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Research Review The Genetic Basis of Tooth Development and Dental Defects

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More than 300 genes have so far been associated with tooth development, mainly in mouse embryos. The majority of them are associated with conserved signaling pathways mediating cellular communication, in particular between epithelial and mesenchymal tissues. Necessary functions of many signals, receptors and transcription factors have been demonstrated in mice, and mutations causing dental defects in humans have been identified in several genes.
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Key words: *TGFβ*; *Wnt*; *BMP4*; *FGF8*; *TNF*; *RUNX2*; *MSX1*; *PAX9*; *AXIN2*; *P63*; *Eda*; *dlx1*; *dlx2*; hypopohidrotic ectodermal dysplasia; cleidocranial dysplasia; EEC syndrome; oligodontia

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INTRODUCTION

The genetic programs regulating embryonic development are gaining more understanding in great detail and new developmental regulatory genes are being discovered at speed in different model organisms such as *Drosophila*, zebrafish and mouse. The genes have been highly conserved during evolution and have similar functions in different animals. It is also typical for a developmental regulatory gene to govern the development of many different structures in the embryo. The specific functions of numerous genes in the formation of many tissues and organs are known, as are the effects of their mutations on normal development. It is obvious that most human congenital malformations are caused by mutations in the developmental regulatory genes.

The genes that regulate tooth development have been studied actively and to date over 300 genes have been associated with the patterning and morphogenesis as well as with cell differentiation in teeth. Schematic expression patterns of these genes can be viewed in a graphical database created by Pekka Nieminen at the University of Helsinki (http://bite-it.helsinki.fi). It is interesting that the majority of the genes in this database have functions in cellular communication and that mutations in many of these genes cause dental defects in man [Thesleff and Pirinen, 2003].

MORPHOLOGY AND MECHANISMS OF TOOTH DEVELOPMENT

Teeth form from the surface ectoderm of the first branchial arch and the frontonasal prominence as well as from the underlying mesenchyme that is derived from the neural crest. The development of individual teeth is preceded by the formation of a thickened epithelial stripe, the dental lamina at the sites of the future dental arches of the maxilla and the mandible. The developmental anatomy and histology of tooth morphogenesis have been described in detail long ago [e.g., textbook by Nanci, 2003, Fig. 1]. Central features of tooth morphogenesis are the formation of the epithelial placode, the budding of the epithelium, the condensation of mesenchyme around the bud, and the folding and growth of the epithelium generating the shape of the tooth crown. The mineralized structures characteristic for teeth, that is, dentin and enamel, are formed by specialized cells, the odontoblasts and ameloblasts differentiating from the mesenchyme and epithelium, respectively.

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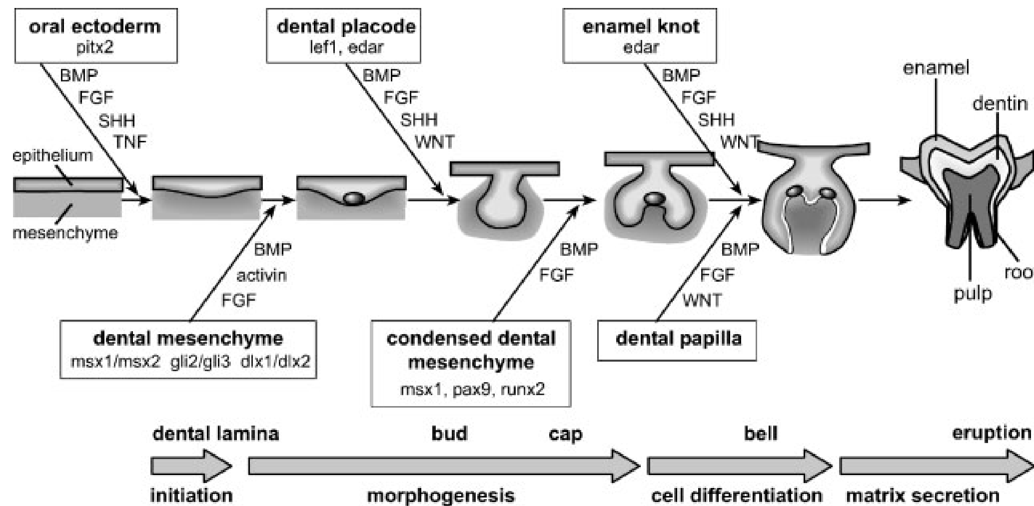

Fig. 1. Tooth development is regulated by conserved signal pathways (FGF, BMP, SHH, WNT, TNF). The signals mediate interactions between the oral ectoderm and mesenchyme and regulate the expression of key transcription factors (shown in the boxes). Mutations of the transcription factors in this figure cause tooth agenesis in mice, and most of them are associated with dental defects in humans.

