Sonic hedgehog is required for vascular outgrowth in the hindbrain choroid plexus

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Critical to the exchange and metabolic functions served by tissues like brain choroid plexi and lung is the coherent development of an epithelial sheet of large surface area in tight apposition to an extensive vascular bed. Here, we present functional experiments in the mouse demonstrating that Sonic hedgehog (Shh) produced by hindbrain choroid plexus epithelium induces the extensive vascular outgrowths and vascular surface area fundamental to choroid plexus functions, but does not induce the more specialized endothelial cell features of fenestrations and bore size. Our findings indicate that these Shh-dependent vascular elaborations occur even in the presence of Vegf and other established angiogenic factors, suggesting either that the levels of these factors are inadequate in the absence of Shh or that a different set of factors may be more essential to choroid plexus outgrowth. Transducing the Shh signal is a perivascular cell—the pericyte—rather than the more integral vascular endothelial cell itself. Moreover, our findings suggest that hindbrain choroid plexus endotheleal cells, as compared to other vascular endothelial cells, are more dependent upon pericytes for instruction. Thus, in addition to Shh acting on the progenitor pool for choroid plexus epithelial cells, as previously shown, it also acts on choroid plexus pericytes, and together serves the important role of coordinating the development of two disparate yet functionally dependent structures—the choroid plexus vasculature and its ensheathing epithelium.

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Introduction

Tight apposition of a highly metabolic epithelium and an intricate vasculature is essential for the functioning of many vital tissues. Examples include the brain choroid plexi, which are ventricular organs specialized for producing and detoxifying cerebrospinal fluid (CSF); the lung, for blood cell gas exchange; and retinal pigmented epithelium with its underlying choriocapillaris for nourishing retinal cells. In each case, the epithelium and vasculature maximize and compact a large surface area for exchange by assuming convolutions in which each case, the epithelium and vasculature maximize and compact a large surface area for exchange by assuming convolutions in which epithelial villi surround each of numerous capillary plexi (Figs. 1A–E). Organ function, in these examples, demands that constituent epithelium and vasculature develop coordinately so that expansion of the epithelium occurs in concert with, and can properly ensheathe and communicate with, the growing vasculature. Adding complexity is that distinct cell lineages separately contribute the epithelial and vascular components; thus, separate progenitor cell populations must be coordinated. Underlying mechanisms are just beginning to be understood.

