Development of Teeth☆

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Introduction

Tooth development, or odontogenesis, includes the processes of tooth formation, eruption, and its integration with the supporting periodontal tissues, jaw bones, and the circulatory and nervous systems. Humans have two sets of dentitions, known as the deciduous (primary or baby) teeth and the permanent (secondary or adult) teeth. Both dentitions undergo the same developmental process to form teeth, although the permanent teeth are formed later than and replace the deciduous teeth.

Teeth are used mainly to bite and chew food, and are therefore hard, calcified organs. However, in the early stages of tooth development, the tooth germs are soft tissue organs composed of epithelial and connective tissues. The former is derived from the early embryonic oral ectoderm whereas the latter is derived from the neural crest.

Once initiated, tooth germs undergo morphogenesis followed by differentiation into distinct types of functional cells, including those that make the two main hard tissues of the teeth: enamel and dentin. In addition, upon completion of the formation of the crown (the portion that is exposed to the oral cavity), the tooth germ continues to form the root (the portion that is embedded in and attached to the jaw bones). The major hard tissue of the root is dentin, which is covered with a thin hard tissue layer, called the cementum. At this stage, the teeth also establish an attachment to the jaws via three layers of supporting tissues, the cementum, periodontal ligament (PDL), and alveolar bone. During this process, a soft tissue of ectomesenchymal origin remains at the center of the teeth, enclosed by the surrounding hard tissues, and becomes the pulp.

Genetic studies have identified mutations in particular genes that are associated with various tooth developmental disorders in humans, which are often found together with other birth defects. By using various experimental models with genetic alteration, scientists have uncovered the underlying molecular mechanisms that control tooth development.

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Studies have shown that tooth development involves interactions between the epithelial and ectomesenchymal tissues mediated by multiple signaling pathways and transcription factors that are shared with development and/or maintenance of other organs. Aberration in the complex signaling networks that regulate tooth development may result in various developmental defects and tooth anomaly. These dental disorders are often associated with other birth defects and syndromes.

Here, we review the morphological changes that occur during development of teeth at the organ and cellular levels, as well as the current understanding of the genetic basis and molecular mechanisms underlying tooth development and associated developmental disorders in humans.

Initiation Stage

Epithelial Thickening

The earliest morphological sign of tooth development is the appearance of the primary epithelial band, which is a U-shaped band of thickened epithelium formed along the maxillary and mandibular dental arch. This epithelial thickening occurs in the facial processes in the fifth week of embryonic development in humans (Ooe, 1957).

Dental Lamina

The primary epithelial band gives rise first to the dental lamina on the inside of the dental arch and shortly afterward to the vestibular lamina on the outside of the dental arch, which both grow into the underlying ectomesenchyme. The dental lamina is where the future tooth germs will form, while the vestibular lamina eventually degenerates to form the future vestibule or sulcus between the cheek and the tooth-forming area. Both the primary epithelial band and dental lamina serve as a foundation for future development of multiple individual tooth germs.

Dental Placode

Dental placodes are localized thickenings in the dental lamina that initiate formation of individualized tooth germs. In humans, the entire deciduous dentition is initiated between 6 and 8 weeks of embryonic development.

At the initiation stage, transcription factors, such as *paired-like homeodomain 2* (*Pitx2*), *forkhead box I3* (*Foxi3*), *distal-less 2* (*Dlx2*), *lymphoid enhancer-binding factor 1* (*Lef1*), and *p63* (Oosterwegel et al., 1993; Mucchielli et al., 1997; Thomas et al., 1997; Shirokova et al., 2013), and signaling molecules that belong to the bone morphogenetic protein (BMP), fibroblast growth factor (FGF), and wingless-related (Wnt) families, such as Bmp2/4/7, Fgf8/9, Wnt4/6/10a/10b, and Sonic hedgehog (SHH), are expressed in the oral epithelium at the tooth-forming sites (Wozney et al., 1998; Lyons et al., 1989; Vainio et al., 1993; Tureckova et al., 1995; Neubuser et al., 1997; Dassule and McMahon, 1998; Sarkar and Sharpe, 1999; Tucker et al., 1998). Scientists have found that, by experimentally inhibiting any of these signaling pathways in the mouse embryo at the initiation stage, tooth development could be arrested at the lamina or early bud stage (Jernvall and Thesleff, 2000; Tucker and Sharpe, 2004; Song et al., 2006; Bei, 2009; Tummers and Thesleff, 2009; Jussila and Thesleff, 2012). Therefore, the early embryonic oral ectoderm is believed to provide the initial signals for tooth development.

