Emerging roles for CNS fibroblasts in health, injury and disease

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Abstract | Recent transcriptomic, histological and functional studies have begun to shine light on the fibroblasts present in the meninges, choroid plexus and perivascular spaces of the brain and spinal cord. Although the origins and functions of CNS fibroblasts are still being described, it is clear that they represent a distinct cell population, or populations, that have likely been confused with other cell types on the basis of the expression of overlapping cellular markers. Recent work has revealed that fibroblasts play crucial roles in fibrotic scar formation in the CNS after injury and inflammation, which have also been attributed to other perivascular cell types such as pericytes and vascular smooth muscle cells. In this Review, we describe the current knowledge of the location and identity of CNS perivascular cell types, with a particular focus on CNS fibroblasts, including their origin, subtypes, roles in health and disease, and future areas for study.

With the advent of single-cell sequencing technologies, it has become increasingly recognized that fibroblasts are present in perivascular spaces, meninges, and choroid plexus of the brain and spinal cord1–3. Recent studies have revealed greater detail about the roles that these fibroblasts play in health and disease in the CNS. For example, in the meninges, fibroblasts secrete cytokines that are important for immune regulation and antigen recognition1. In addition, meningeal and perivascular fibroblasts are the main drivers of fibrotic scarring after neuroinflammation and ablating proliferating fibroblasts to reduce scarring leads to decreases in motor disability4.

Despite these recent advances, we still know very little about the roles of CNS fibroblasts in the development and maintenance of the healthy, adult CNS and how they contribute to disease aside from fibrotic scarring. While we focus mostly on rodent studies in this Review, perivascular fibroblasts have been detected in human tumour samples5 and associated with the vasculature of the human brain6, confirming that these cells are indeed present in the human CNS. Furthermore, perivascular fibroblasts have been shown to be dysfunctional in patients with amyotrophic lateral sclerosis (ALS)6. More in-depth analyses of human CNS fibroblasts will determine whether these cells are potential therapeutic targets for neurological diseases.

In this Review, we summarize what is known about fibroblasts in the CNS and discuss the gaps in our knowledge regarding their subtypes and functions. First, we compare the localizations and markers for many CNS perivascular cell types, including fibroblasts, as these cells are often mistaken for each other owing to a lack of specific molecular markers. Second, we provide an overview of the localization of CNS fibroblasts in perivascular spaces, meninges, and choroid plexus and discuss evidence regarding the developmental origins of fibroblasts in each of these regions. Third, we summarize the known roles of CNS fibroblasts in health and disease with a focus on reticular networks and fibrotic scarring. While perivascular and meningeal fibroblasts have been shown to be the main drivers of fibrotic scarring after experimental autoimmune encephalomyelitis (EAE; a model of the neuroinflammatory disease multiple sclerosis (MS)), rigorous experiments are needed to establish the involvement of these cells in other conditions with fibrotic scarring such as stroke and spinal cord injury (SCI). Finally, we discuss the main unanswered questions in CNS fibroblast biology and how future research studies can enhance our understanding of these important cells.

Localization of fibroblasts in the CNS

Perivascular spaces

Perivascular spaces in the CNS are home to various cell types that have important roles in communicating between the periphery and the brain and spinal cord parenchyma. These fluid-filled spaces are continuous with the subarachnoid space of the meninges, allowing for fluid transfer with the meninges, and are also a major route of solute clearance from the CNS2–11. Perivascular spaces are bordered by the vascular basement membrane produced by endothelial cells and mural cells and the glial basement membrane produced by astrocyte endfeet. In capillaries, these membranes are fused to form...
a composite basement membrane. In penetrating arterioles, precapillary arterioles, postcapillary venules and venules, the glial and vascular basement membranes are separated by a fluid-filled perivascular space also referred to as the Virchow–Robin space. Perivascular cells, which include mural cells (pericytes and vascular smooth muscle cells (vSMCs)), perivascular macrophages and perivascular fibroblasts, have been implicated in many different processes in the CNS, including fibrotic scarring. However, the lack of good markers to distinguish between these cell types has made it difficult to examine the specific roles of each cell type. Below, we describe the locations, functions and molecular markers for each of these cell types (TABLES 1, 2).

Pericytes are located within the endothelial basement membrane of capillaries and extend finger-like processes that incompletely cover the endothelial tube (FIG. 1a). Commonly used markers of CNS pericytes include PDGFRβ and chondroitin sulfate proteoglycan NG2 (encoded by CSPG4), but neither marker is specific to pericytes as they are also expressed by fibroblasts and oligodendrocyte progenitor cells, respectively, and at lower levels in vSMCs. Many of the proposed functions of pericytes have been determined in studies that relied on these markers (specifically PDGFRβ) for their identification. Consequently, many conclusions drawn from these studies may not be specific to pericytes. Sequencing studies have identified additional potential pericyte markers, including KCNJ8 and ABCC9, although these markers are rarely used to identify pericytes.

vSMCs form continuous sheets around the walls of arteries, arterioles, and veins and have a major role in controlling blood flow of these larger vessels (FIG. 1a). Like other perivascular cell types, vSMCs express PDGFRβ, aSMA, CD13, NG2, CD146 and desmin, although they show higher expression of aSMA and desmin but lower expression of NG2 (REF. 17) and PDGFRβ than pericytes.

