Genetic basis of tooth development and dental defects

Irma Thesleff
Research Program in Developmental Biology, Institute of Biotechnology, Viikki Biocenter, University of Helsinki, Finland

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Tooth development is under strict genetic control, and during recent years an increasing number of genes have been identified that are involved in the regulation of tooth morphogenesis. One of the organs in which development is now beginning to be understood at the gene level, the tooth is an example of a typical vertebrate organ starting as an epithelial bud and undergoing complex morphogenesis, regulated by interactions between epithelial and mesenchymal tissue layers. It has become evident that developmental regulatory genes have been conserved to a high degree during evolution and that similar gene networks regulate the development of teeth as of other vertebrate organs. So far, all genes that have been linked with early tooth morphogenesis have developmental regulatory functions in other organs, too. The majority of these genes are associated with the signaling pathways transmitting interactions between cells and tissues. They include genes encoding the actual signals as well as their receptors, mediators of signaling in the cytoplasm and transcription factors regulating gene expression in the nucleus. Deletion of the function of many of these genes in transgenic mice results in arrested tooth development, but all these mutants also show defects in many other tissues. Mutations in several of these genes in humans have been identified as causes of dental defects, mainly hypodontia. □ *tooth development; tooth defects; craniofacial development; ectodermal dysplasia; cleidocranial dysplasia*

Irma Thesleff, Institute of Biotechnology, Viikki Biocenter, POB 56, 00014 University of Helsinki, Finland

Knowledge of the genetic mechanisms underlying animal development has increased exponentially during the last 10 years. Hundreds of genes have been identified which regulate embryonic development, and we are now starting to understand how the different genes function. For many genes, we know in great detail how they affect cell behavior, such as cell proliferation and differentiation, how they influence patterning and morphogenesis, and how they interact with each other in complex pathways and networks. In addition, an increasing number of genes have been identified among the regulatory genes which, when mutated, cause aberrant development and congenital defects.

Genes that regulate tooth development are being identified with increasing speed. More than 200 are included at present in our graphical database illustrating the gene expression patterns during tooth development (Gene expression in tooth, http://bite-it.helsinki.fi). We have started to understand how the genes regulate tooth formation, and how aberrant functions of specific genes cause dental defects.

What are developmental regulatory genes and how do they function?

The morphogenesis of teeth, like the development of the whole embryo, is under strict genetic control. Developmental regulatory genes have been mostly identified in basic biological and genetic studies which have focused on the development of a variety of different animals. The most informative have been the genetic analyses of the development of the fruitfly, *Drosophila*, and also the mouse. The remarkable conclusion from these studies was the unforeseen conservation of the genes regulating development. It has become apparent that the same genes regulate the development of all animals, indicating that they have been conserved during more than 500 million years of evolution. This has caused a revolution in the fields of developmental biology and genetics, and indicates that developmental studies in any animal are highly relevant in all other animals (1).

It is also noteworthy that the same genes regulate numerous developmental processes in the same animal. They are used sequentially throughout development in all parts of the embryo, and their effects depend on the time and tissue where they function; in other words, on the previous history of the target cells. For instance, similar sets of genes govern the development of all organs in all animals, including the teeth, which are only present in vertebrates. Therefore, although flies obviously have no teeth, the information from *Drosophila* studies is applicable to teeth, particularly concerning the interactions between different genes. Most information concerning the genetic control of tooth development, however, has come from studies conducted on mouse embryos.

The majority of regulatory genes are somehow associated with interactions between the cells. Such interactions are central regulators of development, because the microenvironment of the cell has a key role in determining cell behavior. All cells have the same genes, but information from outside the cells affects the decisions of the cell to turn on and off the expression of different

