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Polydendrocyty a jejich úloha v CNS
Polydendrocytes and their role in CNS

Bakalářská práce

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Praha, 2014

Prohlášení:

Prohlašuji, že jsem bakalářskou práci zpracovala samostatně a že jsem uvedla všechny použité informační zdroje a literaturu. Tato práce ani její podstatná část nebyla předložena k získání jiného nebo stejného akademického titulu.

V Praze, dne 16. dubna 2014

Poděkování

Ráda bych poděkovala vedoucí své bakalářské práce Ing. Miroslavě Anděrové, CSc. za ochotu, trpělivost a cenné rady, které mi při vypracování bakalářské práce pomohly.

Abstrakt

Polydendrocyty ($NG2^+$ buňky) jsou nově objevený typ gliových buněk v centrálním nervovém systému (CNS) lišící se od neuronů, oligodendrocytů, astrocytů a mikroglií. Polydendrocyty bývají identifikovány zejména na základě exprese NG2 proteoglykanu a alfa typu receptoru pro růstový faktor odvozený z krevních destiček. Nacházejí se v šedé i bíle hmotě a představují největší populaci buněk schopných proliferace v dospělém CNS. Všeobecně je přijímán názor, že subpopulace polydendrocytů dává vznik oligodendrocytům a to nejen ve vývoji, ale i v dospělém CNS. Některé polydendrocyty mohou navíc dávat vznik protoplasmatickým astrocytům v průběhu embryogeneze a *in vitro* studie prokázaly, že polydendrocyty mohou diferencovat v neurony a astrocyty. Elektrofyziologické studie ukázaly, že polydendrocyty vytváří synaptická spojení s neurony a je možné, že neurony skrze synapse dokáží ovlivňovat jejich schopnost proliferace. Polydendrocyty jsou významné ve studiu remyelinizace po ischemickém poškození a u nemocí souvisejících se ztrátou myelinu, a to především proto, že jsou považovány za zdroj nových oligodendrocytů a i jiných gliových buněk. Tato práce shrnuje základní informace o polydendrocytech. Nejdříve se zaměřuje na markery a na morfologii polydendrocytů a shrnuje znalosti o jejich vývoji a diferenciačním potenciálu. Pozornost je věnována také jejich buněčné fyziologii a na závěr je diskutována úloha polydendrocytů v remyelinizaci.

Klíčová slova: polydendrocyt, $NG2^+$ buňka, gliová buňka, oligodendroglie, prekurzorová buňka oligodendrocytu, NG2 proteoglykan

Abstract

Polydendrocytes ($NG2^+$ cells) are recently discovered glial cells in central nervous system (CNS) distinct from neurons, oligodendrocytes, astrocytes and microglia. Polydendrocytes could be identified mainly by the expression of the proteoglycan NG2 and platelet derived growth factor receptor alpha. They could be found in grey and white matter and represent the largest proliferating cell population in adult CNS. It is accepted that a subpopulation of polydendrocytes gives rise to oligodendrocytes not only in development, but also in adult CNS and after demyelination. A subpopulation gives rise also to protoplasmic astrocytes in embryonic development. In *in vitro* studies was observed that neurons and astrocytes may arise from polydendrocytes. Electrophysiological studies revealed that polydendrocytes form synapses with neurons and that their rate of proliferation could be controlled this way. Polydendrocytes are very important in study of remyelination after ischemia and demyelinating diseases, as they might serve as source of new oligodendrocytes or possibly of another glial cells. This thesis summaries general knowledge about polydendrocytes. Initially, I focus on their immunohistochemical markers and morphology. Next, I summarise findings about their development and fate in both embryonic and adult CNS. A bit more focus is given on their physiology and at the end I give information about their possible role in remyelination.

Keywords: polydendrocyte, $NG2^+$ cell, glial cell, oligodendroglia, oligodendrocyte progenitor cell, NG2 proteoglycan

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Abbreviations

A2B5 - plasma membrane ganglioside

Aldh1L1 - aldehyde dehydrogenase 1 family member L1

AMPA - α-Amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid

AN2 - glycoprotein, mouse homologue of NG2 glycoprotein

BMP - bone morphogenetic protein

BrdU - 5-bromo-2'-deoxyuridine, synthetic analogue of thymidine

CC1 - oligodendroglial differentiation marker

CNPase - 2',3'-cyclic-nucleotide 3'-phosphodiesterase

CNS - central nervous system

Cspg4 - chondroitin sulphate proteoglycan 4

DCX - doublecortin, neuronal migration protein

DNA - deoxyribonucleic acid

E - embryonic day

ECM - extracellular matrix

EGFP - enhanced green fluorescent protein

FCS - fetal calf serum

FGF - fibroblast growth factor

GABA - gamma-aminobutyric acid

GC - galactocerebroside

GFAP - glial fibrillary acidic protein

GluR2 - glutamate receptor subunit 2

GRIP1 - glutamate receptor interacting protein 1

K_A - A-type potassium channel

K_{DR} - delayed rectifying potassium channel

K_{IR} - inwardly rectifying potassium channel

MAG - myelin associated glycoprotein

MBP - myelin basic protein

MCAo - middle cerebral artery occlusion, a model of ischemic stroke

MUPP1 - multi PDZ protein 1

NeuN - neuronal nuclei, neuron-specific nuclear protein

NG2 - neuron/glia antigen 2, chondroitin sulphate proteoglycan

Nkx2.2 - homeodomain transcription factor

NMDA - N-methyl-D-aspartate

O-2A - oligodendrocyte-Type-2 astrocyte progenitor cell

O4 - oligodendrocyte marker

Olig1 - oligodendrocyte transcription factor 1

Olig2 - oligodendrocyte transcription factor 2

OPC - oligodendrocyte progenitor cell

P - postnatal day

P2X - ATP-activated purinoceptor 2

P2Y - ATP-activated purinoceptor 2

PCNA - proliferating cell nuclear antigen

PDGF - platelet derived growth factor

PDGFR - platelet derived growth factor receptor

PDZ - structural domain found in the signalling proteins

PLP - proteolipid protein

PNS - peripheral nervous system

S100β - β-subunit of Ca^{2+}-binding protein

Sox10 - SRY (sex determining region Y)-box 10

SVZ - subventricular zone

TF - transcription factor

TTX - tetrodotoxin

1 Introduction

In the past, the term "glial cells" indicated three cell types – astrocytes, oligodendrocytes and microglia. From 1980s, when polydendrocytes have been discovered, we can assume that there is an additional – fourth – glial cell type. Polydendrocytes are widespread population of mitotically active cells in adult central nervous system. Recent evidence has demonstrated that indeed polydendrocytes give rise to myelinating and nonmyelinating oligodendrocytes, but also to subpopulation of astrocytes during development. The discovery of synaptic association between polydendrocytes and neurons has turned attention toward this cell population. Nowadays, the aim is not only to discover their differentiation potential, but also to find out more about their role in neural network.

