

## The spinal cord ependymal region: A stem cell niche in the caudal central nervous system

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## 1. ABSTRACT

In the brain, specific signalling pathways localized in highly organized regions called niches, allow the persistence of a pool of stem and progenitor cells that generate new neurons and glial cells in adulthood. Much less is known on the spinal cord central canal niche where a sustained adult neurogenesis is not observed. Here we review our current knowledge of this caudal niche in normal and pathological situations. Far from being a simple layer of homogenous cells, this region is composed of several cell types localized at specific locations, expressing characteristic markers and with different morphologies and functions. We further report on a screen of online gene-expression databases to better define this spinal cord niche. Several genes were found to be preferentially expressed within or around the central canal region (Bmp6, CXCR4, Gdf10, Fzd3, Mdk, Nrtn, Rbp1, Shh, Sox4, Wnt7a) some of which by specific cellular subtypes. In depth characterization of the spinal cord niche constitutes a framework to make the most out of this endogenous cell pool in spinal cord disorders.

## 2. INTRODUCTION

It is now well established that in mammals the central and peripheral nervous systems maintain a pool of multipotent precursor cells, which are dispersed throughout the parenchyma or are located in specific regions called niches. Niches are highly organized structures allowing the maintenance of specific signalling pathways and cellular interactions. These architectures provide clues to maintain precursor cells in an undifferentiated state and to tightly regulate their balance between self-renewal and production of more differentiated cells, which then migrate along precise pathways. Based on their extended or limited capacity for long-term self-renewal, adult precursor cells are classified as neural stem or progenitor cells respectively. In the brain, the hippocampus, the recently discovered sub-callosal zone and the subcortical white matter contain neural progenitors whereas bona fide stem cells, capable of sustained proliferation, are preferentially found in the SVZ (1). Stem and progenitor cells have also been identified in the peripheral nervous system, namely in the carotid body, the enteric nervous system and the adult

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dorsal root ganglia (2-4). Owing to the discovery of specific markers, the development of specific techniques such as non adherent cultures (neurospheres) and the identification of growth factors, these cells have been studied extensively over the last two decades. In particular, there has been a growing interest for studying these cells in pathological situations in order to control their number and fate. This could lead to innovating strategies to amplify and manipulate the regenerative capacity of the adult nervous system.

Whereas much attention has been given to stem and progenitor cells in the brain of mammals, much less is known about these cells in the spinal cord. Definite *in vitro* evidence for the presence of these cells was reported in the late nineties (5-7). Compared to the tremendous regenerative capacity of the spinal cord in lower vertebrates, mammals show little capacity to alleviate spinal cord damages, being of traumatic or degenerative origin. Therefore there is a need to compare at different levels (molecular, cellular, tissular) stem/progenitor cells in regenerative and non-regenerative species so as to provide a detailed knowledge of common and distinctive features. This may ultimately provide significant clues to develop rational strategies to repair the spinal cord following injury and degenerative diseases such as amyotrophic lateral sclerosis (ALS).

Here we review our current knowledge on the ependymal region of the adult spinal cord in mammals. We show that far from being a simple layer of ependymocytes, this region is composed of several cell types with specific markers, morphologies, locations and functions. In addition, probably owing to the maintenance of high levels of developmental signalling pathways, this region harbours a pool of stem and progenitor cells, which are readily activated and recruited in several types of spinal cord damage. This niche is organized along a very long tube (40-50 cm in humans) and probably undergoes mechanical forces due to extensive vertebra column bending. The common and distinctive cellular and molecular features of this caudal CNS niche compared to those in the brain are a source for a better understanding of the adult precursor cell diversity. Finally we describe how online gene expression databases such as Gensat (8) can help to define the cellular composition of the ependymal region and to identify canonical signalling pathways and genes which may have important roles for controlling the spinal cord stem/progenitor cell fate in normal and pathological situations.

### 3. CELLULAR COMPOSITION OF THE EPENDYMAL REGION IN MAMMALS

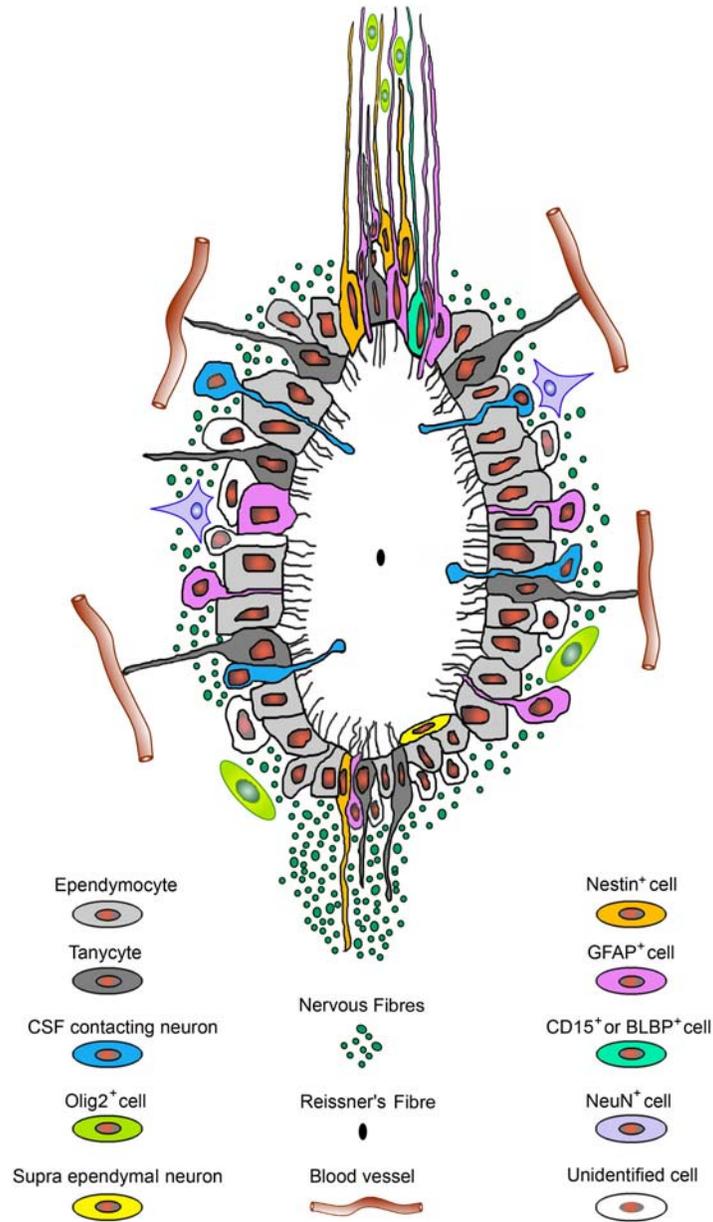
The spinal cord is divided into 6 regions (cervical, thoracic, lumbar, sacra, conus medullaris, terminal filum). A central canal is observed in all regions, but this has been reported to be collapsed or obstructed notably in the caudal regions (9, 10). Bjugn *et al*, suggested that a closed canal could be due to fixation problems (11). The canal is filled with cerebrospinal fluid (CSF) but can also contain a small fibre called Reissner's fibre (diameter 0,4-1  $\mu\text{m}$ ) running along the entire spinal cord length (12).

