Journal of Nepal Dental Association (2010), Vol. 11, No. 1, Jan.-Jun., 88-93

Review Article

The role of neural crest cells and homeobox genes in craniofacial development

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Abstract

The role of neural crest cells in the development of craniofacial development has been a topic of research for the molecular biologist for decades. With the discovery of the homeobox genes, many research and investigations have shown that there is a genetic control for the patterning of the craniofacial region. With the identification of more different types of homeobox gene along with the transcription factors, it is becoming clearer that the homeobox genes have greater role than previously thought. With advancement of the research techniques, more insights of the role of these genes have been explored. These genes in fact are found to be the regulators and thus the master genes of the head and face. So it is now transparent that there is a genetic domination over the development and the patterning of the head and facial region through these genes. This review article tends to give a simplified overview of the role of neural crest cells and homeobox genes in the development of the craniofacial complex.

Key words: Neural crest cells, Homeobox genes, Craniofacial development

Introduction

With the drastic development in the field of craniofacial biology, we have been able to understand in-depth of the craniofacial development in much more detail than ever before. Though the study of embryology in the last part of the century has helped us to understand much of the development of the embryo, it is only in the last few years that has led us to understand the significance of the neural crest cells and the more complex genes activity for the proper development of the head and facial region of the vertebrate. It is now possible to study how the particular genes act on their natural environment, how they express phenotypically and what will be the consequences if their expression is blocked. Without the knowledge of gene activity and the relevant cellular signal transduction pathways, elucidating the mechanism that control development would be impossible. With these advancements in understanding the role of genes, it is now possible to explain the cause of craniofacial defects and also the magnitude of defect if a particular gene is missing. It is therefore utmost important for the clinician to have a superior understanding regarding these developments.

The role of the neural crest

Although neural crest is a transient structure and consists of only a few cells, it plays an important role in the developmental biology. The neural crest forms according to the rostocraudal gradient along the body axis and releases a free moving mesynchymal cell that follow definite migration route at precise time of development finally reaching target embryonic site where they differentiate and develop. The neural crest was first described by His on a chick embryo and ever since its discovery, many studies have been done. During the process of neuralation, ectodermal cells thicken to form the neural plate[1]. The neural crest can be divided into four functional domains[2]. They are:

a) Cranial neural crest (CNC) – Gives rise to various structures of chondrocranium. These cells migrate dorsolaterally to produce the craniofacial mesenchyme that differentiates into the cartilage, bone, cranial neurons, glia, and connective tissues of the face. These cells enter the pharyngeal arches and pouches to give rise to thymic cells, odontoblasts of the tooth primordia, and the bones of middle ear and jaw.

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b) Trunk neural crest – gives rise to pigment synthesizing melanocytes and dorsal root ganglion containing sensory neurons.
c) Vagal and sacral neural crest – giving rise to parasympathetic ganglia of gut.
d) Cardiac neural crest- giving rise to melaonocytes, neurons and connective tissue

Cells from the lateral border of the crest of the neuroectoderm dissociate to form a cell population called the neural crest cells. Neural crest cells are a population of highly pluripotent cells that plays an important role in the development of the vertebrate head. In mammals, neural crest cells are formed during neuralation when cells at the margins of the neural folds undergo an epithelial to mesenchymal transition following an inductive interaction between neural plate and presumptive ectoderm. Neural crest cells migrate extensively throughout the embryo in the four overlapping domains (cephalic, trunk, sacral, and cardiac), in the developing head the cephalic neural crest migrates from the posterior midbrain and hindbrain regions into the brachial arch system. The ectomesemchymal neural crest cells then interact with epithelial and mesodermal cell populations present within the arches leading to the formation of craniofacial bones, cartilages and connective tissues[3]. So in short, we can say that each component of the face that is the forehead, nose, cheeks, lips, jaws, chin etc arises from the coordination of a variety of morphogenetic events which includes cell migration and extracellular matrix remodelling, proliferation and differentiation of neural crest derived mesenchyme into skeletal and connective tissues, the assembly of musculature and the beginning stages of organogenesis[4]. It should be noted that the facial mesenchyme is derived principally from the neural crest cells and not from the embryonic third germ cells which is responsible for the development of most of the other parts of the body[3].