Perivascular macrophages (also known as border- associated macrophages) are part of the immune surveillance system in the brain and lie adjacent to parenchymal blood vessels. Like other perivascular cell types, vSMCs express PDGFRβ, aSMA, CD13, NG2, CD146 and desmin, although these markers, along with their perivascular location, are used to distinguish these cells from microglia.

Perivascular fibroblasts in the CNS are found immediately adjacent to vSMCs on arteries, arterioles, and veins and are morphologically distinct from other perivascular cell types as they have flattened somata and ruffled, sheet-like membrane processes. Fibroblasts throughout the body (including those in the CNS) express type I collagen, a polymer of COL1A1 and COL1A2 proteins (encoded by COL1A1 and COL1A2, respectively), which is commonly used in sequencing and histological studies as a molecular marker of these cells. A mouse Coll1a1-GFP strain has been used to visualize fibroblasts, including perivascular fibroblasts, meningeal fibroblasts and choroid plexus fibroblasts (FIG. 1b). The ER-TR7 antibody is often used to label reticular fibroblasts and their adjoining extracellular network, although the molecular identity of this antigen is unknown. All of these tools can be used to mark CNS fibroblasts in the perivascular spaces, meninges and choroid plexus. Perivascular fibroblasts in the CNS express both PDGFRα and PDGFRβ and it is likely that they have been confused with other PDGFRβ+ cells such as pericytes and vSMCs.

### Meningeal fibroblasts form the distinct layers of the meninges

CNS fibroblasts are found throughout the three meningeal layers: the pia mater, arachnoid mater (collectively known as the leptomeninges) and dura mater. The dura mater is the strong outer layer of the meninges, consists primarily of collagen fibres and contains fenestrated blood vessels that connect this meningeal layer with the periphery. Fibroblasts and collagen fibres attach the outer layer of the dura to the skull and a thin layer of fibroblasts (dural border cells) separates the dura from the arachnoid. Below the dura is the arachnoid barrier layer, which consists of an outer layer of epithelial-like cells connected by tight junctions. The arachnoid barrier layer is part of the blood–cerebrospinal fluid (CSF) barrier and functional studies using horseradish peroxidase show that this layer prevents the free movement of molecules from the dura into the subarachnoid space that lies beneath the arachnoid. The subarachnoid space contains a vascular network and immune cells and is traversed by arachnoid trabeculae that are formed by inner arachnoid fibroblasts and collagen, which connect this layer to the pia. In the pia, a thin layer of fibroblasts and basement membrane separate the pia and the glia limitans, the layer between the parenchyma and meninges that is formed by astrocyte endfoot processes. The blood vessels in the leptomeninges are non-fenestrated, barrier vessels and are surrounded by a vSMC layer and an adventitial layer of fibroblasts that have been referred to as ‘pial’ or ‘leptomeningeal’ cells. The visualization of perivascular fibroblast morphology by two-photon live imaging in Col1a1-GFP mice revealed that perivascular fibroblasts along cerebral and leptomeningeal vessels have an essentially identical cell morphology, suggesting that the perivascular fibroblasts in these two regions are likely the same cell type. However, it is currently unclear whether perivascular fibroblasts in the meninges or those associated with parenchymal vessels are a molecularly distinct cell type from fibroblasts in the pia or arachnoid layers.

| Table 1 | Histological markers of perivascular cell types and meningeal and choroid plexus fibroblasts |
|---------|---------------------------------------------------------------|
| Cell types | Markers | Refs |
| Pericytes | PDGFRβ, NG2, desmin, KCNJ8, ABCC9 and CD13 | 1,7-17 |
| Vascular smooth muscle cells | PDGFRβ, aSMA, CD13, NG2, CD146 and desmin | 1,17 |
| Macrophages | CD163, CD206, LYVE1 and F4/80 | 21,119 |
| Fibroblasts | PDGFRα, PDGFRβ, COL1A1, ERTR7, LAMA1, CD13 and FN | 1,2,4,7,14,26,28,29 |
| Dural fibroblasts | FXD5, FOXP1 and SIX1 | 27 |
| Arachnoid fibroblasts | CRABP2, ALDH1A2 and SLC6A13 | 27 |
| Pial fibroblasts | S100A6 and NGFR | 27 |
Table 2 | Transgenic mice for lineage tracing of perivascular cell types and meningeal and choroid plexus fibroblasts

| Transgenic strain | Labelled cell types | Refs |
|-------------------|---------------------|------|
| Col1a2–CreER<sup>2</sup> | Fibroblasts and a small population (<5%) of pericytes and vSMCs | 4,121,123 |
| Pdgfrb–CreERT<sup>2</sup> | Fibroblasts, pericytes, vSMCs and lymphatic endothelial cells | 113,124 |
| Slc1a3–CreER | Astrocytes, type A pericytes and fibroblasts | 1,87 |
| Cspg4–CreER<sup>TM</sup> | Pericytes, vSMCs and OPCs | 124–125 |
| Acta2–CreER<sup>2</sup> | Pericytes and vSMCs | 126 |
| Col1a1–GFP | Fibroblasts and a small population (<2%) of pericytes and vSMCs | 4,26 |
| Tbx18–CreER<sup>2</sup> | Fibroblasts, pericytes and vSMCs | 27,127 |

OPC, oligodendrocyte progenitor cell; vSMC, vascular smooth muscle cell.