In my thesis, I summaries the general knowledge about polydendrocytes and their properties and functions in CNS during physiological and also pathological conditions. Firstly, I focus on polydendrocytes identification, namely their immunohistochemical markers and morphology. Further, I describe the origin and the fate of polydendrocytes *in vivo* and *in vitro* and factors that contribute to their fate decision are also mentioned. Next, I concentrate on cell physiology of polydendrocytes and on their role in neural network. The role of polydendrocytes in myelin repair is highlighted in the final section.

2 The discovery of polydendrocytes

The discovery of polydendrocytes was made in the early 1980s, when Stallcup and co-authors employed a rabbit serum raised against rat brain tumour cells, whose properties were neither typically neuronal nor glial. One of the epitopes recognised by the serum was chondroitin sulphate proteoglycan 4 (also termed NG2 proteoglycan), the product of *Cspg4* gene [1]. That is why polydendrocytes are also called $NG2^+$ cells as will be used here. The word polydendrocyte was employed in order to describe in one word their multi-processed morphology and their commitment to oligodendrocyte lineage [2]. Currently, there is no doubt about their commitment to oligodendrocyte lineage [3,4], however, whether they are progenitors of other cells in central nervous system (CNS) *in vivo* is still a matter of debate. Immunohistochemical analyses employing NG2 antibody together with antibodies recognizing other markers, such as astrocytic glial fibrillary acidic protein (GFAP), neuronal markers (tetanus toxin) and galactocerebroside (GC, marker of oligodendrocytes), revealed that NG2 is not coexpressed with GC. However, few $NG2^+$ cells were found in population of tetanus-toxin-binding cells and some of them expressed GFAP. Thus, certain subpopulations of $NG2^+$ cells apparently possess some neuronal as well as glial properties [5], which is the

reason why the proteoglycan was termed N (neuron) G (glia) antigen 2 and cells, which express this proteoglycan, were termed $NG2^+$ cells.

3 Identification of $NG2^+$ cells

An important marker for distinguishing $NG2^+$ cells is the receptor for platelet-derived growth factor alpha (PDGFRα) [6–9] (Fig. 1). The PDGFRα binds platelet derived growth factor, a dimeric glycoprotein composed of two A (-AA) chains (PDGF AA), which is a mitotic factor for $NG2^+$ cells and prevents premature differentiation of $NG2^+$ cells *in vitro* [7]. Besides $NG2^+$ cells, also pericytes (mural cells) express NG2 proteoglycan; however, we can distinguish them from $NG2^+$ cells based on their distinct morphology and expression of receptor for platelet-derived growth factor beta (PDGFRβ) instead of PDGFRα [10]. In addition, NG2 was expressed on almost 95% of cells stained with A2B5 antibody, which recognizes a cell surface ganglioside, epitope expressed on oligodendrocyte progenitors. This antibody could be also used as a marker for $NG2^+$ cells [1].

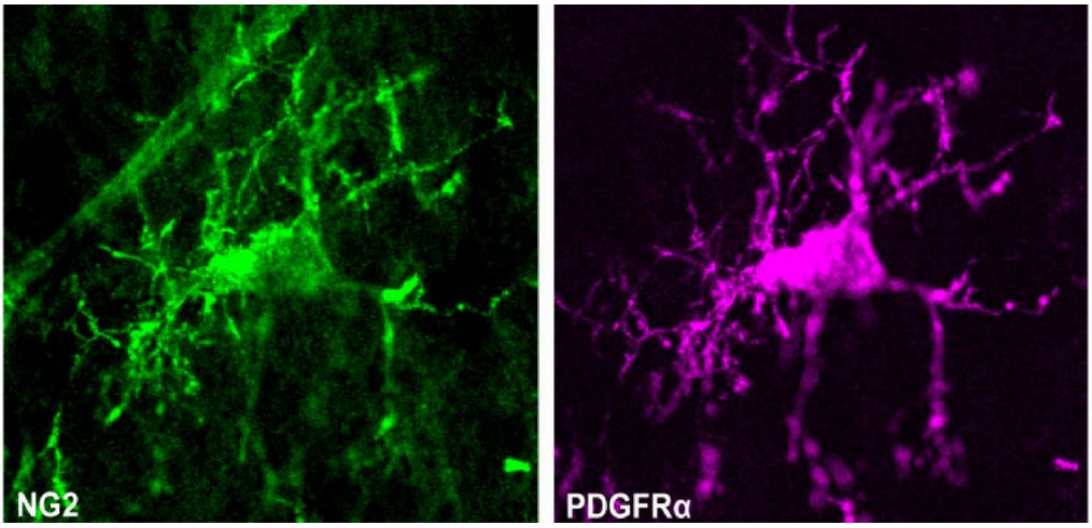

Fig. 1 – Markers of $NG2^+$ cells. (left) Cell immunolabeled for NG2 (right) the same cell immunolabeled for platelet derived growth factor receptor alpha (PDGFRα) (purple). Taken from Komitova et al., 2009 [11].

$NG2^+$ cells comprise 5-10 % of glial cells [12] and their real number depends on the location in the brain. Generally, the white matter contains higher number of $NG2^+$ cells than the grey matter. In peripheral nervous system (PNS), some immature Schwann cells also express NG2 as well as PDGFRα, however, they differ from $NG2^+$ cells in CNS. They do not

express oligodendrocyte transcription factor 2 (Olig2), nevertheless, some of them may originate from $NG2^+$ cells in CNS [13].

4 The NG2 proteoglycan

The NG2 proteoglycan is a chondroitin sulphate proteoglycan which passes once through the membrane. The rat NG2 proteoglycan has its homologue in the human melanoma proteoglycan [14] or in the AN2 protein in mice [15].

Its cytoplasmic tale is short, comprising 76 amino acids [16] with cytoplasmic C-terminus containing a PDZ-binding motif QYWV (glutamine, tyrosine, tryptophan, valine), by which the proteoglycan can interact with proteins that contain PDZ domain, a structural domain of 80-90 amino acids found in signalling proteins, such as multi PDZ protein 1 (MUPP1), glutamate receptor interacting protein 1 (GRIP1) or syntenin-1 [17–19]. These interactions are supposed to be important as they mediate a link between the proteoglycan and structural or signalling components in the cytoplasm [19]. Additional important amino acids in cytoplasmic domain are three threonines, which could be used as the sites for phosphorylation [16], which can stimulated the cell proliferation and motility [20]. The extracellular domain has 2225 amino acids and contains site for collagen binding V [21] or VI [21,22]. The full-length NG2 proteoglycan has 2325 amino acids and about 600 kDa (core protein hold 300 kDa) [16] (Fig.2).

