Chapter

Embryological Development of Human Molars

Fatiha Rhrich and Hakima Aghoutan

Abstract

Dental development is a complex process by which teeth from embryonic cells grow and erupt into the mouth. It is governed by epithelio-mesenchymal interactions. The biological mechanism is the same for all teeth; however, epithelial signaling and homeogenous combinatorics are different from one type of tooth to another. The primary dental blade splits into the vestibular and primary dental blades opposite to the mesenchymal condensation. During dental development, three successive stages are described: bud, cup, and bell. The secondary dental blade responsible for the formation of germs in permanent teeth is formed from the primary dental blade in the bell stage. For the central incisor, lateral incisor, canine, first temporary molar, and second temporary molar, each primary dental blade gives rise to a single secondary dental blade for the corresponding permanent tooth. On the other hand, the primary dental blade of the second temporary molar will cause the formation of four secondary dental blades that will cause the formation of permanent germs of the second premolar, the first permanent molar, the second permanent molar, and the third permanent molar. The objective of this chapter is to focus on the cellular and molecular mechanisms explaining the normal development of molars by presenting the different current data and theories of science illustrating the human molar embryological development.

Keywords: tooth development, molar, morphological appearance, molecular regulation, epithelial-mesenchymal interaction

1. Introduction

Teeth are a topic of interest to paleontologists because they are very well preserved. As a matter of fact, the dental remains have made it possible to study the evolution of mammals by analyzing their morphology. In developmental biology, the mouse model is an interesting model for studying dental development.

Humans have two dentitions (temporary and permanent) and different types of teeth, incisor, canine, premolar, and molar with different morphologies, whereas mice only have two types (incisor and molar) separated by a diastema from which the incisors have unlimited growth. Despite these differences, the dental development process is similar in humans and mice, and regulatory phenomena have been maintained over the evolution.

Teeth, such as mammary glands, hair, and feathers, develop from two adjacent tissues: the epithelium and the mesenchyme, although they all have different morphologies. Indeed, during development, the specific shape of each organ is defined

in relation to epithelial-mesenchymal proliferation and to all the changes that the epithelium undergoes [1].

The embryological aspect of the molars was addressed in order to clarify the etiopathogenic aspect and to adapt therapeutic attitudes according to the diagnosis.

The objective of this chapter is to address the embryology of human molars by focusing on its molecular and morphological characteristics.

2. Phylogenetic aspects

Teeth represent a new morphological feature of mammals [2, 3]. Molars are complex teeth able to become occluded. Interlocking intercuspation between upper and lower molars allows food to be crushed [4]. Evolutionary dietary radiations are related to the great diversity of the current mammalian molars. They are clarified in the fossil record, where new molar organizations are often related to significant line diversifications. Several theories have been advanced to explain the evolution of molars. Like all primates, Man is a placental mammal, and the ancestor of contemporary humans is *Homo sapiens*. For 200 million years, in Therian mammals, the molars have trigonodontal morphology; in other words, the three tubercles are arranged in a triangle [5].

In 1965, the discovery of a fossil of a lower molar made it possible to show that on this Therian branch around 135 million years ago, these molars already existed. They were called tribosphenic by Simpson in 1936 [6]. These mandibular molars have six tubercles, three of which are pointed, high, sharp, and are arranged in a triangle and distal position. The three others tubercles are lower and are arranged in a central basin to receive the main palatal tubercle of the opposite teeth that have only three cusps. The fact of having six tubercles is of physiological interest when taking food.

Nearly 110 million years ago, the oldest placental mammals had a dental formula with 52 teeth, including 3 molars in a decreasing series, the first being the largest. This primitive disposition is found in modern man.

Around 75 million years ago, with the dinosaurs extinction, other species invaded space, and the dental formula was reduced to 44 teeth for all placental mammals including the man.

In the Catarrhini, the loss of one incisor and two premolars leads to a dental formula with 32 teeth found in monkeys of the ancient world (Afro-Eurasia), the Hominids, and the contemporary Men. It has been recognized for 45 million years [7].

In the genus Homo, the 32-teeth morphology does not differ much from the modern men, except for the great variability in size. Root morphology may vary from one group to another. The reduction in the number of cusps observed in humans can be considered as a specialization trait and not as a step backward. However, the reduction in the dental formula in the placentals and primates mainly affected the incisors, premolars, and even canines but not the molars.

