Early Embryonic Facial Development

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The starting constituent for the majority of skeletal structures in the facial region, is a pluripotent population of cells, the cranial neural crest cells (CNCCs). Here we discuss the initial formation and migration of these cells to the developing facial regions and the subsequent morphological changes that occur in the face at the different stages of embryonic development.

I. INTRODUCTION

Tissues of the face are primarily derived from cranial neural crest cells (CNCCs). The CNCCs comprise a population of transiently migrating cells that originate from the dorsal aspect of the neural tube during embryogenesis and subsequently migrate to form most of the skeletal, dermal, mesenchymal and neural structures of the face. A tightly controlled spatial and temporal signaling network is required for the induction, migration, proliferation, and differentiation of CNCCs. During migration, and even after arrival at their final destination, interactions between CNCCs and the adjacent surface ectoderm, neuroectoderm and endoderm are necessary for normal development of the facial region.

II. CRANIAL NEURAL CREST CELLS (CNCCs)

A. Early embryonic development

During the first three days of development*, the fertilized ovum, or the zygote, is located in the fallopian tube and undergoes rapid divisions as it travels down the tube to form the morula. The cells of the morula then organize themselves to form the blastocyst, and by the end of day 5, the fully formed blastocyst comes into contact with the uterine mucosa for implantation.

During the second week of development, the inner cell mass, or embryoblast, of the blastocyst differentiates into two layers, the hypoblast and the epiblast. Together, these layers form an oval shaped bilaminar germ disc.

In the third week of development, this bilaminar germ disc undergoes a process called gastrulation. Gastrulation begins with formation of the primitive streak on the surface of the epiblast. The primitive streak is a narrow groove that extends through the midline, with slightly bulging regions on either side. The presence of the primitive streak will establish bilateral symmetry and create the cranial-caudal axis, determining the site of gastrulation. The cells of the epiblast migrate toward the primitive streak. Upon arrival in the region of the streak, they become flask shaped, detach from the epiblast, and slip beneath it. This inward movement is known as invagination.

Once the cells have invaginated, some displace the hypoblast, creating the embryonic endoderm, and others come to lie between the epiblast and the newly created endoderm to form mesoderm. Cells remaining in the epiblast then form the ectoderm. As more and more cells move between the epiblast and hypoblast layers, they begin to spread laterally and cranially.

These three germ layers, the ectoderm, mesoderm and endoderm, created by the migration of the epiblast cells, will go on to form all of the tissues and organs in the human body.

B. Formation of CNCCs

In the third to fourth weeks of development, the epiblast cells invaginating in the primitive pit move forward and intercalate in the endoderm as the notochordal plate. With further development, the plate detaches from the endoderm, and a solid cord, the notochord, is formed. This forms the midline axis.

Formation of the notochord induces the overlying ectoderm to thicken and form a slipper shaped plate of thickened neuroectoderm, known as the neural plate, in the mid-dorsal region.

The lateral edges of the neural plate abut the non-neural ectoderm and soon elevate to form the neural folds. With further development, in a process referred to as neurulation, the neural folds continue to elevate, approach each other in the midline, eventually forming the neural tube.

As the neural tube forms, a group of cells called neural crest cells are induced to form uniformly at the interface between the surface ectoderm and the neuroectoderm along almost the entire length of the vertebrate embryo neuraxis. In the cephalic or cranial region, the neural crest cells are called cranial neural crest cells or CNCCs.

Signaling by three major groups of genes has been shown to play a significant role in neural crest induction. These genes are the *Bone Morphogenetic Proteins (BMP)* within the neural plate, *WNT* from the non-neural ectoderm, and *Fibroblast Growth Factor (FGF)* from the underlying mesoderm.

These CNCCs then delaminate from the surrounding ectoderm through a process called epithelium to mesenchyme transition, or EMT. Initially the CNCCs are arranged on a basement membrane, exhibiting apico-basal polarity and abundant expression of intercellular adhesion complexes such as E-cadherin and integrins. In order to adopt a mesenchymal phenotype, induced CNCCs must lose cell adhesion by E-Cadherin down-regulation. Transitioning cells then progressively lose polarity while eroding the basement membrane by matrix metalloproteinase production. Cytoskeletal changes mediated by Rho GTPases induce apical constriction and further structural rearrangements to permit passage through the degraded basement membrane, culminating in the delamination from the epithelial layer. Completing the transition, cells activate the expression of additional mesenchymal genes and proteins, such as smooth muscle actin, collagen I and III, vimentin and fibronectin.

C. Migration of CNCCs

The CNCCs then migrate from the neural tube to their target destination. CNCCs migrate collectively and in a directed fashion, influenced by interactions between themselves and other cells. The migration of CNCCs towards the ventral pharyngeal regions follows discrete segregated streams. The forebrain and rostral midbrain neural crest cells colonize the frontonasal and periocular regions; caudal midbrain derived CNCCs populate the maxillary component of the first branchial arch; and, CNCCs emigrate from part of the hindbrain to populate the first branchial arch, forming the mandibular region.

