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Jacobs Journal of Dentistry and Research

Review Article

Molecular Mechanism of Cranial Neural Crest Cell Development

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Received: 07-31-2014

Accepted: 08-28-2014

Published: 09-24-2014

Abstract

The craniofacial region, comprising the face and the skull, is largely the product of cranial neural crest (CNC) cells, in which CNC cells form most of the bone, cartilage and connective tissues. Genetic mutations, environmental factors, and nutritional deficiencies have been identified as risk factors to affect the fate of CNC cells and cause craniofacial deformities. Many signaling pathways and their interactions are involved in the formation of CNC derivatives and disturbances of these cascades are attributed as etiological agents for craniofacial pathologies. Much progress has been made in identifying causative factors associated with craniofacial anomalies; however, the etiologies of craniofacial birth defects are still largely unknown. In this review, we focus on the current understanding of the molecular networks involved in the fate determination of CNC cells during craniofacial development.

Keywords: Neural Crest Cells; Craniofacial Development; Cell Signaling; Genetic Factor; Epigenetic Factor; Environmental Risk Factor; Birth Defect

Abbreviations

BMP: Bone Morphogenetic Protein;
CNC: Cranial Neural Crest;
EMT: Epithelial-To-Mesenchymal Transition;
FAS:Fetal Alcohol Syndrome;
FGF:Fibroblast Growth Factor;
HAT:Histone Acetyltransferases;
HDAC: Histone Deacetylase;
miRNA: microRNA;
NC: Neural Crest;
PA: Pharyngeal Arches;
r: Rhombomere;
RA: Retinoic Acid;
SHH: Sonic Hedgehog;
TCF/LEF1: T-Cell Factor/Lymphoid Enhancer-Binding Factor 1;
TGFβ: Transforming Growth Factor Beta

Introduction

The facial primordia are populated predominantly by CNC-derived cells surrounding a mesodermal core and covered with ectoderm [1]. The formation of craniofacial structures is composed of the processes of induction, delamination, migration and differentiation of CNC cells

[1]. Dysregulation of CNC development leads to congenital birth defects such as Treacher-Collins syndrome, DiGeorge syndrome, and orofacial clefts [2-4]. However, the genetic and epigenetic networks regulating CNC cells during craniofacial development are still largely unknown. Many findings of CNC cell features have been initially identified in chick embryos, which are consistent (or inconsistent in some features) with other species [5]. Because mouse craniofacial development is similar to human craniofacial development, mouse models are very useful to study craniofacial structures (palate, jaw, skull etc.). This review seeks to provide a current understanding of the molecular networks in the fate determination of human and mouse CNC cells. Several other reviews are available that provide excellent summaries of other aspects of CNC cells [5-10].

Fate of Cranial Neural Crest Cells

Origin of Neural Crest Cells

The hindbrain is segmented along the anterior-posterior axis into compartments called rhombomeres [11]. CNC cells migrate ventrally from rhombomeres 1 to 8 (r1 to r8) into five pharyngeal arches (PAs: aka branchial arches) and the frontonasal process (Figure 1).

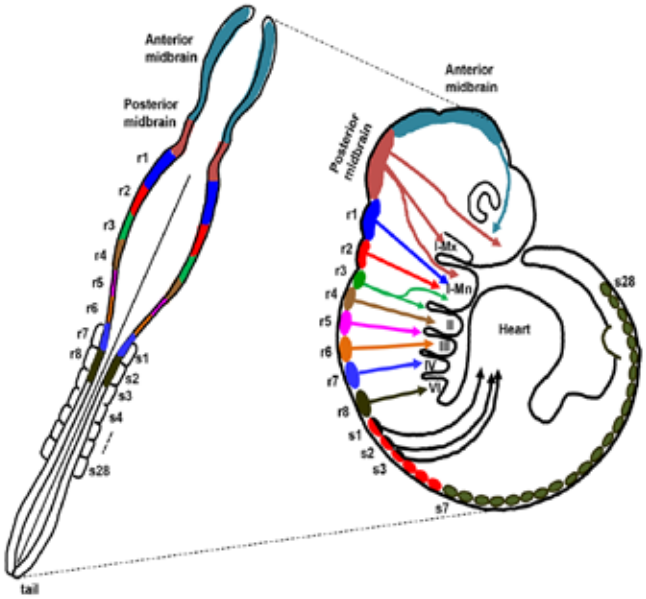

Figure 1. Cranial neural crest (CNC) cell migration in the mammalian head.
CNC cells migrate ventrally from rhombomeres 1 to 8 (r1 to r8) into the frontonasal process and five pharyngeal arches (I to VI; the fifth arch degenerates in humans). Cardiac NC cells arise near somites 1-3 (s1 to s3) and migrate into the cardiac region. Trunk NC cells arise about somite 6 through the tail and migrate into the body to make sympathetic neurons and pigment cells (melanocytes). A subset of trunk NC cells at the level of s18 to s24 form the medulla portion of the adrenal gland. Vagal NC cells (arisen near s1 to s7) and sacral NC cells (arisen posterior to s28) migrate into the body to form the parasympathetic nerves of the gut.

