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Dlx Genes, p63, and Ectodermal Dysplasias

Maria I. Morasso^{*} and **Nadezda Radoja**

and **Nadezda Radoja** are from the Developmental Skin Biology Unit, National Institute of Arthritis and Musculoskeletal and Skin Diseases, National Institutes of Health, Bethesda, Maryland.

Abstract

Many events in vertebrate morphogenesis and organogenesis develop from epithelial/mesenchymal interactions. These processes involve a series of sequential and reciprocal interactions between the thickened epithelial sheets and underlying mesenchymal cells. Much has been learned from in vitro assays and knockout experiments in mice on the early signaling molecules that regulate the initial stages of the epithelial/mesenchymal interactions. In this review, we discuss effectors of these initial signals, specifically the p63 and Dlx families of transcription factors, that play central roles in embryonic patterning and regulation of different developmental processes, and provide a review of some of the mutations in these genes that have been associated with ectodermal dysplasias (EDs).

Keywords

Dlx; p63; development; epithelial/mesenchymal; ectodermal dysplasias; hair; tooth

INTRODUCTION

During embryonic development and organ formation, a series of signals between epithelial cells and underlying mesenchymal cells are responsible for the formation of a variety of appendages/organs (Pispa and Thesleff, 2003) that include, in mammals, hair, nail, sweat and sebaceous gland, mammary gland, and the tooth. Teeth and hair are among the organs in which a substantial amount of information on developmental regulation has been accumulated in recent years.

In the mammalian embryo, the surface ectoderm envelopes the embryo during gastrulation and neurulation, forming a simple epithelium comprising a single cell layer. During tooth and hair development, the first morphologically distinguishable event is the thickening of the surface ectoderm, to form an epidermal placode. In the case of the hair follicle, the epidermal placode arises from a region-specific inductive signal from the dermis (Hardy, 1992). The induced placode invaginates into the dermis, enclosing a cluster of mesenchymal cells. This cluster becomes the dermal papilla and remains associated with the fully formed hair follicle (Millar, 2002). Another structure that has been extensively studied is the tooth (Thesleff and Mikkola, 2002; Tucker and Sharpe, 2004). Tooth morphogenesis also starts as a mesenchymally induced thickening of the surface ectoderm, which subsequently undergoes invagination and surrounds the condensed bud of underlying mesenchymal cells. This associated mesenchyme is derived from branchial arch neural crest (Miletich and Sharpe, 2004) and forms the odontoblasts. The tooth germ continues to develop, with the odontoblast cells differentiating into the dental pulp and dentine layers, while the epithelial cells become the ameloblasts that produce the dental enamel.

^{*}Correspondence to: Maria I. Morasso, Developmental Skin Biology Unit, Bldg. 50, Rm. 1525, NIAMS, National Institutes of Health, Bethesda, MD 20892. E-mail: morassom@mail.nih.gov.

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The signaling molecules shown to be determinants in several aspects of the development of these structures are bone morphogenetic protein (BMP), fibroblast growth factor (FGF), sonic hedgehog (SHH), and Wnt (Pispa and Thesleff, 2003) (Fig. 1).

Anomalies in hair and teeth, as well as other epithelial- and mesenchymal-derived organs, are features of a group of human pathological conditions defined as ectodermal dysplasias (EDs) (Priolo and Lagana, 2001). This review focuses on two families of transcription factors, *Dlx* and *p63* proteins, that are putative effectors of signaling pathways through developmental organogenesis, and in which mutations have been linked to specific EDs.

Dlx Homeobox Genes

Homeobox proteins comprise a large class of transcription factors that are crucial regulators of many developmental processes, ranging from organization of the body plan to differentiation of individual tissues. Binding sites for homeodomain proteins contain a core motif, TAAT, with adjacent bases being responsible for the interactions between specific homeodomain factors and target genes (McGinnis and Krumlauf, 1992; Gehring et al., 1994).

