Angiogenesis (2017) 20:655–662
DOI 10.1007/s10456-017-9570-9

BRIEF COMMUNICATION

PDGFRβ-P2A-CreER^{T2} mice: a genetic tool to target pericytes in angiogenesis

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Received: 3 February 2017 / Accepted: 20 July 2017 / Published online: 27 July 2017

Abstract Pericytes are essential mural cells distinguished by their association with small caliber blood vessels and the presence of a basement membrane shared with endothelial cells. Pericyte interaction with the endothelium plays an important role in angiogenesis; however, very few tools are currently available that allow for the targeting of pericytes in mouse models, limiting our ability to understand their biology. We have generated a novel mouse line expressing tamoxifen-inducible Cre-recombinase under the control of the platelet-derived growth factor receptor β promoter: *PDGFRβ-P2A-CreER^{T2}*. We evaluated the expression of the *PDGFRβ-P2A-CreER^{T2}* line by crossing it with fluorescent reporter lines and analyzed reporter signal in the angiogenic retina and brain at different time points after tamoxifen administration. Reporter lines showed labeling of NG2^{+}, desmin^{+}, PDGFRβ^{+} perivascular cells in the retina and the brain, indicating successful targeting of pericytes; however, signal from reporter lines was also observed in a small subset of glial cells both in the retina and the brain. We also evaluated recombination in tumors and found efficient recombination in perivascular cells associated with tumor vasculature. As a proof of principle, we used our newly generated driver to delete Notch signaling in perivascular cells and observed a loss of smooth muscle cells in retinal arteries, consistent with previously published studies evaluating *Notch3* null mice. We conclude that the *PDGFRβ-P2A-CreER^{T2}* line is a powerful new tool to target pericytes and will aid the field in gaining a deeper understanding of the role of these cells in physiological and pathological settings.

Keywords Mural cells · Pericytes · Mouse models · Platelet-derived growth factor β

Introduction

Pericytes comprise a fundamental part of the mural cell populations that is associated with small caliber vessels. They play key roles during angiogenesis in the development and stability of the vasculature, as well as in the

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Electronic supplementary material The online version of this article (doi:10.1007/s10456-017-9570-9) contains supplementary material, which is available to authorized users.

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formation and maintenance of the blood brain barrier (BBB) [1, 2]. Additionally, abnormalities in pericyte function have been linked to pathologies such as diabetic retinopathy [3], fibrosis [4], Alzheimer's disease [5], and stroke [6]. Pericytes are also commonly detected in the tumor microenvironment, where they have been involved in promoting tumor vessel stability [7] and limiting tumor metastasis [8, 9].

To date, limited tools are available to study pericytes partly due to the lack of a unique marker for their identification [1]. Current strategies to identify pericytes rely on their morphology, their proximity to endothelium, and in the best case, assessment of several well-established makers such as NG2, desmin, CD13, or platelet-derived growth factor receptor β (PDGFRβ). Efforts to study pericyte biology using *NG2-CreER* mice [10] have resulted in efficient linage tracing of pericytes in the developing heart [11]. However, the use of this mouse line is limited in brain tissue, where NG2 is also expressed in oligodendrocyte progenitor cells [10]. PDGFRβ has been suggested as a useful marker for brain pericytes [1]. The use of constitutively active *PDGFRβ-Cre* mice [12] [13] results in efficient targeting of the mural cell populations (pericytes and smooth muscle cells). However, these drivers also target additional progenitor populations that give rise to non-mural linages [12] and, more importantly, blood and lymphatic endothelium, raising concern over their use in vascular studies [14].

In order to study the functions of pericytes during postnatal angiogenesis, and to bypass the confounding effects that constitutive *PDGFRβ-Cre* drivers may have on unrelated progenitor cells during development, we generated a tamoxifen-inducible *PDGFRβ-P2A-CreER*^{T2} mouse driver. Here, we present the characterization of this driver in different angiogenic postnatal vascular beds.

Materials and methods

Mice

All mice received humane care following the guidelines of the National Institute of Health's *Guide for the Care and Use of Laboratory Animals*. Additionally, all animal experiments were approved by the Institution of Animal Care and Use Committee (IACUC) at Columbia University (New York) or the Animal Care Committee (ACC) at the University of Illinois at Chicago.

Gt(ROSA)26Sor$^{tm4(ACTB-tdTomato,-EGFP)Luo}$ (*Rosa-mT/mG*) [15] and Gt(ROSA)26Sor$^{tm9(CAG-tdTomato)Hze}$ (*Rosa-tdTomato*) [16] were obtained from Jackson Laboratories. *PDGFRβ-P2A-Cre*^{T2} mice (recently deposited at The Jackson Laboratory as Stock No. 030201) were generated by replacing the translation stop codon (TAG) of the *Pdgfrb* gene on RP23-106H12 BAC clone with a 4445-bp P2A-CreERT2-FNF cassette by BAC recombineering. A DNA fragment containing 2 kb upstream of P2A-CreERT2-FNF cassette insertion and a 4.8-kb sequence downstream of the P2A-CreERT2-FNF cassette were inserted into plasmid pMCS-DTA (a generous gift from Dr. Kosuke Yusa of Osaka University, Japan) to generate the Pdgfrb-P2A-CreERT2-FNF-gene-targeting vector, pMCS-Pdgfrb-P2A-CreERT2-FNF-DTA. This targeting vector was linearized and electroporated into KV1 (129-C57BL/6 hybrid) ES cells, and targeted ES clones were injected into C57BL/6N blastocysts to generate male chimeras. Male chimeras were bred to ACTB-Flpe females (Jackson Lab Stock No: 005703) to transmit the Pdgfrb-P2A-CreERT2 allele and to remove the neo cassette at the same time (Fig. 1). Primers used to verify the successful removal of the neo cassete: P1: ctctctctgcctccctcagctat, P2: acggacagaagcattttccaggtat, P3: ttgatatcgaattcccgaagttcct, P4: cctccccacctctcctctagtttta.

