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The role of well-defined patterned substrata on the regeneration of DRG neuron pathfinding and integrin expression dynamics using chondroitin sulfate proteoglycans

Gerald N. Hodgkinson, Patrick A. Tresco, Vladimir Hlady*

Department of Bioengineering, University of Utah, Salt Lake City, UT 84112, USA

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ABSTRACT

Injured neurons intrinsically adapt to and partially overcome inhibitory proteoglycan expression in the central nervous system by upregulating integrin expression. It remains unclear however, to what extent varying proteoglycan concentrations influence the strength of this response, how rapidly neurons adapt to proteoglycans, and how pathfinding dynamics are altered over time as integrin expression is modulated in response to proteoglycan signals. To investigate these quandaries, we created well-defined substrata in which postnatal DRG neuron pathfinding dynamics and growth cone integrin expression were interrogated as a function of proteoglycan substrata density. DRGs responded by upregulating integrin expression in a proteoglycan dose dependent fashion and exhibited robust outgrowth over all proteoglycan densities at initial time frames. However, after prolonged proteoglycan exposure, neurons exhibited decreasing velocities associated with increasing proteoglycan densities, while neurons growing on low proteoglycan levels exhibited robust outgrowth at all time points. Additionally, DRG outgrowth over proteoglycan density step boundaries, and a brief β1 integrin functional block proved that regeneration was integrin dependent and that DRGs exhibit delayed slowing and loss in persistence after even transient encounters with dense proteoglycan boundaries. These findings demonstrate the complexity of proteoglycan regulation on integrin expression and regenerative pathfinding.

1. Introduction

Regenerating neurons possess the ability to modulate integrin expression, enabling navigation of various extracellular environments after injury. Notably, elevated integrin expression has been associated with axotomy [1], and in in vitro experiments integrin upregulation is sufficient in some cases to overcome the inhibitory effects of chondroitin sulfate proteoglycans (CSPGs) [2,3], the major inhibitory constituent in central nervous system (CNS) injuries [4,5]. In recent years, many promising CNS repair strategies have focused both on diminishing inhibitory signals at the site of injury and augmenting the intrinsic ability of neurons to extend new processes and to find a path through injured tissue [6–12].

Neurons such as dorsal root ganglia (DRG) have been studied for their ability to regenerate after spinal cord injuries and have served as a model cell type for many CNS injury studies. In vitro and in vivo studies have revealed that DRGs, regardless of age, require a number of conditions for robust regeneration including 1) sufficient growth promoting ECM molecules such as laminin for anchor dependent locomotion [13,14], 2) growth factors such as NGF or NTF [15–17], 3) expression of active ECM anchoring receptors such as integrins [3,18,19], and 4) active expression of second messenger systems such as cAMP [20,21]. DRGs, unlike non-neuronal migrating cell types, posses the capacity to adapt to a wide range of substrate adhesivities such that robust pathfinding can proceed on diffuse concentrations of laminin or even in the presence of varying concentrations of inhibitory proteoglycans [18,22–24]. Various means have also been devised to experimentally increase DRG outgrowth through inhibitory boundaries, including by increasing integrin expression by viral transfection [2], activating existing integrins into an ECM binding conformation [20,22], increasing cAMP intracellular levels [20,21], providing growth factors [15–17], and by removing activity of a recently discovered receptor for CSPGs [25].

Despite of in vitro evidence that neurons adapt to CSPG signals, there are uninvestigated aspects of this phenomenon which, if better understood, could provide insights into more effective

* Corresponding author. 20 S. 2030 E., Rm 108A, Department of Bioengineering, University of Utah, Salt Lake City, UT 84112, USA. Tel.: +1 801 581 5042; fax: +1 801 585 5151.
E-mail addresses: vladimir.hlady@utah.edu, vladimir.hlady@m.cc.utah.edu (V. Hlady).

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