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Role of Genes in Odontogenesis

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Authors' contributions

This work was carried out in collaboration between all authors. Authors RD, UK downloaded the articles an framed the outline of the study. Authors RD, UK, SS and AS wrote the first draft of the manuscript. Author AP managed the literature searches, guided authors RD, UK, SS and AS. The final draft was checked written modified and approved by author AP. All authors read and approved the final manuscript.

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Review Article

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ABSTRACT

With the discovery of the homeobox genes in craniofacial biology researchers across the globe have studied in depth the genetic patterning of the craniofacial region. With respect to craniofacial development –, Barx, Dlx, Gsc, Lim, Msx, Otx, Prx; part of the Hox cluster are important. Barx gene are strongly expressed only in the mesenchyme of the developing molars. Dlx gene expression is noted in the mandibular and maxillary arch ectomesenchyme. Msx genes are expressed in the area of epithelial mesenchymal interactions in the brachial arches in the area of future dentition and also expressed in the formation of skull, facial primordial and sense organs. Msx-1 is seen to be expressed in various stages of tooth formation i.e bud and cap stage of organogenesis. Lim genes which control morphogenesis of the first brachial arch, are expressed in the maxillo-mandibular ectomesenchyme. Prx gene expression is seen in the proximal portion of the mandibular arch. The role of hox genes in the morphogenesis of the jaws and the dentition is immense. Thus it has been proved beyond doubt that the genes have a major role in organogenesis than what human beings have ever envisaged. This review will give the scientific community an overview of all the genes affecting odontogenesis.

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1. INTRODUCTION

The role of homeobox genes in morphogenesis and organogenesis of the craniofacial region has helped us to differentiate the effect of genes and environmental factors [1,2]. These are seen to play a role not only during prenatal or natal period but also during the postnatal period which is strongly under epigenetic control [3-7]. Two third of the genes in humans seem to play an important role in development of craniofacial region.

The ‘‘field’’ and ‘‘clone’’ theories provided models for mechanisms that might be involved in differentiation and patterning of the dentition and are based upon observation and analysis of the human dentitions [8,9]. The detailed cascade of events at the genetic level has been studied extensively [10,11]. Pattern of the craniofacial region is determined majorly by the axial origin of the neural crest cells present within each arch and by regional epithelial mesenchymal interactions mediated by several growth factors like Fibroblast growth factors (FGF), Transforming growth factors, the family of Wnt and Sonic hedgehog [11]. Neural crest specification is precisely monitored by which for regulate downstream target gene expression via the transcription factors. The area of the first branchial arch where teeth are developing contain a homeobox code specific patterning [12,13]. The *‘‘homeobox code’’* controls and expresses regional diversity within the tooth-forming regions of the first branchial arch. Various homeobox-containing genes, such as *Barx, Dlx, Lhx, Msx* and *Pitx* exhibit tempo-spatial expression patterns in the first branchial arch. The *Msx* and *Isl-1* genes are expressed in anterior regions of the first brachial arch where the incisors would develop whereas *Barx, Dlx* and *Pitx* genes are seen only in the proximal areas of the first branchial arch.

In *Msx null* mice, the incisors and molar development is arrested, whereas targeted null mutations in *Barx, Dlx* and *Pitx* result in either an alteration in morphology or agenesis of the molars. The dental placodes development is controlled by ecto-mesenchymal interactions and an array of signaling molecules [14,15]. BMP, FGF and Ectodysplasin (EDA) is necessarty for formation of placode. EDA is responsible for the size of the placode. Suppression of BMP expression can result in transformation of a tooth type(i.e. from incisor to molar). Thus alteration of epithelial-mesenchymal signals results switching of the dentition identity via the the homeobox gene expression. The dentition of rodents varies from that of the humans, as canines and premolars are missing in rodents. Mutations of genes specific to odotogenesis in rodents usually affect all similar type teeth whereas in humans they can affect only specific teeth not necessarily of the same class.