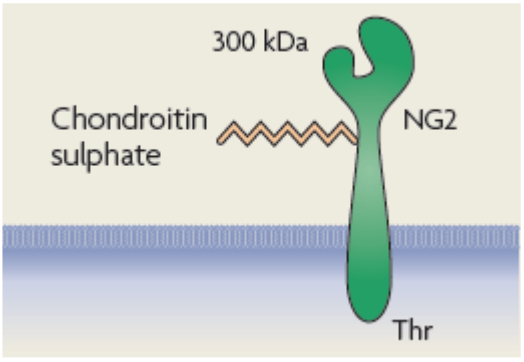

Fig. 2 - Illustration of NG2 proteoglycan. NG2 proteoglycan passes once through the membrane and the core protein has about 300 kDa. Chondroitin sulphate binds proteoglycan on its extracellular domain, while cytoplasmic domain contains threonine residues, sites for phosphorylation. Taken from Nishiyama et al., 2009 [2].

The NG2 proteoglycan has many important roles in $NG2^+$ cells. It is necessary for proliferation, which is activated by the presence of fibroblast growth factor (FGF) and PDGF AA [23]. These growth factors are critical mitogens for $NG2^+$ cells [7,23]. By binding to

actin cytoskeleton, stress fibres and retraction fibres the proteoglycan can regulate the motility and also the morphology of the $NG2^+$ cell [24].

During last decade, an important role of $NG2^+$ glia in CNS regeneration was proposed, however, large number of experiments provide evidence of inhibitory effect of NG2 proteoglycan on neurons [25,26].

5 Morphology of $NG2^+$ cells

Generally, $NG2^+$ cells have darkly stained, irregularly shaped cell bodies with few organelles and fine processes radiated in all directions (Fig. 3); however, there is a slight difference between $NG2^+$ cells in grey and white matter. In grey matter they have stellate morphology, while white matter $NG2^+$ cells have elongated bodies with oriented processes along axonal fibres [27–29]. Electron microscopy revealed that astrocytic processes are often intervened between $NG2^+$ cell and an endothelial cell of the blood vessel [28] as it is with neurons in blood brain barrier.

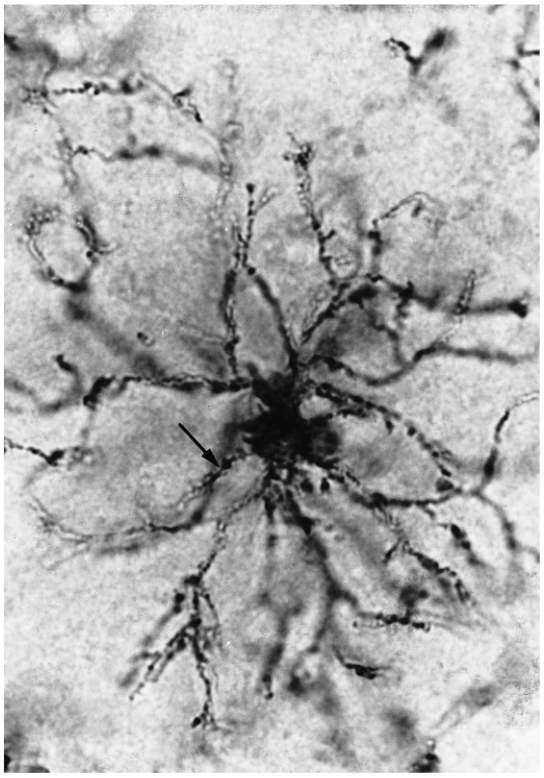

Fig. 3 - Morphology of $NG2^+$ cell. Light microscopy image. Taken from Ong and Levine, 1999 [28].

6 Origin of NG2$^+$ cells and their fate

6.1 NG2$^+$ cells in embryonic development

During embryonic development NG2$^+$ cells arise from neural stem cells in germinal zones for glia and neurons within the spinal cord [27,30,31], ventricular and subventricular zone (SVZ) [8,9,27].

First PDGFRα$^+$/NG2$^-$ cells appear around embryonic day (E) 14 in the motor neuron progenitor domain (pMN domain) in ventral part of the spinal cord, arranged in two columns next to the central canal [8]. The cells migrate and after E16 they appear also in a dorsal part of the spinal cord [30,31]. In ventricular zones, the cells are located in ventral part of lateral ventricles and are also PDGFRα$^+$/NG2$^-$, like those in the spinal cord [9]. They appear around E14 and within a day or two expand, at first in the ventral region and then dorsally [31]. The expression of NG2 proteoglycan is upregulated, when the cell leaves the germinal zone [27] (Fig. 4).

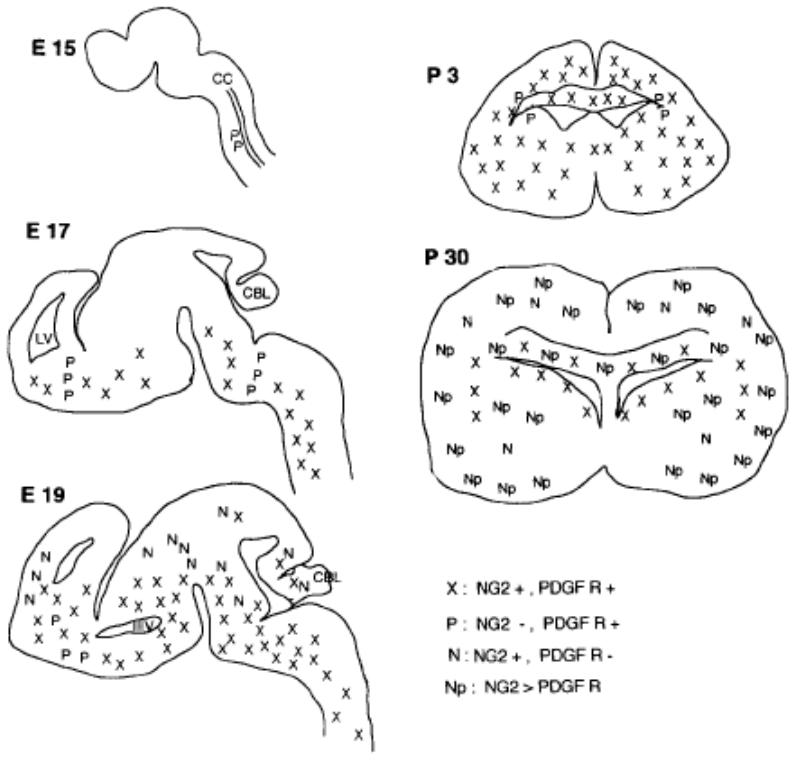

Fig. 4 – Schemes showing the distribution of NG2$^+$ or/and PDGFRα$^+$ cells in developing brain. E15, E17, E19 are sagittal sections. P3, P30 are coronal sections of the cerebrum at the level of striatum. Taken from Nishiyama et al., 1996 [27]. X: PDGFRα$^+$/NG2$^+$ cells, P: PDGFRα$^+$ but NG2$^-$ cells, N: NG2$^+$ but PDGFRα$^-$ cells, Np: cells that express NG2 more than PDGFRα, CC: central canal, CBL: cerebellum, LV: lateral ventricle, IIIV: third ventricle, PDGFRα: platelet derived growth factor receptor alpha, E: embryonic day, P: postnatal day

6.2 The fate of NG2$^+$ cells

One of the most efficient approaches to track the progenies of NG2$^+$ cells is the usage of double-transgenic mice, in which constitutively active Cre recombinase[1] in NG2-expressing cells permanently activates enhanced green fluorescent protein (EGFP) expression, as the expression of markers differs during their maturation.

