● **REVIEW**

Schwann cell Myc-interacting zinc-finger protein 1 without pox virus and zinc finger: epigenetic implications in a peripheral neuropathy

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Abstract

Functionality of adult peripheral nerves essentially relies on differentiation of Schwann cells during post-natal development, as well as fine-tuned re- and transdifferentiation in response to peripheral nerve injury. Epigenetic histone modifications play a major role during the differentiation of embryonic stem cells and diverse organ specific progenitor cells, yet only little is known about the epigenetic regulation of Schwann cells. Just recently, Fuhrmann et al. reported how the transcription factor Myc-interacting zinc-finger protein 1 (Miz1) might contribute to Schwann cell differentiation through repression of the histone demethylase Kdm8. Here, we discuss the potential novel role of Miz1 in Schwann cell differentiation and give a short overview about previously reported histone modifications underlying peripheral nerve development and response to injury.

The deletion of Myc interacting zinc finger protein 1 (Miz1) causes a late onset neuropathy with a spontaneous remission (Fuhrmann and Elsässer, 2015; Sanz-Moreno et al., 2015). Miz1 is a zinc finger transcription factor of 794 amino acids (Peukert et al., 1997) which preferentially binds to a consensus sequence near the initiation region of transcription (Wolf). Binding of other proteins to Miz1 can either stimulate (*e.g.*, Npm, p300, Smad3/4) or inhibit (*e.g.*, Myc, Gfi1, TopBP, Bcl 6) its transactivating function (Herkert and Eilers, 2010). About the first 100 N-terminal amino acids comprise a poxvirus and zinc finger (POZ) domain (Bardwell and Treisman, 1994), while 13 zinc finger (ZF) motifs are scattered over the mid and C-terminal part of the protein with a larger gap between ZF12 and ZF13.

Miz1 binds to DNA as a homotetramer and tetramerisation depends on the POZ domain (Stead and Wright, 2014). Consequently, the deletion of the Miz1 POZ domain abrogates its function as a transcription factor. In a Cre/lox mouse model (Gebhardt et al., 2007) the *Miz1ΔPOZ* genotype was introduced into Schwann cells expressing the Cre recombinase under the control of the desert hedgehog (dhh) promotor (Sanz-Moreno et al., 2015). Mice with *Miz1ΔPOZ* Schwann cells developed a late onset demyelinating neuropathy with severe hind leg paresis around day 90 after birth (P90), with a spontaneous remission of the clinical symptoms from P120 on (Fuhrmann and Elsässer, 2015; Sanz-Moreno et al., 2015).

Tracing back the development of the *Miz1ΔPOZ* neuropathy, Fuhrmann et al. (2018) have shown in a recent paper that the upregulation of demyelinating genes and the repression of myelin genes started not earlier than at P60, and that morphological hallmarks like myelin loops, tomacula, degradation of myelin by Schwann cell autophagy or mac-

rophages were not visible ultrastructurally before P60. This means that between the completion of the postnatal development of the peripheral nervous system (PNS) at P30 and the occurrence of the *Miz1ΔPOZ* phenotype at P60 no obvious pathological features have been observed. As expected from these findings, an RNAseq analysis at P30 revealed a small group of deregulated genes, most likely involved in the very initial pathogenesis of the *Miz1ΔPOZ* neuropathy. Strikingly, using gene ontology analysis, a strong correlation of these genes with the regulation of the cell cycle and cell proliferation became evident (Fuhrmann et al., 2018).

