The genetic basis of craniofacial and dental abnormalities

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Schweiz Monatsschr Zahnmed 121: 636–646 (2011)

Accepted for publication: 22 November 2010

Key words: embryo, development, cleft, tooth agenesis

Summary The embryonic head development, including the formation of dental structures, is a complex and delicate process guided by specific genetic programs. Genetic changes and environmental factors can disturb the execution of these programs and result in abnormalities in orofacial and dental structures. Orofacial clefts and hypodontia/oligodontia are examples of such abnormalities frequently seen in dental clinics. An insight into the mechanisms and genes involved in the formation of orofacial and dental structures has been gradually gained by genetic analysis of families and by the use of experimental vertebrate models such as the mouse and chick models. The development of novel clinical therapies for orofacial and dental pathological conditions depends very much on a detailed knowledge of the molecular and cellular processes that are involved in head formation.

Introduction

Disturbances in normal head development during embryogenesis manifest clinically as malformations that affect orofacial and dental structures.

For example, abnormalities in the growth or fusion of the facial processes may result either in orofacial clefts or severe disorders such as holoprosencephaly, where the midline of the face "collapses". Similarly, tooth abnormalities are usually the result of disturbances in the "molecular dialogue" between the oral epithelium and the underlying mesenchyme during tooth development, which can result in tooth agenesis of varying severity with failure to form the correct number of teeth.

Much of our knowledge on the role of specific genes in the formation of the human head has originated from studying the genome of family members with a particular orofacial or dental disorder. In the past 30 years the discovery and availability of genetic markers across the human genome has allowed the analysis of the genetic code responsible for these disorders. In addition, the techniques of cloning and sequencing greatly contributed in the identification of specific genes that are responsible for these defects.

Furthermore, animal models, and most particularly mouse models, have become invaluable tools for understanding the molecular processes that govern head development. The genetic programs controlling head development appear to be highly conserved between all vertebrates. Together with the sequencing of the mouse and human genomes, of particular help is the technology of gene "knock-out" (deletion/inactivation), by which a specific gene believed to be involved in a human orofacial or dental disorder can be inactivated in the mouse, offering valuable information about malformations observed in humans. The detailed study of the resulting phenotypes constitutes the starting point for the elucidation of the molecular pathways involved in these abnormalities.

In this review, we discuss some of the cellular and molecular processes involved in the development of the orofacial complex and teeth. We focus on the results obtained from studies

in humans and mouse models that have helped to identify the genetic causes of specific abnormalities with emphasis on the subjects of oral clefts and tooth agenesis (oligodontia/hypodontia/anodontia).

Finally, we present the clinical benefits that could derive from the development of new technologies combined with our knowledge on the genetic manifestations of craniofacial and dental defects.

Head formation

The formation of the head occurs at the earliest stages of human embryonic development (Wilkie & Morris-Kay 2001). This process is very complex and under a tight genetic control.

For the development of the head a group of cells with stem cell properties, called cranial neural crest (CNC) cells, are of particular importance. These cells delaminate from the lateral ridges of the neural plate (which will form the neural tube) and then emigrate towards the developing branchial arches. Subgroups of cranial neural crest cells migrate towards specific areas where they intermingle with the existing population of mesodermal cells (Fig. 1). The proliferation of the CNC cells is responsible for the budding of tissues around the future oral cavity. Continuous neural crest stem cell proliferation leads to the formation of a single frontonasal process and of pairs of maxillary and mandibular processes (Fig. 1). As development advances, all these processes join and fuse giving rise to the completed face. Thereafter, in both the maxillary and mandibular processes teeth will form.

The CNC cells will give rise to virtually all head structures with the exception of the muscles, which are formed by a mesodermal cell population. Further specification and organisation of the CNC cells into distinct elements (such as bones and teeth) takes place by means of a continuous "molecular dialogue" with the epithelium that covers the developing face and oral cavity. In this molecular "dialogue" the interaction between CNC cells and epithelium involves proteins that are products of specific genes. These proteins instruct cells to either divide or die (apoptosis), migrate/proliferate or differentiate into more specific cell types such as osteoblasts, odontoblasts, chondrocytes, etc.

Orofacial and dental disorders result when mutations in the sequence of either a gene or a group of genes cause alterations to the expression or function of the encoded protein(s). Gene mutations, but also environmental factors, can affect the expression of genes or interfere with the normal function of their protein products.

Growth and transcription factors

The fate of cells is regulated by signalling molecules. These are chemical substances (hormones, neurotransmitters, etc.) synthesized and secreted by cells for the purpose of extracellular communication with other cells. Upon binding to their respective cell-surface receptors these signalling molecules initiate a chain of molecular events and activate specific transcription factors (proteins that bind directly to DNA). These transcription factors bind to regulatory regions of the genome and direct the expression or repression of specific sets of genes that control cell behaviour (Fig. 2).

The above process is a crucial and repeated event in the developing embryo and the basis for the correct formation of all