To examine the progenies of NG2$^+$ cells at different developmental stages, the transgenic mice that express tamoxifen-inducible Cre recombinase can be employed [32].

6.2.1 Generation of oligodendrocytes

It is generally accepted that NG2$^+$ cells give rise primarily to oligodendrocytes in the white and grey matter, therefore they may belong to oligodendrocyte progenitor cells (OPCs), which are characterized by expression of NG2 proteoglycan and might be a subpopulation of NG2$^+$ cells.

Initially, oligodendrocyte progenitors express PDGFRα, oligodendrocyte lineage transcription factor 2 (TF Olig2) and Sox10 [4,33]. The expression of NG2 starts when the cell leaves the germinal zone [27]. NG2$^+$ cells, which differentiate into oligodendrocyte lineage upregulate expression of O4 [34], downregulate the expression of PDGFRα and NG2 [35,36] and finally, they upregulate expression of GC [34] as well as myelin basic protein (MBP), myelin associated glycoprotein (MAG), proteolipid protein (PLP) and 2',3'-cyclic-nucleotide 3'-phosphodiesterase (CNPase) [4,35,37–39] (Fig. 5).

More than 90% of NG2$^+$ cells express transcription factor (TF) Olig2, which is important for their development into oligodendrocytes [40,41] while translocation of TF Olig2 from nucleus to cytoplasm forces NG2$^+$ cells to differentiate into astrocytes [40,42].

Transcription factor Olig2 is not the only factor crucial for OPC differentiation. During their maturation the expression of TF Olig2 is downregulated while expression of TF Nkx2.2 and Sox10 is upregulated and the TF Olig1 translocates from nucleus to cytoplasm [43]. Each of these events is important for maturation into oligodendrocytes.

[1] Cre recombinase is a tyrosine recombinase enzyme. It is known to catalyse the site specific recombination between two DNA recognition sites (loxP sites).

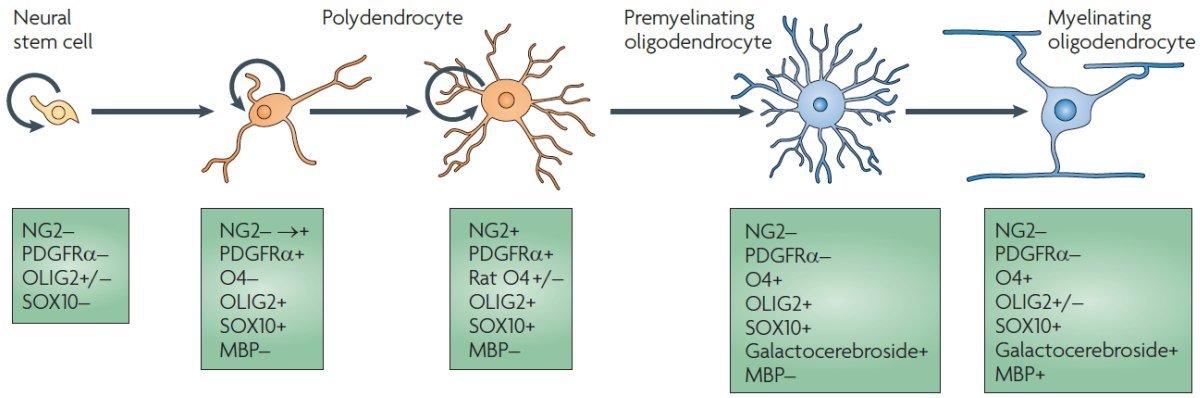

Fig. 5 - Differentiation of NG2$^+$ cells into oligodendrocytes. Neural stem cell express neither PDGFRα (platelet derived growth factor alpha) nor NG2. Nondifferentiated cells have bipolar morphology and are able to proliferate. The number of processes is rising during differentiation. Precursors committed to oligodendrocyte lineage upregulate expression of O4 (oligodendrocyte marker) and downregulate expression of NG2 and PDGFRα. At last, they upregulate expression of GC (galactocerebroside) and MBP (myelin basic protein). Taken from Nishiyama et al., 2009 [2]. The circular arrows indicate proliferation.

6.2.2 Generation of astrocytes

Under certain conditions, NG2$^+$ cells can also give rise to astrocytes, especially in embryonic brain. It was demonstrated that embryonic NG2 expressing cells can give rise to protoplasmic astrocytes in grey matter of the ventral forebrain and spinal cord during embryonic development, but not postnatally (Fig. 6).

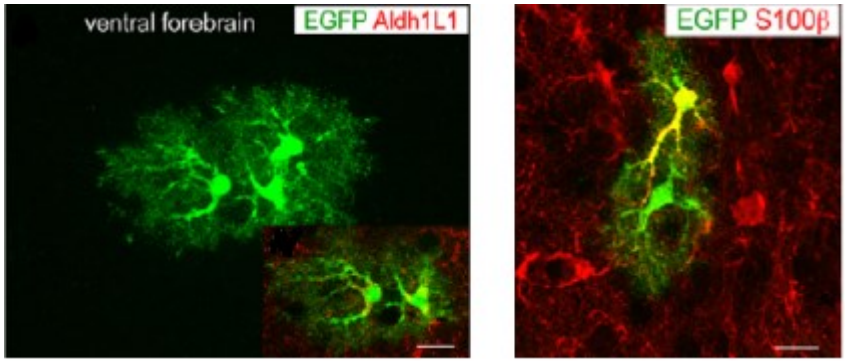

Fig. 6 – Protoplasmic astrocytes generated from NG2$^+$ cells. (left) Mouse ventral forebrain EGFP$^+$ (enhanced green fluorescent protein) NG2$^+$ cells at P14 (after Cre induction at E16.5) immunolabeled with mouse anti-Aldh1L1 (Aldehyde dehydrogenase 1 family member L1) (down - merged image of EGFP fluorescence and Aldh1L1 immunoreactivity) or (right) with anti-S100β (β-subunit of Ca^{2+}-binding protein), showing EGFP$^+$ astrocyte clusters. Taken from Zhu et al., 2011 [44].

Astrocytes generated from $NG2^+$ cells were seen when $NG2^+$ cells from postnatal brain were studied *in vitro*. After cultivation in a medium containing 10% fetal calf serum (FCS), more than 80 % of $NG2^+$ cells expressed glial fibrillary acidic protein (GFAP). These cells were termed type-2-astrocytes. In contrast, only 15 % of the $NG2^+$ cells expressed GFAP after cultivation in serum-free medium and up to 40 % acquired GC under these conditions. Two types of $GFAP^+$ astrocytes could be distinguished based on specific marker expression, A2B5 negative type-1-astrocyte and A2B5 positive type-2-astrocytes. Stallcup and co-authors defined the original $A2B5^+/GFAP^-/GC^-$ cell that generate oligodendrocytes and type-2-astrocytes and called them oligodendrocyte-Type-2 astrocyte progenitor cells (O-2A) [1]. As A2B5 is also marker of $NG2^+$ cells, O-2A cells might be a subpopulation of $NG2^+$ cells with ability to give rise to astrocytes. The similar finding that 10% FCS can potentiate the differentiation of $NG2^+$ cells into type-2-astrocytes was also shown by others [36,45,46]. However, type-2-astrocytes are not thought to exist *in vivo* [47].