Cre-recombinase activity was induced by delivery of a solution of Tamoxifen (Sigma) in Corn Oil (Sigma). For analysis of brain and retina at postnatal (P) day 5, tamoxifen was delivered through maternal milk by administering 250 μg/kg to the nursing mom at P1, P2, and P3.

For experiments involving a time-course activation of Cre-recombinase, 250 μg/kg was administered to the nursing mom in a single dose at P0, and analysis of the retina was performed at P2, P5 and P14.

For experiments involving tumor implantation, and adult time-course activation of Cre, tamoxifen was injected at 6 weeks intraperitoneally 2 mg/mouse/day during 5 consecutive days.

Tumor cell growth and implantation

Lewis lung carcinoma (LLC) cell line was obtained from the American Type Culture Collection (ATCC) and cultured in Dulbecco's modified Eagle medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 100 u/ml of penicillin–streptomycin. 5×10^5 LLC cells were injected subcutaneously in the lower flank of 8-week mice (2 weeks after tamoxifen injection).

Immunofluorescence

After euthanasia, organs were harvested and fixed in a 4% formaldehyde solution in phosphate-buffered saline (PBS).

For retinal staining, eyes were fixed for 1–2 h at 4 °C and then transferred to PBS. Retinas were then dissected and permeabilized in 1% bovine serum albumin (BSA), 0.5% Triton X-100 in PBS overnight (O/N) at 4 °C, and washed with PBLEC buffer (1% Triton X-100, 0.1 mmol/L MgCl2, 0.1 mmol/L CaCl2, 0.1 mmol/L MnCl2 in PBS

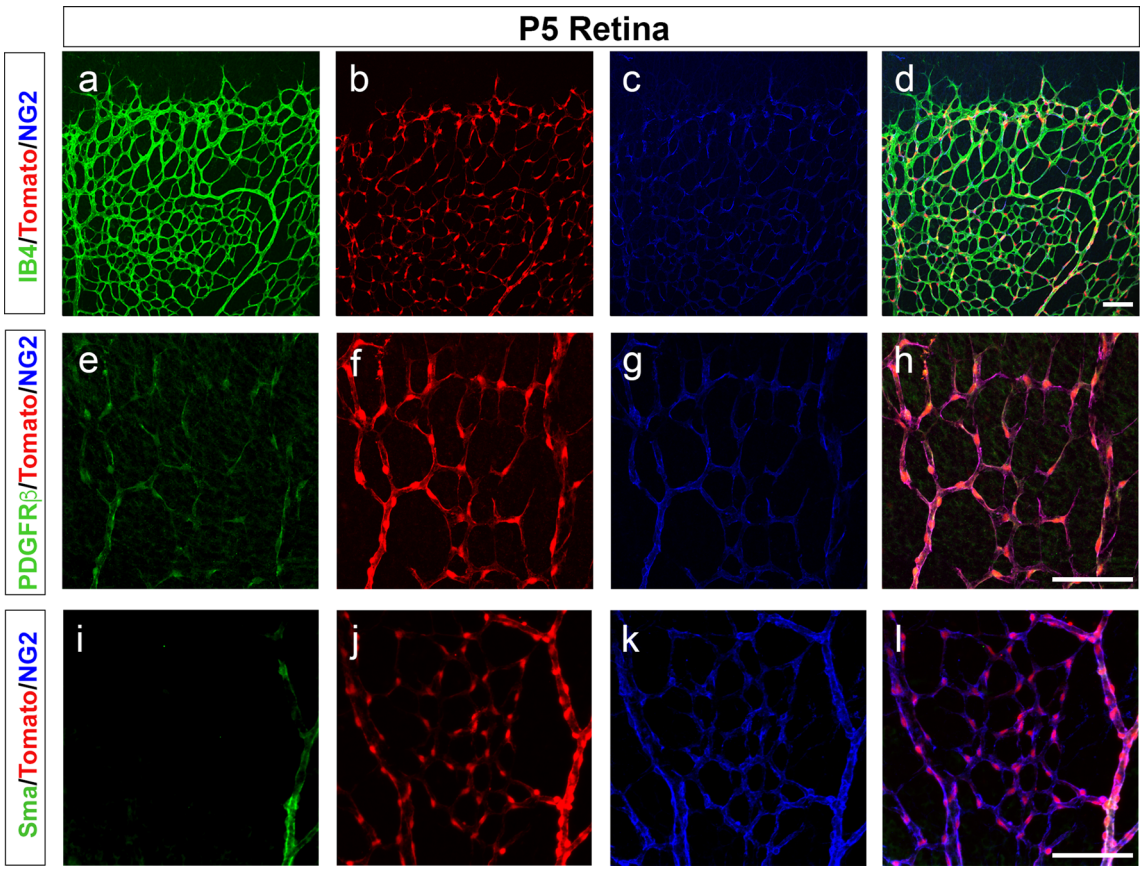

Fig. 1 Retinal characterization of tamoxifen-treated *PDGFRβ-P2A-CreER^{T2}*; *Rosa-tdTomato* mice. Whole-mount retinas from P5 mice. **a–d** Low magnification of the retinal vascular plexus stained in *green* with Isolectin B4 (IB4; **a**), and anti-NG2 (*blue*; **c**) shows expression of the tdTomato reporter (**b**; *red*) co-localizing with NG2 + cells lining the endothelium (**d**). **e–h** Higher magnification of the retinal vascular plexus stained in *green* with anti-PDGFRβ (**e**) and NG2 (*blue*; **g**) shows co-localization of the tdTomato reporter in *red* (**f**) with the NG2 and PDGFRβ (**h**). **i–l** Higher magnification of the retinal vascular plexus stained with anti-smooth muscle cell actin (Sma) in *green* to label smooth muscle cells (**i**) and anti-NG2 (*blue*; **k**) shows the expression of the tdTomato reporter (**j**) in *red* in all the vascular segments, arteries (A), capillaries (C), and veins (V). *White scale bar* represents 100 μm

