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On the role of galectin-4 in (re)myelination and multiple sclerosis

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ON THE ROLE OF GALECTIN-4 IN
(RE)MYELINATION AND
MULTIPLE SCLEROSIS

Charlotte G.H.M. de Jong

On the role of galectin-4 in (re)myelination and multiple sclerosis

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Introduction and scope of this thesis

INTRODUCTION AND SCOPE OF THIS THESIS

Introduction

In the central nervous system (CNS) oligodendrocytes (OLGs) produce myelin membranes that enwrap axons to provide insulation and metabolic support to the axon. The loss of myelin sheaths is called demyelination, and when persistent, such as in multiple sclerosis (MS), leads to axon degeneration and neurological symptoms. MS is a chronic, inflammatory, demyelinating and neurodegenerative disease of the CNS¹ that affects 2.8 million people worldwide [an update from “the Atlas of MS” of the MS International Federation²]. The most common disease course is relapsing-remitting MS (RRMS), a condition in which demyelinating attacks alternate with recovery periods. This relapsing phase, which may last as long as 20 years, turns into a progressive phase, called secondary progressive MS, in approximately half of the patients. In 10-15% of the patients, the disease starts with a progressive course from the onset, known as primary progressive MS, when patients do not experience recovery periods at all^{1,3}. No treatment is available that completely halts the progression or promotes functional recovery. Due to their immunomodulatory abilities, 15 FDA-approved disease-modifying therapies are currently applied, aimed at decreasing the number and extent of the relapses in patients with RRMS⁴. However, most of these therapies are ineffective at the progressive phase, likely as neurodegeneration may occur independently of inflammation⁵⁻⁷.

Remyelination failure is one of the key processes that contribute to neurodegeneration in MS⁸. Remyelination is the natural regeneration of myelin membranes to replace lost or damaged myelin, and a complex process which is still not completely understood. The myelin sheaths generated upon remyelination are thinner than myelin sheaths produced during developmental myelination, but nevertheless functional to reinstate saltatory conduction. Based on numerous studies in experimental rodent demyelination models, it has long been thought that the newly-formed myelin sheaths could only be produced by oligodendrocytes generated from adult oligodendrocyte progenitor cells (OPCs). Recent studies in large animal models

(cats and non-human primates) and zebrafish demyelination models demonstrated that without *de novo* oligodendroglioneogenesis, new myelin sheaths were generated by mature oligodendrocytes^{9,10}, implying that OPCs are not a prerequisite for *de novo* myelin biogenesis. Also, studies in postmortem MS tissue revealed that remyelination is not necessarily mediated by newly-formed oligodendrocytes, but may also occur by pre-existing oligodendrocytes that remain intact when myelin debris is cleared from the lesion¹¹. Notably, in zebrafish, remyelination by newly-formed oligodendrocytes is more efficient than remyelination by pre-existing surviving oligodendrocytes, as pre-existing surviving also mistarget myelin membranes around cell bodies¹⁰. Hence, these recent studies add new insights as to potential cellular sources for remyelination to occur, i.e., both OPCs and mature oligodendrocytes may jointly generate new myelin sheaths that survive within an MS lesion^{12,13}. Importantly, in active MS lesions both OPCs and surviving mature oligodendrocytes are present, while mature oligodendrocytes are almost completely lacking in chronic active and chronic inactive MS lesions¹⁴⁻¹⁶, indicating that remyelination in chronic MS lesions has to proceed via OPC-based remyelination.

Irrespective as to whether OPCs or mature oligodendrocytes represent the cellular source of remyelination, the biogenesis of new myelin membranes is very similar between remyelination and developmental myelination^{17,18}. However, in contrast to developmental myelination, remyelination is preceded by demyelination, which is accompanied by signaling molecules generated by cells that are activated upon injury, namely astrocytes^{19,20}, microglia^{21,22} and peripheral-infiltrated macrophages and that regulate remyelination^{23,24}. In addition, intensive crosstalk between astrocytes and microglia is evident. For example, secreted factors of astrocytes attract microglia for the clearance of myelin debris²⁵, while microglia secrete factors that influence astrocyte reactivity²⁶. The clearance of myelin debris by classically activated microglia and macrophages is a critical phase in the remyelination process, as myelin debris impairs the differentiation of OPCs²⁷. In addition, alternatively activated microglia and macrophages timely secrete pro-OPC differentiation factors thereby positively regulating OPC differentiation^{22,28,29}. Upon demyelination, astrocytes secrete extracellular matrix (ECM) molecules, such as chondroitin sulfate proteoglycans (CSPGs) and fibronectin^{30,31}. These ECM proteins are transiently expressed to aid OPC recruitment and to timely halt OPC differentiation and need to be cleared at the

