

Pathology-induced NG2 proteoglycan expression in microglia

Erika Meyer, Anja Scheller*

Oligodendrocyte precursor cells (OPCs) and microglia are two very fascinating cell types with a multitude of important but different functions. At a first glance, they appear not to share many cellular properties, nor are directly related to one another or derived from a common ancestor. Despite all differences, emerging data show that both cell types express the protein nerve/glia antigen 2 (NG2) after pathological insults (**Figure 1**). For years, it remained controversial whether microglia really could express NG2 upon injury, with contradictory results reported among different disease models. Addressing this question, we could recently show by using triple transgenic knock-in mice and either an acute injury model (stab wound injury) or the middle cerebral artery occlusion combined with immunohistochemistry that a subset of microglia activates the *cspg4* gene in a disease dependent manner leading to a bonafide microglia-specific NG2 protein expression besides OPCs and pericytes. Our data show that the *cspg4* gene not only gets transcribed in microglia based on reporter expression after recombination, but also the protein itself is expressed (Huang et al., 2020).

NG2 is a surface type I transmembrane core protein belonging to the family of chondroitin proteoglycans, has a large extracellular domain, a shorter cytoplasmic part, and is encoded by the *cspg4* gene (**Figure 1**). Multiple proteolytic cleavage as well as phosphorylation and glycosylation sites are important for NG2 protein function (Sakry and Trotter, 2016). Because of its interactions with extracellular matrix proteins, adhesion molecules, and growth factors, NG2 affects numerous cellular phenomena, including those implicated in pathological conditions (Nishihara et al., 2015). In the central nervous system (CNS), NG2 immuno-labelling is an important tool for OPC identification and separation from mature oligodendrocytes. OPCs belong to the oligodendrocyte lineage and are often named NG2 cells to highlight their speciality. They act as oligodendrocyte precursor cells, but also remain proliferative their whole life and are nowadays known as the fourth mature glial cell type with a unique expression of receptors and channels distinguishing them not only from oligodendrocytes but also astrocytes. In total, 5–10% of all neural cells are OPCs, comparable to microglia which share these numbers (Sakry and Trotter, 2016; Du et al., 2021).

As the tissue-resident CNS specific immune cells, microglia reside in the brain throughout life while maintaining their numbers through self-renewal processes. Several studies confirmed their myeloid origin and it was demonstrated that yolk-sac progenitors give rise to embryonic microglia migrating into the CNS while macrophages are throughout life produced from bone marrow tissue (Illes et al., 2020). Microglia and macrophages are often termed myeloid cells and invading macrophages share many properties with amoeboid microglia. Microglia are very motile and sense their direct surrounding area for

physiological and/or pathophysiological changes. Activation of microglia changes their morphology from a ramified form with fine processes to microglia with extended processes and ultimately to an amoeboid shape with retracted processes. Activated microglia have beneficial functions (phagocytosis of cellular debris and release of protective factors) as well as a destructive response (release of pro-inflammatory cytokines) making it hard to determine if they are “friend or foe” (Illes et al., 2020).

Under physiological conditions, microglia and OPCs can interact. Microglia influence the balance between OPCs and oligodendrocytes by influencing OPC proliferation and migration (Du et al., 2021), which are known to be NG2-dependent processes. NG2 interacts directly with extracellular matrix components influencing OPC proliferation and migration. When OPCs and microglia are observed under pathological conditions both cell types are directly involved in the first cellular response after a pathological insult and both react with increased proliferation and migration towards the lesion

side (Sakry and Trotter, 2016; Du et al., 2021). While OPC activation is essentially discussed as a reaction to myelin damage and loss, microglia get activated mainly via purinergic signaling involving the activation of P2Y12 receptors on microglial processes by damage-induced increase of extracellular ATP (Illes et al., 2020). Macrophages, closely related to microglia, also get activated after phagocytosis of myelin debris, therefore showing a similar activation signal as OPCs (Liu et al., 2021).