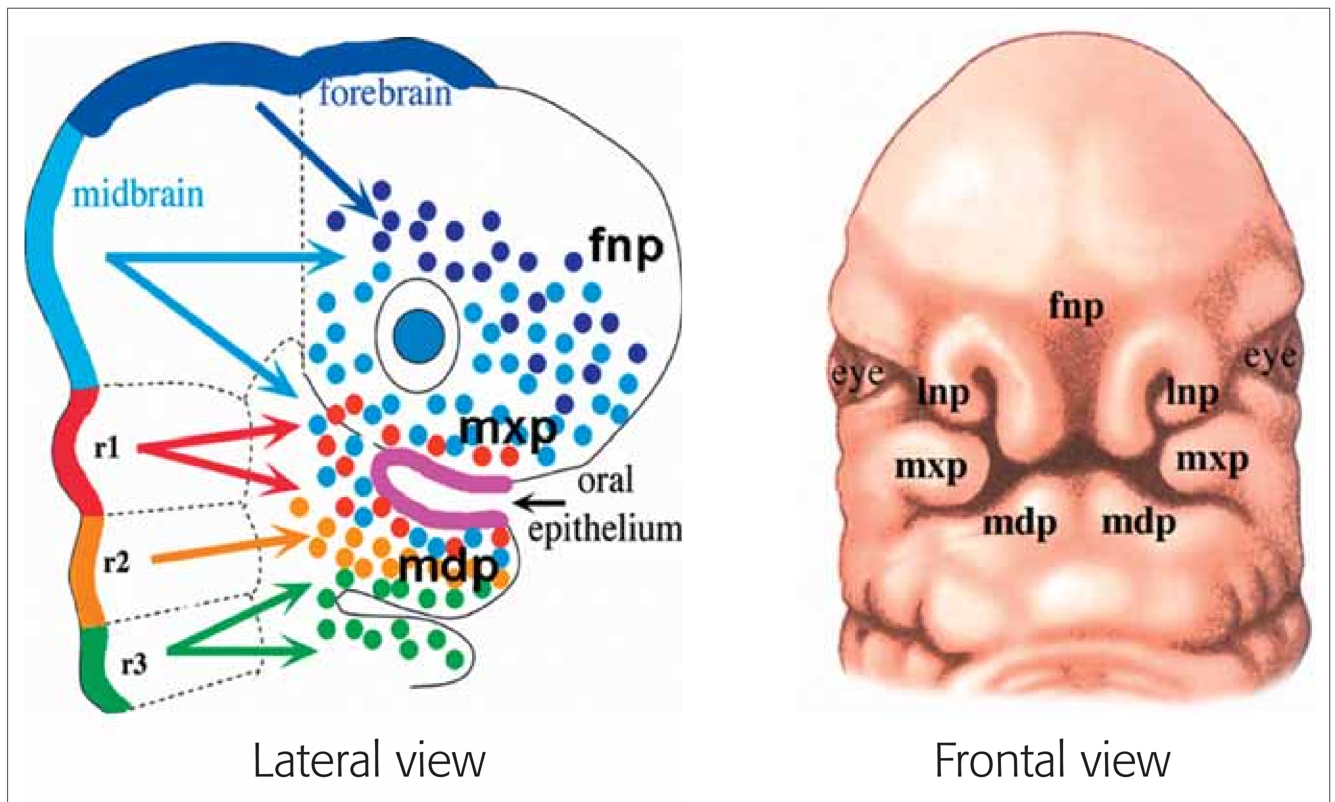

Fig. 1 Migration of neural crest cells and formation of facial prominences
Different groups of neural crest cells (coloured arrows and dots) are somehow instructed to follow each a specific migration path and reach a specific final destination. There, the neural crest cells proliferate and the facial prominences form.
Key: **fnp,** frontonasal prominence; **lnp,** lateral nasal prominence; **mxp,** maxillary prominence; **mdp,** mandibular prominence; **r,** rhombomere.
The frontal view is a redrawn figure from the book "Human Embryology and Developmental Biology" by Bruce M Carlson, 2004.

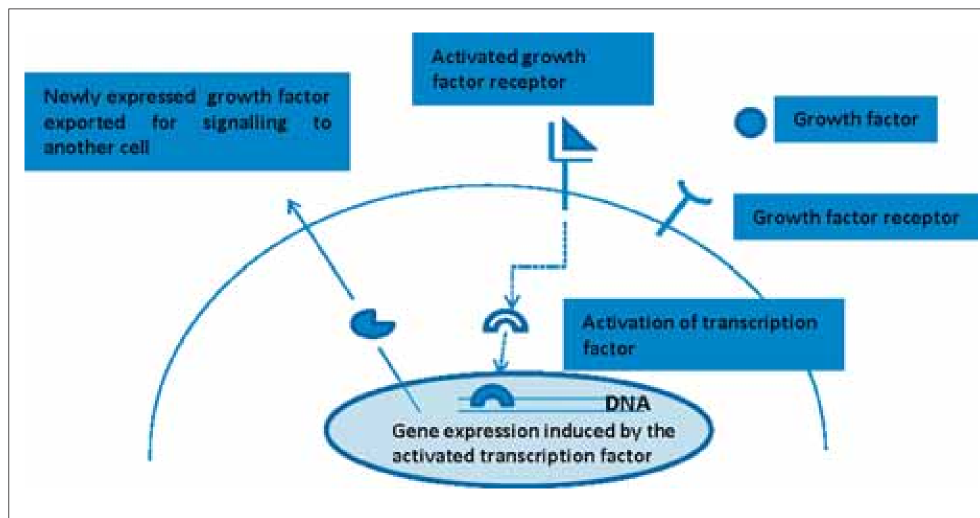

Fig. 2 Signalling carried out by growth factors, growth factor receptors and transcription factors
Different growth factor-growth factor receptor combinations lead to activation/inactivation of a particular transcription factor. Each transcription factor then drives or stops the expression of a particular set of genes including genes for structural proteins, proteins mediating cell migration, cell division or cell death. In many cases transcription factors lead to expression of new growth factors by the cell for signalling to other cells, establishing a cell-to-cell "dialogue".

embryonic structures including the head. Studies on orofacial and tooth development have identified a number of key signalling molecules (mainly growth factors) and transcription factors.

Growth factors are peptide or steroid hormone molecules which stimulate cellular growth, proliferation and differentiation. The growth factors involved in orofacial development belong mainly to four families that are well conserved between different species: the Fibroblast Growth Factor (FGF) family, the Hedgehog (HH) family, the Wingless (WNT) family and the Transforming Growth Factor beta (TGF-β) family, which includes the Bone Morphogenetic Proteins (BMPs) and Activins.

Mutations in genes of these signalling pathways or environmental factors that interfere with the growth factors often affect the developing organism in a predictable manner. For this reason the resulting phenotypic changes are often comparable between humans and other vertebrates such as the mouse.

The FGF signalling pathway

The FGF family of growth factors consists of 25 members. There are four major FGF receptors, but multiple variants exist and split the four main receptors into a total of seven key human FGF receptors: FGFR1b, FGFR1c, FGFR2b, FGFR2c, FGFR3b, FGFR3c, and FGFR4. Each receptor can be activated by specific members of the FGF family.

The FGF signalling pathway is active in both the facial epithelium and mesenchyme and is mainly involved in stimulating cell proliferation. Mutations in FGF molecules or their receptors can result in craniosynostosis (Kim et al. 1998, Johnson et al. 2000), which reflects the role of FGF signalling in the processes of osteogenesis and chondrogenesis.

During palatal formation, FGF molecules and their receptors are expressed in the developing palatal shelves (Lee et al. 2001). In humans, mutations which essentially inactivate the FGFR1 cause Kallmann syndrome. A small percentage of patients with this syndrome present with cleft lip (Dode et al. 2003, Kim et al. 2005). Transgenic mice carrying a deletion of the gene encoding for either the FGF10 factor or the FGFR2b exhibit a cleft palate phenotype (Rice et al. 2004). Interestingly, cleft palate is a frequent feature of many patients (44%) presenting with craniosynostosis syndromes (e.g. Apert syndrome) (Kreiborg & Cohen 1992).