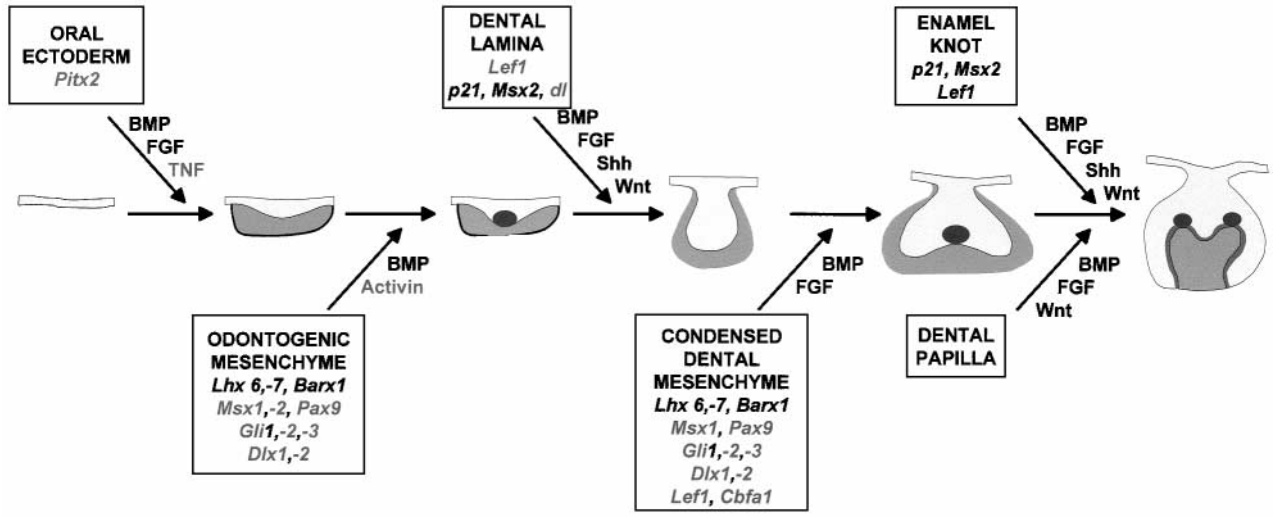

Fig. 1. Genes regulating early tooth morphogenesis. Signaling molecules, their receptors and target genes form pathways and networks regulating development of the tooth from initiation through bud and cap stages. Mutations in several different genes (indicated in grey letters) lead to an arrest in tooth development in mutant mice. Signalling molecules: BMP, bone morphogenetic protein; FGF, fibroblast growth factors; Shh, sonic hedgehog, TNF; tumor necrosis factor.

genes. There are several families of secreted signal molecules that transmit information between cells. They act by binding to specific cell surface receptors, and through molecular cascades in the cytoplasm the signaling results in the activation of transcription factors which enter the nucleus and regulate the expression of genes. This results in the production of new proteins, including new signals, receptors, and transcription factors and thus to a change in the behavior of the target cell, as well as to the continuation of signaling between cells (2). Thus each signaling pathway includes numerous different molecules. The pathways of different signaling molecules interact with each other and so development is regulated by complex signaling networks. It is the numerous genes in the signaling networks that are the central regulators of patterning and morphogenesis in the embryo, and which determine the sizes and shapes of all organs, including the teeth.

Signaling networks in tooth development

Teeth are examples of organs which develop as appendages of the embryonic surface epithelium. The most important events during regulation of the development of all such organs are the so-called inductive interactions between the epithelial and mesenchymal tissues (3). These are mediated by the conserved signaling pathways described above, and in tooth development these have been characterized in great detail (4, 5). Signal molecules of several different families are used sequentially during the advancing development and reciprocally from epithelium to mesenchyme and vice versa. In addition, numerous transcription factors have been identified as the targets of signaling. Although most signal molecules so far analysed mediate interactions between the epithelial and mesenchymal tissues, there are also signals acting within one tissue.

Signaling interactions which determine the location, identity, size, and shape of teeth take place during the early stages of tooth development (Fig. 1). The most studied signals belong to the families of FGF (fibroblast growth factors), TGFβ (transforming growth factors, which include BMPs (bone morphogenetic protein) and activin), Hh (hedgehog), and Wnt. Each family consists of several signals encoded by different genes. They are used reiteratively; the first signals are secreted by the oral ectoderm, which thereby initiates the odontogenic program in the underlying neural crest-derived mesenchyme (6–8). The committed mesenchyme signals back to the epithelium and controls the growth and folding of the epithelium. The mesenchymal signals also induce the formation of signaling centers in the epithelium, in which many genes encoding signal molecules are activated (the enamel knots in cap stage teeth express more than 10 different signals). These centers signal again back to mesenchyme as well as within the epithelium and regulate the advancing development including cusp development in molars (5). Numerous transcription factors have been identified which are turned on in the target tissues as a result of signaling. The first example was our demonstration that BMP from early dental epithelium induced the expression of the homeobox-containing transcription factor *msx1* in the mesenchyme (9). FGF, too, induces *msx1* (4, 10), and, as seen in Fig. 1, today numerous transcription factors have been discovered which are turned on in the mesenchyme as a result of BMP, FGF, as well as the other epithelial signals.

