

## Abstract

Nidogen 1 and nidogen 2 are ubiquitous basement membrane (BM) proteins. Nidogens have been proposed to play a key role in BM formation. However, genetic ablation of both nidogen genes showed that nidogens, while important for certain BMs, are not generally required for BM formation. Although there is redundancy within the mammalian nidogen family, experiments in nidogen 1 and nidogen 2 deficient mice suggest tissue- and isoform-specific functions of the two isoforms. Nidogen 1 deficient mice show neurological phenotypes such as ataxia and progressive hind limb paralysis not seen in the absence of nidogen 2. To characterize the neurological phenotypes of nidogen 1 deficient mice, sciatic nerves of adult nidogen 1 null, nidogen 2 null and control mice were studied. Remarkably, analysis of nidogen 1 deficient nerve sections revealed replacement of nidogen 1 by nidogen 2 and a reduction of laminin 211 in the BMs of Schwann cells (SCs). Morphological and immunofluorescence analysis revealed that nidogen 1 null sciatic nerves contain large areas of non-myelinated axons which are in contact to non-myelinating SCs. In the absence of nidogen 1, electron microscopy showed less regularly organized Remak bundles in comparison to nidogen 2 deficient and control mice. Simultaneously, BM defects of SCs were detected in nidogen 1 null mice. To investigate the role of nidogen 1 during peripheral nerve development, axonal sorting and the myelination were analyzed during postnatal life. Nidogen 1 deficient mice exhibit no axonal sorting defect. However, morphometric, immunoblot and proteome analysis revealed that nidogen 1 null mice display myelination defects when compared to control and nidogen 2 deficient mice. Analysis of transcription factors required for myelination showed that in contrast to wildtype and nidogen 2 deficient mice, nidogen 1 deficient SCs do not down-regulate Oct-6 and fail to up-regulate Egr-2. To decipher the contribution of nidogen 1 during the myelination process, integrin patterns of SCs and signaling pathways were analyzed. Six-weeks-old nidogen 1 deficient mice display higher protein levels of  $\beta$ 1 integrin and analysis of signaling pathways suggest involvement of Akt (Ser473) and mTOR signaling during these processes. *In vitro* analysis of dorsal root ganglia (DRG) cells revealed that *NID1*<sup>-/-</sup> DRG cells do not respond to stimulation by forskolin, a strong inducer of myelination. In addition, *NID1*<sup>-/-</sup> DRG cells display lower levels of phospho-Erk1/2 and phospho-c-JUN after stimulation with Oncostatin M when compared to control and *NID2*<sup>-/-</sup> DRG cells. Interestingly, mice with a mutation in the laminin  $\gamma$ 1 chain (lam $\gamma$ 1N802S), resulting in the loss of nidogen 1 and 2 binding, show similar but more severe neurological phenotypes. Analysis of lam $\gamma$ 1N802S mutant sciatic nerves revealed comparable but also additional changes as seen in the absence of nidogen 1 thus indicating a specific role for the laminin – nidogen 1 interaction in the development and maintenance of the peripheral nervous system which cannot be compensated for by nidogen 2.