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Molecular Genetics of Ameloblast Cell Lineage

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Abstract

Late tooth morphogenesis is characterized by a series of events that determine crown morphogenesis and the histodifferentiation of epithelial cells into enamel-secreting ameloblasts and of mesenchymal cells into dentin-secreting odontoblasts. Functional ameloblasts are tall, columnar, polarized cells that synthesize and secrete a number of enamel-specific proteins. After depositing the full thickness of enamel matrix, ameloblasts shrink in size and regulate enamel maturation. Amelogenesis imperfecta (AI) is a heterogeneous group of inherited defects in enamel formation. Clinically, AI presents as a spectrum of enamel malformations that are categorized as hypoplastic, hypocalcified, or hypomaturational types, based upon the thickness and hardness of the enamel. The different types of AI are inherited, either as X-linked, autosomal dominant or autosomal recessive traits. Recently, several gene mutations have identified to cause the subtypes of AI. How these genes, however, coordinate their function to control amelogenesis is not understood.

In this review, we discuss the role of genes that play definitive role on the determination of ameloblast cell fate and life cycle, based on studies in transgenic animals.

Keywords

ameloblasts; genetics; transgenic; mouse

Introduction

The development of teeth is a complex process that involves sequential and reciprocal interactions between dental epithelium and mesenchyme. It is characterized by events that determine the histodifferentiation of mesenchymal cells into dentin-secreting odontoblasts and of epithelial cells into enamel-secreting ameloblasts (Kollar and Lumsden, 1979; Thesleff and Nieminen, 2005).

The differentiation process of epithelial cells into functional ameloblasts consists of several morphologic and functional changes that occur in time and involve considerable growth, elongation of the cytoplasm, polarization and the appearance of processes that secrete matrix (Fig. 1). These morphologic changes are known as: (i) *the inductive stage* (pre-ameloblasts), where the cells of inner enamel epithelium begin to differentiate into ameloblasts, elongate, their nuclei shift distally (away from the dental papilla), and their cytoplasm becomes filled with organelles needed for synthesis and secretion of enamel proteins; (ii) *the initial-secretory stage*, where the proximal end of the newly formed ameloblasts (near the dental papilla) is flat and the matrix secreted is called rodless enamel matrix; (iii) *the secretory stage*, where the

ameloblasts lengthen, polarize, form conical projections, known as Tomes' processes, and deposit enamel in the form of rods and prism; (iv) the *maturation stage* where the ameloblasts function to resorb much of the water and organic matrix from enamel in order to provide space for the growing enamel crystals (Nanci A and Ten Cate, 2007; Fig. 1).

Animal and human studies that employ the tools of contemporary molecular genetics have identified a number of genes that act at specific stages of the ameloblast life cycle and regulate its patterning and differentiation process. The purpose of this review is to discuss recent findings regarding genes that definitively control the ameloblast life cycle and function, based on animal studies.

I. A non-cell autonomous mechanism controls ameloblast induction

The presumptive ameloblast begins its life cycle as a proliferative cell, low columnar in shape, separated from adjacent mesenchyme by a basement membrane. Once stimulated, the differentiating ameloblast ceases proliferation and begins to grow in height. Tissue recombination experiments using dental and non-dental tissues have shown that ameloblast cytodifferentiation is regulated by a series of reciprocal interactions between epithelium and mesenchyme (Kollar and Baird, 1970; Thesleff and Hurmerinta, 1981). Signals from the dental epithelium first induce the differentiation of underlying mesenchymal cells into odontoblasts. The odontoblasts deposit dentin matrix and signal back to the epithelium, inducing differentiation of epithelial cells into functional ameloblasts (Karcher-Djuricic *et al.*, 1985).

The molecules mediating this induction are members of the transforming growth factor (TGF β) superfamily. BMP2 and TGF β 1 are secreted by odontoblasts and induce ameloblast differentiation *in vitro* (Coin *et al.*, 1999). Recent studies show that BMP4, another TGF β signal, is secreted by the odontoblasts to induce ameloblast differentiation *in vivo*. It is shown that BMP4 releasing beads induce strong *ameloblastin* expression, an ameloblast-specific gene, in cultured teeth and that BMP4 inhibitor, noggin, dramatically inhibits induction of ameloblast differentiation (Wang *et al.*, 2004).