Wisdom tooth agenesis, especially mandibular agenesis, is often considered as a sign of evolution. On the other hand, the presence of supernumerary teeth or hypergenesis is explained as a return to ancestral forms

3. Morphological aspects

3.1 Formation of the odontogenic epithelium

The odontogenic epithelium is formed from the oral epithelium that lines the primary oral cavity called the “stomodeum.” It appears as a localized thickening of

the oral epithelium, and it is formed by several cellular layers resulting from a series of localized mitoses affecting the oral epithelium. The mitotic spindle of dividing cells is oriented perpendicular to the basal membrane that separates the epithelium from the ectomesenchyma.

3.2 Placement of the vestibular and primary dental blades

Epithelial thickening continues to proliferate and sinks into the underlying ectomesenchymal tissue forming a plunging wall (also called a primitive dental blade). This latter splits into two blades: vestibular and dental. The vestibular blade determines the formation of the buccal vestibule, which is the space between the cheek/lip and the dental arch.

3.3 Evolution of dental placodes

In humans, as in rats and mice, the dental blade will give birth to the dental placodes that will be at the origin of the formation of future dental germs. Dental placodes are cellular clusters attached to the dental blade by a net of epithelial cells called the primary dental blade. Each dental arch initially contains 10 dental placodes. From the primary dental blade develops the secondary dental blade, which is at the origin of the 16 permanent teeth per arch.

Each placode will undergo morphological changes that are described as three successive stages: bud stage, cup stage, and bell stage [1].

4. Placement of molar dental germs

Since the three molars are not preceded by temporary teeth, they evolve from the distal end of the initial dental blade, which proliferates in a posterior direction. The primary dental blade of the second temporary molar will cause the formation of four secondary dental blades. For each half of the arch, starting from the anterior area toward the posterior area, each of these four secondary dental blades will give the permanent germ of the following teeth: the first permanent molar, the second permanent molar, and the third permanent molar.

The secondary dental blades that are at the origin of the formation of the 1st and 2nd molar will orient themselves vertically as long as they have space that allows them to orient themselves in the mesenchyma. On the other hand, in most cases for the 3rd molar, orientation problems arise because there is not enough space for its secondary dental blade to be parallel to the other two blades [8].

All dental buds, with the exception of the second and third permanent molars, are present and begin to develop before birth [9]. The chronology of the appearance of molar germs remains variable according to the authors; however, it is often found that the germ of the first molar appears around the 4th or 5th month of intrauterine life. The one of the second molar appears around the 9th month or 1 year after birth.

The germ of the third molar does not appear until around 4 or 5 years of age. Mineralization begins between 7, 9, and 10 years, and the crown is completed between 12 and 16 years. The emergence in the oral cavity is between 17- and 21-year-olds; the tooth will then slide along the distal surface of the second molar to reach the occlusion level. Root building ends between the ages of 18 and 25 years. The place it has depends on the growth in the posterior region of the arch. The main activity of the dental blade is spread over a period of about 5 years. However, the dental blade near the third molar continues to be active until about 15 years of age [9].

A number of anomalies can occur during the development of the tooth. The development of excess dental blade can lead to an increase in the number of dental buds, resulting in too many teeth (supernumerary). A deficient dental blade can lead to a reduction in the number of teeth (hypodontia) [9].

5. Root formation

Molars are multiradiculated teeth. Indeed, the vast majority of the first maxillary molars have three roots. The second maxillary molar has more frequent variations in the number of roots than the first maxillary molar, and the first mandibular molar and the second have two roots in the majority.

Root formation or radiculogenesis or rhizagenesis is the development of the root pulpo-dentinary organ in close relationship with cemenesis, the outline of the dentoalveolar ligament and the construction of the alveolar bone. It begins when the final dimensions are acquired. The Hertwig epithelial sheath is at the origin of root formation, depending on their number, shape, and size [10].

As for the crown, root development is governed by interactions involving the Hertwig epithelial sheath, basement membrane, mesenchymal papilla, and dental follicle.