Migration of the neural crest cells

The neural crest cells migrate away from the neural tube and begin to migrate throughout the embryo. Unlike other cells in which the cell migration occurs in sheets, the migration of the neural crest cells occurs individually. So the mechanism for the neural crest cell migration is different than others and occurs in one of the following or all process. It generally occurs in three stages[5].

1. Initiation: The neural crest cells undergo an epithelial to mesenchymal transition causing the cells to break free from the neural tube and in this process adhesion connection between the neural crest cells mediated by molecules such as N-CAM, N-Cadherin and E-Cadherin are down regulated. Simultaneously there is an increase in the junction between the neural crest and the extracellular matrix. This happens due to an increase in a protein integrin in the cell surface. The amount of the intercellular space increases and finally the neural crest cells become more motile.
2. Dispersion: The neural crest cells migrate through the extracellular matrix to reach its final destination where they proliferate and develop.
3. Cessation of migration: Occurs as a reverse of initiation of migration. The adhesive molecules i.e. N-CAM, N-Cadherin and E-Cadherin are re-expressed. The amount of extracellular matrix is decreased and thus the migration is reduced.

The neural crest cells take either the ventral pathway or the dorsolateral pathway. The cells arising from the cranial or cephalic neural crest cell migrate dorsolaterlly to produce the craniofacial mesenchyme which gets differentiates into the cartilage, bone, cranial neurons, glia and connective tissues.

Homeobox genes

Homeobox genes were discovered independently by Walter J Gehring in 1983 working at the University of Basel, Switzerland and Matthew Scott and Amy Weiner who were working at Indiana University Bloomington. Homeobox is a 180 base pairs long DNA sequence found within genes that are involved in the morphogenesis in animals, plants and fungi. The homeobox encodes a 60-amino acid helix loop helix DNA binding within an encoded transcription factor. The region of the protein is the homeodomain and act as transcription factors that activate or inhibit the transcription of other genes[2]. The homeobox genes were first identified in *Drosophila melanogaster* in which it was clustered in two segments (Antennapedia and bithorax) on chromosome no 3 and hence was known as HOM-C complex. The HOM-C represented the molecular representation of the anterior – posterior embryonic axis of the developing fly[3]. Soon after this, a search began for similar genes in vertebrates. The homeobox genes have been classified in different ways into superclasses, classes, subclasses, or groups but there has been inconsistency the terms. The most widely used groupings are ANTP, PRD, LIM, POU, HNF, SINE, TALE, CUT, PROS and ZF. Also widely recognized gene families include Dlx, Msx, Otx, Hox, PAX, and NK. There are 11 classes of homeobox genes subdivided into 102 homeobox gene 'families' with a total of 300 human homeobox genes loci[6]. A number of genes containing sequences coding for DNA-binding domains homologous to homeobox sequences in *Drosophila* have been isolated in vertebrate and their mechanism of action has been studied. In particular, Hox family genes share with *Drosophila* homeotic genes a genomic organization in gene clusters and an expression pattern that is similar in a number of important aspects. It was found that the homeotic genes

were preserved during evolution[7,8]. Analysis of human DNA sequence showed that it shared more than 90% homology with the peptide sequences specified by the homeobox domain of the *Drosophilia* homeotic gene[9]. Segment specific combinatorial Hox gene expression specifies each rhombomeres identity. The neural crest cells that migrate and form the bulk of the facial mesenchyme arise from the same axial level of neural tube as the rhombomeres whose neurons will innervate that mesenchyme. As the neural crest migrates from the rhombomeres into specific brachial arches it retains the particular combination or code of Hox gene expression that is characteristic of the rhombomeres from each axial level conveys a unique combinatorial Hox code. However, it is interesting to note that the neural crest destined from the first branchial arch from which the maxillary and mandibular process develop, does not express Hox genes related to the homeotic homeobox[10]. It is the subfamily of the Hox genes, that are expressed, more diverged from the ancestral Hox genes[11].

The Hox gene subfamily that are important in respect to craniofacial development are – Muscle segment (Msx), Distal less (Dlx), Orthodenticle (Otx), Goosecoid (Gsc), Bar class (Barx), paired-related (Prx, SHOT) and Lim homeobox[2]. There is a better appreciation of way in which neural crest cells associations are established with the help of these genes and these interactions are mediated by two groups of regulatory proteins: growth factors (e.g. FGF and TGF α) and so called steroid/ thyroid/retinoic acid superfamily[12,13].