pH6.8). The following primary antibodies/lectin were used O/N at 4 °C in PBLEC: biotin-conjugated isolectin B4 (Vector Laboratories) 1:50, anti-NG2 (Millipore) 1:500, anti-desmin (AbCam) 1:500, anti-PDGFRβ (Cell Signaling or R&D Systems) 1:100, anti-smooth muscle actin (Sma) 1:500 (Sigma), and FITC-conjugated anti-Sma (Sigma) 1:200. Of note, anti-Sma antibodies target the protein encoded by the *Acta2* gene. After washes, detection of primary antibodies/lectin was performed with streptavidin-conjugated Alexa Fluor 488 or Alexa 647 (Invitrogen) 1:500 and Alexa Fluor 488-, 594-, or 647-conjugated secondary antibodies (Invitrogen) 1:700 for 2 h at room temperature (R.T.). Samples were post-fixed with 4% formaldehyde and flat-mounted.

For brain and tumor, samples were fixed at 4 °C O/N and then immersed in 30% sucrose solution for 24 h. Samples were then embedded in Tissue-Tek OCT compound (Takura). Immunostaining was performed O/N at 4 °C in cryosections using the following primary antibodies/lectin: biotin-conjugated Isolectin B4 (Vector Laboratories) 1:50, anti-NG2 (Millipore) 1:500, anti-desmin (AbCam) 1:500, anti-PDGFRβ (Cell Signaling or R&D Systems) 1:100, anti-Endomucin (Santa Cruz) 1:200, and the following secondary reagents: streptavidin-conjugated Alexa Fluor 647 (Invitrogen) 1:500 and Alexa Fluor 488-, 594-, or 647-conjugated secondary antibodies (Invitrogen) 1:700 for 1 h at R.T.

All samples were mounted using Vectashield with DAPI (Vector Laboratories). Images were acquired using Zeiss LSM 700, 880 or Nikon A1RMP confocal microscope and analyzed and processed using FiJi.

Results and discussion

In order to generate the *PDGFRβ-P2A-CreER^{T2}* mouse line, we inserted a sequence derived from the porcine teschovirus-1 P2A gene that allows "ribosome skipping"

[17] between the *Pdgfrb* and *CreER^{T2}* coding sequences (Online Resource 1). Thus, the *PDGFRβ-P2A-CreER^{T2}* sequence in the construct allows for production of both PDGFRβ and CreER^{T2} at similar expression levels via ribosome skipping. This approach has been found to be more efficient at producing comparable levels of two functional proteins from one mRNA than use of an internal ribosomal entry (IRES) site [17].

To analyze the efficiency of tamoxifen-mediated induction and the expression pattern of Cre-recombinase in *PDGFRβ-P2A-CreER^{T2}* mice, we crossed it with two different Cre-recombinase mouse reporter lines: *Rosa-mT/mG* [15] and *Rosa-tdTomato* [16]. The *Rosa-mT/mG* reporter mice are engineered to express membrane-targeted tdTomato fluorescent protein in the absence of Cre-recombinase; however, when Cre-recombinase is present, the tdTomato expression cassette is excised allowing for the expression of membrane-targeted eGFP (enhanced green fluorescent protein). *Rosa-tdTomato* reporter mice drive tdTomato expression upon Cre-mediated excision of a floxed STOP cassette [16].

To determine whether *PDGFRβ-P2A-CreER^{T2}* targets recombination to pericytes, we analyzed the postnatal retina, a well-characterized active angiogenesis system that permits the study of mural cells in different vascular compartments: newly formed capillary plexus, mature arteries, and veins. We crossed *PDGFRβ-P2A-CreER^{T2}* to *Rosa-tdTomato* mice and administered tamoxifen by oral gavage to the nursing mom at postnatal (P) day 1, 2, and 3. At P5, we evaluated the expression of tdTomato protein in the retina by whole-mount analysis. Retinas were immunostained with isolectin-B4 (IB4) to identify blood vessels, and with NG2, a marker expressed in pericytes. Expression of the tdTomato reporter was observed associated with the NG2-expressing cells lining the vascular network (Fig. 1a–d) with an efficiency of recombination in these cells of $84.17 \pm 3.48\%$ ($n = 3$). We sought to determine whether the tdTomato reporter signal was observed in cells expressing PDGFRβ, since Cre expression should be driven by the *Pdgfrb* promoter. Immunostaining against PDGFRβ revealed that the distribution pattern of this receptor was associated with NG2$^+$ pericytes in a similar fashion to that of the tdTomato reporter protein (Fig. 1e–h), indicating a genuine expression of Cre-recombinase in PDGFRβ$^+$ cells. As expected considering the previously described pattern of expression of PDGFRβ [18], fluorescent signal from the tdTomato reporter was present in the different vascular segments of the retina: arterioles (identified by Sma), capillaries, and venules (Fig. 1i–l). Of note, we also observed additional weak expression of the reporter in a small subset of retinal glial cells (data not shown). Taken together, these results indicate faithful expression of the *PDGFRβ-P2A-CreER^{T2}* construct and efficient recombination upon tamoxifen induction in PDGFRβ$^+$ cells in the postnatal retina.

