III. Abstract

Oligodendrocytes are the myelinating cells of the central nervous system (CNS) and of central importance for normal nerve conductance because the insulating myelin sheath serves as an electrical insulation for the axons that is essential proper neurological function. Oligodendrocyte differentiation evolves stepwise from bipolar precursors (OPCs) to immature oligodendrocytes bearing multiple processes and ends with membrane sheath-bearing mature oligodendrocytes. The last steps coincide with the coordinated expression of myelin genes. The differentiation of cultured oligodendrocytes can be followed with ease by morphological criteria and a series of established markers. Here the usefulness of the hitherto uncharacterized monoclonal antibody (mAb) 486 as a novel stage specific oligodendrocyte marker is described. The mAb 486 selectively labelled developing white matter tracts in situ across species and mature oligodendrocytes in vitro. The epitope was identified as a short LewisX glycostructure confined to lipids and is different from established antibodies that are commonly used to mark cells of the OPC lineage.

The differentiation from an undifferentiated OPC to a mature oligodendrocyte is tightly controlled by intrinsic and extrinsic factors. I studied the impact of defined extracellular matrix (ECM) constituents as spatiotemporal cues for oligodendrocyte differentiation. The ECM consists of a network of macromolecules that are synthesized and secreted into the extracellular space. I investigated the two related glycoproteins Tenascin C (Tnc), Tenascin R (Tnr), and chondroitin sulfate proteoglycans (CSPGs). The abovementioned molecules were expressed in a defined pattern during the differentiation of oligodendrocytes. Early OPCs expressed Tnc and the CSPG Phosphacan, but not Tnr. In contrast, immature oligodendrocytes expressed Phosphacan and also Tnr, but no longer Tnc. Functionally, all these ECM components exert different roles on differentiation. Degradation of CSPG side chains favoured the transition from an early OPC to an immature oligodendrocyte without changing terminal differentiation and myelin gene expression. In contrast, exposure to purified CSPG substrates prevented terminal oligodendrocyte differentiation. Similarly, the glycoprotein Tnc inhibited the formation of myelin membranes and myelin gene expression. This inhibitory role of Tnc is also reflected in vivo as myelin basic protein (MBP) gene expression was accelerated in Tnc mutant mice. In contrast to Tnc, the related Tnr molecule favoured differentiation in culture, because OPCs from Tnr-deficient mice showed delayed differentiation, which could be rescued with purified Tnr. Therefore, the ECM constituents
studied play unique roles to balance OPC lineage progression and myelin gene expression.

In contrast to the antagonistic roles of Tnc and Tnr on MBP expression, both molecules similarly interfered with the formation of myelin membranes. Both Tnc and Tnr reduced the activation of the small GTPase RhoA. The pharmacological modulation of Rho activity altered the morphology of oligodendrocytes, but this did not affect the timing of differentiation. Therefore, myelin gene expression and membrane formation are separate molecular processes although they normally occur simultaneously during differentiation.

To further uncover how Tnc exerts its inhibitory function on MBP expression, established regulators of oligodendrocyte differentiation were tested whether they also participate in Tnc signalling. Tnc interfered with phosphatidylinositol-3-kinase (PI3K) signalling without increasing apoptosis. Correspondingly, moderate reduction of PI3K signalling induced by pharmacological inhibitors prevented oligodendrocyte differentiation. Thus, PI3K signalling is required for effective differentiation. At the cellular level, Tnc associated with lipid raft microdomains in conjunction with the cell adhesion molecule Contactin (Cntn1) and the tyrosine kinase Fyn. siRNA-mediated depletion of Cntn1 abolished the Tnc-dependent inhibition of maturation. Tnc stimulation also impeded the activation of the tyrosine kinase Fyn via Cntn1, because Fyn inactivation was overcome when Cntn1 was knocked-down. Since Fyn activation is necessary for proper oligodendrocyte differentiation, the data revealed that Tnc inhibits MBP expression via Cntn1 and Fyn. I further found that the signalling adaptor and RNA binding protein Sam68 is expressed in oligodendrocytes and its expression levels accumulate during differentiation. Interfering with differentiation through Tnc, PI3K- or Fyn-inhibitors reduced Sam68 levels whereas Sam68 over-expression increased oligodendrocyte differentiation. Sam68 acts downstream of Fyn because forced Sam68 expression could rescue differentiation when perturbed through the Fyn inhibitor PP2. Finally, Sam68 proved to bind directly to MBP mRNA. Taken together, I have elucidated a regulatory signal transduction pathway from the matrix to the nucleus that mediates Tnc-dependent control of the MBP gene, which may represent an ECM-mediated inhibitory mechanism to avoid untimely, premature differentiation of oligodendrocytes.

In conclusion, my thesis provides detailed insights on the functional role of the neural ECM for oligodendrocyte differentiation that led to a model how the ECM orchestrates oligodendrocyte lineage progression. This model may serve to derive new strategies to overcome the inhibitory actions of neural ECM that is found in many CNS lesions.