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Minireview

Recycling signals in the neural crest

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Abstract

Vertebrate neural crest cells are multipotent and differentiate into structures that include cartilage and the bones of the face, as well as much of the peripheral nervous system. Understanding how different model vertebrates utilize signaling pathways reiteratively during various stages of neural crest formation and differentiation lends insight into human disorders associated with the neural crest.

The vertebrate neural crest is characterized by a high degree of multipotentiality and migratory ability. These cells originate at the border between neural and non-neural ectoderm as the neural tube closes to form the central nervous system. Initially residing within the dorsal neural tube as a relatively homogeneous precursor population, neural crest cells are thought to represent stem cells. They subsequently delaminate from the neural tube epithelium as individual cells and migrate extensively throughout the body, proliferating at the same time. Finally, they differentiate into many different cell types under the influence of growth factors differentially expressed along their migratory pathways and/or at their destinations. Neural crest derivatives include cartilage and bones of the face, glia, melanocytes, smooth muscle, dermis, and connective tissue, as well as sensory, sympathetic, and enteric neurons.

Defects in neural crest development, characterized by mutations in different signaling pathway components that control the neural crest, give rise to various disorders and syndromes in humans. Comparative studies of the signaling pathways used during neural crest development in a range of model vertebrates can provide insights into such disorders. These signals are used during the induction, migration, and differentiation of the neural crest, and the same key molecules are recycled at temporally distinct developmental phases (Figure 1). This means that the same signal can elicit very different cellular responses in pre-migratory, migratory and post-migratory neural crest. The main pathways used are those mediated by three families of signaling molecules: transforming growth factor β (TGFβ), fibroblast growth factors (FGFs) and Wnts. Here we briefly review the known roles of members of these families in *Xenopus*, zebrafish, bird, and mouse embryos, noting some of the human neural crest disorders they may help us to understand. Such disorders include various human skeletal dysmorphology syndromes (Apert syndrome and Beare-Stevenson cutis gyrata syndrome), diseases of the nervous system (neurofibromatosis and Hirschsprung's disease) and pigment disorders (Waardenburg syndrome).

An eye on TGFβ signaling in the neural crest

A good example of the comparative approach to understanding human neural crest disorders is the article in this issue of *Journal of Biology* in which Ittner and colleagues [1] describe a new study in mouse of a developmental eye disorder related to Axenfeld-Rieger's syndrome in humans. The authors have made an elegant examination of the function of TGFβ signaling in the regulation of the ocular neural crest, which is critical for the proper development of the eye. First they delineated the normal contribution of neural crest cells to the eye region using *Wnt1-Cre*-mediated recombination to mark neural crest cells with β-galactosidase; they find neural crest contributions to the optic cup, lens, periocular mesenchyme, primary vitreous, and the corneal stroma and endothelium, but no cells contributing to the epithelium, lens or retina. The effects of a loss of TGFβ signaling on eye development were then assessed by using recombination to delete exon 4 of the *Tgfβ receptor 2* (*Tgfβr2*) gene. The resulting mice exhibit ocular defects remarkably similar to those found in human patients carrying mutations in the genes for the transcription factors *Pitx2* and *FoxC1* , leading to Axenfeld-Rieger's anomaly [2]. These mutant mice have small eyes that lack both the endothelial layer and the ciliary body. Moreover, mesenchyme accumulates between the lens and retina, the vitreous is hypertrophic, and retinal patterning is disturbed. Interestingly, neural crest cells appear to migrate to the appropriate locations in the mutants, suggesting that the defect is in differentiation rather than cell migration. Expression of both *Pitx2* and *FoxC1* is absent in the mutants, consistent with the regulation of these genes by TGFβ signaling, which was confirmed by experiments in cultured cells and in *ex vivo* eye cultures. The study by Ittner *et al.* [1] thus shows that TGFβ signaling is essential for the proper differentiation of the neural crest into ocular structures, and that loss of TGFβ signaling in mice recapitulates Axenfeld-Rieger's syndrome in humans.