5.1 Formation of the Hertwig epithelial sheath

The Hertwig epithelial sheath originates from the reflection zone or cervical loop which is the place where the external and internal adamantin epitheliums (EAE and EAI) meet to form a double epithelial layer. Hertwig epithelial sheath has an annular structure surrounded by a basal membrane that separates it from the pulpal and follicular mesenchyma. This basement membrane has anchoring fibrils on the pulp side. The internal epithelium faces the papilla and the external epithelium faces the dental follicle. The Hertwig epithelial sheath will emit tongues in the centripetal direction that will fuse in the central region of the papilla and form rings from which the roots can be identified. The number of strips emitted is proportional to the number of roots that each molar can have. For example, for the molar which will have two roots, two tongues are formed, and after fusion of two rings, each of the two will be at the origin of the formation of a root. These two leaves remain attached and progress in the underlying connective tissue in the apical direction defining the future shape of the dental root [11].

Root elongation and tissue formation are related to the coordinated proliferation of sheath epithelial cells and surrounding mesenchymal cells [12].

5.2 Formation of root dentin, cement, and apex

Root dentin forms in parallel with the proliferation in the apical direction of the Hertwig sheath. The latter gradually induces odontoblastic differentiation. The pulp parenchyma cells close to the anchor fibrils differentiate into odontoblasts. These odontoblasts produce preentine, which mineralizes to form dentin. The cells of the outer dental epithelium forming the outer layer of the sheath do not differentiate into ameloblasts as is the case for the crown. Then, the basement membrane degrades, and the epithelial blade involutes and gradually dissociates.

Developmental defects of the Hertwig sheath at the apical third of the root are at the origin of the formation of the lateral canals following a stop of dentinogenesis at this site due to the nondifferentiation of pulp fibroblasts into odontoblasts.

The cells of the sheath can undergo three spells: some can form the "Malassez epithelial debris," others can die by apoptosis, while others can undergo epithelial-mesenchymal transformation.

As the sheath disintegrates, follicular cells near the surface of the root dentin differentiate into cementoblasts. These synthesize and deposit the cement matrix in contact with the dentin.

As the root development progresses, the epithelial ring forming the Hertwig epithelial sheath gradually shrinks as a result of a reduction in mitosis, thereby reducing the size of the root tube. This narrowing allows the development of one or more orifices (or foramina), which are the place where vascular and nervous elements intended for the pulp to pass through.

The development of the root ends with the construction of the apex, which is a slow process. In humans, for example, for the 1st permanent molar, this operation is performed until the age of 9–10 years. In the case of permanent teeth, this phenomenon lasts longer and requires more time than the development of the root itself.

6. Molecular aspects

6.1 Epithelial-mesenchymal interactions

In humans, dental development includes the morphogenesis of crowns and roots and results in the formation of the enamel organ, odontoblastic, ameloblastic, and cementoblastic differentiation. Huge advances in research have made it possible to understand the phenomena of molecular regulation of dental development.

Dental development follows a precisely controlled and regulated genetic program. The dental organ consists of an epithelial part that derives from the ectoderm and a mesenchymal part that derives from mesodermal cells on the one hand and cells from neural ridges on the other hand [13–16].

The dental organ develops from a communication between the epithelium and the underlying mesenchyma. The communication language has been preserved throughout the evolution. This communication between the epithelium and the mesenchyma is done through signaling molecules and growth factors [17–19].

The studies carried out on the mouse molar have enabled us to gather a body of knowledge with many similarities to those of humans. However, the experimental data obtained in animals can be extrapolated relatively reliably to understand what is actually happening in humans.

Several families have been described, including:

- TGF-beta (transforming growth factor beta) including BMP (bone morphogenetic proteins) activins and follistatin;
- FGF (Fibroblast growth factors);
- Hedgehog (only Sonic hedgehog (Shh) is known for its role in odontogenesis);
- Wnts [20–24].

These molecules send their message to the nucleus through the signaling pathways and receptors on the cell membrane surface. Transcription factors will then modulate the expression of different target genes and induce changes in cell response and behavior (**Figure 1**) [25].