Fibroblasts are present in the choroid plexus stroma

Each of the brain ventricles contain a choroid plexus that regulates cerebrospinal fluid composition and secretion<sup>43–45</sup>. The choroid plexus consists of an outer epithelial layer and an inner stromal compartment<sup>46</sup> that contains various cell types (Fig. 1a,b). The cells of the epithelial layer are polarized and contain tight junctions, forming an essential component of the blood–CSF barrier separating the permeable blood vessels in the stromal layer and the CSF within the ventricles, which is secreted by the choroid plexus epithelial cells<sup>46,47</sup>. The stroma contains fibroblasts, distinct immune cell populations and a fenestrated vasculature with pericytes and vSMCs<sup>40,46</sup> (Fig. 1a,b). A wide variety of immune cells, including macrophages and dendritic cells, reside in the stroma and it is thought that the choroid plexus along with the meninges serve as an important neuroimmunological gateway<sup>48–50</sup>. Stromal fibroblasts are thought to secrete extracellular matrix (ECM) and might thereby provide structural support in the choroid plexus<sup>41,42</sup> in addition to having other, yet-to-be discovered roles.

CNS fibroblast origins and diversity

The development of CNS fibroblast populations has been most studied in the meninges. Lineage tracing studies in chick and mice show that meningeal fibroblasts in the midbrain, hindbrain and spinal cord are derived from the somatic and cephalic mesoderm, whereas those in the forebrain originate from the neural crest<sup>46–51</sup>. An immature meningeal primordium containing fibroblasts is detected by the fifth week of gestation in humans<sup>52</sup> and between embryonic day 9 (E9) and E10 in mice<sup>57</sup> (Fig. 2).

Recent single-cell transcriptional profiling has provided new insight into the molecular diversity of meningeal fibroblasts and their emergence during development<sup>27</sup>. This work showed that, in E14 mice, pial, arachnoid and dural fibroblasts have distinct gene expression profiles. Pial fibroblasts can be differentiated from both arachnoid and dural fibroblasts by their expression of S100a6 and Ngfr, and they show enriched expression of genes involved in the production of ECM proteins, such as collagens, laminins and glycoproteins, consistent with their role in pial basement membrane maintenance. Arachnoid fibroblasts show enriched expression of various genes, including those encoding sulfate, magnesium and GABA transporters, and other ECM proteins implicated in regulation of ECM assembly. Dural fibroblasts have elevated expression of genes encoding small and large ribosomal subunits, the ion transport regulator FXYD5, and the transcription factors FOXp1 and SIX1. Markers of differentiating meningeal fibroblasts that are conserved across humans and mice include CRABP2 for arachnoid fibroblasts and S100a6 and p75NTR for pial fibroblasts<sup>27</sup>. Developmental studies show that many of the markers specific for the meningeal fibroblast layers are first detected between E12 and E14 in mice<sup>1</sup>, consistent with earlier electron microscopy studies describing distinct meningeal cell layers by E13 in rodents<sup>27</sup> and the sixth week of gestation in humans<sup>53</sup>. In the forebrain, some markers specific for the meningeal fibroblast layer, such as RALDH2 (arachnoid fibroblasts), p75NTR (pial fibroblasts) and E-cadherin (arachnoid barrier cells), are first detected in ventral meninges before being detected in dorsal meninges<sup>27</sup> (Fig. 2a). The expression of many, but not all, meningeal fibroblast markers identified in mouse embryos persists into adulthood, suggesting that meningeal fibroblast populations undergo additional, postnatal maturation in mice<sup>27</sup>. In contrast to the meninges, the appearance of perivascular fibroblasts in the parenchymal vasculature in most brain regions occurs during mouse postnatal development. In mice, type I collagen-expressing perivascular fibroblasts gradually appear in all brain regions around the non-capillary vasculature in the first 3 weeks after birth<sup>54</sup> (Fig. 2b). They first appear close to the pial surface and then later in deeper regions, suggesting that these cells originate from the meninges. Whether perivascular fibroblasts are pial fibroblasts that move into perivascular spaces, as inferred from electron and light microscopy studies of human and mouse brain tissue<sup>13,40,41</sup>, or are cellularly and molecularly distinct from pial fibroblasts remains to be resolved. The association of perivascular fibroblasts with the meningeal and parenchymal vasculature observed in electron microscopy studies and two-photon live imaging of fibroblasts in mice expressing Col1a1–GFP<sup>22</sup> suggests that perivascular fibroblasts are morphologically distinct from pial fibroblasts. Perivascular fibroblasts wrap around large blood vessels and their cell bodies are aligned to vessels in the leptomeninges and brain, whereas pial fibroblast cell bodies and processes in the leptomeninges are aligned parallel to the brain surface<sup>13,27,42</sup>. Perivascular fibroblasts express many of the same markers as pial fibroblasts, including laminin α1 (Ref<sup>41</sup>), PDGFRe<sup>1,53</sup> and type I collagen, suggesting that these two fibroblast populations share some molecular characteristics. To better define the molecular characteristics of perivascular and pial fibroblasts, single-cell RNA sequencing of fibroblasts isolated separately from adult leptomeninges and brain will be required. However, a recent preprint study reporting vessel isolation and nuclei extraction for sequencing (VINE-seq) in humans used annotations from other sequencing studies to detect distinct clusters of perivascular and meningeal fibroblasts<sup>27</sup> and found enriched expression of ECM components in perivascular fibroblasts whereas meningeal fibroblasts showed enriched expression of solute transporters<sup>4</sup>.
a Meninges

