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Cellular and Molecular Characterization of Multipolar Map5-Expressing Cells: A Subset of Newly Generated, Stage-Specific Parenchymal Cells in the Mammalian Central Nervous System

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Abstract

Although extremely interesting in adult neuro-glio-genesis and promising as an endogenous source for repair, parenchymal progenitors remain largely obscure in their identity and physiology, due to a scarce availability of stage-specific markers. What appears difficult is the distinction between real cell populations and various differentiation stages of the same population. Here we focused on a subset of multipolar, polydendrocyte-like cells (mMap5 cells) expressing the microtubule associated protein 5 (Map5), which is known to be present in most neurons. We characterized the morphology, phenotype, regional distribution, proliferative dynamics, and stage-specific marker expression of these cells in the rabbit and mouse CNS, also assessing their existence in other mammalian species. mMap5 cells were never found to co-express the Ng2 antigen. They appear to be a population of glial cells sharing features but also differences with Ng2+progenitor cells. We show that mMap5 cells are newly generated, postmitotic parenchymal elements of the oligodendroglial lineage, thus being a stage-specific population of polydendrocytes. Finally, we report that the number of mMap5 cells, although reduced within the brain of adult/old animals, can increase in neurodegenerative and traumatic conditions.

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Introduction

Parenchymal progenitors have become a hot research topic in neural plasticity since they represent intriguing players in adult neuro-glio-genesis and a promising source of endogenous elements for repair [1,2,3]. Most of them display neural developmental markers of the glial lineage, in the postnatal and adult central nervous system (CNS) being committed to the oligodendrocyte lineage and expressing a chondroitin sulfate proteoglycan (Nerve/glial antigen 2, Ng2; referred to as Ng2+cells [1,4,5]). The Ng2+cells are generally considered as synantocytes [6] or polydendrocytes [5], endowed with multiple functions in physiology and pathology which are still far from being utterly elucidated. A proportion of these cells persist in the adult CNS in a phenotypically immature form [1,5,7], most of which do continue to proliferate throughout life, thus being considered the main cycling population of the mature mammalian CNS [8]. Although parenchymal progenitors physiologically produce mainly glial cells [2], in some mammals/regions they can undergo spontaneous neurogenesis, e.g., in the rabbit striatum [9] and cerebellum [10]. Yet, also in the case of neuronal-committed cells, the primary progenitors remain poorly identified, in contrast with their progeny which is far more visible and characterized in its phenotype [9,10]. The strong interest in better understanding parenchymal progenitors crashes against the many aspects which remain obscure about their identity, real nature, and physiology. Among these problems, a scarce availability of stage-specific markers along with a high heterogeneity linked to different variables (species, age, anatomical region, etc.), make the identification of subpopulations a hard task. More sneakily, what appears difficult is the distinction between real cell populations and various differentiation stages of the same population.

We have recently described a subset of glial-like cells immunoreactive for the microtubule associated protein 5 (Map5) in the rabbit cerebellum [10]. These cells show a morphology (ramified, multipolar) and a molecular signature (e.g., Olig2 expression) reminiscent of synantocytes/polydendrocytes, and some of them are newly generated within the mature cerebellar parenchyma [10]. Intriguingly, they express a cytoskeletal-associated molecule which is typically found in neurons (see Table 1). The Map5 molecule [33], also referred to as Map-1B [17], Map1X [34], or Map1.2 [35], belongs to a family of large and fibrous microtubule associated proteins (Maps) and shows a very wide range of expression in the CNS (summarized in Table 1). Map5 is the first Map detectable in neurons of the developing nervous system [36,37], expressed at high levels in growing axons/growth cones and usually downregulated after cessation of axonal growth [25,38] (reviewed in [39,40]). Nevertheless, the protein

remains expressed in the whole CNS during adulthood, its phosphorylated form reaching high levels within some regions endowed with plasticity [11,12,19], or under conditions that elicit axonal/synaptic plasticity in relation to physiological conditions and in response to injury [11,41,42,43]. Map5 has also been implicated in a number of neurological disorders, such as fragile X syndrome [44,45], giant axonal neuropathy [46] and Alzheimer disease [47,48].