This fibre is seen in several mammalian species including rodents, cat, bovine and primates (13-16). It is composed of a bundle of straight, parallel, longitudinally arranged filaments of 50-100  $\text{\AA}$  in diameter (17). It appears to have a role in controlling CSF flow through the canal (18). The presence of amorphous proteinous material in the canal has also been described (16) but this could be of artefactual origin (12). The ependyma region is surrounded by the lamina X region according to Rexed divisions and by longitudinally arranged myelinated and unmyelinated axons (19, 20). Numerous blood vessels are in close proximity of the ependymal cells (9, 21). In the lumbar and sacral regions, a ventral bundle of fibres (mostly unmyelinated) that represents visceral autonomic afferents is observed. These fibres appear to interact with the ependymal cells (9, 20, 21). In the rat, a dense network of oxytocin-fibres apposed to the ependyma has been observed along the entire spinal cord (22).

The ependymal region is composed of several cell types, which are located either in direct contact with the lumen or in a subependymal position evoking a pseudo-stratified epithelium (Figure 1). However a distinct subependymal layer as observed in the brain is not present. Markers for the different cell types observed around the canal are indicated in Table 1.

#### 3.1. Ependymocytes-Tanocytes

Ependymocytes are the main cell type found around the central canal. These cells have a cuboidal morphology, abut the lumen and contain few 1-4 cilia (mostly 2) (17, 23) compared to multiciliated ventricle ependymal cells. Primate spinal cord ependymocytes may be more ciliated (24). They appear to be frequently interconnected by zonula occludens-like and gap junctions in the apical part and by zonula adherens further down, but their presence may not be revealed in all preparations and species (9, 12, 19, 24). A second frequently cell type observed is tanocyte (also referred to as radial ependymocytes) (19) which is mostly observed in the lateral sides of the central canal region. These ependymal cells, originally observed by Lenhossek and Ramon y Cajal at the turn of the 20<sup>th</sup> century, are characterized by a long basal process terminating on blood vessels (25). They are in contact with the lumen but their soma can be either subependymally or ependymally (23, 26). As in the brain, spinal cord tanocytes abut the basal lamina of blood vessels. Microvilli and cytoplasmic protusions evaginate from their apical side into the lumen and they frequently have a single cilia (23, 26). They are rich in intermediate filament (9). Their double contacts with the CSF and vessels make them ideal cells to transport substances between these two compartments, and they may function in regulating the composition of the CSF. They may also have a clearing function as they can uptake a number of substances. As in the brain, they bridge the CSF to the capillaries thereby providing a potential link between the CSF, blood and the neuroendocrine system. Indeed, ependymocytes and tanocytes show strong immunoreactivity for VIP (vasoactive intestinal polypeptide) (27, 28), a hypotensive and vasodilatory peptide found in the CSF (29, 30). VIP<sup>+</sup> ependymal cell



**Figure 1.** Schematic drawing of the adult mouse ependymal region. Adapted with permission from Sabourin *et al* (32) and Hamilton *et al* (21).

basal processes terminate on capillaries (28) suggesting a control of vascular tone of spinal cord vessels by these cells. The capacity of these cells to monitor and modify their environment is also illustrated by the presence of several receptors for cytokines/chemokines (endothelin receptor, PDGF receptor, CXCR4) (23, 31, 32) (Figure 2) and neurotransmitters (33, 34) together with the expression of cytokines/chemokines/neurotransmitters (CTGF, SDF1, Glutamate) (35-37).

The dorsal and ventral parts of the central canal display a divergent organisation with a higher density of cells with a radial morphology and lying ependymally and

subependymally (21, 23, 32). A third cell type is observed in these regions which has very long processes and expresses GFAP or Nestin intermediate filaments. These cells were described as persistent ependymogial cells in the newborn mouse spinal cord by Ramon Y Cajal. In the dorsal part, these GFAP<sup>+</sup> cells have very long basal processes extending along the dorsal midline up to the dorsal column white matter or pial surface (21, 32, 38). A small number of dorsal subependymal GFAP<sup>+</sup> cells send a process between ependymal cells to reach the lumen (Figure 1). These dorsal GFAP<sup>+</sup> cells do not appear to proliferate even in young animals (2 months) where proliferation within the central canal region is however still

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**Table 1.** Markers for the different cell types within the mammal ependymal region