In parallel, we also crossed *PDGFRβ-P2A-CreER^{T2}* with a different reporter line, *Rosa-mT/mG*. As described above, we administered tamoxifen by oral gavage to the nursing mom at P1, P2, and P3, and analyzed the retinal vasculature at P5. Similar to the results observed with the previous reporter, staining with anti-PDGFRβ showed that eGFP expression from the *Rosa-mT/mG* reporter was associated with PDGFRβ$^+$ cells (Online Resource 2a-d), further validating expression of Cre-Recombinase in PDGFRβ$^+$ cells. Additionally, as observed with the tdTomato reporter, eGFP signal from the *Rosa-mT/mG* reporter was detected in the perivascular cells in arterioles, capillaries, and venules (Online Resource 2e-h). When retinas were stained with anti-NG2 to label pericytes, we observed a clear co-localization of eGFP with NG2$^+$ cells indicating a successful targeting of pericytes (Online Resource 2i-p). However, we observed that efficiency of recombination in pericytes using this reporter was less than that observed using the tdTomato reporter, in this case $41.94 \pm 18.67\%$ ($n = 5$) of NG2$^+$ cells were GFP$^+$. Discrepancies between recombination efficiency in different mouse reporter lines (including *Rosa-tdTomato* and *Rosa-mT/mG*) have already been described by Liu et al. [19], and the differences we observed in efficiency are similar to what has been previously noted. Liu et al. proposed that their results imply that reporter sensitivity inversely correlates with the distance between the LoxP sites, a theory that is consistent with previous observations by Coppoolse et al. [20].

To further our understanding of the *PDGFRβ-P2A-CreER^{T2}* mouse line, we performed a time-course activation of the Cre-Recombinase. We treated *PDGFRβ-P2A-CreER^{T2}*; *Rosa-tdTomato* moms with a single dose of tamoxifen on the day of birth (P0) and analyzed the retinal vasculature of the pups at different times 48 h (P2), 5 days (P5), and 2 weeks (P14) after tamoxifen delivery. Expression analysis of the tdTomato reporter in NG2$^+$ cells in P2 mice revealed that recombination was already highly efficient at this early time point (Fig. 2a–d). Similarly, analysis at 5 days and 2 weeks after tamoxifen administration showed widespread labeling of perivascular cells (NG2$^+$) in the arterioles, capillaries, and venules (Fig. 2e–l). The observed efficient recombination of the *PDGFRβ-P2A-CreER^{T2}* mouse line at the analyzed time points makes it a very attractive tool to study the role of perivascular cells at the different stages of angiogenesis: P2, when the primary vascular plexus is starting to form; P5, when the vascular plexus is actively remodeling into arterioles and venules; and P14, when the deeper plexuses of the retina are developing [21].

We also evaluated the expression of the *PDGFRβ-P2A-CreER^{T2}* mouse line in the retina of adult mice. Six-week-

old *PDGFRβ-P2A-CreER*^{T2}; *Rosa-tdTomato* mice were treated with tamoxifen intraperitoneally for five consecutive days, and the retinal vasculature was evaluated at 8 weeks (2 weeks later). TdTomato signal from the reporter was detected in the perivascular cells of the retina (NG2^{+}), but also in additional cells present in the retina (Fig. 2m–p). Similar to P5 retinas, 8-week-old mice also showed expression of the tdTomato reporter in smooth muscle cells in the retina and in bigger caliber vessels (data not shown). Detection of the reporter in smooth muscle cells was expected, as smooth muscle cells have been described to express PDGFRβ; however, this lack of