Evident through studies of lung development is that epithelial and vascular elaborations occur in register so that the two tissue surface areas keep pace and functionally couple to enable respiration (Galambos et al., 2002; Jakkula et al., 2000; Loscertales et al., 2008; McGrath-Morrow et al., 2005; Schwarz et al., 2004; Warburton et al., 2000). Mechanisms enacted by the lung to regulate this interdependent development involve Shh, fibroblast growth factor (Fgf)-9, and vascular endothelial growth factor (Vegf)-A, each secreted by lung epithelium and promoting vascular development adjacentlly (White et al., 2007). Factors from this adjacent lung mesenchyme then commensurately provide local expansion signals back to the mitotic epithelium to expand in situ and ensheathe the growing vasculature (del Moral et al., 2006; van Tuyl et al., 2007, Yamamoto et al., 2007). Much less is known about mechanisms coordinating cell lineages during choroid plexus development. It is clear that a highly branched and extensive vascular bed, comprised of endothelial cells harboring fenestrations for high permeability, must be tightly ensheathed by an epithelium—the choroid plexus epithelium (CPe)—that is capable of high-rate CSF production (Figs. 1A–E). Thus the production of vascular endothelial cells and CPe must be tightly coordinated. Here the choroid plexus is particularly challenged because CPe cells, being nonmitotic, are not equipped to proliferate in situ in response to signals from the underlying and growing vasculature (Hunter and Dymecki, 2007; Knudsen, 1964). Instead of proliferating locally at sites of vascular expansion, new CPe cells must be produced by a progenitor zone situated at a relative distance—that region of dorsal hindbrain neuroectoderm (Fig. 1D) demarcated by expression of the transcription factor Lmx1a and the secreted factor Gdf7 (Awatramani et al., 2003; Chizhikov et al., 2006; Huang et al., 2008; Jakkula et al., 2000; Loscertales et al., 2008; McGrath-Morrow et al., 2005; Schwarz et al., 2004; Warburton et al., 2000). Mechanisms enacted by the lung to regulate this interdependent development involve Shh, fibroblast growth factor (Fgf)-9, and vascular endothelial growth factor (Vegf)-A, each secreted by lung epithelium and promoting vascular development adjacentlly (White et al., 2007). Factors from this adjacent lung mesenchyme then commensurately provide local expansion signals back to the mitotic epithelium to expand in situ and ensheathe the growing vasculature (del Moral et al., 2006; van Tuyl et al., 2007, Yamamoto et al., 2007). Much less is known about mechanisms coordinating cell lineages during choroid plexus development. It is clear that a highly branched and extensive vascular bed, comprised of endothelial cells harboring fenestrations for high permeability, must be tightly ensheathed by an epithelium—the choroid plexus epithelium (CPe)—that is capable of high-rate CSF production (Figs. 1A–E). Thus the production of vascular endothelial cells and CPe must be tightly coordinated. Here the choroid plexus is particularly challenged because CPe cells, being nonmitotic, are not equipped to proliferate in situ in response to signals from the underlying and growing vasculature (Hunter and Dymecki, 2007; Knudsen, 1964). Instead of proliferating locally at sites of vascular expansion, new CPe cells must be produced by a progenitor zone situated at a relative distance—that region of dorsal hindbrain neuroectoderm (Fig. 1D) demarcated by expression of the transcription factor Lmx1a and the secreted factor Gdf7 (Awatramani et al., 2003; Chizhikov et al., 2006; Huang et al., 2008).
Understanding how vascular endothelial cells and CPe progenitors are regulated to achieve coordinated development is a burgeoning field and our focus here.

During embryogenesis, hindbrain CPe expresses the Shh gene (Awatramani et al., 2003; Bitgood and McMahon, 1995; Huang et al., 2009; Lewis et al., 2004), first in patches associated with CPe that has begun to differentiate into an invaginating cuboidal epithelium (Fig. 1F). Two topographically separate hindbrain territories have been proposed to harbor cells that respond to this CPe-produced Shh and which act to coordinate choroid plexus development. One territory is the choroid plexus mesenchyme (CPm) lying immediately underneath the Shh-expressing CPe (Tannahill et al., 2005); the other is the more distant CPe progenitor pool (Huang et al., 2009). Here we examine the former (largely uncharacterized) territory, first by identifying which cell type within the CPm responds directly to Shh, and second by determining the consequences to this mesenchyme following CPe-specific deletion of Shh.

Collectively, our work demonstrates that in the hindbrain, CPe-produced Shh induces the extensive vascular outgrowths and vascular surface area fundamental to choroid plexus functions but not the more specialized vascular features of fenestrations and bore size. Our findings also suggest that Shh-dependent vascular outgrowths may either require angiogenic factors other than Vegf and the Angiopoietins or that the levels of these angiogenic factors are inadequate to induce or sustain these vascular outgrowths in the absence of Shh. Transducing the Shh signal appears to be the pericyte, a perivascular cell, rather than the more integral vascular endothelial cell itself. Given that endothelial cells in other tissues are capable of transducing Hh signals (Passman et al., 2008; Vokes et al., 2004), our findings suggest that CPm endothelial cells reside in a category more dependent upon pericytes for instruction. Thus, Shh appears to coordinate the development of vascular (CPm) and epithelial (CPe) cell lineages of the hindbrain choroid plexus in part through regulating actions of the pericyte. Identification of the pericyte as the Hh-transducing cell type within the CPm expands the current model of hindbrain choroid plexus development. By delineating the specific Hh-responsive cell populations in the dorsal hindbrain, this work opens an avenue by which to delineate their relative contributions during choroid plexus development.

