Homeobox genes and tooth development: Understanding the biological pathways and applications in regenerative dental science

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\textbf{ABSTRACT}

Objectives: Homeobox genes are a group of conserved class of transcription factors that function as key regulators during the embryonic developmental processes. They act as master regulator for developmental genes, which involves coordinated actions of various auto and cross-regulatory mechanisms. In this review, we summarize the expression pattern of homeobox genes in relation to the tooth development and various signaling pathways or molecules contributing to the specific actions of these genes in the regulation of odontogenesis.

Materials and methods: An electronic search was undertaken using combination of keywords e.g. Homeobox genes, tooth development, dental diseases, stem cells, induced pluripotent stem cells, gene control region was used as search terms in PubMed and Web of Science and relevant full text articles and abstract were retrieved that were written in English. A manual hand search in text books were also carried out. Articles related to homeobox genes in dentistry and tissue engineering and regenerative medicine of odontogenesis were selected.

Results: The possible perspective of stem cells technology in odontogenesis and subsequent analysis of gene correction pertaining to dental disorders through the possibility of induced pluripotent stem cells technology is also inferred.

Conclusions: We demonstrate the promising role of tissue engineering and regenerative medicine on odontogenesis, which can generate a new ray of hope in the field of dental science.

1. Introduction

The tooth is a specialized structure of the craniofacial skeleton, the development of which is an excellent organogenesis model, which entails significant insights into the molecular mechanisms involved in odontogenesis. Understanding the origin of tooth development and genetic pathways at various developmental stages regulating the differentiation and patterning of tooth, in an evolutionary sense of human embryogenesis is an interesting subject. Homeobox genes (HMG), the master regulator might provide an appealing starting point in gaining the knowledge on this direction as they play a pivotal role in the regionalization of the body plans in all bilaterally symmetric animals (Prince, 2002). HMG contain a small conserved regions of DNA consisting of 180 nucleotide base pair (bp) and function by producing proteins that bind to the DNA of downstream genes, thereby regulating their expression (Burglin & Affoiter, 2016; Gehring, 1985). Therefore,
these unique families of developmental genes (HMG) provide the springboard of all subsequent advances in the field of developmental biology (Hrycay & Wellik, 2016; Sharpe, 2007). Transcriptional activities of these genes during morphogenesis regulates the multiple germ layers to coordinate the cell division and other cellular functions such as; cell migration, differentiation, proliferation, and apoptosis through the process of cell to cell communication (Sanchez-Herrero, 2013). The HMG genes are involved in the developmental patterning, which is achieved by the combination of expression of a set of HMG genes (Hox code). The evolution of jaw-bearing gnathostomes form jawless fish (agnathans) was achieved by divergent expression of different set of HMG genes from the Hox code in the first brachial arch (Cohn, 2002). These different set of HMG genes are involved in the development of head of gnathostomes and in dental patterning. Sequences of events occur during dental patterning, which begins with the regional determination of dentition as a whole in maxillary and mandibular jaws (Butler, 1939). This is followed by, patterning at sub-regions of tooth families including; incisors, canine, premolar and molar regions and ultimately the morphologies of individual teeth within each tooth families are formed (Suryadeva & Khan, 2015; Weiss, 2003). Indeed, a number of HMG that act at specific differentiation process have been well implicated (Bei, 2009; Zhang, Lan, Chai, & Jiang, 2009). However, the role of a specific homeobox gene in human dental patterning is rather difficult to consider, basically because of their complex mechanism of action, which are interlinked with other HMG. Nonetheless, characterizing the downstream targets of these genes could provide some insights into their role on regulatory mechanisms.

In the field of dental regenerative medicine homeobox gene therapy could be effectively utilized for regenerating dental tissues or rejuvenating the existing damaged ones using stem cells through tissue engineering approaches. In this regard depending on the clinical requirements, regenerative dentistry focuses on using stem cell properties in consolidation with tooth organogenesis by utilizing high throughput technologies at levels of genomics, transcriptomics, metabolomics, epigenetics and proteomics to manipulate stem cells to become odontogenic fate (Srijaya, Sandhya, Shigeki, & Noor Hayaty, 2016). In such situations, the clinical feasibility of stem cells is best utilized by engineering cells with gene expression cassettes under in vitro conditions. Accordingly for tooth regeneration or repair, homeobox gene expression cassettes can be considered for regulating downstream phase-specific promoters or by delivering Hox proteins which proves to be beneficial (Kachgal, Mace, & Boudreau, 2012).

Hence, our aim is to provide a detail insight into the functional array of genetic switches and the regulatory roles and mechanism of HMG in tooth development. This review will thus be helpful in understanding the highly dynamic changes occurring during the embryonic development of dental patterning in humans, associated with homeobox gene code or clusters and thereby unravel the relevance of these genomic clusters for the proper establishment of tooth structures. Moreover, we have attempted to undermine the gene mutations in-}

2. Evolution and organization domain of homeobox genes

The HMG were originally discovered in the fruit fly (Drosophila melanogaster) and subsequently they were identified in all metazoan ranging from sponges to vertebrates (Barucca, Canapa, & Biscotti, 2016; Capovilla, Brandt, & Botas, 1994; Carroll, 1995; Lewis, 1978; Sivanantharajah & Percival-Smith, 2015) and were also reported in plants and fungi (Jacob & Monod, 1961; Pruner & Meyerowitz, 2016; Sheridan, 1988). At first their conserved sequence element of 180 bp was named as H repeats as they had low level repeat of bases and these were used to isolate other homeotic genes (the functional gene sequence of homeobox gene motif) from random population of DNA segments in DNA libraries. Hence, those H repeats were named as homeobox (Sharpe, 2007). Meanwhile, the term ‘box’ refers to the fact that cross-homology is limited to a short and highly conserved DNA segment, encoding 60 amino acid protein domains called homeodomains (Akin & Nazarali, 2005). The homeomain was first identified in those proteins whose absence or misregulation caused homeotic transformation in Drosophila (Peifer & Wieschaus, 1990; Sivanantharajah & Percival-Smith, 2015). It is a form of homeotic mutation that led to the transformation of one body structure by another, which is normally located elsewhere (Barucca et al., 2016). In human genome, the HOX-families are located in chromosomes 2, 7, 12, and 17 and are called HOXA, HOXB, HOXC, and HOXD, respectively (Quinonez & Innis, 2014). The Hox-families are located in the chromosomes 6, 11, 15, and 2 in the mouse genome and are represented in the same way as the human families.

To note, there is a relationship between the genomic organization of HMG, their expression and biological functions (Chapman et al., 1997). A peculiar genomic organization is closely associated with a regulatory process referred to as ‘collinearity’, where a correlation exists between the order of these genes within each cluster, and a series of their expression pattern happen along the anterior–posterior embryonic axis. This property is referred to as spatial collinearity (Barucca et al., 2016; Gaunt, 2015) indicating that animals displaying highly divergent morphologies also rely on the same genetic systems to pattern their body plans (Barucca et al., 2016; Gaunt, 2015; Montavon & Duboule, 2013). Thus when the developmental biology of an organism is considered, whether it be on the basis of morphogenesis, patterning or differentiation level, all of them rarely seems to escape the reach of HMG (Holland, 2013). Nevertheless, these genes can be considered as the master control genes or transcriptional regulators that can act at the top of genetic hierarchies thereby regulating the aspects of morphogenesis, cell differentiation and patterning (Akam, 1998; Lappin et al., 2006; Suryadeva & Khan, 2015).

3. Is homeobox gene function for dental patterning an informational array?

During embryonic development, a properly coordinated expression of the genes and genetic pathways within their different genomic clusters is critical for patterning the body plans (Prince, 2002; Suryadeva & Khan, 2015). The enticing correspondence between the topological organization of Hox clusters and their transcriptional activation in space and time has served as a paradigm for understanding the relationships between genome structure and function (Montavon & Duboule, 2013). The HMG as we know them today have the properties of fascinating mechanisms of ‘self regulating’ their protein products (Barber & Rastegar, 2010; Schughart, Kappen, & Ruddle, 1988; Sheth, Bastida, Kmita, & Ros, 2014). For instance, Hox proteins exert their regulatory role through specific targets that are involved in organogenesis, cellular differentiation, adhesion, migration, cell cycle and apoptosis (Barber & Rastegar, 2010; Sheth et al., 2014). Several homeodomain proteins can bind to specific regulatory sites in its own cis-flanking regions and these genetic circuits can act like feedback loops (Schughart et al., 1988). Further if the gene undergoes duplication on account of animal diversity or evolution (Schughart et al., 1988), a more complex switching apparatus is created with auto-regulation and cross-regulation properties (Barber & Rastegar, 2010; Schughart et al., 1988). Hence it is a fascinating aspect in developmental biology, how from a relatively small number of conserved HMG and signaling pathways, it is possible to generate an array of organs with a wide range of shapes, tissue organization, and functions. The dental structures and its distinct specific tooth types represent a valuable system to address the issues of differential molecular signatures of such conserved code in tooth development.
Cells constituting the tooth are mostly derived from two embryonic sources: ectodermal epithelium and cranial neural crest derived ectomesenchyme (Koussoulakou, Margaritis, & Koussoulakos, 2009; Kauka et al., 2014; Shiyama, Yanduri, Girish, & Murgod, 2015). The ectodermal component gives rise to ameloblasts that form the enamel of the tooth crown and the ectomesenchyme mediates the odontoblasts and cementoblasts to form the pulp, dentin, cementum and periodontal ligament of the teeth (Beı, 2009; Koussoulakou et al., 2009). In mammals during craniofacial development, teeth develop on the fronto-nasal process, mandibular process and proximal maxillary process (Cobourne & Sharpe, 2003). Cell-labelling studies have demonstrated that the fronto-nasal process is formed by the neural crest cells (NC), which are derived from the mid-brain and forebrain regions. However, the first branchial arch is populated by NC cells that have migrated from the posterior midbrain and anterior hindbrain (Chai et al., 2000; Cobourne & Sharpe, 2003). The first branchial arch gives rise to the maxillary and mandibular processes. Studies have demonstrated that NC cells as the source of instructive patterning which influences the unique developmental fate of branchial arches. It is also believed that these cells prevails more considerable plasticity in providing patterning clues (Biben, Hatzistavrou, & Harvey, 1998; Sandell & Paul, 2013). Hence in dental context the proper migration of neural crest cells during embryogenesis is essential for the development of teeth.

