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Lamprey neural crest migration is Snail-dependent and occurs without a differential shift in cadherin expression

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ABSTRACT

The acquisition of neural crest cells was a key step in the origin of the vertebrate body plan. An outstanding question is how neural crest cells acquired their ability to undergo an epithelial-mesenchymal transition (EMT) and migrate extensively throughout the vertebrate embryo. We tested if differential regulation of classical cadherins—a highly conserved feature of neural crest EMT and migration in jawed vertebrates—mediates these cellular behaviors in lamprey, a basal jawless vertebrate. Lamprey has single copies of the type I and type II classical cadherins (*CadIA* and *CadIIA*). *CadIIA* is expressed in premigratory neural crest, and requires the transcription factor Snail for proper expression, yet *CadIA* is never expressed in the neural tube during neural crest development, suggesting that differential regulation of classical cadherin expression is not required to initiate neural crest migration in basal vertebrates. We hypothesize that neural crest cells evolved by retention of regulatory programs linking distinct mesenchymal and multipotency properties, and emigrated from the neural tube without differentially regulating type I/type II cadherins. Our results point to the coupling of mesenchymal state and multipotency as a key event facilitating the origin of migratory neural crest cells.

1. Introduction

The evolutionary origin of the vertebrates is linked to their acquisition of the neural crest, a multipotent, migratory embryonic cell population that contributes to the development of many vertebrate traits, including the peripheral nervous system, pigment cells, and components of the endocrine system (Donoghue et al., 2008; Green et al., 2015; Square et al., 2016; Trainor, 2013). The neural crest is also responsible for generating the core of the vertebrate "new head"—the cartilage, bone and muscle that forms the pronounced cranium and jaws, features that house the primary sense organs and are hypothesized to have facilitated the invasion of new ecological niches, and distinguish vertebrates morphologically and behaviorally from their closest relatives, the invertebrate chordates (Cattell et al., 2011; Gans and Northcutt, 1983; McCauley and Bronner-Fraser, 2006). The neural crest is therefore exemplary of a developmental and evolutionary innovation that correlates with the adaptive radiation of a major animal clade.

The neural crest forms in vertebrate embryos in a highly stereotyped manner. They become established in the neural plate border between the medial neural plate and lateral epidermal ectoderm (Fig. 1A). A highly conserved gene regulatory network (GRN) of transcription factors and signaling molecules orchestrates the progressive specification of these cells to become bona fide neural crest (Betancur et al., 2010; Meulemans and Bronner-Fraser, 2004; Sauka-Spengler and Bronner-Fraser, 2008a). These specification factors include members of the SoxE family, Tfap2α, Id, Snail/Slug, Myc, Twist, Ets, Myb and several others that are directly responsible for establishing the hallmarks of neural crest cells (Sauka-Spengler and Bronner-Fraser, 2006; Simoes-Costa and Bronner, 2015; Simões-Costa and Bronner, 2013). One key feature of neural crest development is a dramatic change in cell shape and molecular architecture that results in an epithelial-to-mesenchymal transition (EMT), a feature that enables these cells to migrate to specific locations throughout the vertebrate embryo (Bronner, 2012; Duband et al., 1995; Kerosuo and Bronner-Fraser, 2012; Fig. 1B, C). Although EMT is not specific to vertebrates (Kee et al., 2007) or neural crest *per se* (Nakaya and Sheng, 2013; Savagner, 2010), the extent to which neural crest cells migrate as a multipotent and proliferative cell population has no parallel in any other animal embryo. Neural crest EMT has been described at length in embryos of numerous jawed (gnathostome) vertebrates (Ahlstrom and Erickson, 2009; Barriga et al., 2013; Duband et al., 1995; Strobl-Mazzulla and Bronner, 2012), and is therefore thought to be an evolutionarily conserved process. The initiation of neural crest EMT requires fine-tuned control of the spatial and temporal expression of numerous genes (Savagner, 2001; Thiery and Sleeman, 2006), many of

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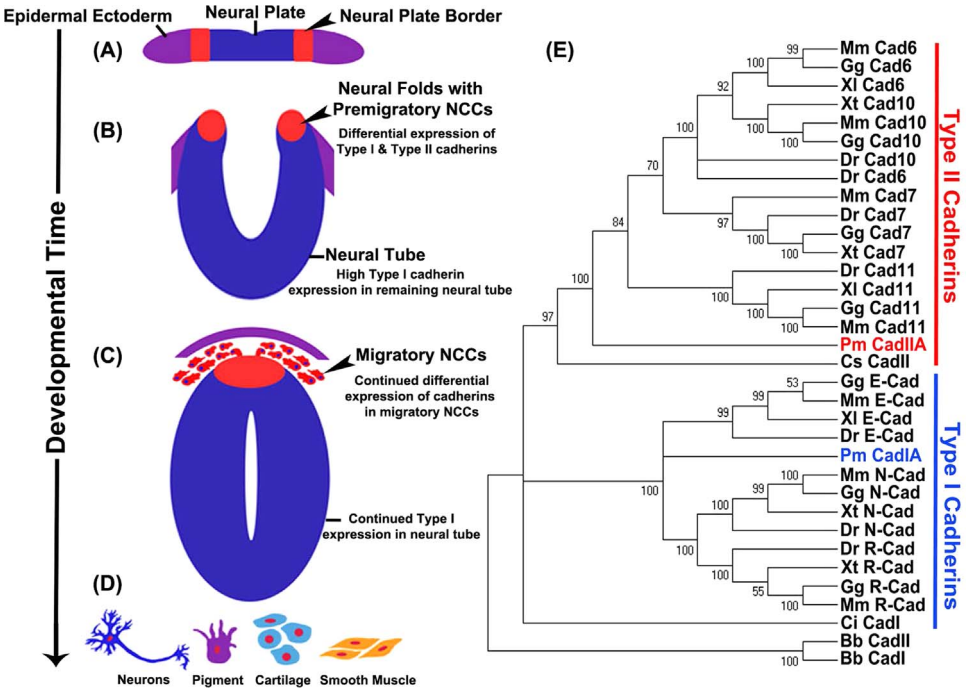