The communication between the two tissues, the epithelium and the mesenchyme, is the key regulatory mechanism governing tooth development. There is a series of reciprocal epithelial-mesenchymal interactions that regulate the initiation and morphogenesis of the tooth as well as the differentiation of the odontoblasts and ameloblasts at the interface of the two tissues. During the last 15 years the "language" which the epithelial and mesenchymal cells use for communication has been uncovered [Thesleff, 2003; Thesleff and Nieminen, 2005; Wang and Thesleff, 2006]. It is the same language that cells use for communication throughout the embryo and in all developmental processes, and this language has been conserved during evolution. It is composed of mainly secreted signal molecules and growth factors. The most studied and universal signals are members of four conserved families, TGFβ (includes BMPs and activins), FGF, hedgehog (in teeth only sonic hedgehog, Shh), and Wnt. In addition to the actual signals, there are numerous molecules involved in the signal pathways, notably the receptors at the cell surface, transcription factors mediating the signal to the nucleus and regulating gene expression. Of specific importance among the genes that are regulated by signals are transcription factors which activate new genetic programs in the embryonic cells (Fig. 1).

INITIATION OF TOOTH DEVELOPMENT

The epithelium guides the very early stages of tooth formation and sends signals to the mesenchyme inducing odontogenic potential in the dental mesenchyme. Studies on mouse embryos have identified FGF8 and BMP4 as signals derived from the oral epithelium, and many important transcription factors have been identified as their targets in the mesenchyme. These include transcription factors in the *msx, dlx*, and *lhx* families. These are all necessary for the advancement of tooth development beyond the initiation stage as shown in knockout mice. More than one member of each family are expressed in the developing teeth and they have compensatory functions. Thus, tooth formation is arrested at the initiation stage only when both *msx1* and *msx2*, or both *dlx1* and *dlx2* are inactivated [Thomas et al., 1997; Satokata et al., 2000].

PLACODE FORMATION AND THE GENETIC BASIS OF ECTODERMAL DYSPLASIA SYNDROMES

A key feature of tooth development is the formation of ectodermal placodes, which are small thickenings of the epithelium at the sites of each tooth family. It is noteworthy, that similar placodes initiate the development of all organs that form as appendages of the ectoderm [Pispa and Thesleff, 2003; Millar and Mikkola, 2006]. These include hairs and nails as well as glands such as mammary, salivary, sweat, and sebaceous glands, and it has become evident that the genes involved in the formation and function of placodes are very similar in different ectodermal organs. Typically, signals in all the four families are required for placode development. Studies mainly on hairs and feathers have identified FGFs and Wnts as activators of placode formation and BMPs as inhibitors [Jung et al., 1998; Millar, 2002] and the available evidence indicates that most of these functions are similar in dental placodes.

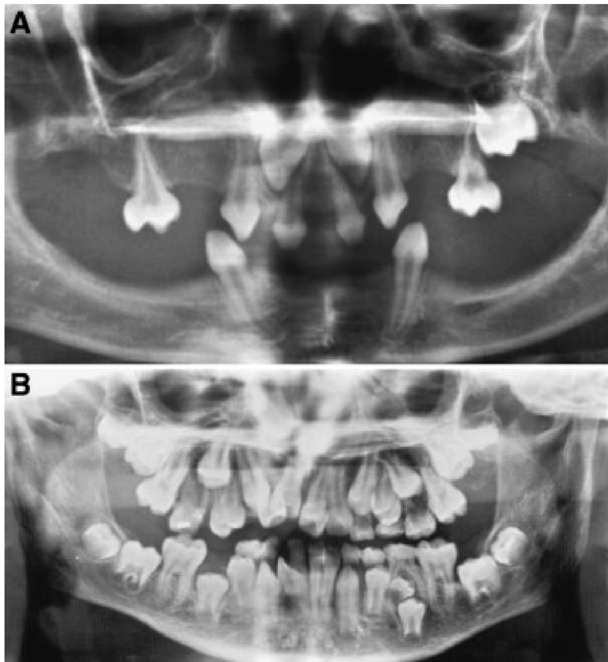

Fig. 2. **A**: Oligodontia (severe tooth agenesis) in a boy with X-linked hypohidrotic ectodermal dysplasia (HED). **B**: Supernumerary teeth in a girl with cleidocranial dysplasia. Courtesy by Sinikka Pirinen.

Ectodermal dysplasia syndromes are defined as conditions in which two or more types of ectodermal organs are affected, and dental defects in these syndromes include typically multiple missing teeth (oligodontia) as well as small and misshapen teeth (Fig. 2). Many genes have been identified in which mutations cause ectodermal dysplasias. Taken the similarities in the early development of various ectodermal organs, it is not surprising that these genes encode molecules regulating placode formation and function.

