Shh and Gli3 activities are required for timely generation of motor neuron progenitors

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ABSTRACT

Generation of distinct ventral neuronal subtypes in the developing spinal cord requires Shh signaling mediated by the Gli family of transcription factors. Genetic studies of $Shh^{-/-};Gli3^{-/-}$ double mutants indicated that the inhibition of Gli3 repressor activity by Shh is sufficient for the generation of different neurons including motor neurons. In this study, we show that although ventral neural progenitors are initiated in normal numbers in $Shh^{-/-};Gli3^{-/-}$ mutants, the subsequent appearance of motor neuron progenitors shows a ~20-hour lag, concomitant with a delay in the activation of a pan-neuronal differentiation program and cell cycle exit of ventral neural progenitors. Accordingly, the $Shh^{-/-};Gli3^{-/-}$ mutant spinal cord exhibits a delay in motor neuron differentiation and an accumulation of a ventral neural progenitor pool. The requirement of Shh and Gli3 activities to promote the timely appearance of motor neuron progenitors is further supported by the analysis of $Ptch1^{-/-}$ mutants, in which constitutive Shh pathway activity is sufficient to elicit ectopic and premature differentiation of motor neurons at the expense of ventral neural progenitors. Taken together, our analysis suggests that, beyond its well established dorso-ventral patterning function through a Gli3-derepression mechanism, Shh signaling is additionally required to promote the timely appearance of motor neuron progenitors in the developing spinal cord.

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Introduction

Neurogenesis in the vertebrate central nervous system is characterized by a series of coordinated events, involving specification, expansion and differentiation of distinct neuronal progenitor cell types. Among these, regulated differentiation of neuronal progenitors is a prerequisite to control the expansion of neuronal progenitor pools as well as the generation of appropriately numbered post-mitotic neurons (Bertrand et al., 2002). Therefore, tight regulation of the length and duration of neuronal progenitor cell cycles is critical for proper neural tube size regulation and appearance of distinct neuronal subtypes at the correct time and location.

In the developing ventral spinal cord, the expression of homeodomain protein Nkx6.1 highlights the early ventral progenitor population which eventually differentiates into the floor plate, motor neurons and several interneuron subtypes (Briscoe et al., 2000; Sander et al., 2000). Establishment of the Nkx6.1+ progenitor domain is regulated by secreted signaling molecule Sonic hedgehog (Shh) (Briscoe et al., 2000). Mice lacking Shh function display severe neural patterning defects including a lack of most ventral neuronal cell types in the spinal cord (Chiang et al., 1996). However, in $Shh^{-/-}$;

$Gli3^{-/-}$ or $Smo^{-/-};Gli3^{-/-}$ double mutants, motor neurons and several classes of ventral interneurons are generated, indicating that inhibiting Gli3 transcriptional repressor activity by Shh signaling is central to the establishment of Nkx6.1+ neural progenitors (Litingtung and Chiang, 2000; Persson et al., 2002; Wijgerde et al., 2002). Indeed, activation of Shh signaling inhibits Gli3 repressor formation by preventing Gli3 proteolytic processing (Litingtung et al., 2002; Wang et al., 2000). The full-length form of Gli3 can also act as a transcriptional activator in certain developmental context. This is demonstrated by the observation that ectopic Hh pathway activation in $Ptch^{-/-}$ mutants is partially dependent on Gli3 function (Motoyama et al., 2003). Moreover, Gli3 apparently shares redundant function with Gli2, another Gli family member, in the development of V3 interneurons (Bai et al., 2004; Lei et al., 2004; Motoyama et al., 2003). Thus, Gli3 is a bifunctional transcription factor and its activity is modulated by Shh signaling.

It has been shown that ventral spinal cord progenitor cells initially express Nkx6.1 at an early somite stage, then subsets of Nkx6.1+ cells express cell-type specific (floor plate, MN progenitor, V3 or V2 interneuron progenitor) transcription factors that eventually dictate their fates (Jeong and McMahon, 2005). This temporal stepwise cell fate determination process appears to be associated with gradual accumulation of Shh ligand in the responding neural progenitors (Chamberlain et al., 2008). Emerging evidence suggests that both the strength and duration of Shh signaling activity affect the final cellular

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response. Specifically, it has been shown in chick spinal cord that prolonged exposure to a defined level of Shh signaling leads to progressively more ventral cell fates (Stamataki et al., 2005; Dessaud et al., 2007). It is therefore important to note that Shh ligand continues to be expressed by the notochord and floor plate well after the completion of spinal dorso-ventral patterning until midgestation, which raises the possibility that Shh signaling may assume an as yet undetermined functional role.

The differentiation of motor neurons in the spinal cord is initiated by the expression of bHLH protein Olig2 within the Nkx6.1 progenitor domain (Mizuguchi et al., 2001; Novitch et al., 2001). Both gain- and loss-of-function studies indicated that Olig2 acts downstream of Nkx6.1 and related Nkx6.2 proteins to promote motor neuron differentiation (Novitch et al., 2001; Vallstedt et al., 2001). Abrogating Olig2 function not only led to lack of motor neuron generation but also prolonged ventral neural progenitor cell proliferation (Lu et al., 2002;

