Regional diversity in oligodendrocyte progenitor cells: implications for remyelination in grey and white matter

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Introduction and scope of thesis

Introduction

The central nervous system (CNS), comprising the brain and spinal cord, is mainly populated by neurons, microglia and macroglia, the latter consisting of oligodendroglial lineage cells and astrocytes. Broadly, electrical signal-conducting neurons are functionally and metabolically supported by macroglia, while microglia continuously survey the CNS and rapidly respond to injury by mediating inflammatory reactions and clearing debris. Oligodendroglial lineage cells consist of oligodendrocyte progenitor cells (OPCs), intermediate maturation stages, and ultimately mature oligodendrocytes (OLGs). Mature OLGs enwrap neuronal axons with membranes, creating multiple stacks of compacted double-lipid bilayers, called myelin. The lipid-rich myelin functionally isolates the axon, thereby facilitating saltatory conduction, and provides metabolic support[1,2]. OLGs and their myelin segments are focally lost in the chronic demyelinating disease multiple sclerosis (MS)[3]. Clinically, this results in disability and irreversible neurological damage[4], while at the cellular level MS is characterized by denuded axons, chronic inflammation[5], astrogliosis[6] and neurodegeneration[7–10]. Persistent demyelination leads to the degeneration of axons, the subsequent degeneration of neurons, and thus progression of the disease. New OLGs can be formed in these demyelinated lesions that regenerate myelin membranes by a process called remyelination. Remyelination is necessary for functional recovery and mitigating irreversible neurodegeneration[3]. Unfortunately, remyelination in MS is often insufficient and, like other repair processes, becomes less efficient upon aging, ultimately failing at later stages[3,11–13]. Studies in experimental models demonstrate that remyelination upon toxin-induced demyelination involves the recruitment of OPCs to the demyelinated area, where they locally differentiate into remyelinating OLGs[3,14–16]. Of interest, OPCs are present in 70% of white matter (WM) MS lesions but fail to remyelinate[15,17,18], implying that not the recruitment but the differentiation of OPCs is a major culprit in MS[3]. With the etiology of MS unknown, and available treatments limited to disease-modifying immunomodulatory drugs[19], opening remyelination-enhancing therapeutic avenues would be most beneficial for the treatment of progressive MS. A better understanding of the process of remyelination is paramount for the development of such therapies.

Where remyelination by OPCs is robust in rodents[3], in MS OPCs are less reactive[20,21] and remyelination may be partially performed by pre-existing mature OLGs[22]. Whether the latter is an endogenous phenomenon or an adaptation to the lack of regenerative capacities of OPCs in MS remains to be elucidated. In addition, a recent study in zebrafish showed that pre-existing OLGs mistarget neuronal cell bodies and are less capable of remyelination than newly-produced OLGs[23]. Nonetheless, both in MS and animal models for remyelination, remyelination is more efficient in grey matter (GM) than in WM[24–27]. This is especially apparent in leukocortical MS lesions that span both GM and WM and which are thought to have the same pathological background and age[26]. This difference in regional remyelination efficiency may be attributed to intrinsic regional differences in oligodendrocyte lineage cells and/or regional differences in local environmental cues directing remyelination. Indeed, distinct populations of macroglia are found in different brain regions[20,28–33] and particularly mature OLGs appear to be transcriptionally heterogeneous both in rodents[34] and in humans[20]. In contrast, postnatal OPCs are transcriptionally similar in rodents[28,34] and postmortem white matter human brain[20,21]. While transcriptional quiescent in white matter MS brain tissue, transcriptionally diverse OPCs have been identified in experimental autoimmune encephalomyelitis[29] (EAE), an animal model that reflects immunological aspects of MS (reviewed in [35]). In addition, functionally OPCs are regionally distinct at normal conditions, and display heterogeneity in proliferation[36,37] and differentiation rate[38,39], injury response[40,41] and electrical properties[37,42,43]. Moreover, when homo- and heterotopically transplanted in the rodent adult healthy CNS, OPCs from WM differentiate equally well in both GM and WM, whereas OPCs from GM remain more immature independent from their environment[44], indicating the relevance of regional OPC diversity for remyelination. Furthermore, remyelination is negatively correlated with age, both in MS patients[3,11–13] and in animal models for remyelination[45,46]. Indeed, OPCs become less reactive to environmental stimuli upon aging[47,48]. Whether OPCs from GM and WM age differently in their respective environments is currently unknown, but may contribute to regional diversity in OPCs and remyelination.

