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Meningeal Multipotent Cells: A Hidden Target for CNS Repair?

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Abstract

Traditionally, the primary role of the meninges is thought to be structural, i.e., to act as a surrounding membrane that contains and cushions the brain with cerebrospinal fluid. During development, the meninges is formed by both mesenchymal and neural crest cells. There is now emerging evidence that subsets of undifferentiated stem cells might persist in the adult meninges. In this mini-review, we survey representative studies of brain-meningeal interactions and discuss the hypothesis that the meninges are not just protective membranes, but instead contain multiplex stem cell subsets that may contribute to central nervous system (CNS) homeostasis. Further investigations into meningeal multipotent cells may reveal a “hidden” target for promoting neurovascular remodeling and repair after CNS injury and disease.

Keywords

The meninges; Multipotent stem cells; Perivascular space; Cerebral ischemia; CNS injury

Introduction

The meninges consist of three different membranes—dura mater, arachnoid, pia mater—and are filled with cerebrospinal fluid (CSF), thus protecting and cushioning the brain (Decimo et al. 2012). Ultrastructure assessments reveal that the meninges not only cover the brain surface but penetrate inside neural tissue to form perivascular space around diving parenchymal blood microvessels (Hallmann et al. 2005). Emerging evidence suggests that meninges may have an important role as migratory scaffolds for cells and participate in central nervous system (CNS) pathology (Cai et al. 2019). In this mini-review, we explore representative studies of meningeal responses during CNS development, attempt to define subsets of undifferentiated stem cell populations that may persist in adult meninges, and discuss the hypothesis of crosstalk between meningeal stem cells and damaged brain for the purpose of restoring cerebral function after CNS injury and disease.

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Declarations

Conflict of interest There is no conflict of interest.

Meninges in CNS Development

In the early embryo, both mesenchymal and neural crest-derived cells appear to form the primary meninx that is a layer of loose mesenchymal tissue surrounding the neural tube and the developing early brain anlage (O'Rahilly & Muller, 1986). The three meningeal layers are established with primarily a single pia meshwork structure, then arachnoid layers, and dura mater. The dura mater is the outer membrane and forms a sac that supports the dural venous sinuses of the falx cerebri, the tentorium cerebella and falx cerebella, and the pituitary gland and the sella turcica (Jacobson & Marcus, 2008). Additionally, the leptomeninges consist of two other membranes which are arachnoid and the inner pia mater, attributing to the space filled with cerebrospinal fluid (CSF).

The meninges cover and deeply penetrate the brain as sheaths of vasculatures and as stroma of the choroid plexus (Jacobson & Marcus, 2008) and also connect to the hippocampal neurogenic zone (Mercier & Arikawa-Hirasawa, 2012). A recent finding demonstrates that, during development, the meninges may provide a neurogenic niche wherein meningeal radial glia-like cells migrate to the caudal cortex through passing underneath the hippocampus, the fornix, and choroid plexus and differentiate into neurons in cortical layers II–IV (Bifari et al. 2017). Another study showed that, when meningeal cells were destroyed and removed by injecting 6-hydroxydopamine into the interhemispheric tissue of newborn hamsters, the formation of dentate gyrus was compromised (Hartmann et al. 1992). Moreover, the meninges were needed for the survival and growth of the developing forebrain (Etchevers et al. 1999) as well as serving as a central source of pericytes and smooth muscle cells to line blood vessels in the forebrain (Etchevers et al. 2001). Although the underlying molecular mechanisms remain to be fully elucidated, the collective literature strongly suggest that the meninges are essential for the CNS development by serving as a source and migratory guide for neurons and vascular cells.

Role of Meninges in CNS Pathophysiology

Adult meninges are known to contain multiple cell types including fibroblasts, mast cells, pericytes, smooth muscle cells, immune cells, telocytes, and endothelial cells. There may be a strong possibility that some of these subsets participate in CNS pathophysiology. For example, meningeal mast cell-originated factors such as IL-6 and CCL7 may exacerbate pro-inflammatory immune cell accumulation such as granulocytes and activated macrophages along with worsening cerebral infarction and brain swelling after stroke (Arac et al. 2014). Moreover, it has been reported that meningeal immune cells can profoundly participate in neurological disorders, and the meninges may play a primary role in neuroinflammation by triggering secondary responses in the parenchyma (Filiano et al. 2015). Perivascular stromal cells, type A pericytes, originating from meninges may communicate with astrocytes and neurons via retinoic acid and thus influence neurorecovery in the ischemic brain (Kelly et al. 2016). Altogether, the emerging science implicates the meninges as a key player in the regulation of parenchymal neuroinflammation in CNS pathophysiology.

