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Migration pathways of sacral neural crest during development of lower urogenital tract innervation

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ABSTRACT

The migration and fate of cranial and vagal neural crest-derived progenitor cells (NCPCs) have been extensively studied; however, much less is known about sacral NCPCs particularly in regard to their distribution in the urogenital system. To construct a spatiotemporal map of NCPC migration pathways into the developing lower urinary tract, we utilized the *Sox10*-H2BVenus transgene to visualize NCPCs expressing *Sox10*. Our aim was to define the relationship of Sox10-expressing NCPCs relative to bladder innervation, smooth muscle differentiation, and vascularization through fetal development into adulthood. Sacral NCPC migration is a highly regimented, specifically timed process, with several potential regulatory mileposts. Neuronal differentiation occurs concomitantly with sacral NCPC migration, and neuronal cell bodies are present even before the pelvic ganglia coalesce. Sacral NCPCs reside within the pelvic ganglia anlagen through 13.5 days post coitum (dpc), after which they begin streaming into the bladder body in progressive waves. Smooth muscle differentiation and vascularization of the bladder initiate prior to innervation and appear to be independent processes. In adult bladder, the majority of Sox10+ cells express the glial marker S100β, consistent with Sox10 being a glial marker in other tissues. However, rare Sox10+ NCPCs are seen in close proximity to blood vessels and not all are S100β+, suggesting either glial heterogeneity or a potential nonglial role for Sox10+ cells along vasculature. Taken together, the developmental atlas of Sox10+ NCPC migration and distribution profile of these cells in adult bladder provided here will serve as a roadmap for future investigation in mouse models of lower urinary tract dysfunction.

1. Introduction

Early in embryonic development, neural crest cells delaminate from the dorsal neural tube and migrate along prescribed paths to eventually differentiate into Schwann cells and glia, as well as peripheral neurons, melanocytes, chondrocytes, adrenal chromaffin cells and other cell lineages (Le Douarin et al., 2008; Shakova and Sommer, 2010). These migratory cells, termed neural crest-derived progenitor cells (NCPCs), express *Sox10* and differentiate to form sensory and autonomic innervation for a variety of organs, including the lung, heart, kidney, and intestine (Freem et al., 2010; Itaranta et al., 2009; Lajiness et al., 2014; Lake and Heuckeroth, 2013; Musser and Southard-Smith, 2013; Obermayr et al., 2013; Verberne et al., 2000). Detailed spatiotemporal maps of neural crest derived innervation for these organs have been particularly informative for understanding disease processes. In contrast, surprisingly little is known at the cellular level about initial colonization of the lower urogenital tract (LUT) by Sox10+ NCPCs. Despite the fact that sacral NCPCs give rise to pelvic ganglia, which provide essential autonomic innervation to the LUT, the principal focus of prior sacral NC analysis has been the contribution of these progenitors to the enteric nervous system (Anderson et al., 2006; Kapur, 2000; Mundell et al., 2012; Wang et al., 2011).

A comprehensive understanding of LUT innervation and the factors that regulate this system have the potential to impact treatment and quality of life for patients who have sustained bladder damage. Injury to the bladder can result from a multitude of insults: congenital disorders, infection, trauma, cancer, or iatrogenic injury occurring during abdominopelvic surgery (Atala, 2011). Significant advances have been made in the field of bladder repair using autologous patient cells to seed bladder scaffolds (Atala et al., 2006). However, efforts to innervate bladder scaffolds have not been successful (Lam Van Ba et al., 2015; Oberpenning et al., 1999).

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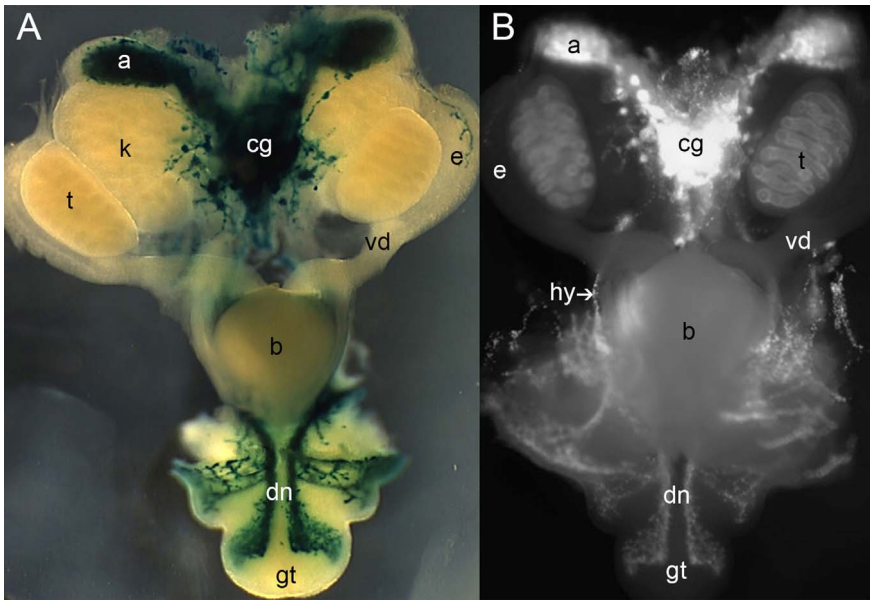

Fig. 1. Distribution of sacral neural crest-derived progenitor cells (NCPCs) in *Sox10*$^{LacZ\text{-}KO/+}$ and *Sox10*-H2BVenus embryos. Ventral views of micro-dissected urogenital tracts are shown in whole mount from (A) a 14.5 dpc *Sox10*$^{LacZ\text{-}KO/+}$ and (B) a 14.5 dpc *Sox10*-H2BVenus embryo (70× magnification). The superior (anterior) surface of the genital tubercle is shown. Abbreviations: a, adrenal gland; b, bladder; cg, celiac ganglia; dn, dorsal nerve; e, epididymis; gt, genital tubercle; hy, hypogastric nerve; k, kidney; t, testis; vd, vas deferens.