During neural tube formation in early development, the neural plate forms the neural crest (NC) during fusion of new neural folds. Specific to vertebrates, the NC can be divided into four main regions (cranial, trunk, vagal and sacral, and cardiac NCs), each of which with their own characteristic derivatives and functions (Figure 2).

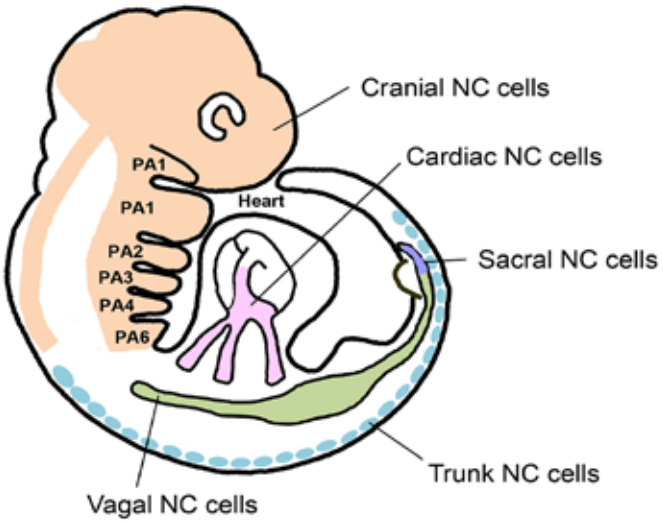

Figure 2. Regions of neural crest (NC) cells in mammals.
Cranial neural crest cells migrate into the head and pharyngeal arches 1-6 (PA1-6). Cardiac NC cells play a critical role in making the division between the aorta and the pulmonary artery. Trunk NC cells give rise to sympathetic neurons and pigment cells (melanocytes), and a subset of trunk NC cells form the medulla portion of the adrenal gland. Vagal NC and sacral NC cells form the parasympathetic nerves of the gut.

NC cells arise in the NC of the dorsal neural tube, a transient structure that forms the roof plate of the brain and spinal cord at the end of the first month of pregnancy in humans [12]. NC cells are a multipotent, migratory cell population that undergoes an epithelial-to-mesenchymal transition (EMT) before migrating away from the developing central nervous system soon after the neural tube closes [12,13]. NC cells from the midbrain and r1 and r2 of the hindbrain migrate to the first PA, forming maxillary and mandibular bones as well as the incus and malleus bones of the middle ear and the frontonasal process generating midfacial skeleton. These NC cells from the midbrain, r1 and r2 also differentiate into the neurons of the trigeminal ganglion and the ciliary ganglion. NC cells from r4 populate the second PA, forming the hyoid bone of the neck as well as the stapes bone of the middle ear. These r4-derived NC cells also form the neurons of the facial nerve. NC cells from r6-r8 migrate into the 3rd and 4th PAs and pouches to form the hyoid bone, and the thymus, parathyroid, and thyroid glands [11,14].

Influence of Genetic Factors during Rhombomere Formation and Patterning

Mutations in genes involved in rhombomere formation

and patterning cause craniofacial anomalies. For example, *Otx2* null mice (*Otx2*$^{-/-}$ mice) show no structure anterior to r3, indicating that *Otx2* plays an essential role in the formation of the rostral head [15]. *Otx2*$^{+/-}$ heterozygous mutant mice also show severe craniofacial defects [15]. Mice with disruption of *Hoxa2* (*Hoxa2*$^{-/-}$ mice) exhibit rhombomere 2 patterning defects [16]. *Pbx* genes are involved in retinoic acid (RA) synthesis during hindbrain segmentation [17]. *Pbx1/Pbx2* and *Hoxa1/Pbx1* double null mice display a prominent posterior expansion of r2 through gene expression of *Raldh2* that is a RA biosynthetic enzyme [17].