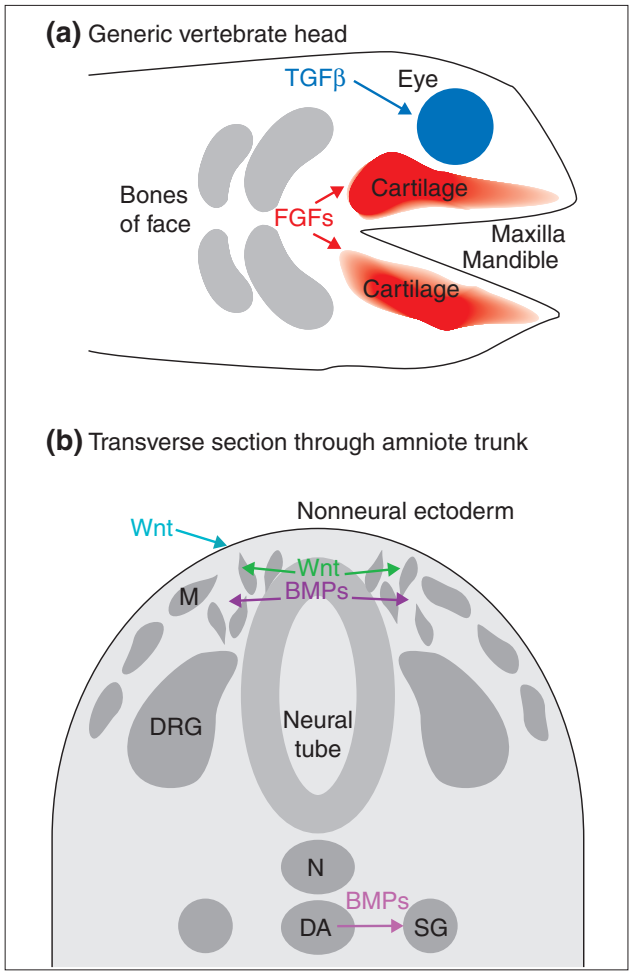

Figure 1
Recycling counts in the neural crest. The reiterative function of various signaling molecules (Wnts, TGFβ/BMPs, and FGFs) is tantamount to the regulation of neural crest development at multiple stages, ranging from the initial phases of induction to migration and subsequent differentiation. Depending upon their developmental stage, neural crest cells respond differently to the same signals. **(a)** Neural crest cells build much of the facial skeleton. TGFβ and FGF molecules signal to ensure proper development of the eye and facial cartilage, respectively. **(b)** In the trunk, Wnts and BMPs work to specify various neural crest derivatives. Early Wnt signals from the nonneural ectoderm are important in neural crest induction, whereas later Wnts specify neural crest cells to become sensory neurons and pigment cells. In addition, BMPs, also members of the TGFβ family, are produced by the dorsal aorta to regulate sympathetic neuron differentiation. DA, dorsal aorta; DRG, dorsal root ganglion; SG, sympathetic ganglion; N, notochord; M, melanocytes.

Interestingly, TGFβ signaling affects other aspects of craniofacial development as well. A role for *Tgfβr2* in the formation of the palate and the skull in mice was demonstrated previously by Ito *et al.* [3]. Using similar methods to Ittner *et al.* [1], cranial neural crest cell progeny were marked with β-galactosidase to examine their contribution to the palatal mesenchyme. Conditional mutation of *Tgfβr2* in the cranial neural crest caused a cleft secondary palate, non-formation of the calvaria (the dome of the skull), and other skull defects. Although migration of the cranial neural crest occurred normally, a study of bromodeoxyuridine incorporation revealed a decreased rate of cranial crest proliferation and a reduction in the level of cyclin D in the mutant palatal mesenchyme, suggesting a role for TGFβ signaling in controlling the rate of cell division in the cranial neural crest. In addition, the neural-crest-derived dura mater, which lines the interior of the skull, was abnormal, causing a lack of parietal bone induction and impaired development of the calvaria. The effect on the skull was dramatic:

there was a 25% reduction in size, with defects in the mandible and maxilla (the lower and upper jaw, respectively). Thus, TGFβ signaling plays a significant role in several aspects of craniofacial development.