4.1. Homeobox genes involved in the establishment of oral-aboral axis

Tooth development in mouse is widely used as a model to investigate the various molecular mechanisms involved with the odontogenesis. The tissue recombination trials using mouse models have shown that the oral epithelium isolated from the mandibular arch of a mouse embryo between embryonic day 9 and 11.5 (E9-E11.5) can induce non-dental, neural crest derived mesenchyme to form a tooth (Hashmi, Mammo, & Inger, 2014; Mina & Kollar, 1987). The early mesenchymal markers for tooth formation are LIM-homeobox (Lhx) domain genes Lhx6 and Lhx7 (Denaxa, Sharpe, & Pachnis, 2009; Grigoriou, Tucker, Sharpe, & Pachnis, 1998). These genes are expressed as early as E9 in the ectomesenchyme of oral portion of the first branchial arch (Grigoriou et al., 1998; Matsumoto et al., 1996). The prime-signaling molecule for inducing the Lhx gene expression is the fibroblast growth factor 8 (Fgf8), which further determines the establishment of proper oral-aboral axis (Denaxa et al., 2009). The Lhx gene is normally not expressed in the second branchial arch mesenchyme. However, induced expression of these genes can be attained in the second branchial arch mesenchyme by infusing epithelium from the oral surface of the first branchial arch (Grigoriou et al., 1998). Thus, it indicates that the oral-aboral polarity is achieved by the local interaction between epithelium and the mesenchyme rather than unfolding of a developmental program inherent in the neural crest (Morita et al., 2016; Sharpe, 2007). Fgf8 acts as the endogenous inducer of both Lhx6 and Lhx7 expression (Tucker, Yamada, Grigoriou, Pachnis, & Sharpe, 1999). Homozygous Lhx6 mutant mice show no craniofacial defects whereas 70% of the homozygous Lhx7 mutant mice show palatal defects (Zhao et al., 1999). Lhx6 and Lhx7 are also expressed during odontogenesis, which will be discussed later.

Goosecoid (Gsc) is another homebox gene expressed within the ectomesenchyme of the first branchial arch from E10.5 (Cobourne & Sharpe, 2003). It consists of two genes Gsc and Gsc2 in humans (Yamada et al., 1995). Unlike Lhx6 and Lhx7 genes expression, which is restricted to the oral half of the ectomesenchyme of the first branchial arch, the Gsc expression takes place only in the aboral half of the first branchial arch (Cobourne & Sharpe, 2003). Consistent with this restricted expression, tooth defects may be revealed only when targeted mutation in Lhx6 and Lhx7 are combined. Similarly, mutations of either of Gsc genes have only skeletal abnormalities but exert normal tooth development (Yamada et al., 1995).

Interestingly, Fgf8 has an indirect role in defining the expression of Gsc in the first branchial arch via induction of Lhx6 and Lhx7, which in turn repress the expression of Gsc to the aboral axis. Although the mechanism that restricts Lhx6/7 expression to oral mesenchyme is independent of Gsc and is more probably related to the distance from the source of Fgf8 (Cobourne & Sharpe, 2003), but the Gsc expression is further lost when Endothelin1 (End1) signaling from mandibular arch epithelium is disrupted (Tucker et al., 1999). End1, which is expressed throughout the mandibular arch ectoderm, mediates the mandibular development by binding with the Endothelin receptor type A (ETA), which is expressed throughout the ectomesenchyme of the mandibular arch. Targeted mutation in End1 and its ETA receptor have a mandibular phenotype similar to that of Gsc mutant affecting skeletal patterning but not tooth development (Tucker et al., 1999). Thus, it was previously proposed that Lhx and Gsc genes were responsible for the establishment of oral-aboral axis. However, double mutants of Lhx6 and Lhx7 show no change in patterning of the first branchial arch as there are no ectopic bone formation seen in the tooth region and moreover there are normal expressions of other markers seen in the ectomesenchyme (Gsc). However, there is correct development of incisors in the proximal region of both maxilla and mandible. These results suggest that there may be other factors other than Lhx6 and Lhx7 responsible for establishment of oral-aboral axis (Denaxa et al., 2009).

4.2. Homeobox genes involved in the tooth germ position

After the establishment of the oral-aboral axis in the first branchial arch, the oral epithelium thickens to produce the correct number of tooth germs in the correct position along the oral surface. The tooth buds form in a row from the dental lamina, which forms the future dental arch on E11. Paired box 9 gene (Pax9) gene, acts as an early mesenchymal marker for determining the exact location/site for tooth germ appearances, which is induced by Fgf8 but is repressed by Bone morphogenetic protein 2 and 4 (Bmp2 and Bmp4) (Hofmann, Drosopoulos, Mcmahon, Balling, & Tickle, 1998; Hlouskova et al., 2015; Stockton, Das, Goldenberg, D'Souza, & Patel, 2000). Thus, Fgf8, Bmp2 and Bmp4 are expressed in non-overlapping areas of oral epithelium, where the Pax9 gene expression is facilitated at sites of Fgf8 presence (Lainoff et al., 2015). The position of teeth is determined by a combination of two different types of signaling molecules, which is produced in wide but overlapping domains, rather than by a single localized inducer molecule (Lainoff et al., 2015; Neubüser, Peters, Balling, & Martin, 1997). Pax9 mutation results in lack of teeth entirely due to arrest of tooth formation in the early bud stage indicating that this gene is required for the tooth analogue to develop further (Peters, Neubüser, Kratowchwil, & Balling, 1998).

Paired-like homeodomain transcription factor 2 (Pitx2) is a key player in cell fate determination during odontogenesis (Venugopalan et al., 2008; Zhang et al., 2013; Zhu et al., 2013). Pitx2 is one of the earliest markers of tooth development and is expressed in the proximal and distal stomodeal ectoderm of both mandibular and maxillary processes, before any sign of odontogenesis (Duvenger & Morasso, 2009). Later, Pitx2 expression is localized at the sites of tooth formation and remains in the dental epithelium throughout tooth development (Duvenger & Morasso, 2009). The expression of Pitx2, is regulated positively by Fgf8 and negatively by Bmp4, in the presumptive dental epithelium. The lack of the Pitx2 gene activity in mice leads to a down-regulation of Fgf8 in the dental epithelium, indicating a positive feedback loop between Pitx2 and Fgf8 (Zhang, Chen, Song, Liu, & Chen, 2005). The failure to express Pitx2 results in Axenfeld-Reiger syndrome, a condition associated with tooth hypoplasia and hypodontia (Semina et al., 1996) (Table 1). Pitx2 also induces expression of Lhx6 and Lhx7 in turn represses Pitx2 (Zhang et al., 2013).
Table 1

Mutations of mice and human odontogenic related homeobox genes causing dental defects identified in knockout mouse models.