Although, these studies indicate the importance of odontoblasts in the induction of epithelium into ameloblast cell fate, an epithelial-dependent, cell autonomous control is also needed for ameloblasts to fully differentiate, mature and deposit enamel matrix. Pre-secretory, secretory and mature ameloblasts express several (i) secreted proteins, such as *ameloblastin*, *amelogenin*, *enamelin*, *tuftelin*, *dentin sialoprotein*, *apin*, *amelotin* (ii) enzymes such as *kallikrein 4* and enamel proteinases, such as *matrix metalloproteinase 20*, (iii) signaling molecules like BMPs, TGF β 1, SHH and WNTs and (iv) transcription factors like *Msx2*, *Sp3*, *Sp6* and *Dlx5* (Zeichner-David *et al.*, 1995; Krebsbach *et al.*, 1996; Aberg *et al.*, 1997; Begue-Kirn *et al.*, 1998; Dassule *et al.*, 2000; Hu *et al.*, 2002; Gritli-Linde, 2002; Mustonen, *et al.*, 2004; Wang *et al.*, 2004; Hart *et al.*, 2004; Bei *et al.*, 2004; Wright, 2006; Moffatt *et al.*, 2006a, 2006b; Wang *et al.*, 2007; Dubrowolski *et al.*, 2008; Lezot *et al.*, 2008; <http://bite-it.helsinki.fi>). Studies using transgenic animals provide functional data showing that disruption of the ameloblast signaling and its mediators result in aberrations of ameloblast differentiation and enamel deposition (Table 1). Below, we discuss these studies.

II. A cell autonomous gene network controls ameloblast life cycle

Signaling molecules

Shh is strongly expressed in pre-ameloblasts and secretory ameloblasts (Dassule *et al.*, 2000; Gritli-Linde, *et al.*, 2002). Conditional inactivation of *Shh* in the epithelium results in defective ameloblasts that exhibit little elongation, organization, polarization and some enamel matrix deposition (Dassule *et al.*, 2000). When *Smoothed* a transmembrane protein essential for all

Hh signaling, is conditionally removed from the dental epithelium, results in a complete failure of epithelial cells to proliferate, to grow, to polarize and to become secretory ameloblasts (Gritli-Linde, *et al.*, 2002). These data suggest that although the dental mesenchyme is necessary for ameloblast fate induction, it is not sufficient, that cell-autonomous epithelial-epithelial interactions play an important role and that Shh may be an endogenous epithelial factor regulating ameloblast cytodifferentiation.

Shh, although important, is not the only cell-autonomous factor that determines ameloblast cytodifferentiation and/or function. Several members of the TGF β superfamily are also expressed in ameloblasts during the pre-secretory and secretory stages. Overexpression of *TGF β 1* in the dental epithelium under the *Dspp* promoter results in hypoplastic enamel and detached pre-secretory ameloblasts from dentin (Haruyama *et al.*, 2006). In contrast to *TGF β 1* transgenic mice where some enamel matrix is deposited, overexpression of *folliculin*, a BMP inhibitor, in the epithelium abrogates ameloblast differentiation. The K14-*folliculin* mice lack enamel, the ameloblasts fail to form and do not express enamel specific markers like *ameloblastin*, *MMP20* and *DSPP* (Wang *et al.*, 2004). The critical role of *folliculin* in ameloblast differentiation is further exemplified by the fact that in *folliculin* knockout mice, functional ameloblasts differentiated on the normally enamel-free surface (Wang *et al.*, 2004). The latter indicates that *folliculin* is essential for enamel-free area formation by preventing ameloblast differentiation (Wang *et al.*, 2004). Experiments on cultured tooth explants suggest that the mechanism by which *folliculin* prevents ameloblast differentiation is by inhibiting the ameloblast-inducing activity of BMP4 from the underlying odontoblasts. They also show that *folliculin* expression is induced by activin from the surrounding dental follicle. Thus, *folliculin* controls ameloblast differentiation in a cell-autonomous manner by integrating the opposing effects of two non cell-autonomous signals that of activin and BMP4 from dental follicle and odontoblasts, respectively (Wang *et al.*, 2004).

Lack of enamel formation and loss of ameloblast differentiation is also observed in mice overexpressing other signaling factors, such as *Wnt3* or *Ectodysplasin* (*Eda-A1*) under the K14 promoter (Millar *et al.*, 2003; Mustonen *et al.*, 2004). Ectopic expression of *Wnt3* in the tooth epithelium causes progressive loss of ameloblasts from postnatal lower incisor teeth while overexpression of *Eda-A1* causes disruption of ameloblast differentiation and enamel formation. The striking similarity of phenotypes observed in transgenic mice over-expressing *Folliculin*, *Wnt* and *Eda-A1* suggests that these genes may share or reside in the same pathway controlling ameloblast differentiation.