FGF signalling is also essential for the progression of tooth development in mice (Celli et al. 1998). Also, studies using transgenic mice indicated that members of the FGF family are likely to be involved in tooth root formation and elongation (Ota et al. 2007).

However, there is no direct indication from genetic mapping studies for a role of FGFs in the common forms of hypodontia (Arte et al. 2001). Mutations in the growth factor FGF23 in humans cause an abnormality called Autosomal Dominant Hypophosphataemic Rickets (ADHR) (White et al. 2000). The clinical presentation of this condition includes teeth with hypomineralized dentine, enlarged pulp chambers and non-carious exposure of the pulp leading to dental abscesses (Pereira et al. 2004).

The Hedgehog signalling pathway

Sonic hedgehog (SHH) is the main member of the Hedgehog family which is associated with abnormal orofacial and tooth development.

SHH is expressed in the ectoderm of the frontonasal and maxillary processes during development. In humans, mutations of SHH result in holoprosencephaly, demonstrating the crucial role of this growth factor in the development of the face (Roessler et al. 1996). In the chick model, the transient loss of SHH signalling results in midfacial clefts analogous to the human cleft lip/palate. In contrast, excessive SHH expression leads to midfacial widening with increased space between the eyes similar to the human condition hypertelorism (Hu & Helms 1999).

SHH is also expressed at all stages of tooth development and has been shown to be important for the initiation of tooth formation (Hardcastle et al. 1998), crown morphology and tooth size (Dassule et al. 2000). Furthermore, SHH is important for the proliferation of the dental epithelial cells and their final differentiation into ameloblasts (Gritli-Linde et al. 2002, Takahashi et al. 2007). Mice with a defect on the SHH receptor exhibit delayed root development and tooth eruption (Nakatomi et al. 2006).

Similarly, transgenic mice that overexpress the SHH protein in epithelia exhibit phenotypes of cleft palate and arrested tooth formation (Cobourne et al. 2009).

In humans, the Gorlin-Goltz (nevoid basal cell carcinoma) syndrome is associated with hypodontia and clefts. This syndrome could be in certain cases caused by mutations in a gene showing high homology to a Drosophila SHH receptor (Hahn et al. 1996).

The WNT signalling pathway

The WNT family of signalling molecules comprises 19 members in humans. The WNT molecules act through specific receptors (Frizzled 1–10) to activate intracellular signals that dictate a variety of cell actions.

During early craniofacial development in the mouse embryo, members of the WNT family are expressed at tooth initiation sites of the oral epithelium, in the mesenchyme of the elevating palatal shelves, and at sites of future bone formation of the maxilla and mandible. WNT expression is often coincident with the expression of molecules of the Hedgehog and TGF-β families (Silva Paiva et al. 2010).

Loss of WNT function in vertebrates causes a wide range of developmental defects.

Targeted inactivation of WNT signalling in mice causes reduced growth of the facial processes (Yamaguchi et al. 1999) or cleft lip with or without cleft palate (Juriloff et al. 2006). Reduction of WNT molecules in the mesenchyme during tooth initiation results in the formation of teeth with a reduced size (Sarkar & Sharpe 2000).

In humans, a mutation of the WNT3 gene has been linked to a rare condition called tetra-amelia (Niemann et al. 2004). Subjects with tetra-amelia lack all four limbs and often have a cleft lip with or without cleft palate.

The TGFβ signalling pathway

The Transforming Growth Factor beta (TGFβ) family of growth factors plays multiple and critical roles during all stages of tooth development. This family also includes the Bone Morphogenetic Protein (BMP) and Activin signalling molecules.

Inactivation of a TGFβ receptor gene (TGFβR2) in mouse neural crest cells resulted in cleft palate and abnormalities in the formation of the cranium (Ito et al. 2003, Wurdak et al. 2005), thus implicating the TGFβ signalling pathway in the molecular cascade that dictates craniofacial development.

Inactivation of another TGFβ receptor (ALK5: Activin receptor-Like Kinase 5) in mice causes unilateral or bilateral clefts of the upper lip (Li et al. 2008), indicating that the TGFβ pathway may be also important in lip formation.

Inactivation of the TGFβ3 gene in mice results in bilateral cleft of the secondary palate due to the non-fusion of the palatal shelf processes (Kaartinen et al. 1995, Kaartinen et al. 1997). Half of the mutants exhibited severe clefting anteriorly that had as a consequence the exposure of the nasal cavity. Characteristically, none of the TGFβ3 mutants presented with any other craniofacial abnormality.

Mutations in TGFβ receptor genes (TGFβR2 or TGFβR3) were identified in members of ten families with an aortic aneurism-type syndrome who also present with craniosynostosis, hypertelorism, bifid uvula and/or cleft palate (Loeys et al. 2005).

With respect to BMPs, several members of the BMP family have been shown to be expressed at various stages of tooth development.

BMP2 and BMP4 are expressed at particular times and in specific regions of the epithelium of the nasal and the maxillary and mandibular processes (Francis-West et al. 1994). In experiments where Noggin, a molecule antagonizing the action of BMP molecules, was applied on the facial processes of developing embryos, the shape and the size of the facial processes were significantly altered (Wu et al. 2004). Overexpression or inhibition of BMP signalling in embryos caused cleft lip (Ashique et al. 2002).

In mice where BMP activity was reduced by overexpression of the BMP antagonist Noggin, many aspects of tooth development were affected. The mice lacked mandibular molars, the maxillary molars were reduced in size, had altered crown shape and reduced number of roots, while the incisors lacked enamel (Plikus et al. 2005).

These results indicate that BMP signalling controls important and diverse cellular events during the different stages of tooth formation.

Oral clefts

The term *oral cleft* refers to the presence of a fissure or fissures in the lip and/or palate resulting in tissue discontinuity. It is one of the most common congenital orofacial defects with a reported incidence of one in 750 live births, which can be higher for certain racial groups such as those of Asian or native American ancestry (Croen et al. 1998).

Children with cleft lip and palate (CLP), can face problems during feeding, show speech and hearing difficulties, and to varying degrees suffer from disturbances in the normal facial and dental development (Hodgkinson et al. 2005). The impact of the condition on the life of the individual and the need for high cost care by a team of several different professionals, including plastic or paediatric surgeons, maxillofacial surgeons, ENT specialists, speech pathologists, dentists and orthodontists have triggered intense research into the genetic causes of this condition. Genetic information could lead to the development of novel preventive and corrective therapies for CLP patients.

