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Restricted response of mesencephalic neural crest to sympathetic differentiation signals in the trunk

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Abstract

Lineage diversification in the vertebrate neural crest may occur via instructive signals acting on pluripotent cells, and/or via early specification of subpopulations towards particular lineages. Mesencephalic neural crest cells normally form cholinergic parasympathetic neurons in the ciliary ganglion, while trunk neural crest cells normally form both catecholaminergic and cholinergic neurons in sympathetic ganglia. In contrast to trunk neural crest cells, mesencephalic neural crest cells apparently fail to express the catecholaminergic transcription factor dHAND in response to BMPs in the head environment. Here, we show that migrating quail mesencephalic neural crest cells grafted into the trunk of host chick embryos colonise the sympathetic ganglia. While many express *dHAND* and form *tyrosine hydroxylase* (*TH*)-positive catecholaminergic neurons, the proportion that expresses either *dHAND* or *TH* is significantly smaller than that of quail trunk neural crest cells under the same conditions. Furthermore, the proportion of quail mesencephalic neural crest cells that is TH^+ in the sympathetic ganglia decreases with time, while the proportion of TH^+ quail trunk neural crest-derived cells increases. Thus, a subset of mesencephalic neural crest cells fails to express *dHAND* or *TH* in the sympathetic ganglia, while a further subset initiates but fails to maintain *TH* expression. Taken together, our results suggest that a subpopulation of migrating mesencephalic neural crest cells is refractory to catecholaminergic differentiation signals in the trunk. We suggest that this heterogeneity, together with local signals that repress catecholaminergic differentiation, may ensure that most ciliary neurons adopt a cholinergic fate.

Keywords: Mesencephalic neural crest; Cholinergic; Catecholaminergic; Quail-chick chimera; Ciliary ganglion; Sympathetic; Parasympathetic; dHAND; Tyrosine hydroxylase

Introduction

The vertebrate neural crest is a transient embryonic cell population that arises at the neural plate border and undergoes extensive migration throughout the embryo to differentiate into a wide variety of different neuronal and non-neuronal derivatives (reviewed in Hall, 1999; Le Douarin and Kalcheim, 1999). Two opposing hypotheses exist to explain how this lineage diversification is achieved. One proposes that the neural crest comprises a homogeneous population of multipotent cells whose differentiation is instructively determined by environmental signals. The second suggests that the neural crest is a heterogeneous population of committed cells whose differentiation occurs in permissive environments. These models are not mutually exclusive and there is evidence to support aspects of both. Multipotent neural crest cells have been identified that differentiate in response to inductive environmental cues (reviewed in Anderson, 1997; Le Douarin and Kalcheim, 1999; Sommer, 2001). Indeed, neural crest stem cells persist throughout development (Morrison et al., 1999, 2000; White et al., 2001). However, there is also substantial evidence to support the specification, though not necessarily irreversible commitment, of subpopulations of neural crest cells very early in development, both before and during migration (reviewed in Anderson, 2000; Dorsky et al., 2000).

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Some of the first evidence for heterogeneity in migrating neural crest cell populations was based on antigenic variation. Mesencephalic neural crest cells give rise to the cholinergic neurons of the parasympathetic ciliary ganglion during normal development (Narayanan and Narayanan, 1978; Noden, 1978). An antigen associated with high-affinity choline uptake is found both in the ciliary ganglion and in a small subpopulation of migrating mesencephalic neural crest cells (Barald, 1988a,b), suggesting that a subpopulation of these cells may already be specified to form cholinergic neurons even during early migration stages. Further support for this hypothesis is given by the observation that mesencephalic neural crest cells in culture form cholinergic neurons 2 days earlier, and as a higher proportion of total neurons, than do trunk neural crest cells under the same culture conditions (Leblanc et al., 1990). These results also suggested the more general point that mesencephalic and trunk neural crest cell populations respond differently to the same environment. Indeed, recent papers confirm that mesencephalic and trunk neural crest cells differ dramatically in their responses to the same survival and differentiation factors in vitro (Abzhanov et al., 2003) and that mesencephalic and trunk neural crest cells differ in the extent of their contribution to different neuronal and non-neuronal derivatives in vivo (Lwigale et al., 2004).