<table>
<thead>
<tr>
<th>Genes in Mice</th>
<th>Mutant</th>
<th>Tooth phenotype</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lhx6 (Chromosome 2: NC_000068.7 (36081950.36105998, complement))</td>
<td>Null</td>
<td>No tooth defect described</td>
<td>Zhao et al. (1999), Liodis et al. (2007)</td>
</tr>
<tr>
<td>Lhx6 (Chromosome 2: NC_000068.7 (36081950.36105998, complement))</td>
<td>Homozygous Lhx6 mutant</td>
<td>No craniofacial defects</td>
<td>Zhao et al. (1999)</td>
</tr>
<tr>
<td>Lhx7 (Chromosome 3: NC_000069.6 (154306288.154330560, complement))</td>
<td>Null</td>
<td>Palatal defects</td>
<td>Zhao et al. (1999)</td>
</tr>
<tr>
<td>Lhx6 and Lhx7</td>
<td>Double mutant</td>
<td>Initiation stage arrest</td>
<td>Grigoriou et al. (1998)</td>
</tr>
<tr>
<td>Lhx6 (Chromosome 2: NC_000068.7 (36081950.36105998, complement))</td>
<td>Double mutant</td>
<td>Initiation stage arrest</td>
<td>Grigoriou et al. (1998)</td>
</tr>
<tr>
<td>Lhx7 (Chromosome 3: NC_000069.6 (154306288.154330560, complement))</td>
<td>Double mutant</td>
<td>No tooth defects but skeletal defects present</td>
<td>Yamada et al. (1995)</td>
</tr>
<tr>
<td>Gsc and Gsc2</td>
<td>Double mutant</td>
<td>Development of maxillary molar teeth stops at lamina stage</td>
<td>Thomas et al. (1997), Qiu et al. (1999)</td>
</tr>
<tr>
<td>Pax9 (Chromosome 12: NC_000078.6 (5691679.56712824, complement))</td>
<td>Null</td>
<td>Bud stage arrest</td>
<td>Peters et al. (1998)</td>
</tr>
<tr>
<td>Pitx2 (Chromosome 3: NC_000069.6 (129199878.129219594, complement))</td>
<td>Null</td>
<td>Bud stage arrest</td>
<td>Peters et al. (1998)</td>
</tr>
<tr>
<td>Barx1 (Chromosome 13: NC_000079.6 (48663057.48665507, complement))</td>
<td>Null</td>
<td>Bud stage arrest</td>
<td>Peters et al. (1998)</td>
</tr>
<tr>
<td>Dlx1 (Chromosome 2: NC_000068.7 (71528113.71533981, complement))</td>
<td>Null</td>
<td>Normal tooth development</td>
<td>Liu, Selever, Lu, and Martin (2003)</td>
</tr>
<tr>
<td>Dlx2 (Chromosome 2: NC_000068.7 (71543408.71546754, complement))</td>
<td>Null</td>
<td>Normal tooth development</td>
<td>Liu et al. (1999), Lu, Pressman, Dyer, Johnson, and Martin (1999)</td>
</tr>
<tr>
<td>Dlx1 and Dlx2</td>
<td>Double mutant</td>
<td>Development of maxillary molar teeth stops at lamina stage</td>
<td>Thomas et al. (1997), Qiu et al. (1997)</td>
</tr>
<tr>
<td>Dlx5 (Chromosome 6: NC_000072.6 (6877801.6882068, complement))</td>
<td>Null</td>
<td>Bud stage arrest. Lethal (after birth) – Molar development stops at bud stage while incisors development stops at lamina stage</td>
<td>Satokata and Maas (1994)</td>
</tr>
<tr>
<td>Mx1 (Chromosome 5: NC_000071.6 (37820491.37824585, complement))</td>
<td>Null</td>
<td>Malformation of mandibular and maxillary molars</td>
<td>Acampora et al. (1999), Depew et al. (1999)</td>
</tr>
<tr>
<td>Mx1/Msx2</td>
<td>Double mutant</td>
<td>Development of all teeth but abnormal morphology of mandibular molars</td>
<td>Acampora et al. (1999), Depew et al. (1999)</td>
</tr>
<tr>
<td>Mx2 (Chromosome 11: NC_000077.6 (95120117.95125293, complement))</td>
<td>Null</td>
<td>Bud stage arrest. Lethal (after birth) – Molar development stops at bud stage while incisors development stops at lamina stage</td>
<td>Satokata et al. (2000), Duverger and Morasso (2009)</td>
</tr>
<tr>
<td>Msx1 (Chromosome 5: NC_000071.6 (37820491.37824585, complement))</td>
<td>Double mutant</td>
<td>Development of all teeth but abnormal morphology of mandibular molars</td>
<td>Mitsiadis and Drouva (2008)</td>
</tr>
<tr>
<td>Msx1 (Chromosome 5: NC_000071.6 (37820491.37824585, complement))</td>
<td>Null</td>
<td>Bud stage arrest. Lethal (after birth) – Molar development stops at bud stage while incisors development stops at lamina stage</td>
<td>Satokata et al. (2000), Duverger and Morasso (2009)</td>
</tr>
<tr>
<td>Msx2 (Chromosome 13: NC_000079.6 (5346681.53472780, complement))</td>
<td>Null</td>
<td>Bud stage arrest. Lethal (after birth) – Molar development stops at bud stage while incisors development stops at lamina stage</td>
<td>Satokata et al. (2000), Duverger and Morasso (2009)</td>
</tr>
<tr>
<td>Mx1/Msx2</td>
<td>Double mutant</td>
<td>Development of all teeth but abnormal morphology of mandibular molars</td>
<td>Mitsiadis and Drouva (2008)</td>
</tr>
<tr>
<td>Msx1 (Chromosome 5: NC_000071.6 (37820491.37824585, complement))</td>
<td>Double mutant</td>
<td>Development of all teeth but abnormal morphology of mandibular molars</td>
<td>Mitsiadis and Drouva (2008)</td>
</tr>
<tr>
<td>Msx2 (Chromosome 13: NC_000079.6 (5346681.53472780, complement))</td>
<td>Null</td>
<td>Bud stage arrest. Lethal (after birth) – Molar development stops at bud stage while incisors development stops at lamina stage</td>
<td>Satokata et al. (2000), Duverger and Morasso (2009)</td>
</tr>
<tr>
<td>Ptx1 (Chromosome 13: NC_000079.6 (55825044.55836193, complement))</td>
<td>Double mutant</td>
<td>Development of all teeth but abnormal morphology of mandibular molars</td>
<td>Mitsiadis and Drouva (2008)</td>
</tr>
<tr>
<td>Dlk3 (Chromosome 11: NC_000077.6 (95120117.95125293, complement))</td>
<td>Null</td>
<td>Bud stage arrest. Lethal (after birth) – Molar development stops at bud stage while incisors development stops at lamina stage</td>
<td>Satokata et al. (2000), Duverger and Morasso (2009)</td>
</tr>
<tr>
<td>Dlx1 (Chromosome 2: NC_000068.7 (71528113.71533981, complement))</td>
<td>Null</td>
<td>Bud stage arrest. Lethal (after birth) – Molar development stops at bud stage while incisors development stops at lamina stage</td>
<td>Satokata et al. (2000), Duverger and Morasso (2009)</td>
</tr>
</tbody>
</table>
### 4.3. Homeobox genes involved in establishment of dental axis

The jaws have a proximal-distal dental axis where the molars develop proximally and the incisors distally. Two models explain tooth patterning i.e. the determination of the tooth type along this axis (a) Clone model states the clone of ectomesenchymal cells are programmed by epithelium to produce the tooth type. However, the cranial neural crest cells populate the first branchial arch are not pre-specified with respect to either proximal-distal position or the odontogenic fate. Recombination experiments of the oral epithelium and ectomesenchyme have showed that if recombination is carried out before E10 the epithelium determines the tooth type whereas if it is carried out after E10.5 the ectomesenchyme determines the tooth type. (b) Field model proposes that there is a regional specified pattern of expression of ectomesenchymal genes in graded fields. At E10, different HMG are expressed in distinct domains in the ectomesenchyme along the dental axis. The Branchial arch homeobox (Barx) family consists of two genes: Barx1 and Barx2 (Cobourne & Sharpe, 2003). The Barx1 homeobox gene is expressed in the proximal mesenchyme of the maxillary and mandibular process where molars develop and later its expression becomes restricted specifically to the mesenchyme of molars, while incisors develop in Barx1 negative areas (Cobourne & Sharpe, 2003). The specificity of Barx1 expression domains in the molar mesenchyme is due to the antagonistic effects of Bmp4 and Fgf8 from the overlying oral epithelium (Zhang et al., 2005). Another set of HMG, Distal-less 1 and 2 (Dlx1 and Dlx2) are co-expressed in a smaller domain of the presumptive molar region (Tucker & Sharpe, 1999). Muscle segment homebox gene (Msx) family is orthologous to the Msh gene of Drosophila, which consists of three genes: Msx1, Msx2 and Msx3 (Davidson & Hill, 1991). Aristaless-Like Homeobox gene family consist of three genes Alx1, Alx3 and Ax14 (McGonnell et al., 2011). Alx3 and Msx1 are expressed in the presumptive incisor region where Barx1 is completely absent (Tucker & Sharpe, 1999).

Transformation of tooth type has been demonstrated by manipulating the homebox gene expression in the presumptive incisor forming ectomesenchyme of the mandibular process. Inhibiting Bmp4 in the distal region of mandibular arch (presumptive incisor region) extends the expression domain of Barx1 and downregulates endogenous expression of Msx1 (which is normally induced by Bmp4 in the overlying ectoderm) results in the formation of molar teeth rather than incisors (Tucker, Khamis, & Sharpe, 1998). Therefore, the cells expressing Alx3 and Msx1 but not Barx1 and Dlx are directed to follow the incisor pathway while cells expressing Barx1 and Dlx but not Alx3 and Msx1 will follow molar pathway. Hence there is no one specific gene responsible for each tooth shape (Sharpe, 2001). The absence of a gene is as important as its presence (Thomas et al., 1997). As the different homeobox gene expressions are overlapping it can result in wide range of subtle differences in the tooth shape. This is important in peripheral regions of overlap between teeth of different classes, which are vulnerable to congenital absence (e.g. upper lateral incisors, lower second premolars and third molars) (McCullum & Sharpe, 2001).

