Sox9 in the developing central nervous system: a jack of all trades?

Julia K. Vogel, Michael Wegner

Sox9 – gliogenesis and beyond: Neurons and glial cells are the major neuroectodermal cell types of the vertebrate central nervous system (CNS). Their generation from common progenitor cells takes place mostly during embryonic and early postnatal development. After closure of the neural tube, neural epithelial progenitor cells (NEPs) establish the ventricular zone (VZ). By asymmetrical cell division, NEPs first give rise to neuronal precursor cells (NPs) that then differentiate into various types of neurons. Later, NEPs predominantly produce glial precursor cells that become either astroglia or oligodendroglia.

The initiation of gliogenesis requires the activity of several transcription factors. One of them is the high-mobility-group-domain protein Sox9. Following its Notch-dependent induction, Sox9 induces further transcriptional regulators and cooperation partners for gliogenesis in NEPs such as Nfa (Deneen et al., 2006; Kang et al., 2012).

When Sox9 is deleted in NEPs of the spinal cord of the mouse embryo, neurons continue to be produced even beyond their regular birth date at the expense of glial cells, whose generation is strongly decreased (Stolt et al., 2003). This has led to the assumption that Sox9 functions as a neuron-glial switch. Additionally, Sox9 has been postulated to be already crucial for the induction of glial competence in NEPs, possibly by prebinding glial regulatory regions and thereby allowing their future activation (Klum et al., 2018). By doing so, Sox9 is required for transforming NEPs into multipotent neural stem cells (Scott et al., 2010). In the mouse cerebellum, Sox9 appeared particularly important for suppression of neurogenesis (Vong et al., 2015).

Sox9, NEPs and neuronal cells: To understand how Sox9 functions relate to each other, we generated a mouse model that allowed temporally controlled, Cre-induced and tetracycline-sensitive CNS expression of a Sox9 transgene, and analyzed spinal cord development (Vogel et al., 2020).

The chosen Brn4::Cre led to precocious Sox9 expression in NEPs of the VZ at embryonic day (E) 9.0, approximately one day before the start of endogenous Sox9 expression (Figure 1). As a consequence of the first premature and then elevated Sox9 expression, several striking alterations were observed in the developing spinal cord, including a strong reduction in overall size and total cell numbers coupled to a dramatic increase in apoptosis. From early stages onwards, the VZ lost its epithelial organization. NEP survival was not impaired.

At E9.0, high Sox9 levels are usually observed in the most dorsal part of the neural tube where premigratory neuronal crest cells reside. In these cells, Sox9 is responsible among others for initiating an epithelial-to-mesenchymal transition as a precondition for emigration (Cheung et al., 2005). It is thus conceivable, that precocious and elevated Sox9 induction inevitably leads to epigenetic changes in chromatin structure that limit Sox9 activity. This interpretation is supported by experiments where Sox9 transgene expression prior to and as a prerequisite of differentiation (Stolt et al., 2003). It is thus conceivable that Sox9 overexpression, the numbers of Sox10-expressing and Olig2-expressing cells were both dramatically increased. However, at early times there were substantially more Sox10- than Olig2-expressing cells. Differences had disappeared at later stages. During early neural crest development, Sox9 acts as a strong, direct inducer of Sox10 (Cheung et al., 2005). Therefore, it seems plausible to assume that high Sox9 levels also initiate Sox10 expression in early NEPs and that Sox10 in turn activates Olig2 expression. In support of such an assumption, Sox10-positive cells were found in closer vicinity to the VZ than Olig2-positive cells in those experiments where Sox9 overexpression was delayed and VZ integrity maintained. Induction of Sox10 prior to Olig2 is actually the reverse of what normally happens during oligodendroglial precursor selection. It may involve regulatory elements and circuits that are involved in maintaining Olig2 expression during later oligodendroglial development, including a Sox10-responsive enhancer approximately 33kb upstream the Olig2 gene (Weider et al., 2015).