Materials and methods

Mouse strains include: Shhcre-gfp (Harfe et al., 2004); R26R (Soriano, 1999); Ptc1lacZ (Goodrich et al., 1997); Tie2-cre (Kisanuki et al., 2001); RC::PFwe (Farago et al., 2006); RC::ePE (Seri and Dymecki, unpublished; cre-responsive eGFP indicator); SmoM2-YFPlox (Jeong et al., 2004);
**Histological staining** involved fixing tissue (4% paraformaldehyde) and processing for paraffin embedding. 6 µm sections were collected and stained using hematoxylin and eosin (Bancroft and Stevens, 1990).

**mRNA in situ hybridization (ISH) and βgal detection**

Antisense riboprobes against Shh, Ihh, Dhh, Ptc1, Gli1, Msx1 (C. Tabin); Ttr (C. Walsh; IMAGE ID10782224); and Otx2 (C. Cepko) were employed. Tissue was processed for ISH (14 µm sections) as described (Hunter et al., 2005). For βgal detection, tissue was prepared as above but fixed in 2% paraformaldehyde. Xgal on 14–30 µm sections was performed as described (Rodriguez and Dymecki, 2000).

**Immunodetection**

Fixed tissue was blocked in 0.5% goat or donkey serum/0.1%Triton X-100/PBS, incubated overnight at 4 °C with: rabbit anti-Otx2 (Chemicon, 1:1000); rabbit anti-Maß (Bethyl, 1:500); rabbit anti-NG2 (W. Stallcup, Burnham Institute, 1:1000); chicken anti-βgal (Abcam, 1:4000); rabbit anti-AFP (Invitrogen, 1:4000); and goat anti-βgal (Serotec, 1:2000). Fresh-frozen tissue was post-fixed in 1% paraformaldehyde, RT for mouse anti-Ki-67 (BD Pharmingen, 1:500) or (Serotec, 1:2000). Fresh-frozen tissue was post-fixed in 1% paraformaldehyde, 80 µm, were cut using a Reichert Ultracut-S microtome, and stained using hematoxylin and eosin (Bancroft and Stevens, 1990). Alternate sections were imaged; every cell in the field counted. Student’s paired t-test performed on mean; error bars—standard deviation.

(g) Hh transcription factor Maß (Kreisler) (Cordes and Barsh, 1994) heterozygotes (Fig. 1K) showed no detectable Maß+ cells in forebrain or midbrain CPe (Figs. S1M, N) suggests that Hh signaling in choroid plexus is restricted to the hindbrain. The main CPM constituents are endothelial cells and pericytes; scarce dendritic cells and poorly described mesenchymal cells. From this, we utilized E12–E14.5 hindbrain CPM in subsequent studies.

To determine which CPM cell type responds directly to Shh, we assayed for expression of the Patched1 (Ptc1) gene, a transcriptional target of and therefore read-out for Hh signaling (McMahon et al., 2000). Detection of Ptc1 mRNA in hindbrain tissue (Fig. 1) or βgal activity in Ptc1lacZ (Goodrich et al., 1997) heterozygotes (Fig. 1K) confirmed an abundant Shh-responsive population in the hindbrain CPM beginning at E12 (Fig. 2A). By contrast, the endothelial cell marker Pecam1/CD31 appeared to mark a population separate from both the Maß+ and βgal+ populations (Fig. 2C). Similarly, in triple transgenic Tie2-cre:RC::ePE (Cre-responsive GFP reporter):Ptc1lacZ animals, endothelial cells (Tie2-cre-expressing cells) marked by GFP appeared distinct from Ptc1lacZ, expressing βgal+ cells (Fig. 2D). These findings suggest that Ptc1-expressing cells in hindbrain CPM are pericytes. Indeed, this was further supported by electron microscopy (EM) observations using CPM from Ptc1lacZ heterozygotes. Pericytes are readily identifiable (Soriano, 1994) and distinguishable from other stromal and vascular elements by EM, and Xgal precipitate, for example indicative of βgal activity in hindbrain CPM but not detectable in any CPe, whether in the hindbrain, lateral or third ventricles (Figs. S1A–F). Others report Shh transcripts persisting in the hindbrain CPE through E18.5 (Huang et al., 2009); however, we were unable to detect Shh mRNA in CPE beyond E15 (Fig. 1). Imaging onset at E12 in hindbrain CPE was further corroborated by βgal expression in double transgenic ShhlacZ-βgal (Harfe et al., 2004); R26R (Soriano, 1999) mice (Fig. S1I); as expected, expression was not seen in forebrain or midbrain CPE (Figs. S1K–L). Given this, we utilized E12–E14.5 hindbrain CPM in subsequent studies.