Insulin gene enhancer protein (Isl1), another member of the LIM homeobox family is specifically expressed in developing incisors, at both mandibular and maxillary processes. Isl1 expression is restricted to the dental epithelium throughout the tooth development and a strong expression is maintained in differentiated ameloblasts (Duverger & Morasso, 2009; Mitsiadis, 2003). It has been reported that Bmp4 is needed for incisor formation, while Fgf8 is responsible for the molar tooth (Mitsiadis, 2003). Thus it explains the coincident expression of Isl1 in presumptive incisor epithelium along with Bmp4. Further, Isl1 plays an important role in regulating distal gene expression during tooth development. Loss of Isl1 in distal epithelium inhibits Bmp4 expression which in-turn results in corresponding loss of Msx1 expression in mesenchyme. This indicates that a positive regulatory loop exists between Isl1 and Bmp4 in distal epithelium affecting the underlying Msx1 expression (Mitsiadis, 2003).
4.4. Homeobox genes involved in the development of maxillary and mandibular molars

Members of the Distal-less (Dlx) family of homeobox transcription factor are also involved in tooth morphogenesis (Duverger & Morasso, 2009). In mammals, there are six Dlx genes organized into three closely linked pairs of inverted, convergently transcribed genes: Dlx1-2, Dlx3-4 and Dlx5-6 with each pair having similar domain of expression (Duverger & Morasso, 2009; Morasso & Radoja, 2005). The Dlx genes are expressed along the proximo-distal (rostro-caudal) axis of the branchial arch in a nested pattern. Prior to tooth formation, Dlx1 and Dlx2 are co-expressed in the proximal mesenchyme of both mandibular and maxillary processes where molars will form, while Dlx2 is expressed in the distal epithelium of the maxillary process where incisors develop (Duverger & Morasso, 2009; Suryadeva & Khan, 2015; Thomas, Rorteus, Rubenstein, & Sharpe, 1995; Thomas et al., 1997; Thomas, Liu, Rubenstein, & Sharpe, 2000). Epithelial Fgf8 and Bmp4 induce the expression of Dlx1 and Dlx2 during initiation of tooth development (Jheon, Seidel, Biehs, & Klein, 2013; Suryadeva & Khan, 2015). Bmp4 induction of Dlx2 expression in epithelium does not require the presence of mesenchyme, which imply that this signaling regulatory pathway acts within epithelial region (Thomas et al., 2000). However, for the mesenchymal expression a different mechanism comes to play which is regulated by Fgf8 expressed in overlying epithelium. On the other hand, Fgf8 inhibits expression of Dlx2 in the epithelium by a signaling pathway that requires the mesenchyme, which suggests the requirement for epithelial-mesenchymal signaling pathways for inhibition of Dlx2 in the epithelium (Thomas et al., 2000). Hence, Bmp4 acts via an intraepithelial signal, whereas Fgf8 provide the mechanism for maintaining the exact epithelial or mesenchymal Dlx2 expression.

However, at the same stage, Dlx5 and Dlx6 are expressed in the proximal mesenchyme of the mandibular process but not in the maxillary process (Duverger & Morasso, 2009; Qiu et al., 1997). However, at the later stage, Dlx genes are expressed in all developing teeth, with a very complex expression pattern. Although Dlx1 is exclusively located in the dental mesenchyme of the developing tooth, Dlx2 is associated with a very dynamic expression pattern in both the dental mesenchyme and the dental epithelium. Whereas, Dlx5 and Dlx6 are restricted to the proximal mesenchyme of the mandibular process before the first signs of tooth formation, but later are expressed in the dental mesenchyme of all teeth (Zhao, Stock, Buchanan, & Weiss, 2000; Duverger & Morasso, 2009).

In transgenic mice with null mutations in either of genes (Dlx1−/− or Dlx2−/−) showed normal teeth development whereas, in double mutant mice (Dlx1−/− and Dlx2−/−) where there is loss of function of both the genes, the developing maxillary molars are arrested at the epithelial thickening stage and does not progress to the bud stage (Duverger & Morasso, 2009; Qiu et al., 1997; Thomas et al., 1997), but the development of incisors and mandibular molars were normal (Duverger & Morasso, 2009; Sharpe, 2007). These results conclude that the Dlx1 and Dlx2 genes are required for the development of the upper molars and not the incisors and lower molars. This also proves that there is functional redundancy present between Dlx1 and Dlx2 for the development of upper molars as all teeth develop normally in mice with null mutation of either of the genes (Dlx1−/− or Dlx2−/−) (Duverger & Morasso, 2009; Sharpe, 2007).

Other genes that are expressed in the presumptive molar mesenchyme are Barx1, Lhx6 and Lhx7. The Lhx6 and Lhx7 are also expressed in the incisor mesenchyme (Denaxa et al., 2009; Hluskó, Sage, & Mahaney, 2011; McColm & Sharpe, 2001). In Dlx1 and Dlx2 double mutants the expression of Barx1 and Lhx7 are absent in a small group of cells underlying the epithelial thickening in the molar region of the maxilla but are normally expressed in the mandible (Hluskó et al., 2011; Sharpe, 2007). Further, Sex determining region Y-box 9 (Sox9) an early chondrogenic marker is expressed in the Barx1 and Lhx7 negative cells of the maxilla at E10.5 (Thomas et al., 1997). This defect in mesenchyme results in reprogramming towards chondrogenic fate instead of odontogenic due to the loss of the Dlx1 and Dlx2 gene functions (Thomas et al., 1997). Thus, the Dlx1 and Dlx2 genes play a significant role in specification of neural crest derived mesenchymal cell population as odontogenic lineages.

It is very interesting that although Dlx1 and Dlx2 are expressed in both mandibular and maxillary teeth, Dlx1/2 deletion affects specifically the teeth of the maxillary jaw. One explanation for the absence of defects in mandibular molars is the compensation by Dlx5 and Dlx6, which are expressed in the mandible but not in the maxilla prior to tooth formation (Duverger & Morasso, 2009; Thomas et al., 1997) whereas, in a Dlx 5/6−/− mutant, a homeotic transformation of the lower jaw into the upper jaw occurs due to the presence of Dlx1 and Dlx2 expression in the mandibular mesenchyme (Duverger & Morasso, 2009; Nanci, 2008; Thomas et al., 1997). The dental phenotype of Dlx5−/− mice, which have multiple defects in craniofacial structures, has not been extensively described (Acampaora et al., 1999; Depew et al., 1999; Duverger & Morasso, 2009). One of the two studies on Dlx5−/− mice reports that, although all teeth were formed, both maxillary and mandibular molars are malformed and have poorly mineralized crowns while both sets of incisors are shortened and misshapen (Depew et al., 1999; Duverger & Morasso, 2009).

Pitx1 is expressed in the ectomesenchyme and dental epithelium of developing incisors and molars, but exclusively in the mandible (Duverger & Morasso, 2009). Strikingly, the Pitx1 expression persists in the dental epithelium throughout all stages of odontogenesis. At the bud and cap stage, Pitx1 expression is seen in all cell layers of the dental epithelium except in the enamel knot (Mitsiadis & Drouin, 2008). Whereas, at E9.5 and E10 stage strong Pitx1 expression persisted in the mandibular mesenchyme. The Pitx1 has a synergistic interaction with other transcription factors such as T-box transcription factor 1 (Tbx1) and Barx1 (Mitsiadis & Drouin, 2008). In Pitx1−/− mutant mouse embryo of E17.5 stage, the Barx1 expression is decreased in the mesenchyme of the mandibular molars while its expression is normal in the mesenchyme of maxillary molars causing abnormal tooth morphology of the mandibular molars (Mitsiadis & Drouin, 2008). Thus, it explains that the odontogenic specification of the neural crest derived mesenchymal cells may be partly controlled by Pitx1 in the mandibular molar region, suggesting a different genetic pathway existing between the maxillary and mandibular molars specification.

