Functions of HDAC8 in Schwann cells during peripheral nerve regeneration

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My project focused aimed at identifying the functions of HDAC8 during the regeneration process. For this purpose, we carried out sciatic nerve crush lesions in mice lacking HDAC8 specifically in SCs at several time points of the regeneration process. Western blot, BrDU proliferation assay and electron microscopy analyses previously done in our lab suggested a faster demyelination and a delayed remyelination in HDAC8KO mice after lesion. Indeed, the simultaneous increase in c-Jun and decrease in Oct6 at 1dpc, the decreased number of degenerative myelin rings and the increased proportion of proliferating SCs found at 12dpc suggested a faster SC de-differentiation in HDAC8KO mice. In contrast, the downregulation of Oct6 until 12dpc and the subsequent decrease in Krox20 levels, together with an increased number of unremyelinated axons at 12dpc suggest a remyelination delay. HDAC8KO mice show a faster toe sensitivity at 12dpc and a faster sensory and motor recovery at 19dpc. However, no significant improvement in functional recovery has been observed at 30 dpc. In addition, Krox20 and P0 levels, myelin sheath morphology and thickness were similar in HDAC8KO mice and control mice at 30dpc.Taken together, those results indicate that SC de-differentiate earlier and suggest that axonal regrowth and functional recovery are fostered in HDAC8KO mice. However, it is possible that the delayed remyelination in HDAC8KO mice is compensated by a faster axonal regrowth in such a way that no difference in myelin morphology and sensory-motor performance is observed at 30dpc.
In the second part of my project, I investigated whether Pax3 and Oct6 regulate each other. Indeed, HDAC8KO mice present a simultaneous decrease in Oct6 and Pax3 levels at 5dpc and 12dp. Western blot analyses on Oct6ΔSCE and Pax3KO mice crushed nerves revealed that Oct6 regulates Pax3 levels starting from 5dpc and that at 12dpc, Oct6 and Pax3 regulate each other. Those observations led us to conclude that the phenotype of HDAC8 KO mice is due at least partly to reduced Oct6 level. Furthermore, the significant decrease in P0 and Oct6 levels at 1mpc in Pax3KO mice indicates that Pax3 acts as positive regulator of myelination. We are also interested in finding out the mechanisms of action of HDAC8. Acetyl-lysine immunoprecipitation coupled to mass spectrometry revealed that CBP is more acetylated in HDAC8KO mice crushed nerves. Consistently, CBP co-immunoprecipiated with HDAC8 in wild-type mice crushed nerves. In conclusion, HDAC8 seems to slow down axonal regrowth and functional recovery after sciatic nerve lesion.

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