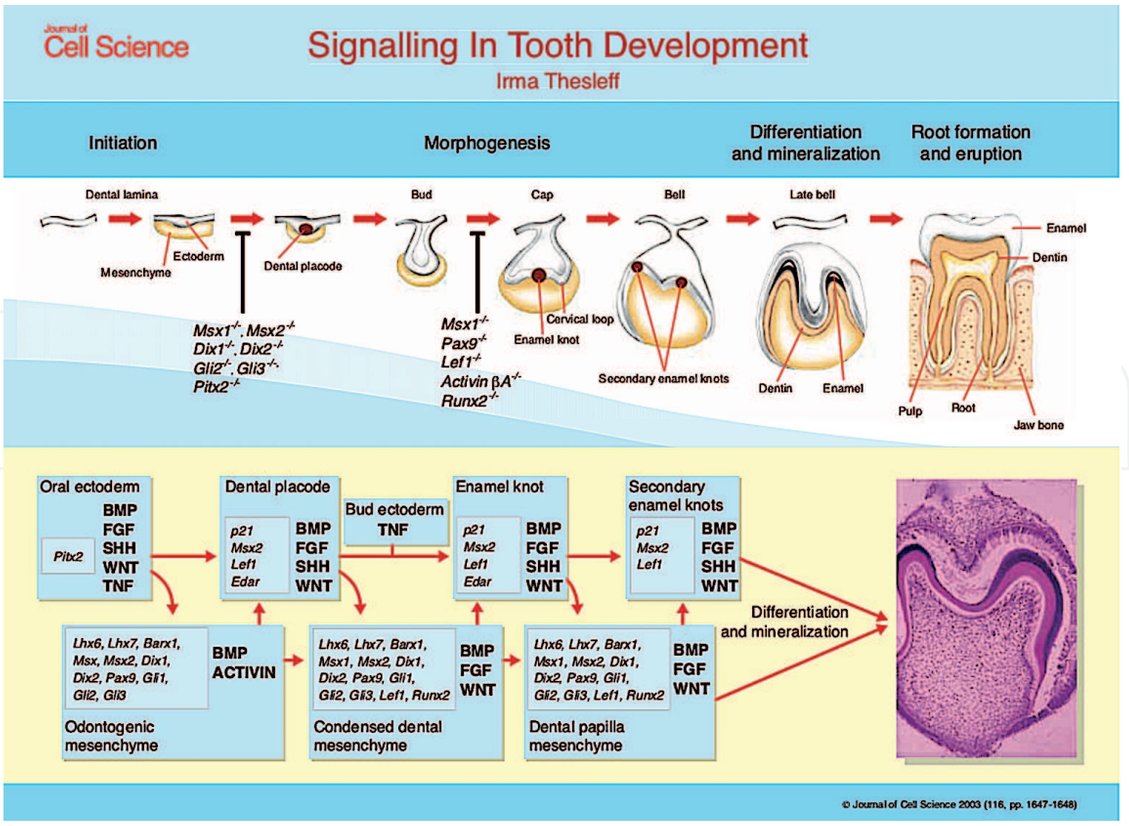

Figure 1.
Signaling in tooth development [25].

Genes represented in "light blue" colored squares or rectangles are responsible, when inactivated, for stopping dental development.

6.2 Determination of the dental region

It should be remembered that the odontogenic epithelium is formed at the first gill arch. The latter undergoes pharyngeal regionalization, resulting in the expression of Fgf8 and 9 (fibroblast growth factors 8 and 9) and Lhx-6 and -7 (LIM homeobox 6 and 7) in the oral part (rostral) and Gsc (goosecoid) in the aboral part (caudal). Indeed, the expression of Fgf8 in the odontogenic epithelium in the oral part of the first pharyngeal arch causes the expression of Lhx-7 in the underlying ectomesenchyma. In the aboral region, there is an important expression of Gsc in the ectomesenchyma. Gsc expression in the caudal region is not responsible for inhibiting Lhx-7 expression in this area; however, Lhx-7 expression in the rostral region will result in blocking Gsc gene expression in this.

In addition to Fgf8, a second BMP4 signaling molecule (bone morphogenetic protein 4) is expressed in the epithelium in the distal and therefore in the median region of the 1st arc.

The activation and inhibition of transcription factors allows the delimitation of the odontogenic territory by BMP4 and Fgf8a double signalling. (**Figure 2**) [19].