Nevertheless, a subpopulation of $NG2^+$ cells could represent cells with astrocytic properties also *in vivo*, as certain subpopulation of $NG2^+$ cells in the brain expresses beta-subunit of the calcium binding protein (S100β) [4,48–51] or glutamine synthetase [51], both known as specific markers of astrocytes.

6.2.3 Generation of neurons

There are already many controversial reports about $NG2^+$ cell differentiation into neurons.

Several *in vitro* studies demonstrated the presence of $NG2^+$ cells, which coexpressed a neuronal specific nuclear protein Neuronal Nuclei (NeuN) in the adult neocortex and which were identified as GABAergic interneurons [52,53] or $NG2^+$ cells in SVZ coexpressing NG2 with transcription factors of interneuron progenitors [53]. Excitable neurons generated from cells immunolabeled for NG2 were also detected in hippocampus and in the piriform cortex of the postnatal brain [54,55]. On the other hand, there are studies that did not reveal any neurons derived from $NG2^+$ cells in SVZ, rostral migratory stream or olfactory bulbs [3,4]. These controversial findings might originate from the fact that neurons also express NG2, for example when they are about to migrate, as NG2 proteoglycan is important in many processes, such as cell adhesion, cell growth, cell migration, and interaction with other extracellular matrix components.

A new approach how neurons can be generated from $NG2^+$ cells was described by Kondo and Raff [56]. After cultivation *in vitro* in the presence of a bone morphogenic protein,

$NG2^+$ cells, as already mentioned, differentiate into type-2-astrocytes. When these type-2-astrocytes are cultivated with basic fibroblast growth factor, they change into neural stem-like cells that are capable of self-renewing and could give rise to type-1-astrocytes, neurons, type-2-astrocytes and oligodendrocytes. Kondo and Raff suggested that chromatin remodelling takes a part in such changes in differentiation potential of $NG2^+$ cells [56].

6.3 $NG2^+$ cells in postnatal and adult brain

$NG2^+$ cells, which originate in the embryonic brain, expand perinatally and persist in the grey and white matter of the mature CNS. This property could be important in brain repair/regeneration, especially after demyelination following white matter injuries. In order to elucidate the proliferation and differentiation potential of postnatal and adult $NG2^+$ cells, newly derived cell clusters, which have arisen from single $NG2^+$ cells, were analysed in brain slices from postnatal day (P) 2, P30 and P60. This analysis revealed that more than 80 % of $NG2^+$ cells in the P2 brain produce clusters containing only oligodendrocytes, while the majority of the $NG2^+$ cells in the P60 brain generate clusters that consist mainly of $NG2^+$ cells or a combination of oligodendrocytes and $NG2^+$ cells. It is obvious that oligodendrocyte (and myelin) production from $NG2^+$ cells continues in the adult CNS, but declines with age [44] (Fig. 7).

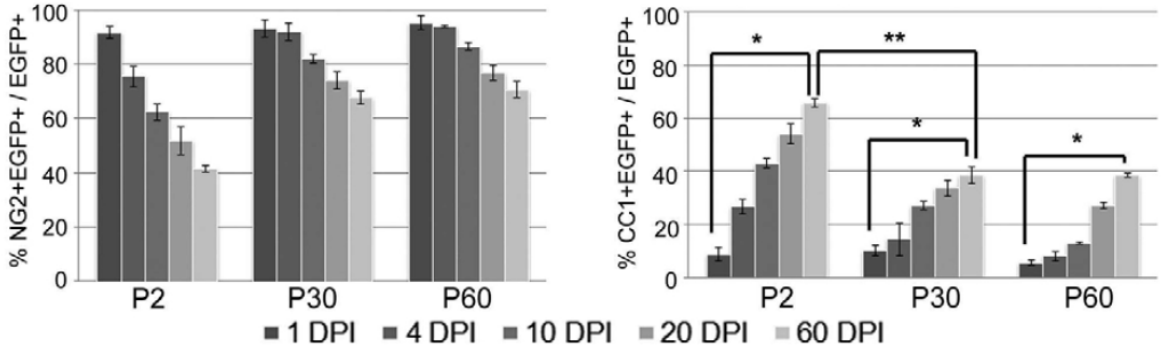

Fig. 7 – Characteristic of postnatal and adult $NG2^+$ cells. (left) The percentage of $EGFP^+$ cells that were $NG2^+$ at 1,4,10,20 and 60 days after Cre induction at postnatal day (P) 2, P30 and P60. (right) The percentage of $EGFP^+$ cells that were $CC1^+$ (mature oligodendrocyte marker) oligodendrocytes at 1, 4, 10, 20 and 60 days after Cre induction at P2, P30 and P60. The proportion of oligodendrocytes among the $EGFP^+$ cells is significantly higher at 60 days after 4-Hydroxytamoxifen injection (dpi) than at 1 dpi and at all time points the proportion of oligodendrocytes among the $EGFP^+$ cells is significantly higher in mice induced at P2 than at P60. Taken from Zhu et al., 2011 [44].

7 The physiological function of $NG2^+$ cells in adult CNS

Using a marker of proliferating cells bromodeoxyuridine (BrdU), the analogue of thymidine which is incorporated into DNA during replication, revealed that 70 % of all $BrdU^+$ cells in adult CNS are $NG2^+$ cells. Thus, $NG2^+$ cells seem to be the most proliferative cells in brain [57], which points toward their importance in regeneration within CNS.

Interestingly, several human gliomas display markers for $NG2^+$ cells, such as PDGFRα and NG2. which might indicate that gliomas might originate from $NG2^+$ cells or it also indicates increased proliferation/migration of gliomas [58].

7.1 Membrane properties

$NG2^+$ cells express a complex repertoire of voltage-gated channels (Fig. 8), such as fast inactivating A-type K^+ channel and delayed K^+ channel, which are both outwardly rectifying and responsible for cell membrane repolarization. Besides, $NG2^+$ cells display voltage-dependent inward rectifying K^+ channels [59,60] which are crucial determinants of the membrane resting potential [61]. The regulation of K^+ currents in cells of the oligodendrocyte lineage plays an important role in determining their proliferative potential and K^+ current phenotype of O-2A cell can be modified by long-term depolarization of their cell membrane [59].

