Seminars in Cell & Developmental Biology 25–26 (2014) 11–21

Contents lists available at ScienceDirect

Seminars in Cell & Developmental Biology

journal homepage: www.elsevier.com/locate/semcdb

Review

Early inductive events in ectodermal appendage morphogenesis

Leah C. Biggs, Marja L. Mikkola*

Developmental Biology Program, Institute of Biotechnology, P.O. Box 56, University of Helsinki, 00014 Helsinki, Finland

ARTICLE INFO

Article history:
Available online 30 January 2014

Keywords:
Skin appendage
Epithelial–mesenchymal interaction
Placode
Bud
Dermal condensate

ABSTRACT

The embryonic surface ectoderm gives rise to the epidermis and ectodermal appendages including hair follicles, teeth, scales, feathers, and mammary, sweat, and salivary glands. Their early development proceeds largely the same through the induction, placode, and bud stages prior to diversification of epithelial morphogenesis which ultimately produces the wide array of mature organs. In this review we summarize the current knowledge on the molecular and cellular processes driving the shared stages of skin appendage development revealed by analysis of mouse mutants. We focus on three mammalian organs: hair follicle, tooth, and mammary gland. We reevaluate the information gained from classic epithelial–mesenchymal tissue recombination experiments in light of current molecular knowledge. We place special emphasis on the signaling pathways that mediate tissue interactions, and attempt to link the signaling outputs to changes in cellular behavior that ultimately shape the developing organ.

Contents

1. Introduction

The vertebrate ectoderm gives rise to a wonderful variety of appendages such as hair follicles, teeth, mammary and sweat glands, scales, and feathers to name a few. Despite the apparent differences in the adult form, function, and regenerative capacity, ectodermal appendages share multiple features during development. Several commonalities in the molecular regulation have been uncovered [1]. Further, the early stages of development are morphologically remarkably similar and proceed via induction, placode and bud stages followed by diverse patterns of epithelial growth (Fig. 1). They all arise from two proximate tissues: the epithelium and the mesenchyme, separated by a basement membrane. The epithelial tissue is of ectodermal origin whereas the source of the mesenchymal tissue varies [1]. Sequential and reciprocal crosstalk between the epithelium and the underlying mesenchyme is a critical and uniting theme. Its importance in all aspects of ectodermal appendage development – induction, patterning, morphogenesis, and differentiation – cannot be overemphasized.

* Corresponding author. Tel.: +358 9 191 59344; fax: +358 9 191 59366.
E-mail address: marja.mikkola@helsinki.fi (M.L. Mikkola).

1084-9521/$ – see front matter © 2014 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.semcdb.2014.01.007

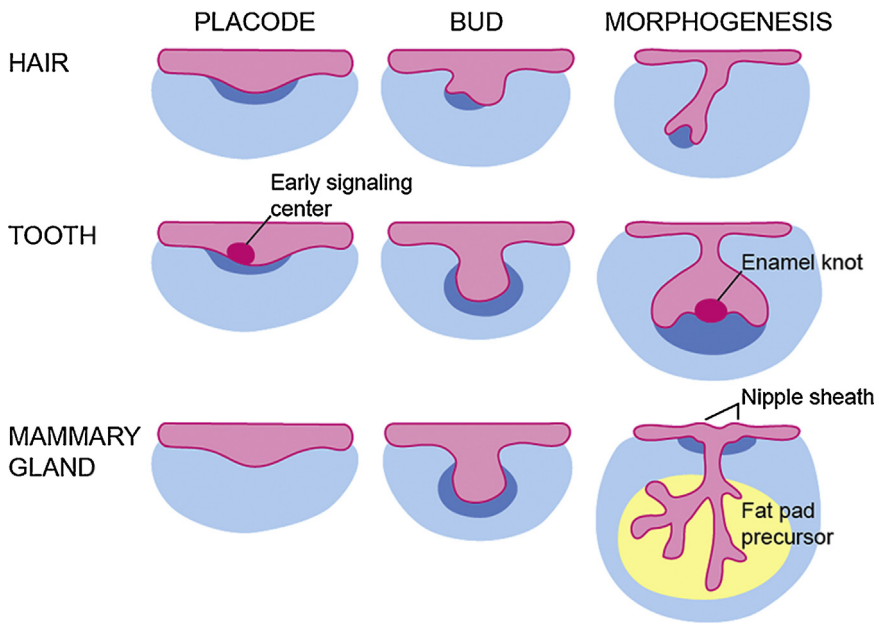

Fig. 1. Development of ectodermal appendages proceeds via shared placode and bud stages prior to diversification of epithelial morphogenesis. In teeth, the epithelial signaling centers express many of the same signaling molecules, but the relationship between the early signaling center and the enamel knot has not been clarified. Expression of many enamel knot markers is also detectable at the tip of the late bud stage tooth (not depicted in the figure). Epithelium, pink; epithelial signaling centers, purple; mesenchyme, blue; condensed mesenchyme, dark blue.

The site and timing of induction for each ectodermal appendage differ. Tooth primordia form in the oral cavity along a horseshoe-shaped region: the dental lamina that marks the future dental arch [2]. In mice, one incisor and one molar tooth rudiment, separated by a toothless diastema region, is apparent in each jaw half at embryonic (E) day E12. The development of the second and third molars differs in that they are generated from the posterior extension of the preceding molar, and thus will not be discussed further in this review. Mammary gland primordia develop along a curved line, the mammary line, that runs between the fore and hind limb on the flanks of the embryo [3]. In mice, five pairs of mammary rudiments become morphologically distinct from the surrounding epidermis between E11 and E12. Accordingly, expression of many placode markers can be detected along the mammary line between E11 and E12, but from ~E12.5 onwards they become restricted to individual mammary primordia [3]. Hair follicle development initiates somewhat later. Murine pelage hair follicles develop in three waves: the first ones, primary hair follicles, are induced at E13.5 and become morphologically pronounced during the following 24 h [4]. The second and third waves of hair placode formation occur at E16.5 and just before birth.

2. Overview of embryonic hair follicle, tooth, and mammary gland development

The first morphological sign of a forming ectodermal appendage is a local epithelial thickening, known as a placode. In all three organs, placodes appear as truly stratified structures (Fig. 2). It should be mentioned that the term placode has not been universally adopted in the field of tooth development research. Instead, stages prior to bud (E11–E12.5) have often been collectively referred to as the epithelial thickening, dental lamina, or simply initiation stage. However, gene expression pattern analyses suggest the presence of two distinct early stages: the continuous dental lamina resolves into separate tooth rudiments between E11 and E12. Many genes are initially (E11.0–E11.5) expressed along the entire dental lamina, but by E12 become evident as discrete spots that mark the epithelium of individual incisor and molar primordia (e.g. *Foxi3*, *Pitx2*) or the early signaling centers therein (e.g. *Shh*, *Bmp2*) [2,5–8] (Fig. 1). Based on morphological and molecular similarities, we regard the E12.0–E12.5 tooth stage equivalent to the placode stage in mammary glands and hair follicles.

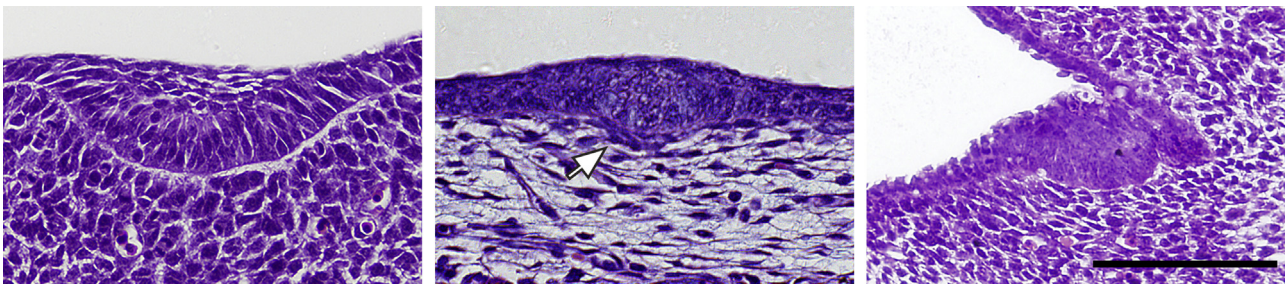

Fig. 2. Hematoxylin-eosin stained sections of a placode stage molar tooth (left), hair follicle (middle), and mammary gland (right). Scale bar, 100 μm. Arrow points to the hair follicle dermal condensate.

After placode formation, the epithelium gives rise to an ingrowth, a bud, which in the case of hair follicle is also called the hair germ. Differences in epithelial morphogenesis become notable after the bud stage. The hair follicle elongates and progresses via peg and bulbous peg stages before reaching its final shape and size postnatally [9]. The dental epithelium transits through cap and bell shapes prior to hard tissue formation [2]. In late bud stage, cells at the tip of the bud cease to proliferate, start to express a multitude of growth factors and thus function as a signaling center known as the enamel knot [2,5]. The enamel knot instructs the surrounding epithelial tissue, the cervical loops, to proliferate and grow deeper into the mesenchyme. The mammary bud epithelium plunges into the dermis and reorganizes into a light bulb-like structure characterized by a narrow neck, a poorly understood morphogenetic phenomenon observed also in developing teeth. After a period of relatively slow growth, the mammary bud elongates at ~E16 into the deeper lying tissue, the precursor of the adult fatty stroma, and undergoes branching morphogenesis [3].

Concurrent with epithelial morphogenesis, the underlying mesenchyme becomes reorganized. The first visible sign of mesenchymal morphogenesis is the condensation of the mesenchymal tissue [1]. This local increase in cellular density is readily evident in histological sections of placode stage hair follicles (Fig. 2). As hair morphogenesis continues, the leading edges of the down-growing follicular epithelium (which bear resemblance to the cervical loops of the tooth epithelium) gradually engulf the dermal condensate (DC) which from thereon is called dermal papilla (DP). In developing molars, quantitative measurements have revealed that placode formation at E12.5 (but not prior stages) is associated with ~50% increase in mesenchymal cell density which is further augmented in the bud stage [10]. We are not aware of quantitative analyses on mammary mesenchyme, but it appears that no specific condensate can be detected at placode stage [3,11]. The condensate becomes evident only at the bud stage (E12.5–E13.0) coinciding with the onset of expression of certain mammary mesenchyme-specific genes such as estrogen receptor α [3,11].