4.5. Homeobox genes involved in tooth initiation

4.5.1. Dental lamina (E11.5)

After E11.5, the induction ability of oral epithelium is lost and the odontogenic potential shifts from the oral epithelium to the mesenchyme, which induces tooth formation when combined with a non-dental epithelium. The tooth development begins morphologically at E11.5 in the mouse embryo with a thickening of the dental epithelium to form the dental lamina (Fig. 1) (Bei, 2009). The epithelial Fgf8 triggers Msx1 and Bmp4. Msx1 induces expression of Bmp4 in the ectomesenchyme (Bei & Maas, 1998; Vainio, Karanovanova, Jowett, & Thesleff, 1993) whereas Fgf8 induces the expression of Activin βA (Ferguson et al., 1998). This is followed by the initiation of bud formation of the epithelium at the sites of teeth around E11.5. Msx1 expression is critical for the proliferation and fate determination of the cranial neural crest cells (Duverger & Morasso, 2009). It is expressed in the mesenchyme of all teeth, from the dental placode stage to the early bell stage (Duverger & Morasso, 2009; MacKenzie, Leeming, Jowett, Ferguson, & Sharpe, 1991). Further Bmp4 also can induce Msx1 in the ectomesenchyme and a feedback loop exists between Bmp4 and Msx1. Moreover once the Bmp4 expression has shifted to the ectomesenchyme their expression becomes independent of the oral epithelium (Tucker & Sharpe, 1999). The shift of the odontogenic potential from the oral epithelium to the ectomesenchyme coincides with the timing of localization of Msx1 in the ectomesenchyme around the tooth buds.
Interestingly, when both Msx1 and Msx2 are knocked out, tooth development stops at the placode stage for both incisors and molars (Bei, Stowell, & Maas, 2004; Duverger & Morasso, 2009; Satokata et al., 2000). Lhx6 and Lhx7 are expressed in the ectomesenchyme with Lhx6 restricted to the proximal parts of maxilla and mandible whereas Lhx7 expressed throughout the proximal-distal axis of both maxilla and mandible. Double mutants of Lhx6 and Lhx7 mice show absence of molar teeth due to failure of normal differentiation of molar ectomesenchyme leading to arrest of molar development at dental lamina stage. This leads to both survival and proliferation of molar ectomesenchyme and dental epithelium compromised. However in mutant mice carrying at least one allele of either of the genes, development of molars take place suggesting that these two genes have a redundant role in odontogenesis (Denaxa et al., 2009).

**4.5.2. Bud stage (E12.5-E13.5)**

Dental epithelium invaginates into the surrounding mesenchyme to form a tooth bud at E12.5-E13.5 (Zhu et al., 2013) (Fig. 1). Epithelial signaling molecules Fgf8 and Bmp4 are responsible for the induction of Msx1 and Msx2 gene expression in the dental mesenchyme (Zhang et al., 2005). The shift in expression of Msx1 is preceded by shift in Bmp4 expression pattern (Alappat, Zhang, & Chen, 2003). Once induced by Bmp4 a positive feedback loop comes into play between Msx1/2 and Bmp4 in the dental mesenchyme maintaining the levels of both genes throughout tooth morphogenesis (Alappat et al., 2003). This same mechanism accounts for the restricted expression of Msx1 in late bud stage of odontogenic mesenchyme. While Bmps can induce both Msx1 and Msx2 in dental mesenchyme, Fgfs can only induce Msx1.

These two pathways appear to be independent of each other and occur in parallel during early odontogenesis (Alappat et al., 2003). Msx1 knockout mice die after birth of major craniofacial defects. The development of the first and second molars is delayed and stops at the bud stage, while the development of incisors is not even initiated (Duverger & Morasso, 2009; Satokata & Maas, 1994). Mutations in MSX1 have been associated with oligodontia of maxillary and mandibular second bicuspids and maxillary first bicuspids (Mostowska, Biedziak, & Trzeciak, 2006) of all tooth types (Qin, Xu, & Xuan, 2013). In Witkop syndrome with presence of tooth agenesis and nail dysplasia, a nonsense mutation of MSX1 has been found (Lidral & Reising, 2002).

**4.5.3. Transition from bud to cap stage (E13.5-E14.5)**

At E13.5 days, the invaginated epithelium becomes thick and induces the proliferation and condensation of the underlying dental mesenchyme (Fig. 1). Presence of Msx1 and Pax9 is critical for tooth morphogenesis. Pax9 does not induce Msx1 expression at E12.5 but begins to induce Msx1 expression at E13.5 in the ectomesenchyme. Both Pax9 and Msx1 induce and enhance expression of Bmp4 in the ectomesenchyme and cause the transition from the bud to cap stage. A heterodimeric complex is formed between Pax9 and Msx1, which activates Msx1 and Bmp4 expression during tooth development. The mutated Pax9 physically interacts with Msx1 but is unable to transcriptionally activate Msx1 and Bmp4. Moreover, mutant Pax9 and Msx1 when coexpressed are also not able to activate Bmp4. Therefore in mutant Pax9 and Msx1, either alone or together, fails to transactivate Bmp4 therefore leading to failure of transition of tooth development from bud stage to cap stage (Ogawa et al., 2006). This can be rescued by transgenic expression of Bmp4. Heterozygous Pax9 mutation is.

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(Satokata & Maas, 1994). Interestingly, when both Msx1 and Msx2 are knocked out, tooth development stops at the placode stage for both incisors and molars (Bei, Stowell, & Maas, 2004; Duverger & Morasso, 2009; Satokata et al., 2000).

Lhx6 and Lhx7 are expressed in the ectomesenchyme with Lhx6 restricted to the proximal parts of maxilla and mandible whereas Lhx7 expressed throughout the proximal-distal axis of both maxilla and mandible. Double mutants of Lhx6 and Lhx7 mice show absence of molar teeth due to failure of normal differentiation of molar ectomesenchyme leading to arrest of molar development at dental lamina stage. This leads to both survival and proliferation of molar ectomesenchyme and dental epithelium compromised. However in mutant mice carrying at least one allele of either of the genes, development of molars take place suggesting that these two genes have a redundant role in odontogenesis (Denaxa et al., 2009).

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**Fig. 1. Molecular signaling during tooth development.**

Schematic representation of early oral cavity, showing presumptive incisor and molar region further detailing the molecular factors at different stages of tooth development along with signaling molecules and homeobox genes expressed in the epithelial and mesenchymal components of developing tooth.

**Bold font:** Signaling molecules; **italic font:** genes.

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![Molecular signaling during tooth development.](image-url)
observed in humans with familial oligodontia but non-syndromic involving the permanent molars (Frazier-Bowers et al., 2002; Nieminen et al., 2001) and sometimes other teeth such as premolars, canine and incisors with reduced size of teeth (Lammi et al., 2003). The hypodontia of premolars might be the result of the interaction between Pax9 with the Msx1 as shown in the mice (Ogawa et al., 2006). Missense mutation of PAX9 shows milder phenotypes than the nonsense and frameshift mutations thus providing a clear genotype-phenotype correlation (Bailleul-Forestier, Molla, Verloes, & Berdal, 2008). A novel PAX9 mutation associated with syndromic tooth agenesis has been reported in a male having defects of hair, but lacked ectodermal symptoms of nails and skin (Mostowska, Badurska, Rakowska, Lianeri, & Jagodziński, 2013).