Cell type	Markers	Species	Comment	References
<b>Ependymocytes</b>				
	Cadherin 13	m	stronger in young animals	32
	Cadherin 13	m	stronger in young animals	32
	GFAP	h, occasionally in mouse	increase after SCI	21, 33, 53
	Nestin	m, h	few dorsal or ventral cells, stronger in young animals and after SCI	21, 53, 90, 91
	PSA-NCAM	m, r		32, 41, 47
	S100b	m, r		21, 41, 91
	Vimentin	m, r, s	stronger in young animals	21, 32, 38, 69, 130, 131
	VIP	r		28
<b>Signalling and Receptors</b>				
	BMP4	m	weak but increase after SCI	88
	CD15	m, h	dorsal cells	32, 53
	CD44	m		32
	CD133	m, r		32, 91
	CTGF	r		35
	CXCL12/SDF1	m		36
	DAN	m		32
	Endothelin Receptor B	m, r		31, 32
	GPR17 (P2Y receptor)	m		33
	Musashi	m		23
	Notch1	m, r	weak but increase after SCI	72, 88
	Numb	m	weak but increase after SCI	88
	Jagged	m		32
	mGluR1	r		34
	PDGFRa	m		23
	SHH	m	weak but increased after SCI	88
<b>Transcription factors</b>				
	FoxJ1	m		23
	HES1	m		32
	Msx1/2	m	weak but increase after SCI	88
	Nkx6.1	h, m	weak	32, 53, 64
	Olig2	h, m	very weak	Hugnot, unpublished
	Pax6	r		72
	Sox2	h, m, r		23, 32, 53, 91
	Sox3	m		23
	Sox4	m	few cells in young animal	32
	Sox9	m		23, 32
	Zeb1	m	weak	32
<b>Enzymes</b>				
	prostaglandin F synthase II	r		132
<b>Radial ependymal cells or tanycytes</b>				
	CTGF	r		35
	Endothelin Receptor B	r		31
	mGluR1	r		34
	Nestin	m		23
	Prostaglandin F synthase II	r		132
	VIP	r		28
<b>Radial dorsal cells or dorsal tanycytes</b>				
	BLBP	m	subpopulation	32
	CD15	m	subpopulation	32
	GFAP	m, r, s		21, 32, 38
	Nestin	m		21, 23
	Sox2	m		21, 32
	Vimentin	m		21
	VIP	r		28
	Zeb1	m	strong	32
<b>Cerebral-fluid contacting neurons</b>				
	Aromatic-L-amino acid decarboxylase	r		133
	Dex	m, r		32, 36, 41
	GABA	r		20
	GABAa R	r		41
	GABAb R	r		49
	Gap43	r		20
	Glutamic acid decarboxylase	r		20, 48
	HuC/D	r	young animals	41
	Map2	r		20
	Methionine-enkephalin-	r	few cells	45

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	Arg6-Gly7-Leu8			
	Nkx6.1	m		32
	P2X2	r		20
	PKD2L1	m		51
	PSA-NCAM	r, m		20, 41
	Synaptotagmin	r		20
	Synaptophysin	r		20
	VIP	c		43
<b>Supraependymal cells</b>				
	b-III tubulin	r		52
	Map2	r		52
	PSA-NCAM	r		52
	VIP	c		43
<b>Unidentified ependymal cells</b>				
	Sox4	m	young animals	32
	RhoB	r		134
<b>Unidentified subependymal cells</b>				
	NeuN	m, r	different from cerebral-fluid contacting neurons	21, 41
	Olig2	m		21
	PSA-NCAM	h	ventral cluster	53
	c-ret	r		135

c: cat; h: human; m: mouse; r: rat; s: sheep

present (21). In the ventral part these cells are less frequent and have a shorter basal process. Sub-ependymal GFAP<sup>+</sup> cells sending a process toward the lumen can occasionally be observed laterally (32). Considering their radial morphology and their contact with the lumen and the pial surface, these cells can be considered as a subtype of tanycytes (dorsal tanycytes). Like in the brain (39), it is likely that several types of tanycytes are present around the canal but distinctive markers are scarce so far. A subset of dorsal cells with a radial morphology has been shown to express BLBP and CD15, two markers for neural stem cells (32) (Figure 1).

### 3.2. Cerebral-fluid contacting neurons (CSF-cNs)

These neurons are very common and well-described in several lower vertebrates especially fish and amphibians (see for review (40)). In mammals, their presence has been reported in rats (9, 20, 41), mice (12, 32, 36, 42), cats (43, 44) and monkeys (24, 43) with a preferred caudal position. These cells are sporadically distributed around the canal with a soma in an ependymal or subependymal position (Figure 1). They send a single thick dendritic-like process terminated by a large bulge (several  $\mu\text{m}$  large) in the lumen and occasionally have a single cilium (20). They make contact with ependymocytes (zonula adherens and zonula-occludens) in their apical part and form synapses in their basal part (12, 16, 20). In the rat, GABAergic CSF-cNs in the low thoracic part, send unmyelinated axons forming bundles surrounding the ependyma (but with a preferred ventral location) and in close association with the basal part of the ependymal cells (20). These axons seem then to converge ventrally to localize against the walls of the ventral median fissure at the lumbar level. However no terminal field was found for these fibres. In the rat, few CSF-cNs display immunoreactivity for methionine-enkephalin-Arg6-Gly7-Leu8 (45). In the cat, a seemingly different type of CSF-cN, expressing VIP, has been observed at the thoracic and sacral levels. These cells appear to send axons, sometimes in contact with the lumen, that terminate in the ventral median fissure and ventral surface (43).

The function of these CSF-cNs remains elusive. They represent the main neuronal population in the spinal cord of the prechordate amphioxius and are abundant in

