



Significance of Neural Crest in Tooth Development: The Molecular Signature

¹VP Jayasekharan, ²Jacob Kurien, ³Eapen Cherian, ⁴Renji K Paul, ⁵Aravind S Raju

ABSTRACT

The neural crest originates from cells located along the lateral margins of the neural plate. Neural crest cells arise as the result of an inductive action by the non-neural ectoderm adjacent to the neural plate and possibly by nearby mesoderm as well. As the neural tube forms, a group of cells separate from the neuroectoderm. These cells have the capacity to migrate and differentiate extensively within the developing embryo and they are the basis of structures such as spinal sensory ganglia, sympathetic neurons, Schwann cells, pigment cells and meninges. Specific interactions occur during the development of tooth and recent research has concentrated more on the molecular aspects of these interactions. Thus, it is highly imperative to understand and digress the complex mechanisms involved in these processes.

Keywords: Epithelial-Mesenchymal transition, Neuroectoderm, Placode.

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INTRODUCTION

The neural crest cells are a unique population of cells that arise from the dorsal part of the forming neural tube during neurulation. These cells undergo an epithelial to mesenchymal transformation as they detach from the neural tube. Neural crest cells differentiate first in the mesencephalic zone of the future brain. In the spinal cord portion of the neural tube, the neural crest cells at the end detach after the neural folds have fused. Neural crest cells at the very caudal end of the neural tube are formed from the medullary cord after the caudal neuropore closes on.¹ The neural crest cells break free from the neural plate or neural tube by changing their shape and properties from those of typical neuroepithelial

cells to those of mesenchymal cells. In the head region, incipient neural crest cells send out processes that penetrate the basal lamina underlying the neuroepithelium just before neural tube closure.

The neural crest provides cells for the development of future organs and tissues. After the induction of neural crest, the neural crest cells are formed within neural plate border regions, which rise as neural folds, and ultimately converge to form the dorsal midline of the neural tube, and it is from here that the neural crest cells will emerge in an anterior to posterior sequence. During and after migration, cells differentiate into a wide range of derivatives that are grouped into: (A) ectomesenchymal category and (B) non-ectomesenchymal category.

In the last stage, the mammalian teeth develop from two type of cells: stomodeal ectoderm, which form ameloblasts and cranial neural crest-derived (ecto) mesenchymal cells, which finally form odontoblasts and cementoblasts.²

BRANCHIAL ARCH FORMATION

The accumulation and proliferation of cranial neural crest cells, together with proliferation of the ectoderm cells, produce a series of swellings that constitute the facial primordia. Mammalian teeth develop on two of these primordia:

1. Frontonasal process.
2. First branchial (pharyngeal) arch which forms mandible and proximal maxilla.

These primordial structures form as cranial neural crest cells from the developing brain and accumulate beneath ectoderm, covering what will become the face and oral cavity. In addition, more caudal swellings are also created which form the 2nd, 3rd, 4th branchial arches, each of which develop into a defined set of structures.³

SCENARIO FOR THE ORIGIN OF NEURAL CREST

Neural crest evolved from Rohon-Beard cells, a class of primary sensory neurons that occur in dorsal spinal cord of chordates, projecting axons peripherally onto lateral migration pathway between somites and epidermis that is also used by vertebrate neural crest cells. Under the developing scenario, peripheral Rohon-Beard cells would need to dedifferentiate and divide to form sensory neurons and glia of

^{1,4,5}Senior Lecturer, ²Professor and Head, ³Reader

¹⁻³Department of Oral Pathology and Microbiology, St Gregorios Dental College, Kothamangalam, Kerala, India

^{4,5}Department of Orthodontics, St Gregorios Dental College Kothamangalam, Kerala, India

Corresponding Author: VP Jayasekharan, Senior Lecturer Department of Oral Pathology and Microbiology, St Gregorios Dental College, Kothamangalam, Kerala, India, Phone: 8547055944, e-mail: jaypatho@gmail.com



the dorsal root ganglia and neurons of the central nervous system are not known to dedifferentiate.