Tooth Germ Morphogenesis Stages

Individual tooth germs undergo morphogenesis through the bud, cap, and bell stages. These stages are termed according to the shape of the tooth germ viewed from frontal sections.

Bud Stage

The bud stage is characterized by the budding of the dental epithelium from the dental lamina into the underlying ectomesenchyme, with the ectomesenchymal cells condensing around the epithelial bud. The bud stage tooth germs of the deciduous teeth are first observed in the 8th week in utero.

Classical embryological studies using mouse tissue recombination assays have shown that, in contrast to the early embryonic oral epithelium, the bud stage oral epithelium can no longer induce tooth formation when recombined with non-dental mesenchymal tissues, whereas the tooth mesenchyme at and after this stage can induce tooth formation when recombined with various embryonic epithelial tissues (Mina and Kollar, 1987).

Extensive studies using mouse models have shown that evolutionarily conserved transcription factors expressed in the dental mesenchyme, including *muscle segment homeobox 1/2* (*Msx1/2*), *paired box 9* (*Pax9*), *LIM homeobox 6/7* (*Lhx6/7*), *Dlx1/2*, and *runt-related transcription factor 2* (*Runx2*), are essential for tooth development around the bud stage. Mutant mice with disruption of the *Msx1*, *Pax9*, or *Runx2* gene show tooth developmental arrest at the bud stage (Satokata and Maas, 1994; Peters et al., 1998), whereas compound mutant mice with loss of function of the *Msx1/2*, *Dlx1/2*, or *Lhx6/Lhx7* (only molars) genes show arrest at the dental lamina stage (Otto et al., 1997; Thomas et al., 1997; Denaxa et al., 2009). Expression of these transcription factors in the developing

tooth mesenchyme is regulated by BMP and FGF signaling pathways and is required for activation of key mesenchymal signals that are essential for subsequent tooth germ morphogenesis.

The major signaling pathways, including the BMP, FGF, and Wnt signaling pathways, are also required for the bud stage tooth germ to progress to the cap stage. Genetic inactivation of *Bmp receptor type 1a* (*Bmpr1a*), which encodes a subunit of the BMP receptors, in either the dental epithelium or mesenchyme resulted in tooth developmental arrest at the bud stage (Andl et al., 2004; Liu et al., 2005; Li et al., 2011a, b). Tissue-specific deletion of *Bmp4* in the dental mesenchyme or overexpression of the BMP antagonist Noggin in the dental epithelium led to a less severe tooth developmental defect, causing bud stage arrest of mandibular molars while other tooth germs continued development (Plikus et al., 2005, Jia et al., 2013). Whereas inactivation of *InhibinβA* (*Inhba*, also known as *ActivinβA*), another member of the TGFβ superfamily, led to bud stage arrest of all tooth germs except the maxillary molars (Ferguson et al., 1998), simultaneous inactivation of both the *Bmp4* and *InhibinβA* genes in the dental mesenchyme resulted in arrest of all teeth at the early bud stage (Kwon et al., 2017).

Inactivation of the *FGF receptor-2* (*Fgfr2IIIb*) gene also resulted in tooth developmental arrest at the bud stage (De Moerlooze et al., 2000). Whereas FGF8, expressed by the dental epithelium, induces *Fgf3* expression in the dental mesenchyme via the key transcription factor Msx1 (Bei and Maas, 1998), tissue-specific deletion of *Fgf8* in the oral epithelium or genetic inactivation of both the *Fgf3* and *Fgf10* genes resulted in tooth developmental arrest at the initiation stage (Trumpp et al., 1999; Wang et al., 2007).

Canonical Wnt signaling is also essential for early tooth germ morphogenesis. Concurrent with the shift of odontogenic potential from the oral epithelium to dental mesenchyme, expression of *Lef1*, a direct target of canonical Wnt signaling, is initially activated in the dental placodes and subsequently also activated in the dental mesenchyme at the bud stage (Kratochwil et al., 1996). Tissue-specific inactivation of β-catenin, the intracellular transducer of canonical Wnt signaling, in either the dental epithelium or mesenchyme resulted in tooth developmental arrest at the bud stage (Liu et al., 2008; Chen et al., 2009).