3. Lessons from tissue recombination experiments

Much of our understanding on the mechanisms that govern the induction and patterning of ectodermal appendages is grounded in classic tissue recombination studies. In these experiments, epithelial and mesenchymal tissue layers were enzymatically separated from each other and recombined into new composites where the age, body location, and source (species) of the two tissues could be varied [12,13]. For prolonged culture, the tissue chimeras were usually grafted into the anterior eye chamber or kidney capsule of mice, or the chorioallantoic membrane of chick embryos.

It has been known for a long time that the cap stage tooth mesenchyme can elicit odontogenic potential in heterologous epithelia [14,15]. A combination of dental papilla mesenchyme and plantar epithelium of the foot gave rise to teeth whereas the reverse produced keratinized plantar-like skin only [14]. Mina and Kollar generated a series of chimeras in which isochronic epithelia and mesenchyme from the first (odontogenic) and second (non-odontogenic) branchial arches (BA) were recombined [16]. Between E9 and E11, chimeras produced from first BA epithelium and second BA mesenchyme induced tooth formation at high frequency. At E12 only few, and at E13 none such chimeras gave rise to teeth. The opposite result was observed when first BA mesenchyme was recombined with second BA epithelium: no tooth formation took place in E9–E11 chimeras whereas E12–E13 recombinants readily formed teeth [16]. These findings were substantiated by Lumsden whose data suggest that only neural crest derived "ecto-mesenchyme", but not mesodermal mesenchyme, is competent to respond to the initial epithelial cue(s) [17]. He also showed that isochronic first BA mesenchyme-limb bud epithelium chimeras from E9 to E10 embryos failed to produce teeth whereas in E11 recombinations, teeth did form [17]. Thus, although the timing when the prospective dental mesenchyme acquires tooth-inductive capacity differed slightly between the two reports (~E12.5 vs. ~E11.5) [16,17], collectively these studies reveal that the tooth-inductive potential resides initially in the epithelium, and then shifts to the mesenchyme, the timing of which seems to coincide with the appearance of the condensed dental mesenchyme [10].

In hair follicle research, transplantation assays have been used extensively to test the inductive capacity of the hair-specific mesenchyme. After the pioneering studies using rat vibrissae [18], numerous studies have unequivocally shown that the DC/DP has hair inducing capacity, even when associated with epithelium that normally does not produce hair [19; for review see 20]. These findings are concordant with Kollar's tissue chimera experiments: recombination of E12 snout or E14 dorsal mesenchyme (i.e. DC-containing mesenchyme) with E15 plantar (non-hair forming) epidermis led to hair follicle formation [21]. In contrast, no hair formed in chimeras consisting of E14 back skin epidermis and E15 plantar mesenchyme [21]. These data show that DC but not placodal epithelium possesses the hair fate reprogramming ability. Heterologous mouse-chick recombination assays showed that E14 back skin mesenchyme induces appendage formation even in non-feather forming epidermis [22].

These and other similar experiments [12] have commonly been interpreted to indicate that the initiating signal for hair follicle development arises in the dermis. It should be noted, however, that the inductive capacities of naive (prior to placode/DC formation) epidermis and dermis have not been rigorously tested, or the results have been inconclusive. In particular, E12–E13 back skin-plantar skin recombinants would be highly informative. Dhouailly reported that unlike murine E14.5 dorsal dermis, E12.5 dermis did not induce appendage formation when recombined with non-feather forming epidermis from midventral apterium although the control experiment (E12.5 mouse dermis + E12.5 mouse epidermis) was successful [22]. In the reverse chimeras (mouse E12.5 back skin epithelium + non-feather forming mesenchyme) "small atypical epidermal nodules" formed but their exact nature remained obscure. Taken together, once a morphologically distinct mesenchymal condensate has formed, it bears the hair-inductive potential. Further, the possibility that a similar early epithelial to mesenchymal shift in the inductive potential as observed in teeth exists cannot be currently excluded.

Tissue chimera studies assaying the inductive potential of mammary mesenchyme in non-mammary epithelium have also been performed [13,23]. Recombination of E13 condensed mammary mesenchyme with dorsal or mid-ventral epidermis of E13 mouse and rat embryos induced development of mammary ducts that underwent functional differentiation when grafted in vivo [24]. Thus, as in developing teeth and hair follicles, the condensed mesenchyme bears the inductive capacity. Whether the mammary bud mesenchyme can elicit mammary development in heterologous epithelia also at earlier stages – prior to condensation – remains to be tested.

4. Epithelial morphogenesis

4.1. Signaling pathways involved in placode and bud formation

The Wnt, fibroblast growth factor (Fgf), hedgehog (Hh), and transforming growth factor β (Tgfβ), in particular the bone morphogenetic protein (Bmp) and activinβA, pathways have been

identified as key regulators of nearly all organ systems (for a detailed description of these signaling cascades see [25–29]). In addition, the tumor necrosis factor (Tnf) family ligand ectodysplasin (Eda) and its receptor Edar have a recognized role in ectodermal appendage morphogenesis [30]. We will focus on these pathways in the following discussion.

4.1.1. The Wnt/β-catenin pathway

The canonical Wnt pathway is mediated by β-catenin which becomes stabilized upon receptor activation and complexes with Tcf/Lef1 family of transcription factors for signal transduction [25]. The importance of Wnt/β-catenin pathway in initiation of ectodermal appendage development was first uncovered by mouse mutants deficient in Lef1, which display reduced number of poorly developed hair follicles, absence of most mammary buds, and bud-stage arrested teeth [31]. Further analyses using mice overexpressing the soluble Wnt inhibitor Dickkopf1 (Dkk1) either constitutively under the keratin14 (K14) promoter or inducibly under the K5 promoter (K5-rtTA; tetO-Dkk1) revealed that suppression of canonical Wnt signaling leads to absence of all signs of hair and mammary placode formation [32–34]. Epithelial deletion of β-catenin caused a hair phenotype identical to Dkk1 overexpression revealing the necessity of epithelial Wnt signaling in placode morphogenesis [33,35]. Deletion of β-catenin in mammary epithelium has only been achieved after placode formation. This results in smaller mammary buds, but their further development was not assessed [36]. Tooth development was arrested at the dental lamina-placode and placode-bud transition stage in Dkk1 expressing and β-cat loss-of-function (l-o-f) models, respectively [37]. The difference could be due to earlier Wnt inhibition in the former model, or may indicate a role for mesenchymal β-catenin. Whether canonical Wnt pathway is required for dental lamina formation could not be assessed due to the presence of residual signaling activity at E11.5 in both mouse models.

Wnt/β-cat pathway gain-of-function (g-o-f) studies have also been very informative. Forced epithelial activation of β-catenin advances induction of hair placodes and eventually programs the entire epidermis to hair follicle fate [38,39]. In the oral cavity, it provokes formation of multiple ectopic epithelial invaginations giving rise to supernumerary teeth [37,40]. The effect of epithelial β-catenin g-o-f on mammary placode formation has not been described, but mouse mutants lacking negative regulators of the canonical pathway (Sostdc1, Lrp4) display enlarged mammary placodes/buds and ectopic placode-like structures [36,41]. Genetic rescue experiments confirmed that these defects were primarily caused by elevated Wnt/β-catenin signaling [36]. Collectively, l-o-f and g-o-f studies suggest that a Wnt ligand(s) lies high upstream in the hierarchy of placode regulators, and could even perhaps be the key inducer of ectodermal appendage development. Keeping this in mind together with the presumption that the primary inductive cue for hair development is thought to arise in the mesenchyme, it was somewhat surprising that embryonic hair morphogenesis progresses seemingly normally in mice lacking expression of dermal Wntless (Wls; a.k.a. Gpr177), a protein essential for Wnt secretion [42,43]. This finding argues that a mesenchymal Wnt ligand is an unlikely prime inducer of hair formation.

The consequences of epithelial loss of Wls have been analyzed in embryonic hair and teeth. When Wls was deleted in the embryonic epidermis, hair placode induction was fully inhibited [42,43]. Data from teeth also stress the importance of epithelial Wnts. Whereas loss of Wls in neural crest-derived tissues including dental mesenchyme does not cause retarded tooth growth until bell stage (unpublished data reported in [44]), epithelial deletion leads to a much earlier developmental arrest [44]. However, tooth development proceeds somewhat further than in epithelial β-catenin l-o-f mice. Although this difference may indicate redundancy between epithelial and mesenchymal Wnt ligands, it could as well reflect differential Cre efficiency and/or stability of the transcripts/proteins encoded by the deleted genes. Together, data obtained from manipulating various Wnt pathway components indicate that epithelial Wnt/β-cat signaling, likely activated by epithelial Wnts, is needed for both placode formation and budding morphogenesis.

The importance of mesenchymal Wnt/β-catenin activity for placode formation has also been assessed by l-o-f and g-o-f approaches. In the mammary region, Dermo1-Cre mediated deletion of β-catenin does not interfere with placode/bud formation [45], but the great variability observed in Cre activity hampers data interpretation. No mammary mesenchyme specific g-o-f studies have been reported. In developing teeth, deletion of β-catenin with Prx1-Cre (active in the incisor region only) does not halt incisor morphogenesis but occasionally results in splitting of the placode [46]. Deletion using Osr2-Cre allows molar morphogenesis up to bud stage [47]. Unfortunately, in both murine models, inactivation of β-catenin is initially mosaic precluding conclusions on the effect of an early depletion of mesenchymal Wnt/β-catenin activity on tooth morphogenesis. On the other hand, mesenchymal stabilization of β-catenin with Osr2-Cre induces formation of tooth bud-like structures in the palate. These invaginations express several markers of the dental epithelium, but lack many mesenchymal ones and apparently do develop beyond aberrant bud stage [47]. Thus, both epithelial and mesenchymal Wnt/β-catenin activity can trigger tooth induction – this may suggest that the Wnt pathway is imperative both in the initial epithelial odontogenic potential, as well as in the later ability of the mesenchyme to induce tooth formation.

In the skin, En1-Cre driven inactivation of mesenchymal β-catenin fully inhibits hair placode formation. However, it also leads to loss of several fibroblast lineage markers in the upper dermis raising the possibility that the defect in hair follicle formation could be secondary to loss of proper dermal identity rendering these cells incapable to support hair formation [42]. Tbx18-Cre mediated deletion of β-catenin leads to near complete ablation of Wnt/β-catenin signaling in forming DCs at E14.5. In this model, loss of β-catenin does not interfere with primary hair placode formation, but results in aborted morphogenesis soon after likely due to downregulation of several DC-derived paracrine factors such as *activin βA* and *Fgf7/10* [48; see also below]. In contrast to epithelial β-catenin g-o-f mutants [38,39], forced mesenchymal stabilization of β-catenin does not induce ectopic or premature hair follicle initiation but increases the size of hair placodes and associated DCs [42].