Induction and Specification of NC Cells

WNT (wingless in *Drosophila*), fibroblast growth factor (FGF) and bone morphogenetic protein (BMP) signaling plays a pivotal role in the process of NC cell induction at the neural plate border in ectoderm as neurulation begins [18,19]. Specification of NC cells is governed by transcription factors such as Msx1/2, Pax3/7, Zic1, Dlx3/5, Hairy2, Id3, and Ap2 [19,20]. These processes specify a border between the neural plate and the non-neural ectoderm. FGF and WNT signaling first induces the formation of NC progenitors at the border of the neural plate, and then BMP, WNT and Notch signaling maintains the progenitors through the expression of *Snail2, Foxd3, Sox9/10,Msx1/2, Pax3/7, Zic1, Dlx3,* and *Hairy2* [20].

EMT and Migration of NC Cells

Soon after specification of NC progenitors, a second set of transcription factors, including Snail2, FoxD3, Sox9/10, Twist, c-Myc, and Ap2 play key roles in the processes of EMT and NC cell migration [20,21]. NC progenitor cells, located near the dorsal midline of the neural tube, undergo EMT to become migratory NC cells which traverse along stereotypical paths throughout the embryo [22,23]. WNT, FGF, BMP, and transforming growth factor beta (TGFβ) signaling activate transcription factors to induce EMT [24,25]. Noggin, a BMP inhibitor, prevents delamination of premigratory NC cells until *Noggin* transcription is downregulated in the dorsal tube, resulting in activation of BMP and consequent *Wnt1* production [26]. This initiation of NC migration conventionally begins prior to neural tube closure in mammals [11]. Through the coordinated action of the *Sox9, Foxd3* and *Snail* genes (targets of WNT signaling), it is apparent that WNT signaling is crucial for both EMT and migration [27]. *Snail* genes play a key role in the delamination and migration of NC cells [28]. The absence of *Snail2* leads to the accumulation of epithelial cells and results in a failure of NC cell migration [29]. In humans, deletion of *SNAI2* (aka *SLUG*) or *Sox10* leads to Waardenburg syndrome although *Slug* is not essential for facial development in mice [30-32].

Differences between Cranial and Trunk NC Cells

Cranial and trunk NC cells are differently regulated in EMT, cell migration, and differentiation [33]. During migration of CNC cells, and even after arrival at their final destination, CNC cells migrate superficially and interact with the adjacent surface ectoderm, neuroectoderm and endoderm during craniofacial development [34,35]. Although CNC cells acquire their anterior-posterior identities before migration, the patterning and the fate of CNC cells is regulated by reciprocal signals from a niche in which CNC cells thrive mainly at a post-migratory stage [34,36,37]. For example, BMP and WNT signaling promotes the fate of sensory neurons [38,39]. Endothelin and WNT signaling stimulates CNC cells to differentiate into melanocytes in skin and adrenergic neurons [38,40]. In contrast to the other NC cells, CNC cells can give rise to odontoblasts, osteoblasts and chondrocytes in addition to the other cell types that trunk NC cells can differentiate into [33,41]. CNC cells migrate into the head and neck and form a wide array of derivatives, including craniofacial bones and cartilages, odontoblasts, melanocytes, connective tissue of the face and neck, peripheral and enteric nerves, smooth muscle and endocrine cells etc. (Figure 3) [11,22,23].

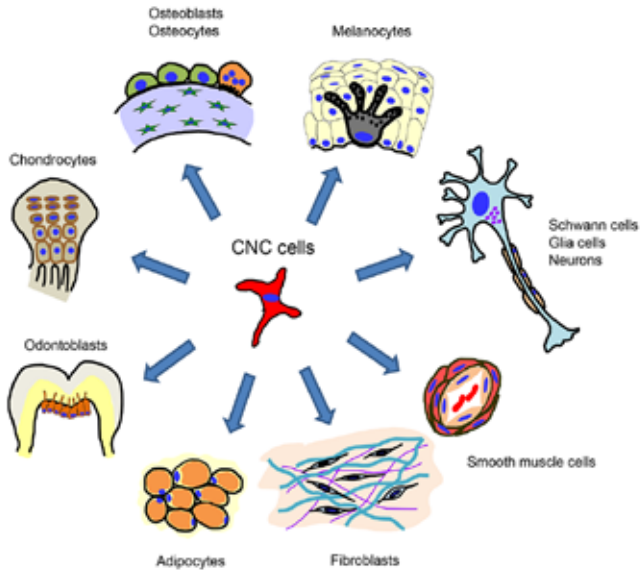

Figure 3. Cranial neural crest (CNC)-derived lineages.
CNC cells can give rise to osteoblast, osteocytes, chondrocytes, odontoblasts, adipocytes, fibroblasts, smooth muscle cells, Schwann cells, glia cells, neurons, and melanocytes.