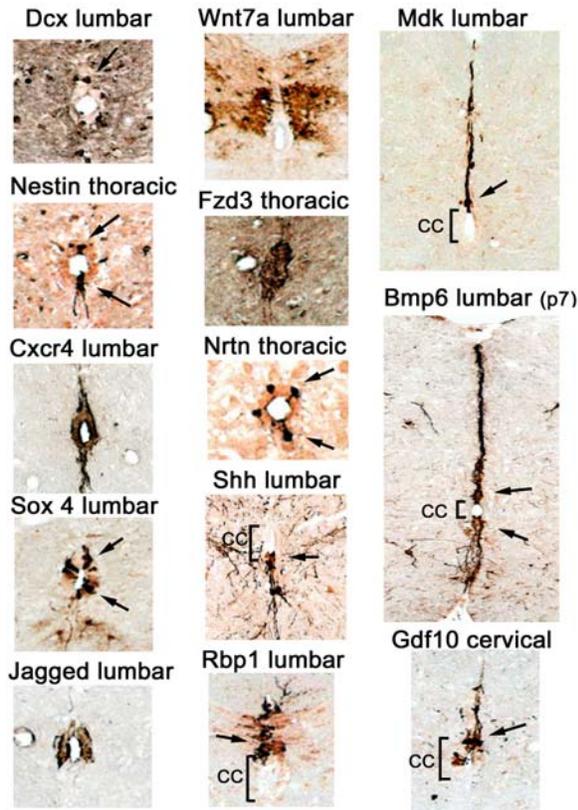
lower vertebrate caudal CNS (40). In the latter, these cells have been considered as mechanosensory neurons according to the presence of stereocilia, akin to sensory-cells of the inner ear and the lateral line organ in fish. These neurons may be sensitive to CSF flow or pressure or may be able to detect motions of the vertebrate column and spinal cord (40). In these species, CSF-cNs send their axons to the external CSF-space and may also be endowed with neurohormones secretory properties. They may represent a phylogenetically ancestral system to monitor and modify CSF composition. In mammals, these cells are less abundant and the presence of stereocilia has not been reported suggesting a different function. In *Xenopus*, GABAergic spinal CSF-cNs are born shortly after neural tube closure and thus they may have a role during development (46). As they co-express Nkx6.1 in the adult lumbar mouse spinal cord (32), they may be derived from the V3 ventral neural tube domain during development. Even in adults, these cells continue to express PSA-NCAM (41, 47), Dcx (32, 41) (Figure 2) and GAP43 (20), three proteins involved in plasticity and migration, suggesting that they are endowed with some degree of immaturity.

In rodents, this neuronal system appears to be mainly GABAergic (20, 48). They have functional GABA<sub>A</sub> and b receptors (20, 41, 49) and depending on the cells, GABAergic stimulation has an inhibitory or excitatory effect by eliciting hyperpolarisation or depolarisation respectively, which may reflect a different degree of differentiation (41). These neurons also express functional P2X2 ATP receptor and ATP-induced currents (20, 41, 50). More recently, the specific expression of the polycystic kidney disease-like channel (PKD2L1) has been reported in these cells (51). These channels are stimulated by acidic pH and represent the main element for taste responses to sour stimuli in the tongue. Likewise, in the spinal cord, CSF-cNs are excited by a drop in pH suggesting that these cells may be involved in the homeostatic circuitry responsible for monitoring and reporting the CSF pH (41, 51).

### 3.3. Supra-ependymal cells

These cells have been detected in the rat using a specific histological preparation. They lie within the central

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**Figure 2.** Brightfield images of immunohistochemistry against EGFP performed on adult (or P7 for BMP6) spinal cord sections derived from transgenic mice bearing a BAC promoter of the indicated gene. These images are reproduced with courtesy of The Gene Expression Nervous System Atlas (GENSAT) Project, NINDS Contracts N01NS02331 & HHSN271200723701C to The Rockefeller University (New York, NY) (8). When necessary, the central canal region (CC) is indicated on some pictures.

canal apposed to the luminal face of Vim<sup>+</sup> ependymocytes. They are predominantly found in the lumbar part and appear to be neuronal-like cells expressing PSA-NCAM, Map2 and  $\beta$ -III-tubulin (52). Similar cells are present on the surface of the third and fourth ventricles and can be induced to proliferate by EGF/FGF2 infusion. Neuronal-like cells expressing VIP have also been observed in the cat central canal (43). The role of these cells, if any, remains elusive.

### 3.4. Human ependyma

Very few data are available regarding the human ependymal region especially in the adult. In fact, the spinal cord central canal is often found to be occluded with age (100% after 65 years) (53-55) and the ependymal region appears disorganised with the frequent presence of rosettes or micro-canals. Like in rodents, this region retains immature features in adults such as the expression of Nestin, Sox2, CD15, Nkx6.1 and PSA-NCAM (53, 56). As observed in the foetal and post natal ependyma (56) different cell types can be distinguished in the adult ependyma sometimes with specific locations. In foetal, post

natal and adult tissue, CD15 cells are mainly present in the dorsal half of the central canal. Nestin cells are found to be restricted to the dorsal and ventral midlines at the foetal and neonate stages, an organisation not observed in the adult spinal cord. The presence of these Nestin<sup>+</sup> cells is detected inconsistently in adults and their number is increased in patients with amyotrophic lateral sclerosis and spinal cord tumours (56). Strong Nestin and PSA-NCAM stainings are also observed associated with a cell cluster lying ventrally outside the canal of the adult spinal cord (53). Compared to rodents and reminiscent of the reported difference between the SVZ in rodents and humans (57), the human central canal is surrounded by a hypo-cellular region containing a high density of GFAP gliofilaments and nervous fibres (53). In addition, contrasting with rodents where GFAP cells are scarce in the ependyma, a substantial number of ependymal cells express this marker in humans (53).

## 4. EMBRYONIC ORIGIN OF THE EPENDYMA AND POST-NATAL DEVELOPMENT

### 4.1. Embryonic development

The adult spinal cord is derived from the caudal neural tube. This structure is initially composed of proliferative neuro-epithelial cells surrounding a central lumen. These cells are heterogeneous showing different proliferative and differentiation potentials. Early during development, the caudal neural tube acquires a rostro-caudal and dorso-ventral specification by the action of morphogenetic molecules such as retinoic acid, SHH, Wnt and BMP. These molecules will induce restricted and complex expressions of a variety of transcription factors notably of the homeodomain family. Different combinatorial expressions of Hox genes will specify the rostro-caudal regionalisation of the spinal cord (58) whereas Pax, Nkx genes and other genes will be involved in dorsal-ventral patterning (59). In rodents, the walls of the dorsal part of the canal will appose before E15 leaving a ventral cavity (60). A similar apposition of dorsal luminal walls is observed in cats (61). The cavity roof is then composed of Nestin<sup>+</sup> radial glial cells with long processes maintaining contact with the pial surface (62, 63). These cells persist around 2 weeks post-natally in the rat and may be the origin of the dorsal radial GFAP<sup>+</sup> or Nestin<sup>+</sup> cells seen in the adult spinal cord (21, 32).