2. ROLE OF BMP IN TOOTH DEVELOPMENT

Various genetic pathways in Drosophila embryogenesis are conserved during vertebrate development. Patterning mechanisms in the fly imaginal disc is reciprocal signaling between secreted growth factors and cell populations. These signaling molecules consist of BMP, Fibroblast growth factors (FGFs), *Hedgehog,* TGF-β families and *Wnt*. We consider the BMPs first as the initial studies had localized BMP-4 as the first signaling molecule in the developing mammalian tooth germ. The BMPs are homodimeric proteins induce bone formation *in vitro* and *in vivo* [16] and consists of eight members, who based upon amino acid similarity are divided into three subclasses. BMP heterodimers such as BMP-2, BMP-4, and BMP-7 mRNA are up-regulated in the developing molar tooth germ, whereas BMP-4 and BMP-7 are expressed in both dental epithelium as well as dental mesenchyme, further complicating the BMP family. BMP-2 and BMP-4 being 95% identical and can interact with any of the two serine threonine kinase Type I receptors *(Alk-3* and *Alk-6)* similar to BMP-7 [17]. ActRII and ActRIIB, Type 11 receptors, can bind both activin and BMP-7. Type I BMP-receptors (*Alk-3)* is expressed in dental epithelium at El 2.5 [18]. Interestingly, dpp plays a vital role in the regulation ectodermal-mesodermal signaling and signaling across various germ layers in Drosophila [19,20]. Knockout lines of BMP-2 [21,22], or BMP-7 [23,24] express major defects during embryogenesis, although the BMP-2 and BMP-4 knockout mice die prior to tooth formation. The expression of BMP-2 and BMP-4, concomitant with *Msx-l* and *Msx-2*, in mouse dentition from E-1O to E-14 has been extensively analyzed wherin BMP-2 expression appears mesially in the molar epithelium [25]. The length of the molar anlage increases during subsequent development and

the length of the BMP-2 is confined to the middle of the epithelial bud and expression domain shortens so that at E13. This indicates a regulatory role and association between BMP-2 and *Msx-2*, expressed in the region of enamel knot. BMP-2 is not expressed in the dental mesenchyme between Ell and E13 as compared to BMP-4which is seen in the dental epithelium and mesenchyme [26]. BMPs are endogenous inducing signals in early tooth patterning and the expression patterns of BMP and *Msx* genes are inter-related, suggestive of a monogenic pathway. Teeth of Bmp2 conditional knock out mice displayed profound phenotypes with asymmetric and malformed incisors as well as abrasion of incisors and molars [26].

3. EXPRESSION OF *SHH* IN EARLY TOOTH PRIMORDIAL

Shh, a member of the hedgehog signalling proteins, has been regulating the polarity of the floorplate, neural tube, somites and limbs [27]. *Shh* null mutant mice die before birth with the presence of extensive defects in the above mentioned areas and are cyclopic [28-30]. *Patched (Ptc)* is a transmembrane protein receptor for the *Shh* ligand that is thought to act with *Smoothened (Smo)* [31,32]. The current model of the Shh signalling pathway is that *hh* binds to *ptc*, which normally represses *smo*, releases this inhibition, thereby allowing *smo* to activate the transcription of downstream target genes via the *cubitus interruptus (ci)* transcription factor [33]. *Ci* is a member of the *Gli* family of zinc finger transcription factors [34] and is essential for development [35]. *Gli-2* and *Gli-3* mutant mice do not survive after birth and have extreme skeletal abnormalities. Loss of *Gli-2* is associated with abnormal development of the neural arches and defects of the palate, teeth, limbs, sternum, vertebral column and the skull [36]. *Gli-3* homozygous mutant mice have craniofacial defects in cranial vault formation, cleft palate and shortening of the tibia. *Shh* is also seen to be expressed in the mesenchyme of dental placode in the developing incisor tooth germ. Ectopic expression of hedgehog activates ectopic *dpp* expression, proposing that Dpp mediates many activities attributed to *hh*. Tiggywinkle, recently identified in zebrafish is the fourth gene in addition to the tree family members of hedgehog *Desert hedgehog* and *Indian hedgehog* (members of Hedgehog family) also exist in vertebrates and are expressed at various sites of epithelial-mesenchymal interactions in the mouse embryo [37]. *Sonic* expression is seen in the incisor tooth- forming regions of dental lamina [38]. Hedgehog directly or indirectly represses patched function thus leading to the activation of *dpp* and *wg* expression [39,40]. Sonic gene expression is rarely seen in the molar germ area of the dental lamina [41]. The enamel knot is presumed to play a vital role in directing cuspal patterning.

