Video Article

Assessing Signaling Properties of Ectodermal Epithelia During Craniofacial Development

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Abstract

The accessibility of avian embryos has helped experimental embryologists understand the fates of cells during development and the role of tissue interactions that regulate patterning and morphogenesis of vertebrates (e.g., [1, 2, 3, 4]). Here, we illustrate a method that exploits this accessibility to test the signaling and patterning properties of ectodermal tissues during facial development. In these experiments, we create quail-chick [5] or mouse-chick [6] chimeras by transplanting the surface cephalic ectoderm that covers the upper jaw from quail or mouse onto either the same region or an ectopic region of chick embryos. The use of quail as donor tissue for transplantation into chicks was developed to take advantage of a nucleolar marker present in quail but not chick cells, thus allowing investigators to distinguish host and donor tissues [7]. Similarly, a repetitive element is present in the mouse genome and is expressed ubiquitously, which allows us to distinguish host and donor tissues in mouse-chick chimeras [8]. The use of mouse ectoderm as donor tissue will greatly extend our understanding of these tissue interactions, because this will allow us to test the signaling properties of ectoderm derived from various mutant embryos.

Protocol

1. Preparing the Donor Tissue

1. Prepare culture media, sharpen glass pins, sharpen tungsten needles.
2. Collect embryo from shell, wash in ice-cold PBS.
    1. Using a 10 mL syringe and an 18 gauge needle remove 1.0 mL of albumin from the pointed end of the egg shell.
    2. Make a small hole on the top of the shell using the point of scissors, and then cut a circular opening to expose the embryo
3. Remove head and place into DMEM (serum free, room temperature)
4. Dissect Frontonasal Process from the embryo (or other donor site such as maxillary or mandibular process)
5. Digest in 2.4U/ mL dispase in PBS on ice for 20mins
6. Cover tissues with DMEM with 1% BSA to stop digestion
7. Use sharpened tungsten needle to separate ectoderm, mesenchyme, and neuroectoderm
8. Store graft tissue in DMEM 1%BSA on ice until host is ready for transplantation

2. Preparing the Host

1. Expose the embryo
    1. Using a 10 mL syringe and an 18 gauge needle remove 1.0 mL of albumin from the pointed end of the egg shell.
    2. Make a small hole on the top of the shell using the point of scissors. Place a piece of tape over the hole, and then cut a circular opening to expose the embryo.
2. Using 2 pairs of forceps, grasp the amnion and gently tear to make a hole.
3. Rotate the head by placing a pair of forceps on the right eye and applying pressure. While holding the head steady, use a tungsten needle to remove the host ectoderm to accommodate the graft.
4. Transfer the graft to the host using a glass pipette.
5. Position the graft to replace the removed ectoderm. Insert a glass pin in each corner of the grafted tissue to pin the graft in place. To make glass pins, pull a microcapillary tube to a fine point over an alcohol flame.
6. Place tape tightly over the hole and return embryo to the incubator for appropriate length of time for analysis.
7. see also: [9]

3. Representative Results:

To assess chimeras, embryos should be collected and processed for analysis of the distribution of host and donor tissues. For detection of quail cells, immunohistochemistry using the QcPN antibody (described in: [3]) is employed, and for detection of mouse cells, *in situ* hybridization on paraffin sections is used [6]. Donor cells should be restricted to the epithelium, and there should be no evidence of donor mesenchymal tissues (Figure 1). The presence of isolated donor cells in the mesenchyme indicates the presence of contamination neural crest cells which will confound any morphological interpretation due to the influence of these cells on many aspects of facial development (e.g., [10]). Once convinced that the graft technique is free of contaminating mesenchyme, further morphological or molecular outcome measures can be used to assess the chimeras. For our purposes, we detected the presence of ectopic cartilages and bones that corresponded to duplications of the upper jaw that were induced by the transplanted tissues (Figure 2), and molecular changes in the mesenchymal tissues in response to the grafted ectoderm (Figure 3)[5,6].