Another ion channel in $NG2^+$ cells is tetrodotoxin (TTX)-sensitive voltage-dependent Na^+ channel [60–62], whose expression is downregulated after the cell differentiation [61] and its importance is discussed later. Various Ca^{2+} channels, such as L- and T-type voltage-gated Ca^{2+} channels are present in $NG2^+$ cells and they mediate inward currents. An increase in intracellular concentration of Ca^{2+} can evoke Ca^{2+}-induced Ca^{2+}-release from intracellular stores. Another way how Ca^{2+} can enter cytoplasm is through Ca^{2+} permeable α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors [48]. AMPA receptors in $NG2^+$ cells could be either permeable or impermeable for calcium ions. Their permeability to calcium and other cations is determined mostly by the GluR2 subunit. The presence of a GluR2 subunit in AMPA receptor results in channel impermeability to calcium [63].

Inotropic glutamate receptors, such as AMPA and NMDA receptors [60–62,64] belong to the most important receptors expressed by $NG2^+$ cells together with $GABA_A$ receptors. Efflux of Cl^- induced by $GABA_A$ receptor activation was described to cause cell depolarisation [61,65].

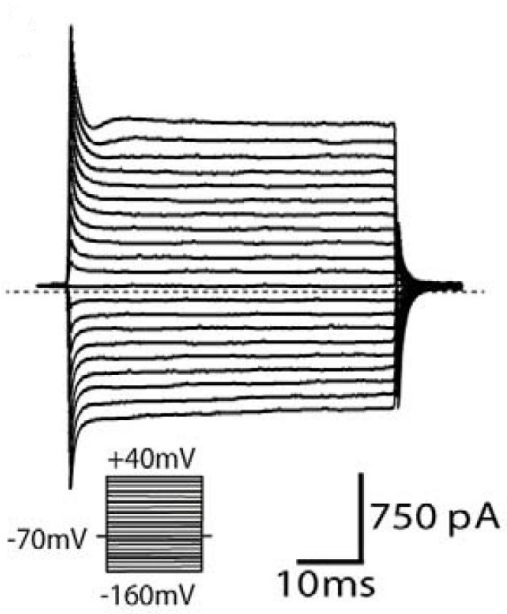

Fig. 8 - NG2$^+$ polydendrocytes displaying a complex current pattern. Current pattern measured after depolarizing the cell membrane from a holding potential of -70 mV to +40 mV and hyperpolarizing to -160 mV (see the inset, bottom). Polydendrocytes showed a complex current pattern, that is, time- and voltage-independent K^+ currents, K_{DR} (delayed rectifier), K_A (A type) and K_{IR} (inward rectifying) currents. The dashed line marks zero current. Taken from Honsa et al., 2012 [66].

In addition, NG2$^+$ cells in culture were shown to express purinergic receptors [67], nicotinic acetylcholine receptors [68,69], metabotropic glutamate receptor[48] or α and β adrenergic receptors [70,71], among others. Interestingly, using transgenic mice, in which GFAP promoter-driven EGFP expression predominantly visualizes brightly fluorescent astrocytes, a subpopulation of weakly fluorescent cells with short thin processes was discovered in the hippocampus. These cells expressed ionotropic glutamate receptors and they did not express glutamate transporters. A subpopulation of these glutamate receptor-bearing cells was AN2-positive [72] and they also expressed a complex current pattern typical for NG2$^+$ cells. Despite their astrocytic properties (EGFP expression under the GFAP promoter) they possibly represent one of the NG2$^+$ cell subpopulations [73] termed GluR cells.

The passive membrane properties of NG2$^+$ cells described in hippocampal CA1 region showed a negative resting membrane potential (mean: -77,2 ± 1,9 mV), a membrane capacitance inferior to 50 pF (mean: 29 pF) and input resistance higher than 90 MΩ (mean: 479 ± 49 MΩ) [68]. NG2$^+$ cells don't possess gap junctions, unlike remaining glial cells [74].

7.2 NG2$^+$ cells form synapses with neurons

Whole-cell path clamp experiment performed on NG2$^+$ cells from hippocampus showed that they make synapses with neurons (Fig. 9). The evidence of synapses between neuron and NG2$^+$ cell is based on the fact that stimulation of preceding neurons mediated depolarization in NG2$^+$ cells [48].

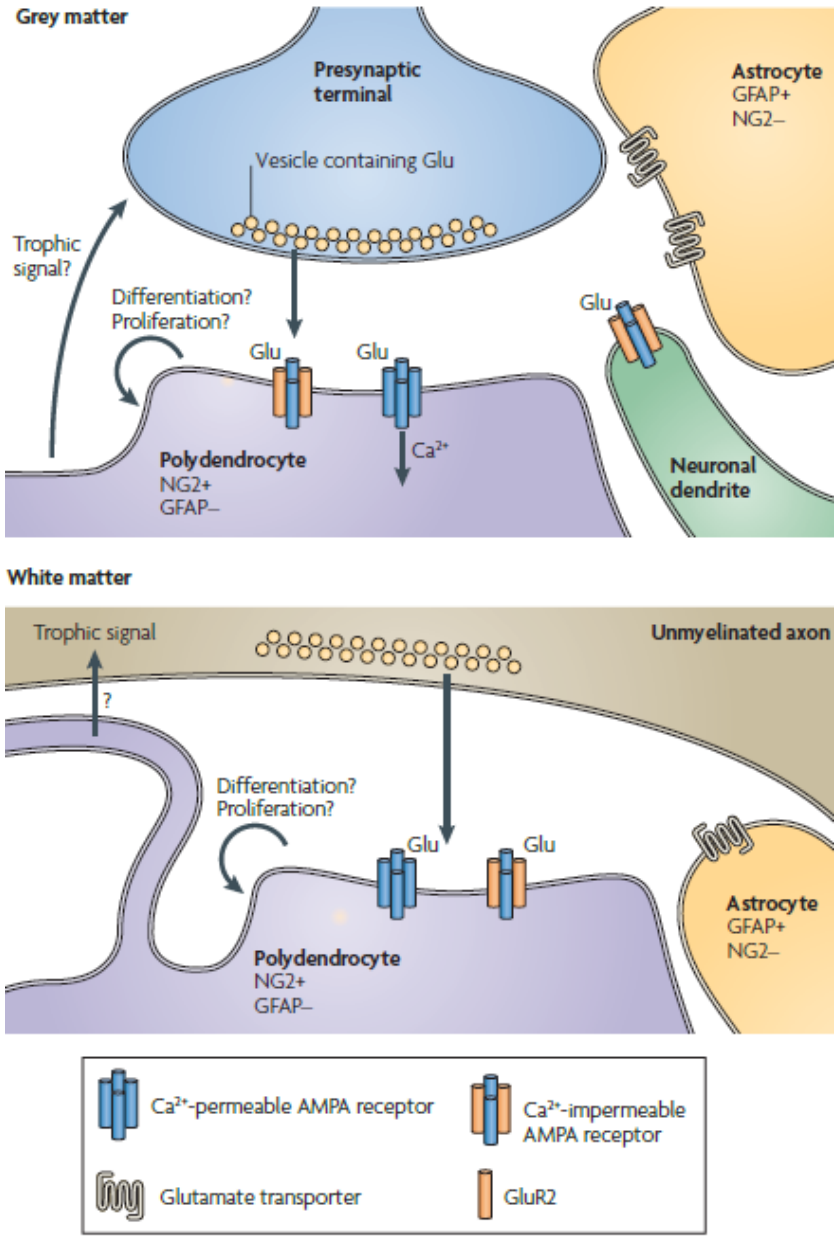

Fig. 9 - Model of synapse between NG2$^+$ cell and neuron. (up) In grey matter NG2$^+$ cells take signals from neuron terminals. (Some glutamate receptors possess GluR2 subunit and are impermeable to Ca^{2+}, whereas some are permeable to Ca^{2+}). (below) In white matter, NG2$^+$ cells receive glutamate released from unmyelinated axons [62] and nodes of Ranvier [75]. It is not known whether the synapses are bidirectional and whether NG2$^+$ cells release trophic factors to neurons. Taken from Nishiyama et al., 2009 [2].