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**Results and discussion**

Pericytes but not endothelial cells in the hindbrain CPM express Ptc1, a transcriptional target of the Hedgehog pathway

Toward determining which CPM cell type responds directly to Shh, we established the most suitable temporal window for investigation by re-examining Hh expression in the dorsal hindbrain. We confirmed Shh expression in hindbrain CPe from E12 to E14.5 (Figs. 1F–H). HH family members Desert hedgehog and Indian hedgehog were not detectable in any CPe, whether in the hindbrain, lateral or third ventricles (Figs. S1A–F). Others report Shh transcripts persisting in the hindbrain CPE through E18.5 (Huang et al., 2009); however, we were unable to detect Shh mRNA in CPE beyond E15 (Fig. 1). Imaging onset at E12 in hindbrain CPE was further corroborated by βgal expression in double transgenic ShhlacZ-βgal (Harfe et al., 2004); R26R (Soriano, 1999) mice (Fig. S1I); as expected, expression was not seen in forebrain or midbrain CPE (Figs. S1K–L). Given this, we utilized E12–E14.5 hindbrain CPM in subsequent studies.
transient population that is no longer present by ∼E14.5 (when most if not all βgal+ cells appear NG2+).

Removal of Shh from hindbrain CPe results in a rudimentary choroid plexus with stunted vasculature

To determine the consequences to CPm following selective loss of CPe-produced Shh, we characterized embryos and postnatal mice (Gdf7<sup>cre</sup>:Shh<sup>fl/fl</sup>/Shh<sup>fl/fl</sup>) animals, here referred to as conditional knockout (CKO) animals that lacked functional Shh alleles specifically in the CPe lineage. To attain specificity in this lineage, we used Gdf7<sup>cre</sup> (Lee et al., 2000), because the Gdf7 gene is transcribed by CPe progenitors and mature CPe cells and is thus a suitable driver (Currie et al., 2005; Landsberg et al., 2005; Hunter and Dymecki, 2007). CKO embryos were devoid of CPe-produced Shh mRNA at all stages (Fig. S2), indicating efficient Cre-mediated removal of the floxed Shh gene sequences (Dassule et al., 2000; Lewis et al., 2001) when partnered with Gdf7<sup>cre</sup>. By contrast, Shh transcripts were readily detectable in all non-Gdf7-expressing territories such as hindbrain floor plate (Fig. S2), verifying the specificity of Shh<sup>fl/fl</sup> deletion. Commensurate with loss of CPe-produced Shh was loss of Ptc1 transcripts from CPM of CKO animals (Fig. S2), further indicating effective loss of CPe-produced Shh. This loss of Ptc1 transcripts also served to identify CPe as the critical source of Shh for the CPm, as opposed to the floor plate or ventral midbrain/isthmus territories. Loss of Shh and Ptc1 transcripts were confirmed by reverse transcriptase (RT)-PCR using cDNA generated from harvested choroid plexi (Fig. S2).

