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Stage-dependent plasticity of the anterior neural folds to form neural crest

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ABSTRACT

The anterior neural fold (ANF) is the only region of the neural tube that does not produce neural crest cells. Instead, ANF cells contribute to the olfactory and lens placodes, as well as to the forebrain and epidermis. Here, we test the ability of the ANF to form neural crest by performing heterotopic transplantation experiments in the chick embryo. We find that, at the neurula stage (HH stage 7), the chick ANF retains the ability to form migrating neural crest cells when transplanted caudally to rostral hindbrain levels. This ability is gradually lost, such that by HH9, this tissue appears to no longer have the potential to form neural crest. In contrast to the ANF, hindbrain dorsal neural folds transplanted rostrally fail to contribute to the olfactory placode but instead continue to generate neural crest cells. The transcription factor GANF is expressed in the ANF and its morpholino-mediated knock-down expands the neural crest domain rostrally and results in the production of migratory cells emerging from the ANF; however, these cells fail to express the HNK1 neural crest marker, suggesting only partial conversion. Our results show that environmental factors can imbue the chick anterior neural folds to assume a neural crest cell fate via a mechanism that partially involves loss of GANF.

1. Introduction

Cranial ectodermal placodes are thickened regions of ectoderm in the head of vertebrate embryos that contribute to the sense organs (nose, ears, lens of the eye) as well as neurons of cranial sensory ganglia. Together with neural crest cells, the ectodermal placodes form the entire peripheral nervous system of vertebrates. Of the cranial placodes, the olfactory placode is one of the most unusual since it forms not from lateral ectoderm but from the anterior neural fold (ANF) (Couly and Le Douarin, 1985). The ANF contains a mixture of precursors for epidermis, olfactory and lens placodes, as well as portions of the forebrain (Bhattacharyya et al., 2004; Cobos et al., 2001) and, interestingly, is the sole region of the neural tube that does not give rise to neural crest cells, which form from the neural folds at all axial levels caudal to the ANF. After its specification at stage 10 (Bhattacharyya and Bronner-Fraser, 2008), the olfactory precursors move to the ventral ectoderm at the level of the forebrain region where the olfactory placode thickens and invaginates to form the olfactory vesicle. A subset of the placode cells subsequently differentiates into olfactory sensory receptor neurons, critical for perception of smell, and project axons to the olfactory bulb in the central nervous system (Buck, 2000). Olfactory sensory neurons are unusual and particularly interesting since they have the rare property of being generated throughout life, even in mammals (Bermingham-McDonogh and Reh, 2011; Graziadei and Graziadei, 1979).

Ectodermal placodes and neural crest share many similar properties. Both originate from neurogenic ectoderm at the border between neural ectoderm and presumptive epidermis (Baker and Bronner-Fraser, 1997a; Webb and Noden, 1993). Both have progeny that undergo an epithelial-to-mesenchymal transition (EMT) and are capable of migration. In addition, they share some common derivatives, e.g. sensory neurons and neuroendocrine cells (Baker and Bronner-Fraser, 1997b). Of the cranial placodes, the olfactory placode shares the most similarities with neural crest cells. Not only does it originate from the anterior neural folds, but like neural crest, it also gives rise to sensory neurons and neuroendocrine cells. These similarities suggest an embryonic and perhaps evolutionary relationship between these two populations.

This raises the intriguing possibility that the territories that contribute to these two populations may be interchangeable and that environmental factors at different axial levels may play a role in determining whether neural fold cells have the ability to form neural crest. To test this, here, we challenge the differentiative ability of the chick ANF to form neural crest when grafted to more caudal levels of the neural axis. These heterotopic grafting

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experiments demonstrate that ANF cells can contribute to a neural crest cell fate during a critical period. Molecular analysis suggests that the loss of the transcription factor, GANF, may be partially involved in acquisition of neural crest identity in these cells. These results are consistent with findings in other species suggesting flexibility in ANF fate at early stages (Villanueva et al., 2002; Carmona-Fontaine et al., 2007; Li et al., 2009).

2. Materials and methods

2.1. Embryos

Fertilized Colorado Red, White Leghorn chicken eggs, obtained from local farms, were incubated at 38 °C for 26–31 h to obtain stage HH7 embryos, or 36–42 h to obtain stage HH 9–10 embryos (Hamburger and Hamilton, 1951). Green fluorescent protein (GFP)-transgenic chick eggs were obtained from Dr. Susan Chapman at the University of South Carolina, and used within 3 days of receipt. Transgenic quail eggs were obtained from Dr. Rusty Lansford at Caltech. The Colorado Red or White Leghorn eggs were used as tissue hosts and transgenic chick, electroporated chick, or quail embryos were used as the tissue donors.

Embryos were collected onto Whatman paper rings and placed into sterile Ringers containing 5 mL of penicillin/streptavidin per 500 mL of Ringers. The embryos were screened under a fluorescent Leica MZFLIII dissecting microscope, illuminated with an X-cite series 120 fluorescent lamp and a fiber-optic lamp for reflected lighting. All selected donors were transferred into a dish of clean Ringers with penicillin/streptavidin.