- Calvarial bone
- Periosteal dura
- Dural border
- Arachnoid barrier
- Inner arachnoid
- Subarachnoid space
- Pia
- Glial limitans
- Astrocyte

Perivascular fibroblast

- Perivascular space
- vSMC
- BAM
- Pericyte
- Astrocyte

Choroid plexus

- Epithelium
- Fenestrated blood vessel
- Fibroblast
- Pericytes and vSMCs
- Stroma
- Macrophage
- Parenchyma

b Meninges

- Perivascular fibroblast

Choroid plexus

- Col1a1-GFP
- Type I collagen
- DAPI
Although numerous studies have provided insights into the developmental origins, diversity and functions of choroid plexus epithelial cells, investigation of the development of stromal cells, particularly choroid plexus fibroblasts, is limited. Ultrastructural studies in rodents and chick describe the choroid plexus stromal compartment as a connective tissue that emerges from the embryonic head mesenchyme and is thought to be developmentally related to leptomeningeal tissue, although a lack of lineage-tracing studies means that its precise developmental origin is unclear\(^1\). A seminal study of human embryonic choroid plexus described an increasing abundance of fibroblasts and collagen fibrils in the stroma throughout development, suggesting the gradual differentiation of stromal fibroblasts\(^4\). However, the potential mechanisms controlling stromal fibroblast differentiation have not been uncovered, although it has been suggested that the stroma engages in cellular crosstalk with the developing choroid plexus epithelium and vascular plexus\(^5\).

Bulk and single-cell transcriptional profiling of fibroblasts in the meninges and perivascular spaces in healthy, adult mice revealed the presence of various subtypes of CNS fibroblasts. Comprehensive single-cell sequencing of brain vascular and perivascular cells in healthy mice found two clusters of perivascular fibroblasts, denoted F1 and F2 \(\text{(REF.}^1\text{)}\). Both clusters express high levels of ECM proteins such as collagens and a comparison of CNS fibroblasts with lung fibroblasts found that 45 of the 50 transcripts most enriched in CNS fibroblasts compared with other CNS vascular cells were also found in lung fibroblasts\(^1\). Single-cell sequencing of spinal cord fibroblasts in healthy mice and in those with EAE also found two primary populations of fibroblasts as well as different states of activation of these cell populations in disease\(^1\). Further studies using techniques such as spatial single-cell sequencing and in situ hybridization are needed to better characterize these fibroblast subtypes and their localization within the CNS.

Similarly, a recent single-cell RNA sequencing analysis to molecularly profile the choroid plexus described fibroblasts that express Col1a1 and Pdgfra and show enriched expression of ECM protein transcripts in the choroid plexuses from each ventricle. Similar to the epithelium, choroid plexus fibroblasts in different ventricles (such as the lateral, third and fourth ventricles) displayed differential gene enrichment, suggesting that these fibroblasts may be patterned on the rostral–caudal axis and may have different functions in each ventricle\(^2\).

Several single-cell sequencing studies of the whole brain have also identified fibroblast cell clusters that represent different fibroblast subtypes, which likely contain a combination of fibroblasts from the meninges, perivascular spaces and choroid plexus\(^3\). In some of these studies, these cells were described as vascular leptomeningeal cells, although other names have been used to characterize the same populations\(^4\). Owing to the low abundance of fibroblasts in the brain, these whole brain analyses have only assessed a small number of cells for each cluster, which makes analysing diversity in these populations difficult using these datasets.

**CNS fibroblast roles in health**

The roles of CNS fibroblasts in healthy adults is just beginning to be studied. In the periphery, the foremost role of fibroblasts is to provide structural support to connective tissues through the secretion of ECM components, particularly type I and type III collagens\(^5\). Fibroblasts produce a wide variety of cytokines and growth factors and, when needed, can also differentiate into other cell types, such as fat cells or cartilage cells, in some tissues\(^2\). Peripheral fibroblasts are also crucial for angiogenesis as they secrete matrix proteins that facilitate tube formation and vascular endothelial growth factor (VEGF), which promotes angiogenesis\(^5\). They also sense and respond to mechanical stress by remodelling tissue as required and altering their membrane potential\(^5\).