Through extensive whole transcriptome gene expression profiling analysis of early tooth epithelium and mesenchyme tissues combined with mathematical modeling of the reciprocal epithelial-mesenchymal interactions, O'Connell et al. identified a Wnt-Bmp feedback circuit as the major regulator of expression of major signaling molecules that control early tooth germ morphogenesis, including BMP4, SHH, FGFs, and Wnts in the epithelium and FGFs, BMP4, and Activin-A in the mesenchyme (O'Connell et al., 2012). Tissue specific inactivation of the *adenomatous polyposis coli* (*Apc*) gene, which encodes a negative regulator of canonical Wnt signaling, in the oral epithelium induced constitutive activation of canonical Wnt signaling and resulted in formation of supernumerary teeth, even in the absence of Msx1 or Pax9 (Wang et al., 2009; O'Connell et al., 2012). More recently, a series of studies have demonstrated that the BMP4-Msx1 pathway and Activin signaling converge on the regulation of expression of several secreted Wnt antagonists, including Dkk2 and Sfrp2, in the developing tooth mesenchyme (Jia et al., 2013, 2016; Kwon et al., 2017). Moreover, pharmacological inhibition and/or genetic inactivation of the secreted Wnt antagonists Dkk2 and Sfrp2/Sfrp3 could rescue tooth morphogenesis in mice lacking Bmp4, Msx1, or Activin-A in the tooth bud mesenchyme (Jia et al., 2016; Kwon et al., 2017).

Cap Stage

The cap stage is characterized by the epithelial component of the tooth germ resembling a cap sitting on top of a spherical ectomesenchyme aggregation. This epithelial component is termed the enamel organ, which will differentiate into ameloblasts that make the enamel of the tooth. The spherical ectomesenchyme aggregation is termed the dental papilla, which will make the dentin and pulp. Another group of ectomesenchymal cells, termed the dental follicle or sac, encapsulates the enamel organ and the dental papilla. Thus, by the cap stage, the tooth germ is composed of the enamel organ, dental papilla, and the dental follicle.

Blood vessels are found around the tooth germ in the dental follicle and entering the dental papilla at the cap stage. Nerve fibers are also found around the tooth germ in the dental follicle but not in the dental papilla until the bell stage when dentinogenesis begins.

An important structure that is present at the cap stage is the enamel knot, which is a cluster of non-dividing cells expressing *p21*, also known as *cyclin-dependent kinase inhibitor 1* (*Cdkn1*), in the enamel organ. The primary enamel knot (PEK) forms just prior to transition from the bud stage to the cap stage at the distal tip of the tooth bud and serves as a signaling center, where BMPs (Bmp2/4/7), FGFs (Fgf3/4/9/20), Wnts (Wnt3/6/10a/10b), and SHH signals are locally secreted. The current view is that the enamel knot promotes growth of its surrounding dental epithelial cells and thus regulates the folding of the enamel organ to form the "cap" and subsequently the "bell" shape, eventually organizing the cuspal patterning of the crown. Each tooth germ has one PEK at the cap stage, which is distinguished from the secondary enamel knots (SEKs) that subsequently form at the tips of the future cusps in the bell stage molar tooth germs. Both FGF and Shh signaling pathways play a crucial role in tooth morphogenesis during the cap-to-bell transition (Dassule et al., 2000; Jeong et al., 2004; Wang et al., 2007).

Bell Stage

The bell stage is characterized by the enamel organ growing into a bell shape as its undersurface deepens and encases the dental papilla, while the dental sac remains encapsulating both the enamel organ and the dental papilla.

Two important events take place at this stage: histodifferentiation and morphodifferentiation. Histodifferentiation refers to differentiation of cells of the enamel organ into four distinct cell groups based on their morphology and function: the inner enamel epithelium (IEE), stratum intermedium, stellate reticulum, and outer enamel epithelium (OEE). The IEE cells are column-shaped

epithelial cells that compose the undersurface of the enamel organ and are positioned adjacent to the dental papilla. Stellate reticulum cells are star-shaped cells situated in the center of the enamel organ and synthesize and secrete glycosaminoglycan into the intercellular space, which draws water into the intercellular space. Intercellular water separates the cells apart, while intercellular adhesions remain through desmosomes, resulting in a star-shaped appearance of the stellate reticulum cells. Stratum intermedium is a stratified layer of cells situated between the IEE and the stellate reticulum. Stratum intermedium cells show high activity of alkaline phosphatase and act, together with the IEE cells, as a single functional unit in enamel formation. The OEE cells are cube-shaped epithelial cells on the periphery of the enamel organ. The OEE and the IEE are continuous and their junction forms the rim of the bell, termed the cervical loop. Cervical loop cells continue to proliferate through the crown stage and play crucial roles in root formation.