In rodents and chicks, the adult ependyma is mostly derived from neuro-epithelial cells lining this ventral cavity (64). The latter express Nkx6.1 and encompass the embryonic pMN domain which expresses the olig2 transcription factor (64). Indeed, tracing experiments using Olig2-cre<sup>TM</sup> mice have indicated that at least some of ependymal cells are generated from Olig2<sup>+</sup> cells located in the ventral pMN domain at E9.5 in mice (65). Thus in addition to producing motoneurons, oligodendrocytes, and subtypes of astrocytes, the pMN appear to generate some ependymal cells. This is also supported by retroviral lineage tracing performed in chicks embryonic spinal cord which demonstrated that 18% of clone containing motoneurons also contains cells contributing to the central canal region although the identity of these cells (ependymocytes or CSF-cNs) was not

specified (66). Using marker analysis, Fu *et al*, concluded that spinal cord ependymal cells in mice and chicks are derived from Nkx6.1<sup>+</sup> Nkx2.2<sup>+</sup> ventral neuroepithelial cells (64). Nkx6.1 expression is still expressed in the adult ependymal region both by the CSF-cNs (32) and by ependymocytes (64). In the human spinal cords, ependymal cells also express Nkx6.1(53). A common origin of the ependyma region with the region generating oligodendrocytes during development may account for the high propensity of ependyma cells to generate oligodendrocytes *in vitro* and during SCI (23, 67). Nkx6.1 expression is controlled by SHH signalling and its downstream target gli2 during development (64, 68). Likewise, Nkx6.1 expression in the adult ependyma may be controlled by this kind of signalling. In addition to containing cells born during early CNS development, recent birth-dating experiments using BrdU have indicated that a substantial number of cells in the ependymal region are produced in rats around E18 but also post-natally (32, 62). Regarding the CSF-cNs, there are very few data on the origin of these cells. BrdU incorporation indicates that they are generated between E7-E17 in rats (41) and are not the result of a post-natal or adult spinal cord neurogenesis (20, 32, 41).

### 4.2. Post-natal development

The post-natal development of the ependymal region has been studied in several mammals. In cats, embryonic-like cells remaining in the dorsal part of the central canal after birth disappeared after 2-3 months (61). Compared to adult animals, the ependymal region shows an increased level of several markers (for instance Vimentin and cadherin13) and the presence of few Sox4<sup>+</sup> cells (32, 69) (fig 2). Ependyma aging is associated in the rat with an increase of some carbohydrate expression (70). Cellular proliferation declines until 12-13 weeks corresponding to the end of spinal cord elongation (32, 64). Proliferating ependymocytes, identified by S100 $\beta$  or Nkx6.1 labelling, have been detected by Ki67 staining and BrdU incorporation at 8 and 6 weeks after birth respectively (21, 64). It is likely that a production of new ependymocytes is necessary for extending the length of the central canal. Proliferation in the adult ependyma region has been repeatedly reported but it is unclear if young adult (10 weeks) or true adult animals (>13 weeks) were used (62, 71-74). Variation in animal strain and husbandry conditions (like infections) may also influence the rate of cellular proliferation around the canal.

## 5. STEM AND PROGENITOR CELLS IN THE EPENDYMAL REGION

The persistence of stem cells in the adult spinal cord was reported using adherent or non adherent culture (classical neurosphere assay) conditions in the late nineties (5-7). Using microdissection and cytometry analysis, these cells were found located mainly in the ependymal region (23, 32, 75). Progenitor cells with a more limited proliferation potential are also present in the parenchyma (32, 67, 72, 75, 76). The presence of stem cells around the central canal was suggested by previous work in the early sixties showing a remaining mitotic activity in the

ependymal region (74). Extensive expression analysis performed in this region confirmed the persistence of several markers typically expressed by immature neural cells such as Sox2, CD15, CD133, Nestin, BLBP and PSA-NCAM (Table 1). These can be expressed by most of the ependymal cells (for instance Sox2, CD133) or by cellular subpopulations (for instance Nestin, Dcx, BLBP). The immature feature of these cells is probably linked to the persistence of specific stem cell signaling pathways, such as Notch, epithelial-mesenchymal-transition (EMT) and BMP signalings (32, 72, 73) in the ependymal region. The precise identity of the cells able to form neurospheres, a classical assay for identifying stem cells *in vitro*, is still a matter of debate. Using transgenic mice in which EGFP is placed under the control of the hGFAP promoter, Sabourin *et al* (32) showed that Zeb1<sup>+</sup> GFAP<sup>+</sup> radial glial-like cells, mainly located in the dorsal part of the canal, are able to form passageable multipotent neurospheres, reminiscent of the situation observed for neural stem cells in the SVZ (77). Through a similar approach but with a gene expressed in ependymocytes (FoxJ1, a transcription factor involved in cilium formation), Meletis *et al* (23) provided evidence that stem cells are a subpopulation of ependymocytes. Yet, recent data from the same group suggests that although forebrain ependymocytes are endowed with some stem cell properties (multipotentiality), their self-renewal is limited in contrast to GFAP-derived stem cells (78). So it is likely that similarly to other stem cell niches, the spinal cord central canal region harbors several types of stem and progenitor cells endowed with different capacities to differentiate and self-renew. New markers and new transgenic animals will reveal the complete diversity and potential of the cells around the central canal.