Muscle segment (Msx)

The Msx homeobox gene (Human ANTP class NKL subclass) family plays a crucial role in the development of craniofacial development[6]. The vertebrate Msx genes were initially cloned from mice and were identified as homologous to Drosophila muscle segment homeobox gene (Msh)[14]. Three subtypes are present Msx 1, Msx 2 and Msx 3 (Msx 2PI) in which Msx 1 and Msx 2 are expressed in craniofacial development including the brachial arches especially in the region of epithelial mesenchymal organogenesis including the developing teeth[15]. In studies done in murine, it is found that Msx 1 and Msx 2 are detected in the development and formation of skull and meninges, the digital aspects of the facial primordial, the associated sense organs and the teeth[11,16]. Both the Msx 1 and Msx 2 are expressed in the sutural mesenchyme and duramater but the expression of Msx 1 is present in the postnatal stages of skull morphogenesis as well but the Msx 2 expression is found to get declined[17]. During the tooth development Msx 1 is expressed in the bud stage and in the morphogenetic cap stage. Msx 1 becomes localized in the mesenchymal cells of the dental follicle and the papilla and Msx 2 becomes more expressed in the enamel organ besides expressing in dental papilla and the follicles[18]. It is found that Msx 2 plays role in the expression in the formation of the extracellular matrix and ameloblast differentiation[19]. In the late stage of morphogenesis, Msx 1 expression is clearly absent in root sheath epithelium which may support that Msx does not support root morphogenesis[20]. Msx 1 also plays an important role in the development of the palate specially the anterior portion of the palatal shelves[15]. Wolf-Hirschhorn syndrome (WHS) is a congenital human syndrome resulting from a deletion of Msx1 locus on chromosome 4. It manifests as midline fusion defects, ear defects, supernumery teeth and microcephaly. It may also cause tooth agenesis, nail dysgenesis, mental retardation, cardiac defects and variety of skeletal deformities[21].

Distal-less (Dlx)

Distal-less genes (Human ANTP class NKL subclass)[6] as the name suggest requires for the development of the limbs. There are six known Dlx genes in humans and named as Dlx 1 to Dlx 6. Dlx 3 and Dlx 4 are now renamed as Dlx 7 and Dlx 8. The Dlx genes like the Msx genes are expressed in both migrating neural crest cells and in spatially restricted regions of the first branchial arch. In contrast to the Msx genes, the expression of Dlx 1 and Dlx 2 in the maxillary and mandibular arch mesenchyme is restricted to the proximal regions where the future molar teeth will develop specially for the ectodermal and mesenchymal compartments of the developing tooth[18,22]. Experiments in mice have shown that inactivation of Dlx 5 and Dlx 6 genes results in craniofacial defects including the defect of calvaria (exencephaly), reduction in the size of the eyes, cleft and dysmorphogenesis of the nasal, maxillary and mandibular structures[23].

The activities of the Msx and Dlx genes are mediated by homeoproteins they encode which has the affinity to bind the T-A-A-A-T sequence of the DNA. It is found that the transcriptional of Msx and Dlx proteins have a reciprocal inhibition activity. The repression by Msx is blocked by Dlx proteins whereas the activation of Dlx proteins is inhibited by Msx proteins. This action shows that functional antagonism through heterodimer formation may provide a mechanism for regulating homeoprotein functions[24].

Goosecoid (Gsc)

Goosecoid (Human PRD class) encodes a protein that acts as a transcription factor and was previously isolated from Xenopus[6]. Experiments on mice with targeted deletion of Gsc genes led to many craniofacial defects. Nasal capsules and mandible are found to be affected with defect the Gsc genes. It has also been found that the Gsc genes have some short of autonomous in its function[25]. In other studies Goosecoid seems to be

required only in the later period during embryogenesis because mice devoid of the genes survived but died soon after birth due to severe craniofacial and rib defect. The craniofacial defect included hypoplastic mandible, absence of coronoid and angular process, defects in the maxillary bones, palatine process and pterygoid plates[3,26].