Cell-cell adhesion molecules

At the secretory stage, the ameloblasts are characterized by strong cell-cell adhesion formation and different molecules of the cell-cell adhesion apparatus, including the *E-cadherin*, *catenins*, the *integrins* $\alpha\beta5$, $\alpha\beta4$, *connexin 43* and *laminins* are strongly expressed by pre-ameloblasts and secretory ameloblasts (Pasqualini *et al.*, 1993; Meyer *et al.*, 1995; Salmivirta *et al.*, 1996; Garrod *et al.*, 1996; Green *et al.*, 1996; Fausser *et al.*, 1998). Mouse and human mutations provide further evidence for the role of some cell-cell adhesion molecules on ameloblast differentiation and function.

Laminin 5 isoforms, for example, are highly expressed in secretory ameloblasts (Yoshida *et al.*, 1998). Mutations in human LAMININ-5 isoforms result in variants of Herlitz junctional epidermolysis bullosa (EB) or dystrophic EB, groups of recessive inherited disorders characterized by dermal-epidermal separation (Aberdam *et al.*, 1994; Kivirikko *et al.*, 1995, McGrath *et al.*, 1995; Christiano *et al.*, 1997). In some cases of EB caused by mutations in LAMININ-5, its receptor *Integrin* $\alpha\beta4$ or *type VII collagen*, enamel hypoplasia is observed (Vidal *et al.*, 1995; Christiano *et al.*, 1996; 1997). When *laminin 5a3* is mutated in mice, among

other epithelial abnormalities, ameloblast differentiation is affected resulting in reduced enamel deposition. The ameloblast reach the secretory stage and secrete little enamel matrix (Ryan *et al.*, 1999). A potential mechanism by which Laminin 5 might control ameloblast differentiation is by interaction with its receptor, integrin $\beta 4$. It is shown that this interaction is important for stabilizing the architecture of epithelial cells by means of their hemidesmosome assembly (Baker *et al.*, 1996). Absence or reduction in *laminin5a3* expression, therefore, would be expected to result in profound alterations in ameloblast structure and function, since positional information and strong cell-cell adhesions become critical prerequisites for the structural integrity of ameloblasts (Meyer *et al.*, 1995). Consistent with this, in the *Msx2* mutant teeth where *laminin 5a3* is absent in ameloblasts, profound changes in the structural integrity of ameloblasts and in the enamel deposition process is observed (Bei *et al.*, 2004 and Fig. 2).

Mutations in the human *connexin 43* gene, a member of gap junction-specific family of genes, the connexins, result in oculodentodigital dysplasia (ODDD). ODDD is a dominant negative inherited disorder affecting different organs including the teeth that exhibit hypoplastic enamel. When the human Cx43G138R point mutation is inserted in to the mouse *Cx43* gene, the transgenic mice exhibit all ODDD phenotypes and hypoplastic enamel (Dobrowolski *et al.*, 2008). Additional studies suggest that this mutation is associated with gap junctional dysfunction and increased cellular ATP release in cardiomyocytes that result in abnormal function and arrhythmia, providing thus a potential mechanism by which Cx43 may operate in ameloblasts, as well.

Ameloblast-specific genes

Several human mutations in ameloblast-specific genes like *amelogenin*, *ameloblastin*, *enamelin*; proteolytic enzymes *enamelysin* (*MMP20*), *Kallikrein 4* and the non-extracellular matrix protein FAM83H have identified to cause the different subtypes of Amelogenesis Imperfecta (AI) indicating their essential role for the correct deposition and maturation of dental enamel. (Aldred *et al.*, 2002; Wright, 2006; Lee *et al.*, 2008; Kim *et al.*, 2008). Amelogenesis imperfecta (AI) is a heterogeneous group of inherited defects in enamel formation. Clinically, AI presents as a spectrum of enamel malformations that are categorized as hypoplastic, hypocalcified, or hypomaturational types, based primarily upon the thickness and hardness of the enamel (Witkop, 1989). The different types of AI reflect differences in the timing during amelogenesis, when the disruption occurs and are inherited, either as an X-linked, autosomal dominant or autosomal recessive traits.