Morphodifferentiation refers to morphogenesis of the enamel organ, via folding of the IEE layer, into a three-dimensional structure that resembles the future tooth crown. During the transition from the cap stage to the bell stage, the PEK disappears by apoptosis and SEKs begin to form within the enamel organ at the future locations of the cusps. Similar to the PEK, SEKs consist of non-proliferative cells and act as signaling centers that regulate differential rates of proliferation in the IEE and folding of the enamel organ, thus determining the positions and size of the tooth cusps in molar teeth (Jernvall et al., 1994). Several secreted molecules, including ectodysplasin-A (Eda), Fgf3, follistatin (Fst), sprouty 2/4 (Spry2/4), and sclerostin domain containing 1 (Sostdc1), play crucial roles in cuspal patterning (Pispa et al., 1999; Laurikkala et al., 2003, Wang et al., 2004a, b, 2007; Kassai et al., 2005; Klein et al., 2006). Fst, Spry2/4, and Sostdc1 are antagonists of the BMP, FGF, and Wnt signaling pathways, respectively, indicating that cuspal patterning involves the major signaling pathways and is regulated by complex and fine-tuned activator-inhibitor balance within and around the enamel knots.

Another important event that takes place at the bell stage is the breakup of dental lamina, which until then connects the enamel organ to the oral epithelium. Connection between the enamel organ and the oral epithelium is reestablished later when teeth begin to erupt.

Crown Formation

The crown-forming stage is characterized by deposition of the two main mineralized tissues, enamel and dentin, which are produced by the ameloblasts and odontoblasts, respectively. These events require terminal histodifferentiation of the IEE and dental papilla into ameloblasts and odontoblasts, respectively, via progressing through the presecretory stage, secretory stage, and maturation stage. Therefore, this stage is also called the advanced or late bell stage, or maturation stage.

Histodifferentiation and enamel/dentin formation begin at the interface between the IEE and the dental papilla. Starting at the points where the future cusps will develop, the IEE cells differentiate into preameloblasts, showing polarization and cease of cell proliferation. Their nuclei become located away from the dental papilla while the Golgi complex become located closer to the dental papilla. These changes induce differentiation of adjacent undifferentiated ectomesenchymal cells in the dental papilla to become column-shaped preodontoblasts. Preodontoblasts further differentiate into odontoblasts and synthesize and secrete a collagen type I-based organic matrix termed predentin, and begin to migrate away from the adjacent preameloblasts while leaving behind their odontoblast processes, cytoplasmic extensions, in the dentin matrix. This results in formation of the dentinal tubules. Differentiation in the IEE and dental papilla cells progress along the cusp slope.

Once the odontoblasts are completely differentiated, they secrete the first layer of predentin matrix, which triggers the terminal differentiation of preameloblasts into ameloblasts. Ameloblasts secrete an organic matrix on the newly formed dentin layer that almost immediately mineralizes into the initial layer of the enamel. Ameloblasts migrate away from the dentin and continue to deposit enamel on their way. In addition to predentin, the odontoblasts secrete non-collagenous proteins, such as the Dentin Sialophosphoprotein (Dspp), through the odontoblast processes to facilitate mineralization of the predentin.

Signaling Pathways that Regulate Odontoblast Differentiation

Both BMP/transforming growth factor-β (TGFβ) and canonical Wnt signaling pathways are important in odontoblast differentiation.

Mutant mice with conditional deletion of the BMP/transforming growth factor-β (TGFβ) pathway components, such as *Bmp4*, *Tgfβr2*, or *Smad4*, resulted in defects in odontoblast differentiation and dentin production (Oka et al., 2007; Gluhak-Heinrich et al. 2010; Li et al., 2011a, b; Wang et al., 2013; Yun et al., 2016; Kim et al., 2015). Conditional deletion of *Runx2*, a key transcription factor in osteoblast differentiation, also resulted in defective odontoblast differentiation (Miyazaki et al., 2008).

Wnt10a, *Axin2*, and *Dkk1* are expressed in odontoblast cells (Fjeld et al., 2005; Yamashiro et al., 2007; Lohi et al., 2010). Experiments have shown that Wnt10a inhibits proliferation and promotes differentiation of the odontoblasts (Liu et al., 2013; Yamashiro et al., 2007). In addition, overexpression of *Lef1* or constitutive stabilization of β-catenin in the dental mesenchyme resulted in premature differentiation of odontoblasts and excessive formation of dentin (Yokose and Naka, 2010; Kim et al., 2011). On the other hand, conditional deletion of *Wntless* (*Wls*), a transmembrane protein that regulates Wnt secretion, resulted in reduced dentin formation and wider pulp chamber due to decreased Wnt signaling activity in odontoblasts (Bae et al., 2015).