Different studies have reported NG2 immunoreactivity in Iba1 positive microglia and/or macrophages in different pathology models like multiple sclerosis (Gao et al., 2010; Kucharova and Stallcup, 2017), Parkinson’s disease (Kitamura et al., 2010), stroke [middle cerebral artery occlusion (Sugimoto et al., 2014; Huang et al., 2020)], stab wound injury (Jones et al., 2002), and peripheral nerve injury [sciatic nerve crush; (Nishihara et al., 2015)]. The expression of NG2 in microglia was controversially discussed in the field for 20 years based on the properties of the NG2 protein itself. The existence of extracellular cleavage sites leads to the shedding of the extensively large extracellular domain (290 kDa) by matrix metalloproteases and secretases. It was discussed that this ectodomain could be released by OPCs and then bound to microglia would lead to “false positive” microglia with only the appearance of microglial NG2 expression (Sakry and Trotter, 2016). Nevertheless, more and more

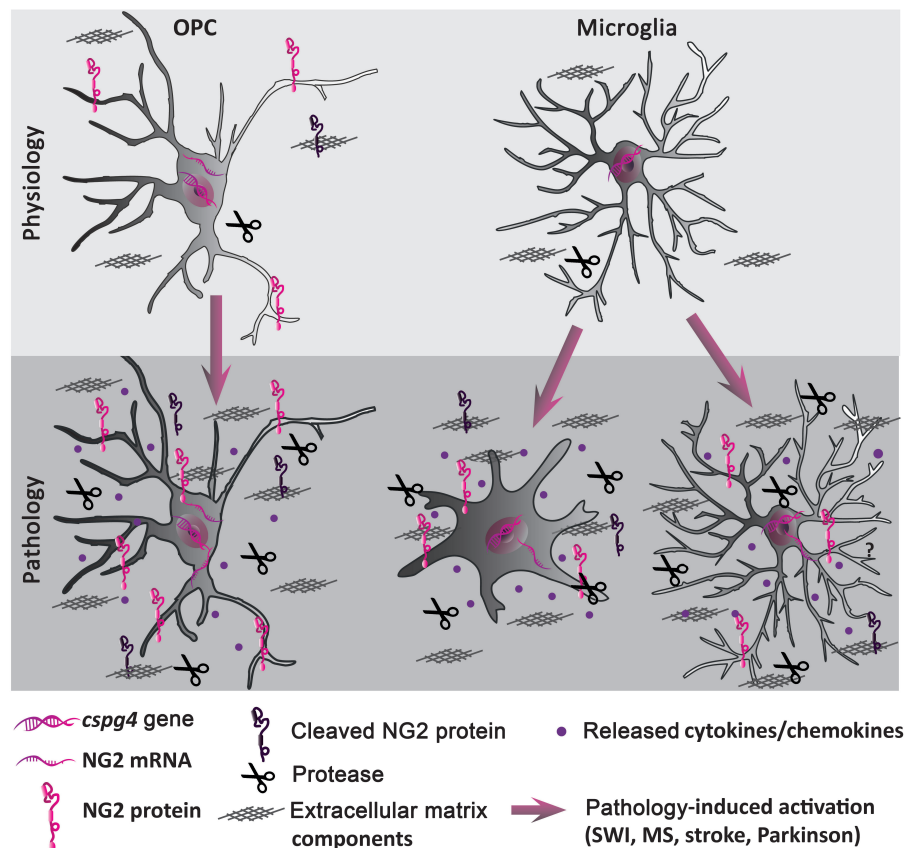


Figure 1 | NG2 expression under physiological and pathophysiological conditions in OPCs and microglia. NG2 is expressed under physiological conditions by OPCs while microglia do not express NG2, nor can the RNA be found (macrophages are omitted in the figure to simplify the graph). NG2 is cleaved and the large extracellular domain interacts with extracellular matrix components. Under pathological conditions, OPCs increase transcription and translation of NG2, leading to a higher NG2 expression on the surface of activated OPCs. Under pathological conditions, microglia also can act as a source of NG2, and its expression has been found in both amoeboid microglia (left) as well as activated stellate microglia with prolonged processes (right). Little is known about the function of NG2 in microglia, but an increased proliferation and decreased phagocytotic activity are discussed. MS: Multiple sclerosis; NG2: nerve/glia antigen 2; OPC: oligodendrocyte precursor cell; SWI: stab wound injury.