Thus, detailed understanding of the normal events that occur in development of LUT innervation may lead to strategies for regeneration of damaged or diseased neural inputs in the bladder.

We previously reported the distribution of neural elements in the fetal mouse urogenital tract (Wiese et al., 2012); however, much remains unknown about the initial stages when LUT innervation begins. Sacral NCPCs have been reported migrating around the distal hindgut on their way to the urogenital sinus as early as 11.5 days post coitus (dpc), and neuronal differentiation within pelvic ganglia is ongoing at 15.5 dpc (Anderson et al., 2006; Wang et al., 2011; Wiese et al., 2012). It has not yet been determined when autonomic pelvic ganglia first coalesce or when neurogenesis in these ganglia first initiates. Because regenerative strategies aimed at compensating for deficits of bladder innervation would benefit from understanding basic processes in the normal development of LUT nerves, we undertook a study of sacral NCPC migration during development of bladder innervation. Using our *Sox10*-Histone2BVenus (*Sox10*-H2BVenus) reporter strain (Corpening et al., 2011), we specifically examined when neuronal progenitors first enter the urogenital sinus mesenchyme that will become the primitive bladder, when markers of differentiating neurons and glia first appear within the structures of the LUT, and whether there are temporal variations in migration of NCPCs into the bladder that might suggest key regulatory stages. We concurrently documented the distribution of Sox10+ NCPCs in late fetal and adult bladders to establish a normal baseline that may prove informative in the analysis of mouse models of bladder dysfunction. Based on our initial observations of NCPC migration into the bladder and the potential interdependence between innervation and bladder muscle development and vascularization, we examined the distribution of NCPCs relative to the timeline of fetal smooth muscle and vascular development in the normal mouse bladder. We observed that the processes of innervation, vascularization and smooth muscle development appear to initiate independently of one another. Our initial survey of the distribution of Sox10+ cells in the adult bladder suggests heterogeneity of these cells within the bladder wall and sets the stage for future analysis of discrete neural crest-derived lineages in normal maturation and disease of the LUT.

2. Results

2.1. NCPCs that populate the urogenital tract are revealed by Sox10 expression

Initial studies characterizing the migration patterns of sacral NCPCs focused on progenitors expressing a dopamine beta-hydroxylase transgene (Dβh-nLacZ) and their differentiation as they approached the hindgut (Anderson et al., 2006; Kapur, 2000). While those studies identified Dβh+ cells within pelvic ganglia by 13 dpc, more comprehensive labeling of NCPCs would be advantageous for visualizing migration patterns throughout the developing genitourinary system. To assess the feasibility of using a *Sox10* transgenic reporter for detection and characterization of NCPCs in the genitourinary system, we compared the expression pattern of our previously described transgenic line *Sox10*-H2BVenus to that of a knock-in allele for *Sox10* that expresses LacZ (*Sox10*$^{LacZ\text{-}KO/+}$) (Britsch et al., 2001). The *Sox10*-H2BVenus transgene line faithfully recapitulates *Sox10* expression in rostral neural crest populations, including cranial ganglia, otic vesicles, branchial arches, dorsal root ganglia, cervical ganglia, and vagal enteric neural crest (Corpening et al., 2011). Thus, we expected transgene expression patterns to mirror endogenous *Sox10* among sacral NCPC as well. Intact genitourinary tissues were micro-dissected from *Sox10*$^{LacZ\text{-}KO/+}$ and *Sox10*-H2BVenus embryos at 14.5 dpc and either stained for LacZ activity or imaged for fluorescence of the *Sox10*-H2BVenus reporter in whole mount (Fig. 1). Nearly identical expression patterns were observed, with strong expression in the adrenal glands, celiac ganglia, and numerous nerves tracts of both *Sox10*$^{LacZ\text{-}KO/+}$ and *Sox10*-H2BVenus tissues. The consistency between expression of *Sox10*$^{LacZ\text{-}KO/+}$ and *Sox10*-H2BVenus in the genitourinary tract extends prior studies that demonstrated that the 28O11 BAC backbone used to drive heterologous transgene reporters recapitulates expression of the endogenous *Sox10* gene (Corpening et al., 2011; Deal et al., 2006). While the majority of expression sites were comparable between the two *Sox10* lines, one difference we observed was the presence of *Sox10*-H2BVenus signal in the Sertoli cells of the testes. Sox10 has previously been identified in Sertoli cells (Harding et al., 2011; Polanco et al., 2010); however, no comparable LacZ staining was seen in *Sox10*$^{LacZ\text{-}KO/+}$ testes. While this discrepancy may result from the testes