Takebayashi et al., 2002; Zhou and Anderson, 2002). The latter property is associated with the ability of Olig2 to promote cell cycle exit by activating a generic neuronal differentiation program (Mizuguchi et al., 2001; Novitch et al., 2001). Thus, precise regulation of Olig2 expression is critical not only for motor neuron generation but also for the balanced development of ventral progenitor pools. While Shh is capable of inducing Olig2 expression, this is thought to be indirect and mediated through Nkx6.1 expression (Briscoe and Novitch, 2008; Novitch et al., 2003; Rowitch et al., 2002). Moreover, recent studies have identified retinoic acid as an obligate signal in activating Olig2 expression and motor neuron differentiation (Novitch et al., 2003). In this study, we show that the timely appearance of Olig2 expression in the Nkx6.1+ progenitor domain also depends on Shh signaling. The delay of Olig2 expression in $Shh^{-/-};Gli3^{-/-}$ double mutants leads to a defective pan-neuronal differentiation program and accumulation of ventral neural progenitors. Thus, our
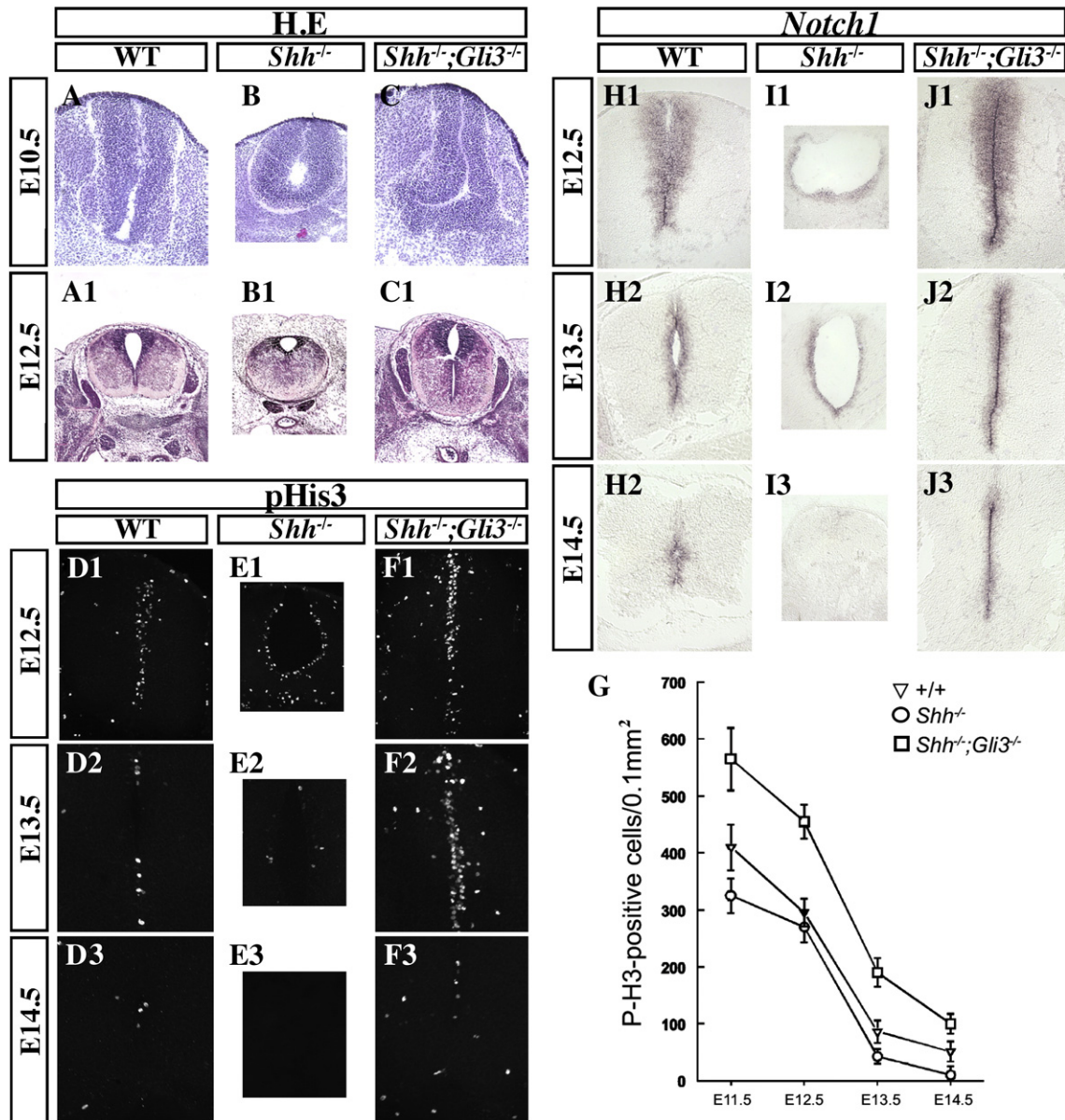

Fig. 1. Increased cell numbers and augmented mitotic activity in $Shh^{-/-};Gli3^{-/-}$ mutant spinal cord. (A–C, A1–C1) H&E-stained cross-sections of E10.5 and E12.5 wildtype (A, A1), $Shh^{-/-}$ (B, B1) and $Shh^{-/-};Gli3^{-/-}$ (C, C1), showing a larger spinal cord size in $Shh^{-/-};Gli3^{-/-}$ mutant. (D1–D3, E1–E3, F1–F3) Distribution of pHis3 protein in wildtype (D1–D3), $Shh^{-/-}$ (E1–E3), and $Shh^{-/-};Gli3^{-/-}$ (F1–F3) embryos at various stages in the lumbar region. Note that there are more mitotic pHis3+ cells in the $Shh^{-/-};Gli3^{-/-}$ mutant spinal cord ventricular zone compared with wildtype and $Shh^{-/-}$ (G). (H1–H3, I1–I3, J1–J3) Neuronal progenitor marker Notch1 in wildtype(H1–H3), $Shh^{-/-}$ (I1–I3) and $Shh^{-/-};Gli3^{-/-}$ (J1–J3). Note the consistent expansion of Notch1 expression domains in $Shh^{-/-};Gli3^{-/-}$ mutant spinal cord.