2.2. Transplants

For transplantation experiments, the anterior neural fold (ANF) of the donor embryo was carefully microdissected with a glass needle. To this end, an incision was made at the anterior tip of the neural fold, followed by a second incision at a distance of about 100 μm posterior to the first. After making two mediolateral cuts, the tissue was released from the neural fold by scraping carefully along the sides. After dissection, the ANF was pipetted into a glass capillary tube, and set aside.

Host eggs at HH8 (3–5 somites) were windowed with a small pair of sharp scissors. A small amount of blue food dye (FD&C Blue, Spectra Color Corp, Kearny NJ) (8 mg/mL food dye in Ringer's solution containing 1% penicillin and streptomycin) was injected sub-blastodermally to aid in visualization. The vitelline membrane was opened with a glass needle. To facilitate experimental manipulation, the embryo was raised to the top of the egg by adding back 3–5 mL of the egg white.

The donor anterior neural fold tissue was pipetted onto the side of the host embryo. A similarly sized opening was made in the host, at the level of the midbrain/rostral hindbrain, by removing and discarding an approximately 100 μm portion of caudal midbrain or rostral hindbrain. The anterior neural fold was carefully inserted into the opening in the host midbrain/rostral hindbrain neural fold, closing it off. After experimental manipulation, a few drops of Ringer's solution was deposited carefully around the embryo, 5–8 mL of egg white were removed to lower the embryo. The egg was sealed with clear adhesive tape and re-incubated at 38 °C in a humidified incubator until the host embryo reached HH 13–14. Embryos were fixed into 4% paraformaldehyde overnight at 4 °C and rinsed into 3–6 changes of buffer (phosphate buffered saline (PBS) with 0.1% Tween) over a period of 24 h at 4 °C before further processing.

Two types of grafts were performed as positive controls. First, anterior neural folds from stage HH7 donors were transplanted into HH8 control embryos at the level of the anterior neural folds. Secondly, midbrain/hindbrain neural folds from HH7 donors were grafted into HH8 hosts. We used slightly different aged donor and hosts because the embryo develops in an anterior to posterior progression such that the ANF at HH7 is approximately at the same state of development as the hindbrain at HH8. In addition, the HH8 hosts survived the transplantation much better than at earlier stages.

2.3. Immunohistochemistry

Embryos were processed for cryosectioning. Briefly, embryos were equilibrated for 1 h in a 5% sucrose solution, followed by 6 h to overnight in a 15% sucrose solution. Embryos were next embedded overnight at 37 °C in gelatin and sectioned at 10 μm on a MicromHM550 cryostat. Slides were blocked in antibody buffer (PBS+0.1% Tween+5% (by volume) goat serum+0.2% BSA) for 1–2 h at room temperature before the primary HNK1 antibody (hybridoma culture media diluted 1:50 in antibody buffer) was applied overnight at 4 °C to recognize migrating neural crest, followed by fluorescently labeled (Alexa 594) secondary antibody for 12 h at 4 °C (1:1000 dilution). Sections were imaged on a Zeiss Axioskop Plus, using the Axiovision (Rel 4.6) software and image processing was done using Adobe Photoshop CS4 Extended Version11.0.

2.4. Morpholino and RFP electroporation

Morpholino oligomers (MO) (Gene-Tools, Philomath OR) were prepared in water at 1.5 mM with a double stranded plasmid (ECR1-pTK, 1 mg/ml) serving as a carrier. The MO were electroporated as described previously (Sauka-Spengler and Barembaum, 2008). The control MO was injected on the right ventral side and the GANF MO on the left ventral side of the stage 4–5 embryos ex ovo on filter paper rings in the space between the vitelline membrane and the embryo. The embryos were electroporated using 5 pulses for 50 ms each at 5.2 V with 100 ms in between each pulse, then incubated at 37 °C. After 18 h the embryos were collected and fixed in paraformaldehyde for 18 h at 4 °C and afterwards washed in PBS+0.1% Tween then dehydrated in methanol and stored at −20 °C. For transplant experiments, the embryos were electroporated on both sides with either the GANF or the control morpholinos, and incubated until they reached stage HH7. The embryos were used as donors for transplant experiments using non-electroporated embryos as hosts. The sequence of the GANF MO is CTGCACACAGCGATGTACTTGCCAT. The control MO is the Gene-tools standard control MO CCTCTTACCTCAGTTACAATTTATA.

In some cases, donor chick embryos were electroporated with red fluorescent protein (RFP) expression construct into order to label the donor ANF. Electroporations were performed as described above for morpholino and grafting was done as described above.

2.5. In situ hybridization

Whole mount in situ hybridization was done using standard protocols (Wilkinson, 1992). The Sox10 construct was kindly provided by Yi-Chuan Cheng. After color development, the two sides of each embryo were compared.

3. Results

3.1. Grafts of labeled anterior neural fold (ANF) to ANF contribute to the olfactory epithelium, eye, forebrain and epidermis

According to the fate map of the early avian embryo, the dorsal and most anterior portion of the forming neural tube, the anterior