The role of fibroblasts surrounding blood vessels in the perivascular space of the CNS is less clear. There is little evidence that these perivascular fibroblasts continually differentiate and angiogenesis is not widespread in the adult human CNS. Some researchers have proposed that fibroblasts covering pial vessels facilitate fluid exchange between the CSF and perivascular spaces\(^5\) and play roles in the development of the glymphatic system\(^6\), but more in-depth studies are needed to confirm these hypotheses.

Fibroblasts in the meninges provide structural support within and between the different meningeal layers. This role in maintaining the integrity and separation of the meningeal layers is vitally important as these layers have varying degrees of vascular leakage and therefore contact with the periphery\(^5\). As the meninges are home to a variety of immune cells and peripheral fibroblasts are known to recruit, maintain and interact with immune cells in various organs, including lymph nodes\(^6\), it is likely that there is signalling crosstalk between meningeal fibroblasts and neighbouring immune cells. Indeed, a recent study demonstrated that CXCL12 secretion by fibroblasts in the dura stroma contributes to the recruitment of T cells to dural sinuses, where these lymphocytes recognize antigens in the CSF\(^5\). More in depth experiments, both in vivo and in vitro, are needed to decode the full profile of signalling interactions of meningeal fibroblasts with immune cells.

The specific functions of choroid plexus fibroblasts in the healthy adult brain are also largely unknown, although some insights have been obtained from in vitro and transcriptomic studies of choroid plexus...
stromal cells. A study using cultured rat stroma found that a large proportion of intracellular adhesion molecule 1-positive (ICAM1+) fibroblast-like cells express the neural stem cell marker nestin and that these cells form clusters with choroid plexus myeloid cells, an interaction that was also observed in the choroid plexus in postnatal rats. A recent single-cell analysis of choroid plexus cells also highlighted multiple potential functions of choroid plexus fibroblasts. The enriched expression of genes encoding ECM components, such as collagen, fibronectin and prolyl-4-hydroxylase, indicates that choroid plexus fibroblasts might be an essential scaffold in the stroma. The expression of nestin in a subset of choroid plexus fibroblasts suggests a degree of heterogeneity in this population of cells and that they might be progenitors of other stromal cell types. Overall, the proximity of choroid plexus fibroblasts to the choroid plexus epithelium, vasculature and immune milieu could enable numerous cell–cell interactions that are important for choroid plexus maintenance and function.

CNS fibroblasts may also play a part in sensing changes in their environment, specifically changes in blood flow and tissue stiffness, via mechanotransduction. Cardiac fibroblasts are known to sense mechanical stress through membrane receptors such as integrins and respond by secreting ECM proteins, thereby remodeling local tissue. Blood flow throughout the body is sensitive to environmental factors and fibroblasts in the CNS could respond to these changes over time by subtly remodeling their local tissue environment and signaling to nearby smooth muscle and endothelial cells. The exact responses of fibroblasts and the consequences of this mechanical sensing need to be confirmed as these cells are present at crucial sites where they can monitor both the periphery and the CNS. Whether CNS fibroblasts are involved in maintaining the vascular basement membrane is not known but their high expression of ECM proteins suggests that this is likely. The potential and confirmed roles of fibroblasts throughout the CNS are summarized in Table 3.

CNS fibroblasts in disease

Meningeal inflammation

Following peripheral inflammation, a fibroblastic reticular network forms in lymph nodes that serves as an immune cell niche. A similar network forms in the meninges during neuroinflammation, most notably in MS. These networks consist of T cells, B cells and other immune cells interacting with fibroblasts, all of which are held together by ECM secreted by the fibroblasts. In mice with EAE, T helper 17 cells support the formation of this network in the meninges, specifically, IL-17 and IL-22 secreted by T helper 17 cells upregulate the expression of fibrotic genes by CNS fibroblasts.
The overall role of this meningeal network in disease progression has only recently been studied. In MS, the meningeal network is present near areas of grey matter demyelination, implicating this structure in lesion formation\(^{1,71,78}\). In mice with EAE, injecting blocking antibodies against IL-17 and IL-22 reduced the size of this meningeal network and led to a decrease in the EAE motor disability score\(^{75}\). However, the exact signalling contribution from the individual cells in this network, including fibroblasts, is unclear, as cell type-specific deletions have not been tested.

A similar fibroblastic reticular network in the meninges has also been described after CNS infections in mice, including coronavirus\(^{75}\) and lymphocytic choromeningitis virus, in which the latter was shown to infect ER-TR7\(^{+}\) stromal cells\(^{86}\). Furthermore, this network also forms after infection with mouse hepatitis virus (MHV) strain A59 and stromal cells in the network produce the chemokine CCL19 that recruits anti-MHV CD8\(^{+}\) cells\(^{77}\). Many questions remain about the conditions that lead to the formation of this meningeal network, its overall role in disease progression (as it appears to be beneficial to recovery from infection but harmful in demyelination), and the exact role of fibroblasts in its formation and in signalling to maintain the immune cell niche. Transcriptional profiling of fibroblasts in this network could help to reveal the signalling mechanisms that are upregulated in fibroblasts in the network compared with those in the healthy meninges.