During late stages of spinal cord development, numbers of differentiating oligodendrocytes were also elevated. However, the observed increase was surprisingly modest. Oligodendroglial cells normally downregulate their Sox9 expression prior to and as a prerequisite of differentiation (Stolt et al., 2003). It is thus conceivable that Sox9 overexpression by itself precludes efficient terminal differentiation. Alternatively, the embryonic spinal cord may simply not yet contain all permissive or provide the inductive signals required for oligodendrocyte differentiation.

We also observed an increased generation of astroglial cells. However, the increase in astroglial numbers was not as impressive as the rise in oligodendroglial cells independent of whether Sox9 expression started at E9.0.
or E12.5. This was unexpected as Sox9 is normally an efficient inducer of astroglial fate and identity in VZ cells outside the oligodendrogial-generating region within the ventral VZ (Deneen et al., 2006). Considering that there are many more VZ cells outside than inside this region, we had assumed astrogliogenesis to prevail over oligodendrogliogenesis in our mouse model.

That this is not the case may again be due to the robust Sox9-dependent Sox10 induction; Sox10 in turn not only promotes the oligodendroglial identity but also inhibits astroglial fate and properties in developing spinal cord glia by antagonizing Nfia function (Glasgow et al., 2014). Under experimental conditions, Sox10 can even convert astroglia into oligodendroglia (Mokhtarzadeh Khanghahi et al., 2018). An efficient generation of astroglial cells by Sox9 therefore requires conditions where Sox9 no longer induces strong Sox10 expression. It could for instance be envisaged that at some point during spinal cord development, Sox9 in the developing wildtype spinal cord (left) and functional consequences of premature and increased Sox9 expression (right) during the period from neuration (top) to birth (bottom).

Normal onset of gliogenesis and Sox9 expression levels in NEPs during embryogenesis are indicated in the middle. Time of appearance, relative number and increased susceptibility to apoptosis are shown for each cell type. NEPs: Neural epithelial progenitor cells.

Summary and perspectives: Sox9 overexpression in the developing spinal cord has shown that its function is strongly influenced by timing, the exact expression levels and the cellular context. Depending on these parameters, Sox9 expression has different consequences. This accounts for the various functions previously ascribed to Sox9 during CNS development (Stolt et al., 2003; Scott et al., 2010; Vong et al., 2015). Premature expression in NEPs is not compatible with maintenance of their epithelial character. High amounts of Sox9 expression furthermore prevent proper neuronal development and instead promote both oligodendrogial and astroglial development by counteracting shut-off and promoting expression of glial genes. Again, timing and cellular context appear important as conditions that lead to Sox10 induction favour oligodendrogenesis, while efficient astrogliogenesis can only occur in the absence of Sox10.

While our analysis has yielded substantial insights into early developmental functions of Sox9 including its impact on NEP and glial fate specifications, there is little information yet on its role in already specified astroglial or oligodendroglial cells. Therefore it will be interesting in future experiments to study macroglial development in mice in which Sox9 is only transiently expressed during early times and then turned off again, or in mice in which Sox9 expression is confined selectively to later stages of astroglial or oligodendroglial development. All this can be done in our mouse model by making use of different Cre transgenes and exploiting the possibility of modulating Sox9 expression by tetracycline treatment.

This work was supported by grants from the Deutsche Forschungsgemeinschaft, No. We1326/14 and We1326/15 to MW.

Julia K. Vogel, Michael Wegner*
Institut für Biochemie, Emil-Fischer-Zentrum, Friedrich-Alexander-Universität Erlangen-Nürnberg, Erlangen, Germany
*Correspondence to: Michael Wegner, PhD, michael.wegner@fau.de, https://orcid.org/0000-0002-4586-3294 (Michael Wegner); https://orcid.org/0000-0002-9890-5670 (Julia K. Vogel).

Received: April 6, 2020
Peer review started: April 10, 2020
Accepted: May 22, 2020
Published online: October 9, 2020

https://doi.org/10.4103/1673-5374.295327
How to cite this article: Vogel JK, Wegner M (2021) Sox9 in the developing central nervous system: a jack of all trades? Neural Regen Res 16(4):676-677.

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Plagiarism check: Checked twice by iThenticate.

Peer review: Externally peer reviewed.

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C-Editors: Zhao M, Li XP; T-Editor: Jo Y