Spinal cord stem cells, identified by their ability to form neurospheres are present along the entire rostro-caudal axis but the lumbar region appears to be enriched for these cells (7). In adult human, Nestin<sup>+</sup> Sox2<sup>+</sup> neurospheres can also be formed by cells derived from the central region of the lumbar spinal cord. However these cannot be passaged suggesting that these cells are more related to progenitors than bona fide stem cells (53). Even more caudally, the *filum terminale*, the terminal end of the spinal cord which is mainly composed of ependymocytes surrounded by axons and glial cells, has also been recently shown to be a source of neural stem/progenitor cells. In culture, *filum terminale*-isolated-cells self-renew and proliferate to form neurospheres, and exhibit tripotent differentiation into neurons, astrocytes, and oligodendrocytes (79). Importantly, neurospheres derived from different levels of the adult spinal cord show different differentiation properties (80) and express variable combinations of developmental Hox genes mimicking the Hox gene pattern found in the embryonic spinal cord (32, 58). This means that adult spinal cord stem cells are not equivalent along the rostro-caudal axis which should be taken into consideration to develop stem cell based strategies for spinal cord repair. Along the dorso-ventral axis, neurospheres forming cells are preferentially derived from the dorsal central canal part which is in agreement with the specific presence of immature radial glial like cells in this region (21, 32). Neurospheres derived from the adult

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spinal cord display features of radial glial cells such as the expression of BLBP, RC2 and Glial (81) but also of oligodendrocyte lineage cells (Olig2, NG2, Nkx2.2, PDGFR $\alpha$ , MBP) (32, 81, 82). This coincides with a higher propensity of these cells to differentiate into oligodendrocytes vs neurons *in vitro* (67) but also *in vivo* after spinal cord injury (SCI) (23). Unexpectedly, these neurospheres also have mesenchymal features as evidenced by the expression of smooth-muscle actin (Acta2), fibronectin and several epithelial-mesenchymal transition transcription factors (slug, Zeb1, Zeb 2) (32). Zeb1 was found to be required for neurosphere growth *in vitro*. Importantly, default differentiation of these spinal cord stem cells leads to a predominant GABAergic neuronal phenotype (21, 32), however after exposure to embryonic spinal cord morphogens (SHH and retinoic acid), HB9<sup>+</sup> electrophysiological active motoneurons can be obtained (82, 83). These can also be generated from stem cells isolated from animals with SCI suggesting that these cells retain their motoneuronal differentiation potentiality, at least *in vitro*.

This caudal stem cell niche shares several features with the well-characterized SVZ niche. First the ependymal region is surrounded by an abundant vasculature (Figure 1) and the cellular proliferation within the niche occurs in close proximity to the vessels (21). This is consistent with the now well-described interactions between neural stem/progenitor cells and endothelial cells (the so-called neurovascular niche) (84). Second, the spinal cord niche maintains a pool of radial glial-like cells, mainly dorsally, which express radial glial markers (Nestin, BLBP, CD15). During CNS development, radial glial cells are transient cells which generate neurons or neuronal progenitors notably through asymmetric divisions (85). Thus their persistence in specific regions of the adult CNS may account for the competence of these niches to generate neurons *in vitro* or/and *in vivo*. Third, the spinal cord niche expresses a high level of DAN, an anti BMP protein (32). In the SVZ, ependymocytes express noggin, another BMP antagonist (86). BMPs are stem-cell differentiating factors (87) and it is likely that the expression of BMP antagonists may participate to the maintenance of neural stem cells in adulthood. Fourth, this caudal niche maintains a high level of Notch signalling as evidenced by expression of the Notch1 receptor (72, 88), its ligand Jagged (32) (fig 2) and of Hes1, a key effector of this pathway (32). This signalling has been well described as having a key role in maintaining an adult stem cell pool in the SVZ but also in other non CNS niches (89).

Whereas the role of stem cell niches of the forebrain in memory and learning is being elucidated, functions of the spinal cord stem and progenitor cells remain elusive. In adults, cells within the spinal cord niche are not or slowly proliferating and no associated gliogenesis or neurogenesis has been reported. Proliferation studies hint at a main post-natal function to provide new cells during spinal cord elongation (32). The ependymal region would then enter a quiescent state and maintain a stem/progenitor pool. Interestingly, physical exercise (treadmill training and wheel running) can reactivate proliferation within the niche

and increase Nestin staining (90, 91). Thus as observed in the brain, activation of the spinal cord niche is influenced by behaviour. More work is needed to explore the fate and location of newly formed cells after training. One possibility would be that stem and/or progenitor cells generate new CSF-cNs upon training. Work by Marichal *et al* showed that these cells are at different stages of maturation raising the possibility that they are in a "standby mode" and under some circumstances (e.g., injury or training) may complete their maturation to integrate spinal circuits (41). Physical exercise and locomotor learning induce modifications in the spinal cord (92-96) and one exciting possibility would be that some of the central canal cells can contribute to this plasticity.

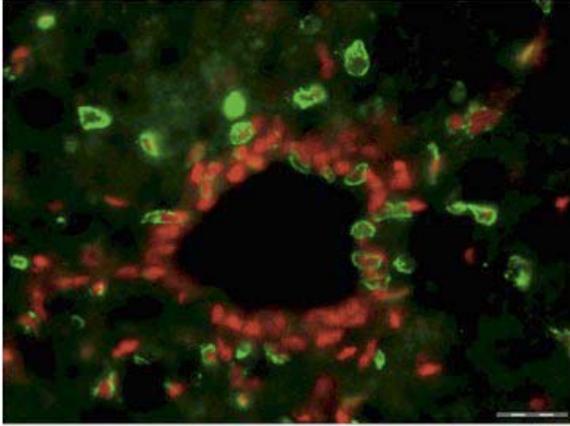
## 6. EPENDYMA IN SPINAL CORD INJURY AND DISEASES

### 6.1. Spinal cord injury

Spinal cord injury is a major cause of irreversible paralysis, with no effective therapy as yet. The demonstration of the presence of a pool of stem/progenitor cells in the adult mammalian spinal cord has led to extensive research aiming at characterizing and recruiting these cells in the context of SCI and cell replacement therapies.