Since activation of epithelial β-catenin is currently the only genetic manipulation known to cause precocious hair induction, and only epithelial Wnts are required for hair follicle induction, does this indicate that an epithelial Wnt ligand is the long-sought primary inductive cue for hair development? While there are currently no strong data to argue against, it is equally possible that the mesenchyme provides the first signal, perhaps one that renders the epithelium competent to respond to epithelial Wnts, either directly or indirectly. An interesting twist to the Wnt saga was the identification of R-spondins (Rspo1-4) as secreted agonists of the canonical Wnt pathway and G-protein-coupled receptor 4/5/6 (Lgr4/5/6) as their receptors [49]. Although the detailed expression pattern of Lgr4/5/6 and Rspo1-4 in ectodermal appendages awaits analysis, at least Lgr4 and Lgr6 are expressed in the epithelium during early stages of hair development whereas *Rspo* transcripts seem to be located both in the epithelium and the mesenchyme [50,51; www.genepaint.org; www.eurexpress.org]. K5-Cre mediated epithelial deletion of *Lgr4* leads to severely reduced number of primary hair placodes [52], but the Rspo(s) involved have not been identified. An attractive hypothesis is that a mesenchymal Rspo could function as (part of) the first inductive

cue to potentiate autocrine Wnt/β-catenin signaling in the epidermis.

4.1.2. The Eda pathway

Eda, its receptor Edar, and the intracellular adaptor protein Edaradd form a linear pathway leading to downstream activation of the transcription factor NF-κB [30]. Edar is focally expressed in placodes, and the pathway is exclusively active in the epithelium. K14-promoter driven overexpression of Eda (K14-Eda mice) increases hair placode size, whereas inactivation of the pathway abrogates primary hair placode formation [53–56]. Detailed histological and placode marker analyses have revealed the presence of rudimentary epithelial structures termed pre-placodes in Eda pathway mutants [57–59]. This initial placode specification requires Wnt/β-catenin activity, but the maintenance and further morphogenesis of pre-placodes depend on Eda [33]. Stabilization of epithelial β-catenin can bypass the need for Eda in primary hair placodes, but forced activation of Eda/Edar cannot rescue placode formation in the epithelial β-catenin l-o-f model [33,38]. Further, a complex interdependence between the two pathways has been revealed: Wnt/β-catenin upregulates expression of *Edar* in placodes whereas Edar/NF-κB is needed to maintain focal epithelial Wnt activity and expression of *Wnt10a/b* [33]. In mice mutant for both Eda and Troy, a Tnf receptor related to Edar, secondary hair placodes also fail to form [60]. This is an intriguing finding in light of recent data showing that Troy suppresses canonical Wnt signaling in the intestinal epithelium [61]. Whether this function of Troy is conserved in hair placodes is not known.

In contrast to primary hair placodes, Eda/NF-κB is dispensable for tooth and mammary placode formation [56,60,62,63]. In K14-Eda mice, multiple supernumerary mammary placodes are induced along the mammary line giving rise to nipples with associated ductal trees in the adult [53,64]. In addition, a supernumerary tooth is induced at high frequency anterior to the first molar [64]. Whether it forms from a de novo placode similar to ectopic mammary placodes, or as result of an anterior extension of the first molar via a process resembling the generation of second and third molars, is not known. Thus, although not necessary, excess Eda pathway activation can provoke dental/mammary cell fate [53,64,65]. One plausible explanation for the absence of an early dental/mammary phenotype in Eda null embryos could be the presence of another NF-κB inducer. However, expression analysis of an NF-κB reporter transgene in *Eda* null background does not to support such a possibility [60,63].

Tooth buds of *Eda* null embryos are smaller, indicating a role in budding morphogenesis [62]. Several placode markers are also expressed at reduced levels in mammary placodes and buds and thus a presence of a subtle mammary bud phenotype cannot be excluded [8,63]. The function of Eda in hair bud formation is less clear. Conditional murine models in which *Eda* transgene expression was induced in *Eda* null background suggest that Eda is needed only until the early dermal papilla stage [66]. Analysis of tail hair formation (which also fail to form in the absence of Eda) in another in vivo model where the inductive properties of Eda were tested by administering recombinant Eda protein to *Eda* null mice suggests that Eda is needed only very briefly for placode formation but not for the following morphogenesis [67].

4.1.3. The Fgf pathway

Ligand engagement of any of the seven mammalian Fgfr isoforms (encoded by four Fgfr genes) may activate several downstream pathways including the Ras-Raf-Mapk and PI3K-Akt pathways [26]. Dissecting the exact role of Fgf signaling in ectodermal appendage development has been hampered by redundancy at the level of both ligands and receptors. Data from in vitro experiments in which E10–E11 mandible explants were treated with SU5402, a pan-Fgfr inhibitor, suggest that Fgf signaling is not essential for the expression of dental lamina/placode markers [68]. However, in this study the morphological development was not assessed. The only *Fgfr* detectable in the dental epithelium at early developmental stages is the IIIb isoform of Fgfr2 (*Fgfr2b*) [69]. In mice with germline deletion of *Fgfr2b*, teeth develop up to placode stage [70,71]. A similar result was obtained when both isoforms of *Fgfr2* were conditionally inactivated with the K14-Cre driver [72]. Thus, Fgf signaling seems to be dispensable for tooth placode formation, yet indispensable for budding morphogenesis.

As in the oral cavity, expression of *Fgfr2b* is also confined to epithelium in the embryonic skin. In contrast to teeth, however, Fgfr2b plays an early essential role in mammary placode formation. Mice null for *Fgfr2b*, or its ligand *Fgf10*, lack all mammary placodes except the fourth [73,74]. Expression of *Fgf10* is unaffected in Dkk1 overexpressing embryos suggesting that Fgf10 lies upstream of, or is parallel to, the Wnt/β-catenin pathway [34]. It is not known whether placode 4 forms independently of Fgfs, or whether loss of Fgf10/Fgfr2b signaling is compensated for by another Fgf ligand-receptor pair.

A positive role for Fgfr2b in early hair morphogenesis was inferred from the reduced number of hair buds observed in *Fgfr2b* null embryos [70,75], although the exact developmental stage affected has remained elusive. Somewhat surprisingly, a more recent study showed that the expression of Fgfr2b is downregulated in nascent hair placodes [76]. Further, exposure of naïve embryonic skin explants to Fgfr2b agonists either prevents hair placode induction (Fgf7) or has no effect (Fgf10). In line with the in vitro study, transgenic overexpression of Fgf7 (K14-Fgf7) suppresses hair formation [77] and epithelial deletion of both *Fgfr1* and *Fgfr2* using a K5-Cre transgene expressed from E15.5 onwards (i.e. presumably active during induction of secondary and tertiary hair follicles that constitute >95% of pelage hairs) has no obvious deleterious effect on hair numbers or morphogenesis [78]. Further studies are needed to define the role of epithelial Fgfrs in early hair follicle morphogenesis.

4.1.4. The Hedgehog pathway

Hh binding to its receptor Patched (Ptch1 or Ptch2) relieves Ptch mediated repression of the transmembrane protein Smoothened (Smo), an obligate component of the Hh signaling cascade. Smo activation converges on Gli1-3 proteins, which are key transcriptional effectors of the canonical Hh pathway [27]. Of the three mammalian hedgehog proteins, Sonic hedgehog (Shh) is the only one implicated in ectodermal appendage morphogenesis. Expression of *Shh* is epithelial in developing appendages, but in mammary buds *Shh* transcripts are barely detectable [1,79]. In contrast to teeth and hair follicles [80–82], no Gli1+, Ptch1+ cells have been detected at any stage of mammary gland development indicating absence of canonical Hh signaling, thereby distinguishing the mammary gland from other ectodermal appendages [83].

Shh null mice have severe craniofacial abnormalities and therefore are not informative in tooth development studies, but *Shh* has been conditionally deleted using the K14-Cre driver [84]. In this model, some residual *Shh* expression was still detected at placode stage precluding analysis of Shh's function at earlier stages. Molar placodes and buds were less deep but wider indicating that Shh somehow organizes placodal cells, but the mechanism is not known [84]. In addition, Shh seems to act as an epithelial mitogen [80]. An early function was indicated also by analysis of mice lacking Gli2 and Gli3. Compound mutant embryos analyzed at E13.5–E14.5 show no sign of molar development and only a rudimentary central incisor bud [80]. Unfortunately, interpretation of these data is complicated by the fact that Gli2/3 have both activator and repressor functions [27; see also below] and that the effects of Shh may be in part mediated by the mesenchyme [80]. Comparison of conditional

Shh or *Smo* mutant mice, generated using the same K14-Cre strain, support this conclusion: *Smo* deficient embryos have a milder tooth phenotype than those lacking *Shh* [84,85].

In hair follicles, Shh appears to mark more committed placode cells as no expression of *Shh* or *Ptch1* has been detected in primary hair pre-placodes that form in Eda/NF-κB mutant mice [54,55,57,86]. Shh is not required for hair placode formation, because hair morphogenesis proceeds up to the placode/bud transition stage in *Shh* null embryos [81,82]. This defect is phenocopied by loss of Gli2 and is associated with reduced epithelial proliferation in both mutants [81,87]. Gli2 deficiency can be rescued by epidermal Gli2 expression confirming a key role for epithelial Gli2 in hair bud growth [87].

Hh proteins are dispensable for mammary gland development [79,88], yet mice lacking functional Gli3 (*extra-toes* mutant) fail to induce placode 3 and 5, and buds 2 and 4 are hypoplastic [74,83,89]. These two findings may seem contradictory at first glance. However, there is strong genetic evidence showing that Hh signaling needs to be off to allow induction of mammary placodes 3 and 5, a state achieved by the repressor function of Gli3 [83]. Reduced *Fgf10* expression has been proposed to explain the mammary phenotype of *Gli3* mutant mice [74].

4.1.5. The Tgfβ pathway

Cellular responses elicited by the large family of Tgfβ ligands are mediated by a transmembrane receptor complex formed by type I and type II receptors. Signal transduction is propagated through the phosphorylation of either Smad1/5/8 (Bmps) or Smad2/3 (Tgfβ1–3 and activins) that partner with Smad4 for canonical signal transduction [28,29]. Several regulators of the Tgfβ pathway have been linked with the early inductive events guiding hair and tooth development, but so far no mouse mutants with an early mammary gland phenotype have been reported.