The evolutionary developmental origin of neural crest, specifically, the sensory neurons and glia of dorsal root ganglia, is to be found in migration of mitotically active Rohon-Beard progenitor cells from dorsal neural tube. Neural crest cells would be considered a subset of Rohon-Beard precursors that delaminated from nascent spinal cord and migrate into the periphery.⁴

THE MIGRATION OF NEURAL CREST

After leaving the neuroepithelium, the neural crest cells first encounter a relatively cell-free environment rich in extracellular matrix molecules. In this environment, they undergo extensive migrations along several well-defined pathways. These migrations are determined by intrinsic properties of the neural crest cells and features of the external environment encountered by the migrating cells.⁵ The migration of neuroectodermal cells lying along the crest of each neural fold occurs ventrolaterally on each side of the neural tube, forming an irregular elongated mass between the neural tube and overlying surface ectoderm. In addition, cranial and more caudal neural crest cells give rise to some identical cell types (neurons) but also some different cell types.⁶

Before neural crest emigrates from the neural tube, they undergo a process called epithelial mesenchymal transition (EMT) that will confer to them the ability to migrate. A similar EMT process in different cellular machineries also occurs. When the EMT is complete, the neural crest cells delaminate from neuroepithelia and migrate along specific pathways.⁷

CRANIAL NEURAL CREST CELLS

Neural crest cells from the caudal prosencephalon (forebrain) and mesencephalon (midbrain) regions give rise to the parasympathetic ganglion of cranial nerve III, a portion of the connective tissue around the developing eyes and optic nerves, the muscles of the iris and ciliary body, and part of the cornea of the eye, they also contribute, along with head mesoderm, to the head mesenchyme cranial to the level of mesencephalon. Neural crest cells from the mesencephalon and rhombencephalon (hindbrain) regions also give rise to structures in the developing pharyngeal arches of the head and neck. The rhombencephalic neural crest cells also contribute to some of the cranial nerve ganglia. Specifically, rhombencephalic neural crest cells give rise to some neurons and all glial cells in the sensory ganglia of cranial nerves V, VII, IX and X. The remaining neurons in sensory ganglia of cranial nerves V, VII, IX and X arise from small ectodermal placodes, called epibranchial or epipharyngeal placodes.

The special sensory nerves and associated glia and ganglia also arise from placodes. Cranial nerve I arises from the olfactory placodes, cranial nerve II arises from optic cup (distal end of which thickens as the placode-like rudiment of neural retina) and cranial nerve VIII (vestibulocochlear nerve) and vestibulocochlear ganglion arises from the otic placode.⁸

Neural crest is inhibited from forming in the anterior neural ridge by the signaling molecule Dickkopf 1, a Wnt inhibitor that is secreted by prechordal mesoderm. Neural crest cells emerging from the diencephalon posteriorly through r2 do not express any Homeobox gene, (Hox gene). These genes are characterized by possession of a particular DNA sequence, the homeobox, encoding a variable protein domain called as homeodain, whereas the cells emerging from the hindbrain region from r3 and posteriorly express a well-ordered sequence of Hox genes.

It is known that some of the neural crest cells associated with r3 and r5 undergo apoptosis because of the presence of the apoptosis-inducing molecule BMP-4. A close relation exists between the pattern of migration of rhombomeric neural crest cells and the expression of products of the Hox-b gene complex. Hoxb-2, Hoxb-3, Hoxb-4 products are expressed in a regular sequence in the neural tube and the neural crest-derived mesenchyme of the second, third and fourth pharyngeal arches. Isthmic tissue emits a high concentration of FGF-8 which suppress Hoxa-2, the selector gene for determining the character of the second arch. The introduction of FGF-8 alone into region of future second arch suppresses its formation, and it takes on the character of first arch. The cranial mesoderm is presently suspected to play a formerly unexpected role in regulating the fate of migrating neural crest cells. Cranial neural crest cells differentiate into a variety of tissues, including connective tissue and skeletal tissues. These tissues constitute much of the soft and hard tissues of the face.⁵

THE NEURAL CREST—MOLECULAR LEVEL SIGNATURE

Expression of neural crest cell marker genes are preceded by expression of other genes important in neural crest cell induction which are expressed in neural plate, *msx1* and *msx2* which are expressed in neural folds and non-neural ectoderm and *Dlx*, which is expressed in non-neural ectoderm only. Together these factors induce expression of a number of genes and transcription factors including *Ap2*, *cMyc*, *Foxd3*, *Id*, *Twist* and *Sox 9* and *Sox 10* in a subset of neural plate border cells to form premigratory neural crest cells (Table 1).

Both gene and gene-network surveys for neural crest have shown *Bmp2* and *4* expressed in non-neural ectoderm, and that *Pax3/7*, *msx1/2*, *Zic* and *slug/snail* are expressed at