Map5 expression is not restricted to neuronal populations [27,28,29,30,32]. Only limited, heterogeneous information is available concerning its localization in other cell types (Table 1). It has mainly been reported in oligodendrocytes and Schwann cells that produce myelin in the central and peripheral nervous system [28,41]. In particular, it is elevated in oligodendrocytes that initiate ensheathment of axons in the normal brain [27], and in Schwann cells during development and nerve regeneration [41]. Map5 is generally absent in astrocytes, although its expression in some subtypes, e.g., type1 astrocytes [29], is still controversial. Since most of these studies were carried out in culture [28,29,30,31,32], very little is known about the glial localization of Map5 in vivo.

Here we focused on the distribution of Map5 in the CNS of different mammalian species, with particular reference to a population of multipolar, glial-like cells (here referred to as mMap5 cells) in young/adult mice and rabbits. We characterized their morphology, phenotype, regional distribution, and stage-specific marker expression, as well as their occurrence in other mammals. Finally, we investigated the behavior of mMap5 cells in traumatic and neurodegenerative injury conditions in mice.

Results

Distribution of microtubule associated protein 5 (Map5) in neuronal and glial cell populations of the rabbit and mouse CNS

Firstly, we tested the cellular and regional distribution of Map5 in mature neurons of the rabbit and mouse CNS to check if the staining obtained with our antibodies was consistent with previous reports. The pattern of expression of Map5 in vivo, was analyzed at three different ages (3.5, 12, and 36 months) in rabbits, and two (40 days and 3 months) in mice, corresponding to peri-puberal and adult ages. Consistently with previous reports, Map5 was largely present in most CNS neurons (see references in Table 1 and Fig. 1A). This neuronal staining was detectable in apical dendrites and cell bodies but the nuclei were immunonegative. Large projection neurons, such as cerebral cortex pyramidal neurons, cerebellar Purkinje neurons, and spinal cord motorneurons were heavily immunostained for Map5 (Fig. 1A, top). As previously described in rodents [11,16], cerebral cortex layers II, III, V were more intensely labeled (not shown) and staining in the olfactory bulb was restricted to the olfactory nerve and external plexiform layers (Fig. S1). No qualitative differences in the amount and distribution of Map5 in neurons were detectable at the different ages explored (see below for further age-related features).

In the whole CNS, the polyclonal anti-Map5 antiserum employed in our study and recognizing both unphosphorilated and phosphorilated isoforms, can be used interchangeably on rabbit and mouse tissues, yielding an identical staining pattern (Fig. S1). Once assessed the reliability of staining in mature neurons, we observed that Map5 is strongly expressed in several populations of neuroblasts which persist in the postnatal and adult brain (Fig. 1A, middle and bottom). They include newly generated neuronal precursors of germinal layer-derived neurogenic sites (the forebrain subventricular zone, SVZ, and the dentate gyrus of the hippocampus), neurogenic parenchymal progenitors of the rabbit striatum [9], and chain of neuroblasts in the transitory subpial layer (SPL), a secondary germinative zone of the postnatal rabbit cerebellum [49] (Fig. 1A). By contrast, Map5 is not present in immature or differentiating neurons, such as the PSA-NCAM+/DCX+ immature neurons in the piriform cortex (with rare exceptions; see Fig 1A, bottom) and the PSA-NCAM+/DCX+ newly generated neurons in the rabbit cerebellum (green arrowheads in Fig. 1A).

Beside its wide distribution in neuronal cells, Map5 is also detectable in a population of multipolar, glial-like cells widespread in most CNS grey and white matter (here referred to as mMap5 cells in order to distinguish them from the Map5+neuronal elements; Fig. 1B and red arrows in panel A). These cells, whose shape is reminiscent of parenchymal progenitors known as synantocytes/polydendrocytes [5,6], were far more visible in the rabbit than in mouse, in the latter the ramified, multipolar processes appearing not completely stained (Fig. 1B). In order to assess this difference we quantified the total length of mMap5 cell processes in the grey (cerebral and cerebellar cortex) and white matter (corpus callosum) of the two species (Fig. S2A). Results indicated that rabbit mMap5 cell process ramifications are longer in both grey matter regions analyzed (cerebral cortex: rabbit 285,7±23,5 μm versus mouse 182,1±15,6 μm, $P<0,05$; cerebellar cortex: rabbit 485,9±29,5 μm versus mouse 127,1±12,3 μm, $P<0,005$), whereas they are similar within the corpus callosum (rabbit 93,7±0,5 μm versus mouse 57,0±13,5 μm, $P>0,05$). Such difference was more prominent in the cerebellar cortex with respect to cerebral cortex.