One study has proposed that intrinsic differences between different neural crest cell populations may underlie the formation of noradrenergic sympathetic neurons versus cholinergic parasympathetic neurons in response to bone morphogenetic proteins (BMPs) (White et al., 2001). Neural crest stem cells (NCSCs) isolated from rat sciatic nerve primarily form cholinergic parasympathetic neurons when transplanted into the trunk of host chick embryos, while migrating rat trunk NCSCs primarily form noradrenergic sympathetic neurons under the same conditions (White et al., 2001). BMPs from the dorsal aorta have long been known to be important for the induction of noradrenergic sympathetic neurons from trunk neural crest cells (reviewed in Goridis and Rohrer, 2002). BMPs induce the expression of a hierarchy of transcription factors in trunk neural crest cells, including Mash1, Phox2a/2b and dHAND (Hand2) (reviewed in Brunet and Pattyn, 2002; Goridis and Rohrer, 2002). These transcription factors in turn control the expression of catecholaminergic markers such as tyrosine hydroxylase (TH) and dopamine β-hydroxylase (DBH) (reviewed in Brunet and Pattyn, 2002; Goridis and Rohrer, 2002). Rat sciatic nerve NCSCs seem to be less sensitive to BMP2 than migrating rat NCSCs (White et al., 2001). This, together with the observation that cholinergic neurons differentiate at lower BMP2 concentrations than noradrenergic neurons, was suggested to underlie the preferential differentiation of rat sciatic nerve NCSCs into cholinergic neurons in the chick trunk (White et al., 2001).

In contrast, it has also been proposed that local environmental signals in the head repress *dHAND* and consequently catecholaminergic differentiation in the ciliary ganglion (Müller and Rohrer, 2002). BMPs near the eye are necessary and sufficient for the formation of cholinergic ciliary ganglion neurons from mesencephalic neural crest cells (Müller and Rohrer, 2002). BMPs induce *Cash1* (chick *Mash1*) and *Phox2a*/*2b*, but not *dHAND*, in mesencephalic neural crest cells, although a subpopulation of developing ciliary neurons transiently expresses the catecholaminergic markers *TH* and *DBH* (Müller and Rohrer, 2002). Overexpression of BMP4 in the head failed to elicit increased *TH* expression in the ciliary ganglion, while overexpression of *dHAND* (which presumably overrides the local repressive signals postulated by the authors' hypothesis) strongly increased the number of TH^+ cells in the ciliary ganglion (Müller and Rohrer, 2002). dHAND seems to be essential for maintaining the noradrenergic phenotype, suggesting that in the absence of dHAND, neurons adopt a cholinergic fate in response to BMPs (Müller and Rohrer, 2002). The authors propose that local environmental signals repress *dHAND* in the ciliary ganglion (Müller and Rohrer, 2002). The main difference between the BMP-mediated induction of noradrenergic sympathetic neurons from trunk neural crest cells, versus that of cholinergic parasympathetic neurons from mesencephalic neural crest cells, may be the failure of mesencephalic neural crest cells in the head to express *dHAND* in response to BMPs (Müller and Rohrer, 2002). The lack of *dHAND* expression and the virtually total downregulation of *TH* and *DBH* by E8 represent major differences between the differentiation of mesencephalic and trunk neural crest cells into ciliary and sympathetic neurons, respectively.

Taken together, these results suggest at least two hypotheses to explain the different responses of mesencephalic and trunk neural crest cells to BMPs at sites of autonomic neurogenesis in the head and trunk, respectively. Firstly, the mesencephalic neural crest cells that populate the ciliary ganglion may differ from trunk neural crest cells in that they are biased towards a cholinergic fate, or less likely to adopt a catecholaminergic fate, perhaps because they fail to express *dHAND* in response to BMPs. Alternatively, local environmental signals in the head could repress *dHAND* expression in the mesencephalic neural crest cells that populate the ciliary ganglion, leading to cholinergic differentiation. Thirdly, perhaps both these mechanisms act in concert to ensure that the great majority of ciliary neurons adopt a cholinergic fate.

Previous heterotopic grafting experiments support the hypothesis that local signals repress catecholaminergic differentiation in the head, since after transplantation into the trunk, both premigratory cephalic neural crest cells and postmigratory ciliary ganglion cells adopt a catecholaminergic phenotype in the sympathetic ganglia and adrenal glands (Coulombe and Bronner-Fraser, 1986; Dupin, 1984; Le Douarin and Teillet, 1974; Le Douarin et al., 1978; Le Lièvre et al., 1980; Sechrist et al., 1998). However, these were qualitative studies: no statistical comparison was made between the proportion of mesencephalic versus trunk