A lingering literature suggests that adult meninges may also contain multipotent cells including neural stem/progenitor cells (NSPCs) that are relatively quiescent under normal

condition but transiently activated after CNS injury. For instance, injury-induced meningeal spinal cord cells increased some stemness markers such as Oct4, Nanog, Nestin, Dcx, Pax6, and Klf11 (Decimo et al. 2011). After stroke, a subset of nestin-positive cell population in the leptomeninges may migrate into the cortical parenchyma and showed neurogenic potential along with expressions of PDGFR β and NG2 (Nakagomi et al. 2011). These findings may support a role for the meninges as a heretofore under-appreciated stem cell niche in response to CNS injury. However, the origin, the fate and the role of meningeal stem cells are not fully characterized.

Potential Origin of Multipotent Stem Cells in the Meninges

During development, both mesenchymal and neural crest-derived cells form dura mater and leptomeninges, respectively (Decimo et al. 2012). Cranial neural crest cells contribute to the formation of perivascular pericytes, smooth muscle cells (Etchevers et al. 2001). Additionally, neural crest stem cells are originally derived from the surface ectoderm and capable of differentiating into motor and sensory neurons (Chai et al. 2003). It is known that neural crest stem cells (prototypical markers: CD271, TFAP2, Slug, Twist1, Sox9, Sox10 etc.) are indeed present in bone marrow, carotid body, cornea, dental tissues, dorsal root ganglion, gut, heart, palatum, and skin (Achilleos & Trainor, 2012).

The neural crest is a unique and transient embryonic cell population that originates in the ectoderm within margins of the neural tube. Recent findings have shown that trunk neural crest may be a source of mesenchymal stem cells and be able to produce mesenchymal derivatives (Morikawa et al. 2009). More recently, it has been demonstrated that transplantation of human neural crest stem cells derived from the inferior turbinate into a rat Parkinson's disease model induced recovery of dopamine neurons in the substantia nigra and reduced neurologic deficits (Muller et al. 2015). iPSC-derived neural crest stem cells mixed with hydrogel survived and integrated, and differentiated into neuronal lineage in repaired spinal cord (Saadai et al. 2013). In addition, skin-derived precursors give rise to both neural and mesodermal cell types, and myelinated glial cells, Schwann cells, which may contribute to white matter repair of the injured or diseased nervous system (Biernaskie & Miller, 2010).

Collectively, if they truly exist, neural crest-derived stem cells can be a novel target for promoting functional recovery after tissue injury. In the context of the damaged or diseased CNS, is it possible that neural crest-originated stem cells comprise a source of meningeal multipotent stem cells that may be leveraged for neurorecovery?

As a proof of principle study, we examined CD271, a prototypical marker of neural crest origin (Dupin & Coelho-Aguiar, 2013) in the meninges. A whole-body cryosection in E14.5 rats revealed that CD271 expressed in the broad area including meninges, heart, and intestines (Fig. 1a). Similarly, CD271-positive population was found in postnatal P1 rat meninges (Fig. 1b). Intriguingly, cultured CD271 cells following magnetic-activated cell sorting (MACS) were co-expressed by TFAP2A and Sox10 (Fig. 1b–d). Subsequently, we confirmed the multipotency of CD271 cells differentiating into neurons and mesenchymal derivatives (Fig. 1e–g). Collectively, meningeal CD271 cells were capable of differentiating

into the ectodermal and the mesodermal cell populations as demonstrated in neural crest-originated stem cells (Fig. 1h) (Lee et al. 2007).