Signaling Pathways that Regulate Ameloblast Differentiation

Bmp signaling is important for differentiation of ameloblasts. Conditional deletion of *Bmp4* and/or *Bmp2* in the ameloblast cells resulted in defective differentiation and formation of hypoplastic enamel (Feng et al., 2011; Xie et al., 2016). Ameloblast differentiation also depends on Bmp4 signals secreted by the adjacent odontoblast layer (Gluhak-Heinrich et al., 2010).

Shh signaling is also important for ameloblast polarization and enamel formation. Mice lacking either Shh or its signaling transducer Smoothened (Smo) in the dental epithelium exhibit defects in cell polarity and organization in the ameloblast layer and disorganized enamel (Dassule et al., 2000; Gritli-Linde et al., 2002). Ameloblasts and enamel formation also depend on genes expressed in the adjacent SI layer, such as cell-cell adhesion molecules Nectin1/3 and cell membrane protein Perp (Jheon et al., 2011; Neupane et al., 2014; Yoshida et al., 2010).

Root Formation

The tooth root is essential for tooth function. It consists of root dentin and root canals, where dental pulp cells, including undifferentiated mesenchymal cells, blood vessels, and nerves that support the dental pulp tissue reside. Outside, the root is covered with cementum and provides attachment to the alveolar bone via fibrous connective tissue structures termed the periodontal ligaments (PDL). The main tissues that contribute to root formation are the Hertwig's epithelial root sheath (HERS), dental papilla, and dental follicle (reviewed by Li et al., 2017).

Upon completion of crown formation, the cervical loop cells continue to proliferate and become the HERS. The HERS extends toward the apex of the future root, enclosing the dental papilla, while continuously interacting with the surrounding ectomesenchymal cells. Ectomesenchymal cells of the dental papilla adjacent to the inner layer of the HERS undergo differentiation into odontoblasts and form the root dentin.

The HERS is also important for cementum and PDL formation. As root formation progresses, the dental follicle cells come in contact with the HERS while the HERS itself becomes perforated into a mesh network structure. This allows the dental follicle cells to come in contact with the root dentin surface and induces them to differentiate into cementoblasts, which produce cementum matrix proteins, and fibroblasts, which produce the PDL. In addition, some HERS-derived cells differentiate into cementocytes (Huang et al., 2009). Further on, the HERS rapidly disintegrates and becomes small clusters of epithelial cells, known as the epithelial cell rests of Malassez (ERM). In adults the ERM persists in the PDL space around the root surface.

The HERS determines the number of tooth roots by forming a pair of tongue-shaped extensions that fuse with each other (Orban 1980, Ten Cate, 1996). The joining of a pair of HERS extensions occurs at the base of the future dental pulp chamber, forming a continuous HERS bridge that results in formation of the root furcation.

Signaling Pathways that Regulate Tooth Root Formation

Tooth root development occurs through epithelial-mesenchymal interactions and involves the major signaling pathways, including Wnt, Bmp/Tgfβ, Fgf, and Shh pathways.

Tissue-specific inactivation of the canonical Wnt signaling transducer *β-catenin* in the ectomesenchyme resulted in severe root formation defects (Kim et al., 2013), and other studies showed that disruption of Wnt signaling in the ectomesenchyme affected root elongation (Han et al., 2011; Bae et al., 2015; Yang et al., 2015). Interestingly, constitutive activation of canonical Wnt signaling in the ectomesenchyme also caused root elongation defects (Kim et al., 2011, 2012).

Bmp signaling is important in the dental epithelium for proper HERS formation and elongation. Adjacent to the HERS, Bmp2/3/7 ligands are expressed in the early stage odontoblasts (Yamashiro et al., 2003). Tissue-specific overexpression of the Bmp signaling antagonist Noggin or deletion of the *Bmpr1a* or *Smad4* gene in the dental epithelium resulted in severe disruption of root formation and elongation (Plikus et al., 2005; Huang et al., 2010; Lapthanasupkul et al., 2012; Rakian et al., 2013; Yang et al., 2013; Li et al., 2015). On the other hand, Tgfβ signaling is important in the ectomesenchyme, rather than in the dental epithelium, for root formation (Oka et al., 2007, Gao et al., 2009; Wang et al., 2013; Li et al., 2015).