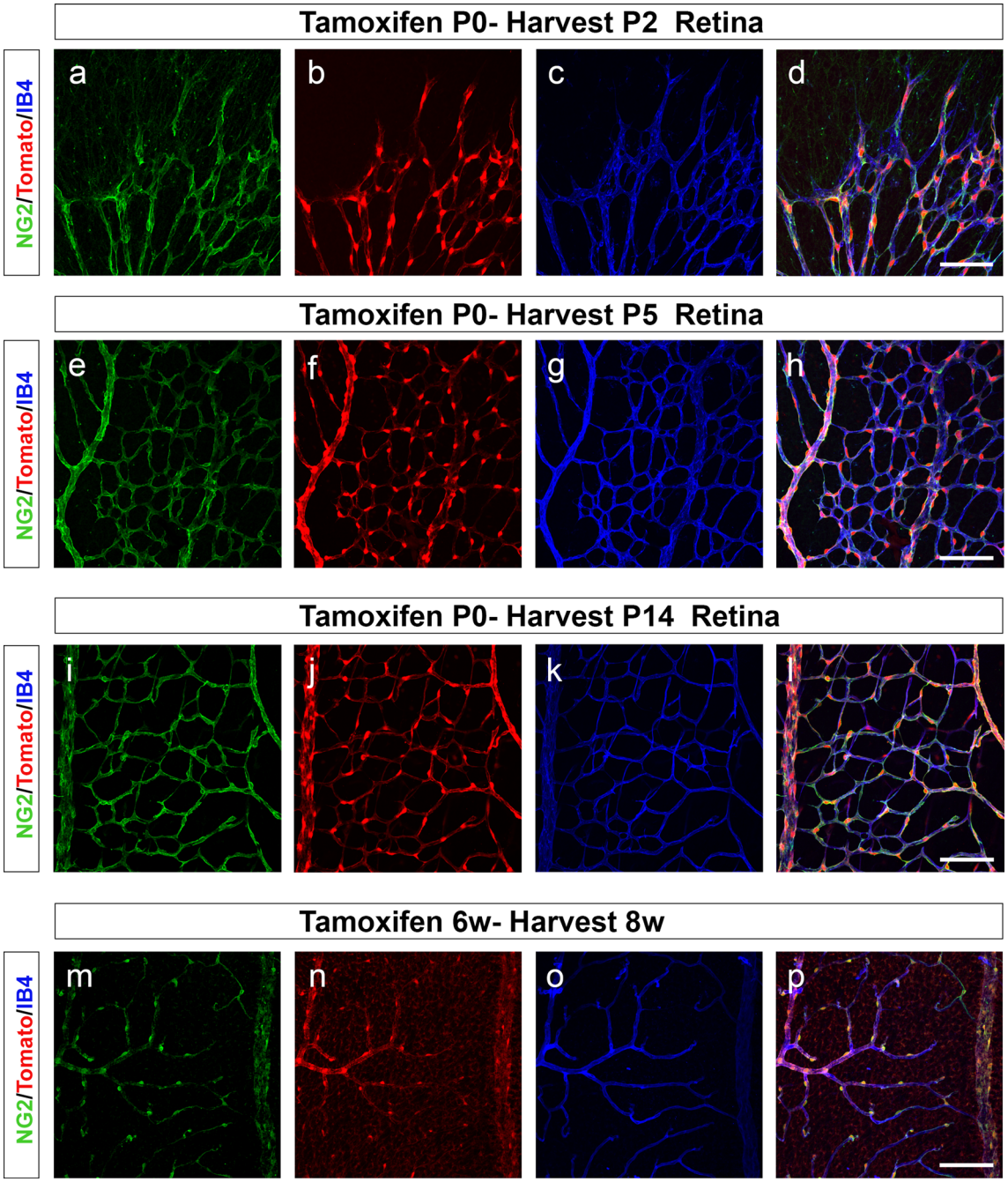

Fig. 2 Time course of recombination of tamoxifen-treated *PDGFRβ-P2A-CreER*^{T2}; *Rosa-tdTomato* mice. **a–l** Whole-mount retinas from mice treated with tamoxifen at birth (P0) and analyzed at P2 (**a–d**), P5 (**e–h**), and P14 (**i–l**); stained in *green* with anti-NG2, and in *blue* with Isolectin B4 (IB4) show expression of the tdTomato reporter (*red*) co-localizing with NG2+ cells lining the endothelium. **m–p** Whole-mount retina from mice treated with tamoxifen at 6 weeks and analyzed at 8 weeks stained in *green* with anti-NG2 (**m**), and in *blue* with IB4 (**o**) shows expression of the tdTomato reporter (**n**; *red*) co-localizing with NG2+ cells lining the endothelium (**p**). *White scale bar* represents 100 μm

pericyte specificity is a potential limitation of the model since the PDGFRβ promoter is active in both pericytes and smooth muscle cells. These results indicate that, while the *PDGFRβ-P2A-CreER^{T2}* mouse line is very efficient at targeting perivascular cells also in the adult retina, the expression of this Cre-driver in other populations should be taken into account when using this line in experimental settings.

Considering the importance that pericytes have in the brain vasculature, we characterized the pattern of expression of *PDGFRβ-P2A-CreER^{T2}* in the murine brain. Brain sections from tamoxifen-induced *PDGFRβ-P2A-CreER^{T2}*; *Rosa-tdTomato* mice at P5 showed high expression of the tdTomato reporter in NG2$^+$, PDGFRβ$^+$ cells lining the blood vessels in the cortex at P5 (Fig. 3a–d), indicative of Cre expression in pericytes. Since NG2 at this time point is also expressed by other brain populations, we further confirmed the pericytic nature of the perivascular tdTomato$^+$ cells by co-staining with anti-desmin, which also showed co-localization with the reporter (Fig. 3e–h). Of note, in addition to the tdTomato reporter expression in perivascular cells, we observed scattered signal, though weaker, in other populations in the brain (data not shown).

We also analyzed P5 brain sections from tamoxifen-induced *PDGFRβCreER^{T2}* mice crossed with the *Rosa-mT/mG* reporter. We observed perivascular expression of the eGFP protein and co-localization with anti-desmin staining (Online resource 3) similar to that observed with the tdTomato reporter. Consistent with the differential reporter efficiency observed in the retina, lower efficiency of recombination was also observed in the brain with the *Rosa-mT/mG* reporter when compared to *Rosa-tdTomato.* Taken together, our overall analysis of the reporter expression in the brain from reporter mice crossed with *PDGFRβ-P2A-CreERT2* indicates successful targeting of the brain pericytes.

Tumor angiogenesis remains one of the most clinically relevant angiogenic processes, and pericytes are a critical component of the tumor microenvironment. Similar to developmental angiogenesis, pericytes are recruited by tumor vessels during tumor growth and angiogenesis [22]. We therefore interrogated whether we could target the pericyte populations in tumors using *PDGFRβ-P2A-CreER^{T2}* mice. For this purpose, 6-week *PDGFRβ-P2A-CreER^{T2}*; *Rosa-tdTomato* adult mice were treated with tamoxifen, and 2 weeks later Lewis lung carcinoma (LLC) tumors were grown subcutaneously in the lower left flank. After dissection, we evaluated tumor sections by immunostaining with anti-Endomucin to label the tumor endothelium. Our results revealed tdTomato reporter signal in perivascular populations lining Endomucin$^+$ tumor vessels (Fig. 4), indicating an efficient recombination of pericytes in this tumor model. However, while our data using LLC tumors points to successful targeting of tumor pericytes utilizing our novel *PDGFRβ-P2A-CreER^{T2}* mouse line, there is a considerable degree of heterogeneity in the pericyte coverage and marker expression in different tumor types [1, 23], and hence further studies might be needed to determine the specificity of the *PDGFRβ-P2A-CreER^{T2}* mouse line in alternative tumor models.