Many studies have indicated that suppression of epithelial Bmp signaling is necessary for hair placode morphogenesis to take place [58,90,91]. This is achieved by noggin, a potent inhibitor expressed in the dermal condensate. Noggin null mice lack secondary and tertiary hair placodes yet primary placodes are induced [91,92]. Excess noggin increases hair placode density ex vivo and hair follicle density in vivo, whereas administration of Bmp4, normally expressed in the dermal condensate, precludes hair placode induction in embryonic skin explants [58,90,91,93]. It has been proposed that Ctgf and follistatin, two Tgfβ antagonists induced by Edar, could compensate for loss of noggin in primary hair placodes [58,91], but genetic evidence for their role in hair placode formation is lacking.

The negative effects exerted by epithelial Bmp signaling on hair placodes are not fully understood, but include at least inhibition of Wnt/β-cat signaling via downregulation of Lef1 and suppression of *Edar* expression [58,90,94]. In addition, Bmps generate a Wnt/β-catenin inhibited zone around hair and mammary placodes by inducing the expression of *Sostdc1* [58,95]. In *Sostdc1* null embryos, primary hair placodes are slightly enlarged and multiple supernumerary vibrissae form [36,41]. On the other hand, mice lacking follicular Bmp signaling (achieved via K14-Cre mediated deletion of Smad1 and Smad5) display fewer and poorly developed hair follicles at birth suggesting a positive role for Bmps in hair bud downgrowth [96].

The function of Bmps in dental placode and bud morphogenesis appears quite different from that reported in hair follicles. A positive role for Bmp4 in particular was proposed two decades ago when it was realized that its expression shifts from the epithelium to mesenchyme simultaneously with the shift in odontogenic potential. Further, in tooth mesenchyme explants Bmp4 induces its own expression and that of several genes essential for tooth development [2,97]. Several murine models with suppressed Bmp signaling have been reported, but they all present with a relatively late bud-to-cap transition arrest [1,2,98]. In the dental epithelium, Smad4 functions redundantly with p38 Mapk signaling to mediate Tgfβ signaling [99], but so far no role for epithelial Bmp signaling prior to cap stage has been confirmed.

Only recently, a role for Bmps in tooth placode morphogenesis was genetically confirmed by generating a K14-Cre inducible noggin transgenic mouse [100]. In this model, prominent noggin overexpression was achieved already at the dental lamina stage (E11.5) which led to arrested molar development at the lamina-placode transition. Noggin is a secreted molecule, and therefore it remained uncertain whether the target tissue was the epithelium, mesenchyme, or both. When Smad4 is deleted in the dental mesenchyme using Wnt1-Cre (active in neural crest cells) dental lamina appears unaffected [101]. The *Smad4* mutant embryos die at E11.5-E12.5 impeding further analysis, but organ culture experiments suggest that the mutant teeth do not progress beyond the lamina stage, similar to noggin overexpressing mice [101]. Although inactivation of Smad4 inhibits signaling by all Tgfβ family members, the mesenchymal *Smad4* deficient phenotype most likely reflects loss of Bmp signaling because other putative Smad4 activators either have no known role in early tooth development (Tgfβ1–3) or the epithelium is their likely target tissue (activin A; see below) [102].

A growing body of evidence indicates that the Wnt and Bmp pathways regulate one another [38,46,103]. Notably, the noggin transgenic tooth phenotype is similar to that of Dkk1 overexpressing embryos [37,100]. However, these models are not phenocopies of one another. For example, expression of *Shh* is completely abolished in Dkk1 transgenic embryos, but unaffected by noggin overexpression. Also many mesenchymal markers including *Bmp4* are only slightly affected in noggin mutants but abolished in Dkk1 mice [37,100]. Further, a recent genome-wide gene expression profiling study suggests that a Wnt-Bmp feedback circuit is the key regulator of epithelial-mesenchymal interactions in developing teeth [103], but the full nature of these interactions remains to be uncovered.

In addition to Bmps, at least two other Tgfβ superfamily members, Tgfβ2 and activin A, are involved in early ectodermal appendage morphogenesis. Activin A, consisting of two polypeptides encoded by the *activin βA* (*inhba*) gene, is expressed in the mesenchymal condensate both in teeth and hair follicles, but based on target gene expression its actions are limited to the epithelium [102]. In *activin βA* null mice, development of incisors and mandibular molars proceeds only up to a small bud stage yet mesenchymal condensation is unaffected [102]. The reason for the growth arrest has remained inexplicable. *Activin βA* null mice display a differentiation defect in vibrissae, but the pelage hair phenotype has not been reported [104]. Of note, hair follicles of *Tgfβ2* null embryos exhibit a profound delay in hair morphogenesis. Tgfβ2, also emitted from the mesenchyme, positively regulates epithelial proliferation in the hair bud [105,106].

4.2. Cellular mechanisms driving placode and bud formation

As described in Section 4.1, the details of the molecular regulation of placode and bud formation are being uncovered with greater precision. A central question is how this molecular information is translated into coordinated changes in cellular behavior that ultimately drives epithelial morphogenesis. Does the use of common signaling cascades such as the Wnt/β-catenin pathway indicate that all ectodermal appendage placodes form via the same mechanism? Or, does the apparently divergent use of, e.g. Fgfr2 rather point to at least partially distinct mechanisms? One possible scenario is the existence of a unifying theme with distinct variations in each organ.

The cellular mechanisms most commonly discussed in the context of ectodermal appendage placodes are change in cell shape

from cuboidal to columnar, focally increased cell proliferation, and cell migration [1,3,12,107]. Mechanisms involved in tooth placode formation are particularly poorly studied, but both changes in mitotic spindle orientations and cell proliferation have been proposed to be involved [1,100]. We have recently set up confocal time-lapse imaging of embryonic skin explants to visualize hair placode formation in live tissues (Ahtiainen et al., unpublished data). Our data suggest that primary hair placodes form via directed cell migration and cell intercalation resulting in a truly stratified thickening of the epithelium. Quantitative analysis of the Fucci cell cycle indicator transgenes [108] do not support cell proliferation as the cellular mechanism driving placode formation, but instead suggest that proliferation needs to be suppressed for placode morphogenesis to take place (Ahtiainen et al., unpublished data). This conclusion is in line with data showing that treatment of skin explants with potent mitogens such as Fgf7 or epidermal growth factor receptor agonists prevents placode formation [76,109].

Several studies also implicate cell migration as the main cellular mechanism associated with mammary placode formation [3]. This was first proposed by Balinsky based on comparison of mitotic indices in early mammary rudiments and the surrounding non-mammary epithelium in mouse and rabbit embryos [110,111], and later by Propper who observed cells with motile characteristics atop the mammary ridge in the rabbit embryo by scanning electron microscopy [112]. A more recent study also showed near absence of BrdU incorporation in forming mammary placodes [89]. In *Lrp4* and *Sostdc1* mutant mice, excess mammary epithelial cells (judged by Wnt reporters and other placode markers) form and are maintained for a longer time in-between mammary placodes [36,41]. Interestingly, these cells show greatly reduced cell proliferation, similar to mammary placode cells [36]. This finding is concordant with our unpublished results on the E13.5–E14.5 back skin where excess Wnt protein, or overexpression of Eda, confers interplacodal cells certain placode characteristics such as increased motility and decreased proliferation. Collectively, these data indicate that acquisition of hair/mammary placode cell fate downstream of Wnts and/or Eda correlates negatively with cell proliferation but positively with cell motility.

After hair, tooth, and mammary placodes have emerged they invaginate to form bud structures. An interesting question is whether and how budding morphogenesis differs from placode formation. The rapid growth of hair and tooth buds strongly suggests cell proliferation as the driving force, and it seems that multiple pathways including Wnt/β-catenin, Shh, Fgf, and Tgfβ are involved in bud formation, and/or downgrowth. Analysis of the skin phenotype of epidermal Gli2 and other Shh pathway g-o-f studies indicate that Shh signaling functions to promote epithelial cell proliferation, likely through the D-type cyclins [87,113; and references therein]. BrdU incorporation assays suggest that the block in tooth placode to bud transition in *Fgfr2b* null embryos is due to reduced epithelial cell proliferation [71,72] further hinting that budding is driven by proliferation.

In contrast to teeth and hair follicles, budding morphogenesis seems to proceed very differently in mammary glands. Very low mitotic indices have been reported not only at the placode stage but at least until E14.5 (late bud stage) indicating that cell proliferation has only a minor contribution in mammary bud formation [89,110,111]. Therefore it seems plausible that continuous influx of epithelial cells likely accounts for the observed (slow) growth of mammary buds. In addition, basal cells are reported to change shape and increase in size thereby contributing to bud growth [89]. Two models for cell migration in forming buds have been proposed: (1) movement, perhaps even long distances, along the mammary line, and (2) centripetal, but short-distance, migration from all directions toward the bud [3]. BrdU pulse-chase experiments support the second model [89]. Regardless which model turns out to be correct, one interpretation of the current data is that mammary placode and bud morphogenesis is a continuum of a single cellular process mainly driven by migration. However, 3-dimensional (3D) live imaging will be necessary to confirm the involvement of cell migration in mammary placode and bud formation.

Modulation of cell–cell and cell–extracellular matrix interactions (ECM) also play important roles in ectodermal appendage morphogenesis. In hair follicles, E-cadherin levels decrease and in particular P-cadherin levels increase already at early stages of placode formation indicating alterations in adherens junctions [86,114]. Modification of adhesion molecules is generally regarded as a prerequisite for epithelial cells to become motile. An intriguing possibility is that the change in E-cadherin/P-cadherin balance serves that purpose in hair placode morphogenesis. However, downmodulation of E-cadherin plays a role at least during budding morphogenesis. In secondary and tertiary follicles, noggin and Tgfβ2 support bud formation by downregulating E-cadherin via Lef1 and Snail, respectively [94,106], and transgenic overexpression of E-cadherin suppresses hair follicle formation [94].