Morphologic and phenotypic characterization of mMap5 cells

In immunocytochemical specimens, multipolar Map5+cells (mMap5 cells) and Ng2+cells showed a similar, yet not identical, morphology (Fig. 2A). Processes revealed with Map5 are quite smooth, without the beads, swelling and varicosities that characterize the fine processes of Ng2+cells [1] (see Fig. 2A and Discussion). Since observations carried out on mMap5 cells in different CNS regions revealed various shapes, we performed a quantitative analysis of cell somata diameters, by measuring their minimum (min) and maximum extent (max) in mMap5 cells and Ng2+cells, within three brain regions (cerebral cortex, cerebellar cortex and corpus callosum; Fig. S2B). Comparison of the data obtained revealed the following trend: mMap5 cell somata were more frequently round-shaped in the cerebellar cortex (min 8,3±0,6 μm; max 9,4±0,5 μm; $P>0,05$), and, to a lesser extent, in the cerebral cortex (min 7,9±0,8 μm; max 10,3±0,8 μm; $P<0,001$), whereas Ng2 cell somata were more elongated (cerebral cortex: min 5,8±1,0 μm; max 10,4±1,2 μm; cerebellar cortex: min 5,9±0,9 μm; max 10,8±1,2 μm; $P<0,001$ in both regions). On the other hand, somata of both cell types were prevalently elongated in the white matter of the corpus callosum (mMap5 cells: min 5,8±1,1 μm; max 11,3±2,1 μm; $P<0,005$, and Ng2+cells: min 5,8±1,0 μm; max 10,7±1,5 μm; $P<0,005$).

Double staining with NeuN, β-tub, GFAP and Iba1, consistently excluded their neuronal, astroglial or microglial nature (Fig. 2B). This fact, along with morphological features described above, confirmed that mMap5 cells strongly resemble to a population of glial-like, synantocyte/polydendrocyte-like cells. They were widely distributed in all regions examined, interspersed among the Ng2+cells (Fig. 2C; detectable in mice but not in rabbits with immunocytochemistry, see Table 2 and [10]. As to their amount, mMap5 cells were far less numerous than the Ng2+cells, with an average proportion of 1:30 (semi-quantitative

Table 1. Distribution of Map5 in the mammalian CNS as described in literature.

Species/region/method	Age	Cell type/distribution	Function	Refs
Neuronal				
Rat CNS	P0–P20, adult	most neurons (complementary to Map1A)	developmental/adult plasticity	[11]
	P3–P25, adult	most neurons	adult plasticity	[12]
Rat CNS and PNS	2,5 months	most neurons	plasticity, regeneration	[13]
Rat PNS	E18–P30, adult	peripheral nerves	axonal growth in nerve regeneration	[14]
Rat brain	3–24 months	most neurons		[15]
	adult	synaptic localization	synaptic plasticity	[16]
Rat brain and cultures	newborn	most neurons, some glial cells		[17]
Rat spinal cord	E9-postnatal	neuroblasts	morphogenic events	[18]
Rat olfactory bulb	adult	olfactory nerve, mitral and periglomerular cells	neurite outgrowth	[19]
Mouse brain	P5–P90	barrel cortex neurons	Plasticity	[20]
Mouse brain and cultures	E16	neurons, neuroblasts		[21]
Cat brain	P3–P28, adult	most neurons	axon/dendritic growth, microtubules stabilization	[22]
Human brain	13GW-17 years	most neurons	axonal growth	[23]
	18–33GW	ganglionic eminence, axonal bundles	axonal growth	[24]
Rat cell cultures	newborn	symphatethic neurons	axon extension	[25]
Mouse Map1b mutant cell cultures	E18–19	hippocampal pyramidal neurons	axon formation	[26]
Glial				
Rat brain and cultures	P7-adult	immature oligodendrocytes	myelination	[27]
Primary glial cultures		oligodendrocytes		[28]
		oligodendrocytes and type1 astrocytes		[29]
		oligodendrocytes, (negligible in astrocytes)	Formation/stabilization of myelin-forming processes	[30,31]
CG4 cell line		oligodendrocytes		[32]