6.3 Determination of dental identity

Mammalian teeth are meristic series. The determination of different morphology was explained by two theories:

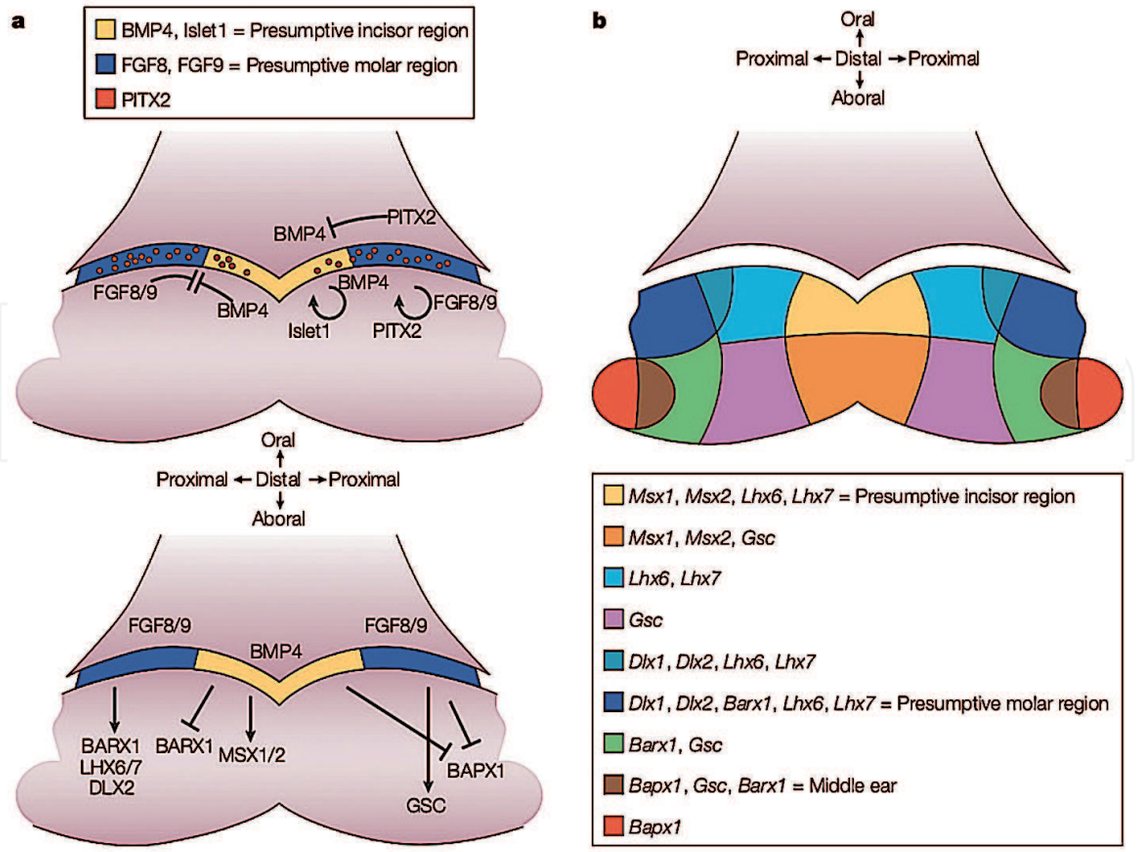

Figure 2.
Pattern of gene expression in the developing tooth [19]. (a) Signaling within the epithelium and between the epithelium and the mesenchyme at embryonic day (E) 10.5. The diagram shows an isolated mandibular arch. Positive autoregulatory loops and mutual repression within the epithelium lead to the formation of strict boundaries of gene expression, which sets up the presumptive incisor and molar fields. Members of the bone morphogenetic protein (BMP) and fibroblast growth factor (FGF) families of protein in the epithelium induce and inhibit the expression of various homeobox genes. This results in a complex pattern of gene expression in the mesenchyme, across both the proximal–distal and oral–aboral/rostral–caudal axes. (b) The odontogenic homeobox code model of dental patterning. The nested expression pattern of homeobox genes in the mandible produces a homeobox code that defines tooth type. Bapx1 (bagpipe homeobox gene 1 homolog); Barx1 (BarH-like homeobox 1); Dlx (distalless homeobox); Gsc (goosecoid); Lhx (LIM homeodomain genes); Msx (homeobox, msh-like); Pitx (paired-related homeobox gene).