Mouse mutations exist for four ameloblast-specific genes, the *Amelx*, *Enamelin*, *Ameloblastin* and *Mmp20* genes. When *Amelx* and *Mmp20* genes are deleted from the mouse genome result in the development of enamel defects (Gibson *et al.*, 2001; Caterina *et al.*, 2002). The *amelogenin* mutant incisors exhibit disorganized hypoplastic enamel containing a rough knobby surface, while the (*MMP20*, *enamelysin*) mutant enamel is hypoplastic with improperly processed amelogenin. Consistent with the mouse studies, 14 different *AMELX* human mutations are known to cause the enamel malformation disease, *amelogenesis imperfecta* (AI) (Lench *et al.*, 1995; Collier *et al.*, 1997; Hart *et al.*, 2002; Kim *et al.*, 2004; Wright *et al.*, 2003). In addition, the human *MMP20* is located to chromosome 11 and an autosomal-recessive form of AI was recently discovered in a family that had a mutation in the intron 6 splice acceptor (AG to TG) (Kim *et al.*, 2005). The *ameloblastin* null mice develop severe enamel hypoplasia (Fukumoto *et al.*, 2004). The dental epithelium initially differentiates into enamel-secreting ameloblasts. Subsequently, the cells are detached from the matrix, lose polarity, resume proliferation and, thus, their status is reversed from differentiated to undifferentiated one, suggesting that ameloblastin, a cell adhesion molecule, maintains the differentiation state of ameloblasts at the secretory stage, by binding to ameloblasts and by inhibiting their proliferation (Fukumoto *et al.*, 2004, 2005). In contrast to ameloblastin null

mice, the enamel knock out/LacZ knock in mice exhibit a later defect in amelogenesis. The mice secrete enamel matrix but its organization and mineralization process are severely disturbed resulting in the formation of an irregular mineralized crust arising from the fusion of small mineralization foci in the deeper part of the accumulated enamel matrix (Hu *et al.*, 2008).

Transcription Factors

Transcription factors are critical for early tooth development, but recent data shows that several of them are essential for late stages of tooth morphogenesis including amelogenesis. Specificity protein 3 (Sp3) is a ubiquitously expressed protein that belongs to the Sp-family of transcription factors. This family consists of several members, Sp1-6, that are characterized by their ability to bind G-rich elements, such as GC-boxes and related motifs found widely distributed among promoters and enhancers of several genes. *Sp3* homozygous null mice exhibit a hypoplastic phenotype in both, dentine and enamel matrices (Bouwman *et al.*, 2000). *Sp6* null mice, also known as *Epfⁿ* mice, among different phenotypes exhibit enamel deficiency. In these mice rapid proliferation and differentiation of the inner dental epithelium are inhibited and, thus, the dental epithelium retains the progenitor phenotype (Nakamura *et al.*, 2008). Transfection of *Epfⁿ* promoted dental epithelial cell differentiation into ameloblasts and activated promoter activity of the enamel matrix *ameloblastin* gene, suggesting that *Sp6* promotes ameloblast differentiation by controlling the rate of proliferation of inner enamel epithelium (Nakamura *et al.*, 2008; Ruspita *et al.*, 2008).

Mice lacking the homeobox gene *Msx2* exhibit defects in amelogenesis. In the *Msx2* knock out mice the ameloblasts reach the secretory stage of their differentiation process and sparse amounts of enamel matrix are deposited (Satokata *et al.*, 2000; Bei *et al.*, 2004; Fig. 2). In a case of amelogenesis imperfecta, cleft lip and palate and polycystic kidney disease sequence analysis of the human homolog of *MSX2* gene identified a missense mutation of T447C that resulted in the conversion of methionine to threonine at 129, further indicating the important role of *Msx2* during amelogenesis (Suda *et al.*, 2006). Interestingly, *Msx2* is selectively required for the expression of the extracellular matrix gene, *Laminin 5 a 3*, which, as mentioned above, play an essential role during ameloblast differentiation, suggesting that *Laminin 5 a 3* and *Msx2* genes may function within the same genetic pathway (Bei *et al.*, 2004).

In addition, the similarity in the odontogenic phenotype between *Msx2* and Sp3/Sp6 and their overlapping expression in ameloblasts raises the possibility that these transcription factors interact closely within a molecular cascade that operates in these cells, regulating amelogenesis. Consistent with this idea, *Msx2* and Sp3 transcription factors interact *in vitro* and *in vivo* (Bei, unpublished). The tooth phenotype and the enamel defects in *Msx2* mutants are also similar to those observed in transgenic mice expressing urokinase-type plasminogen activator in the enamel organ (Zhou *et al.*, 1999). Of interest, in the urokinase transgenic mice, a reduction of *laminin5* expression is observed in the basal basement membrane and in the apical pole of ameloblasts. Since the urokinase-type plasminogen activator gene is expressed, like *Msx2*, in both secretory ameloblasts and stratum intermedium cells, suggests that a potential genetic relationship between these two genes exist, as well.