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data prove NG2 expression not only based on immunohistochemistry but RNA expression and recombination studies using different Cre DNA recombinase applications (Kucharova and Stallcup, 2017; Huang et al., 2020). NG2 expression in microglia/macrophages was analyzed in a multitude of different pathological animal models probably leading to the still controversial results on the number of NG2 expressing microglia (Jones et al., 2002; Gao et al., 2010; Kitamura et al., 2010; Sugimoto et al., 2014; Nishihara et al., 2015; Huang et al., 2020). The still ongoing controversy can also be explained by the use of antibodies with different binding sites as well as the different animal models (rats versus different transgenic and wild-type mice). In addition, the different pathologies were analyzed in different white and grey matter CNS regions (cortex, corpus callosum, and spinal cord).

The precise mechanisms regulating NG2 expression are still unknown. However, a growing body of evidence suggests that several regulatory factors underlying pathological processes, like methyltransferases, transcription factors, and miRNAs, play a role in regulating NG2 expression (Ampofo et al., 2017).

But what are the potential benefits or disadvantages of a bonafide pathologically induced NG2 expression in microglia and macrophages? NG2 expression seems to be dependent on the severity and the type of pathology as well as the investigated species and CNS area. Not much so far is known about the function of microglia and macrophage-specific expression of NG2. In macrophages, NG2 expression is induced after myelin debris uptake leading to impaired phagocytotic capacity while at the same time proliferation of NG2 positive macrophages increases (Liu et al., 2021). This is comparable to OPCs showing increased proliferation after activation by myelin fragments. Loss of NG2 in OPCs was associated with decreased differentiation to oligodendrocytes and reduced myelin repair as well as migration (Kucharova and Stallcup, 2017). The emergence of myelin debris is a general appearance after a pathological insult and not limited to oligodendrocyte-specific diseases like multiple sclerosis, thereby affecting microglia/macrophages as well as OPCs overall. The overall loss of NG2 reduced proliferation not only in OPC but also in microglia/macrophages after spinal cord injury (Sakry and Trotter, 2016; Liu et al., 2021). On the other hand, NG2 loss in myeloid cells has different consequences after demyelination, namely reduced macrophage recruitment with less uptake of myelin debris by macrophages and microglia and reduced recruitment of NG2 positive OPCs to the lesioned area with a subsequently reduced myelin repair. In summary, both cell types show defects in myelin repair as a final consequence. However, it needs to be further elucidated whether this is a NG2-dependent process or merely an incidental finding.

Another question is whether the microglia-specific NG2 protein is comparable to OPC-specific NG2 in terms of glycosylation and cleavage properties (Sakry and Trotter, 2016). Pathological insults frequently involve the disruption of the blood-brain barrier, and NG2 upregulation in OPCs is induced not only by myelin fragments but also by increased levels of cytokines and chemokines on the lesion side. The latter also activates a strong microglial response, resulting in the initiation of NG2 expression as a consequence (Huang et al.,

2020; Du et al., 2021). Various proteases, including matrix metalloproteinases, cleave the large extracellular domain of NG2 in OPCs. Upregulated NG2 expression after pathology in OPCs leads to enhanced release of the cleaved NG2 ectodomain, influencing, along with changes in the extracellular matrix components, the glial scar formation (Figure 1) (Nishihara et al., 2015). While on the one hand scar formation helps to minimize the lesion side, on the other hand, it prevents the outgrowth of regenerating axons giving also OPCs a Janus-like fate comparable to the pro- and anti-inflammatory effects of microglia (Jones et al., 2002; Huang et al., 2020; Illes et al., 2020; Du et al., 2021; Liu et al., 2021). It is interesting to speculate that the microglial specific NG2 protein is also cleaved as shown for macrophages after a pathological insult, subsequently influencing the extracellular matrix components in the vicinity of microglia. The first cleavage of NG2 in macrophages (at the N-terminal region by matrix metalloproteinases) differs from the typical OPC cleavage. Subsequent additional shedding of the cleaved NG2 follows, leading to the known NG2 fragments also released from OPCs (Nishihara et al., 2015). Therefore, NG2 expression and cleavage could lead to a new, so far unknown function of microglia/macrophages in the establishment and/or maintenance of the glial scar.