4. ROLE OF *HOX* GENES IN TOOTH DEVELOPMENT

Analysis of *Hox* gene expression in embryonic regions which are segmented, has revealed that a "*Hox code*", is responsible for the differentiation as well as patterning of individual rhombomeres [42]. A *Hox* code has also been proposed to account for differences in digital identity along the anteroposterior axis of the developing limb. Patterning the mammalian dentition A *Hox* code might participate in by specifying the positional identities of the individual tooth anlagen along the mesial-distal axis. Different theories have been put forth to understand the existence of heterodonty in the mammalian dentition. One theory states that different types of teeth are determined by morphogenetic field or tooth-forming locations in the dental lamina. [8] whereas another theory suggests a clone of pre-determined ecto-mesenchymal cells to form the pattern of different types of [9].

5. THE ROLE OF *MSX* GENES IN ODONTOGENESIS

Members of *Msx* homeobox gene family expressed at various locations of epithelial-mesenchymal interactions during embryogenesis play an important role in odontogenesis. *Msx-1*-deficient mice exhibit an arrest in odontogenesis at bud stage, while *Msx-2*- deficient mice exhibit late anomalies in tooth development. Yang et al have shown that Smad1/5 are essential for BMP-induced expression of Msx1 in dental mesenchymal cells [43]. There is now compelling evidence that an atypical canonical BMP signaling pathway regulates the expression of *Msx1* which inturn determines the fate of dental mesenchyme during early tooth development [43]. The *Msx* gene family three in number which are physically unattached in the mammalian genome were identified on the basis of homeobox sequence homology to the fruit fly Drosophila *Msh* or *muscle segment homeobox* gene [44-47]. The third murine family member, *Msx-3*, is expressed only in the dorsal neural

tube thus resembling the expression pattern of the prototypical Drosophila *Msh* gene [48,49]. *Msx-l* and *Msx-2* have arisen by two successive gene duplication events, acquiring their organogenic expression properties in the process *and Msx-3* constitutes the prototypical *MSsh* orthologue. It has been shown that in the natal life *Msx-1* and *Msx-2* together are initially expressed in the mesoderm of primitive streak and later in the precardiac regions and neural tube. They both are expressed at almost all the sites of epithelial-mesenchymal tissue interactions during mid-gestation [50-53], including the developing incisor and molar tooth germs [54]. *In situ* hybridization experiments of staged mouse embryos have revealed that *Msx-l* and *Msx-2* are expressed in the developing molar tooth germ in patterns which correlate with discrete morphologic steps in odontogenesis. *Expression of Msx-1* is at its peak during the morphogenetic cap stages, expression of which neutralizes just before the differentiation of the ameloblasts and odontoblasts. Thus it can be concluded that *Msx-1* does not play a role in root morphogenesis in the developing tooth.

Msx-2 is initially expressed in the mesenchyme underneath the area of dental placode formation which resembles an marker for dental initiation. At El 1.5, *Msx-2* is co-expressed with *Msx-l* in the dental mesenchyme. While *Msx-l* is expressed in the mandibular mesenchyme in a mesial-to-distal gradient, *Msx-2* expression is confined to the mesenchyme around the tooth-forming regions. The mesenchymal expression of *Msx-2* is more restricted than that of *Msx-l*. *Msx-2* expression and the array of tooth-initiating signalling arising from the ectomesenchyme directed towards the overlying epithelium coincides with each other. There is early expression of *Msx-2* in the molar epithelium but after E 1I there is no expression in the molar region whereas there is absence of *Msx-2* expression in the diastema region which is later seen at E10. Research suggested that this down-regulation of *Msx-2* mRNA expression in the diastema region could be an evolutionary mechanism for tooth extinction [55]. Thus *Msx-2* expression is seen during the enamel knot the internal enamel epithelium as well as the dental papilla mesenchyme. *Msx-l* expression is seen in the diastemal mesenchyme, the palatal rugae and the developing molar as well as incisor tooth germ. Expression of both *Msx-l* and *Msx-2* seem to be related to each other, dynamic in nature, but with varying patterns of expression during odontogenesis [56].