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evaluation on 407 Ng2+cells counted in 9 confocal images, from 3 animals, each containing at least one mMap5 cell; Fig. 2C). Double staining with Map5 and Ng2 in mouse did not reveal any overlapping (Fig. 2D), thus indicating that mMap5 cells are not a subpopulation of Ng2 progenitors (although this fact does not exclude they belong to the same lineage at a different differentiative stage).

To further study the phenotype of the mMap5 cell population, double and triple staining with different antigens of progenitor cells and oligodendroglial lineage differentiation stages were performed (Fig. 3). In all CNS regions examined the mMap5 cells were double stained with nuclear transcription factor Olig2 (Fig. 3), thus corresponding to elements of the oligodendroglial lineage. By using Olig2/Map5 double staining as a basis for recognizing the mMap5 cell population, the percentage of co-expression with other antigens were calculated (histograms in Fig. 3B). In addition to Olig2, the vast majority of the mMap5 cells do co-express the nuclear transcription factor Sox10, and a substantial proportion also express Sox2 and Sox9 (Fig. 3B; raw data in Fig. S3). The pattern of marker expression strongly indicates that mMap5 cells might represent a specific stage of maturation along the oligodendroglial lineage, even in the absence of overlapping between the Map5 and Ng2 markers. This is confirmed by the fact that a proportion of mMap5 cells (4–5% in cerebral cortex, 8 Map5+/GPR17+cells out of 179 Map5+cells, and corpus callosum of the adult mouse, 5 Map5+/GPR17+cells out of 122 Map5+cells; 56–84% in cerebellar grey matter, 55 Map5+/GPR17+cells out of 98 Map5+cells, and white matter, 42 Map5+/GPR17+cells out of 50 Map5+cells of the peripuberal rabbit) is immunoreactive for the new Ng2 cell marker GPR17 receptor [52] (Fig. 3B and S3), which has been shown to be present in 25–30% of Ng2+ cells in mice [53]. In most double staining GPR17 appear to label the membranes of the whole process ramifications, as previously described in more mature, premyelinating stages [53].

Finally, we performed a careful analysis on double staining with Map5 and RIP (a monoclonal antibody which recognizes the CNPase specific of mature oligodendrocytes; [54]. In most specimens, there was no co-localization (Fig. 3C), although a faint expression of Map5 was detectable in some oligodendroglial cells (Fig. 3C'). This suggests that Map5 expression precedes oligodendrocytes terminal differentiation.

Experiments with cell proliferation antigens and BrdU pulse labeling followed by different survival times

Since mMap5 cells display most antigens expressed at different stages of the oligodendrocyte precursor cell lineage [55,56], we performed a set of experiments with cell proliferation markers in order to gain more insight on their putative origin from cycling progenitors. Double staining carried out in different CNS areas with Map5 and Ki67 antigen (a cyclin expressed in cells during the G1, S, G2 and M phases, but not in G0, thus in all proliferating