- The gradient theory proposed by Butler [26] which stipulates the presence of morphogenetic fields and that the determination of the shape of the tooth is a function of its position in the field independent of local factors.

- The theory of clones proposed by Osborn [27] which stipulates that ectomesenchyma is already differentiated into three cellular clones, incisal, canine, and molar clones, before its migration. The proposal of this second concept suggested that the two theories are competing.

In 1995, the theory of odontogenic homeocode was developed by Sharpe [22], which represents a synthesis of the two theories: gradients and clones and shows that the latter two are complementary. These two concepts were explained in the light of the discovery of new genes and signaling molecules (**Figure 3**) [26–28].

The identity of each tooth, including the molars, is characterized by its homeocode, which represents the combination of homeogens that defines the position and identity of the tooth. Indeed, different homeogens are expressed by the neural crest cells of the ectomesenchyma under the instructive induction of the oral epithelial cells. These homeogens are divergent and therefore of the nonhox type.

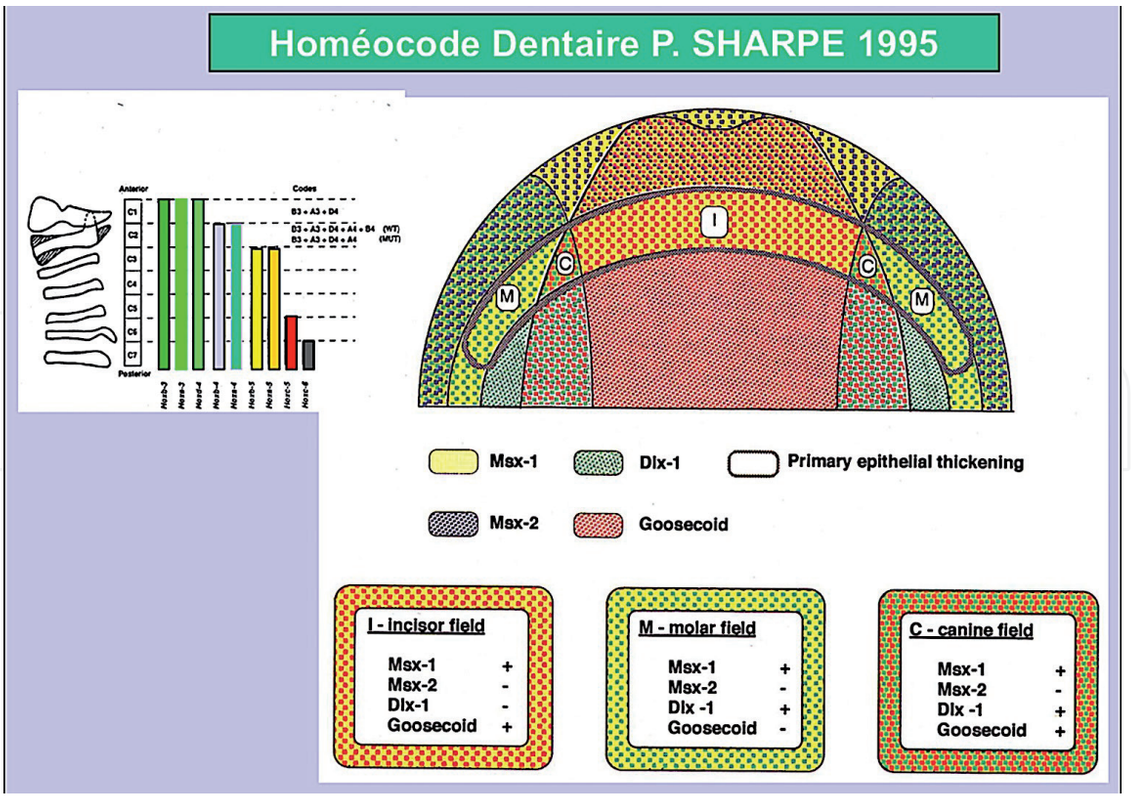

Figure 3.
(A) Regional field theory. (B) Clone theory. (C) Homeobox [26–28].