As a proof of principle that the *PDGFRβ-P2A-CreER^{T2}* mouse line is an efficient tool to drive genetic modifications in perivascular cells, we used it to specifically remove Notch signaling in this population. Notch signaling plays an important role in mural cells, which primarily express the Notch3 receptor [1]. Studies evaluating *Notch3* null mice revealed decreased smooth muscle cell coverage of the arteries likely due to an abnormal maturation of these cells [24, 25]. For our studies, we crossed *PDGFRβ-P2A-CreER^{T2}* mice with *Rbpj$^{flox/flox}$* mice. Rbpj is a transcription factor acting downstream of Notch and is critical to mediate its signaling [26]. Tamoxifen was given to the nursing moms at P1, P2, P3, and we evaluated the retina of *PDGFRβ-P2A-CreER^{T2}*; *Rbpj$^{flox/flox}$* mice and control littermates (*PDGFRβ-P2A-CreER^{T2}; Rbpj$^{flox/wt}$*) at 6 weeks of age. As expected, we detected a marked lack of Sma$^+$ cells in the arterioles of *PDGFRβ-P2A-CreER^{T2}*; *Rbpj$^{flox/flox}$* when compared to *PDGFRβ-P2A-CreER^{T2}*; *Rbpj$^{flox/wt}$* littermates (Online Resource 4). These results show how inducing the loss of Notch signaling in perivascular cells using the *PDGFRβ-P2A-CreER^{T2}* mouse line results in a similar phenotype (reduced presence of Sma$^+$ cells) to that observed in the global *Notch3* null mice, and further validate the use of the *PDGFRβ-P2A-CreER^{T2}* to efficiently target specific genes in perivascular cells.

In summary, we have generated a novel transgenic mouse line expressing Cre-recombinase in a tamoxifen-dependent fashion under the control of the PDGFRβ promoter. Our results comparing two different reporter lines underscore the importance of validating the excision of the floxed gene of interest, since efficiency may vary with different targets. Nevertheless, we have shown how the *PDGFRβ-P2A-CreER^{T2}* line provides a powerful tool to target pericytes in developmental and pathological settings. Additionally, successful tamoxifen-mediated Cre induction was observed in early postnatal and also in adult mice, highlighting the versatility of this mouse line for its use in multiple vascular angiogenic environments.