Integrin mediated cell–ECM interactions have been analyzed in hair follicles and teeth. The predominant epidermal integrins are α3β1 and α6β4, both of which bind laminin-511 which is abundant in the hair follicle and tooth basement membrane. Epithelial deletion of integrin β1 leads to severe defects in basement membrane organization, reduced epithelial proliferation, and failure in hair follicle invagination [115,116]. Mice null for the α5 subunit of laminin-511 show reduced epithelial proliferation and arrested hair follicle development at the bud stage [117,118], further highlighting the importance of the basement membrane in budding morphogenesis. In developing teeth, loss of the α5 subunit does not completely stall tooth morphogenesis, but gives rise to smaller tooth buds, possibly due to reduced epithelial cell proliferation [119].

5. Mesenchymal morphogenesis

5.1. Molecular regulation of mesenchymal condensate formation

The appearance of the mesenchymal condensate somewhat varies in developing ectodermal appendages being evident at a very early stage of placode formation in hair follicles, somewhat later in teeth, and only at the bud stage in mammary glands. Compared to the wealth of genetic information on early epithelial morphogenesis, knowledge on the molecular regulation underlying mesenchymal condensate formation is very rudimentary but at least one common theme is emerging: involvement of the Fgf family. In addition, some organ specific pathways have been uncovered.

Recent identification of Wnt/β-catenin and Edar pathway target, Fgf20, provides great insight into the formation of hair follicle dermal condensates (DCs) [86]. Fgf20 is expressed in the epithelium of all hair follicle types and in primary hair placodes already at E13.5, prior to detectable DC markers. Its deletion results in absent primary and reduced numbers of secondary DCs. Tertiary DCs do form though, indicating the presence of a compensatory pathway in this hair follicle type. None of the classical markers of DC/DP are expressed in primary DCs and no evidence of a morphological DC could be detected in the absence of Fgf20 [86]. Primary hair placodes do form in a relatively regular pattern in *Fgf20* null embryos. This evidence clearly states that the DC inductive signal originates in the epidermis. Further, it shows for the first time that mesenchymal and epidermal morphogenesis and patterning can be uncoupled at the early stages of hair development [86]. In embryos with stabilized epithelial β-catenin, the entire upper dermis appears condensed and expresses DC markers [38,39]; deletion of *Fgf20* in this background does not preclude precocious placode

induction but completely abolishes DC marker expression [86]. Thus, Fgf20 is necessary for DC induction but whether it is also sufficient is not yet known.

Fgf signaling activity also contributes to DC formation in chick. The spontaneous mutant, *scaleless*, lacks dermal condensates and ultimately scales and feathers in certain genetic backgrounds. Recently, the mutation was mapped to the *FGF20* locus [120]. These animals also exhibit absence of placodes although some epithelial genes show a low level of focal, but transient, expression [121,122]. The severity of the *scaleless* phenotype depends on the genetic background, but the most affected cases seem to indicate a broader function for FGF20 in feathers than in hair follicles.

Loss of Fgf20 has no obvious effect on early tooth morphogenesis [123]. However, the Fgf pathway has also been implicated in mesenchymal condensation in developing teeth. An experiment in which dental epithelium was placed atop a monolayer of E10.5 BA1 mesenchymal cells was used to test the capacity of epithelia of different stages to induce mesenchymal condensation [10]. Transcriptional profiling identified five signaling molecules, *Bmp4*, *Cxcl12*, *Fgf8*, *Shh*, and *Wnt5A*, whose temporal expression pattern correlated well with the condensate-inducing ability. Of the five candidate proteins only Fgf8 was able to attract mesenchymal cells in vitro [10]. However, the endogenous Fgf involved has not been confirmed by genetic means. Several Fgfs (including Fgf9, -15, -17, and -20) [124,125] are expressed in the dental epithelium at E11.5 and may have overlapping functions with Fgf8. *Fgf8* has been conditionally inactivated in BA1 epithelium, but at an earlier stage using Nestin-Cre, which results in loss of expression by E9.0 and extensive mesenchymal cell death indicating a role for Fgf8 in cell survival as well [126].

Currently, the molecule(s) that induce condensation of the mammary gland mesenchyme is not known, and so far there are no data to support or exclude Fgf participation. Instead, there is evidence for involvement of the β-catenin and parathyroid hormone-related protein (PTHrP) pathways. PTHrP is expressed in the epithelium while its receptor, Pthr1, is expressed broadly in the mesenchyme [127]. If either of these genes is ablated, mesenchymal compaction is compromised and mammary development stops after this initial condensation, and the mesenchyme in turn fails to maintain the mammary-specific epithelial gene expression profile [45,127]. Inactivation (albeit mosaic) of mesenchymal β-catenin after placode stage using Dermo1-Cre results in a PTHrP-mutant like phenotype with fewer layers of condensed mesenchymal cells [45]. This was proposed to be the result of reduced cell proliferation; however, it remains unclear whether mesenchymal condensation per se was affected or if the phenotype was due to fewer cells being available for condensate formation. In both β-catenin and PTHrP mutants, expression of several markers of condensed mesenchyme including androgen receptor and tenascin C is lost [45,127]. Ultimately, Wnt and PTHrP pathways were shown to interact: mesenchymal Wnt activation was shown to fully depend on PTHrP signaling thereby explaining the similarities of the phenotypes [45]. Although the mechanism whereby PTHrP enables mesenchymal Wnt signaling is not understood, the ability of PTHrP to upregulate the expression of *Rspo1* provides one possible link [45].

It is not yet clear whether this function of mesenchymal β-catenin is conserved in other ectodermal appendages. In developing teeth, inactivation of mesenchymal β-catenin also abolishes the expression of certain mesenchymal genes, yet neither l-o-f or g-o-f models generated so far implicate a role for β-catenin in condensation of the dental mesenchyme [46,47]. In the skin, En1-Cre mediated deletion of dermal β-catenin leads to loss of all signs of hair formation including DC, yet forced activation of dermal β-catenin fails to program the upper dermis into DC fate [42]. Deletion of β-catenin in the forming primary DCs via Tbx18-Cre results in downregulation of several DC markers, arrest in follicular morphogenesis, and apparent loss of DC [48] indicating a role in DC maintenance. However, interpretation of dermal β-catenin l-o-f models is challenging as some manipulations may lead to premature deletion which may impede survival and/or specification of the dermis known to depend on canonical Wnt signaling [128], or too late deletion which may not be efficient enough to ensure complete inactivation at the time of DC formation (see also Section 4.1.1).

Studies in hair follicles have indicated that distinct pathways may regulate the formation and maintenance/maturation of the mesenchymal condensate. Deletion of platelet-derived growth factor-A (Pdgf-A), expressed throughout the epithelium, results in smaller DPs [129]. However, the DP-phenotype may not be specific, but secondary to generalized dermal hypoplasia caused by reduced dermal proliferation in the absence of Pdgf-A [129]. Additionally, absence of Shh leads to smaller DCs that do not mature to DP [81,82,129]. Dermal knockout of the Shh effector Smo ultimately results in disintegration of the DC and eventual arrest of hair follicle development confirming that Shh acts directly on DC cells [113]. Smo activation seems to feed back to the epithelium by upregulating noggin [113].

5.2. Cellular mechanisms associated with mesenchymal condensate formation

The mesenchymal condensates not only differ in the timing of their induction with respect to epithelial morphogenesis, but also in their proliferative status. While the hair follicle-associated condensate is famous for showing little proliferative activity, mammary, and in particular, tooth mesenchymal cells are highly mitotic [10,89,107,130]. This, however, does not necessarily indicate that the cellular mechanisms driving their aggregation need to be distinct. In teeth, the mitotic indices of the condensing cells adjacent to the tooth germ and the more distally located non-condensing cells are similar from E10.5 to E13.5 [10]. There is no significant difference in BrdU incorporation of mammary and nearby dermal mesenchymal cells prior to (E11.75) or at the onset (E12.5) of condensation either [89]. The hair condensate expresses cyclin-dependent kinase inhibitor *p21* from early stages and appears mitotically inert as soon as it is morphologically discernible [86,107]. These data suggest that local variations in cell proliferation are unlikely to drive mesenchymal condensation in these three organs. However, it has also been proposed that hair follicle DC cells gain quiescence only after condensation and that proliferation is in fact essential for DC formation [12]. An attractive alternative mechanism for proliferation is chemotaxis toward a placode-produced cue. Additionally, enhanced chemokinesis combined with local changes in adherence to substratum could result in condensate formation. An interesting related question is whether condensation occurs first and functional differentiation of the mesenchyme second, or vice versa.

The evidence available so far points to the involvement of directed cell migration in compaction of the tooth mesenchyme. E10.5–E11.5 dental epithelium overlaid on E10.5 BA1 mesenchymal cells was shown to promote chemokinesis. Additional experiments showed that these epithelia attract distant cells to the epithelial–mesenchymal interface but also repulse the nearby cells [10]. Fgf8 and semaphorin-3f (Sema3f), also produced by the dental epithelium, were identified as the stimulatory and repulsive migratory factors, respectively. These opposing cues were proposed to account for the relatively sharp border between condensed and non-condensed mesenchyme in vivo [10].

Condensation of dental mesenchymal cells is associated also with reduction in cell volume [10]. In a series of in vitro experiments

it was shown that a mere decrease in cell size as a result of increased cellular density substantially augments the expression of several dental mesenchymal markers including *Bmp4*. The authors proposed that Fgf8 and Sema3f manifest their pattern-generating actions mechanically rather than chemically, and that altered mechanotransduction leads to functional differentiation of the dental mesenchyme [10]. The relative contribution of epithelial signals such as Wnts, Shh, and Bmp4, as well as that of tissue mechanics in generation of the tooth specific mesenchyme are areas of future research.

The cellular mechanisms associated with hair follicle DC formation have not been identified, and the downstream effects exerted by Fgf20 are unknown. In vitro studies in chick have examined the effects of other Fgf family members on mesenchymal cell behavior. Fgf4, expressed in the feather placode in vivo, has been shown to act through p-ERK as a chemoattractant for mesenchymal cells both in vitro and ex vivo [131]. Both in mouse and chick skin, p-ERK activity localizes to the DC during early hair/feather morphogenesis [86,131]. Also Fgf2 has been implicated to form dermal condensates through cellular migration [132]. Fgf2 is normally expressed in the feather placodes but is absent in *scaleless* skin. Ectopically applied Fgf2 locally rescues feather formation in *scaleless* embryonic skin explants [133]. Collectively, these findings hint to a role for Fgf20 in mesenchymal cell motility.

6. Concluding remarks

In this review, we aimed to summarize the current knowledge on the signaling cascades regulating early ectodermal appendage development. Although we focused on the 'major' pathways, new data on less studied signals such as semaphorins and chemokines [10,134] indicate that the puzzle is not yet complete. It is also evident that mesenchymal events are less well understood, in particular in the mammary gland. This is in part due to the paucity of suitable tools for conditional gene inactivation.