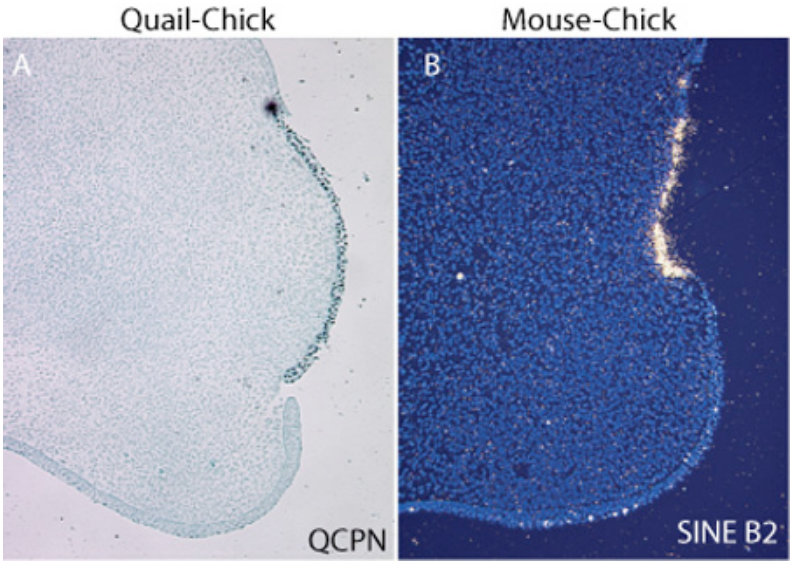

Figure 1. Assessment of chimerism (A) Anti-QcPN staining is used to detect quail cells in quail-chick chimeras. There is no evidence of staining in the mesenchyme. (B) *In situ* hybridization is used to detect the expression of SINE B2 transcripts in mouse-chick chimeras. Expression is restricted to the ectoderm comprising the graft.

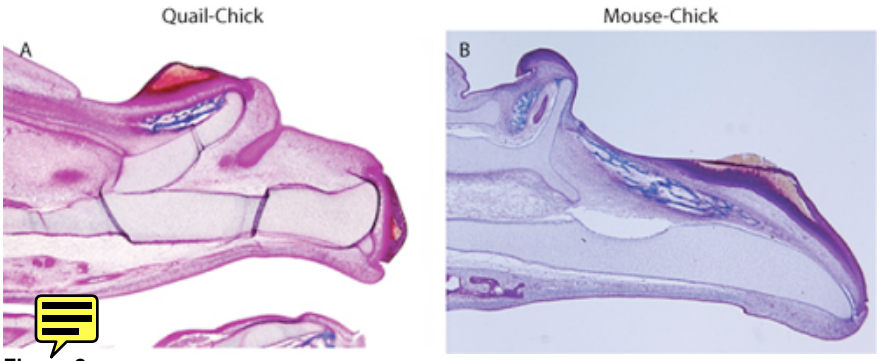

Figure 2.

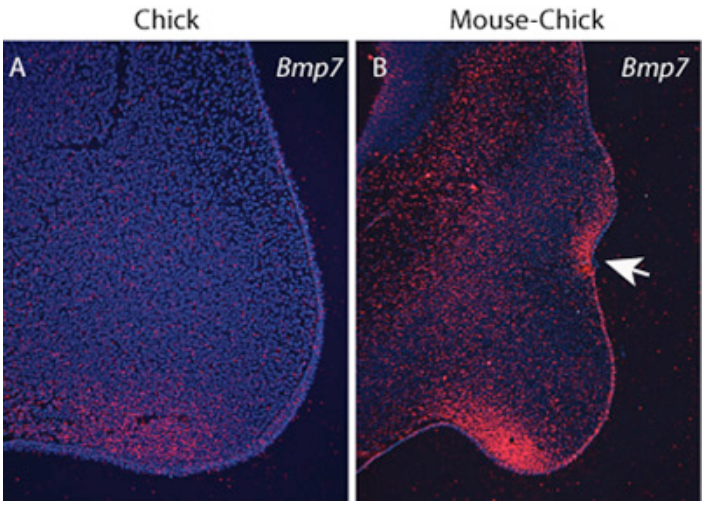

Figure 3.

Disclosures

No conflicts of interest declared.

Discussion

Using this transplantation method has allowed us to determine that the ectoderm contains signaling information that regulates dorsoventral polarity and proximodistal extension of the upper jaw. The similarity of outcomes when using quail or mouse ectoderm, and the conservation of molecular signals in this tissue among many species [6,11] indicates that this is a highly conserved signaling center among vertebrates. Furthermore, other investigators have used similar techniques to test the signaling properties of different regions of surface cephalic ectoderm and have uncovered the presence of multiple signaling centers located in the ectoderm that contribute to morphogenesis of the face [12]. This method allows us to further our understanding of the role that different tissues, as well as regional differences within tissues, play during facial

development. Finally, by combining the strength of mouse genetics with the strength of the avian-chimera system we will be able to elucidate the molecular mediators of the epithelial-mesenchymal interactions during development, and we will be able to identify the mechanisms by which these interactions regulate development.

Acknowledgements

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