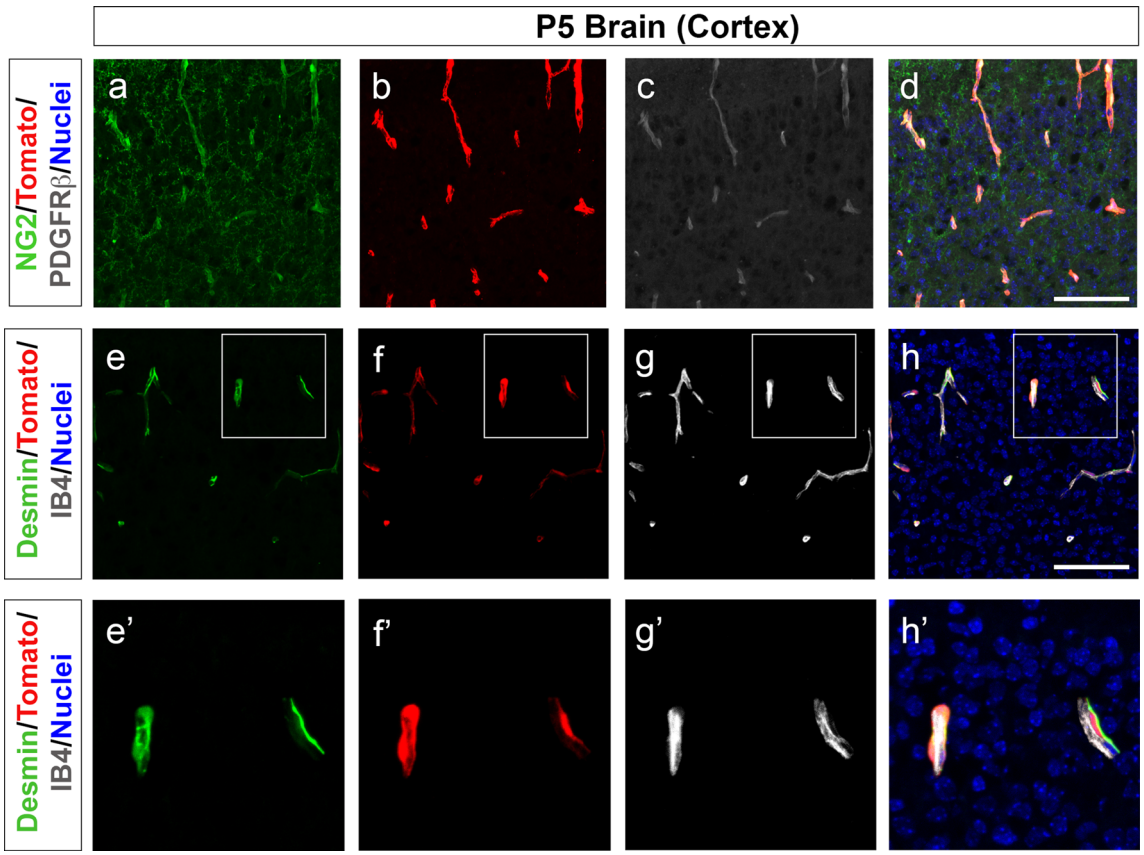

Fig. 3 Reporter expression in brain from tamoxifen-treated *PDGFRβ-P2A-CreER*^{T2}; *Rosa-tdTomato* mice. Images of the cortex from brain sections from P5 mice. **a–d** Anti-NG2 staining (*green*) labels pericytes and other glial cells (**a**), tdTomato reporter expression is shown in *red* (**b**), and anti-PDGFRβ in *white* (**c**). Merge image **d** shows co-localization of anti-NG2, tdTomato reporter and anti-PDGFRβ together with Nuclei staining (DAPI) in *blue*. **e–h** Anti-Desmin (*green*) staining labels pericytes (**e**), tdTomato reporter expression is shown in *red* (**f**), and IB4 is used to label endothelial cells in *white* (**g**). Merge image **h** shows co-localization of anti-Desmin, tdTomato reporter and IB4 together with Nuclei staining (DAPI) in *blue*. *White scale bar* represents 100 μm

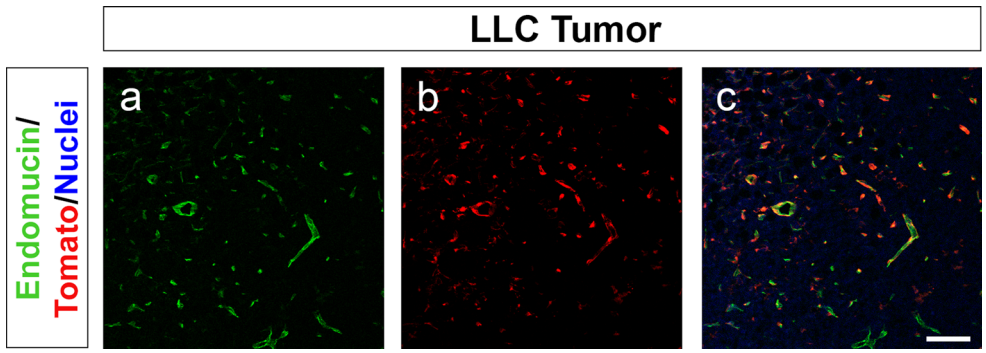

Fig. 4 Characterization of LLC tumors implanted in tamoxifen-treated *PDGFRβ-P2A-CreER*^{T2}; *Rosa-tdTomato* mice. Images from sections of Lewis Lung Carcinoma (LLC) implanted tumors. **a–c** Tumor sections were stained with anti-Endomucin (*green*), to visualize the endothelial cells (**a**). tdTomato reporter expression in *red* (**b**), and Merge (**c**) image of anti-Endomucin and tdTomato reporter together with Nuclei staining (DAPI) in *blue*. *White scale bar* represents 100 μm

Acknowledgements We would like to thank Valeriya Borisenko for her assistance in mouse husbandry, and Hui-Chuan Hung for her assistance in the microinjection to generate the germline chimera and mouse breeding. This work was funded by NIH Grant R01HL112626 (J.K.), and NCI P30CA013696-40 (C.S.L).

Author contributions' C-SL, ML, and JK conceptualized and designed the *PDGFRβ-P2A-CreER*^{T2} mice. ML generated the PDGFRβ-P2A-CreER^{T2} targeting vector. FL performed the gene targeting and identified the targeted ES cells for the generation of the *PDGFRβ-P2A-CreER*^{T2} mice. HC, BP, and TN collected and

interpreted the data on the characterization of the *PDGFRβ-P2A-CreER^{T2}* with the two reporter mouse lines. HC drafted the manuscript.

Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

Human and animal rights This article does not contain any studies with human participants performed by any of the authors. All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. All procedures performed in studies involving animals were in accordance with the ethical standards of the institution or practice at which the studies were conducted as described above.