Our understanding of the cellular mechanisms driving ectodermal appendage morphogenesis is still in its infancy. However, the recent advances in live imaging techniques that allow time-lapse monitoring of developing embryonic tissues in 3D will provide novel tools to address these important questions. Data available so far seem to indicate that similar mechanisms account for placode morphogenesis and diverge for bud formation in different ectodermal appendages, but additional studies are needed to confirm these conclusions. Further, there is an additional level of complexity that we did not discuss earlier: not all placode cells are equal as placodes consist of both basal and suprabasal cells, a fact that may have a profound effect on their behavior and later fate [135].

Interestingly, other examples of cellular heterogeneity exist. Two molecularly distinct cell populations are found within tooth placodes: the signaling center cells (Foxi3$^+$, Shh$^+$ cells), located in the lingual aspect of the thickened molar epithelium, and the surrounding placodal cells (Foxi3$^+$, Shh$^-$ cells) [5,8] (Fig. 1). Although direct comparisons are difficult, it seems that the expression of many signaling molecules is restricted to the signaling center [5–8]. In the mammary placodes, published expression patterns do not suggest the presence of a signaling center similar to that observed in dental placodes, perhaps reflecting the differences in budding morphogenesis. In primary hair placodes, some markers (e.g. *Shh*) are expressed more centrally and in fewer cells than others (e.g. *Dkk4*) [136]. Lineage tracing has shown that *Shh+* cells give rise to all follicular cells, but not to interfollicular epidermal cells [137]. As far as we know, no lineage tracing data exist for any of the more broadly expressed placode markers. Future studies are needed to reveal how the cellular heterogeneity is established in ectodermal appendage placodes and whether this has any functional consequences.

Acknowledgements

We thank Isa Uski for preparing figure 1, and Vera Shirokova, Maria Voutilainen, and Maria Jussila for providing pictures for figure 2.

References

[1] Mikkola ML, Millar SE. The mammary bud as a skin appendage: unique and shared aspects of development. J Mammary Gland Biol Neoplasia 2006;11:187–203.
[2] Jussila M, Thesleff I. Signaling networks regulating tooth organogenesis and regeneration, and the specification of dental mesenchymal and epithelial cell lineages. Cold Spring Harb Perspect Biol 2012;4:a008425.
[3] Propper AY, Howard BA, Veltmaat JM. Prenatal morphogenesis of mammary glands in mouse and rabbit. J Mammary Gland Biol Neoplasia 2013;18:93–104.
[4] Sennett R, Rendl M. Mesenchymal–epithelial interactions during hair follicle morphogenesis and cycling. Semin Cell Dev Biol 2012;23:917–27.
[5] Jernvall J, Thesleff I. Reiterative signaling and patterning during mammalian tooth morphogenesis. Mech Dev 2000;92:19–29.
[6] Dassule HR, McMahon AP. Analysis of epithelial–mesenchymal interactions in the initial morphogenesis of the mammalian tooth. Dev Biol 1998;202:215–27.
[7] Keränen SV, Aberg T, Kettunen P, Thesleff I, Jernvall J. Association of developmental regulatory genes with the development of different molar tooth shapes in two species of rodents. Dev Genes Evol 1998;208:477–86.
[8] Shirokova V, Jussila M, Hytönen MK, Perälä N, Drögemüller C, Leeb T, et al. Expression of Foxi3 is regulated by ectodysplasin in skin appendage placodes. Dev Dyn 2013;242:593–603.
[9] Schneider MR, Schmidt-Ullrich R, Paus R. The hair follicle as a dynamic miniorgan. Curr Biol 2009;19:R132–42.
[10] Mammoto T, Mammoto A, Torisawa YS, Tat T, Gibbs A, Derda R, et al. Mechanochemical control of mesenchymal condensation and embryonic tooth organ formation. Dev Cell 2011;21:758–69.
[11] Sakakura T. Mammary embryogenesis. In: Neville MC, Daniel CW, editors. The mammary gland: development, regulation, and function. New York: Plenum Press; 1987. p. 37–66.
[12] Sengel P. Morphogenesis of skin. Cambridge, UK: Cambridge University Press; 1976.
[13] Parmar H, Cunha GR. Epithelial–stromal interactions in the mouse and human mammary gland in vivo. Endocr Relat Cancer 2004;11:437–58.
[14] Kollar EJ, Baird GR. Tissue interactions in embryonic mouse tooth germs II. The inductive role of the dental papilla. J Embryol Exp Morphol 1970;24:173–86.
[15] Kollar EJ. The development of the integument: spatial, temporal, and phylogenetic factors. Am Zool 1972;12:125–35.
[16] Mina M, Kollar EJ. The induction of odontogenesis in non-dental mesenchyme combined with early murine mandibular arch epithelium. Arch Oral Biol 1987;32:123–7.
[17] Lumsden AG. Spatial organization of the epithelium and the role of neural crest cells in the initiation of the mammalian tooth germ. Development 1988;103(Suppl.):155–69.
[18] Oliver RF. The induction of hair follicle formation in the adult hooded rat by vibrissa dermal papillae. J Embryol Exp Morphol 1970;23:219–23.
[19] Reynolds AJ, Jahoda CA. Cultured dermal papilla cells induce follicle formation and hair growth by transdifferentiation of an adult epidermis. Development 1992;115:587–93.
[20] Yang CC, Cotsarelis G. Review of hair follicle dermal cells. J Dermatol Sci 2010;57:2–11.
[21] Kollar EJ. The induction of hair follicles by embryonic dermal papillae. J Invest Dermatol 1970;55:374–8.
[22] Dhouailly D. Dermo–epidermal interactions between birds and mammals: differentiation of cutaneous appendages. J Embryol Exp Morphol 1973;30:587–603.
[23] Propper A, Gomot L. Tissue interactions during organogenesis of the mammary gland in the rabbit embryo. C R Acad Sci Hebd Seances Acad Sci D 1967;264:2573–5.
[24] Cunha GR, Young P, Christov K, Guzman R, Nandi S, Talamantes F, et al. Mammary phenotypic expression induced in epidermal cells by embryonic mammary mesenchyme. Acta Anat 1995;152:195–204.
[25] Niehrs C. The complex world of WNT receptor signalling. Nat Rev Mol Cell Biol 2012;13:767–79.
[26] Turner N, Grose R. Fibroblast growth factor signalling: from development to cancer. Nat Rev Cancer 2010;10:116–29.
[27] Ingham PW, Nakano Y, Seger C. Mechanisms and functions of Hedgehog signalling across the metazoa. Nat Rev Genetics 2011;12:393–406.
[28] Mueller TD, Nickel J. Promiscuity and specificity in BMP receptor activation. FEBS Lett 2012;586:1846–59.