onset of remyelination ³². In addition, to astrocyte and microglia-derived signaling cues, signaling factors that play a role in the timing of developmental myelination, such as Jagged1 and PSA-NCAM ^{33,34} are re-expressed upon demyelination ³⁵⁻³⁷. This extensive cellular crosstalk and timely sequence of actions, i.e., clearing of myelin debris, OPC recruitment and OPC differentiation, are essential for successful remyelination. In MS, remyelination ultimately fails despite the presence of OPCs in most lesions ^{14,15,38-41}, implying that either stimulating extrinsic or intrinsic factors are lacking or that inhibitory signals are dominant. Indeed, some factors, including PSA-NCAM and Jagged1, that are transiently re-expressed upon demyelination are present in and around MS lesions lacking remyelination ^{35,42}. Similarly, ECM remodeling is dysregulated in MS, resulting in the persistent presence of and alterations in ECM proteins, such as high-molecular hyaluronan and fibronectin aggregates, that impair remyelination ^{39,43}. Hence, a central problem of remyelination failure appears the presence of factors in MS lesions that prevent myelin biogenesis and therefore successful remyelination.

As glycosylated proteins and lipids are often involved in cellular interactions and signaling, it is reasonable to assume that proteins that bind these glycoconjugates, which are known as lectins, are involved in the tight regulation of (re)myelination. Galectins are a family of proteins that bind β -galactosides and contain one or two conserved carbohydrate-recognition domains (CRDs). There are three types of galectins: prototypical, tandem-repeat or chimera ^{44,45}. While some galectins are widely distributed, others are highly tissue and species specific, and the expression of some galectins is altered at pathological conditions. Extracellularly, galectins bind to glycans, ECM proteins or glycosylated cell surface receptors thereby inducing differentiation, intracellular signaling or causing modulation of inflammatory processes ⁴⁶⁻⁴⁸. Our previous findings revealed a role of neuronal galectin-4, as a soluble negative regulator in the timing of OPC differentiation ⁴⁹, indicating that galectin-4 may be a potential target or tool to modulate OPC differentiation, thereby overcoming remyelination failure in MS.

Scope of this thesis

This thesis is dedicated to improve our understanding of underlying mechanism(s) of galectin-4 in (re)myelination (failure) and to acquire knowledge that will enable to devise therapeutic means to counteract remyelination failure in MS. In **chapter 1**, current knowledge on the endogenous and exogenous role of galectins during developmental myelination and remyelination and its potential for developing new approaches to treat MS is outlined. More specifically, the role of galectins in modulating immune responses in the CNS and glial cells behavior during development and in experimental models relevant to (re)myelination are described, as well as the potential contribution of galectins to MS pathology. In **chapter 2**, we extended our previous findings on galectin-4 as a negative regulator of OPC differentiation, and explored whether galectin-4 fulfills a role in remyelination. Using experimental models for de- and/or remyelination we examined whether galectin-4 is re-expressed on demyelinated axons and whether galectin-4 is present on axons in distinct MS lesion types. Given the unexpected finding in **chapter 2** that in contrast to developmental myelination, galectin-4 is present on distinct cell types upon demyelination and in MS lesions, we aimed in **chapter 3** to reveal cellular sources for galectin-4, and examined galectin-4 at the mRNA level in experimental models and in cultured oligodendrocytes, neurons, microglia and bone marrow-derived macrophages. An intriguing previous finding is that brain galectin-4 is 4 kDa smaller than galectin-4, localized in the intestine⁴⁹. Therefore, we also investigated the possibility that distinct isoforms of galectin-4 might exist in intestine and CNS. As manipulating or competing with galectin-4 counterreceptors or modulating post-galectin-4 binding effects may be therapeutic approaches to modulate OPC differentiation, we next aimed at identifying galectin-4 binding sites at the cell surface of oligodendrocytes. In addition, identification of oligodendroglial galectin-4 counterreceptors may uncover why galectin-4 prevents OPC maturation. Of relevance, galectin-4 displays two different CRDs, allowing it to bind and cross-link two different types of β -galactoside-containing proteins and/or lipids in the oligodendrocyte plasma membrane. Hence, the focus of **chapter 4** was to elucidate galectin-4 counterreceptors at the cell surface of developing oligodendrocytes *in vitro*. Using affinity precipitation, proteomics and/or competition assays, we considered the possibility that lipids and/or proteins could be functional counterreceptors for galectin-4. Galectins bind to ECM glycoproteins

⁵⁰ or act on the same receptor as ECM proteins ⁵¹, indicating that galectin-4 may interfere with ECM-receptor interactions. Therefore, as remyelination-impairing fibronectin aggregates are present in MS lesions ³⁰, we also examined in chapter 4 the simultaneous effect of galectin-4 and fibronectin on OPC differentiation and myelin membrane formation. Finally, in **chapter 5** the work in this thesis is summarized and perspectives and directions for future research on whether a galectin-4-based strategy may promote remyelination in MS lesions are provided.