**Fibrotic scarring**

Scars in the CNS consist of two major parts: an outer glial scar consisting of reactive astrocytes that migrate and organize their processes around the injury border beginning 1–2 days after the injury and an inner fibrotic scar at the core of the injury or inflammation that seals the damage site and forms in the days and weeks following the injury or inflammation trigger\(^{78,79}\) (Fig. 5). The glial scar has been extensively characterized and can have an overall beneficial role in damage repair\(^{71,81}\). The inner fibrotic scar has been much less studied, although it has also been implicated in damage repair and recovery after injury or inflammation in the CNS. While these two scars occur in distinct areas after an injury to the CNS, they exhibit some overlap in inflammatory lesions with scarring\(^{74}\). In addition, the major fibrotic scar component type I collagen can induce glial scar formation following injury\(^{46}\); however, reducing fibrotic scar formation following inflammation does not prevent the formation of the glial scar\(^{4} \). Microglia have also been shown to be an integral component of the scar following injury and, in neonatal mice, these cells organize scar-free injury repair by secreting ECM proteins that form bridges to bind damaged tissue and peptidase inhibitors to prevent scar buildup\(^{85,86}\). Below, we summarize the main CNS injuries and diseases in which fibrotic scarring has been implicated and describe the cell types that have been proposed to form the scar, the role of the scar in recovery following specific triggers, and the mechanisms that lead to CNS fibroblast proliferation and collagen production. Many of these aspects remain incompletely understood; studies probing the cellular origin, role and mechanisms of fibrotic scarring in many CNS diseases would be useful to fully understand how scarring influences CNS damage repair.

**Spinal cord injury.** SCI is the most widely studied trigger for CNS fibroctic scarring in both humans and animal models. Several studies have suggested that this scar arises from type A pericytes that upregulate collagen expression following injury. For example, a study in Scl1a3–CreER mice, in which Cre expression driven by the promoter of Scl1a3 (the gene encoding GLAST, thought to be most active in astrocytes), leads to fluorescent labelling of scar-forming cells, these cells were classified as pericytes on the basis of their expression of PDGFR\(^{\beta}\) and perivascular localization\(^{95}\). Another study using Colla1-GFP mice suggested that CNS fibroblasts form the fibrotic scar as the GFP\(^{+}\) collagen-producing cells do not express pericyte markers such as NG2 [Ref.\(^{26}\)]. Light sheet microscopy images of an injured mouse spinal cord suggest that the GFP\(^{+}\) cells likely originate from the meninges although there are no definitive data regarding the proportion of the scar that arises from meningeal versus perivascular fibroblasts\(^{89}\). Single-cell sequencing studies revealed that, in addition to astrocytes and pericytes, fibroblasts also express Scl1a3 and, therefore, these two studies might have identified the same fibroblast cell population but named them differently\(^{1}\).

The fibrotic scar is thought to negatively affect disease progression in SCI as it blocks progenitor cells from entering the injury core to facilitate axon regeneration; consequently, therapies have been proposed to target this scar to stimulate recovery\(^{96,97}\). However, completely blocking fibrotic scar formation in mice leads to a failure to seal the injury site, resulting in an open lesion\(^{97}\).
By contrast, reducing the size of the scar without completely blocking its formation (by combination knock-out of HRas, NRas and a cell-specific deletion of KRas to prevent fibroblast proliferation) results in improved recovery from SCI in mice as shown in motor tests and functional assessment of optogenetically stimulated regenerating axons. However, the Ras pathway is not specific to fibroblasts and is therefore unlikely to be used in a clinical setting. Other studies have shown that inhibiting microtubule formation prevents fibrotic scarring and dampening immune cell signalling can also be used to reduce scarring and enhance disease recovery.

Traumatic brain injury. Traumatic brain injury (TBI), like spinal cord injury, is a physical injury to the CNS that often results in fibrotic scarring. As in SCI, studies have reported different cellular origins of the fibrotic scar in TBI. For example, PDGFRβ+ cells accumulate in the injury core in rodents, with these cells identified as either pericytes or meningeal fibroblasts. Inhibiting PDGFRβ signalling after TBI decreases scar formation in mice although how this manipulation affected overall tissue recovery was not studied. Scar-forming cells also highly express TGFβ receptors following TBI and inhibiting TGFβ signalling after TBI in rodents reduces scar formation and promotes the regeneration of dopaminergic neurons. Reducing scar formation by the pharmacological inhibition of type IV collagen deposition at the injury site also promotes axonal regeneration following brain injury.

Stroke. Stroke is caused by loss of blood flow to specific areas of the CNS. The resulting hypoxia leads to blood–brain barrier breakdown, tissue damage and CNS scarring. In Col1a1-GFP mice, both PDGFRβ+ and GFP+ cells, which were referred to as stromal cells, increased in number in the lesion core surrounded by fibrotic proteins following middle cerebral artery occlusion, a model of stroke. In Pdgfrβ+/– mice following middle cerebral artery occlusion, fibrotic scar formation is reduced but the overall infarct volume is larger, implicating PDGFR signalling in fibrotic scar formation and the fibrotic scarring process in managing infarct size in this model. Whether this change in infarct sizes results in changes in disease recovery and tissue repair remains unknown. In a mouse model of meningeal cerebrovascular injury induced by transcranial ultrasound, blocking myeloid cell recruitment led to a lack of vascular repair and increased fibrosis in the injury site, suggesting that immune cell...
entry into vascular lesions is crucial for wound repair and that altering this process can lead to fibrosis. The signals that lead to this fibrosis remain unknown.