[29] Dunphy KA, Schneyer AL, Hagen MJ, Jerry DJ. The role of activin in mammary gland development and oncogenesis. J Mammary Gland Biol Neoplasia 2011;16:117–26.
[30] Mikkola ML. TNF superfamily in skin appendage development. Cytokine Growth Factor Rev 2008;19:219–30.
[31] van Genderen C, Okamura RM, Farinas I, Quo RG, Parslow TG, Bruhn L, et al. Development of several organs that require inductive epithelial–mesenchymal interactions is impaired in LEF-1-deficient mice. Genes Dev 1994;8:2691–703.
[32] Andl T, Reddy ST, Gaddapara T, Millar SE. WNT signals are required for the initiation of hair follicle development. Dev Cell 2002;2:643–53.
[33] Zhang Y, Tomann P, Andl T, Gallant NM, Huelsken J, Jerchow B, et al. Reciprocal requirements for EDA/EDAR/NF-κB and Wnt/β-catenin signaling pathways in hair follicle induction. Dev Cell 2009;17:49–61.
[34] Chu EY, Hens J, Andl T, Kairo A, Yamaguchi TP, Brisken C, et al. Canonical WNT signaling promotes mammary placode development and is essential for initiation of mammary gland morphogenesis. Development 2004;131:4819–29.
[35] Huelsken J, Vogel R, Erdmann B, Cotsarelis G, Birchmeier W. β-catenin controls hair follicle morphogenesis and stem cell differentiation in the skin. Cell 2001;105:533–45.
[36] Ahn Y, Sims C, Logue JM, Weatherbee SD, Krumlauf R. Lrp4 and wise interplay controls the formation and patterning of mammary and other skin appendage placodes by modulating Wnt signaling. Development 2013;140:583–93.
[37] Liu F, Chu EY, Watt B, Zhang Y, Gallant NM, Andl T, et al. Wnt/β-catenin signaling directs multiple stages of tooth morphogenesis. Dev Biol 2008;313:210–24.
[38] Närhi K, Järvinen E, Birchmeier W, Taketo MM, Mikkola ML, Thesleff I. Sustained epithelial β-catenin activity induces precocious hair development but disrupts hair follicle down-growth and hair shaft formation. Development 2008;135:1019–28.
[39] Zhang Y, Andl T, Yang SH, Teta M, Liu F, Seykora JT, et al. Activation of β-catenin signaling programs embryonic epidermis to hair follicle fate. Development 2008;135:2161–72.
[40] Järvinen E, Salazar-Ciudad I, Birchmeier W, Taketo MM, Jernvall J, Thesleff I. Continuous tooth generation in mouse is induced by activated epithelial Wnt/β-catenin signaling. Proc Natl Acad Sci USA 2006;103:18627–32.
[41] Närhi K, Tummers M, Ahtiainen L, Itoh N, Thesleff I, Mikkola ML. Sostdc1 defines the size and number of skin appendage placodes. Dev Biol 2012;364:149–61.
[42] Chen D, Jarrell A, Guo C, Lang R, Atit R. Dermal β-catenin activity in response to epidermal Wnt ligands is required for fibroblast proliferation and hair follicle initiation. Development 2012;139:1522–33.
[43] Fu J, Hsu W. Epidermal Wnt controls hair follicle induction by orchestrating dynamic signaling crosstalk between the epidermis and dermis. J Invest Dermatol 2013;133:890–8.
[44] Zhu X, Zhao P, Liu Y, Zhang X, Fu J, Ivy Yu HM, et al. Intra-epithelial requirement of canonical Wnt signaling for tooth morphogenesis. J Biol Chem 2013;288:12080–9.
[45] Hiremath M, Dann P, Fischer J, Butterworth D, Boras-Granic K, Hens J, et al. Parathyroid hormone-related protein activates Wnt signaling to specify the embryonic mammary mesenchyme. Development 2012;139:4239–49.
[46] Fujimori S, Novak H, Weissenböck M, Jussila M, Gonçalves A, Zeller R, et al. Wnt/β-catenin signaling in the dental mesenchyme regulates incisor development by regulating Bmp4. Dev Biol 2010;348:97–106.
[47] Chen J, Lan Y, Baek JA, Gao Y, Jiang R. Wnt/β-catenin signaling plays an essential role in activation of odntotogenic mesenchyme during early tooth development. Dev Biol 2009;334:174–85.
[48] Tsai SY, Sennett R, Rezza A, Clavel C, Grisanti L, Zemla R, et al. Wnt/β-catenin signaling in dermal condensates is required for hair follicle formation. Dev Biol 2014;385:179–88.
[49] de Lau WB, Snel B, Clevers HC. The R-spondin protein family. Genome Biol 2012;13:242.
[50] Van Schoore G, Mendive F, Pochet R, Vassart G. Expression pattern of the orphan receptor LGR4/GPR48 gene in the mouse. Histochem Cell Biol 2005;124:35–50.
[51] Snippert HJ, Haegebarth A, Kasper M, Jaks V, van Es JH, Barker N, et al. Lgr6 marks stem cells in the hair follicle that generate all cell lineages of the skin. Science 2010;327:1385–9.
[52] Mohri Y, Kato S, Umezawa A, Okuyama R, Nishimori K. Impaired hair placode formation with reduced expression of hair follicle-related genes in mice lacking Lgr4. Dev Dyn 2008;237:2235–42.
[53] Mustonen T, Ilmonen M, Pummila M, Kangas AT, Laurikkala J, Jaatinen R, et al. Ectodysplasin A1 promotes placodal cell fate during early morphogenesis of ectodermal appendages. Development 2004;131:4907–19.
[54] Headon DJ, Overbeek PA. Involvement of a novel Tnf receptor homologue in hair follicle induction. Nat Genet 1999;22:370–4.
[55] Laurikkala J, Pispa J, Jung HS, Nieminen P, Mikkola M, Wang X, et al. Regulation of hair follicle development by the TNF signal ectodysplasin and its receptor Edar. Development 2002;129:2541–53.
[56] Schmidt-Ullrich R, Aebischer T, Hulsken J, Birchmeier W, Klemm U, Scheidereit C. Requirement of NF-κB/Rel for the development of hair follicles and other epidermal appendices. Development 2001;128:3843–53.
[57] Schmidt-Ullrich R, Tobin DJ, Lenhard D, Schneider P, Paus R, Scheidereit C. NF-κB transmits Eda A1/EdaR signaling to activate Shh and cyclin D1 expression, and controls post-initiation hair placode down-growth. Development 2006;133:1045–57.
[58] Mou C, Jackson B, Schneider P, Overbeek PA, Headon DJ. Generation of the primary hair follicle pattern. Proc Natl Acad Sci USA 2006;103:9075–80.
[59] Fliniaux I, Mikkola ML, Lefebvre S, Thesleff I. Identification of dkk4 as a target of Eda-A1/Edar pathway reveals an unexpected role of ectodysplasin as inhibitor of Wnt signalling in ectodermal placodes. Dev Biol 2008;320:60–71.
[60] Pispa J, Pummila M, Barker PA, Thesleff I, Mikkola ML. Edar and Troy signalling pathways act redundantly to regulate initiation of hair follicle development. Hum Mol Genet 2008;17:3380–91.
[61] Fafilek B, Krausova M, Vojtechova M, Pospichalova V, Tumova L, Sloncova E, et al. Troy, a tumor necrosis factor receptor family member, interacts with lgr5 to inhibit wnt signaling in intestinal stem cells. Gastroenterology 2013;144:381–91.
[62] Pispa J, Jung HS, Jernvall J, Kettunen P, Mustonen T, Tabata MJ, et al. Cusp patterning defect in Tabby mouse teeth and its partial rescue by FGF. Dev Biol 1999;216:521–34.
[63] Voutilainen M, Lindfors PH, Lefebvre S, Ahtiainen L, Fliniaux I, Rysti E, et al. Ectodysplasin regulates hormone-independent mammary ductal morphogenesis via NF-κB. Proc Natl Acad Sci USA 2012;109:5744–9.
[64] Mustonen T, Pispa J, Mikkola ML, Pummila M, Kangas AT, Pakkasjarvi L, et al. Stimulation of ectodermal organ development by ectodysplasin-A1. Dev Biol 2003;259:123–36.
[65] Chang SH, Jobling S, Brennan K, Headon DJ. Enhanced Edar signalling has pleiotropic effects on craniofacial and cutaneous glands. PLoS ONE 2009;4:e7591.
[66] Cui CY, Kunisada M, Esibizione D, Douglass EG, Schlessinger D. Analysis of the temporal requirement for eda in hair and sweat gland development. J Invest Dermatol 2009;129:984–93.
[67] Swee LK, Ingold-Salamin K, Tardivel A, Willen L, Gaide O, Favre M, et al. Biological activity of ectodysplasin A is conditioned by its collagen and heparan sulfate proteoglycan-binding domains. J Biol Chem 2009;284:27567–76.
[68] Mandler M, Neubuser A. FGF signaling is necessary for the specification of the odontogenic mesenchyme. Dev Biol 2001;240:548–59.
[69] Kettunen P, Karavanova I, Thesleff I. Responsiveness of developing dental tissues to fibroblast growth factors: expression of splicing alternatives of FGFR1, -2, -3, and of FGFR4; and stimulation of cell proliferation by FGF-2, -4, -8, and -9. Dev Genet 1998;22:374–85.
[70] De Moerlooze L, Spencer-Dene B, Revest J, Hajihosseini M, Rosewell I, Dickson C. An important role for the IIIb isoform of fibroblast growth factor receptor 2 (FGFR2) in mesenchymal-epithelial signalling during mouse organogenesis. Development 2000;127:483–92.
[71] Veistinen L, Aberg T, Rice DP. Convergent signalling through Fgfr2 regulates divergent craniofacial morphogenesis. J Exp Zool B Mol Dev Evol 2009;312B:351–60.
[72] Hosokawa R, Deng X, Takamori K, Xu X, Urata M, Bringas Jr P, et al. Epithelial-specific requirement of FGFR2 signaling during tooth and palate development. J Exp Zool B Mol Dev Evol 2009;312B:343–50.
[73] Mailleux AA, Spencer-Dene B, Dillon C, Ndiaye D, Savona-Baron C, Itoh N, et al. Role of FGF10/FGFR2b signaling during mammary gland development in the mouse embryo. Development 2002;129:53–60.
[74] Veltmaat JM, Relaix F, Le LT, Kratochwil K, Sala FG, van Veelen W, et al. Gli3-mediated somitic Fgf10 expression gradients are required for the induction and patterning of mammary epithelium along the embryonic axes. Development 2006;133:2325–35.
[75] Petiot A, Conti FJ, Grose R, Revest JM, Hodivala-Dilke KM, Dickson C. A crucial role for Fgfr2-IIIb signalling in epidermal development and hair follicle patterning. Development 2003;130:5493–501.
[76] Richardson GD, Bazzi H, Fantauzzo KA, Waters JM, Crawford H, Hynd P, et al. KGF and EGF signalling block hair follicle induction and promote interfollicular epidermal fate in developing mouse skin. Development 2009;136:2153–64.
[77] Guo L, Yu QC, Fuchs E. Targeting expression of keratinocyte growth factor to keratinocytes elicits striking changes in epithelial differentiation in transgenic mice. EMBO J 1993;12:973–86.
[78] Yang J, Meyer M, Müller AK, Böhm F, Grose R, Dauwalder T, et al. Fibroblast growth factor receptors 1 and 2 in keratinocytes control the epidermal barrier and cutaneous homeostasis. J Cell Biol 2010;188:935–52.
[79] Michno K, Boras-Granic K, Mill P, Hui CC, Hamel PA. Shh expression is required for embryonic hair follicle but not mammary gland development. Dev Biol 2003;264:153–65.
[80] Hardcastle Z, Mo R, Hui CC, Sharpe PT. The Shh signalling pathway in tooth development: defects in Gli2 and Gli3 mutants. Development 1998;125:2803–11.
[81] St-Jacques B, Dassule HR, Karavanova I, Botchkarev VA, Li J, Danielian PS, et al. Sonic hedgehog signaling is essential for hair development. Curr Biol 1998;8:1058–68.
[82] Chiang C, Swan RZ, Grachtchouk M, Bolinger M, Litingtung Y, Robertson EK, et al. Essential role for Sonic hedgehog during hair follicle morphogenesis. Dev Biol 1999;205:1–9.
[83] Hatsell SJ, Cowin P. Gli3-mediated repression of Hedgehog targets is required for normal mammary development. Development 2006;133:3661–70.
[84] Dassule HR, Lewis P, Bei M, Maas R, McMahon AP. Sonic hedgehog regulates growth and morphogenesis of the tooth. Development 2000;127:4775–85.
[85] Gritli-Linde A, Bei M, Maas R, Zhang XM, Linde A, McMahon AP. Shh signaling within the dental epithelium is necessary for cell proliferation, growth and polarization. Development 2002;129:5323–37.