CKO animals harbored a stunted, rudimentary hindbrain choroid plexus as compared to control littermates (Figs. 3A–D). The voluminous folds and villus outgrowths characteristic of wild-type choroid plexi were severely reduced. The CPm vasculature was correspondingly attenuated. Transcardial perfusion with fluorescein-labeled Griffonia-Bandeiraea simplicifolia lectin-1 (BSL-1)—a reagent that stably labels the luminal surface of vascular endothelial cells—allowed for visualization of CPm vasculature en masse. The CPm vascular branches extending from the central vascular stalk were greatly reduced in length in CKO tissue as compared to controls (Figs. 3E, F). Vessel bore size, though, appeared unaffected (data not shown)—a parameter worth investigating given that exogenous Shh induces neovascularization of wider bore than controls (Polà et al., 2001). Another feature of CPm vasculature is capillary fenestrations (Dohrmann, 1970)—thin diaphragms within capillary walls that permit enhanced transfer kinetics between blood and CPe. Vascular fenestrations were indeed identified in electron micrographs of adult CKO tissue (Fig. S3), although features such as fenestration size and distribution may be mildly aberrant. At E14.5, neither control nor CKO choroid plexi yet showed fenestrations (data not shown). Together these findings indicate that acquisition of fenestrations is a later developmental event largely independent of CPe-produced Shh.

While a rudimentary structure, the CKO choroid plexus harbored the major constituent cell types, including vascular endothelial cells, pericytes, and CPe cells (Figs. 3C, D, G, H). Lateral and third ventricle choroid plexi from CKO animals appeared indistinguishable from controls (data not shown), as was predicted given that Shh is not normally expressed in these structures (Figs. S1G, H).

Because Shh activity can influence cell proliferation and survival (Agarwala et al., 2001; Echelard et al., 1993; Garcia-Lecea et al., 2008; Litingtung et al., 1998), we explored the proliferative capacity of CPm cells and cell death following loss of CPe-produced Shh. CKO CPm harbored ∼45% fewer cells overall (number of DAPI-positive nuclei in the CPm; data not shown) with a similar reduction in the number of proliferative cells as measured by the cell cycle antigen Ki-67 (Fig. 3I, left); however, the percentage of Ki-67+/DAPI+ cells was unaffected (Fig. 3I, right). Immunodetection of cleaved caspase-3 for apoptotic cells revealed no differences between CKO and control choroid plexi; nor did TUNEL assays (data not shown). H&E stained tissue revealed no evidence of necrotic cell death.

Distinctions of choroid plexus endothelial cells

Our findings suggest a model in which CPe-produced Shh, signaling directly to CPm pericytes, induces CPm cell proliferation and vascular outgrowth, the latter by way of endothelial cell proliferation and/or cell shape remodeling. Given that endothelial cells in other organ systems have been reported to undergo angiogenesis in direct response to Shh (Passman et al., 2008; Vokes et al., 2004), we considered whether hindbrain CPm endothelial cells had such competence, notwithstanding their lack of Ptc1 expression under physiological conditions (Fig. 2). Lack of such competence would characterize further the endothelial cell subtype present in the CPm and would further support a model in which pericytes are the critical Shh-responder. We expressed in endothelial cells the constitutively active form of Smoothened (SmoM2 (Jeong et al., 2004; Xie et al., 1998))—Smo being a cell-autonomous mediator of Hh signaling. The Tie2·cre:SmoM2·YFP<sup>fl/fl</sup> genotype was lethal embryonically (∼E16.5) with various defects and vascular abnormalities (Figs. 3N, O). The hindbrain choroid plexus, however, appeared indistinguishable morphologically from single transgenic littermate controls