III. Significance

Mutations in, both, mouse and humans reveal the involvement of several classes of genes in ameloblast life cycle and its function, a process that emerges as a model for elucidating the molecular regulatory cascades that operate in other developmental systems, as well. Most of these genes appear to function in the context of evolutionarily conserved genetic hierarchies. As such, the availability of model organisms for the systematic investigation of the conserved genetic and molecular cascades, that regulate enamel formation, could lead to treatments of

human diseases affecting amelogenesis (amelogenesis imperfecta), could further our knowledge in the field of tooth regeneration and help us extrapolate our findings to other developmental systems that form via similar mechanisms.

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Figure 1. Ameloblast life cycle

(A) H&E stained wild type incisor teeth showing the life cycle of ameloblasts. (i): morphogenetic stage; (ii)&(iii): initial secretory (no Tomes' process) and secretory ameloblasts (with Tomes' processes that secrete enamel) and (iv): maturation stages. Abbreviations: a: ameloblasts; o: odontoblasts; e: enamel matrix; d: dentin. (B): TEM of ameloblasts at the secretory stage of their life cycle. The ameloblasts at this stage are characterized by an extensive rER, a Golgi complex located in the center of the cytoplasm, condensed and packaged into membrane-bound secretory granules that migrate to the distal part of the cell. After the first deposition of enamel matrix (structure-less enamel layer) the ameloblasts are migrating proximally and processes, known as Tomes' processes, are developed. The cytoplasm of the ameloblast continues into the process but distinct border between them is marked by the presence of the distal terminal web and junctional complex. Abbreviations: M: mitochondria; nu: nucleus; rER: secretory ameloblasts; sg: secretory granules; Tp: Tomes' process; er: enamel rod; e: enamel; TW: terminal web; d: dentin.

Figure 2. *Msx2* mutant ameloblasts exhibit defective cell-cell adhesion

(A) Wild type ameloblasts at the secretory stage are elongated, highly polarized cells, tightly connected to each other. (B) *Msx2* mutant ameloblasts are polarized and enamel matrix is formed. In contrast to wild type, *Msx2* mutant ameloblasts exhibit a marked absence of cell-cell adhesive junctions (see arrows between adjacent ameloblasts). Abbreviations: nu: nucleus; rER: ribosomal endoplasmic reticulum; si: stratum intermedium (**Modified from** Bei *et al.*, 2004).

Table 1
Enamel defects caused by mutations in transgenic mice.

Gene	Mutation	Enamel Phenotype	Reference
<i>Msx2</i>	null	Enamel hypoplasia	Satokata <i>et al.</i> , 2000 Bei <i>et al.</i> , 2004
<i>Sp3</i>	null	Enamel hypoplasia	Bouwman <i>et al.</i> , 2000
<i>Sp6(Epfn)</i>	null	Enamel hypoplasia	Nakamura <i>et al.</i> , 2008 Ruspita <i>et al.</i> , 2008
<i>Shh</i>	K14 conditional knock-out	No enamel	Dassule <i>et al.</i> , 2000
<i>Smoothened</i>	K14 conditional knock-out	Enamel hypoplasia	Gritli-Linde <i>et al.</i> , 2002
<i>Gdnf</i>	null	No enamel	deVincente <i>et al.</i> , 2002
<i>Periostin</i>	null	Incisor enamel defect	Rios <i>et al.</i> , 2005
<i>TGFβ1</i>	DSPP conditional knock-out	Enamel hypoplasia	Haruyama <i>et al.</i> , 2006
<i>Eda</i>	K14 transgenic	No enamel	Mustonen <i>et al.</i> , 2004
<i>Follistatin</i>	K14 transgenic	No enamel	Wang <i>et al.</i> , 2004
<i>Follistatin</i>	null	Ectopic enamel	Wang <i>et al.</i> , 2004
<i>Wnt3</i>	K14 transgenic	No enamel	Millar <i>et al.</i> , 2003
<i>Amelx</i>	null	Enamel hypoplasia	Gibson <i>et al.</i> , 2001
<i>Ameloblastin</i>	null	No enamel	Fukumoto <i>et al.</i> , 2005
<i>Lama3</i>	null	Enamel hypoplasia	Ryan <i>et al.</i> , 1999
<i>Enamelin</i>	null	Enamel hypoplasia/aplasia	Hu <i>et al.</i> , 2008
<i>Mmp20</i>	null	Enamel hypoplasia	Caterina <i>et al.</i> , 2002
<i>Connexin 43</i>	dominant negative	Enamel hypoplasia	Dobrowolski <i>et al.</i> , 2008