Usually the proliferation of Schwann cell precursors and Schwann cells peaks around birth, ceases during the postnatal PNS development and is completely shut off around P30 (Brown and Asbury, 1981; Stewart et al., 1993). In contrast, the amount of cycling *Miz1ΔPOZ* Schwann cells was significantly elevated at P30, compared with control Schwann cells, and the percentage of cycling *Miz1ΔPOZ* Schwann cells continuously rose until P90 and reached up to 3–4% of the total Schwann cell population (Fuhrmann et al., 2018). This confirmed that the upregulation of the cell cycle relevant genes was associated with a progressive increase in Schwann cell proliferation. So far, it is not clear whether there is a subpopulation of Schwann cells which remains in the cell cycle and expands, or whether already arrested Schwann cells reenter the cell cycle. Interestingly, although Schwann cell proliferation was still present in older *Miz1ΔPOZ* animals, it was 4–5 fold lower than at P90, indicating that the spontaneous remission and regeneration is accompanied by a reduction in the proliferation phenotype.

When testing which of the genes deregulated at P30 in *Miz1ΔPOZ* animals are direct target genes of Miz1, chromatin immunoprecipitation (ChIP) analysis exposed the two
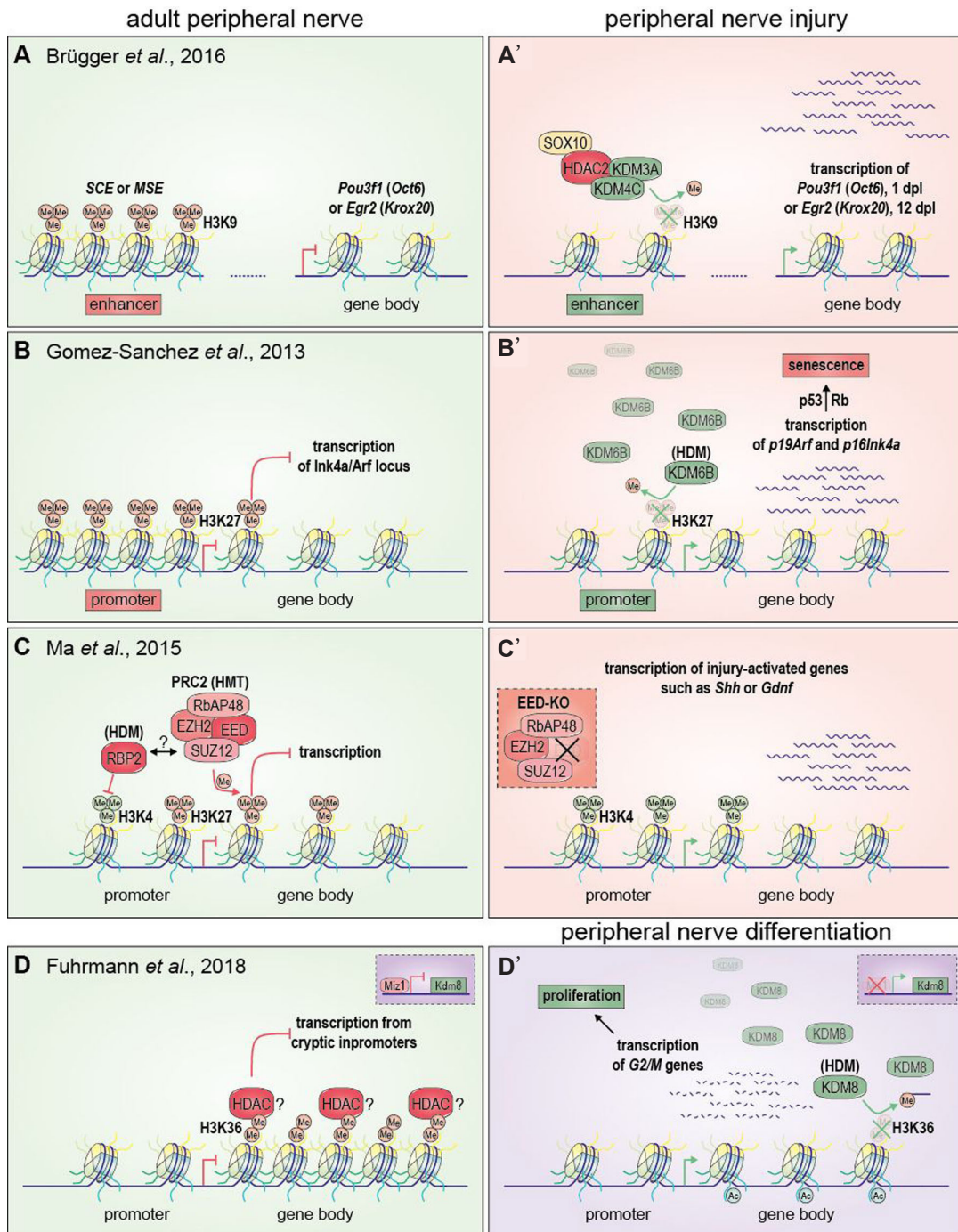
Figure 1 Implications of histone methylation in Schwann cells upon peripheral nerve injury and development.
(A) The Schwann cell enhancer (SCE) element of the *Pou3f1* gene (also referred to as *Oct6*) and the myelinating Schwann cell element (MSE) of *Egr2* (better known as *Krox20*) is tri-methylated at lysine (K) 9 of histone H3 (H3K9me3) in adult Schwann cells. (A') One day post nerve lesion (dpl) histone demethylase complex 2 (HDAC2) recruits the transcription factor Sox10 and the histone demethylases (HDM) Kdm3a and Kdm4c to the *Pou3f1* SCE element, resulting in H3K9me3 demethylation, enhancer activation and increased *Pou3f1* expression. The same complex also activates *Egr2* expression *via* the MSE enhancer 12 dpl. (B) H3K37me3 silences promoter regions of the *p19Arf* gene in adult Schwann cells (B'). Following nerve injury or oncogenic challenges, H3K27me3 is removed by the HDM Kdm6b, leading to increased *p19Arf* gene expression and activation of Schwann cell senescence *via* the ARF and Rb pathway, thereby preventing uncontrolled Schwann cell proliferation. (C) Similarly, H3K27-tri-methylation in promoter and gene regions blocks transcription in adult Schwann cells of many genes, which are exclusively expressed in repair Schwann cells or upon Schwann cell development (*e.g.*, *Shh* or *Gdnf*). The di- and tri-methyaltion of H3K27 is mediated by the multi-subunit histone methyltransferase (HMT) complex Prc2, consisting of RbAP48, Suz12, Ezh2 and Eed subunits. Simultaneously, many promoters display activating H3K4me3 histone marks. H3K4me3 might be restricted to promoter regions by the HDM Rbp2, which is able to interact with Prc2. (C') In injured nerves H3K27-tri-methylation is lost in injury-associated gene promoters and the H3K4me3 expands. A comparable situation can be introduced *via* experimental gene knock out of the Eed subunit of the Prc2 HMT complex (dashed box). (D) In many G_2/M-cell-cycle associated genes H3K36-di-methylation increases throughout the gene body towards the 3'-end. The same distribution is reported for H3K36me3 in different cell types. Here, H3K36me3 recruits HDACs, which convey a repressive, closed chromatin state, to prevent spurious initiation of transcription from cryptic promoters. (D') In *Miz1ΔPOZ* mice increased expression of the HDM Kdm8 results in demethylation of H3K36me2 and elevated expression of G_2/M-associated genes. During postnatal development of control Schwann cells *Kdm8* is progressively repressed (probably through Miz1), indicating that H3K36-di-methylation contributes to the silencing of G_2/M-genes and the exit of Schwann cells from the cell cycle, possibly through the recruitment of Hdacs.