Fig. 2. Pericytes, and not endothelial cells, respond directly to CPe-produced Shh. (A–B) Co-localization of βgal (Ptc1<sup>cre</sup>) and membrane-localized NG2, a pericyte marker, in E12.5 and E14.5 CPm. (C–D) Endothelial cells and Ptc1<sup>cre</sup> cells represent separate CPm cell populations. (E) Xgal precipitate in Ptc1<sup>cre</sup> CPm as visualized by EM. Schematized in (F); endothelial cell, EC; red blood cell, RBC. (G–H) Co-immunolocalization of βgal (Ptc1<sup>cre</sup>) and MafB but not of endothelial cells and MafB.
— no aberrant CPM vascular outgrowths were observed in Tie2-cre;SmoM2-YFPlox/lox animals. Tie2 driven Cre activity was robust and specific to endothelial cells as indicated by SmoM2-YFP colocalization with Pecam1 (Figs. 3L, M). Collectively, these findings are consistent with CPM endothelial cells lacking the inherent competence to respond via proliferation to Hh signaling under conditions of...
intrinsic constitutive Smo activity, and thus argue further that Shh responsiveness in the CPm is a job reserved for the pericyte, not the endothelial cell. Moreover these findings distinguish CPm endothelial cells from endothelial cells elsewhere in the embryo, perhaps placing CPm endothelial cells in a category more dependent upon instruction from pericytes.

Given the attenuation of CPm vasculature in CKO animals, and that pro-angiogenic factors, including Vegf and Angiopoietin-1 (Ang-1), have been proposed to lie downstream of Shh during certain angiogenic events (Lawson et al., 2002; Nagase et al., 2005; Pola et al., 2001), we investigated angiogenic gene expression. Using VegflacZ heterozygotes (Miquerol et al., 1999), we first confirmed that the Vegf gene is expressed in CPm cells (Fig. 3P). Next we analyzed ~E14.5 CKO animals carrying the VegflacZ allele and found that animals deficient in CPm-produced Shh expressed lacZ from the Vegf locus (Fig. 3Q)—there were of course fewer CPm cells but those present exhibited ßgal activity. This indicates a Shh-independent component of Vegf expression. Moreover, should there exist a Shh-dependent component, it would be indirect given that CPm cells (the source of Vegf) do not themselves respond directly to Shh (they are negative for Ptc1 mRNA. Transcripts for the three predominant Vegf isoforms, as well as the angiogenic genes Ang-1, Ang-2, Tie-1, Tie-2, and VE-cadherin were present in both CKO and wild-type choroid plexi (Fig. 3R). Thus, expression of these pro-angiogenic genes by the hindbrain choroid plexus, at least to some detectable level, does not require CPm-produced Shh. Further supporting Shh-independent expression is the finding that in straight VegflacZ heterozygotes, ßgal activity was observed in incipient hindbrain CPm cells at ~E11.5, prior to the onset of Shh production by CPm, and through E17.5, by which time Shh production by the CPm is diminished (Figs. S4A, B). Because CPm vascular defects associated with Shh loss occur despite expression of this gene cohort with the angiogenic properties of their encoded products, we propose either that critical expression levels of this gene cohort are indeed Shh-dependent and required for vascular outgrowth or that other factors may be essential to drive these Shh-dependent vascular elaborations. For example, it is possible that these specific angiogenic genes require Shh for augmented (not absolute) expression and subsequent angiogenic effects in the hindbrain choroid plexus.

**Coordinated attenuation: less vasculature expansion, fewer CPm cells produced**

Along with the reduction in vasculature and mesenchymal constituents in the CKO choroid plexus, we found a commensurate reduction in hindbrain CPm cells (Fig. 4A), validating recent findings (Huang et al., 2009) that show a reduced rate of CPm production by the Lmx1a-portion of the rhombic lip following loss of CPm-produced Shh. While indeed reduced in number, we found that CKO CPm cells were otherwise indistinguishable morphologically from controls with respect to cell size, shape, and packing density (Figs. 4B, C). CKO CPm cells did, however, exhibit some abnormalities; for example, a reduction in detectable Msx1 transcripts was observed (Figs. 4D, E). Because Msx1 is frequently a transcriptional target of signaling triggered by bone morphogenetic proteins (Bmps), this finding raises the possibility that Bmp signaling in the CPm is defective in the absence of CPm-produced Shh. While Bmp4 expression has been reported as unaffected following loss of CPm-produced Shh (Huang et al., 2009), it is possible that other signaling events or other Bmp-interacting factors themselves are affected in the CKO choroid plexus leading to compromised Msx gene expression in the CPm. This would likely be a secondary effect given that mature CPm cells themselves do not appear to respond directly to Shh (Fig. 1; Tannahill et al., 2005; Huang et al., 2009). Future experiments will investigate a link between Hh and Bmp signaling during hindbrain choroid plexus development.