Currents emerging in stimulated $NG2^+$ cells were blocked by AMPA/kainate receptor antagonist and such AMPA receptor mediated currents were not observed until P5. These glutamate-evoked currents were also blocked by the Na^+ channel antagonist, tetrodotoxin [62,76].

Activation of AMPA and NMDA glutamate receptors or ATP-activated purinoceptors (P2X and P2Y) induces Na^+ entry [67], which may ultimately mediate an increase in intracellular Ca^{2+} concentration important to synaptic signal transduction. In oligodendrocyte progenitors (O-2A), which belong to $NG2^+$ cells, increases in intracellular Na^+ induced by activation of non-NMDA glutamate receptors resulted in a reduction of outwardly rectifying voltage-gated K^+ currents and strongly attenuated their proliferation [59]. When such K^+ currents are attenuated, the cell gets a signal to arrest the cell cycle in G1 phase [77], the cell proliferation is stopped and the cell differentiation is stimulated instead [78,79]. Similar block in G1 phase could be caused by activation of β-adrenergic receptors [71]. Last but not least, activation of AMPA receptors can stimulate cell migration mediated by cytoplasmic transmembrane receptors integrins which bind laminin or fibronectin in the extracellular matrix (ECM) [80].

Synapses of $NG2^+$ cells with neurons are lost when they start to differentiate into oligodendrocyte. The way this is achieved is primarily based on the downregulation of glutamate receptors [81] (Fig. 10). On the other hand, $NG2^+$ cells keep synaptic contact during cell division [82].

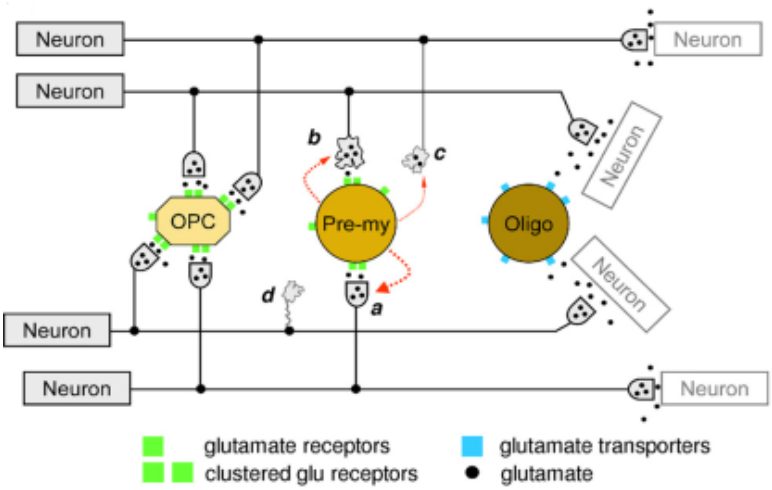

Fig. 10 - Three developmental stages of oligodendroglial cells considering synapses with neurons (oligodendrocyte progenitor cells, OPC; premyelinating oligodendrocyte, Pre-my; mature oligodendrocyte, Oligo). OPC has synapse with neurons with clustered glutamate receptors on postsynaptic membrane. Pre-my transmits a signal to presynaptic neurons (red arrows), which activate the disassembly of the presynaptic terminal. Myelinating oligodendrocyte downregulates glutamate receptors and upregulate the expression of glutamate transporters. Taken from Kukley et al., 2010 [81].

7.3 Heterogeneity of $NG2^+$ cells in adult CNS

Majority of $NG2^+$ cells (about 60 %) in an adult brain don't divide and as the number of $NG2^+$ cells doesn't increase, dividing cells either die or differentiate and downregulate NG2 proteoglycan expression [4].

We can distinguish three categories of $NG2^+$ cells with different functions in the adult brain - young OPCs, adult OPCs and synantocytes. Synantocytes possess stellate morphology and they contact axons at Ranvier's nodes; however, they have low capacity to myelinate. Both OPCs are able to generate oligodendrocytes; however, adult OPCs have longer cell cycle than their young counterpart [29] (Table 1). The duration of the $NG2^+$ cell cycle is strongly age-dependent. The $NG2^+$ cells from P2 became oligodendrocytes within 10 days, but those from P60 differentiated into oligodendrocytes within 20 days or longer [44].

Table 1 - Heterogeneity of O-2A progenitors in perinatal and adult brain. Taken from Wolswijk et al., 1991 [83].

	Source O-2A Progenitors	
Characteristic	Perinatal Optic Nerves	Adult Optic Nerves
Morphology	Bipolar	Unipolar
Antigenic phenotype	$O4^-$ $vimentin^+$	$O4^+$ $vimentin^-$
Cell cycle time	20 ± 6 hours	59 ± 5 hours
Rate of migration	25 ± 5 μm/h	4 ± 1 μm/h

Another division of $NG2^+$ cells is based on their ability to fire action potential. One subpopulation of $NG2^+$ cells has no ability to fire action potential and does not receive synaptic input from neurons, while another subpopulation possess voltage-gated Na^+ channels and when depolarized, these cells are able to produce action potential-like event and to receive synaptic inputs from neurons. In the white matter at P7, about 46 % of OPCs expresses voltage-dependent Na^+ and K^+ channels. It seems to be the only difference between these two populations as both are able to proliferate and their morphology is very similar. It is possible that cells lacking the voltage-gated Na^+ channels could be more mature differentiating oligodendrocytes as they have lower expression of NG2 and have lower percentage of BrdU incorporation [55]. However, the OPCs that were able to fire the action potentials didn't express neuronal markers, such as NeuN, and therefore they don't seem to be a part of neuronal lineage [55,84]. There are also reports, that deny any possibility of generating action potential by $NG2^+$ cells [62] and some reports describe a subpopulation of $NG2^+$ cells, which can fire single action potential, but never depolarize over 0 mV [38,60].