genes *Kdm8* and *Znhit3* as directly bound by Miz1 at the transcription start site, with *Znhit3* as the only gene which was downregulated (Fuhrmann et al., 2018). Znhit3, the mammalian homolog of the yeast Hit1p, is part of the assembly pathway of Box C/D small nucleolar ribonucleoparticles (snoRNPs) (Bizarro et al., 2014) which are involved in post-transcriptional rRNA modifications like 2′-O-methylation of ribose (Watkins and Bohnsack, 2012), but its function in Schwann cells remains to be elucidated. Lysin (K-) demethylase 8 or Jumonji C domain-containing demethylase 5 (Kdm8/Jdmd5) is a histone H3 demethylase with specificity for H3K36me2 (Klose et al., 2006; Kooistra and Helin, 2012). This demethylase has been described in the context of G_2/M cell cycle transition, promoting the proliferation of cells by enhancing the expression of *Cyclin A1* (Hsia et al., 2010), by repression of *Cdkn1b* (encoding p21^{cip}) (Ishimura et al., 2012) or by negatively regulating p53 function (Huang et al., 2015). In control Schwann cells *Kdm8* expression was continuously downregulated during the postnatal development, parallel to the shutdown of proliferation (Fuhrmann et al., 2018). In contrast, in *Miz1ΔPOZ* Schwann cells expression of *Kdm8* progressively increased until P30 (Fuhrmann et al., 2018). These data, together with the observation that cells from the Schwann cell line mSC80 proliferated faster in the presence of Kdm8, allow the hypothesis, that under normal conditions Kdm8, and hence H3K36 demethylation, is involved in the exit of Schwann cells from the cell cycle. Postnatal repression of Kdm8 was accompanied by a peak expression of Zbtb17 (encoding Miz1) suggesting that Miz1 participates in *Kdm8* repression. Taken together, the scenario of the *Miz1ΔPOZ* neuropathy could start with an insufficient repression of *Kdm8* during the postnatal development of the PNS, leading to an impaired cell cycle exit in a subset of Schwann cells which consequently keep on proliferating after P30. Alternatively, reduced H3K36me2 could favor a G_0/G_1 transition in already arrested *Miz1ΔPOZ* Schwann cells.

The exact link between consistently cycling Schwann cells and the onset of demyelination is not clear. However, during Wallerian regeneration after nerve injury, repair Schwann cells, which exhibit a similar phenotype as *Miz1ΔPOZ* Schwann cells (Fuhrmann and Elsässer, 2015; Sanz-Moreno et al., 2015), induce dedifferentiation and demyelination of Schwann cells which are located distally from the initial injury site, indicating that repair Schwann cells can induce dedifferentiation in a paracrine mode (Jessen et al., 2015). Following this notion, the onset of the *Miz1ΔPOZ* neuropathy might occur when a certain threshold of cycling Schwann cells is achieved, explaining the late onset of the *Miz1ΔPOZ* neuropathy. In this model Miz1 is necessary to repress *Kdm8* expression during the postnatal development. As mentioned above, Miz1 represses genes in cooperation with different binding partners, but the corepressor in Schwann cells is still unknown.