Members of the TGFβ superfamily, most notably bone morphogenetic proteins (BMPs), have been implicated in other aspects of neural crest development, ranging from their initial induction to subsequent differentiation (see [4-6] for reviews). BMP activity has, for example, been proposed to delimit the boundary of the neural plate and the position of the neural crest. In *Xenopus* and zebrafish, a gradient of BMP is present in the ectoderm (from which the neural plate derives), with high BMP promoting ectoderm fate and low BMP promoting neural fate. Intermediate levels of BMP activity have been proposed to specify the neural plate border and neural crest. Support for this hypothesis comes from zebrafish mutants with defects in genes encoding components of BMP pathways: *swirl* (mouse equivalent *bmp2b*), *snailhouse* (*bmp7*), and *somitabun* (*smad5*) [7,8]. Mutations in *swirl* result in loss of BMP signaling and a decrease in neural crest progenitors; *snailhouse* or *somitabun* mutants have moderate or low BMP activity, respectively (similar to the intermediate levels of the normal BMP gradient), and show expansion of the neural crest domain [8]. Similarly, injection of BMP4 antagonists into *Xenopus* embryos leads to enlargement of the neural crest domain, whereas BMP overexpression causes crest reduction [9]. It is likely, however, that BMPs influence the position and size of the domain rather than causing induction.

BMP involvement in neural crest development in birds differs in some respects from frog and zebrafish. In birds, addition of BMP to explants of an intermediate region of the open neural plate (the tissue between the ventral portion and the dorsal portion) results in neural crest formation [10], although this action of BMP may be secondary to a Wnt signal [11], as BMP4 is not expressed in the early ectoderm *in vivo* at the right time to initiate neural-tissue-specific gene expression. Rather, it is expressed later in the neural folds and neural tube, where it may act to maintain gene expression during the neural crest development program [10-13]. An important and established action of BMPs in birds is to mediate the epithelial to mesenchymal transition that allows neural crest cells to delaminate from the trunk neural tube. Burstyn-Cohen *et al*. [14] showed that neural crest delamination occurs at a specific point in the cell cycle and that Wnt acts downstream of BMP to mediate delamination at the G1/S transition.

In addition to defining the boundaries of the neural crest and mediating delamination, BMPs later influence neural crest cell differentiation. When added to clonal neural crest cultures, BMPs bias multipotent precursors to differentiate into sympathetic neurons, whereas other growth factors, such as neuregulin, bias sister cells toward glial differentiation [15].

The reappearing Wnts

The Wnt signaling pathway is used reiteratively in all stages of neural crest development, from induction [11], through delamination and proliferation [14] to eventual differentiation [16] (for review see [17]), with neural crest cells responding differently to Wnt signals depending upon their developmental stage. In *Xenopus*, addition of Wnts to neuralized animal caps upregulates neural crest markers, implicating Wnts in early neural crest induction [18]. In the chick, Wnt6 is expressed in the nonneural ectoderm adjacent to the elevating neural folds, and blocking the canonical Wnt-β-catenin signaling pathway prevents neural crest formation. Conversely, adding soluble Wnt to intermediate neural plates promotes *de novo* neural crest induction, showing that Wnt signals are both necessary and sufficient for crest formation [11]. Rather than functioning alone, however, Wnts are likely to be part of a multistep induction process [9].

In addition to its role in induction, Wnt signaling can also control decisions regarding neural crest fate. Using a *cre/loxP* system to generate mice expressing constitutively active β-catenin in neural crest cells, Lee *et al.* [19] demonstrated that canonical Wnt signaling regulates sensory cell fate specification. These mutant mice had drastically reduced numbers of neural crest cells populating lineages other than the sensory lineage - namely the cardiac outflow tract, melanocyte lineage, peripheral nerves, and head. Concomitantly, Lee *et al*. [19] found that activated β-catenin caused neural crest cells to adopt a sensory neuron fate (as indicated by ectopic expression of *ngn2*, *ngn1* and *neuroD*) at the expense of sympathetic neurons (as indicated by loss of *mash1* and *ehand*). Conversely, sensory neurons failed to form in cultures of β-catenin-deficient neural crest stem cells, confirming that it is indeed the canonical Wnt pathway (as opposed to noncanonical Wnt signaling) that is important for sensory fate decisions.