Cleft palate (CP) with or without cleft lip (CL) can occur isolated (70% of cases), or as part of developmental syndromes that are the result of chromosomal or teratogenic conditions (Stanier & Moore 2004). Multiple genes and environmental factors are implicated in the generation of CP/CLP. Knock-out mice with remarkable CLP phenotypes have helped to identify growth factors and transcription factors that are important for the development of the facial primordia. This growth contributes to the formation of the lip and palate, the approximation of these tissues and finally their fusion (Fig. 3).

Genetic abnormalities associated with oral clefts in humans

Defects of growth factors or their receptors have been shown to cause isolated (non-syndromic) or syndromic oral clefts in humans. Linkage and sequencing studies of families have identified mutations in the FGF8 and FGFR1 genes causing non-syndromic cleft lip and palate (Riley et al. 2007).

TGFβ3 has also been shown to be involved in oral cleft formation in humans (Lidral et al. 1998).

We have found that in mice, conditional inactivation of a TGFβ receptor (TGFβR2) in the neural crest cell population also causes cleft palate (Wurdak et al. 2005). The mutant embryos show facial asymmetry, micrognathia, smaller tongue and palatal shelves that are not elevated over the tongue (Fig. 4). These findings suggest that TGFβR2 plays a very important role in orofacial development and further investigation is required to clarify its function in the normal development of the palate.

We have also recently found that inactivation of the growth factor BMP7, another member of the TGFβ family, causes defects in tooth development and clefts in the soft and hard palate (Zouvelou et al. 2009) (Fig. 4).

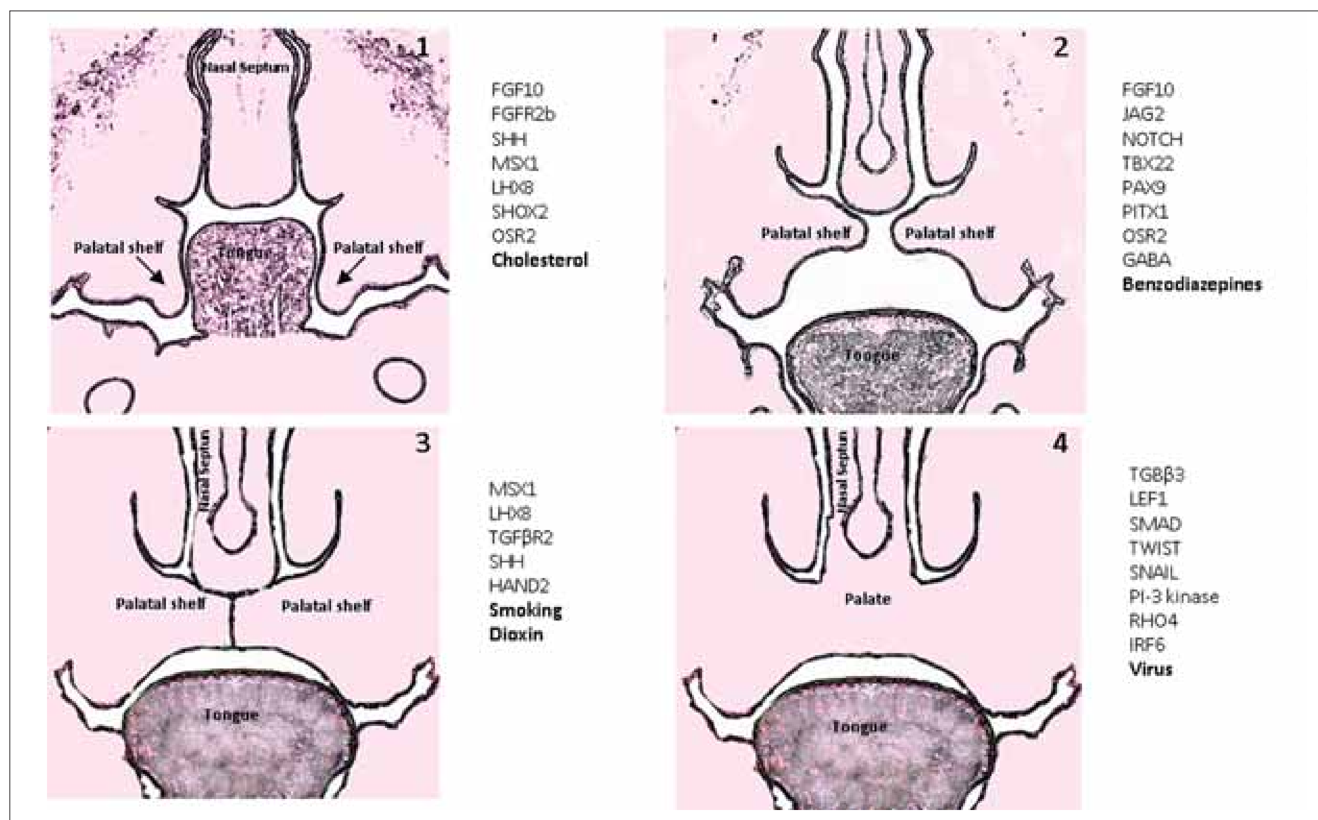

Fig. 3 Developmental process of formation of the palate
The four stages of secondary palate formation are:
1. Growth of palatal shelves from the internal aspect of the maxillary processes
2. Lowering of the tongue and horizontal elevation of the palatal shelves over the tongue
3. Growth and approximation between the palatal shelves in the midline.
4. Fusion of the palatal shelves and of the nasal septum to form a continuous secondary palate.

The signalling molecules and environmental factors (in bold) which play a role at each stage are shown.