Table 1: Key neural crest—related genes

<i>Gene</i>	<i>Encodes</i>	<i>Expressed as</i>	<i>During</i>	<i>Function</i>
Bmp	Growth factor	Non-neural ectoderm	Gastrulation	Neural crest induction
Wnt	Growth factor	Non-neural ectoderm	Gastrulation	Neural crest induction
Zic	Transcription factor	Neural fold and plate	Neurula	Neural plate border specifier
Dlx	Transcription factor	Non-neural ectoderm	Neurula	Neural plate border specifier
Ap2	Transcription factor	Premigratory neural crest	Segmentation	Neural specifier
cMyc	Transcription factor	Premigratory neural crest	Segmentation	Neural crest specifier
Sox 9	Transcription factor	Premigratory neural crest	Segmentation	Neural crest specifier
Sox 10	Transcription factor	Premigratory neural crest	Segmentation	Neural crest specifier
Slug/snail	Transcription factor	Premigratory neural crest	Segmentation	Neural crest cell delamination

neural plate border. Majority of genes implicated in a neural crest gene regulatory network are expressed in mesodermal rather than regular neural plate derivatives.⁴

The molecular mechanism of the emigration of neural crest cells include two groups of proteins, namely:

Ephrins

Eph receptors and ephrins are membrane-bound proteins that function as a receptor-ligand pair. A total of 13 Eph receptors and eight ephrins have been identified in mammals. The Eph receptors belong to the superfamily of receptor tyrosine kinases and as such they autophosphorylate upon binding to their cognate ephrin ligands and subsequently activate subsequent downstream signaling cascade. At the cellular level, both possess a catalytic activity and activate signal transduction pathways after interaction with Eph receptors (reverse signaling).

Semaphorins

Semaphorins are secreted, transmembrane, and GPI linked protein, defined by cysteine-rich semaphoring protein domains that have important roles in a variety of tissues. Humans have 20 semaphorins, they are grouped into eight classes on the basis of phylogenetic tree analyses and the presence of additional protein motifs. Semaphorins can alter the cytoskeleton and the organization of actin filaments and the microtubule networks. These effects occur primarily through binding of semaphorins of heterophilic receptors, neuropilin and plexin. It has been shown that attenuation of semaphoring signaling by expression of soluble neuropilin-Fc results in neural crest cells invading adjacent mesenchymal territories that are normally crest free.⁹

Cranial neural crest cells as a group also give rise to other cell types that populate the head and neck. These include the pia mater and arachnoid—the inner and middle of the three meninges—of the occipital region; the dura mater, the outermost layer of the three meninges, arise largely or exclusively from head mesoderm. In addition, some cranial neural crest cells invade the surface ectoderm as they migrate away from

the neural tube to form the melanocytes of the skin of the head and neck.⁸ Vagal crest cells enter the anterior region of the foregut and begin to populate the gut. One potential reason why neural crest cells in the trunk are unable to enter the gut is that the cells in the mesentery near gut express Slit-2, the molecule that also prevents neurons from crossing the midline of the CNS. Trunk neural crest cells express the slit receptor Robo, causing them to avoid cells that express slit.⁵

Slit/Robo

This molecule has been involved in trunk neural crest migration. Slit is a secreted protein that binds to the Robo receptor, they were initially identified in *Drosophila* as playing an important role in axonal guidance. All slit proteins share a common domain structure, consisting of a series of four leucine-rich repeats, 7 to 9 epidermal growth factor-like domains, a laminin G-domain, and a C-terminal cysteine-rich domain. Slits are expressed in the dermamyotome and migrating early crest cells express Robo 1 and Robo 4. Slit-2 is expressed at the entrance of gut and only trunk neural crest cells express Robo receptors.¹⁰

FUNCTIONS OF NEURAL CREST CELLS

1. The neural crest cells may carry certain preacquired molecular signals as they migrate into branchial arch so they can induce epithelium to form various craniofacial structures.
2. The cranial neural crest cells acquire positional identity at the time they reach their final destination and contribute to the formation of various craniofacial structures. Certain growth and transcription factors have been implicated as important regulators for epithelial – mesenchymal interactions through which multipotent mural crest derived cells become progressively restricted to form neural crest derivatives and eventually develop into individual cell types.
3. The cranial neural cells significantly contribute to tooth morphogenesis. Combinatorial actions of homeobox

genes (Hox) expressed in neural crest-derived facial mesenchyme have been implicated to specify tooth shape, size, position.

4. The cranial neural crest-derived ectomesenchymal cells are responsible for the formation of Meckel's cartilage.¹¹

CONCLUSION

Human development involves several stages and complex mechanisms. Every step in evolution and development is related to molecular changes and genetic sequence of activity. The morphological appearances of all the tissues and organs represent the synthesis of variety of genes working in a co-ordinated manner and at times opposing each other. The ability to study and contemplate on these issues has been the major weak point of research toward development, and thus various diseases mechanisms and pathogenesis has not been dealt with in a detailed manner. However, recent advancements and improvements in technology has provided the research field with ground breaking results and thus further insights into the tunnel of molecular mechanisms could be made. As a result, the earliest of formative stages of cells can be studied in a coherent manner enabling us to assimilate knowledge on development of tissues and further, disturbances in physiological mechanisms.

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