Modulation of Shh signaling is important during transition from crown formation to root formation. Shh signaling is active in the cervical loop at the bell stage before HERS formation, but is dramatically reduced once the HERS is formed (Nakatomi et al., 2006; Li et al., 2015). The Smad4-mediated Bmp/Tgfβ signaling pathway promotes root formation by regulating Shh signaling in the cervical loop (Li et al., 2015). On the other hand, constitutive activation of Hh signaling in the ectomesenchyme around the HERS also disrupts root elongation (Nakatomi et al., 2006; Liu et al., 2015).

Nfic regulates the Smad2/3-mediated Tgfβ signaling and promotes Shh signaling in the odontoblasts (Steele-Perkins et al., 2003; Lee et al., 2009; Liu et al., 2015). On the other hand, Shh could induce *Nfic* expression in the ectomesenchyme (Huang et al., 2010). The Parathyroid hormone (PTH) signaling regulates root formation via inhibition of *Nfic* expression in the ectomesenchyme (Ono et al., 2016).

Studies on continuously growing teeth, such as the mouse incisors or the vole molars, suggest that regulation of *Fgf10* expression in the ectomesenchyme surrounding the cervical loop is required for the formation of HERS and initiation of root formation (Tummers and Thesleff, 2003; Yokohama-Tamaki et al., 2006). In addition, tissue culture experiments show that insulin growth

factor 1 (IGF1) and hepatocyte growth factor (HGF) may also promote proliferation in HERS during root formation (Fujiwara et al., 2005; Sakuraba et al., 2012).

Tooth Eruption

Once root formation is initiated, the tooth begins to move vertically toward the oral cavity in order to erupt into a functional position and form the occlusal plane. Eruption occurs until the tooth reaches its final position in the oral cavity. At the beginning, the overlying alveolar bone is resorbed and other connective tissues are broken down to facilitate vertical movement of the tooth. During eruption, the crown passes through the gingival epithelium. The reduced enamel epithelium, which covers the crown and is composed of the ameloblast layer and the other three layers of the enamel organ, fuses with the gingival epithelium to form a solid mass of epithelial cells overlying the crown. Cells in the center of this mass degenerate to facilitate tooth eruption. As the tooth passes through the gingival epithelium, the dentogingival junction forms from the reduced enamel epithelium and gingival epithelium.

Periodontal Tissue Formation

The periodontal tissues are tissues supporting the tooth and are composed of the cementum, PDL, alveolar bone, and the gingiva. They begin to develop during root formation mainly from the dental follicle-originated cells. Particular dental follicle cells that penetrate between the disintegrated HERS cells and come in contact with the root predentin/dentin surface differentiate into cementoblasts, which secrete an organic matrix that is mineralized to form the cementum layer on the surface of the root (described above in Root Formation). Recent studies suggest that HERS cells can also differentiate into cementoblasts (Huang et al., 2009; Xiong et al., 2013; Bosshardt et al., 2015). The dental follicle cells also differentiate into osteoblasts, which form the alveolar bone surrounding the tooth, and fibroblasts, which form the PDL that anchors the tooth to the alveolar bone.

Development of the Permanent Dentition

In human, the deciduous and permanent dentitions essentially develop in the same manner, although at different times. The tooth germs of the permanent incisors, canines, and premolars develop from the dental lamina, which has already given rise to the deciduous tooth germs. Thus, these permanent teeth are termed successional teeth. Replacement of the deciduous dentition by the successional dentition involves exfoliation of the deciduous teeth. The tip of the dental lamina, from which the deciduous tooth germ once developed, continues to extend distally via proliferation until it forms the bud of the successional tooth on the lingual aspect of the deciduous tooth germ. In contrast, permanent molars do not have deciduous predecessors and they develop from the posterior, or distal, extension of the dental lamina when the dental arch has grown long enough. Therefore, permanent molars form posterior to the deciduous dentition when sufficient jaw growth has occurred to accommodate these tooth germs.

The successional permanent teeth are initiated at 20 weeks in utero $\sim$ 10 months after birth; the permanent molars develop at 20 weeks in utero (first molar) $\sim$ 5 years of age (third molar).

Developmental Disorders of Teeth

As normal tooth development requires precise regulation of multiple molecules, signaling pathways, and cellular events, any aberration in these components may affect the formation of the tooth, resulting in tooth developmental disorders with alteration in number, size, or shape of the dentition. Moreover, these disorders may be isolated findings but can also be commonly associated with other developmental defects or as part of a syndrome (reviewed by Klein et al., 2013).