Molecular Mechanisms during Fate Determination of CNC Cells

Growth factor signaling has a wide range of impacts on the control of cell proliferation, differentiation and migration. These signaling activities are tightly regulated during development and the alternation causes birth defects. Especially this is so true for craniofacial development because CNC cells are very sensitive (partly because they are multipotent cells?) to growth factors. Thus, it is very

important to know the appropriated regulation of growth factor signaling in CNC cells.

TGFβ Signaling

TGFβ activates SMAD2/3 to control downstream target genes [42]. During embryogenesis, TGFβ signaling regulates a vast range of biological functions including cell proliferation, differentiation, and extracellular matrix formation [43-45]. In NC cells, TGFβ is responsible for initiation of EMT and plays a role in the invasion and migration of NC cells [42,46]. TGFβ is also important for later developmental stages in CNC derivative tissues such as skull, maxilla, palate, mandibuler, and tongue [47-49].

BMP Signaling

BMPs, members of the TGFβ superfamily of signaling proteins, are important for craniofacial development [50,51]. BMP ligands bind to type I and type II receptors for BMP, leading to activation of SMAD1/5/8 [52]. BMP signaling, activated at the neural plate border, plays a role in NC cell induction [53]. Thereafter, BMP has multiple roles including the axial patterning, left-right symmetry, and cell-fate determination of NC cells [18,54]. BMP signaling regulates craniofacial skeletal formation by balancing self-renewal and differentiation of CNC progenitor cells [55-58]. In addition, the BMP antagonist Noggin plays multiple roles including cell proliferation and differentiation in CNC development [59].

FGF Signaling

The FGF signaling pathway is involved in several aspects of craniofacial development [60]. For example, FGF signaling plays a role in the fate determination of CNC cells during chondrogenesis [61]. Mutations in FGF signaling molecules lead to craniofacial malformations including midfacial anomalies and craniosynostosis [62]. Loss of *Fgf8* results in a failure of bone and cartilage development [63-65].

WNT Signaling

WNT ligands initiate the translocation of β-catenin to the nucleus, and then β-catenin regulates gene expression of targets together with transcription factors of the T-cell factor/lymphoid enhancer-binding factor 1 (TCF/LEF1) family [66]. WNT/β-catenin signaling controls multiple steps of NC development, ranging from NC induction, migration, and proliferation, to differentiation [67]. Disruption of WNT/β-catenin signaling in CNC cells (*Ctnnb*$^{F/F}$*;Wnt1-Cre* mice) leads to severe craniofacial deformities due to defects in cell survival and/or differentiation [68]. WNT1, WNT3A and WNT8 can induce canonical WNT/β-catenin signaling. In contrast, the non-canonical (β-catenin-independent) pathway, comprised of WNT4, WNT5A and WNT11, can activate the planar cell polarity (PCP) pathway which guides cell movement during embryogenesis [21].

Sonic Hedgehog Signaling

Sonic hedgehog (SHH), which is expressed in the foregut endoderm, facial ectoderm and neuroepithelium, is a morphogen that is critical for specification, proliferation, survival and patterning of NC cells [39]. Mice with deficiency of *Gli3*, a mediator for SHH signaling, exhibit ectopic ossification in the interfrontal suture together with craniosynostosis; this phenocopies the severe craniofacial malformations characteristic of Grieg cephalopolysyndactyly syndrome [69]. The conditional deletion of Smoothened receptor in NC cells results in decreased proliferation and increased apoptosis [70]. Recent studies indicate that disruption of primary cilia formation expands SHH signaling activity [71].

Endothelin Signaling

Endothelin-1 (Edn1/ET1) is a small ligand that is secreted from the ventral portion of the PAs [72]. Endothelin-1 signaling through G protein-coupled endothelin A receptor (Ednra), which is expressed in NC cells, is important for NC cell dorsal-ventral patterning, especially in the first PA [72,73]. Loss of *Edn1, Ednra* or *Ece1* (an endothelin converting enzyme which converts latent Edn1 to the active form) results in craniofacial defects, following disruption of the patterning and differentiation of NC cells [73]. Constitutive activation of *Ednra* throughout the pharyngeal mesenchyme induces the replacement of the maxillary components by a second set of mandibular elements, resulting from a disruption of Edn1/Ednra-Dlx5/6-Hand2 signaling pathway [74]. Mutations in genes involved in the endothelin pathway lead to auriculocondylar syndrome [75].