References

1. Armulik A, Genove G, Betsholtz C (2011) Pericytes: developmental, physiological, and pathological perspectives, problems, and promises. Dev Cell 21(2):193–215. doi:10.1016/j.devcel.2011.07.001
2. Winkler EA, Bell RD, Zlokovic BV (2011) Central nervous system pericytes in health and disease. Nat Neurosci 14(11):1398–1405. doi:10.1038/nn.2946
3. Hammes HP, Lin J, Renner O, Shani M, Lundqvist A, Betsholtz C, Brownlee M, Deutsch U (2002) Pericytes and the pathogenesis of diabetic retinopathy. Diabetes 51(10):3107–3112
4. Lin SL, Kisseleva T, Brenner DA, Duffield JS (2008) Pericytes and perivascular fibroblasts are the primary source of collagen-producing cells in obstructive fibrosis of the kidney. Am J Pathol 173(6):1617–1627. doi:10.2353/ajpath.2008.080433
5. Sagare AP, Bell RD, Zhao Z, Ma Q, Winkler EA, Ramanathan A, Zlokovic BV (2013) Pericyte loss influences Alzheimer-like neurodegeneration in mice. Nat Commun 4:2932. doi:10.1038/ncomms3932
6. Liu S, Agalliu D, Yu C, Fisher M (2012) The role of pericytes in blood-brain barrier function and stroke. Curr Pharm Des 18(25):3653–3662
7. Erber R, Thurnher A, Katsen AD, Groth G, Kerger H, Hammes HP, Menger MD, Ullrich A, Vajkoczy P (2004) Combined inhibition of VEGF and PDGF signaling enforces tumor vessel regression by interfering with pericyte-mediated endothelial cell survival mechanisms. FASEB J 18(2):338–340. doi:10.1096/fj.03-0271fje
8. Keskin D, Kim J, Cooke VG, Wu CC, Sugimoto H, Gu C, De Palma M, Kalluri R, LeBleu VS (2015) Targeting vascular pericytes in hypoxic tumors increases lung metastasis via angiopoietin-2. Cell Rep 10(7):1066–1081. doi:10.1016/j.celrep.2015.01.035
9. Xian X, Hakansson J, Stahlberg A, Lindblom P, Betsholtz C, Gerhardt H, Semb H (2006) Pericytes limit tumor cell metastasis. J Clin Invest 116(3):642–651. doi:10.1172/JCI25705
10. Zhu X, Hill RA, Dietrich D, Komitova M, Suzuki R, Nishiyama A (2011) Age-dependent fate and lineage restriction of single NG2 cells. Development 138(4):745–753. doi:10.1242/dev.047951
11. Volz KS, Jacobs AH, Chen HI, Poduri A, McKay AS, Riordan DP, Kofler N, Kitajewski J, Weissman I, Red-Horse K (2015) Pericytes are progenitors for coronary artery smooth muscle. Elife. doi:10.7554/eLife.10036
12. Cuttler AS, LeClair RJ, Stohn JP, Wang Q, Sorenson CM, Liaw L, Lindner V (2011) Characterization of Pdgfrb-Cre transgenic mice reveals reduction of ROSA26 reporter activity in remodeling arteries. Genesis 49(8):673–680. doi:10.1002/dvg.20769
13. Foo SS, Turner CJ, Adams S, Compagni A, Aubyn D, Kogata N, Lindblom P, Shani M, Zicha D, Adams RH (2006) Ephrin-B2 controls cell motility and adhesion during blood-vessel-wall assembly. Cell 124(1):161–173. doi:10.1016/j.cell.2005.10.034
14. Ulvmar MH, Martinez-Corral I, Stanczuk L, Makinen T (2016) Pdgfrb-Cre targets lymphatic endothelial cells of both venous and non-venous origins. Genesis 54(6):350–358. doi:10.1002/dvg.22939
15. Muzumdar MD, Tasic B, Miyamichi K, Li L, Luo L (2007) A global double-fluorescent Cre reporter mouse. Genesis 45(9):593–605. doi:10.1002/dvg.20335
16. Madisen L, Zwingman TA, Sunkin SM, Oh SW, Zariwala HA, Gu H, Ng LL, Palmiter RD, Hawrylycz MJ, Jones AR, Lein ES, Zeng H (2010) A robust and high-throughput Cre reporting and characterization system for the whole mouse brain. Nat Neurosci 13(1):133–140. doi:10.1038/nn.2467
17. Kim JH, Lee SR, Li LH, Park HJ, Park JH, Lee KY, Kim MK, Shin BA, Choi SY (2011) High cleavage efficiency of a 2A peptide derived from porcine teschovirus-1 in human cell lines, zebrafish and mice. PLoS ONE 6(4):e18556. doi:10.1371/journal.pone.0018556
18. Powner MB, Vevis K, McKenzie JA, Gandhi P, Jadeja S, Fruttiger M (2012) Visualization of gene expression in whole mouse retina by in situ hybridization. Nat Protoc 7(6):1086–1096. doi:10.1038/nprot.2012.050
19. Liu J, Willet SG, Bankaitis ED, Xu Y, Wright CV, Gu G (2013) Non-parallel recombination limits Cre-LoxP-based reporters as precise indicators of conditional genetic manipulation. Genesis 51(6):436–442. doi:10.1002/dvg.22384
20. Coppoolse ER, de Vroomen MJ, van Gennip F, Hersmus BJ, van Haaren MJ (2005) Size does matter: cre-mediated somatic deletion efficiency depends on the distance between the target lox-sites. Plant Mol Biol 58(5):687–698. doi:10.1007/s11103-005-7705-7
21. Fruttiger M (2007) Development of the retinal vasculature. Angiogenesis 10(2):77–88. doi:10.1007/s10456-007-9065-1
22. Abramsson A, Berlin O, Papayan H, Paulin D, Shani M, Betsholtz C (2002) Analysis of mural cell recruitment to tumor vessels. Circulation 105(1):112–117
23. Eberhard A, Kahlert S, Goede V, Hemmerlein B, Plate KH, Augustin HG (2000) Heterogeneity of angiogenesis and blood vessel maturation in human tumors: implications for antiangiogenic tumor therapies. Cancer Res 60(5):1388–1393
24. Domenga V, Fardoux P, Lacombe P, Monet M, Maciazek J, Krebs LT, Klonjkowski B, Berrou E, Mericskay M, Li Z, Tournier-Lasserve E, Gridley T, Joutel A (2004) Notch3 is required for arterial identity and maturation of vascular smooth muscle cells. Genes Dev 18(22):2730–2735. doi:10.1101/gad.308904
25. Liu H, Zhang W, Kennard S, Caldwell RB, Lilly B (2010) Notch3 is critical for proper angiogenesis and mural cell investment. Circ Res 107(7):860–870. doi:10.1161/CIRCRESAHA.110.218271
26. Guruharsha KG, Kankel MW, Artavanis-Tsakonas S (2012) The Notch signalling system: recent insights into the complexity of a conserved pathway. Nat Rev Genet 13(9):654–666. doi:10.1038/nrg3272