A variety of epigenetic modifications have been shown in Schwann cells during development and nerve injury, concerning histone acetylation/deacetylation, histone methylation/demethy-lation and DNA methylation/demethylation (Jacob, 2017; Ma and Svaren, 2018). Specifically, in response to nerve injuries, the Jmjc domain histone demethylases Kdm3A and Kdm4c build a complex with the histone deacetylase 2 (Hdac2) and the transcription factor Sox10. This protein complex demethylates the repressive H3K9me2/3 histone mark at an enhancer element of *Pou3f1* (*Oct6*) one day post lesion (dpl), leading to a higher expression of *Oct6* and, subsequently, to an inhibition of Jun expression and attenuated demyelination (Brügger et al., 2017) (**Figure 1A, A'**). On the other hand, the same complex also de-represses the *Egr2* (*Krox20*) enhancer at 12 dpl and promotes P0 re-expression and remyelination. Strikingly, this indicates that recruitment of histone deacetylases, such as Hdac2, does not always correlate with transcriptional repression. Furthermore, the proliferation of Schwann cells is limited upon oncogenic stimuli or nerve injury by the demethylation of H3K27me2/3 at the promotors of p19^{Arf,} p16^{Ink4a} and p15^{Ink4bA} by enhancing the expression of these cell cycle inhibitor genes and by induction of senescence (Gomez-Sanchez et al., 2013) (**Figure 1B, B'**). Similar to histone demethylases (HDM) which function as erasers on histone methyl modifications, histone methyl transferases (HMT) can play a part as writers in regulating chromatin. For example the polycomb repression complex 2, which catalyzes the methylation of H3K27, is part of an epigenetic pathway that represses genes which are not expressed in Schwann cells (such as *Shh*) or only during Schwann cell differentiation (such as *Gdnf*) (Ma et al., 2015) (**Figure 1C, C'**). Prc2 establishes the repressive H3K27me3 mark in promoter regions and the gene body. Simultaneously, the promoter regions also exhibit active H3K4me3 marks, which are possibly restrained to promoter regions by the HDM Rbp2, which is able to interact with Prc2 (Pasini et al., 2008). Following nerve injury or knock out of an essential Prc2 subunit, the H3K27me3 repressive mark is lost resulting in increased H3K4 trimethylation and expression of repair Schwann cell specific genes (*e.g.*, *Shh* or *Gdnf*). The function of *Kdm8* for the proliferation of Schwann cells during development by controlling the H3K36 methylation status adds a new facet of epigenetic regulation in this cell type (Fuhrmann et al., 2018) (**Figure 1D, D'**). The work indicates, that *Kdm8* demethylates H3K36me2, which is increasingly distributed in the distal 3' gene body of cell cycle associated genes (*e.g.*, *Psrc1*, *Iqgap3*, *Top2a* or *Ckap2*), similar to the distribution observed for H3K36me3 (Carrozza et al., 2005; Keogh et al., 2005). According to the established mode of transcriptional regulation (studied in yeast) H3K36 is trimethylated by Setd2, which interacts with the RNA-Polymerase, during the process of active transcription. Subsequently, H3K36me3 recruits HDACs thereby introducing a closed chromatin state to prevent spurious transcription from cryptic promoters. H3K36me2 might have a preceding function because trimethylation of H3K36 depends on the presence of H3K36me2. Demethylation of H3K36me2 may prevent HDAC recruitment and favor transcription of cell cycle associated genes during development.

The function of H3K36 methylation in Schwann cell biology and the pathogenesis of neuropathies is unknown and the impact of *Kdm8* overexpression in Schwann cells for the development of a demyelinating neuropathy needs further investigation using appropriate mouse models. Also, so far *Kdm8* mutations or variations have not been described in Charcot-Marie-Tooth (CMT) patients. However, as mentioned above, other histone 3 methylations have been shown to be involved in the regulation of proliferation and differentiation of Schwann cells (Gomez-Sanchez et al., 2013; Ma et al., 2015; Brügger et al., 2017). Moreover, in cancer cells, derived from other cell types, cancer cell behavior has been linked to epigenetic modifications of histone H3 including its methylation status. Histone demethylases are now targets for therapeutic approaches, *e.g.*, in acute myeloid leukemia (Castelli et al., 2018), and a variety of inhibitors have been developed for the monoamino oxidase like lysine-specific demethylase 1 and 2 (LSD1 and 2/Kdm1A and B) and the Jumonji C (JmjC) histone demethylases (Jambhekar et al., 2017). A few of them have already been used in mouse models and the best documented inhibitor is probably GSK-J1/4 developed with its highest specificity for KDM5B, KDM5C, KDM6A, KDM6B (Kruidenier et al., 2012). GSK-J1/4 has successfully been used for the reduction of human glioma tissue in a mouse xenograft model (Hashizume et al., 2014) demonstrating the potential of Kdm inhibitors for therapeutic *in vivo* approaches, suggesting that Kdm8 could become a new target in the therapy of neuropathies.

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