8 NG2$^+$ cells as remyelinating cells

Based on the observations that NG2$^+$ cells can give rise to oligodendrocytes in adult brain, the possibility that they could potentially serve as a source of remyelinating cells is highly studied. The remyelinating capacity of NG2$^+$ cells is usually investigated following chemically-induced demyelinations, spinal cord stab wound injuries or in the multiple sclerosis brain. One of the animal models of focal ischemia, middle cerebral artery occlusion[2] (MCAo), was applied by Honsa and colleagues in experiment on adult mice dorsal cortex [66] (Fig. 11). It turned out that three days after MCAo the number of EGFP$^+$ cells (where NG2 promoter-driven EGFP expression is seen in NG2$^+$ cells and their progeny) in the gliotic tissue dramatically increased (2,5-fold) when compared to control animals, and these cells expressed nestin, the intermediate filament, which is upregulated in immature proliferating glia. Changes in the morphology and electrophysiology were significant in these proliferative NG2$^+$ cells. Cells had larger cell bodies and shorter processes. Seven days after MCAo the number of EGFP$^+$ cells was still significantly increased, but these proliferative cells had more complex morphology with multiple processes (like more mature NG2$^+$ cells). Although, the number of EGFP$^+$ cells was increased, the decreased expression of nestin shows that these cells were less proliferative. Many EGFP$^+$ cells did not express NG2; however, almost 17 % of EGFP$^+$ cells expressed GFAP and also resembled the reactive astrocytes morphology (thicker and more extended main cellular processes) and some were positive for marker of reactive astrocytes, vimentin, and as an illustration that NG2$^+$ cells might give rise to neurons, in 5 % of EGFP$^+$ cells was detected marker of newly derived neuron or neuroblast, DCX. Two weeks after MCAo the number of EGFP$^+$ cells was still increased, but proliferation (nestin expression) declined. Yet 14 % of EGFP$^+$ cells were GFAP positive and about 9 % were DCX positive. In addition, in contralateral uninjured hemisphere was also increased number of EGFP$^+$ cells [66] as the ischemia affects greater area of CNS. Increased number of proliferative NG2$^+$ cells after ischemia (stab wound injury) was observed in many other experiments [85–87]. Even if the NG2$^+$ cells possibly give rise to astrocytes following ischemia, they are not considered to be a major source of reactive astrocytes after neocortical stab wound injury [88]. As mentioned above, changes in electrophysiology occur after ischemia in NG2$^+$ cells. The most significant change is increase in membrane resistance as well as in K_{DR}

[2] Middle cerebral artery occlusion leads to reduction of cerebral blood flow in both the striatum and the cortex. One can choose a permanent occlusion or transient occlusion (the artery is occluded for a period of time before reperfusion).

and K_A currents conductance, while the conductance of K_{IR} currents was decreased [66,87]. Increase in the outwardly rectifying K^+ currents has been shown to reflect the proliferative activity of OPCs [59] and it was found that all cells with increased K_{DR} and K_A currents conductance were stained for a proliferating cell nuclear antigen (PCNA) [87].

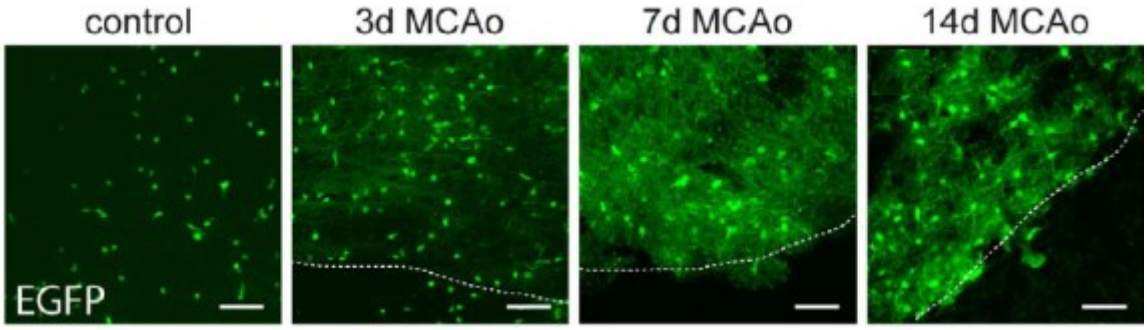

Fig. 11 - Changes in the number of enhanced green fluorescent protein positive ($EGFP^+$) cells (NG2 driven EGFP expression) evoked by MCAo. Brain sections showing the increasing number and different distribution of $EGFP^+$ cells 3, 7 and 14 days after MCAo when compared to controls (left). Borders of the ischemic tissue are highlighted with the dashed lines. Taken from Honsa et al. [66].

In focus on chronic demyelinating diseases, like multiple sclerosis, the most commonly asked question is why remyelination fails. For a long time was assumed that $NG2^+$ cells are depleted, however, experiments identified that $NG2^+$ cells persist in tissue. There was no difference in remyelination following three episodes of demyelination at the same site after ethidium bromide-induced demyelination, when $NG2^+$ cells have the opportunity to remyelinate CNS in the intervening period of time [89]. However, Keirstead and colleagues showed that remyelination of the demyelinated area was associated with a decrease in number of $NG2^+$ cells in lesion surroundings [90]. Similarly, in mice on cuprizone (toxic for oligodendrocytes) diet up to 12 weeks the number of oligodendrocytes never fully returned to control numbers and many oligodendrocytes were apoptotic on long-term cuprizone diet. The apoptosis of oligodendrocytes might be caused by the presence of microglia/macrophages in long-term cuprizone diet mice [91]. One of the reasons of remyelination failure might be an inability to proliferate at sufficient rate, slower migration rate and lower differentiation ability of $NG2^+$ cells in affected CNS [92,93]. The decrease in the efficiency of CNS remyelination is known to be age-associated [93,94]. Another factor that might be responsible is linked to heterogeneity of $NG2^+$ cells, to their ability to differentiate into myelinating oligodendrocytes [92]. As mentioned above some $NG2^+$ cells don't generate myelinating oligodendrocytes, but persist in form of synantocytes in CNS [29].

For cell-based treatments of demyelinated tissues is important the finding that transplanted glial cells migrate over a greater distances and remyelinate lesions more rapidly than endogenous remyelinating cells. While endogenous cells migrate for a distance of 1-2 mm into an area of demyelination during the first month, transplanted cells could be found up to 6 mm from their point of implant after the same time [95]. Similarly, transplanted neonatal OPCs repopulate OPC-depleted tissue 3-5 times more rapidly than endogenous OPCs [96].

9 Conclusion

Insights discussed above focus on identification, lineage restrictions and function of $NG2^+$ cells in the CNS. However, we are still far from a complete understanding. Nowadays, the research of $NG2^+$ cells concentrate especially on questions concerning heterogeneity, response to demyelination or on function in neuronal network. Findings about the greater efficiency of transplanted cells in remyelination indicate a possibility of cell-based treatment of demyelinating diseases. The effect of several growth factors, transcriptional factors, chemokines and other regulators on proliferation, migration or differentiation of $NG2^+$ cells is highly studied these days and could lead to another possible way, how to induce OPCs efficiency in remyelination.

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