Similar to developing rats, CD271 cells were present in adult meninges (Fig. 2a). After focal cerebral ischemia, CD271 cells were profoundly increased in the perivascular area and the leptomeninges in the ipsilateral hemisphere (Fig. 2b, c). Next, CD271 cells were isolated from meninges-enriched tissues at 72 h after focal cerebral ischemia by magnetic-activated cell sorting (Fig. 2g). Immunocytochemistry demonstrated that cultured CD271 cells expressed CD57, TFAP2, and Sox10 (Fig. 2d). Consistent with ones in developing meninges, CD271 cells were capable of differentiating into neurons, pericytes/smooth muscle cells, and adipocytes (Fig. 2e). Collectively, our preliminary studies revealed that neural crest cell-like multipotent stem cells may indeed be present in both developing and adult meninges.

Concluding Remarks

The brain is surprisingly plastic after injury, and multi-cellular processes of remodeling in neuronal, glial, and vascular compartments of the neurovascular unit should mediate functional recovery after central nervous system (CNS) injury and disease (Carmichael, 2010). However, it is now recognized that beyond cell–cell signaling within the brain per se, crosstalk between the brain and tissues outside the brain compartment per se may also be important for neurorecovery (Iadecola & Anrather, 2011).

The meninges are primarily known as brain protective membranes, but these membranes may also play a key role as a stem cell niche or as a scaffold for cell migration into the brain parenchyma. Because adult meninges still contain neural crest-originated stem cells, the meninges may be an attractive compartment in which to seek novel therapeutic approaches by targeting endogenous stem cell responses to boost neurovascular cell migration, proliferation, and differentiation. Future studies are warranted to rigorously investigate the presence of neural crest stem cells in adult meninges and assess these responses in animal models of stroke, brain injury, and neurodegeneration.

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Fig.1.

Meningeal CD271 cells have multipotency in developing rats. **a, b** The whole section in E14.5 (**a**, scale: 1 mm) and postnatal P1 (**b**, scale: 200 nm) Sprague Dawley (SD) rats revealed that CD271 was expressed in the meninges. **c** In a postnatal P1 rat, meninges are separated from brain and CD271-positive cells were isolated by MACS and cultured for 5–7 days. **d** Immunostaining confirmed that CD271 cells expressed TFAP2A, Ki67 and Sox10. Scale: 50 μ m. **e–g** Under the serum-free conditions, the addition of nerve growth factor (NGF; 50 ng/mL) and brain-derived neurotrophic factor (BDNF; 50 ng/mL) induced expressions of neurofilament and Tuj1 (**e**, scale: 50 μ m), whereas with adding TGF- β (5 ng/mL) and FBS (10%) in DMEM/F12 media, protein markers of PDGFR- β and SMA were increased (**f**, scale: 50 μ m.). Oil Red O-positive cells were produced by mesenchymal stem cell adipogenic differentiation medium (**g**). **h**. Meningeal CD271 cells may have multipotency. Serum exposure may convert meningeal CD271 cells into mesenchymal derivatives

Fig. 2.

Multipotent CD271 cells increase in the perivascular space after focal cerebral ischemia in rats. **a** In normal adult male SD rats (12–13 weeks), CD271-positive cells were dominantly present in meninges and also bone marrow (BM). Scale: 500 nm. **b** Male SD rats were subjected to 100 min transient focal cerebral ischemia by following the standard intraluminal filament MCA occlusion model, i.e., by inserting and then withdrawing the filament from the surgical entry point in the ECA. Immunostaining showed that CD271 cells were increased in the perivascular space and the meninges after focal cerebral ischemia at day 3 post-stroke. Scale: 100 μ m. **c** CD271 cells were found in proximity to brain endothelial cells (CD31) at day 3 post-stroke. Scale: 100 μ m. **d** CD271-positive cells were isolated from the meninges-enriched tissue by MACS. Isolated cells were grown on Geltrex-coated plates with DMEM/F12 containing 1% N2, 2% B27, FGF2 (100 ng/mL), and IGF1 (100 ng/mL). After culturing CD271-positive cells for 7–10 days, immunostaining confirmed expressions

of neural crest stem cell markers such as CD271, TFAP2A, HNK-1/CD57, and Sox10. Scale: 50 μ m. **e.** Isolated CD271 cells showed multipotency similar to cells isolated from developing meninges. Scale: 50 μ m

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