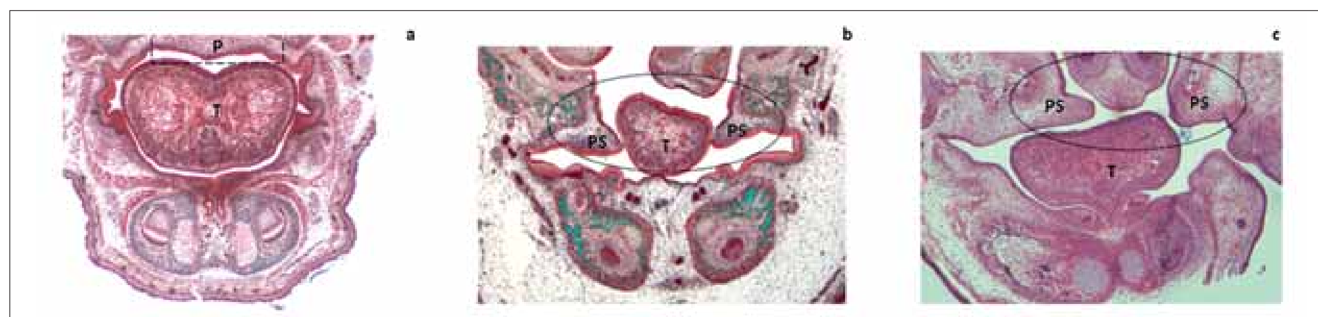

Fig. 4 Developmental disturbances in the formation of the palate in mouse models
a. Wild type mouse embryo (head histological section, coronal view) with normal formation of the palate (indicated by the square).
b. Mouse embryo (head histological section, coronal view) not expressing a TGFβ receptor (TGFβR2) at a particular developmental stage clearly demonstrates defects in palatal formation with inability of the palatal shelves to elevate over the tongue and fuse (circled area).
c. Mouse embryo (head histological section, coronal view) not expressing the growth factor BMP7 at a particular developmental stage demonstrates defects in palatal formation and lack of palatal shelf fusion (circled).
(Key: **T,** tongue; **P,** palate; **PS,** palatal shelf).
In humans, cleft of the palate is a relatively frequent clinical pathology.

In addition to growth factors, transcription factors are important in the process of palatogenesis and lip formation.

In humans, a nonsense mutation in MSX1 was detected in subjects with CP/CL/CLP combinations (van de Boogaard et al. 2000) that represent approximately 2% of non-syndromic cleft cases (Jezewski et al. 2003). Absence of the transcription factor MSX1 causes lack of growth of the palatal shelves leading to the formation of a complete CP in mice (Satokata & Maas 1994).

CP with ankyloglossia is an X-linked inherited condition. Point mutations in the T-box transcription factor TBX22 are found in 8% of cleft palate patients (Braybrook et al. 2002).

TBX22 is expressed in the developing palatal shelves and base of the tongue. A short frenulum may be responsible for the restricted tongue movement and may prevent full elevation of the palatal shelves.

IRF6 is another transcription factor involved in non-syndromic cases of CL/CLP. IRF6 is also involved in the Van der Woude syndrome where CL and/or CP occur with the additional feature of pits in the lower lip (Kondo et al. 2002). IRF6 is considered as a major gene causing approximately 12% of CL or CLP phenotypes (Zucchero et al. 2004).

Clinical relevance of genetic findings

Currently, craniofacial abnormalities such as oral clefts, holoprosenchephaly and craniosynostosis can be detected by modern ultrasound examination as early as at 16 weeks of gestation (Ghi et al. 2002). In oral clefts, which are particularly relevant to clinical dentistry, the only therapy available for the closure of the cleft(s) is surgical intervention. However, cleft repair rarely produces ideal facial aesthetics (Nollet et al. 2007), while the growth of the upper face is often deficient (Nollet et al. 2008).

Identification of the molecular players and unravelling of the genetic pathways that dictate palatogenesis and lip formation could offer new and exciting possibilities for the prevention and therapy of CLP.

Knowledge of the genetic mutations that cause inherited CLP allows the genetic examination and counselling of future parents with traits of oral cleft in the family.

Stem cell therapy is another future possibility of correcting CLP before birth. During the formation of orofacial structures (5 to 12 weeks of pregnancy) the immature immune system of the foetus allows the introduction of stem cells free of the genetic abnormality. The transplantation and engraftment of suitable stem cells could in principle result in the correction of the malformation (Jones & Trainor 2004).

Stem cell technology combined with tissue engineering could also provide solutions for improvements in the treatment of oral clefts in children. When surgically correcting oral clefts, the surgeon frequently faces the problem of tissue shortage. Currently, intense research is under way for the isolation of suitable stem cells from amniotic fluid and adult tissues, including the dental pulp, which could be potentially used to treat orofacial abnormalities (Pittenger et al. 1999, Gronthos et al. 2002, de Coppi et al. 2007, Bluteau et al. 2008). The possibility of isolating stem cells and "directing" them in the laboratory towards attaining specific fates (e.g. become bone cells, muscle cells, etc.) is exciting and promising. Such programmed cells can be seeded on suitably engineered scaffolds to regenerate an appropriate craniofacial tissue of the desired shape and dimensions (Alsberg et al. 2001, Mao et al. 2006, Panetta et al. 2008).

In addition, research on the mechanisms of scar-free wound repair in the embryo could together with tissue engineering achieve faultless results.

During the healing phase, after cleft palate closure, precursor stem cells and fibrocytes (blood-borne cells capable of entering wound sites) differentiate into myofibroblasts in response to growth factors and mechanical tension at the site of the wound (van Beurden et al. 2005). The contractile action of myofibroblasts in conjunction with a periosteal osteogenic reaction at the surgical repair site is thought to result in significant wound contraction and the formation of an immobile scar. This subsequently leads to restricted maxillary growth and abnormalities in tooth development and eruption (Wijdeveld et al. 1991).

The use of growth factors to inhibit the differentiation of fibroblasts into myofibroblasts, to hinder the action of myofibroblasts or to reduce their numbers by delivering apoptotic signals could potentially lead to modulation of palatal wound healing and reduction of scarring (van Beurden et al. 2005).

Mouse models are used in *in vitro* organ culture systems for the detailed investigation of the mechanisms of palatal formation. This technique (Fig. 5; T. Kouskoura, preliminary results) allows the detailed study of the processes involved in the development of the palate (proliferation, apoptosis, fusion, etc.) and the effect of growth factors or chemicals on palate formation in a well controlled environment. This greatly aids our understanding of the specific aetiology of the cleft phenotype