In addition, remyelination is a highly orchestrated process by which the sequential OPC activation, proliferation, migration and differentiation is regulated by timely

and transient actions of other cell types, including astrocytes, resident microglia and in MS also infiltrating macrophages[3,49,50]. Demyelinated lesions contain myelin debris, which is detrimental to OPC differentiation[51]. Microglia and macrophages first adopt a pro-inflammatory profile and clear the lesion of myelin debris by phagocytosis[52] (reviewed in [49]). Then, by the uptake of myelin and the activation of intracellular lipid signaling pathways, they convert to an anti-inflammatory phenotype that secretes pro-remyelinating factors[53–56] (reviewed in [49]). This shift in activation state is necessary for successful remyelination to occur[52]. Given the diversity in mature OLGs, it is not unthinkable that also the composition of myelin differs regionally. Although not yet assessed on purified myelin, human WM homogenates seem enriched in lipids, while GM homogenates are enriched in protein. Of importance, regional differences in OPCs, potential differences in myelin composition and aging will affect the process of remyelination, therefore likely being reflected in MS pathology. Elucidating OPC diversity between GM and WM, and its putative contribution to the differences in remyelination efficiency in GM and WM, may pave the way for the production of novel therapeutics aimed at enhancing remyelination in GM and WM MS lesions.

Scope of this thesis

The work described in this thesis focuses on putative differences between OPCs in grey matter (gmOPCs) and white matter (wmOPCs), both functionally and on the transcriptomic level, and how this may contribute to regional differences in GM and WM (re)myelination. **Chapter 1** elaborates on current knowledge of macroglial diversity, i.e., heterogeneity and plasticity of oligodendrocyte lineage cells and astrocytes, in the GM and WM of the brain, and outlines the influence of macroglia diversity on regional differences in successful remyelination, and remyelination failure in MS.

Using primary rat-derived neonatal OPCs, we explored in **chapters 2**, **3**, and **4** regional differences between gmOPCs and wmOPCs, including their response to for MS relevant environmental cues, and how this affects their behavior. OPCs have to proliferate, migrate, differentiate and elaborate myelin membranes in order to successfully remyelinate the denuded axons[3,14–16]. Therefore, in **chapter 2** potential differences in proliferation, migration, differentiation and myelin membrane formation between gmOPCs and wmOPCs were examined. Furthermore, OPCs in the adult brain revert to a more immature state upon remyelination[57], prompting us to investigate gmOPC and wmOPC morphology and their maturity at the gene expression level. To assesses whether a potential differential response of gmOPCs and wmOPCs to proinflammatory cytokines that are present in MS lesions may contribute to regional differences in (re)myelination, the effect of TNFα and IFNγ on OPC behavior and morphology was studied[58]. In **chapter 3** we investigated in more detail whether and how primary rat gmOPCs and wmOPCs differ in their response to extracellular stimuli. We focused on the primary cilium, which is a cell-signaling hub processing extracellular signals, and is transiently expressed on differentiating OPCs[59]. Primary cilium formation on differentiating cultured neonatal gmOPCs and wmOPCs was quantified, and potential differential responses of gmOPCs and wmOPCs to signals processed via the primary cilium, such as Sonic hedgehog and Wnt, were determined. Furthermore, the effect of primary cilium knockdown on signaling responses and behavior of these cells and differences in primary cilium formation on oligodendrocyte lineage cells in the cortex and corpus callosum upon de- and remyelination upon cuprizone-induced demyelination was examined.

Myelin debris is another micro-environmental cue that is crucial in orchestrating remyelination. Exposure to myelin debris may inhibit OPC differentiation directly, or uptake of myelin debris by microglia and macrophages may induce a more pro-regenerative phenotype that indirectly facilitates OPC differentiation. Whether GM and WM myelin has differential effects on OPCs and/or microglia and macrophages is not known yet. Therefore, we investigated in **chapter 4** whether rat and MS myelin from GM and WM differ in their ratios of myelin-specific proteins and myelin-typical lipids, and whether myelin from different regions as coating distinctly modifies gmOPC and wmOPC proliferation and differentiation. Also, as myelin debris uptake alters the phenotype of microglia and macrophages[55,56], we in addition investigated whether rat and MS myelin from GM and WM alters their (pre-existing) activation state and pro-regenerative properties by qPCR, and whether conditioned medium of microglia or macrophages after regional myelin uptake may subsequently affect OPC differentiation.

In addition to differential response to micro-environmental cues that are present in demyelinated and/or MS lesions, regional differences in cellular aging may also contribute to regional differences in remyelination efficiency. Indeed, remyelination, like other regenerative processes, is hampered by aging[45,46]. Therefore, in **chapter 5**, an *ex vivo* transcriptomics study was carried out to assess how progenitor cells of the rat CNS are affected by aging, and whether this differs between GM and WM. To this end, RNA from freshly isolated, i.e., not *in vitro* cultured, A2B5-bound progenitor cells from the postnatal day (P) 7, i.e., an age after which OPCs hardly redistribute[60] and P250, i.e., resembling a human age of appx. 25 years when myelination is complete and demyelinating diseases may manifest[61,62], rat brain was subjected to a 3'-RNAsequencing study and transcriptomic differences analyzed. Of importance, A2B5 is commonly used to isolate rat OPCs[63–67]. **Chapter 6** provides an overview of the findings presented in this thesis and discusses its relevance to regional differences in remyelination efficiency in MS pathology and future perspectives for development of remyelination-based therapies in MS.