Table 1. Syndromes with hypodontia (missing teeth) in which the gene defect has been identified

Syndrome	Associated defects	Gene	Type of molecule
Oligodontia	None	*MSX1*	Transcription factor
Oligodontia	None	*PAX9*	Transcription factor
Rieger syndrome	Eye and umbilical defects	*PITX2*	Transcription factor
Hypohidrotic ectodermal dysplasia	Hypoplastic hair and glands	*EDA* (ectodysplasin)	TNF signal
Hypohidrotic ectodermal dysplasia	Hypoplastic hair and glands	*EDAR*	TNF receptor
EEC	Ectodermal dysplasia, ectrodactyly, cleft palate	*P63*	Transcription factor
Diastrophic dysplasia	Osteochondrodysplasia	*DTDST*	Sulfate transporter
CLPED1	Cleft lip/palate, ectodermal dysplasia	*PVRL1* (nectin-1)	Cell adhesion molecule

Detailed descriptions of the syndromes as well as original articles describing gene defects can be found in the WWW database: Search OMIM (Online Mendelian Inheritance in Man) http://www3.ncbi.nih.gov/Omim/searchomim.html

Mutations in many different genes can cause disruption to tooth development

It is conceivable that most if not all genes associated with the signaling networks are important for normal tooth development. The vital function of many of these genes for tooth development has been revealed by genetic manipulation of mouse development, i.e. the production of transgenic mice. In so-called knock-out mice the function of a specific gene is inhibited and its importance for development can thus be analysed. It is not surprising that deletion of the function of several different genes may lead to arrested tooth morphogenesis.

The first gene demonstrated to be essential for tooth development in mice was *msx1* (11) and later mutations in the human *MSX1* gene were shown to cause autosomal-dominant oligodontia (12). In *msx1* knockouts tooth development is arrested at the bud stage. *Msx1* is expressed in the dental mesenchyme, and its deletion results in inhibition of the expression of *Bmp4* and *Fgf3*, which act as reciprocal signals to epithelium. It was shown recently that BMP4, when added to cultures of *msx1* mutant tooth germs, could rescue their development (13). This example shows how the understanding of molecular hierarchies involved in the reciprocal signaling pathways offers the possibility to rescue defective development: Deletion of one component of the pathway (*msx1*) arrests development, but this can be compensated by the introduction of its downstream target (BMP4).

Other genes that have been knocked out in mice leading to arrested tooth development include the transcription factors *dlx1,-2, gli2,-3, lef1, pax9, pitx2*, and *cbfa1* as well as the signal molecule *activin βA* (5) (Fig. 1). It is conceivable that mutations in these genes like those of *MSX1* may cause hypodontia in humans, too (Table 1). The *CBFA1* gene is an interesting exception in its mechanism of action. Deletion of *cbfa1* function in knockout mice results in complete loss of bone, as well as arrest of tooth development at an aberrant cap stage (14). However, heterogenous loss of function of the gene in humans is the cause of cleidocranial dysplasia syndrome (15), in which bone is hypoplastic, but instead of having missing teeth the patients have supernumerary teeth. The mechanism of action of the *CBFA1* gene in tooth development remains to be clarified.