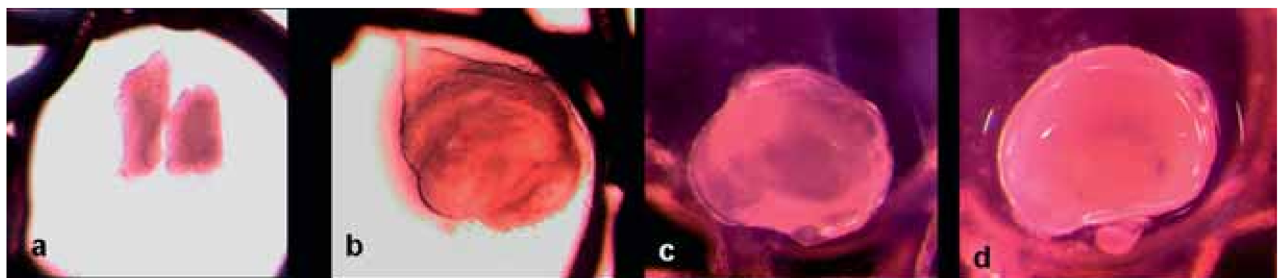

Fig. 5 ***In vitro* organ culture system of developing mouse palates**
Developing palatal shelves are collected from mouse embryos at appropriate stages and are cultured *in vitro.* This system allows us to study the process of palate formation in detail and to directly assess the function of growth factors/growth factor inhibitors.
Microdissected palatal shelves are positioned with their midline aspects (fusing surface) apposed and their oral surface facing up, on filters supported by a metal grid (a). The grid (grid size: 0.65 mm) is placed in a culture chamber filled with fluid medium up to the grid surface. This is taken as time point 0 h (0 hours).
After 72 h of culture the palatal shelves have fused (b) and after more prolonged culture the expected morphology of a fully formed secondary palate is obvious (c: 168 h; d: 240 h).
The appearance of palatal rugae (circled area), a landmark of the oral epithelium of the palate, was observed in many cases at 240 h of culture (e).

and also provides an excellent model to test novel cell- or drug-based therapies.

Tooth agenesis in humans

Tooth agenesis refers to failure of tooth development. In humans, tooth agenesis may involve one or more teeth (or classes of teeth). Failure of development of the third molars is the most common agenesis (10–25%). Patients congenitally lacking less than six teeth (excluding the frequently missing third molars) suffer from hypodontia, while if six or more teeth are missing the condition is termed oligodontia. About 80% of hypodontia patients lack only one to two teeth; approximately 1% of the general population suffers from oligodontia (Lidral & Reising 2002).

Usually, the permanent teeth are congenitally missing, while the lack of primary teeth is relatively rare (0.5–0.9%) and associated with lack of the permanent successor teeth (Vastardis 2000). In most cases, oligodontia is linked to genetic disorders (Vastardis, 2000). Lack of teeth can be an isolated abnormality or part of a syndrome (such as Rieger syndrome, Witkop syndrome and Ectodermal Dysplasia).

Tooth formation is another example of the crucial role of growth factors and transcription factors during embryonic development. At six weeks of gestation a series of molecular events takes place in the forming oral cavity. Cells of the epithelial layer interact with cells of the underlying mesenchyme. Growth factors and transcription factors provide the "molecular language" for this tissue-tissue communication. In this manner, cells of the two tissues instruct each other about the place and the form of the teeth. Thereafter, subgroups of these cells migrate, proliferate and differentiate into cell types forming the enamel, dentine, dental pulp and periodontium (Mitsiadis & Graf 2009).

A schematic representation of these processes and the already known growth factors and transcription factors involved in odontogenesis is shown in Figure 6.

Genes associated with tooth agenesis in humans

Oligodontia and microdontia (small teeth) are common dental features within subjects affected by ectodermal dysplasia syndromes. In the EEC (Ectrodactyly, Ectodermal dysplasia, Clefting) type of ectodermal dysplasia an additional feature can be the presence of CL and CP.

Genetic analyses indicated that the transcription factor p63 is responsible for this type of ectodermal dysplasia in humans. p63 is important for the correct FGF and BMP signalling during early stages of tooth formation (Celli et al. 1999).

Inactivation of p63 in mice leads to arrest of tooth development at early stages (Mills et al. 1999).

Subjects with X-linked hypohidrotic ectodermal dysplasia exhibit oligodontia, and a candidate gene was cloned after positional gene analysis in humans (Kere et al. 1996).

The mouse homologue of this gene (Tabby) (Ferguson et al. 1997) encodes for a signalling molecule (called EDA). Knockout Tabby mice present similar phenotype to that of EED patients (Mikkola & Thesleff 2003). Injection of the missing EDA protein to pregnant Tabby mice permanently corrected the dysplasia (Gaide & Schneider 2003).

The Rieger syndrome is characterised by severe oligodontia in association with midfacial hypoplasia (Childers & Wright 1986). The transcription factor PITX2 is the responsible gene for the Rieger syndrome in humans. PITX2 knock-out mice