Collectively, this work demonstrates that in the hindbrain, CPm-produced Shh induces the extensive vascular outgrowths and surface area fundamental to choroid plexus functions, but does not induce the more specialized vascular features like fenestrations and bore size. Further, our findings suggest that the Shh-dependent vascular outgrowths likely require tightly-regulated levels of angiogenic genes and/or require angiogenic factors other than Vegf and Angiopoietins. We have reported, for the first time, the pericyte as the cell type transducing Hh signaling in the CPm—this follows on a recent report defining a novel Hh-responsive CPm progenitor domain continuous with the hindbrain rhombic lip (Huang et al., 2009). Together, these data highlight Hh signaling as a mechanism by which epithelial (CPm) and vascular endothelial (CPm) cell lineages are coordinately regulated in the developing hindbrain choroid plexus. As modeled in Fig. 5A, Shh produced by the hindbrain CPm cells signals directly (solid blue arrows) to pericytes within the CPm (this report) and to CPm progenitor cells of the rhombic lip (white asterisk; Huang et al., 2009). Subsequently, Hh signaling may invoke unknown factors from pericytes and indirectly.
and have implications for other tissues in which epithelial functions, in general, and choroid plexus development, in particular, pericytes. These epithelial cells reside in a category more dependent upon neighboring endothelial cells in other tissues and thus suggests that CPm endo-transducing the Hh signal. This contrasts the apparent capabilities of have shown that CPm endothelial cells do not appear capable of and CPe cell production, versus that via CPe progenitors. Further, we does Shh signaling via pericytes make toward vascular outgrowth lineages (pericyte versus CPe progenitor domain) during choroid plexus development in the absence of CPe-produced Shh, vascular outgrowth is stunted and CPe cell production is diminished in parallel.

(dashed green arrows) affect angiogenesis, production of CPe cells by the rhombic lip, and/or properties of mature CPe cells. Also possible is that Hh signaling via the CPe progenitor domain indirectly affects these aspects of hindbrain choroid plexus development (dashed gray arrows). In the absence of CPe-produced Shh (Fig. 5B), Hh signaling via the pericyte and CPe progenitor pool is blocked, vascular outgrowth is stunted, and CPe cell production by the rhombic lip is diminished. With the identification of the CPe pericyte as the second Hh-responsive population within the dorsal hindbrain (the first being the CPe progenitor domain of the rhombic lip) (Huang et al., 2009), and as pericyte-specific driver lines become available, it will be informative to disrupt Hh signaling in pericytes and to delineate the contributions of the separate cell lineages (pericyte versus CPe progenitor domain) during choroid plexus development—in other words, what relative contribution does Shh signaling via pericytes make toward vascular outgrowth and CPe cell production, versus that via CPe progenitors. Further, we have shown that CPM endothelial cells do not appear capable of transducing the Hh signal. This contrasts the apparent capabilities of endothelial cells in other tissues and thus suggests that CPM endothelial cells reside in a category more dependent upon neighboring pericytes. These findings expand our understanding of pericyte functions, in general, and choroid plexus development, in particular, and have implications for other tissues in which epithelial–vascular co-development is essential. Evolutionary significance is also suggested because choroid plexus expansion provides a capacity to meet greater brain metabolic demands through enhanced production and detoxification of CSF (and the growth factors, hormones, and metabolites it contains), possibly an important permissive step for nervous system enlargement and gains in circuit complexities.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ydbio.2010.01.032.

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