**Multiple sclerosis.** MS is a neuroinflammatory disease characterized by concentrated areas of inflammation and demyelination. ECM deposits occur around blood vessels in MS and a recent study in Coll1a1-GFP mice with EAE found an increase in the number of GFP+ cells in the spinal cord parenchyma following symptom onset. In mice with EAE, lineage tracing studies demonstrated that the fibrotic scar arises from the proliferation of CNS fibroblasts and not that of pericytes or bone marrow-derived cells, although the relative contribution of meningeal and perivascular fibroblasts to the fibrosis remains unknown. This is an especially intriguing question in inflammation, in which there may not be physical damage to the pial or glial barrier. Reducing fibroblast proliferation and the resulting scar formation in mice with EAE by fibroblast-specific expression of the herpes simplex virus thymidine kinase led to a modest improvement in motor symptoms and an increase in oligodendrocyte lineage cells in inflammatory, demyelinating lesions, suggesting that the fibrotic scar limits the ability of potential reparative cells to enter the lesion. In addition, blocking IFNγ signalling in CNS fibroblasts reduced the area of fibrotic scar formation.

**Microbial infection.** Infection leads to inflammation and, in severe cases, tissue damage and fibrotic scarring. Following *Staphylococcus aureus* infection in the mouse brain, an abscess forms that is surrounded by a fibrotic wall. A small population of bone marrow-derived fibrocyte-like cells in the area of the fibrotic wall are positive for markers of fibrosis such as type I collagen. Whether and how bone marrow-derived cells contribute to fibrosis following other CNS triggers, such as injury or degeneration, remains unknown. However, even in cases where bone marrow-derived cells have been detected in areas of CNS scarring, their contribution to the scar seems to be minimal and most evidence strongly supports the conclusion that CNS fibrotic scarring arises from cells intrinsic to the CNS.

**Retinopathies.** Retinopathies are diseases that damage the retina usually as a result of altered retinal blood flow. Fibrotic scarring occurs in the retina in conditions such as diabetic retinopathy and retinopathy of prematurity and can lead to tractional retinal detachment and blindness. The proposed origin of this fibrotic scar varies with the location of the fibrosis and the extent of tissue damage to specific layers of the retina. For example, when there is a physical injury to the retinal pigment epithelium, these pigmented epithelial cells have been proposed to migrate to the surface of the retina and deposit scar tissue. In other retinal conditions, Müller glia (resident retinal glia) have been proposed to extend their processes to the surface of the retina and deposit scar tissue. Although the retina contains an extensive vasculature, the presence of perivascular fibroblasts in the retina has not been reported. A single-cell transcriptomic study of the murine retina detected a few cells classified as fibroblasts, although these cells were reported to be contaminants. More in-depth sequencing studies are needed to confirm whether fibroblasts are present in the retina of mice and humans and play a role in retinal scarring.

**Neurodegenerative diseases.** CNS fibroblasts and the presence and role of fibrotic scarring have not been extensively studied in neurodegenerative diseases. ECM components, such as proteoglycans, are deposited alongside amyloid plaques in Alzheimer disease and ECM remodelling occurs in ALS but has been attributed to astrocytes. Even if fibroblasts are not engaged in scarring in these diseases, they could influence disease outcomes through signalling with nearby vascular cells. A preliminary study using VINE-seq to profile human vascular cells from the hippocampus and cortex of healthy individuals and patients with Alzheimer disease found dysregulation in IFNγ pathway and SMAD signalling genes in fibroblasts in Alzheimer disease. In addition, in the pre-symptomatic stages of ALS, the fibroblast marker genes *SPP1* and *COLA6A1* are enriched and their protein products accumulate in perivascular spaces. Increased expression levels of these genes predicted shorter survival times in patients with ALS, indicating that perivascular fibroblasts contribute to dysfunction early in disease progression.

**Choroid plexus stroma and immune cells.** Choroid plexus stromal compartment dysfunction has been documented in cases of CNS injury, aging, and disease. In a 1931 study of post-mortem choroid plexus from 61 head trauma cases, nearly every case had marked oedema of the choroid plexus stroma and the presence of phagocytic cells. Given the close proximity of choroid plexus fibroblasts to the immune cells and vasculature of the stroma, it is likely that choroid plexus fibroblasts facilitate or contribute to oedema and immune cell activation following head trauma, although a potential mechanism has not been elucidated. Although this study found common brain injury pathologies, such as oedema, immune cell infiltration and choroid plexus epithelial vacuolization, it does not describe a common feature of choroid plexus dysfunction in many other diseases and disorders: fibrosis. For example, sclerosis of the choroid plexus stroma occurs in chronic hydrocephalus and extensive fibrosis, calcification and aberrant vasculature of the choroid plexus stroma occur with ageing, a fibrotic phenotype that is even more pronounced in patients with Alzheimer disease. It is likely that choroid plexus fibroblasts become activated in these cases to contribute to choroid plexus fibrosis and scarring but the mechanisms of activation remain unknown. By contrast, in a mouse model of the human autoimmune disorder systemic lupus erythematosus, the basement membranes of the choroid plexus epithelium and stromal vasculature are ‘thickened’ and the stromal compartment seems to lack ‘interstitial material’ that typically separates the two basement membranes. These observations could suggest a loss of choroid plexus fibroblasts in autoimmune disorders, although this has not been shown in other autoimmune diseases or models.
**Conclusions**