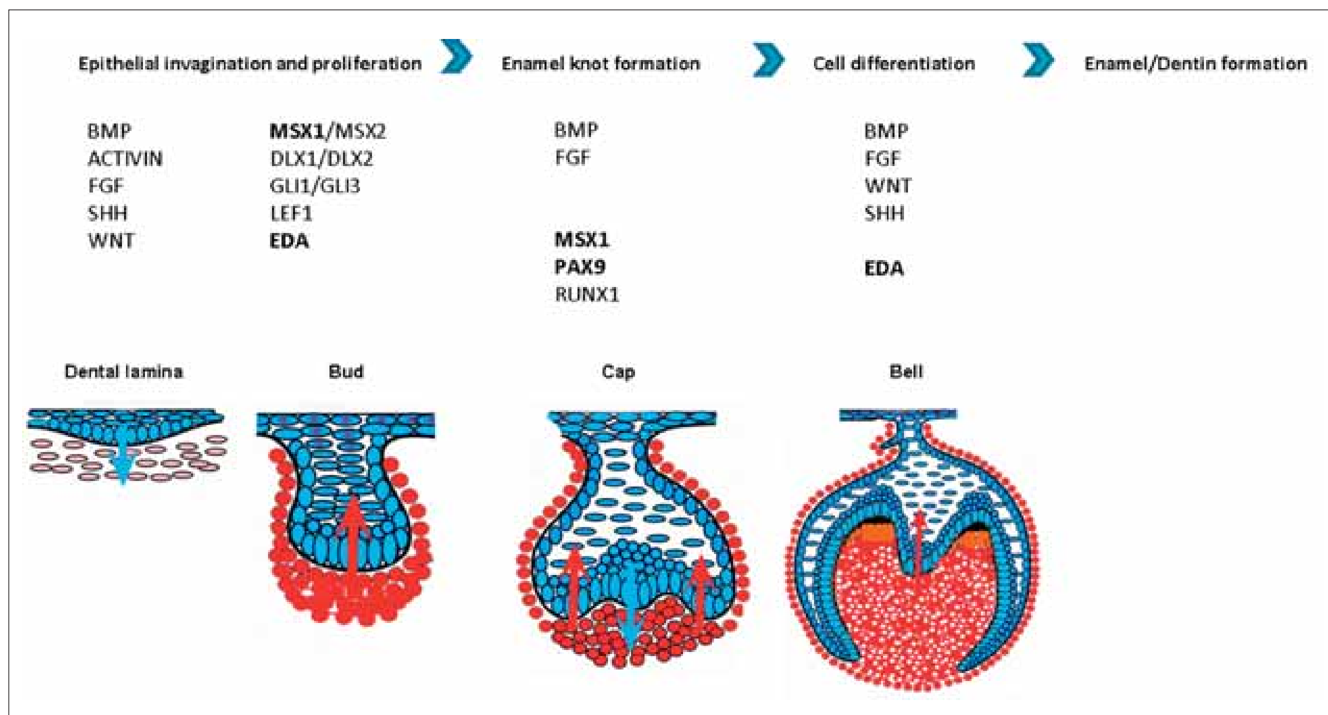

Fig. 6 Process of odontogenesis (tooth formation)
The basis of tooth development is a constant communication between cells of the epithelium and cells of the underlying neural crest derived mesenchyme. This is carried out by means of signalling molecules, which activate (or inactivate) transcription factors. This leads to expression of different groups of gene each time. The same principle is found in the formation of other structures of the head, including formation of the palate.
The molecules known to be involved at each stage of the process of odontogenesis are shown, with those involved in human tooth malformation shown in bold.

exhibit a phenotype with abnormal development of the maxilla and mandible, cleft palate, and absence of teeth (Lu et al. 1999).

The transcription factors MSX1 and PAX9 are responsible for partial tooth agenesis in humans. MSX1 is induced by BMP and FGF molecules and its mutation leads to selective absence of upper lateral incisors and/or upper and lower second premolar teeth (Vastardis et al. 1996, Stockton et al. 2000, Lidral & Reising 2002). MSX1 mutations were also detected in non-isolated tooth agenesis, where hypodontia outside the area of the cleft occurs in association with CLP (van den Boogaard et al. 2000). MSX1 mutation is also involved in a syndromic form (Witkop syndrome) affecting both teeth and nails (Jumlongras et al. 2001).

In mice, deletion of the MSX1 gene results in CP, abnormalities in facial bones and tooth agenesis (Satokata & Maas 1994). PAX9 is induced by FGF molecules and its mutation leads to agenesis of molar teeth (Nieminen et al. 2001, Frazier-Bowers et al. 2002, Das et al. 2003).

Mice deficient for PAX9 present a phenotype which is similar to that observed in MSX1 knock-out mice (Peters et al. 1998).

Clinical relevance of the genetic findings

Identification of the genes responsible for syndromes linked to oligodontia will help prenatal diagnosis and genetic counselling. For example, a successful early (11 weeks of gestation) DNA-based prenatal diagnosis has been applied in a family suffering from the EEC type of ectodermal dysplasia (South et al. 2002).

The gradual identification of the genes responsible for tooth formation could also find remarkable applications in clinical dentistry.

The treatment options available for patients congenitally lacking teeth or for patients who lose their teeth later in life include different types of artificial prostheses. However, the ideal form of tooth replacement is with a biological structure closely resembling the missing tooth. It is hoped that the knowledge on the molecules that drive tooth development and the advances in stem cell research will allow the engineering of new teeth suitable for clinical application. The transition from the laboratory to clinical trials is much easier in dentistry than in medicine where more vital organs are making this exercise too risky.

In fact, recent research has shown that a mouse tooth germ grown *in vitro* from combined embryonic epithelial and mesenchymal tooth cells developed into a fully functional tooth, which subsequently erupted and came into occlusion (Ikeda et al. 2009). This is a significant development and shows that as our knowledge on the genetic programs responsible for tooth formation increases, the possibility of directing stem cells towards forming an entire functional organ by mimicking embryonic developmental processes may become a reality in the near future (Ohazama et al. 2004).

Conclusions

The development of novel clinical therapies for orofacial abnormalities, such as clefts and tooth agenesis, depends very much on a detailed knowledge of the genetic processes involved in the formation of orofacial structures. Intense research in the field of developmental biology aims to lead to clinical benefits. Laboratory development of new products for clefts and hypodontia could provide strategies for therapies in the future.

Résumé

Le développement embryonnaire de la tête, qui inclut la formation des structures dentaires, correspond à un processus complexe et délicat guidé par des programmes génétiques spécifiques. Les changements génétiques et les facteurs environnementaux peuvent perturber l'exécution de ces programmes et aboutir à des anomalies des structures oro-faciales et dentaires. Les fentes oro-faciales ainsi que l'hypodontie et l'oligodontie sont des exemples d'anomalies fréquemment observées en clinique dentaire. Un approfondissement des mécanismes et des gènes impliqués dans la formation des structures oro-faciales et dentaires a été progressivement obtenu par l'analyse génétique de familles et par l'utilisation de modèles expérimentaux de vertébrés tels que la souris et le poulet.

Les fentes oro-faciales, en particulier la fente labiale accompagnée ou non d'une fente du palais ainsi que les fentes palatines seules, se développent à cause de l'expression inappropriée ou défectueuse de protéines, et également à cause de leur absence. Quand cela se produit, les tissus embryonnaires qui contribuent à la formation des lèvres ou du palais ne se développent pas, ne se rapprochent pas les uns des autres et ne fusionnent pas, ce qui entraîne la formation d'une fente/fissure. Dans les cas de fentes humaines, des défauts de certaines protéines (telles que MSX1, TBX22 et IRF6) ont été confirmés par analyse génétique. Lorsque des modèles de souris ne possédant plus ces protéines ont été créés et analysés, il a été mis en évidence la présence de fentes similaires chez ces animaux. En particulier, l'absence de MSX1 chez la souris affecte la croissance des processus palatins, l'absence de TBX22 empêche l'abaissement de la langue et l'élévation des processus palatins, alors que l'absence de IRF6 affecte la fusion de ces mêmes processus palatins.