[86] Huh SH, Närhi K, Lindfors PH, Häärä O, Yang L, Ornitz DM, et al. Fgf20 governs formation of primary and secondary dermal condensations in developing hair follicles. Genes Dev 2013;27:450–8.
[87] Mill P, Mo R, Fu H, Grachtchouk M, Kim PC, Dlugosz AA, et al. Sonic hedgehog-dependent activation of Gli2 is essential for embryonic hair follicle development. Genes Dev 2003;17:282–94.
[88] Gallego MI, Beachy PA, Hennighausen L, Robinson GW. Differential requirements for shh in mammary tissue and hair follicle morphogenesis. Dev Biol 2002;249:131–9.
[89] Lee MY, Racine V, Jagadpramana P, Sun L, Yu W, Du T, et al. Ectodermal influx and cell hypertrophy provide early growth for all murine mammary rudiments, and are differentially regulated among them by Gli3. PLoS ONE 2011;6:e26242.
[90] Botchkarev VA, Botchkareva NV, Roth W, Nakamura M, Chen LH, Herzog W, et al. Noggin is a mesenchymally derived stimulator of hair-follicle induction. Nat Cell Biol 1999;1:158–64.
[91] Pummila M, Fliniaux I, Jaatinen R, James M, Laurikkala J, Schneider P, et al. Ectodysplasin has a dual role in ectodermal organogenesis: inhibition of Bmp activity and induction of Shh expression. Development 2007;134:117–25.
[92] Botchkarev VA, Botchkareva NV, Sharov AA, Funa K, Huber O, Gilchrest BA. Modulation of BMP signaling by noggin is required for induction of the secondary (nontylotrich) hair follicles. J Invest Dermatol 2002;118:3–10.
[93] Plikus M, Wang WP, Liu J, Wang X, Jiang TX, Chuong CM. Morpho-regulation of ectodermal organs: integument pathology and phenotypic variations in K14-Noggin engineered mice through modulation of bone morphogenic protein pathway. Am J Pathol 2004;164:1099–114.
[94] Jamora C, DasGupta R, Kocieniewski P, Fuchs E. Links between signal transduction, transcription and adhesion in epithelial bud development. Nature 2003;422:317–22.
[95] Laurikkala J, Kassai Y, Pakkasjärvi L, Thesleff I, Itoh N. Identification of a secreted BMP antagonist, ectodin, integrating BMP, FGF, and SHH signals from the tooth enamel knot. Dev Biol 2003;264:91–105.
[96] Kandyba E, Hazen VM, Kobielak A, Butler SJ, Kobielak K. Smad1&5 but not Smad8 establish stem cell quiescence which is critical to transform the premature hair follicle during morphogenesis towards the postnatal state. Stem Cells 2014;32:534–47.
[97] 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.
[98] Andl T, Ahn K, Kairo A, Chu EY, Wine-Lee L, Reddy ST, et al. Epithelial Bmpr1a regulates differentiation and proliferation in postnatal hair follicles and is essential for tooth development. Development 2004;131:2257–68.
[99] Xu X, Han J, Ito Y, Bringas Jr P, Deng C, Chai Y. Ectodermal Smad4 p38 MAPK are functionally redundant in mediating TGF-beta/BMP signaling during tooth and palate development. Dev Cell 2008;15:322–9.
[100] Wang Y, Li L, Zheng Y, Yuan G, Yang G, He F, et al. BMP activity is required for tooth development from the lamina to bud stage. J Dent Res 2012;91:690–5.
[101] Ko SO, Chung IH, Xu X, Oka S, Zhao H, Cho ES, et al. Smad4 is required to regulate the fate of cranial neural crest cells. Dev Biol 2007;312:435–47.
[102] Ferguson CA, Tucker AS, Christensen L, Lau AL, Matzuk MM, Sharpe PT. Activin is an essential early mesenchymal signal in tooth development that is required for patterning of the murine dentition. Genes Dev 1998;12:2636–49.
[103] O'Connell DJ, Ho JW, Mammoto T, Turbe-Doan A, O'Connell JT, Haseley PS, et al. A Wnt-bmp feedback circuit controls intertissue signaling dynamics in tooth organogenesis. Sci Signal 2012;5:ra4.
[104] Matzuk MM, Kumar TR, Vassalli A, Bickenbach JR, Roop DR, Jaenisch R, et al. Functional analysis of activins during mammalian development. Nature 1995;374:354–6.
[105] Foitzik K, Paus R, Doetschman T, Dotto GP. The TGF-β2 isoform is both a required and sufficient inducer of murine hair follicle morphogenesis. Dev Biol 1999;212:278–89.
[106] Jamora C, Lee P, Kocieniewski P, Azhar M, Hosokawa R, Chai Y, et al. A signaling pathway involving TGF-beta2 and snail in hair follicle morphogenesis. PLoS Biol 2005;3:e11.
[107] Wessells NK, Roessner KD. Nonproliferation in dermal condensations of mouse vibrissae and pelage hairs. Dev Biol 1965;12:419–33.
[108] Sakaue-Sawano A, Kurokawa H, Morimura T, Hanyu A, Hama H, Osawa H, et al. Visualizing spatiotemporal dynamics of multicellular cell-cycle progression. Cell 2008;132:487–98.
[109] Kashiwagi M, Kuroki T, Huh N. Specific inhibition of hair follicle formation by epidermal growth factor in an organ culture of developing mouse skin. Dev Biol 1997;189:22–32.
[110] Balinsky B. On the developmental processes in mammary glands and other epidermal structures. Trans R Soc Edinb 1949–1950;62:1–31.
[111] Balinsky BI. On the prenatal growth of the mammary gland rudiment in the mouse. J Anat 1950;84:227–35.
[112] Propper AY. Wandering epithelial cells in the rabbit embryo milk line A preliminary scanning electron microscope study. Dev Biol 1978;67:225–31.
[113] Woo WM, Zhen HH, Oro AE. Shh maintains dermal papilla identity and hair morphogenesis via a Noggin-Shh regulatory loop. Genes Dev 2012;26:1235–46.
[114] Hardy MH, Vielkind U. Changing patterns of cell adhesion molecules during mouse pelage hair follicle development 1. Follicle morphogenesis in wild-type mice. Acta Anat (Basel) 1996;157:169–82.
[115] Brakebusch C, Grose R, Quondamatteo F, Ramirez A, Jorcano JL, Pirro A, et al. Skin and hair follicle integrity is crucially dependent on β1 integrin expression on keratinocytes. EMBO J 2000;19:3990–4003.
[116] Raghavan S, Bauer C, Mundschau G, Li Q, Fuchs E. Conditional ablation of β1 integrin in skin. Severe defects in epidermal proliferation, basement membrane formation, and hair follicle invagination. J Cell Biol 2000;150:1149–60.
[117] Gao J, DeRouen MC, Chen CH, Nguyen M, Nguyen NT, Ido H, et al. Laminin-511 is an epithelial message promoting dermal papilla development and function during early hair morphogenesis. Genes Dev 2008;22:2111–24.
[118] DeRouen MC, Zhen H, Tan SH, Williams S, Marinkovich MP, Oro AE. Laminin-511 and integrin β-1 in hair follicle development and basal cell carcinoma formation. BMC Dev Biol 2010;10:112.
[119] Fukumoto S, Miner JH, Ida H, Fukumoto E, Yuasa K, Miyazaki H, et al. Laminin α5 is required for dental epithelium growth and polarity and the development of tooth bud and shape. J Biol Chem 2006;281:5008–16.
[120] Wells KL, Hadad Y, Ben-Avraham D, Hillel J, Cahaner A, Headon DJ. Genome-wide SNP scan of pooled DNA reveals nonsense mutation in FGF20 in the scaleless line of featherless chickens. BMC Genomics 2012;13:257.
[121] Goetinck PF, Sekellick MJ. Observations on collagen synthesis, lattice formation, and morphology of scaleless and normal embryonic skin. Dev Biol 1972;28:636–48.
[122] Houghton L, Lindon CM, Freeman A, Morgan BA. Abortive placode formation in the feather tract of the scaleless chicken embryo. Dev Dyn 2007;236:3020–30.
[123] Häärä O, Harjunmaa E, Lindfors PH, Huh SH, Fliniaux I, Åberg T, et al. Ectodysplasin regulates activator-inhibitor balance in murine tooth development through Fgf20 signaling. Development 2012;139:3189–99.
[124] Kettunen P, Furmanek T, Chaulagain R, Kvinnsland IH, Luukko K. Developmentally regulated expression of intracellular Fgf11-13, hormone-like Fgf15 and canonical Fgf16, -17 and -20 mRNAs in the developing mouse molar tooth. Acta Odontol Scand 2011;69:360–6.
[125] Porntaveetus T, Otsuka-Tanaka Y, Basson MA, Moon AM, Sharpe PT, Ohazama A. Expression of fibroblast growth factors (Fgfs) in murine tooth development. J Anat 2011;218:534–43.
[126] Trumpp A, Depew MJ, Rubenstein JL, Bishop JM, Martin GR. Cre-mediated gene inactivation demonstrates that FGF8 is required for cell survival and patterning of the first branchial arch. Genes Dev 1999;13:3136–48.
[127] Wysolmerski JJ, Philbrick WM, Dunbar ME, Lanske B, Kronenberg H, Broadus AE. Rescue of the parathyroid hormone-related protein knockout mouse demonstrates that parathyroid hormone-related protein is essential for mammary gland development. Development 1998;125:1285–94.
[128] Ohtola J, Myers J, Akhtar-Zaidi B, Zuzindlak D, Sandesara P, Yeh K, et al. β-Catenin has sequential roles in the survival and specification of ventral dermis. Development 2008;135:2321–9.
[129] Karlsson L, Bondjers C, Betsholtz C. Roles for PDGF-A and sonic hedgehog in development of mesenchymal components of the hair follicle. Development 1999;126:2611–21.
[130] Tobin DJ, Gunin A, Magerl M, Handijski B, Paus R. Plasticity and cytokinetic dynamics of the hair follicle mesenchyme: implications for hair growth control. J Invest Dermatol 2003;120:895–904.
[131] Lin CM, Jiang TX, Baker RE, Maini PK, Widelitz RB, Chuong CM. Spots and stripes: pleomorphic patterning of stem cells via p-ERK-dependent cell chemotaxis shown by feather morphogenesis and mathematical simulation. Dev Biol 2009;334:369–82.
[132] Song HK, Lee SH, Goetinck PF. FGF-2 signaling is sufficient to induce dermal condensations during feather development. Dev Dyn 2004;231:741–9.
[133] Song H, Wang Y, Goetinck PF. Fibroblast growth factor 2 can replace ectodermal signaling for feather development. Proc Natl Acad Sci USA 1996;93:10246–9.
[134] Lefebvre S, Fliniaux I, Schneider P, Mikkola ML. Identification of ectodysplasin target genes reveals the involvement of chemokines in hair development. J Invest Dermatol 2012;132:1094–102.
[135] Nowak JA, Polak L, Pasolli HA, Fuchs E. Hair follicle stem cells are specified and function in early skin morphogenesis. Cell Stem Cell 2008;3:33–43.
[136] Heath J, Langton AK, Hammond NL, Overbeek PA, Dixon MJ, Headon DJ. Hair follicles are required for optimal growth during lateral skin expansion. J Invest Dermatol 2009;129:2358–64.
[137] Levy V, Lindon C, Harfe BD, Morgan BA. Distinct stem cell populations regenerate the follicle and interfollicular epidermis. Dev Cell 2005;9:855–61.