The adjacent epithelium and mesenchymal cell layers differentiate into enamel-secreting ameloblasts and dentin-secreting odontoblasts, respectively at E18, which is the late bell stage (Koussoulakou et al., 2009; Slavkin, Trump, Mansour, Matsosian, & Mino, 1974). Msx1 is down-regulated at the early bell stage whereas towards the late bell stage, Msx2 is expressed in both ameloblasts and odontoblasts (Duverger & Morasso, 2009). In Msx2−/− mice, though all their teeth develop, they exhibit malformations and degeneration of incisors and molars, which leads to an inability to chew solid food (Aioub et al., 2007; Duverger & Morasso, 2009; Satokata et al., 2000). Further, the absence of Msx2 causes alterations in Bmp4 and laminin α3 subunit expression, leading to defects in enamel knot and ameloblasts (Beı et al., 2004; Duverger & Morasso, 2009). Laminin 5α3 is expressed in the basal membrane of the dental epithelium before the differentiation of the ameloblast. However, it disappears when the ameloblast differentiate (Yoshiba et al., 1998). Msx2−/− mice are capable of achieving terminal differentiation of the ameloblast however the integrity of their junctional complex is affected leading to the inner enamel epithelium, which is rounded and detached ameloblast with loose intercellular junctions (Beı et al., 2004; Molla et al., 2010). In epidermolysis bullosa the mutation of LAMAS results in skin fragility and enamel dysplasia (Brooks, Bare, Davidson, Taylor, & Wright, 2008) due to the destruction of dermal and dental epithelia. In another interaction Msx2 represses the expression of amelogenin gene indirectly through C/EPβα (Xu, Matsumoto, Sasaki, Harada, & Taniguchi, 2010; Zhou, Lei, & Snead, 2000). Msx2 also plays a role in the transcriptional modulation of enamel and ameloblastin, which encodes enamel matrix proteins. These enamel matrix proteins are also decreased in Msx2−/− mice (Molla et al., 2010). Moreover a feedback loop between ameloblastin and Msx2 expression exists (Fukumoto et al., 2004; Sonoda et al., 2009). As stated earlier Pitx2 induces Lhx6 and Lhx8 in return represses the expression of Pitx2. Lhx6 also auto-regulates itself. At E16.5 there is high expression of Lhx6 in both the dental epithelium and the ectomesenchyme. Lhx6 is required for regulating incisor stem cells thus controls cell proliferation at the cervical loop and for pre-ameloblast differentiation. Therefore homozgyous mutant Lhx6 mice show smaller upper and lower incisors and molar teeth, pre-ameloblast defects and defects in root development (Zhang et al., 2013). Moreover, PITX2 is also involved in normal amelogenesis, where High Mobility Group Nucleosomal Binding Domain 2 (Hmgm2) expression decreases leading to activation of Pitx2, which in turn activates amelogenin expression thus resulting in normal deposition and mineralization of enamel. However, in transgenic mice with high levels of Hmgm2 leads to inhibition of Pitx2 similar to Pitx2 loss-of-function resulting in enamel hypoplasia as seen in Axenfeld-Rieger syndrome. In Axenfeld-Rieger syndrome, PITX2 mutation leads to inactivation of amelogenin expression resulting in decreased enamel deposition and hypoplastic enamel layer (Li et al., 2014). DLX3 is primarily expressed in the neural crest-derived mesenchymal component of the developing tooth during the bud and cap stage. However, in the late bell stage, DLX3 expression shifts from the mesenchyme to be predominantly expressed in the inner enamel epithelium (IEE) and pre-ameloblasts. The outer enamel epithelium (OEE) does not show any DLX3 expression (Duverger et al., 2012; Morasso & Radoja, 2005; Zhao et al., 2000). The IEE gives rise to the ameloblasts, which are responsible for enamel formation. Therefore, the pattern of expression of DLX3 in the developing tooth of mice correlates consistently with the thin-pitted enamel of TrichoDento Osseus (TDO) phenotype in humans (Price, 1998; Price, Wright, Kula, Bowden, & Hart, 1998; Wright, 2006). Furthermore, it is proven that the mutation in DLX3 in humans causes reduced thickness and diminished microhardness of the enamel which explains the severe attrition and interdental spacing observed in affected individuals (Hyun & Kim, 2009). Moreover, the IEE gives to the Hertwig’s epithelial root sheath (HERS), which establishes the root morphology and the mutation in DLX3 results in the failure of the HERS to invaginate at the appropriate time thus leading to taurodontism as seen in human TDO individuals
HMG may help to elucidate their functional role in dental anomalies (Molla et al., 2010). Therefore in Msx2+/− mutants the enamel thickness was increased with the external prismatic layer and a shift of the enamel deposition. Moreover it is strongly expressed in enamel free areas such as the HERS and epithelial rests of Malassez cells (Molla et al., 2010). It also acts as a repressor of amelogenin promoters (Zhou et al., 2000). In enamel matrix deposition Msx2 controls the enamel thickness and rod morphology (Molla et al., 2010). Therefore in Msx2+/− mutants the enamel thickness was increased with the external prismatic layer and a shift of the rod:inter-rod ratio towards the rods. Amelogenin expression in ameloblasts was also increased. Homozygous mutants of Msx2+/− showed reduced expression of amelogenin and enamelin genes (structural genes for enamel matrix) with reduction of the enamel matrix proteins and disappearance of the ameloblasts. Alteration in cell–cell junction laminin 5α3 (as seen in human epimyelodysis bullosa) and cytokeratin 5 genes are also present. Therefore, these homozygous mutant mice showed characteristics as seen in isolated and syndromic enamel dysplasia. Whereas, in the roots the epithelial rest of Malassez cells accumulated and formed hypertrophic islands leading to over and aberrant expression of enamel protein root dentine defects and hypercementosis (Molla et al., 2010).

Dlx2 is expressed in the molar and incisor root epithelia during initial root formation and in a subpopulation of cementoblasts. Thus it may be involved in root morphogenesis and cementogenesis (Lezot et al., 2000, 2008). Further Dlx2 overexpression leads to shortened tooth roots and increased deposition of cementum apart from alteration in the alveolar bone and periodontal ligament (Dai et al., 2017). Moreover, the Dlx2 overexpression increased the expression levels of other genes associated with odontogenesis such as Msx2, Sox9, TgfβR1, TgfβR2 and Smad4. However the significance of these overexpression need to be further studied (Dai et al., 2017). Overexpression studies of HMG may help to elucidate their functional role in dental anomalies similar to the HMG knock out studies.

Alterations of these precisely regulated molecular and cellular sequences of development lead to dental anomalies, i.e. anomalies of teeth number, shape and size, of hard structures (enamel and dentin), of root formation and eruption. These malformations are observed in mouse models mimicking human diseases having the clinical phenotypes as described above in the text and listed in Table 1.

5. Odontogeneic homeobox circuit: signaling mechanisms

HMG typically switch on cascades of other genes by binding with their DNA in a sequence-specific manner (Beukeboom & Perrin, 2014). However, the specificity of a single homeodomain protein is usually not enough to recognize its desired target genes. Hence, homeodomain proteins mostly act in the promoter region of their target genes as complexes with other transcription factors (Beukeboom & Perrin, 2014) (Fig. 2). Such complexes have higher target specificity than a single homeodomain protein. This paradigm is particularly evident in the development of organs and tissues depending on the invagination of ectodermally-derived epithelial cells into the underlying mesenchyme including; hair, whisker follicles, teeth and mammary glands which all follow similar mechanism (Hudson, Taniguchi-Sidle, Boras, Wiggan, & Hamel, 1998). For instance, the dental papilla of teeth expressing Alx homeobox 4 (Alx4) gene is located in mesenchyme and their condensations is additionally dependent on the expression of Lymphoid enhancer-binding factor 1 (Lef1) homeobox protein during murine embryonic development (Hudson et al., 1998). Further, Alx4 strongly activates transcription from a promoter region containing the homeodomain binding site, P2 (Hudson et al., 1998). For optimal activation of this transcription factor requires specific sequences in the N-terminal portion of Alx4 in addition to proline rich region of the downstream PL (paired like)-homeodomain (Hudson et al., 1998). Additionally, the homeodomain protein complex activities are also influenced by diffusible protein signaling molecules consisting mainly of growth factors. These protein signaling molecules acts synergistically and/or antagonistically with HMG thus controlling their overlapping and distinct zone expression (Zhou et al., 2015). As studied by Zhou et al. (2015), the role of Lhx6 and 8 as cell fate regulators, which are involved in odontogenesis, are expressed in the ectomesenchymal region of the maxillary and mandibular processes is finely controlled by Fgf signaling molecules in a distinctive time dependent manner. Coupled with the activity of Lhx6 and 8 in response to Fgf8 are further extended to downstream genes (Msx1, Msx2, Gsc) initiating dentinogenesis (Fig. 2). On the other hand it was demonstrated that Lhx6 has a repressive effect on Pitx2 with synergistic activation of Lef1 and β-catenin co-factors (Zhang et al., 2013). Further findings from another study confirms the role of Dlx3 and Runt related transcription factor (Runx2) along with the clock gene Nuclear receptor subfamily 1, group D, member 1 (Nrd1) on the secretion and maturation of ameloblasts in a distinct time dependent manner (Athanassiou-Papaefthymiou et al., 2011).

Subsequently, the odontogenesis process involves further transformation of the undifferentiated cells into their differentiated forms, which is directed by various molecules and agents like proteins, retinoids or micro RNA influencing the proliferating cells through induction/deduction mechanisms of HMG (Morkmued et al., 2016). Among these, the notable role of BMP regulatory the Msx1 gene in the dental mesenchyme demonstrates the implication of BMP signaling (Yang, Yuan, Ye, Cho, & Chen, 2014). Similarly, the role of non-canonical BMP and Wnt/β-catenin signaling for orchestrating early tooth development was demonstrated by Yuan et al. (2015). Again, retinoids were found to be correlated with the expression of HMG. These retinoids plays a significant role as morphogens in normal craniofacial development and as a teratogen in abnormal craniofacial development (Maas & Bei, 1997; Morkmued et al., 2016). For instance, an excess of retinoid at E14.5 can lead to the reduced activity of Runx2/3, Dlx3/5, and Bmp2/3 which encircle tooth bud essentially targeting mesenchymal cells. In yet another report, the role of micro RNA was emphasized for facilitating Pitx2-β catenin based regulatory pathway for epithelial cell differentiation to mesenchymal cells to amelogenin expressing epithelial cells (Sharp et al., 2014). Again the regulatory circuit of Pitx homeoproteins for Bmp4 expression in tooth development further explains their regulatory input for odontogenesis (Jumlongras et al., 2012). Thus, most likely, a combinatorial effect of various signals or molecules rather than the actions of a specific HMG is involved with odontogenesis process (Bei, 2009).

Alternatively, the HMG acts as selector molecules responsible for tooth type selection, which are differentially expressed among the cells that have the potential to form teeth, prior to any signals for the tooth initiation (Theseleff & Sharpe, 1997; Weiss et al., 1998). Such selector molecules would represent a pre-pattern and shape differences within each tooth type. Accordingly, several factors coordinately mediate the dynamic interactions which lead to the cell growth or its inhibitions in these developing tissues (Tucker et al., 1998). However, the challenge is to identify the nature of such interactions.