Gene mutations causing dental defects usually affect other tissues as well

Because the same developmental regulatory genes are used for many purposes in the embryos, it is possible that a mutation in one gene results in defects in many different tissues and organs. In fact, there are no examples of regulatory genes which would be specific solely for tooth development. In knockout mice, gene function is deleted in both copies of a gene, and dental defects are always accompanied by malformations in other tissues as well. In fact, the mice mostly die early *in utero* or perinatally due to impaired function of some other vital organ. In humans, too, the deletion of both copies of developmental genes in most cases causes early lethality, but inactivation of only one copy of the gene may lead to milder phenotypes, as in the cases of *MSX1* and *PAX9* mutations causing only dental defects (12, 16). However, it is possible that careful inspection of these patients would reveal additional defects in some of the many organs that are affected in mice in which the genes have been knocked out (11, 17).

Dental defects are seen in numerous syndromes in association with malformations of a variety of different organs (Table 1). This is of course understandable as the genes regulating tooth development are used for the development of all organs. Dental defects are most commonly seen in syndromes affecting various derivatives of the skin. This is because the highest degree of conservation in developmental mechanisms and developmental regulatory genes is seen between teeth and other organs developing as ectodermal appendages (Fig. 2). These syndromes are called ectodermal dysplasias, and recently the gene (*EDA*) behind the most common of them, the anhidrotic (hypohidrotic) ectodermal dysplasia, was identified (18). The features of this syndrome include sparse and thin hair, severe hypodontia and a reduction in the number and function of many glands, in particular sweat glands.

Positional cloning of the *EDA* gene causing X-linked hypohidrotic ectodermal dysplasia and its mouse equiva-

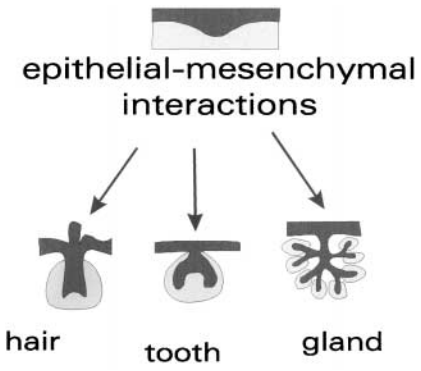

Fig. 2. The early development of organs affected in ectodermal dysplasia syndromes shows remarkable morphological resemblance. Interactions between the epithelial and mesenchymal tissues regulate their morphogenesis, and most of the genes regulating tooth development (Fig. 1) also regulate the development of hairs and glands. Mutations in the genes encoding the TNF signal ectodysplasin (*EDA, Tabby*) and its receptor edar (*downless*) cause hypohidrotic ectodermal dysplasia.

lent *Tabby* led to the identification of a novel tumor necrosis factor (TNF), called ectodysplasin (19, 20). Interestingly, the cloning of the gene of the mouse mutant *downless*, which has a phenotype identical to *Tabby*, revealed a novel TNF receptor, edar (21). This gene was shown to be responsible for an autosomal form of human hypohidrotic ectodermal dysplasia (22). This is a good example of how mutations in two genes in the same pathway, i.e. the signal and its receptor, may lead to identical phenotypes.

We have recently analysed the mechanisms of action of ectodysplasin and edar, and shown that they mediate interactions within the epithelium rather than between epithelium and mesenchyme (23). We have also shown that the expression of edar, the TNF receptor, is regulated by mesenchymal activin signals, whereas ectodysplasin, the TNF ligand is regulated by Wnt signals. This indicates that the TNF signaling pathway is tightly linked to the other signaling networks regulating the development of teeth and other ectodermal organs. Interestingly, the receptor (*downless*) is expressed in the epithelial signaling centers in both teeth and hairs. Hence, the deficient function of the signaling centers appears to be the cause of the hypoplastic development of teeth and other organs in patients with ectodermal dysplasia syndromes.