This odontogenic homeocode theory involves four homogenous genes: muscle segment homeodomain-homeobox 1 (Msx-1), muscle segment homeodomain-homeobox 2 (Msx-2), distal-less homeobox 1 (Dlx1), and goosecoide. In the molar sector, Msx-1 and Dlx-1 are expressed and Msx-2 and goosecoide are not expressed. In the canine sector, Msx-1, Msx-2, and goosecoide are expressed, and Dlx-1 is not expressed; in incisal sector, Msx-1 and goosecoide are expressed, Msx-2 and Dlx-1 are not expressed.

In the concept of morphogenetic fields, the consideration of various genetic factors and their epigenetic modulation influences dental development [29].

According to Mitsiadis' work in 2006, the three models, gradients, clones, and homeocodes, could be grouped into a single model to explain dental identity. Indeed, dental identity, including molars, is given by the presence of morphogenetic fields defined by the diffusion of growth factors. The odontogenic epithelium expresses gradients of signaling molecules that are mainly Fgf, Bmp, Shh, and Wint that will diffuse to the underlying mesenchymal tissue containing neural peak cells. Depending on the location and instruction received by these cells, they will express a set of divergent genes in relation to concentrations of signaling molecules. The locally defined tooth type is related to the locally expressed divergent homeogen combinatorics of these ridge cells (**Figure 4**) [30].

The Mitsiadis model combines the three concepts: morphogenetic fields, clone, and odontogenic homeocode.

These three models should be viewed as complementary rather than contradictory and propose that this unifying view can be extended into the clinical setting using findings on dental patterning in individuals with missing teeth. The proposals are compatible with the unifying etiological model developed by Brook in 1984 based on human epidemiological and clinical findings. Indeed, this new synthesis can provide a sound foundation for clinical diagnosis, counseling, and management of patients with various anomalies of dental development, as well as suggesting hypotheses for future studies.

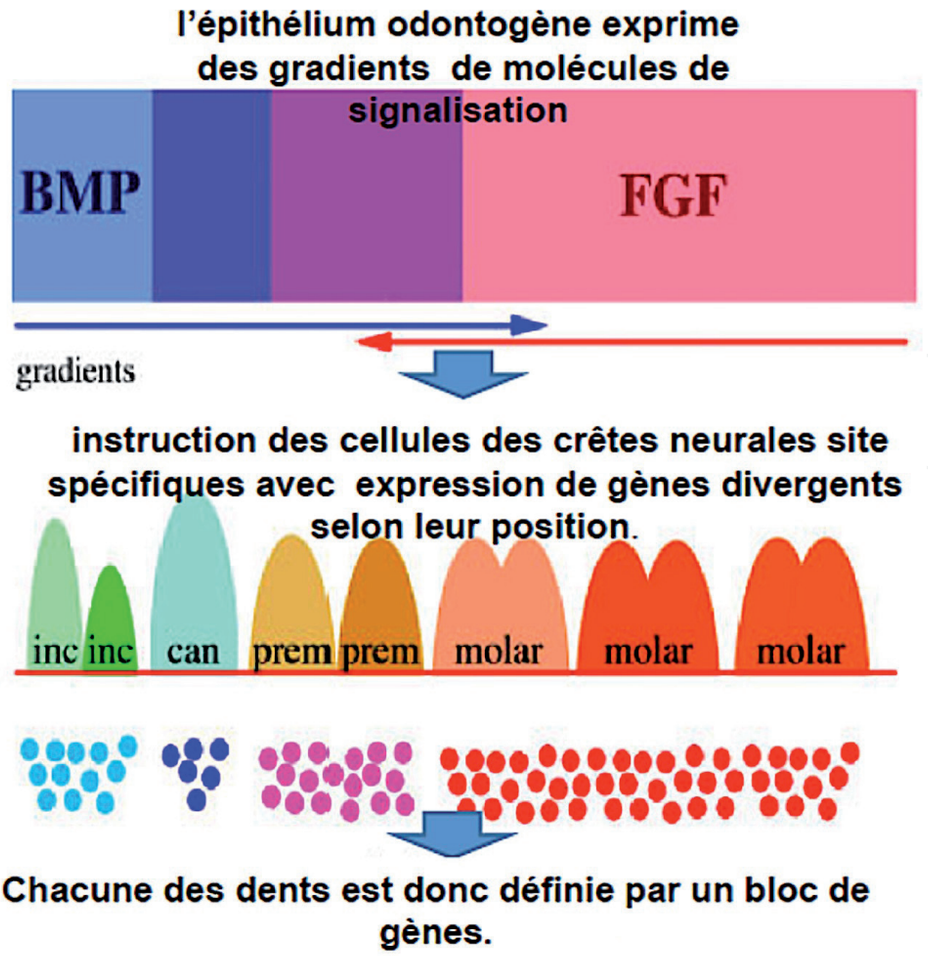

Figure 4.
Dental identity determination (adapted from Ref. [30]).