Tooth Agenesis and Hypodontia/Oligodontia/Anodontia

Tooth agenesis is defined as the congenital absence of teeth due to failure of tooth formation. Mutant mouse model studies showed that developmental arrest at the early stage of tooth development, usually the bud stage, leads to tooth agenesis. Tooth agenesis is one of the most common developmental anomalies, and clinically it is divided into hypodontia (one to six teeth missing), oligodontia (more than six teeth missing), and anodontia (all teeth missing), depending on the number of missing teeth (excluding the third molars, one or more of which is missing in nearly 20% of people).

Mutations in the *MSX1*, *PAX9*, *AXIN2*, *EDA*, and *WNT10A* genes have been associated with non-syndromic hypodontia (Vastardis et al., 1996; Stockton et al. 2000; Lammi et al., 2004; van den Boogaard et al., 2012; Yang et al., 2013). Hypodontia can also result from environmental factors, such as trauma, radiation or chemotherapy (Schalk-van der Weide et al., 1993; Näsman et al., 1997). Therefore, non-syndromic hypodontia can occur sporadically or in a familial form. More than 100 different craniofacial syndromes include hypodontia as a clinical feature. Some of the examples and associated genes are Van der Woude

syndrome (VWS, OMIM 119300) (*IRF6*), ectodermal dysplasia (*EDA* and *WNT10A*), oral-facial-digital syndrome type I (OFD type 1, OMIM 311200) (*OFD1*), Axenfeld-Rieger syndrome (ARS, OMIM 180500) (*FOXC1* and *PITX2*), and holoprosencephaly (HPE, OMIM 236100) (*SHH*). In addition, patients with orofacial clefting, especially cleft lip and palate, often exhibit selective tooth agenesis (reviewed by Phan et al., 2016).

Supernumerary Teeth

Supernumerary teeth are extra teeth that can form anywhere in the dental arch (reviewed by Wang and Fan, 2011). The most common form is a mesiodens, which forms between the two maxillary central incisors (Hyun et al., 2009), but supernumerary teeth can also be found in the premolar and at the posterior end of the dentition (Hyun et al., 2008; Kawashita and Saito, 2010). Supernumerary teeth occur at higher frequency in males than in females (Brook, 1984). Some of the syndromes and associated genes, including cleidocranial dysplasia (CCD, OMIM 119600) (*RUNX2*) and familial adenomatous polyposis (FAP, OMIM 175100) (*APC*), are associated with supernumerary tooth formation.

Anomalies that Affect the Size and Shape of Teeth

Aberration of morphogenesis during the cap and bell stages are believed to result in tooth anomalies with abnormal tooth size and shape. Macrodontia, or abnormally larger teeth, is a rare anomaly that is associated with hormonal imbalance, such as pituitary gigantism (Groper, 1987). Microdontia, or abnormally smaller teeth, commonly affect the maxillary lateral incisors and the third molars.

A double tooth is a union of two adjacent teeth by their dentin and/or pulp. It may be either a gemination or a fusion. Gemination is a splitting of a single tooth germ, while fusion is when two distinct tooth germs fuse together (Shashirekha and Jena, 2013; Hattab, 2014). Concrescence is a joining of two adjacent teeth by cementum but not dentin.

Taurodontism (meaning 'bull teeth') is an apical enlargement and displacement of both the dental pulp and the furcation in multirooted teeth, accompanied by reduced/absent constriction at the cemento-enamel junction (also discussed later in Root Developmental Defects). Taurodontism is associated with genetic mutations in the genes *DLX3* and *WNT10A* (Price et al., 1999; Dong et al., 2005). It is also closely associated with hypodontia, both non-syndromic and syndromic (reviewed by Klein et al., 2013).

Examples of formation of extra cusps are the cusp of Carabelli, talon cusp, dens evaginatus, and dens invaginatus. The cusp of Carabelli can be found on the palatal surface of the mesiolingual cusp of the maxillary molars. A talon cusp can be found on the cingulum of anterior teeth. Dens evaginatus can be found on the buccal cusp of premolars, and less likely in molars. Den invaginatus (dens in dente, meaning 'tooth in tooth'), on the other hand, is an invagination of the enamel surface into the crown, varying in depth.

Root Developmental Defects

Developmental disorders in the tooth root are either an isolated disorder or associated with general tooth dysplasia (reviewed by Luder, 2015). Examples of isolated disorders are premature arrest of root formation, root dilaceration, malformation, short root anomaly, and taurodontism. Affected teeth may show abnormal length, shape, dentin thickness, and pulp chamber size and be associated with mechanical trauma, infection, or radiation/chemotherapy during root formation. On the other hand, taurodontism (described above in Anomalies that Affect the Size and Shape of Teeth) is associated with genetic mutations.