Indeed, it has been proposed that the key signaling pathways including; BMP, FGF, SHH and WNT ligands and their receptors stimulates the tooth development through epithelial–mesenchymal interactions (Bei, 2009; Mitsiadis & Luder, 2011). In most cases, disruption of genes that are part of these signaling pathways results in severe aberrations of tooth development (Bei, 2009). Further, the inhibitors of
these signaling pathways also contribute to control tooth development. Thus when the inhibitors or mediators of these signaling pathways are disturbed either through loss of function of its inhibitors or by over-expression of its effectors leads to many dental defects (Bei, 2009) as shown in Table 2. For example, Dact2 proteins are found to repress the expression of Pitx2 transcription through Wnt/β-catenin signaling pathway during tooth morphogenesis (Li, Florez, Wang, Cao, & Amendt, 2013). On the other hand, the genes Ectodysplasin A (Eda) and Ectodysplasin A receptor (Edar) which are the members of the ectodysplasin pathway are mediator for Wnt signaling. Hence it is imperative for the correct formation and patterning of tooth (Bei, 2009). Mutation in this can cause hypo/oligodontia which is non-syndromic tooth agenesis (Suryadeva & Khan, 2015). Further report by Zhang et al. (2005) shows evidence that EdaA1 expression in dental epithelium is induced by

![Fig. 2. Odontogeneic homebox signaling mechanisms.](image)

Schematic representation of homeodomain signal transduction by combining with promoter and other activator proteins of DNA to form protein complex. The binding of signaling factor with the receptor molecules present in extracellular membrane of cells results in assembly and activation of a group of intracellular signaling proteins including transcription factor leading to gene activation which activates the new genetic programs in the cell body. This signaling mechanisms within each cell layers (epithelial or mesenchyme) activates mutually leading to regulation and repression of epithelial and mesenchymal signaling.

<table>
<thead>
<tr>
<th>Mutated genes/proteins</th>
<th>Mediators/Inhibitors</th>
<th>Dental defects</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Loss of Ectodin</td>
<td>Inhibitor of BMP signaling</td>
<td>Supernumerary teeth</td>
<td>Kassai et al. (2005)</td>
</tr>
<tr>
<td>Loss of Apc (Adenomatous polyposis coli)</td>
<td>Mediator of WNT signaling by organising the complex that degrades β-catenin</td>
<td>Multiple tooth buds and not expressed in the oral epithelium.</td>
<td>Kuraguchi et al. (2006)</td>
</tr>
<tr>
<td>Lrp4 mutations</td>
<td>Modulates and integrates both the BMP and the canonical WNT signalling by binding the secreted BMP antagonist protein</td>
<td>Supernumerary incisors and molars as well as fused molars</td>
<td>Ohazama et al. (2008)</td>
</tr>
<tr>
<td>Loss of primary cilia intraflagellar transport (IFT) protein, IFT88/polaris</td>
<td>Mediator of SHH signaling</td>
<td>Formation of ectopic teeth, through increase of Shh activity in the toothless region of the embryonic jaw primordia, the diastema region</td>
<td>Liu, Wang, and Niwander (2005), Ohazama et al. (2009)</td>
</tr>
<tr>
<td>Upregulation Gnar1 protein</td>
<td>Inhibitor of SHH signaling</td>
<td>Ecptic diastema teeth</td>
<td>Ohazama et al. (2009)</td>
</tr>
<tr>
<td>Inactivation of Spry2 (Spry2) and/or Spry4 (Spry4)</td>
<td>Inhibitors of FGF signaling</td>
<td>Supernumerary teeth in the diastema</td>
<td>Klein et al. (2006, 2008)</td>
</tr>
<tr>
<td>Loss of Sp6</td>
<td>Mediators of Sp6 which functions through upregulation of Lef1, a target of WNT signaling, whose activation leads to extra teeth</td>
<td>Supernumerary teeth</td>
<td>Nakamura et al. (2008)</td>
</tr>
<tr>
<td>Loss of Odd-skipped related 2 (Osr2)</td>
<td>Mediators of Osr2 which leads to up-regulation and expansion of the odontogenic field that is driven by the BMP4-Mxe1-BMP4 pathway in the mesenchyme</td>
<td>Develop supernumerary teeth lingual to their molars</td>
<td>Bei, Kratochwil, and Maas (2000)</td>
</tr>
</tbody>
</table>
Wnt6 and downregulation of Eda is seen in its mutant types. Therefore any mutations to these members can cause various forms of hypohidrotic ectodermal dysplasia (HED), which specifically affects the ectodermal appendages (Mustonen et al., 2003).

Thus the proper implementation of the signaling pathway for cell migration, cell differentiation and ultimately development requires the highly coordinated actions of multiple genes. Notably, these complex molecular signaling interactions and cytodifferentiation phenomena account for the perturbations in the behavior of neural crest mesenchyme or its connective tissue matrix which give rise to many odontal dysmorphology. Consequently, these odontogenic circuit need to be tightly regulated in both time and space, and with respect to their quantitative outputs (Spitz & Duboule, 2008).

6. Feasibility of tooth repair and regeneration using homeobox stemness properties of stem cells

In recent years, the notable role of genetic components as a cause of dental defects and disorders is under scrutiny (Srijaya et al., 2012). Further, the tooth damaged by bacterial infection, traumatic injury, tooth wear or altered tooth development due to chemotherapy or radiotherapy related to oral cancer treatment is very challenging to repair and reconstitute the lost dental tissue (Otsu et al., 2012). In clinical perspective, these two challenging issues of genetic component and complex structural integration have been a dilemma for clinicians all around the globe and have been in search for a possible solution. From a clinical perspective, these two challenging issues of genetic component and complex structural integration have been a dilemma for clinicians all around the globe and resulting in a wide ranging search for a possible solution.

However, in recent years there is a growing tendency to explore the possibility of stem cells as an efficient technology for applying to regenerative dentistry and treat dental diseases or disorders. The pressing role of stem cells in tissue homeostasis and repair throughout life is a well-known fact (Mitsiadis & Graf, 2009). Pronounced interest in the application of stem cells for regenerative endodontology arises from the potential to influence their fate and consequently their functions during tissue repair and/or regeneration (Mitsiadis & Graf, 2009). However, the concept of utilizing these technology is still in its infancy for odontal disorders and diseases.

When encountering a dental injury, the reparative mechanism involves a series of highly conserved genetic programs resembling embryogenesis whereby, the injured tissues or necrotic cells are replaced by cells having stem cell properties, or their differentiated counterparts (Mitsiadis & Graf, 2009). The basic four components crucial for tissue regeneration are; appropriate cells, signals, blood supply, and scaffold that are needed to target the tissue at the site of defect (Srijaya et al., 2012) (Fig. 3). These four elements play a fundamental role in the reconstruction and healing of lost tissues. Stem cells populations from different body sources, although are immuno-phenotypically similar, they display different growth factors and differentiation abilities. Moreover, the fate between self-renewal properties and differentiation of cell type is strongly regulated by both intrinsic cell determinants and signals along with the growth factor from their microenvironment (Mitsiadis & Graf, 2009). Therefore, selecting a suitable cell sources and knowledge on their microenvironment niche is a pre-requirement for cell-based therapeutic strategies for regeneration and repair of tissues. In the context for tooth regeneration, dental tissues derived stem cells (DSCs) will be the appropriate cells to provide the systematic framework for new tissue growth and differentiation because of their inherent biological properties leveraged for dental tissue regeneration (Srijaya et al., 2012) (Fig. 3). Considering the potential biological and therapeutically favorable characteristics of DSCs in terms of easy availability, immature cell source, quick isolation procedure, multipotency, immunomodulatory properties and efficient reprogramming potential which are the core elements of cell-based therapies makes them a suitable candidate.

Again in the field of dental regenerative medicine, homeobox gene therapy can be advantageous for tissue engineering by artificially up-regulating or down-regulating certain genes in the in vitro or in vivo conditions depending upon the required regenerative or regenerative needs. The expression of HMG particularly MSX (Fujii et al., 2015) and Dlx (Qu et al., 2014) has been demonstrated in DSCs which play a significant role in the maintenance of stemness and proliferation of DSCs which is further asserted in our lab while performing DNA methylation studies (Srijaya et al., unpublished data). Apart from MSX and Dlx in our on-going research studies we have also affirmed the important devoir of Pitx2 and Pax9 in the stemness properties of DSCs (Cao et al., 2013; Thesleff & Tummers, 2008; Tziasakis & Kodonas, 2010) (Srijaya et al., unpublished data). The enhanced expression of these genes in DSCs makes them stands out from other mesenchymal stem cells (MSCs) sources at their molecular level.

