ABSTRACT

In vertebrates, the peripheral nervous system is derived from two distinct embryonic cell populations, the neural crest and ectodermal placodes. Neural crest cells arise from the hinges of the invaginating neural plate, while ectodermal placodes form in pairs from discrete, usually thickened, head ectoderm lateral to the neural tube. While these two populations generally contribute to different structures in the nervous system, the exception is where they converge to form the cranial sensory ganglia of the trigeminal (V), facial (VII), glossopharyngeal (IX), and vagal (X) cranial nerves. The dual embryonic origin of cranial sensory ganglia has intrigued investigators for some time, but surprisingly little is known about the neural crest–placode relationship. The process of cranial gangliogenesis exemplifies a fascinating problem on how cell–cell interactions drive assembly of complex structures in the developing embryo.

To investigate this process, I have combined gene expression characterizations, embryological experiments, in vitro culture studies, and in vivo molecular perturbations (gain- and loss-of-functions by electroporation mediated DNA and oligos transfer) to uncover the cellular and molecular events underlying neural crest–placode ganglion assembly in the trigeminal region of the chick embryo. The results show that these cells are in contact and highly intermingled throughout ganglion formation. Ablation of either precursor tissue results in severe ganglion defects. Taken together, the data demonstrate the essential role of neural crest–placode interactions for proper gangliogenesis and the reciprocal nature of their relationship. Their interactions likely involve bi-directional signaling by which each population affects and coordinates with one another.

As candidate mediators, I investigated the potential role of Slits and Robos. The concurrent expression of Slit1 on migratory neural crest cells and its cognate receptor Robo2 on placodal cells raised an intriguing possibility that this ligand–receptor pair may mediate signaling from neural crest to placodal cells. This would represent one form of their cell–cell interactions. Loss of function of either the ligand Slit1 or its cognate receptor Robo2 in vivo resulted in severely disorganized placodal ganglia that were similar phenotypically to the effects of neural crest ablation. More specifically, inhibition of Robo2 resulted in aberrant placodal ingression, axonal projections, and ganglion organization and coalescence. The results suggest that neural crest cells regulate and coordinate assembly of placodal cells for proper trigeminal gangliogenesis through Slit1–Robo2 signaling in chick.

A striking defect in ganglion coalescence by blocking Slit1–Robo2 function

suggested that cell adhesion may have been affected. Thus, as a possible downstream mechanism, I next tested the function of the cell adhesion molecule N-cadherin. The results show that N-cadherin is expressed by placodal neurons, and its function is required for placodal aggregation and may be regulated by Slit1–Robo2. N-cadherin expression is modulated by Slit1–Robo2 and also can partially rescue Robo2 loss-of-function. Moreover, since neural crest and placodal neurons are highly intermixed, condensation of ganglia may require adhesion of not only placode–placode, but also crest–crest and crest–placode cells. The data suggest that another adhesion molecule Cadherin-7 may complement the role of N-cadherin in driving ganglion coalescence. Finally, the similar expression and function of these molecules in the epibranchial regions suggest that the mechanisms of Slit1–Robo2 and N-cadherin may be general for all cranial ganglia of dual origin.

In summary, the results from my thesis establish a critical role for Slit1–Robo2 signaling and cadherin-mediated cell adhesion for cranial ganglia formation. They provide the first molecular basis for neural crest–placode cell–cell signaling during cranial gangliogenesis, and highlight the critical interplay of cell–cell communication and cell adhesion in animal development.