There are also root developmental defects associated with general tooth dysplasias, such as double teeth, hypophosphatasia, regional odontodysplasia, dentinogenesis imperfecta (DI), and dentin dysplasia (DD) (DI and DD are discussed later in Disorders of Hard Tissue Development).

Disorders of Hard Tissue Development

As the formation of enamel and dentin involves several stages: secretion, mineralization, and maturation, aberration at any stage may lead to alteration in the structure of the hard tissues of the teeth.

Amelogenesis Imperfecta

Amelogenesis imperfecta (AI) is a hereditary developmental disorder of enamel formation in the absence of a systemic involvement. Affected teeth have abnormal color (yellow, brown, or gray), are more susceptible to dental caries (cavities), tooth attrition, calculus apposition, and gingival hyperplasia, and are often accompanied by anterior open bite.

AI is classified into three patterns depending on which stage is affected: hypoplastic, hypocalcified, and hypomaturation. (1) Hypoplastic AI teeth result from inadequate secretion of the enamel matrix, which is still normally mineralized. Therefore, affected teeth have inadequate amount of enamel and commonly show multiple scattered pits on the surface of the enamel. (2) Hypocalcified AI teeth are initially formed with proper amount of enamel, but due to absence calcification, they show lower

density, which is easily found in radiographs, and their enamel are soft and easily lost. (3) Hypomaturation AI teeth are also initially formed with proper amount of enamel, which is calcified to a certain extent, but undergo defective maturation of the crystal structure of the enamel, resulting in soft enamel that is easy to chip.

Mutations in several extracellular matrix (ECM) protein coding genes or ECM modifying enzyme coding genes have been associated with AI, such as the *amelogenin, X-linked* (*AMELX*), *enamelin* (*ENAM*), *matrix metallopeptidase 20* (*MMP20*), and kallikrein related peptidase 4 (*KLK-4*) (Lagerström et al., 1991; Rajpar et al., 2001; Kim et al., 2005; Hart et al., 2004).

Dentinogenesis Imperfecta

Dentinogenesis imperfecta (DI) is a hereditary developmental disorder of dentin formation in the absence of a systemic involvement. DI teeth have bulbous opalescent crown with brown or gray discoloration with thin roots, the dentin layer of which shows irregular dentinal tubules and atypical granular dentin matrix. DI teeth are more susceptible to enamel loss and tooth attrition.

DI is classified into three patterns, types I, II, and III, depending on its clinical presentations. DI type I (OMIM 166200) is associated with mutations in the *collagen type I alpha 1/2 chain* (*COL1A1/A2*) genes and osteogenesis imperfecta, while types II (OMIM 125490) and III (OMIM 125500) are isolated and associated with mutations in the *DSPP* gene. Whereas both types I and II DI show complete obliteration of the pulp cavity by dentin, DI type III shows enlarged pulp cavities (MacDougall et al., 2006; Kim and Simmer, 2007).

Dentin Dysplasia

Dentin dysplasia (DD) is another hereditary developmental disorder of dentin formation, which shows loss of organization of dentin during development. It is not associated with systemic involvement or DI. There are two types of DD. Type I, or radicular, DD teeth show dentin formation defects in the root that result in short roots while their crowns appear normal. Therefore, affected teeth exhibit severe mobility and are susceptible to premature exfoliation and root fracture. The pulp chambers are severely obliterated by dysplastic dentin or pulp stones, resulting in a crescent-shaped pulp. Periapical inflammatory lesions may be accompanied in the absence of dental caries. Type II, or coronal, DD teeth have normal root length and show many features that resemble DI. Affected deciduous teeth show bulbous crowns, cervical constriction, thin roots, and early obliteration of the pulp. On the other hand, affected permanent teeth show enlarged and apically extended pulp chambers.

Concluding Remarks

The past decades of extensive research have provided the current understanding of tooth development and the mechanisms of associated developmental disorders. Throughout the development of teeth, epithelial-mesenchymal interaction plays an important role in orchestrating the cellular events that take place at different time points, enabling multiple cell types to carry out their precise roles in order to form teeth without error. This complex molecular regulatory network is tightly maintained by conserved signaling pathways and transcription factors. However, the underlying biochemical mechanisms of tooth development remain poorly characterized. Therefore, one of the future directions in tooth development research would be to understand the essential gene regulatory networks that drive the tooth developmental program. By combining genome wide chromatin immunoprecipitation and gene expression profiling analyses with bioinformatics analysis, scientists will be able to identify novel and unprecedented molecular regulatory network models and provide new insights into the developmental program of odontogenesis.

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