Notably the molecules of the Wnt, Notch, and Bmp signaling pathways have been well-known to control the fate of stem cells by molecular cross-talk among stem cells and control their microenvironment niche interactions in both humans and mice (Mitsiadis & Graf, 2009). During the process of dental repair a recapitulation of developmental signals take effects leading to the recruitment, migration and differentiation of stem cells (Li et al., 2014). HMG especially (MSX, Dlx, Pitx and Pax9) inevitably plays the significant role as orchestrators for the plethora of different molecules involved like, growth factors, cytokines and adhesion molecules and their complex interactions (Ikeda & Tsuji, 2008). For instance, findings have revealed about the significant role played by Wnt7b in human tooth development by promoting cell differentiation whose expression remains strong in the epithelium (Li et al., 2014). In humans, transmission of Wnt signals are carried through at least 3 distinct intracellular signaling pathways: the canonical “Wnt/β-catenin” pathway and 2 non-canonical pathways “Wnt/Ca2+” and “Wnt/planar cell polarity” (Li et al., 2014). A recent report (Sharp et al., 2014) has identified the involvement of Pitx2: Wnt/β-catenin regulatory pathway to be involved in epithelial cell differentiation and conversion of mesenchymal cells to amelogenin expressing epithelial cells via miR-200a in mice. In effect, HMG Pitx2, Wnt/β-catenin signaling and microRNAs (miRs) regulates the differentiation of dental stem cells during embryonic development. Wnts, which comprise to the family of secreted ligands, mediates many receptor-mediated pathways. Likewise, the activation of Wnt/β-catenin pathway results in accumulation of catenin leading to nuclear translocation and further downstream transcriptional activation by complexes of β-catenin, LEF/Tcf and Pitx2 transcription factor (Sharp et al., 2014). Thereby Pitx2 activates miR-200a-3p expression and miR-200a-3p reciprocally represses Pitx2 and β-catenin expression. Accordingly, Pitx2 and β-catenin synergistically interact to activate genes for odontogenesis whereas; miR-200a-3p directs the differentiation process. Thus, clearly it explains the involvement of HMG network of events affecting the biological behavior of cells. A disruption of any of these events could lead to an abnormal tissue formation and a reduced tissue repair properties of stem cells. Further, Qu et al. (2014) have reported about the regulation of BMP4 signaling for enhancing Dlx2 in their in vitro based studies for osteogenesis induction in dental derived stem cells from apical papilla. A separate study have also pointed out the role of HMG as a “angiogenesis switch” corresponding to their major role in the formation of cellular components of blood vessel walls (with the presence of HOX5, HOX11, HOXB1, HOXB7 and HOX C) (Chen & Gorski, 2008; Gorski & Walsh, 2000; Gorski, LePage, & Walsh, 1994; Miano et al., 1996; Patel, Gorski, LePage, Lincecum, & Walsh, 1992) role of HOX3 in differentiation of endothelial progenitor cells to endothelial cells and promoting pro-angiogenic factors suggesting their importance for angiogenesis mechanisms during tissue regeneration process (Rachgal et al., 2012). This findings caters the mechanistic direction of directed differentiation of DSCs for tissue engineering by the activation of appropriate signaling pathways.
In addition to signaling pathways, the association of HOX proteins (control cell positional identity) has been assigned for selecting suitable stem cell populations residing in different tissues and anatomical sites for efficient use of stem cells in regenerative medicine. Accordingly, a recent study has reported the possibility of using HOX (proteins that control cell positional identity) and TALE subfamilies of HMG as suitable markers to identify distinct stromal cell populations in humans (Picchi et al., 2013). Therefore specificity of HOX profiling can be used for selecting the most appropriate cell source for cell-based therapeutic strategies for regeneration and repair of specific tissues (Picchi et al., 2013).

7. Possible clinical applications

At current scenario, tissue engineering in dental science is mainly focused on differentiation potential of various stem cells, studying the gene mutations and possible gene correction in vitro for an implantable tooth structures from stem cells thus providing solutions to certain prevailing dental problems or other dental tissue deformation. For instance, in terms of bone defects recently, Aastrom company has attempted to use stem cells (ixmyelocel-T), for re-growing the craniofacial tissues especially bone tissue and they proved to be successful in terms of quick, more effective and less invasive procedure than traditional bone regeneration treatments (Kaigler et al., 2013). However, immense research has been carried out in regenerative medicine for the use of various types of stem cells ranging from embryonic stem cells (ESCs), mesenchymal stem cells to induced pluripotent stem cells which are generated through enforced expression of defined transcription factors, which can reset the fate of somatic cells to an embryonic stem-cell-like state (Srijaya, Ramasamy, & Kasim, 2014). In-fact, these defined transcriptional factors (like octamer-binding transcription factor Oct3/4, Sry-related HMG box Sox2, Krüppel-like transcription factor Klf4 and Myelocytomatosis oncogene, Myc), and other related homeobox gene product like Nanog homeobox (Nanog) are the significant contributors to this breakthrough finding of iPS technology (Takahashi & Yamanaka, 2006). Interestingly studies are further proving the role of other HMG involved in the reprogramming potential especially using dental derived stem cells. For instance, in a breakthrough research by Tamaoki et al. (2014), reported that in their studies they observed the easiness of reprogramming immature dental pulp derived stem cells (DPSCs) to iPSCs by just using two transcription factors (Oct3/4 and SOX2) compared to adult DPSCs which required all the four defined transcriptional factors. Downstream investigation for this findings indicated that the high expression of DLX4 homeobox gene in immature DPSCs is promoting this reprogramming potential. This explains the improved reprogramming efficiency using iPSCs which required all the four defined transcriptional factors. Downstream investigation for this findings indicated that the high expression of DLX4 homeobox gene in immature DPSCs is promoting this reprogramming potential. This explains the improved iPS technology (Takahashi & Yamanaka, 2006). Strikingly, such tissue engineering strategies using such cell therapy approach can be successfully applied for a wide variety of oro-dental structures including: regeneration of resorbed root, cervical, or apical dentin, periodontal

Fig. 3. Model of stem cell therapy application for tooth repair and regeneration.
An overview of stem cell therapy application for inducing tissue repair and regeneration.
regeneration and gingiva which can facilitate and restore the physiological structural integrity (Srijaya et al., 2012). In addition, the concept of personalized therapeutics for oro-dental genetic disorders is also possible by ex vivo genetic modification of patients own cells using induced pluripotent stem cell (iPSC) technology (Srijaya et al., 2014). The prospect of such pluripotent stem cell generation using iPSC technology will be valuable, preferably for the disease modeling potentials to comprehensively evaluate the genetic disorders.

Since the sequencing of the human genome, the quest for identifying the genome location, which is more prone or likely to develop certain diseases, has been an interesting area. A recent discovery of Sex determining region Y-box2 (Sox2) control region in mice has opened a clue to this investigation, which is a “gene control region” (Vastardis, Karimbux, Gutha, Seidman, & Seidman, 1996). This region imposes significant direction towards the switching on/off mechanisms of genes by controlling and regulating the particular gene activity and their prescribed function (Vastardis et al., 1996). This control region is located elsewhere in the genome region and not necessarily nearby to the gene of interest. For the emerging field of human regenerative medicine this discovery could be a remarkable break through, as it could be helpful for unravelling the control regions of Sox2 HMG gene, which also shares the highly conserved DNA binding domains of homeobox and is one of the crucial gene for maintaining self-renewal and pluripotency of embryonic or pluripotent stem cells. This further explains the positive or negative functional responses of various genes or transcription factors that act either synergistically or counteractively along with their control regions.

This wealth of functional HMG in humans could be utilized for therapeutic targets by up re-regulation or down regulation of these genes to stabilize the microenvironment and impede cell differentiation leading to desired tissue restoration. In practice, such tissue engineering mechanisms could be achieved by using siRNAs (Small Interfering RNA) for over-expression of HMG in vitro grown cells (Northcott, Northev, Barnes, & Weaver, 2015). Otherwise down-regulated HMG, which is often a result of silencing by miRNAs (micro RNA) could be boosted by inhibiting or repressing those miRNAs (Northcott et al., 2015). In other ways targeted gene therapy could also be used for engineering such therapeutic cells for the restoration of the repressed HMG. Thus, such modes of gene restoration approach can yield more effective therapeutic strategies to regenerative medicine. On the other hand, the complex regulatory networks of numerous other transcription factors involved in the stem-ness and differentiation property of stem cells is yet to be explored. Therefore, considering the implications of such interactive molecular genetics of HMG in regulating the cellular responses and advances in tissue engineering techniques, more fruitful advantage can be expected for their use in cell therapies and gene corrections in oro-dental disorders in the near future.

8. Conclusions

As regulatory elements of several transcriptional factors, HMG are very significant in the physiological context with its involvement of several mechanisms and signaling pathways. Any mutations can lead to severe morphological alterations. For that reason, it is important to elucidate how these genes and their products interact with the complex functional pathways and work together in a defined condition to generate the diverse and precise patterned structures of the functional teeth. Therefore, it is a demanding effort to dissect the functional specificity of these complex genes to refine their downstream targets. These attempts can also contribute towards the molecular characterization of stem cells using homeobox gene markers and their feasibility for tooth repair and regeneration. Future investigations should also involve the identification of factors and signals controlling the gene modifying loci of transcriptional factors, which plays a critical role during embryonic development. Finally, it is anticipated that substantial understanding of tooth regenerative therapies can ultimately provide an alternative for the current dental treatments, which are mostly based on semi-synthetic materials. The potential replacement of such semi-synthetic materials by stem cells for dental tissue regeneration can provide appropriate structural integrity, structural and functional recovery. Such developments not only will provide essential oral functions of missing or defective teeth, which is a kind of social handicap due to mastication, speech and aesthetic problems but can improve the socio-cultural interactions of an individual resulting high impact on emotional well-being thereby representing a good life quality.

Competing interests

The authors declare that they have no competing interests.

Authors’ contributions

AR and TCS wrote the manuscript. All authors reviewed, edited and approved the final manuscript.

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