References

1. Gilbert SF. Developmental biology, 6th ed. MA Sunderland, Sinauer Associates, Inc; 2000.
2. Thesleff I. The genetic basis of normal and abnormal craniofacial development. Acta Odontol Scand 1998;56:321–5.
3. Thesleff I, Vaahtokari A, Partanen AM. Regulation of organogenesis. Common molecular mechanisms regulating the development of teeth and organs. Int J Devel Biol 1995;39:35–50.
4. Maas R, Bei M. The genetic control of early tooth development. Crit Rev Oral Biol Med 1997;8:4–39.
5. Jernvall J, Thesleff I. Iterative signaling and patterning during mammalian tooth morphogenesis. Mech Devel 2000;92:19–29.
6. Mina M, Kollar E. The induction of odontogenesis in non-dental mesenchyme combined with early murine mandibular arch epithelium. Arch Oral Biol 1987;32:123–7.
7. Lumsden AGS. Spatial organisation of the epithelium and the role of neural crest cells in the initiation of mammalian tooth. Development 1988;103 Suppl:155–69.
8. Tucker AS, Matthews KL, Sharpe P. Transformation of tooth type induced by inhibition of BMP signaling. Science 1998; 82:1136–8.
9. Vainio S, Karavanova I, Jowett A, Thesleff I. Identification of BMP-4 as a signal mediating secondary induction between epithelial and mesenchymal tissues during early tooth development. Cell 1993;75:45–58.
10. Kettunen P, Thesleff I. Expression and function of FGFs-4, -8 and -9 suggest functional redundancy and repetitive use as epithelial signals during tooth morphogenesis. Devel Dyn 1998; 211:256–68.
11. Satokata I, Maas R. Msx1 deficient mice exhibit cleft palate and abnormalities of craniofacial and tooth development. Nature Genet 1994;6:348–56.
12. Vastardis H, Karimbux N, Guthua SW, Seidman JG, Seidman CE. A human MSX1 homeodomain missense mutation causes selective tooth agenesis. Nature Genet 1996;13:417–21.
13. Bei M, Kratochwil K, Maas R. BMP4 rescues a non-cell-autonomous function of Msx1 in tooth development. Development 2000;127:4711–8.
14. D'Souza RN, Åberg T, Gaikwad J, Cavender A, Owen M, Karsenty G, et al. Cbfa1 is required for epithelial-mesenchymal interactions regulating tooth development in mice. Development 1999;126:2911–20.
15. Mundlos S, Otto F, Mundlos C, Mulliken JB, Aylsworth AS, Albright S, et al. Mutations involving the transcription factor CBFA1 cause cleidocranial dysplasia. Cell 1997;89:773–9.
16. Stockton DW, Das P, Goldenberg M, D'Souza RN, Patel PI. Mutation of PAX9 is associated with oligodontia. Nature Genet 2000;24:18–19.
17. Peters H, Neubuser A, Kratochwil K, Balling R. Pax9-deficient mice lack pharyngeal pouch derivatives and teeth and exhibit craniofacial and limb abnormalities. Genes Devel 1998;12:2735–47.
18. Kere J, Srivastava AK, Montonen O, Zonana J, Thomas N, Ferguson B, et al. X-linked anhidrotic (hypohidrotic) ectodermal dysplasia is caused by mutation in a novel transmembrane protein. Nature Genet 1996;13:409–16.
19. Srivastava AK, Pispa J, Hartung AJ, Du Y, Ezer S, Jenks T, et al. The Taby phenotype is caused by mutation in a mouse homologue of the EDA gene that reveals novel mouse and human exons and encodes a protein (ectodysplasin-A) with collagenous domains. Proc Natl Acad Sci USA 1997;94:13069–74.
20. Mikkola ML, Pispa J, Pekkanen M, Paulin L, Nieminen P, Kere J, et al. Ectodysplasin, a protein required for epithelial morphogenesis, is a novel TNF homologue and promotes cell-matrix adhesion. Mech Devel 1999;88:133–46.
21. Headon DJ, Overbeek PA. Involvement of a novel TNF receptor homologue in hair follicle induction. Nature Genet 1999;22:370–4.
22. Monreal AW, Ferguson BM, Headon DJ, Street SL, Overbeek PA, Zonana J. Mutations in the human homologue of mouse dl cause autosomal recessive and dominant hypohidrotic ectodermal dysplasia. Nature Genet 1999;22:366–9.
23. Laurikkala J, Mikkola M, Mustonen T, Åberg T, Koppinen P, Pispa J, et al.. TNF signaling via the ligand-receptor pair ectodysplasin and edar controls the function of epithelial signaling centers and is regulated by Wnt and activin during tooth morphogenesis. Dev Biol. In press.