Mutations in the transcription factor p63 cause the EEC syndrome featured by ectodermal dysplasia as well as ectrodactyly and cleft lip and palate [Celli et al., 1999]. A typical patient has a severe dental phenotype with multiple missing and misshapen teeth. The p63 knockout mice lack all ectodermal organs and die at birth [Mills et al., 1999]. Detailed analysis of the tooth and hair phenotype in these mice has shown that development is arrested prior to placode development. The dental lamina was present and was composed of a normal thickened epithelium but the placodes failed to form [Laurikkala et al., 2006]. Similarly, hair placodes were completely absent. It was shown that only the ΔN isoform of p63 is expressed in the embryonic teeth and epidermis, and that it is required for the mediation of several signal pathways regulating placode formation. In particular, ΔNp63 function was necessary for FGF, BMP, and Notch1 signaling.

The positional cloning of genes behind hypohidrotic ectodermal dysplasia (HED), led to the discovery of a novel TNF (tumor necrosis factor) pathway, the ectodysplasin (Eda) pathway, regulating ectodermal organ development [Mikkola and Thesleff, 2003]. The characteristic features of HED are oligodontia (Fig. 2), thin and sparse hair and severe lack of sweat glands, but other ectodermal defects, for example, in nails and salivary glands, are also common. Mutations in the TNF signal molecule Eda cause the X-linked HED whereas mutations in the genes encoding the Eda receptor Edar, and the signal mediator molecule Edaradd, are responsible for two autosomal forms of HED with a similar phenotype [Kere et al., 1996; Headon and Overbeek, 1999]. In addition, the gene behind HED-ID, a syndrome with all features of HED and associated with immunodeficiency was shown to encode IKKgamma (NEMO), also an intracellular component of the Eda signal pathway, and apparently of some other TNF pathways [Döffinger et al., 2001].

The role of the Eda pathway in the development of teeth and other ectodermal organs has been analyzed in detail in mice [Mikkola and Thesleff, 2003]. The mouse model for X-linked HED, the *Tabby* mouse has a tooth phenotype characterized by lack of third molars and sometimes incisors and misshapen crowns of the first molars. The Tabby mouse also lacks the first wave of hair follicles and has defects in many ectodermal glands. In addition, transgenic mice overexpressing Eda in the ectoderm have been informative concerning the role of the Eda pathway and the pathogenesis of the ectodermal defects. The accumulated information indicates that Eda signaling is required for the formation and growth of ectodermal placodes. The Edar receptor is expressed in all placodes, and when it is overactivated the placodes grow bigger than normal. This results in the stimulation of ectodermal organ development seen as longer hairs, increased sweat excretion, and extra mammary glands as well as extra teeth [Mustonen et al., 2003]. The teeth form in front of the molars and are apparently premolars, which were lost early during rodent evolution. It was also shown that the application of Eda protein on embryonic mouse skin in culture stimulates placode growth and high concentrations cause fusions of the enlarged placodes. Interestingly, Eda does not stimulate cell proliferation but rather causes a change in the fates of ectodermal cells from an epidermal to placodal fate [Mustonen et al., 2004]. Intriguingly, the injection of Eda protein to pregnant *Tabby* mice rescued the hair and tooth phenotype of their offspring [Gaide and Schneider, 2003]. Hence, it appears that the stimulation of placode formation at an early stage is sufficient to rescue the development of the organs. This finding obviously may lead to novel possibilities to prevent human X-linked HED in the future.

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THE BUD-TO-CAP STAGE TRANSITION AND THE GENETIC BASIS OF TOOTH AGENESIS

The budding of the dental epithelium is accompanied by the formation of a condensate of dental mesenchymal cells around the bud, and this is followed by the induction of a signaling center at the tip of epithelial bud, the enamel knot. The enamel knot expresses more than ten signal molecules belonging to all four families and it is required for the transition of the epithelial bud to a cap, marking the onset of tooth crown development (Fig. 1). This transition is very critical for tooth morphogenesis and it is regulated by sequential interactions between the epithelium and mesenchyme. Again, all the four signal families are involved in mediation of the interactions. Shh is an epithelial signal necessary for epithelial proliferation, but its direct effect appears to be on mesenchyme where it apparently triggers the formation of a reciprocal signal acting back on the epithelium [Gritli-Linde et al., 2002]. Wnt and BMP signals regulate the formation of the enamel knot. Mesenchymal BMP4 regulates the arrest of cell cycle in the enamel knot by inducing p21 expression, and Wnts are required for *Fgf4* expression in the knot [Jernvall et al., 1998; Bei et al., 2000; Kratochwil et al., 2002]. FGFs and their receptors are expressed in both epithelium and mesenchyme and regulate in reciprocal fashion the proliferation in the adjacent tissue [Kettunen et al., 1998, Wang and Thesleff, 2006].