Fig. 1. (A-D) Canonical model for neural crest development and cadherin expression during EMT, illustrated as cross sections through a generalized vertebrate embryo, and (E) phylogeny of chordate type I (blue) and type II (red) classical Cadherins. After gastrulation there are three established dorsal cellular domains (A): medial neural plate (blue), lateral epidermal ectoderm (purple) and neural plate border (presumptive neural crest cells, NCCs) in between (red). All three domains express type I cadherins. As neurulation is completed (B) specified neural crest cells are brought dorsally and begin to delaminate from the underlying neuroepithelium by differentially regulating expression of type I and type II cadherins, whereas the neuroepithelium maintains strong expression of only type I cadherins (B). Because cadherins tend to bind in a homophilic fashion, cell populations that express different cadherin proteins at their surface no longer retain strong intercellular adhesion to their neighbors, resulting in detachment of premigratory neural crest cells (pmNCCs) from the surrounding neuroepithelium. By contrast, neuroepithelial cells remain attached to one another as a result of sustained type I cadherin expression. Sustained, differential expression of type I and type II cadhreins, concomitant with expression of other pro-mesenchymal genes leads to NCC migration from the dorsal neural tube (C) to various regions in the embryo where many subsequently undergo a mesenchymal to epithelium transition and differentiate into a variety of cell types (D). (E) Lamprey has only a single copy of the type I and type II cadherin genes compared to gnathostomes.

which also play important roles earlier in neural crest development (*e.g., Twist, Snail*). The proteins encoded by these genes mediate detachment of the neural crest from the underlying neural epithelium and initiate migration, primarily by promoting reorganization of the cytoskeleton (Clay and Halloran, 2011, 2010). After migration, neural crest cells lose their mesenchymal morphology, and undergo terminal differentiation (Betancur et al., 2010; Hall, 2008) into many of the cell types that define vertebrates (Fig. 1D).

Key to the process of neural crest EMT in jawed vertebrates is the concerted activity of a suite of signaling molecules and transcription factors to repress activity of genes that promote an epithelial phenotype and activate genes that promote migration (Savagner, 2001, 2010). At the molecular level, the onset of EMT is characterized by modulation at the cell surface of cadherin intercellular adhesion proteins that may correlate with epithelial *versus* mesenchymal states (Dady et al., 2012; Taneyhill, 2008; Fig. 1A–C). The differential expression of type I and type II cadherins in the neural crest domain is thought to be one of the principal mechanisms that controls the onset of neural crest migration in gnathostomes. This can occur by direct transcriptional repression of certain cadherins in the dorsal neural tube, whereas the remaining non-migratory neuroepithelium retains uniform levels of type I cadherin (usually N-Cadherin) expression (Gheldof and Berx, 2013; Rogers et al., 2013; Scarpa et al., 2015; Taneyhill and Schiffmacher, 2013; Wheelock et al., 2008; Fig. 1A–C). At the transcriptional level, key factors that mediate these changes in cadherin expression include members of the Twist, Sip, Zeb, and Snail families, all of which play evolutionarily conserved roles in EMT among metazoans (Fairchild et al., 2014; Lander et al., 2011; Linker et al., 2000; Theveneau et al., 2007). Classical models of neural crest EMT have often described switches from type I “epithelial” cadherins (*E-Cadherin*, *N-Cadherin*) to type II “mesenchymal” cadherins (*Cadherin-6*, *Cadherin-7*, and *Cadherin-11*) (Clay and Halloran, 2014; Coles et al., 2007; Nakagawa and Takeichi, 1998b; Vallin et al., 1998). However, there is now evidence that expression of both type I and type II cadherins occurs in both premigratory and migratory neural crest, and that certain cadherins may not always strictly correlate with epithelial or mesenchymal fates (Abbruzzese et al., 2016; Campbell and Casanova, 2015). For example, type I cadherins, such as E-Cadherin (Huang et al., 2016b), persist in and may even be required for early migration of cranial neural crest in *Xenopus*, whereas expression of a type II cadherin, Cadherin-6b, is repressed to facilitate neural crest emigration in the chick midbrain (Coles et al., 2007). By contrast, Cadherin-6 (Clay and Halloran, 2014) and Cadherin-6b (Park and Gumbiner, 2010b) are expressed in premigratory and early migratory neural crest in the fish hindbrain and chick trunk, respectively. Taken together, these findings indicate that modulation of cadherin expression relative to the rest of the neural tube, rather than a singular cadherin “switch” *per se*, may be an important step in driving neural crest EMT and migration. Thus, regardless of clade-specific variation in exact mechanisms and expression patterns, the regulation of cadherin expression in the dorsal neural tube allows neural crest cells to detach from the neighboring neuroepithelium, mobilize their cytoskeleton, and begin collective migration (Kuriyama and Mayor, 2008; Liu and Jessell, 1998a, 1998b; Perez-Alcala et al., 2004).

The regulation of EMT by differential cadherin expression is a highly conserved feature of neural crest development. However, it is not clear when this key regulatory step evolved. Evolutionary-developmental studies in a basal jawless (agnathan) vertebrate, the sea lamprey, *Petromyzon marinus*, suggest that some key steps in the control of neural crest development might not be conserved across vertebrates. In particular, there are marked differences in the neural crest GRN of lamprey and gnathostomes which suggest that the