6. *RUNX-2, OSX, AND DSPP* IN TOOTH DEVELOPMENT

Transcription factor *Runx-2* is essential for odontoblast and osteoblast differentiation and regulates bone as well as tooth-related gene expressions. *Runx-2* expression determines the lineage of odontoblasts as well as osteoblasts from mesenchymal cells [57]. The temporal-spatial *Runx-2* expression cascade during osteogenesis and odontogenesis has been described [58,59]. For example, *Runx-2*-deficient mice showed odontogenesis progressing only upto the cap/early bell stages, whereas *Runx-2* gene mutations displayed dental anomalies in humans, like supernumerary teeth, abnormal tooth eruption, and enamel hypoplasia [60]. Osterix (*Osx* or *Sp7*) is an osteoblast-specific transcription factor which is expressed in mesenchymal cells of the tooth germ [61]. *Osx* knock-out mice have shown that cortical bone and bone trabeculae formation is abolished as well as expression of type I collagen and osteoblast marker genes is reduced in mesenchymal cells in *Osx* null mice. *Osx* transcripts are not detected in skeletal elements of *Runx-2* null mice, indicative that *Osx* acts as a downstream gene of *Runx-2* in the cascade of osteoblast differentiation signaling pathway. The effect of *Osx* on its target genes is involved in various signaling pathways which are independent of *Runx-2* [62]. Although odontoblasts as well as osteoblasts originate from mesenchymal cells having several common characteristics, bone and dentin display variable biological/ physical functions [63]. Differential *Runx-2* expression patterns between osteoblasts and ameloblasts during tooth formation have been observed previously however; the *Osx* expression pattern during odontogenesis has not been described. Furthermore, the complex interactions amongst *Runx-2*, *Osx,* and *Dspp* during odontogenesis and craniofacial osteogenesis remains unclear and unresolved.

During the cap stage (E14), mRNA expression of *Runx-2* was largly expressed in mesenchymal cells in alveolar bone, dental papilla and follicle whereas *Osx* is almost co-expressed in these same areas. *Runx-2* and *Osx* mRNA expression is seen only in the mesenchyme and is barely seen in dental epithelium. In addition, there is no *Dspp* signal in dental and osteogenic mesenchyme. During the bell stage (E16), *Osx and Runx-2* mRNA are expressed in differentiating osteogenic mesenchyme, ameloblasts, odontoblasts and dental pulp cells;

along with a weak *Dspp* signal in odontoblasts, ameloblasts, dental pulp cells and surrounding tissues. At E18, *Runx-2* expression is drastically down-regulated in the odontoblasts, ameloblasts, and dental pulp cells, apart from the cells near the mesenchyme within alveolar bone of the developing incisor and molar. Its signal is apparent in differentiating alveolar bone osteoblasts. *Osx* mRNA expression in the osteoblasts coincided with the *Runx-2* mRNA expression whereas its expression remains intense in odontoblasts. During this stage, the *Dspp* mRNA expressional is clear in differentiating and differentiated odontoblasts (pre-ameloblasts in the incisor and molar). At PN1, *Runx-2* mRNA expression is at a greater level in osteoblasts, but its expression is weak in odontoblasts and dental pulp cells of the developing incisor and molar. At PN5, *Dspp, Osx and Runx-2* mRNA expression patterns are quiet similar to those at PN1. However, the *Osx* mRNA expression is more intense in odontoblasts where *Dspp* mRNA expression is also very high. *Osx* mRNA expression is also seen in bone, cementoenamel junction and roots, concomitant with *Runx-2* expression. Notably, high *Osx* and *Dspp* mRNA expression levels are seen concomitant in odontoblasts at the later stages of tooth development [63].