NG2 expressing microglia and macrophages conclusively appear after different types of CNS insults. Many aspects were already investigated in certain pathologies, but more questions need to be addressed in the future. Why do microglia/macrophages express NG2? Are different expression levels of NG2 influencing microglia/macrophage activity? Is the microglial NG2 protein different from OPC-specific NG2 in terms of phosphorylation and glycosylation? Does the cleavage of microglia/macrophage specific NG2 influence cellular activity, extracellular matrix components as well as proliferation, migration and/or differentiation of neighboring cells thereby influencing the glial scar? Overall, more research is needed and many secrets about the role of NG2 released by different cells in glial scar formation and overall glial activation must be discovered in the future!

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Date of submission: April 7, 2022

Date of decision: June 15, 2022

Date of acceptance: July 5, 2022

Date of web publication: September 16, 2022

<https://doi.org/10.4103/1673-5374.353488>

How to cite this article: Meyer E, Scheller A (2023) Pathology-induced NG2 proteoglycan expression in microglia. *Neural Regen Res* 18(4):801-802.

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Open peer reviewers: Rui-Yuan Pan, Beijing Institute of Basic Medical Science, China; Qingxiu Zhang, Nanjing University Medical School Affiliated Nanjing Drum Tower Hospital, China.

Additional files: Open peer review reports 1 and 2.

References

- Ampofo E, Schmitt BM, Menger MD, Laschke MW (2017) The regulatory mechanisms of NG2/CSPG4 expression. *Cell Mol Biol Lett* 22:4.
- Du X, Zhang Z, Zhou H, Zhou J (2021) Differential modulators of NG2-glia differentiation into neurons and glia and their crosstalk. *Cell Mol Neurobiol* 41:1-15.
- Gao Q, Lu J, Huo Y, Baby N, Ling EA, Dheen ST (2010) NG2, a member of chondroitin sulfate proteoglycans family mediates the inflammatory response of activated microglia. *Neuroscience* 165:386-394.
- Huang W, Bai X, Meyer E, Scheller A (2020) Acute brain injuries trigger microglia as an additional source of the proteoglycan NG2. *Acta Neuropathol Commun* 8:146.
- Illes P, Rubini P, Ulrich H, Zhao Y, Tang Y (2020) Regulation of microglial functions by purinergic mechanisms in the healthy and diseased CNS. *Cells* 9:1108.
- Jones LL, Yamaguchi Y, Stallcup WB, Tuszynski MH (2002) NG2 is a major chondroitin sulfate proteoglycan produced after spinal cord injury and is expressed by macrophages and oligodendrocyte progenitors. *J Neurosci* 22:2792-2803.
- Kitamura Y, Inden M, Minamoto H, Abe M, Takata K, Taniguchi T (2010) The 6-hydroxydopamine-induced nigrostriatal neurodegeneration produces microglia-like NG2 glial cells in the rat substantia nigra. *Glia* 58:1686-1700.
- Kucharova K, Stallcup WB (2017) Distinct NG2 proteoglycan-dependent roles of resident microglia and bone marrow-derived macrophages during myelin damage and repair. *PLoS One* 12:e0187530.
- Liu Y, Hammel G, Shi M, Cheng Z, Zivkovic S, Wang X, Xu P, He X, Guo B, Ren Y, Zuo L (2021) Myelin debris stimulates NG2/CSPG4 expression in bone marrow-derived macrophages in the injured spinal cord. *Front Cell Neurosci* 15:651827.
- Nishihara T, Remacle AG, Angert M, Shubayev I, Shiryayev SA, Liu H, Dolkas J, Chernov AV, Strongin AY, Shubayev VI (2015) Matrix metalloproteinase-14 both sheds cell surface neuronal glial antigen 2 (NG2) proteoglycan on macrophages and governs the response to peripheral nerve injury. *J Biol Chem* 290:3693-3707.
- Sakry D, Trotter J (2016) The role of the NG2 proteoglycan in OPC and CNS network function. *Brain Res* 1638:161-166.
- Sugimoto K, Nishioka R, Ikeda A, Mise A, Takahashi H, Yano H, Kumon Y, Ohnishi T, Tanaka J (2014) Activated microglia in a rat stroke model express NG2 proteoglycan in peri-infarct tissue through the involvement of TGF- β 1. *Glia* 62:185-198.

P-Reviewers: Pan RY, Zhang Q; C-Editors: Zhao M, Zhao LJ, Wang Lu; T-Editor: Jia Y