.9	1.9
Pdgfrb-Cre/R26R #4	72.3	5.6	6.3
Pdgfrb-Cre/R26R #5	71.0	3.3	0.2
Average$\pm$SD	61.9 $\pm$ 17.9	7.5 $\pm$ 9.1	5.3 $\pm$ 4.3

The percentage of vessel area displaying β -galactosidase staining was quantified for the media of unmanipulated carotid arteries and separately for the media and intima of the remodeling carotid artery of 5 three month old Tg(Pdgfrb-Cre)^{35Vli}/Gt(ROSA)26Sor^{tm1Sor} mice.