7. TOOTH PHENOTYPES IN *GLI-2* AND *GLI-3* MUTANTS

Gli-2 null mutants have tooth anomalies which are predominantly related with the maxillary incisors only as the anatomy of the molars is normal and unaffected in the *Gli-2* null mutants. Rarely are the mandibular incisor morphology affected wherein an ectopic mandibular incisor is seen medial to one of the normal incisor germs. *In situ* hybridization with *Msx-1* and *Ptc* showed that the epithelial bud was definitely having an odontogenic potential . The effect of the *Gli-2* null mutation had a variable effect on the maxillary incisors in a few embryos with partial fusion of the two maxillary incisors being the most common phenotype. In three of the *Gli-2* mutant embryos, both maxillary incisors remained in close proximity to each other whereas in another maxillary incisors was missing. On careful analysis of the histology of the single central maxillary incisors at E13.5, it was seen that the incisors had resulted through the fusion of two maxillary incisors and were not mesiodens; the basic histology remained mostly unaffected. The mesenchymal condensations appeared normal at E13.5 whereas the enamel knots are present at E14.5, highlighted by the presence of *FGF-4* and *Shh.* The site of presence of ameloblasts in particular is not normal wheras a mutant maxillary incisor that has not fused but is close together has correct positioning of ameloblasts. Tooth development appeared normal in *Gli-3* null mutants. We examined the Phenotypes in *Gli-2; Gli-3* double mutants, to determine whether there is a functional redundancy of them (*Gli-2* and *Gli-3)* in tooth development. *Gli2-/-; Gli3+/-*mutants had mandibular incisors that were smaller than normal whereas molars and maxillary incisors were absent. At E12.5 two central epithelial thickenings were visible, but these were fused and the development did not occur beyond this stage. Only a few *Gli2-/-; Gli3-/-* mutants could survive up to day E14.5. Observation of one E13.5 and one E14.5 *Gli2-/-; Gli3-/-* embryo showed no visible signs of tooth development beyond a rudimentary bud stage which is equivalent to aprroximately E13.0. In gtC101 background, b-gal staining marked both the epithelial and condensing mesenchymal cells in the developing tooth which was similar to that in wild-type buds suggestive of interactions between the epithelium and mesenchyme. Molar tooth development did not occur in *Gli2-/-; Gli3-/-* embryos suggestive of their degree of invovement than the incisors [64,65].

8. EPITHELIAL-MESENCHYMAL INTER-ACTIONS IN TOOTH DEVELOPMENT OF *GLI-2* AND *GLI-3* MUTANTS

The early interactions between mesenchymal cells and epithelium that are essential for initiation and formation of tooth bud could occur in the mutant embryos; protein expression as well as genes involved in these interactions is as follow: *Lef-1* expression, essential for tooth development, has been shown in epithelial thickenings, expression of *Msx-1* and BMP-2/ 4 in tooth bud mesenchyme is involved in signal transduction whereas expression of *activin* b*A* in mesenchyme prior to epithelial invagination is essential for formation of incisors and molars. Expression of each of these genes in *Gli2-/-;Gli3+/--* embryos is found to be normal [64-66].

9. EXPRESSION OF *SHH* PATHWAY GENES IN *GLI* MUTANTS

The expression of *Gli-1* and *Ptc* are found to be altered considerably in *Gli2-/-*embryos. *Gli-1* expression, at E11.5 and E15.5, is down regulated in the epithelial component of all the

tooth germs, but not in the mesenchymal component. The expression of *Ptc* in *Gli-2* mutants is complicated as that of *Gli-1*. *Ptc* expression is downregulated in the epithelium only at the stages examined except at E13.5-E14. Corresponding parts of *Gli2* mutants hybridised with *Ptc and Gli-1* expressed that *Gli-1* and *Ptc* expression is void from the epithelium in similar areas. *Gli2-/-; Gli3+/-* embryos at E13.5, *Ptc* and *Gli-1* expression is weaker to a slight extent in the epithelium [64-66].

10. CONCLUSION

The entire process of embyogenesis, from the neural crest cell migration and expression of the homeobaox gene is a complex interplay between genetic and epigenetic factors. Induction, patterning and programmed cell death during odontogenesis is under the influence of the cascade of growth factors as well as the regulatory molecules. Thus, genetics play a major role in odontogenesis and in the future a vast plethora of genes would still be researched with the advanced technology of full genome. The ulitization of this knowledge for tissue engineering of teeth in a labortary and implantation in humans cannot be ruled out. This review will give the scientific community an overview of all the genes affecting odontogenesis.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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