● PERSPECTIVE

Schwannomas provide insight into the role of p75^{NTR} and merlin in Schwann cells following nerve injury and during regeneration

Peripheral nerve injury leads to Wallerian degeneration of severed axons, leaving the Schwann cell (SC) sheath behind. Denervated SCs may then either survive and remyelinate a regenerating axon, or they may undergo cell death. Because SCs provide trophic support and guidance cues to regenerating nerve fibers, SC loss severely hampers nerve regeneration (Hall, 1986). Thus, significant work has sought to characterize the molecular mechanisms underlying SC fate following peripheral nerve injury. A deeper understanding of these mechanisms has recently been derived from a somewhat unexpected source; cell survival signaling in benign schwannoma tumors has yielded insight into survival signaling in denervated SCs. This recent evidence implicates a pathway involving the single transmembrane neurotrophin receptor p75^{NTR} and Moesin-ezrin-radixin-like protein (merlin, also known as schwannomin or neurofibromin 2), a membrane-cytoskeleton scaffolding protein linking the cellular membrane to the actin cytoskeleton.

Merlin is the product of the neurofibromatosis type 2 (NF2) gene and functions as a tumor suppressor by mediating contact inhibition. NF2 mutations were first identified in two families affected by hereditary NF2; merlin has since then become recognized for its central role in SC tumorigenesis. Individuals with NF2 accumulate multiple schwannomas over their lifetime including bilateral vestibular schwannomas (VS). NF2 loss-of-function mutations have been identified in the majority of sporadic VSs as well, supporting merlin's central role in schwannoma pathogenesis.

Merlin's tumor suppressor function is controlled by phosphorylation. When it is in the unphosphorylated state, merlin inhibits cell proliferation and when it is in the phosphorylated state it is growth permissive. One of the mechanisms by which merlin suppresses cell growth is by suppressing the cell surface expression of tyrosine kinase receptors for SC growth factors. Interestingly, following nerve injury, merlin becomes phosphorylated which correlates with increased ErbB2 localization in cell membrane lipid rafts that serve as hubs for cell signaling (Brown and Hansen, 2008). The extent to which merlin regulates other, non tyrosine kinase cell membrane receptors involved in SC biology is still being explored.

Recent evidence, however, has emerged that implicates merlin as a key mediator of p75^{NTR} receptor expression and signaling in SCs. p75^{NTR} is a single transmembrane receptor, a member of the Fas/TNF family that when not coupled to any co-receptor only weakly binds mature neurotrophins (in mammals, the four neurotrophins are brain-derived neurotrophic factor (BDNF), nerve growth factor (NGF), neurotrophin (NT-3 and NT-4/5) but strongly binds pro-neurotrophins (for example proBDNF, proNGF). A number of co-receptors for p75^{NTR} have been identified including TrkA, TrkB, TrkC, sortilin, and Nogo (Meeker and Williams, 2015). The complexes formed when these co-receptors associate with p75^{NTR} change the affinity profile and elicit differing cellular responses depending on the identity of the co-receptor. In neurons, p75^{NTR}/Trk complexes strongly binds mature neurotrophins to promote neuronal survival, while p75^{NTR}/sortilin binds proneurotrophins to promote neuronal death, and p75^{NTR}/Nogo affects growth cone dynamics and neuronal pathfinding (Teng et al., 2010; Meeker and Williams, 2015). SCs show an increase in cell death following treatment with proneurotrophins (Provenzano et al., 2011; Ahmad et al., 2015). The roles of co-receptors for p75^{NTR} in Schwann cell death (*e.g.*, sortilin), and the mechanisms by which they are regulated, remain to be fully elucidated.