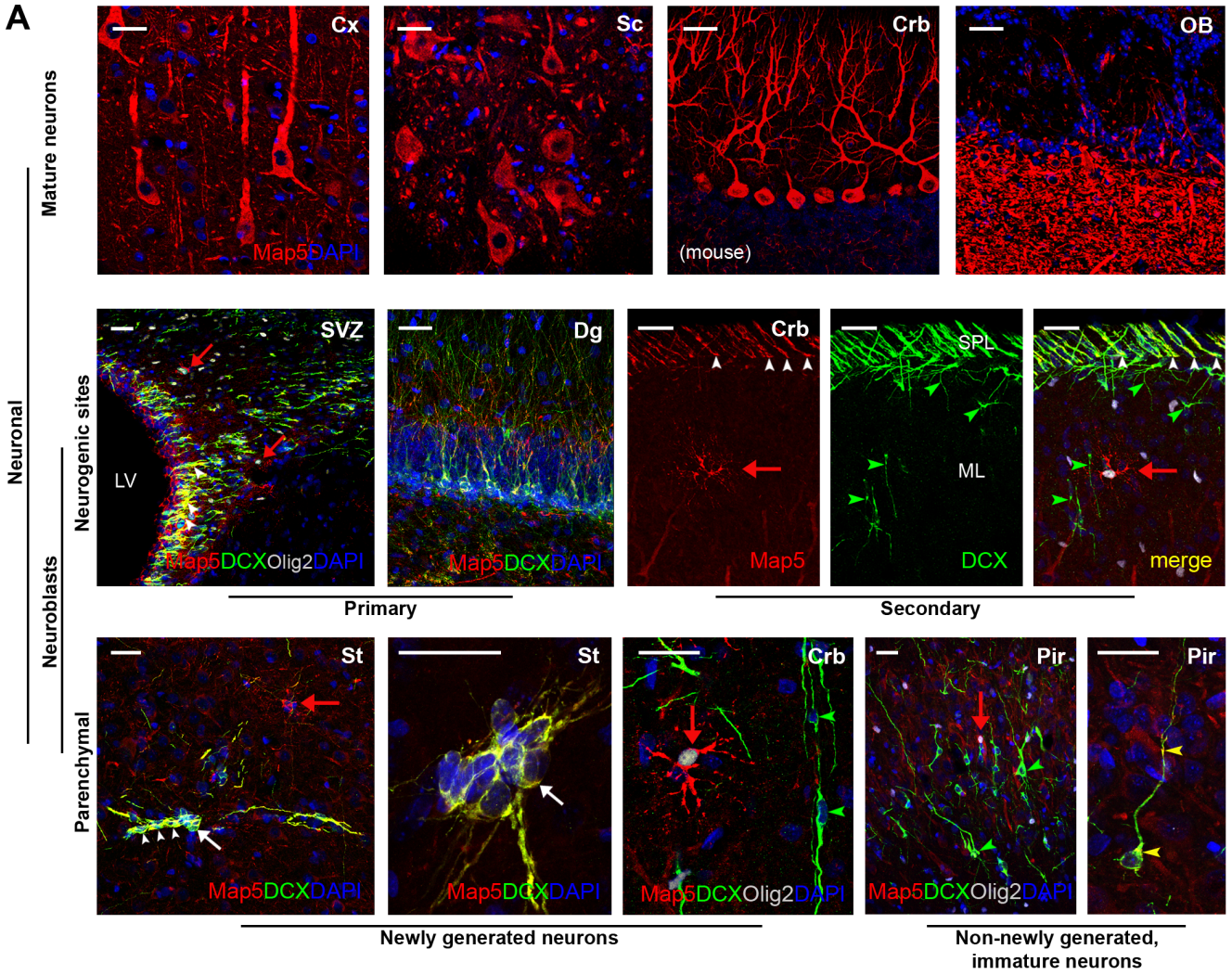

Figure 1. Map5 distribution in neuronal and glial cells of the rabbit and mouse CNS. A, Map5 is abundant in most populations of mature neurons (top), neuroblasts occurring in germinal layer-derived neurogenic sites or transitory germinative zones (e.g., rabbit subpial layer, SPL; middle), and in neural progenitors of the brain parenchyma (e.g., rabbit striatum, St; bottom, left). White arrows: clusters of newly generated neuroblasts; white arrowheads: chains of neuroblasts. Newly generated neurons in the cerebellum (Crb) and immature neurons of the piriform cortex (Pir) are generally Map5-negative (green arrowheads; bottom, right), apart from some immature neurons which show low level of Map5 staining (yellow arrowheads). Micrograph in panel A are from peripuberal and adult rabbit tissue, except those marked for mouse. For the Map5 staining on the SVZ ependymal wall, see Fig. S4B. B, In addition to its neuronal localization, Map5 decorates a population of multipolar cells with the morphology