Notch Signaling

Notch proteins are trans-membrane proteins that play roles in NC cell migration, differentiation, and fate determination through cell-cell interactions [76]. Following ligand binding, Notch receptors are cleaved to trigger Notch pathway activation [77,78]. Mutations in *JAGGED1*, a ligand in the Notch signaling pathway, cause Alagille syndrome which is characterized by biliary, cardiac and craniofacial anomalies [79,80]. Mice with a conditional deletion of *Jagged1* in CNC cells exhibit midfacial anomalies that recapitulate the midfacial hypoplasia of Alagille syndrome [81].

Crosstalk among Growth Factor Signaling Pathways

Recent studies indicate that crosstalk among growth factor signaling pathways is important for CNC development [44,73,82,83]. For instance, BMP is required to induce

endothelin signaling in patterning the dorsal-ventral axis of the craniofacial skeleton [84]. TGFβ signaling interacts with FGF and SHH signaling during craniofacial development [49,85]. Primary cilia integrate hedgehog and WNT signaling during tooth development [86]. Within networks of cell signaling pathways (cell autonomous and non-cell autonomous mechanisms), both genetic and epigenetic factors influence to the fate determination of CNC cells.

MicroRNAs Involved in the Fate Determination of CNC Cells

Despite advances in human genome sequencing technology, the causes of nearly 70 percent of all birth defects still remain unknown [87]. MicroRNAs (MiRNAs) are short (~22 nucleotides) noncoding RNA molecules that regulate gene expression at the post-transcriptional level and fine-tune the expression of ~30% of all mammalian protein-encoding genes [88]. MiRNA expression is regulated in a temporal and spatial specific manner to regulate hundreds of different mRNAs and control the fate of CNC cells. Recent studies indicate that loss of all mature miRNAs in CNC cells results in increased apoptosis and defects in osteogenic differentiation, causing severe craniofacial deformities in mice [89-91].

Epigenetic Factors Involved in the Fate Determination of CNC Cells

Posttranslational modifications of histones by acetylation, phosphorylation, methylation, and sumoylation have been demonstrated to regulate craniofacial development. Mutations in genes affecting histone acetylation and deacetylation, which is catalyzed by histone acetyltransferases (HATs: ex. MYST4) and histone deacetylases (HDACs), result in orofacial cleft [92-94]. Mutations in demethylation, which is regulated by histone demethylase (ex. KDM6A, PHF8), and mutations in sumoylation (*SUMO1*) also result in orofacial clefts [95,96]. HDAC8 specifically controls patterning of the skull by repressing a number of homeobox transcription factors in CNC cells [94]. In humans, a chromosomal translocation disrupting the *MYST4* HAT results in a Noonan-syndrome-like phenotype including craniofacial deformities [92].

Environmental Risk Factors

In addition to above genetic factors, there are some environmental risk factors probable contributing to CNC development [97]. For example, cigarette smoking with its associated hypoxia, maternal consumption of alcohol, diets deficient in zinc, myoinositol, and folic acid have been considered as risk factors for craniofacial birth defects [97-99]. Mouse models for fetal alcohol syndrome (FAS) demonstrate an anterior neural plate deficiency, which leads to close approximation of olfactory placodes towards the midline [100]. Imbalances in RA levels, either through disruption of RA synthesis or exposure to excessive RA *in utero*, have also likewise been noticed in cases of craniofacial anomalies [101].

Perspective

One of the interesting observations is that some mouse models and human syndromes have severe malformations specifically in the craniofacial region [88]. These results suggest that CNC cells are more sensitive to aberrations than are cells from other regions during embryogenesis. Because CNC cells can more efficiently produce bone, cartilage, and connective tissues in craniofacial regions than bone marrow mesenchymal stem cells [102], it is important to understand the fundamental biology in CNC cells. The principles learned from mouse models promise to be fertile ground for future studies of craniofacial development, and will provide strategies for craniofacial tissue regeneration and therapy. In addition, identification of target molecules may provide the basis for genetic tests and proper nutrient intake recommendations aimed at identifying higher-risk pregnancies and prevention of craniofacial anomalies. Given the early developmental onset of structural birth defects, preventive treatment could be effective to reduce its impact on both human health and economy.

Acknowledgment

We thank Dr. Yoshihiro Komatsu and Dr. Jeremy Schaefer for critical reading. We apologize to authors whose relevant work we did not include due to the space limitation. J.I. is supported by start-up fund from the UTHealth School of Dentistry.

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