Recent work on signaling upstream of p75^{NTR} demonstrated that merlin regulates p75^{NTR} expression and that p75^{NTR} signaling is dysregulated in merlin-deficient VS cells. In normal SCs, p75^{NTR} expression increases dramatically as the SCs lose axonal contact either following acute axotomy or more gradual nerve degeneration (*e.g.*, auditory nerve degeneration following aminoglycoside induced deafening) (Taniuchi et al., 1986; Provenzano et al., 2011). Merlin becomes phosphorylated following both acute and gradual nerve injury, and temporally correlated with phosphorylation there is an increase in p75^{NTR} expression (Ahmad et al., 2015). However, until recently, it was not clear whether merlin phosphorylation was causally related to p75^{NTR} expression levels. Interestingly VS cells, like denervated SCs, are not in contact with axons; they also lack functional merlin. Therefore perhaps not surprisingly VS cells, like denervated SCs, have elevated p75^{NTR} levels compared to SCs in uninjured nerves (Ahmad et al., 2014). Further, SCs from a transgenic mouse line bearing a dominant-negative merlin mutation (P0ΔSch121-39) also exhibit higher baseline p75^{NTR} levels, even in uninjured nerves. These observations raise the possibility that loss of merlin function, either by mutation or phosphorylation, leads to elevated p75^{NTR} expression. Because VS cells lack endogenous merlin they provide a model to test whether or nor merlin status is involved in p75^{NTR} expression. Replacement of wild-type merlin reduces p75^{NTR} expression in VS cells, confirming that merlin regulates p75^{NTR} expression. Significantly, replacement of a phosphomimetic merlin mutant (S518D) failed to similarly suppress p75^{NTR} expression (Ahmad et al., 2015). These results further establish that merlin phosphorylation is causally linked to the increase in p75^{NTR} expression.

An interesting paradox is that schwannoma cells survive in the absence of axonal contact despite elevated elevated p75^{NTR} levels. Similarly, SCs from nerves in P0ΔSch121-39 mice survive despite elevated p75^{NTR} levels before injury and are resistant apoptosis following nerve injury with even higher levels of p75^{NTR} expression (Ahmad et al., 2015). These observations suggest that merlin not only regulates p75^{NTR} expression levels but also influences the ability of p75^{NTR} to induce apoptosis in SCs. This possibility was confirmed using a transgenic mouse line with an inducible merlin knock-out. Cre mediated Nf2 excision rendered SCs resistant to p75^{NTR}-mediated cell death (Ahmad et al., 2015). Given that p75^{NTR} activation increases cell death in non-neoplastic SCs, what explains the continued survival of schwannoma cells in the face of high p75^{NTR}? Recent evidence suggests that dysregulation in c-Jun terminal kinase (JNK) and nuclear factor kappa B (NF-κB) signaling may explain this unexpected difference in behavior between non-neoplastic SCs and VS cells.

When p75^{NTR} binds pro-neurotrophins, intramembrane cleavage by γ-secretase releases the intracellular domain that then activates JNK and thereby promotes cell death (Kenchappa et al., 2006). However, merlin also suppresses JNK activity; in merlin-deficient VS cells JNK is dysregulated in a way that contributes to cell survival and proliferation (Yue et al., 2011). JNK activity is significantly elevated in VS cells and replacement of merlin suppresses JNK activity. Further, inhibition of JNK with small-molecule or peptide JNK inhibitors as well as suppression of JNK expression with siRNA oligonucleotides reduces VS cell proliferation and increases VS cell death (Yue et al., 2011). Thus, elevated JNK activity in VS cells promotes cell survival in contrast to the pro-death response in non-neoplastic SCs. One mechanism by which JNK promotes VS cell