Le développement des dents correspond à un autre processus qui peut être fortement affecté par des défauts des gènes codants pour des protéines-clés. Chez l'homme, l'absence de dents peut survenir comme une anomalie isolée ou peut être l'une des diverses anomalies observées dans des syndromes tels le syndrome de Rieger, le syndrome Witkop ou la dysplasie ectodermique. Chez l'homme, le syndrome de Rieger est caractérisé par une oligodontie sévère et une hypoplasie de la face moyenne, et est causé par des défauts du gène codant pour la protéine PITX2. Les souris knock-out, qui n'expriment plus PITX2, montrent des anomalies du développement du maxillaire et de la mandibule, ainsi qu'une agénésie dentaire. Des défauts du gène MSX1 engendrent le syndrome de Witkop (caractérisé par des dents manquantes, coniques ou petites et des ongles altérés), alors que MSX1 est également impliqué dans des cas isolés (non liés à un syndrome) d'absence de dents chez l'homme. Les modèles de souris qui n'expriment plus MSX1 montrent des anomalies des os du visage ainsi qu'une agénésie dentaire. Chez l'homme, l'oligodontie et la microdontie apparaissent aussi comme une caractéristique de la dysplasie ectodermique. Les analyses génétiques montrent que le gène p63 est lié à la dysplasie ectodermique de type EEC (ectrodactylie, dysplasie ectodermique, fentes). Lorsque le même gène a été inactivé chez la souris, le développement dentaire s'est vu arrêté de façon précoce, entraînant une agénésie dentaire.

Le développement de nouvelles thérapies cliniques pour les pathologies oro-faciales et dentaires dépend fortement de la connaissance détaillée des processus moléculaires et cellulaires

impliqués dans la formation de la tête. Des efforts sont faits pour développer des technologies basées sur des protéines importantes pour la signalisation cellulaire et l'expression de gènes. Ces protéines pourraient être utilisées pour moduler le comportement cellulaire et réduire la cicatrisation après la réparation chirurgicale des fentes, ou bien être appliquées dans des techniques d'ingénierie tissulaire ayant pour but de développer des tissus oro-faciaux et même des organes, comme les dents, pour une utilisation en transplantation.

Zusammenfassung

Die embryonale Kopfentwicklung, einschliesslich der Bildung von dentalen Strukturen, ist ein komplexer und schwieriger Prozess, der durch spezifische genetische Programme geführt ist. Genetische Veränderungen und Umweltfaktoren können die Ausführung dieser Programme stören und führen zu Anomalien in orofazialen und dentalen Strukturen. Orofaziale Spalten und Hypodontie/Oligodontie sind Beispiele für solche Auffälligkeiten, die häufig in Zahnkliniken gesehen werden.

Das Verständnis für die Mechanismen und Gene, welche bei der Bildung der orofazialen und dentalen Strukturen eingebunden sind, wurde schrittweise durch die genetische Analyse von Familien und die Verwendung von experimentellen Tiermodellen, wie Maus und Küken, gewonnen.

Orale Spalten, die Lippenspalten mit oder ohne Gaumenspalte oder alleinige Gaumenspalten umfassen, können durch falsch exprimierte, defekte oder fehlende Schlüsseleiweisse verursacht werden. Eine Spalte entsteht, wenn Wachstum, Annäherung oder Fusion der zur Lippen- oder Gaumenbildung beitragenden embryonalen Gewebe ausbleibt. Mittels genetischer Analyse konnten beim Menschen Veränderungen in einigen solchen Schlüsseleiweissen (z. B. MSX1, TBX22 und IRF6) nachgewiesen werden. Als Mausmodelle, in denen diese Eiweisse fehlen, generiert und analysiert wurden, sah man, dass orale Spalten auch in der Maus auftreten. Präzise gesagt beinträchtigt das Fehlen von MSX1 das Wachstum der Gaumenlappen, das Fehlen von TBX22 das Absenken der Zunge und die Anhebung der Gaumenlappen, während das Fehlen von IRF6 den Fusionsprozess der Gaumenlappen selbst betrifft.

Auch die Zahnentwicklung ist ein Prozess, der durch das Fehlen bestimmter Schlüsseleiweisse stark beeinträchtigt werden kann. Beim Menschen können fehlende Zähne als isoliertes Ereignis auftreten oder aber Bestandteil der pathologischen Veränderungen sein, die im Rahmen eines Syndromes auftreten, wie z. B. des Rieger-Syndroms, des Witkop-Syndroms oder der ektodermalen Dysplasie. Das Rieger-Syndrom ist durch schwere Oligodontie und Mittelgesichtshypoplasie charakterisiert und wird durch Defekte in PITX2 verursacht. Knock-out-Mäuse, denen das PITX2 fehlt, zeigen abnormale Entwicklung des Ober- und Unterkiefers sowie Zahnagenese. Defekte in MSX1 liegen dem Witkop-Syndrom zugrunde, welches durch fehlende, konische oder kleine Zähne sowie beinträchtigte Nagelbildung charakterisiert ist. MSX1 kann aber auch in isolierten, nicht syndromischen Fällen von fehlenden Zähnen involviert sein. Mäuse, in denen MSX1 entfernt wurde, zeigen abnormale Gesichtsknochenbildung und Zahnagenese. Beim Menschen können Oligodontie und Mikrodontie auch als Bestandteil der ektodermalen Dysplasie auftreten. Genetische Analysen haben ergeben, dass das p63-Gen mit einem Subtyp der ektodermalen Dysplasie, dem EEC-Syndrom (Ektrodaktylie, ektodermale Dysplasie, Lippen-Kiefer-Gaumen-Spalte), verbunden ist. Inaktivierung dieses Genes in der Maus verursacht einen frühen Stopp in der Zahnentwicklung und dadurch Zahnagenese.

Die Entwicklung neuartiger Therapien für klinische orofaziale und zahnärztliche pathologische Anomalien hängt sehr stark von einer detaillierten Kenntnis der molekularen und zellulären Prozesse der embryonalen Kopfbildung ab.

Anstrengungen sind im Gange, neue Technologien zu entwicklen, die auf solchen für Signaltransduktion und Genregulierung wichtigen Eiweissen basieren. Diese Eiweisse könnten dazu verwendet werden, das Verhalten von Zellen zu beeinflussen und Narbenbildung nach operativem Eingriff an einer Spalte zu reduzieren. Diese Eiweisse könnten aber auch für sogenanntes Gewebe-Engineering verwendet werden, mit dem Ziel kraniofaziale Gewebe oder Organe wie Zähne herzustellen und für Transplantationen zu verwenden.

Acknowledgements

The corresponding author is grateful to Professor Dr. Hansueli Luder for helpful discussion, thorough reading and correction of the manuscript and to Dr. Gilles Bluteau for his kind help with the final preparation of this work.

All animal experiments have been carried out according to the highest ethical standards (animal license 219/2007) and approved by the Cantonal Veterinary Department.

The work of Dr. Thaleia Kouskoura in this publication has been funded by the SSO Forschungsfonds.

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