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Modulating Sema3A signal with a L1 mimetic peptide is not sufficient to promote motor recovery and axon regeneration after spinal cord injury

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We examined whether Sema3A, which is upregulated at the site of spinal cord injury, exerts a direct effect on axons. We used ASNKL peptide that prevents specifically the inhibitory effect of Sema3A on L1/Neuropilin1 (Nrp1)-expressing axons. In the naïve mouse spinal cord, L1 is located on a subset of corticospinal axons, whereas Nrp1 is barely detectable. After contusion injury, Nrp1 is found on L1-negative immune cells, whereas its expression does not increase on severed axons. L1-expressing axons sprout extensively into the lesion site but no difference in axon density could be detected in the lesion area of mice treated with ASNKL. In agreement, these mice did not recover a better motor function than controls. Similarly, culture of neurons sensitive to ASNKL on cryosections of lesioned spinal cords revealed no effect of Sema3A. Our data indicate a limited direct effect of Sema3A on axonal growth at the site of a contusion injury, and suggest that alternative mechanisms underlie positive effects of Sema3A inhibition on motor recovery.

Keywords: Mouse; Spinal cord injury; Semaphorin 3A; L1 CAM; Motor function recovery

Introduction

Both intrinsic growth capacities and extrinsic factors contribute to limit adult central nervous system (CNS) axon regeneration. The inhospitable milieu of the injured CNS results, in part, from the presence of myelin-specific inhibitory factors (Schwab and Bartholdi, 1996). In addition, CNS injury induces a glial scarring response that leads to increased expression of many molecules thought to inhibit regeneration (Silver and Miller, 2004). Furthermore, the recruitment of inflammatory cells and cytokines has a more complicated effect on CNS regeneration (Yiu and He, 2006). Most of the guidance molecules playing a crucial role in development and guidance of neuronal projections, such as Ephrins, Slits, Netrins and class 3 Semaphorins persist in the spinal cord after development is completed (Wehrle et al., 2005; Fabes et al., 2006; Harel and Strittmatter, 2006; Manitt et al., 2006). Their distribution, especially after injury, provides regenerating axons with a drastically altered signaling environment.

Semaphorins are a large family of secreted and membrane bound proteins involved in axon guidance, cell migration and cell death (Kruger et al., 2005; Mann et al., 2007). While Semaphorins are known to be expressed at sites of CNS injury, until recently, evidence for a significant contribution of guidance cues of the Semaphorin family in inhibition of axon regeneration were lacking. Using xantofulvin, a pharmacological inhibitor of Sema3A, Kaneko et al. showed enhanced functional recovery after total spinal cord transection in rats. Pleiotropic effects including enhanced regeneration and/or preservation of injured axons, robust Schwann cell migration, decrease in apoptotic cell number and marked enhancement of angiogenesis are thought to be responsible for the observed recovery (Kaneko et al., 2006).

Sema3A, initially identified based on its growth cone collapse-inducing activity (Luo et al., 1993) repels axons by binding to receptors composed of a binding subunit Neuropilin 1 (Nrp1) and a signal transducer of the PlexinA family, thus activating intracellular pathways that control actin dynamics (Castellani and Rougon, 2002; Tran et al., 2007). We have previously shown that Nrp1 and the Immunoglobulin superfamily cell adhesion molecule (Ig CAM) L1, associate through their extracellular domains, uncovering a link between L1 and Nrp1/Sema3A signaling. This is further supported by the finding that wild-type, but not L1-deficient axons, when exposed to soluble L1 or a 5 amino acid peptide (ASNKL) derived from L1 Ig1 domain and mimicking L1/Nrp1 binding site, switched their response to Sema3A from repulsion to attraction (Castellani et al., 2000, 2002). This peptide thus represents a pharmacological

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tool to directly and specifically manipulate Sema3A responses from cells co-expressing L1 and Nrp1.

Here, we used this tool with the aim to better decipher the multiple cellular effects of Sema3A after spinal cord contusive injury in mice. We show that *in vitro*, both Sema3A-mediated inhibition of axon growth and growth cone collapse are blocked by ASNKL. We show that Sema3A is expressed in the lesion scar in mice, and that some spinal axons express Nrp1/L1, therefore making it possible to modulate their response to Sema3A with ASNKL peptide. However, delivery of the peptide *in vivo* does not improve motor recovery, nor does it increase axonal density in the lesion site in this model. Results from cryoculture assays using adult spinal cord sections showed that the complex environment of the injured spinal cord prevents embryonic neurons from responding to a modulation of Sema3A signal by ASNKL. Together our results suggest that modulation of Sema3A signaling on neurons is not