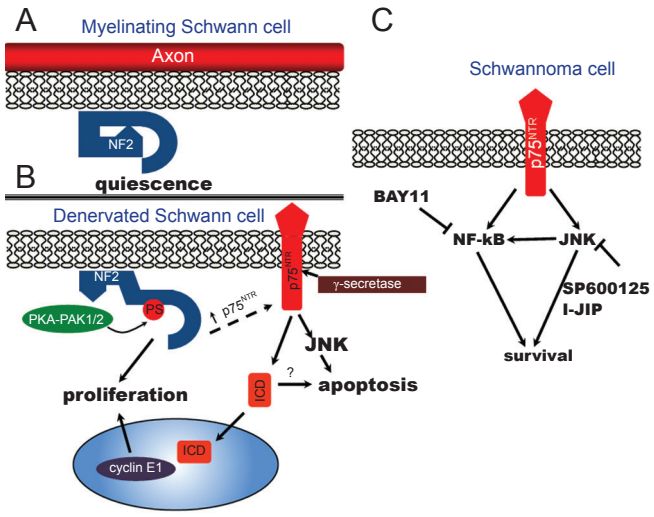

Figure 1 Schematic of merlin and p75^{NTR} signaling in myelinating and demyelinated Schwann cells *versus* schwannoma cells.
(A) Merlin is dephosphorylated in myelinating Schwann cells, which promotes cellular quiescence. (B) When nerve injury occurs, Schwann cells are denervated and merlin is phosphorylated. Denervated Schwann cells may either proliferate or undergo apoptosis. Activation of p75^{NTR} by binding to proneurotrophin promotes apoptosis by gamma secretase cleavage resulting in an intracellular domain that promotes apoptosis. p75^{NTR} also activates the JNK pathway. (C) Signaling downstream of p75^{NTR} is deranged in schwannoma cells. JNK activation by p75^{NTR} promotes survival through a nuclear factor kappaB (NF-κB) dependent pathway and also through an NF-κB independent pathway. NF-κB is also directly activated by p75^{NTR}. Inhibitors of NF-κB, such as BAY11-7082 (BAY11) and of JNK, such as SP600125 or Inhibitor of JNK-based on JNK-interacting protein-1 (I-JIP), have been shown to reduce schwannoma cell survival *in vitro*, confirming that NF-κB and JNK represent potential therapeutic targets.

survival is by suppression of reactive oxygen species in the mitochondria (Yue et al., 2011). Further, JNK inhibition increases oxidative stress in VS cells following γ-irradiation and sensitizes VS cells to radiation though they are otherwise relatively resistant to radiation (Yue et al., 2011). Thus, JNK inhibitors represent excellent potential therapeutic treatments for VSs (**Figure 1**).

Interestingly, treatment of VS cells with proneurotrophins rescues them from apoptosis due to JNK inhibition (Ahmad et al., 2014). This suggests that a pro-survival response is mounted when p75^{NTR} binds proneurotrophins in VS cells in contrast to the pro-death response in non-neoplastic SCs. Indeed, treatment with proneurotrophins activates NF-κB in VS cells (Ahmad et al., 2014). JNK inhibition decreases basal NF-κB activity; however, the increase in NF-κB in response to proneurotrophins occurs even in cells with suppressed JNK (Ahmad et al., 2014). Thus, p75^{NTR} is capable of activating NF-κB *via* a JNK-independent pathway in VS cells. Inhibition of NF-κB by transduction with an adenoviral vector that expresses inhibitor-κB (IκB) overcomes the ability of proneurotrophins to rescue VS cells from apoptosis (Ahmad et al., 2015). Taken together these observations demonstrate that p75^{NTR} activates NF-κB *via* a JNK independent pathway to provide a pro-survival response in VS cells (Gentry et al., 2000). Significantly, network analysis recently implicated aberrant NF-κB activation as a root cause of proliferation in VS cells, and NF-κB inhibitors have been shown to reduce VS cell proliferation *in vitro* further confirming the vital role of this pathway in VS tumorigenesis (Dilwali et al., 2015).

In summary, recent publications have begun to elucidate how merlin regulates responses of SCs to nerve injury and how dysregulation of these responses in the absence of merlin likely contributes to SC tumorigenesis. When SCs lose contact with axons, merlin becomes phosphorylated leading to increased p75^{NTR} expression and ultimately to SC apoptosis and loss. However, in the absence of functional merlin, SCs become resistant to p75^{NTR}-mediated apoptosis. Further, p75^{NTR} signaling elicits a pro-survival response in schwannoma cells, likely contributing to their ability to proliferate and survive in the absence of axons. This pro-survival response may contribute to the relative resistance of VS cells to chemotherapeutic agents such as kinase inhibitors. Thus, simultaneously targeting p75^{NTR} and/or NF-κB may sensitize VS cells to other classes of chemotherapeutic agents. Interestingly, the mechanisms by which VS cells escape cell death also inform our understanding of normal SC behavior following nerve injury.

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