Barx genes

Barx genes (Human ANTP class NKL subclass) consist of transcription factor that exhibits regionalized expression within the ectomesenchyme of the first branchial arch[6]. As tooth development proceeds, Barx expression becomes more localized exclusively to the mesenchymal regions around the developing molars to produce specific folding pattern of the dental epithelium that produce molar cusps[18,27]. It is also found that the Bar class genes are expressed during development of central nervous system and peripheral nervous system. They are expressed in the telencephalon, diencephalon, mesenchephalon, mesencephalon, hindbrain and spinal cord and in the cranial and dorsal root ganglion. Barx1 and Barx2 shows complementary patterns in their expression. Barx1 appears in the mesenchyme of the maxillary and the mandibular process where as Barx2 expresses in the ectodermal lining of these tissues[28]. Therefore Barx1 along with Barx2 have been associated with cleft lip and palate[29].

Lim genes

Lim genes (Human LIM class) have been found to play an important role in the cell type specification and differentiation during embryogenesis[6]. These are found to be related with the expression of the ectomesenchyme of the maxillary and the mandibular process and also suggested to control patterning of the first brachial arch. Experiments have shown that homeodomain proteins of Lim genes are important for craniofacial development and patterning of mammalian dentition[30]. The defect of Lim gene has been shown to result in the formation of cleft palate in experimental animals which has led to the suggestion that isolated nonsyndromic form of cleft palate in humans is caused by these gene defects[31].

Prx genes (Pair related gene)

Prx1 and Prx2 (Human PRD class) are closely related paired class homeobox genes that are expressed in very similar patterns predominantly in mesenchyme[6]. In a study done by Derk Ten Berge on rats, it was seen that mutation of Prx1 and Prx2 genes caused defects in the external, middle and inner ear, reduction or loss of skull bones, a reduced or sometimes cleft mandible, and limb abnormalities[32]. It has been found that Prx1 and Prx2 coordinately regulate gene expression in cells that contribute to the distal aspects of the mandibular arch mesenchyme and that Prx1 and Prx2 play a role in the maintenance of cell fate within the craniofacial mesenchyme[33].

Shot genes (Paired related gene)

Shot genes (Human PRD class) are also closely related paired homeobox genes that are found in two isomers-SHOTa and SHOTb[6]. It is related to the development of aorta, female genitalia, diencephalon, nasal capsules, palate, eyelid and the limbs. Experiments done in mouse shows that defect in SHOT genes may be a candidate for the result of Cornelia de Lange syndrome[34].

Other transcription factors

Sonic Hedgehog (Shh)

Many studies have implicated that Shh plays an important role in regulating the craniofacial morphogenesis. The expression of Shh has been found in various stages of development. This nature of Shh indicates that the normal and abnormal craniofacial patterning is modulated by the expression of this transcription factor. Although most Shh null embryos die early, those that survive to later embryonic stages manifest malformations of the brain, vertebral column and limbs, as well as cyclopia, overall growth retardation and malformations involving the first pharyngeal arch derivatives. Shh is also required in the chondrogenesis in the skull and in the lower face. For example, differentiation of condensation into Meckel's cartilage in the mandible has been shown to be Shh dependant[35]. Shh is found to be expressed in the facial ectoderm. Experiments in which Shh was removed from the frontonasal process by blocking with antibody resulted in the disruption of fusion that led to facial clefting and midfacial hypoplasia[36,37].

Endothelins

Endothelin-1 (ET-1) encodes a vasoactive peptide expressed in vascular endothelial cells and is thought to play a role in the regulation of blood pressure. But experiments on mice with the disruption of ET-1 seem to have no effect on the cardiovascular system but it resulted in the reduction in the size of the tongue, micrognathia, and cleft palate. Targeted disruption of ET-1 in mice produced craniofacial defect similar to the condition in human known as CATCH-22 which is characterized by abnormal facies and cardiovascular defects[38].

Conclusion

It is now clear that the role of homeobox genes in the craniofacial development is immense. Further studies in future may suggest more specific genes. The practical application of this knowledge in the diagnosis and treatment planning will be beneficial. With future advancement in genetic engineering, genetic treatment of craniofacial defects cannot be ruled out.

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