Of particular interest and clinical significance during these stages of morphogenesis are three transcription factors in the condensed dental mesenchyme: *Msx1, Pax9*, and *Runx2* (Fig. 3). Their expression is regulated by epithelial signals. *Msx1* is induced by BMP and FGF, and *Pax9* as well as *Runx2* by FGF [Vainio et al., 1993, Bei and Maas, 1998; Peters et al., 1998; Åberg et al., 2004]. The functions and target genes of the three transcription factors are being actively investigated, and it is apparent that among other genes they regulate reciprocal signals acting back on epithelium and regulating enamel knot formation and epithelial proliferation [Wang and Thesleff, 2006]. Knocking out any one of the three genes in mice arrests tooth development at the bud stage. In *Msx1* null mutants the arrest occurs already prior to mesenchymal condensation, whereas in *Pax9* deficient mice some condensation takes place, and in *Runx2* mutants condensation in normal and even a rudimentary enamel knot forms. However, in none of the mutant mice teeth proceed to cap stage [Bei and Maas, 1998; Peters et al., 1998; Åberg et al., 2004].

In humans, heterozygous loss of function of either *MSX1* or *PAX9* causes oligodontia [Vastardis et al., 1996; Stockton et al., 2000]. The dental phenotypes differ from each other in some aspects, in particular the *PAX9* mutations affect mostly molar teeth. The phenotypes of both *MSX1* and *PAX1* mutations are apparently due to haploinsufficiency. This was supported recently by an allelic series of *Pax9* mutant mice in which the gene dosage was gradually reduced [Kist et al., 2005]. These mice represent the mouse model for human oligodontia. It was shown that *Pax9* is required during multiple stages of tooth development and that the minimal *Pax9* gene dosage required for the formation of individual teeth increased from anterior to posterior in molar teeth.

Heterozygous loss of function of *RUNX2* in humans causes cleidocranial dysplasia [Mundlos et al., 1997]. The patients have bone dysplasia and extra teeth. The tooth phenotype is puzzling since the complete lack of teeth in *Runx2* knockout mice would predict a similar phenotype as in the *MSX1* and *PAX9* heterozygotes, that is, oligodontia. Interestingly, the supernumerary teeth in association with cleidocranial dysplasia develop as parts of a third dentition [Jensen and Kreiborg, 1990]. Hence, Runx2 is a positive regulator of the primary teeth but a negative regulator of secondary teeth. Since the mice do not develop a secondary dentition, it is not well suited for the analysis of tooth renewal. However, recent data on the development of a rudimentary *Shh* expressing bud in the epithelium of *Runx2* mutant and heterozygous mice have supported the role of Runx2 as an inhibitor of tooth renewal [Wang et al., 2005].

Another human gene that has been associated with tooth renewal is *AXIN2*. Mutations in this gene were identified in a family with oligodontia and colorectal cancer [Lammi et al., 2004]. The phenotype of oligodontia, however, was different from those

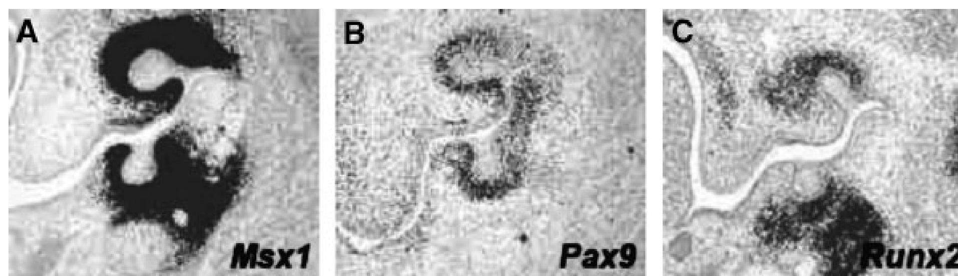

Fig. 3. Expression of *Msx1* (**A**), *Pax9* (**B**), and *Runx2* (**C**) in the dental mesenchyme of bud staged teeth in mouse embryos (in situ hybridization analysis performed on frontal sections of the jaws showing the upper and lower molar buds). Development is arrested at this stage in *Msx1*, *Pax9*, and *Runx2* null mutant mice.

caused by *PAX9* and *MSX1* mutations since it affected almost exclusively secondary teeth. The deciduous teeth developed normally suggesting that Axin2 may be required for tooth renewal. Axin2 functions in the Wnt signal pathway as a feedback inhibitor. A role of Wnt signaling in tooth renewal would be in line with similar functions of Wnts in hair cycling [Gat et al., 1998]. Because the molecular mechanisms regulating the embryonic morphogenesis of ectodermal organs are similar, it is conceivable that also the mechanisms involved in adult regeneration are shared between teeth and other organs forming as appendages of the ectoderm [Huysseune and Thesleff, 2004].

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