At the cellular and molecular levels, a number of mechanisms have been shown to operate in the collective and directed migration of CNCCs. These mechanisms include contact inhibition of locomotion, co-attraction, chemoattraction and inhibition. When two CNCCs meet each other, there is a change in migratory direction and collapse of protrusions in a phenomenon called contact inhibition of locomotion or CIL. Leading edges of groups of CNCCs, in contrast, are polarized, contain lamellipodia (or sheet like extensions of the cytoplasm) and migrate from each other. Thus, cells exposed to a free edge can migrate away from the cluster leading to the directional migration of the whole group. The effect of CIL on cell polarization is thus vital for forward directed movement of CNCCs toward the target destination. Additionally, cells within the group are also attracted to each other in a process called co-attraction. When a CNCC leaves the migratory CNCC stream to which it belongs, it is quickly attracted back to it. The complement factor, C3a, and its receptor C3aR are expressed by CNCCs and together act to maintain cohesive clusters of migrating CNCCs. During migration, CNCCs are also exposed to positive guidance cues which are usually located along the CNCC pathways or within the target regions. The chemoattractant, Stromal Cell-Derived factor 1 (*SDF1)*, is able to influence the migratory direction of CNCCs. Molecules such as *VEGF*, *FGF2/8*, *PDGFs*, and Class6-semaphorins also attract CNCCs to specific locations or create permissive environments for migration. CNCC migration also relies on an available permissive extracellular matrix, mostly comprising fibronectin, laminins and some collagens, which line the migratory routes. Further patterning of CNCCs into

distinct streams and their precise targeting to specific tissues are also controlled by a plethora of negative guidance cues which are usually located at the border of each CNCC stream and includes molecules such as Class 3 semaphorins and Ephrins.

Along the migratory route, the loosely connected CNCCs stop, retract the slender cytoplasmic projections that extend beyond the leading edge of lamellipodia in these migrating cells (filopodia) and then divide. As they reach their target site and enter the branchial arch they undergo a final morphological change and extend multiple filopodia in all directions in order to spread out and fill the space within the arch.

D.Proliferation of CNCCs

Once CNCCs arrive at their target destination in the facial and branchial regions, they proliferate in a highly regimented fashion and the facial structures begin to take shape. The instructions for this proliferative activity are intrinsically programmed with a facial patterning “blueprint” and from instructions from their local microenvironment.The resulting crosstalk involves common signalling pathways from a local milieu of the superficial ectoderm, the neuroepithelium and the pharyngeal endoderm. This molecular dialog affects CNCC proliferation, differentiation, and survival.

III. MORPHOGICAL ASPECTS OF FACIAL DEVELOPMENT

By the fourth week of development, the embryo is characterized externally by five facial swellings: the frontonasal prominence in the center and the paired mandibular and maxillary prominences of the first branchial arch. These structures are formed in part from migration and proliferation of CNCCs from different regions of the neural tube.

In a 5-week-old embryo, localized thickenings of ectoderm on both sides of the frontonasal prominence originate under the inductive influence of the ventral portion of the forebrain. The nasal placodes form the olfactory epithelium while the optic placodes form the lenses of the eyes. Mesenchyme in the margins of the nasal placodes proliferates to form the nasomedial prominence and the nasolateral prominence in horseshoe-shaped elevations. As the tissue surrounding the placodes thickens and elevates, the nasal placodes appear to become recessed within depressions in the surrounding tissue. These are the nasal pits which are the primordia of the external nares and the nasal cavities. In addition, the maxillary prominences enlarge and grow forward and medially.

In a 6-week-old embryo, the two mandibular prominences fuse in the midline to form the tissues of the lower jaw. The mandibular prominences and maxillary prominences are continuous at the angle of the mouth, thus defining its outline. From the upper corners of the mouth, the maxillary prominences grow below the lateral nasal prominences and towards the medial nasal prominences. Between the merging maxillary and the lateral nasal prominences lie the naso-optic furrows. From each furrow, a solid ectodermal rod of cells sinks below the surface and canalizes to form the nasolacrimal duct.

In the 7-week-old embryo, the maxillary prominences grow and fuse with the lateral nasal prominence to form the alanasi and lateral border of nostril. They then fuse with the expanding medial nasal prominence to form the upper lip. The nasolateral and nasomedial prominence fuse with each other and form the external nares. Merging of the maxillary and mandibular prominences forms the cheeks and the corners of the mouth. As a result of medial growth of the maxillary prominences, the two medial nasal prominences merge not only at the surface but also at a deeper level to form the intermaxillary segment. The resulting segment comprises a labial component, which forms the philtrum of upper lip, an upper jaw component, which carries the four incisor teeth, and a palatal component, which forms the triangular primary palate. During the 6^{th} week, two shelf-like outgrowths, the palatine shelves, appear from the maxillary prominences and are directed obliquely downward on each side of the tongue. In the 7^{th} week, however, the palatine shelves ascend to attain a horizontal position above the tongue and fuse forming the definitive palate, the secondary palate.

This flurry of activity in the first 7 weeks after fertilization culminates in the frontonasal prominence contributing to the formation of the forehead, middle and the sides of the nose, the philtrum of the upper lip and the primary palate; the maxillary prominences contributing to the formation of the sides of the face, upper lips and the secondary palate; and the mandibular prominences giving rise to the lower jaw.

By the 7^{th} week of human embryonic development, therefore, most of the facial structures found post natally can be observed. In the next few months of intrauterine development, the initial cartilaginous skeleton of face is replaced by bone and there is an overall increase in shape and size of the different structures of the face. From childhood to adulthood, further growth and remodeling of the facial bones results in adult facial morphology.

Note: * age of embryo is based on “developmental age”– time period after fertilization

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