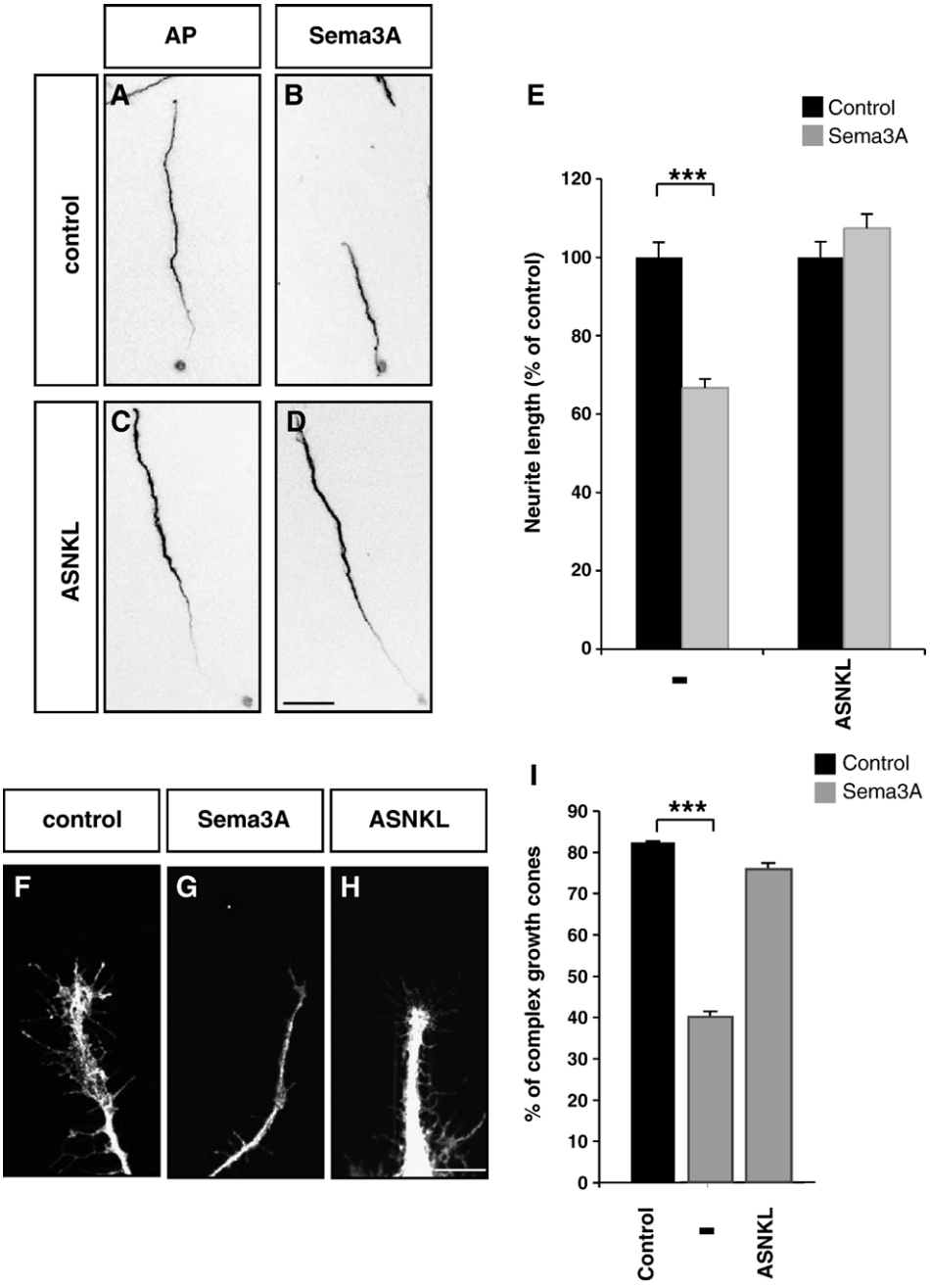

Fig. 1. ASNKL peptide blocks inhibition of axon growth and growth cone collapse mediated by Sema3A. Embryonic cortical neurons were cultured for 48 h in the presence of AP-conditioned medium (A, C) or Sema3A-AP (B, D) from HEK-transfected cells with (C, D) or without (A, B) ASNKL peptide at 10^{-6} M and stained with anti-SMI-31. Cell bodies are at the bottom of the picture for each condition. Scale bar: 40 μm. (E) Quantification of Sema3A effects in the presence or absence of ASNKL peptide. Data are presented as mean ± SEM normalized to values obtained in AP conditions. Sema3A reduces by 30% neurite length in control conditions while it has no effect in the presence of ASNKL peptide. Photomicrographs of growth cones stained with Tuj1 in the presence of control medium (F), Sema3A (G) with ASNKL peptide (H) at 10^{-6} M. Scale bar: 5 μm. (I) Quantification of complex growth cones for each condition. Results are presented as mean ± SEM from 3 independent experiments. Sema3A increases the percentage of collapsed growth cones and ASNKL peptide prevents this effect.