Many earlier studies have described the role of ependymal cells after injury in lower vertebrates such as urodele amphibians, eels and lizards, where successful regeneration is observed (97-99). In the eel, a complete spinal cord transection is followed by an active proliferation, migration and differentiation of the cells surrounding the central canal. They rapidly form a new canal, bridging the rostral and caudal cord portions, suggesting their capacity to self-organize into circular, lumen-containing structures. They also exhibit phenotypical plasticity, with higher Nestin/Vimentin expression and decreased S-100 labeling compared to uninjured tissue, reflecting most likely a new function (98). A similar process is observed during urodele regeneration (97). In the juvenile turtle spinal cord, the presence of a stem cell niche located all around the central canal has also been described (100-102). These cells display properties of neurogenic precursors (they express BLBP and Pax6) and are intermingled with other cells that express early neuronal markers and fire action potentials, indicating they are immature neurons immersed in a neurogenic environment. These endogenous spinal cord precursors are organized in functional domains delimited by Connexin-43.

In mammals, the first images of ependymal cell proliferation in injured spinal cord were obtained around 30 years ago in rats (103, 104) and rabbits (105). Post-injury ependymal cell proliferation (Figure 3) was then confirmed with PCNA/Ki67 markers and BrdU incorporation, in various models of SCI: contusion (106, 107), dorsal hemisection (73, 108), minimal injury model that preserves the integrity of the central canal (109), and compression injury (91, 110, 111). In normal and injured spinal cords, ependymal cell proliferation can be further increased by administration of epidermal growth factor (EGF) and



**Figure 3.** Double immunofluorescent staining for Sox-2 (Rhodamine) and BrdU (FITC) of the central canal region, 7 days after a low thoracic spinal cord compression injury in an adult rat. BrdU was injected once a day during the first 3 days following the lesion. Scale bar: 20 $\mu$ m

fibroblast growth factor 2 (FGF2) which concomitantly promotes functional recovery after SCI (75, 112, 113).

The ependymal region exhibits high post-injury plasticity. Many authors report a significant but transient increase of Nestin, a neural precursor marker, within the ependymocytes after SCI or ischemia (73, 91, 106, 108, 111, 114-116). Cell fate determinants, like BMP4, Msx2, Notch1, Numb, Pax6 and Shh do also exhibit higher expression levels after injury (72, 88), suggesting an attempt to recapitulate developmental features and thus a likely potential of endogenous self-repair capacity.

The fate of ependymal cell progeny as well as its modulation has also been the centre of interest of many studies, aiming at improving behavioral recovery and successful axonal regrowth after SCI. First, it definitively appears that ependymal cells do not undergo apoptosis after SCI, as assessed by TUNEL assay. Secondly, thanks to *in vivo* fluorescent labeling strategy of ependymal cells, allowing their further identification, it is now well established that following SCI, ependymal cells migrate towards the injury site, by a mechanism that may involve the SDF1/CXCR4 signaling which is present in these cells (36) (fig 2). According to some studies, they seem to preferentially differentiate into GFAP<sup>+</sup> astrocytes contributing to scar formation (23, 73, 106, 109) and to some extent into myelinating Olig2 oligodendrocytes (23) making them interesting candidates for myelin degeneration diseases like MS (see below). It thus seems that following SCI, ependymal cells do mostly differentiate into macroglia cells and not into neuronal cells, despite an increased expression of the transcription factor Pax-6 (72, 73, 117). This could be due to the increase of the Notch1 signaling pathway, which may restrict the production of new neurons in the injured spinal cord (72, 88).

### 6.2. Amyotrophic lateral sclerosis

Amyotrophic lateral sclerosis is a neurodegenerative disease characterized by the progressive

loss of motoneurons, leading to progressive paralysis and irretrievably to death. Recent progress in this field has demonstrated that other cell types including astrocytes and microglia contribute to the pathophysiology of this devastating disease (118-120). In this context, stem cell therapy represents an attractive therapeutical approach, given the known pluripotentiality of these cells. However, despite transient beneficial outcomes, these cell transplantations are still confronted to numerous limitations (for review, see (121)).

The existence of neural stem and progenitor cells within the CNS, and the demonstration that pathological processes like those involved in the Alzheimer's or Huntington's diseases promote neurogenesis (78, 122), have recently opened new research perspectives (123). This could also occur in the context of ALS disease and indeed, Chi *et al* (124) provided evidence that motoneuron degeneration stimulates stem and progenitor cell proliferation, migration and neurogenesis in a mouse model of ALS. Interestingly, proliferating cells were mostly described within the ependymal zone of ALS-mice during the onset and the progression stages of the disease. These cells further migrate out of the ependymal zone, to progressively reach the dorsal horns of the spinal cord and, as disease progresses, the ventral horns, where they were preferentially found in the vicinity of dying motoneurons. These results suggest that degenerating motoneurons release factors promoting ependymal cell migration and/or differentiation. The molecular identification of these factors would allow researchers to explore new therapeutical strategies aimed to stimulate *de novo* neurogenesis to fully replace the degenerated neurons in ALS disease.

### 6.3. Multiple sclerosis

Multiple sclerosis (MS) is an inflammatory disease of the CNS characterized by progressive demyelination and axonal degeneration leading to permanent neurological disability. Experimental remyelination strategies represent an important research axis in this pathology. Some of them are based on the proliferative response of endogenous NG2<sup>+</sup> oligodendrocyte precursor cells (OPCs) following demyelination lesion induced either via ethidium bromide injection (125) or in MOG-EAE model of MS (126). But OPCs might not be the only interesting endogenous cell type to be recruited for remyelination. Indeed, ependymal cells in the spinal cord have been shown to re-express RC1, a radial glial antigen, in an experimental model of ethidium bromide-induced demyelination. These RC1<sup>+</sup> cells give rise to a fraction of RC1<sup>+</sup> perilesional astrocytes, which are necessary for oligodendrocyte maturation and spinal cord remyelination (127). In addition, in another model of chemical-induced demyelination, the proliferation of ependymal cells can be increased by exogenous administration of the Shh protein, a key glycoprotein implicated in oligodendrogenesis (128). More recently, newly-formed cells with neuronal like appearance have been observed in the spinal cord of EAE-rats. These cells, identified as being derived from the ependyma, proliferate, migrate towards the neuroinflammatory area and differentiate into cells expressing the neuronal markers  $\beta$ -

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III tubulin and Neu-N. They moreover exhibit electrical activity, as they fire action potentials similar to those of immature neurons. The fact that these cells express specific neuronal markers and do incorporate BrdU confirms that they are likely to be newborn neurons (129).