Wnt signaling is also important for the proliferation of neural crest cells and their prescursors. Loss of both Wnt1 and Wnt3a in the mouse leads to a reduction of neural crest derivatives in the head, including trigeminal, vagal or glossopharyngeal neurons, as well as alterations in the head skeleton [20]. The cervical dorsal root ganglia are also reduced in size by 60%. Taken together, these results suggest that Wnts are important as mitogens or survival factors that facilitate the expansion of the neural crest.

Wnt signals are used yet again at later stages to support the differentiation of various neural crest lineages. In zebrafish, Wnt signaling is necessary and sufficient for the formation of pigment cells (melanophores and xanthophores forming the zebrafish stripes); the precursors of these are medial neural crest cells that initially reside in the dorsal neural keel (the structure which develops from the infolding neural epithelium and eventually forms the neural rod), adjacent to cells producing Wnt1 and Wnt3a signals [16]. Overexpression of activated β-catenin in individual neural crest cells causes them to adopt a pigment fate, whereas overexpression of Wnt inhibitors results in the cells becoming neurons and glia. In zebrafish, the gene *nacre* provides a direct link between Wnt signaling and pigment cell formation. This homolog of the vertebrate gene *MITF* encodes a transcription factor directly activated as a result of Wnt signaling that regulates the expression of pigment genes such as *TRP-1* [21]. The importance of *nacre* is shown by the finding that its overexpression in non-pigment cells drives them towards a pigment cell phenotype, while its loss abrogates pigment cell differentiation.

Making a face with FGFs

Together with TGFβ and Wnts, proper FGF signaling is critical for the development of neural crest-derived structures, in particular the facial skeleton and cartilage elements. To study this aspect of crest development, Petiot *et al.* [22] introduced wild-type or mutant (constitutively active) FGF receptor (FGFR) constructs into the neural tube of quail embryos at stages before crest migration, using the technique of *in ovo* electroporation. The mesencephalic neural crest, which gives rise to facial structures, was then dissected and cultured in the absence of FGF2. Under these conditions, cartilage formation (chondrogenesis) occurred in neural crest that had received the mutant FGFR constructs, but not in neural crest that had received the wild-type constructs, thus showing that FGF signaling is required for chondrogenesis. This effect was also seen in cultures of cranial neural crest cells isolated after the onset of migration that were subjected to electroporation with the same constructs [22].

Conservation of this role of FGF signaling has been confirmed by various experiments in zebrafish embryos. For instance, Walshe and Mason [23] found that zebrafish treated with the FGFR inhibitor SU5402 for 24 hours following the onset of neural crest migration lost almost all the cartilage comprising the pharyngeal skeleton and neurocranium. FGF3 is normally expressed in the embryonic endodermal pouches and the pharyngeal ectoderm, and its knockdown using antisense morpholino oligonucleotides affected cartilage development in a dose-dependent fashion. In the presence of the morpholino, the first, second and seventh branchial arch cartilage derivatives consistently showed defects, while cartilage derived from arches 3-6 was either absent or extremely abnormal. Morpholinos against *Fgf3* and *Fgf8*, which are both expressed in the endoderm adjacent to the hindbrain, resulted in a near complete loss of cartilage. These results, in combination with those of Petiot *et al.* [22] and other researchers [24], indicate the importance of FGF signaling in the development of head cartilage. This is also relevant to humans, as missense mutations in FGFR genes result in several human skeletal dysmorphology syndromes [25,26].

The processes of induction, delamination, migration and differentiation of the neural crest all rely on the recycled deployment of and responses to signaling molecules such as Wnts, TGFβs/BMPs, and FGFs. Comparing the involvement of these signaling pathways in different model organisms provides researchers with a means of understanding the conserved mechanisms that regulate this multipotent cell population. This, in turn, provides insight into the molecular basis of various human disorders and syndromes that arise during aberrant neural crest development.

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