6.4 Molecular factors involved in root formation

The root development process involves a set of signaling cascades. Various growth factors, including BMPs (bone morphogenetic proteins), EGF (epidermal growth factor), IGF (insulin-like growth factor), FGF (fibroblast growth factor), transcription factors Msx1, Msx2, Runx-2, Sonic Hedgehog (Shh), enamel proteins (secreted by HGH cells), and other proteins such as follistatin and activin A, are involved in the root development process. Indeed, they are involved in the growth and/or differentiation of odontoblasts and cementoblasts and/or in the mineralization of dentin and/or cementum [21, 31–36].

7. Signaling center (primary and secondary enamel knots)

Dental morphology is controlled by an epithelial signaling center called the enamel node. The node of the enamel is a particular and transient histological structure formed by a cellular cluster that appears at the basal part of the internal dental epithelium. The node of the primary enamel is present in the dental germs of all types of teeth including incisors.

Because the enamel nodes link cell differentiation to morphogenesis, Thesleff suggests that the latter can be considered as central regulators of dental development [37].

During molar development, the node of the secondary enamel is formed during the bell stage at the location of future cusp areas. At this point, the

expression of signaling molecules precedes the folding and growth of the dental epithelium [38, 39].

The Slit1 gene is expressed in the nodes of the primary and secondary enamel during the formation of molar cusps [40].

8. Genes and dental problems

The approaches provided by Line and Mitsiadis have advanced the clinic's understanding of dental identity establishment based on gradient, clone, and homeocode theories [29, 30].

The multifactorial model involving genetic, epigenetic, and environmental determinants has provided better explanations and helped to understand missing and supernumerary teeth in monozygotic twins [41].

In humans, dental problems are observed during pathologies of dental development or syndromes.

Mutations in genes known as divergent homeobox genes encoding transcription factors such as MSX1 and PAX9 (paired domain box gene 9) are at the origin of oligodontia. Indeed, a mutation in the homeobox of the MSX1 gene (substitution of an arginine by a proline in the homeodomain region) is associated with the agenesis of third molars, indicating the involvement of MSX1 in the dentition pattern [42–44].

Also, mutations in the PAX9 gene cause oligodontia characteristic of molars [45–48]. The severity of dental agenesis appears to be correlated with the ability of the mutated PAX9 protein to bind to DNA [49].

A misdirection mutation during the sequencing of the PAX9 gene may explain a different phenotype of hereditary oligodontia observed in humans, which affects not only molars but also other tooth lines; and is characterized by tooth small size in both types of dentition. This mutation is characterized by a replacement of the amino acid arginine by tryptophan in a region entirely preserved in all genes of the matched sequenced box [50].

In humans, Pitx2 expression deficiency associated with Rieger syndrome is characterized by oligodontia [51].

9. Conclusion

The biological process is the same for all teeth, including molars, regardless of their identity, but epithelial signaling and homeogenic combination differ from one tooth type to another.

The study of first molar of the mouse has allowed us to better understand and follow the stages of dental development in humans. The general pattern remains the same, unlike the training time, the complexity of the dental system, the presence of two types of teeth in humans, and unlimited incisors growth in mice.

The multidisciplinary approach between fundamental and clinical research is essential to clarify the relationship between molecular involvement and clinical manifestations.

Understanding the molecular mechanisms of dental anomalies, including those affecting human molars, helps to propose diagnostic hypotheses and thus to improve patient management.

Future research should focus on synergizing molecular and genetic approaches to further analyze the action mechanisms of key genes involved in the development of human molars.

Conflict of interest

The authors declare that they have no conflicts of interest with the contents of this article.

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