CNS fibroblasts contribute in important ways to CNS development and disease pathology, progression and recovery. Their role in contributing to CNS fibrosis makes them important drug targets for the treatment of neurological diseases and conditions. However, many unanswered questions remain about the functions of these cells in health and disease (BOX 1). It is unknown whether there are significant transcriptional and functional differences between fibroblasts in the meninges, perivascular spaces and choroid plexus. Techniques such as spatial transcriptomics and two-photon microscopy can be utilized to further probe the location-specific properties of these cells. In addition, whether these cells have functions other than structural support in the healthy adult CNS remains unknown. Fibroblasts occur at interfaces between the CNS and the periphery and might thus monitor and respond to changes in blood flow, tissue stiffness and immune cell composition.

In the choroid plexus, comprehensive studies are needed to further our understanding of the identity and functions of choroid plexus fibroblasts, including using lineage tracing tools to elucidate the developmental origins of these cells and molecular tools, such as single-cell RNA sequencing, to further elucidate their functional diversity. Choroid plexus dysfunction, particularly fibrosis, has been extensively documented in CNS injury, ageing and disease but there has been very little focus on the mechanisms of fibrosis in the choroid plexus in these contexts. Understanding how fibroblasts become activated in these contexts will give greater insight into their pathogenesis and may explain other features of choroid plexus dysfunction such as aberrant vasculature and immune cell activation.

While the role of CNS fibroblasts in fibrotic scar formation has been the most widely studied function of these cells in disease, there are still many open questions about fibrosis such as whether fibrosis occurs following neurological injury in neurodegenerative diseases, the role of fibroblast proliferation and fibrosis in repair after stroke, and the full profile of signalling mechanisms that lead to scar formation. Although PDGFRβ, TGFβ and IFNγ signalling in fibroblasts have been shown to be involved in CNS fibrosis, manipulating one signalling pathway has only a partial effect on this process, suggesting that combinations of these pathways and/or other pathways likely contribute to scar formation. In addition, fibrosis can interfere with the function of neural implants, such as electrodes used for the treatment of epilepsy and Parkinson disease, and studies manipulating fibroblast activity by modulating these signalling pathways could identify ways to improve the longevity of these devices.

It is possible that the fibrotic scar is formed by different cell types in response to different triggers, which upregulate different signalling mechanisms in cells in the perivascular spaces. Genetic lineage tracing illustrated that fibroblasts are the predominant origin of the scar following neuroinflammation, but a different cell type, such as pericytes, or a combination of fibroblasts and pericytes, could be activated to upregulate collagen production following hypoxia or injury. It is also possible that CNS fibroblasts are the primary cell type that forms the fibrotic scar after all triggers in tissues in which they are present. We hypothesize that this scenario is more likely, as the type A pericytes that have been identified as the scar-forming cells in SCI have not been clearly detected in single-cell sequencing studies in mice. These pericytes may actually be CNS fibroblasts as their defining characteristics (PDGFRβ expression, GLAST expression and perivascular localization) are shared by fibroblasts. Either way, repeating lineage tracing experiments in cases of CNS injury and hypoxia would resolve this question.

It is largely unknown how CNS fibroblasts contribute to diseases in ways other than secreting fibrotic matrices and supporting reticular networks. In the periphery, fibroblasts are key players in inflammatory signalling: they can recruit immune cells to sites of injury by releasing chemokines such as monocyte chemoattractant protein 1, influence leukocyte transendothelial migration and promote immune cell survival through reducing apoptosis. RNA sequencing analysis showed that the expression of cytokines by spinal cord fibroblasts is upregulated in disease and fibroblasts are a source of retinoic acid following stroke, indicating that they could play important roles in recruiting and eliciting damage responses from other cells in injury sites. In addition, as CNS fibroblasts express amyloid precursor protein (APP) and reside near the locations of amyloid deposition in cerebral amyloid angiopathy, these cells could be involved in the development and propagation of this disease. As current data show that fibrotic scarring impedes axon regeneration, it will be interesting to know whether other potential contributions of fibroblasts to disease have purely negative effects on recovery. Preventing collagen expression in fibroblasts without influencing their proliferation or signalling could delineate other roles for these intriguing cells in disease.

Finally, to determine the involvement of CNS fibroblasts in human disease and how the responses of CNS fibroblasts compare between humans and mice, further characterization of human tissue from different CNS pathologies is needed. While the presence of fibroblasts...
In the human meninges is well documented, less is known about whether perivascular fibroblasts are associated with parenchymal vessels. Fibrotic scarring in human pathologies such as spinal cord injury has been clearly demonstrated but whether the contribution of fibroblasts to this process occurs by similar or different mechanisms in humans than in mice remains to be discovered.

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