Abstract

Oligodendrocytes (OLs) are the myelinating cells of the central nervous system (CNS). The importance of OLs is highlighted by demyelinating diseases such as Multiple Sclerosis (MS). MS is the most common autoimmune disease, affecting mostly young adults, and more than 2.5 million people are affected worldwide. During the course of illness, OLs are the targets of immune attacks and acute focal inflammatory demyelination leads in the end to neurodegeneration. In a healthy organism, OLs form myelin membranes, which wrap around axons in order to form insulating segmented sheaths which allow for the rapid, saltatory conduction of action potentials along the nerve fibres. In MS plaques, remyelination can occur, but only with limited efficiency and is mostly insufficient. For this reason, it is important to understand the biology of OL differentiation, myelination and the underlying molecular mechanisms. Thus, this could allow the directed manipulation of OLs leading to an enhanced remyelination efficiency.

For proper remyelination, oligodendrocyte precursor cells (OPCs) have to migrate to demyelinated lesion sites, then differentiate into mature, myelin-producing OLs and wrap axons with their myelin sheaths.

In this regard, Rho GTPases were shown to play an important role in the regulation of OPC migration, differentiation and myelination, as they drive cytoskeletal rearrangements, which are needed for morphological changes of OLs and the wrapping of myelin membranes around axons. Earlier studies demonstrated that the differential activation of RhoA, Rac1 and Cdc42 is a highly concerted process, necessary for OL differentiation and myelination. Amongst others, the guanine nucleotide exchange factor (GEF) Vav3, belonging to the family of Dbl-related GEFs, activates the GTPases RhoA, RhoG, Rac1 and Cdc42. Although it is known that Vav3 is expressed in the CNS and was shown to have diverse and important functions in the neural stem cell compartment, the developing retina and neurons in the CNS, nothing is known about its function in OLs so far.

In the present thesis, I investigated the role of Vav3 during OL differentiation and myelination as well as remyelination using a recently described Vav3 knockout mouse strain. I could show that the differentiation of Vav3-deficient OPCs towards mature OLs was increased in vitro, accompanied by the formation of larger myelin membranes. Furthermore, differentiation in the rostral part of the corpus callosum in Vav3-deficient mice of postnatal...
day 8 was also increased. However, this accelerated differentiation of Vav3-deficient OPCs was compensated at later developmental stages.

Surprisingly, I observed that remyelination in organotypic cerebellar slice cultures was impaired in slices derived from Vav3 knockout mice. These findings were confirmed in experiments using artificial, electrospun microfibres as a target for OL myelination. A diminished myelination by Vav3-deficient OLs was demonstrated, as less sheaths and shorter internodes were formed per single cell. Moreover, remyelination depending on Vav3 was investigated in the model of toxin-induced demyelination using cuprizone. The analysis of remyelination on an ultrastructural level, using electron microscopy, revealed an impaired remyelination in Vav3 knockout mice. In order to investigate the activity of Rho GTPases upon the loss of Vav3, the method of Förster resonance energy transfer (FRET) was used. For this purpose, wildtype and Vav3-deficient OLs were transfected with RhoA, Rac1 and Cdc42 constructs, which allow FRET to occur when the GTPase is activated by the binding of GTP. Here, we could uncover an upregulated Rac1 activity, accompanied by a downregulated RhoA and Cdc42 activity. For Rac1 it was already reported that it is important for the assembly of the actin cytoskeleton. Moreover, investigations of a Cdc42 knockout mouse attributed a key role to Cdc42 for myelination. The downregulation of Cdc42 activity in Vav3-deficient OLs might be the reason for an impaired remyelination efficiency.

In conclusion, the results of the present thesis characterize Vav3 as an important regulator of OL differentiation and myelination. Uncovering the underlying signaling pathway might serve for the development of new strategies that could overcome deficits in MS remyelination process.