### 7. ONLINE GENE EXPRESSION DATABASES FOR CHARACTERISATION OF THE SPINAL CORD NICHE

Further analysis is needed to characterize the spinal cord niche. The diversity of cells, their interactions and the specific signalings acting to maintain a pool of stem/progenitor cells are still ill-defined. Besides, there is a need for defining specific markers or a combination of them to readily purify and study the different cell types notably through the generation of fluorescent transgenic mice. This can be done by performing one-by-one gene analysis in the niche using immunohistology and *in situ* hybridization. Yet, the rapid development of CNS gene expression databases can greatly assist in this time-consuming but essential task. These databases are based on collections of brain and spinal cord sections where the gene expression pattern is revealed by *in situ* hybridization (Allen brain [www.brain-map.org](http://www.brain-map.org), BGEM [www.stjudebgem.org](http://www.stjudebgem.org), Geneatlas [www.geneatlas.org](http://www.geneatlas.org)) and by GFP expression in transgenic mice bearing BAC promoters (Gensat [www.gensat.org](http://www.gensat.org)). SAGE libraries have also been generated from different CNS regions (Mouseatlas [www.mouseatlas.org](http://www.mouseatlas.org)). Image quality is high enough to screen at the cellular level the expression of genes in specific CNS regions. To illustrate how these tools can help get highly relevant information, we screened the GENSAT database for genes expressed in the spinal cord ependymal region (8). We could confirm many published observations such as the restricted expression of Nestin by dorsal and ventral cells (21), the strong expression of Jagged by most ependymal cells (32), the restricted expression of Dcx by CSF-cNs (32, 36, 41) and of Sox 4 by unknown ependymal cells (32) (fig 2). More interestingly, we observed the expression of the Wnt receptor Fzd3 by ependymal cells and of the strong expression of the Wnt7a ligand by unknown cells located dorsally around the central canal. CXCR4, the receptor for SDF1 was strongly expressed in the ependymal region. Several cytokines and morphogens were also identified as being expressed by specific cell types. BMP6 is expressed by dorsal and ventral radial cells, SHH is expressed by ventral ependymal cells, GDF10 by dorsal ependymal cells, Mdk (midkine) by few dorsal radial cells, Nrtn (neurturin) by subependymal cells surrounding the canal, most probably CSF-cNs. These expression patterns highlight the complexity of the central canal region and show the maintenance in adult of several embryonic morphogens (Wnt, SHH, BMP) as observed in other adult niches. Furthermore they confirm the dorso-ventral dissymmetry of the ependyma region (21, 32) as some genes are mostly expressed in the dorsal or ventral part (Shh, Mdk, Gdf10, Rbp1). These results are generated by high throughput analysis which could lead to artefacts thus they need to be replicated and confirmed by other techniques. However, they constitute a robust basis to

explore further the cellular diversity and signalings within the spinal cord niche.

### 8. CONCLUSION

The spinal cord stem cell niche constitutes an original model to study how and why stem and progenitor cells are maintained in the adult CNS. This niche shares common features with the brain niches (cellular diversity, highly organised structure, maintenance of embryonic signalings, close interaction with vessels, activation upon training) but display also specific traits such as the absence of associated glio- and neuro-genesis in the normal situation. The comparison, at the molecular, cellular and architectural levels, of neurogenic and non neurogenic niches could generate important clues to unravel the mechanisms governing adult gliogenesis and neurogenesis. As in the brain, this caudal CNS niche appears to contain stem cells, proliferation-restricted progenitors and differentiated cells with different identities. The purification of these cells and their molecular comparison would provide meaningful insight to untie the gene networks underlying the distinct properties of these cells.

Besides being an interesting model for studying adult stem cells, the rapid activation of the ependymal region in several spinal cord lesions call for a better and thorough characterization of this region. The mechanisms underlying the proliferation, delamination, and migration of ependymal cells toward the lesion site need to be analysed. Equally, how these cells contribute to the glial scar and oligodendrogenesis should be dissected out so as to influence the fate of these cells toward spinal cord regeneration. Although adult spinal cord stem cells are still able to generate neurons *in vitro*, especially motoneurons (82, 83), they do not appear to do so upon spinal cord damage. Thus mechanisms that biased their differentiation toward gliogenesis at the expense of neurogenesis should be explored further to redirect their fate.

So far, the spinal cord central canal region has usually been considered as a homogenous cell layer, which is not the case. The activation of this region in spinal cord lesion is now well documented, but conversely very little is known about the specific behaviour of the different cell types encountered in this region (Figure 1), for instance the CSF-cNs. The rapid development of fluorescent or cre-recombinase-expressing transgenic mice which target specific cell subpopulations will give very significant insight on these open issues.

Finally, most of our knowledge on adult neural stem cells is based on work performed in rodents. However there are important anatomical and cellular differences between these species especially as regards the niche architecture and the presence of bona fide stem cells (53, 57). Although more complicated due to ethical and availability constraints, further work on the human CNS should be performed to move forward the possible use of these endogenous stem/progenitor pools into clinical applications for ALS, MS or SCI.

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**Abbreviations:** ALS: amyotrophic lateral sclerosis, BLBP: brain lipid binding protein, CNS: central nervous system, CSF: cerebrospinal fluid, CSF-cN: cerebrospinal fluid contacting neurons, DRG: dorsal root ganglion, EGF: epidermal growth factor, FGF: fibroblast growth factor, GFAP: glial fibrillary acidic protein, HuC/D: human neuronal protein, MS: multiple sclerosis, PSA-NCAM: poly-sialylated neural cell adhesion molecule, SCI: spinal cord injury, SDF1: stromal-derived factor 1, SSH: sonic hedgehog, SVZ: sub-ventricular zone, VIP: vasoactive intestinal polypeptide

**Key Words:** Spinal cord, Neural stem cells, Ependyma, Ependymal region, Spinal cord injury, Central canal, Multiple sclerosis, Amyotrophic lateral sclerosis, Review

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