The Distal-less (*Dll*) homeobox-containing gene, initially characterized in *Drosophila*, was shown to be essential for the proximodistal patterning of insect limbs (Cohen et al., 1989). Panganiban et al. (1997) examined the expression of *Dll* in protostomes and deuterostomes, finding it to be a common feature of appendage outgrowth from arthropods to mammals. The vertebrate distal-less (*Dlx*) genes share the highly conserved homeodomain region with the *Drosophila* *Dll* gene and constitute an evolutionary-conserved group of homeobox-containing factors that play a fundamental role in the early patterning of embryonic structures. In the mouse and human genomes, the *Dlx* family comprises six members, which are grouped into pairs (*Dlx1* and *Dlx2*, *Dlx5* and *Dlx6*, *Dlx3* and *Dlx4*) (the latter also reported as *Dlx7* and *Dlx8*), and organized into three closely linked, convergently transcribed loci. Each pair is located near one of the four *Hox* clusters, *Dlx1* and *Dlx2* linked to *HoxD*, *Dlx3* and *Dlx4* linked to *HoxB*, *Dlx5* and *Dlx6* linked to *HoxA* (Simeone et al., 1994; Stock et al., 1996; Nakamura et al., 1996; McGuinness et al., 1996; Morasso et al., 1997) (Fig. 2A). It has been proposed that adjacent duplication of an ancestral *Dlx* gene, followed by two rounds of genome duplication and a subsequent loss of the *Dlx* pair linked to *HoxC*, accounts for the present arrangement found in the mammalian *Dlx* genes (Stock et al., 1996; Sumiyama et al., 2003).

The conserved homology of the vertebrate *Dlx* genes extends to the genomic structure, which presents common exon–intron organization. All *Dlx* genes consist of three exons and two introns. The region encoding the homeobox is split between exons 2 and 3, and this splicing site is also conserved in the *Drosophila* *Dll* gene (Fig. 2A). Several of the *Dlx* genes have multiple transcripts due to usage of alternative transcriptional initiation sites or alternative splicing (McGuinness et al., 1996; Nakamura et al., 1996; Liu et al., 1997). Potentially of great functional importance, homology outside of the homeodomain plus chromosomal location indicates that the *Dlx* genes can be placed into two clades of paralogous groups: *Dlx1/4/6* and *Dlx2/3/5*.

Dlx genes are expressed in discrete domains in both neural and nonneural components of the surface ectoderm, the latter demarcating the regions that will give rise to body appendages, such as apical ectodermal ridge of the limb buds, genital tubercle, branchial arches, and the ectodermal and mesenchymal components of the developing teeth. *Dlx* genes are also involved in the processes of osteogenesis, hematopoiesis, epidermal stratification, and placental development (Dolle et al., 1992; Bulfone et al., 1993; Robinson and Mahon, 1994; Simeone et al., 1994; Morasso et al., 1995, 1996, 1999; Qiu et al., 1995, 1997; Stock et al., 1996; Hassan et al., 2004; reviewed by Bendall and Abate-Shen, 2000; Merlo et al., 2000; Panganiban and Rubenstein, 2002). Expression of only four of the genes, *Dlx1*, *Dlx2*, *Dlx5*, and *Dlx6*, has been

detected in the central nervous system (Price et al., 1991; Robinson et al., 1991; Dolle et al., 1992; Bulfone et al., 1993; Liu et al., 1997; Yang et al., 1998a; Eisenstat et al., 1999).

All the *Dlx* genes are expressed in ectomesenchymal cells derived from the cranial neural crest. The migratory neural crest cells populate the branchial arches, which then give rise to most of the facial connective tissues and bone (Depew et al., 2002; Francis-West et al., 2003). Within the branchial arches, the *Dlx* genes are expressed in nested patterns along the proximodistal axis, where in the proximal area only *Dlx1* and *Dlx2* are expressed, and in the intermediate region, *Dlx1*, *Dlx2*, *Dlx5*, and *Dlx6* are detected, whereas the distal regions express all six genes. The overlapping pattern suggests that there are both redundant and distinct functions for each *Dlx* gene in craniofacial morphogenesis (Qiu et al., 1997; Depew et al., 2002).

The expression of tightly linked *Dlx* genes is highly overlapping; e.g., mouse *Dlx1* and *Dlx2* and mouse *Dlx5* and *Dlx6* are partially redundant in function (Simeone et al., 1994; Chen et al., 1996; Qiu et al., 1997; Robledo et al., 2002). Linked *Dlx* genes share cis-regulating sequences, intergenic regions that contain enhancer elements, that control their expression pattern through embryogenesis (Ellies et al., 1997; Zerucha et al., 2000; Ghanem et al., 2003; Sumiyama and Ruddle, 2003). The linkage has enabled the simultaneous, targeted deletion of a bigene pair, with the redundant and distinct functions of the *Dlx* genes being confirmed by analysis of the *Dlx1*, *Dlx2*, *Dlx1/2*, *Dlx5*, and *Dlx5/6* mutants (Qiu et al., 1997; Acampora et al., 1999; Depew et al., 1999; Robledo et al., 2002; Merlo et al., 2002). Disruption or ablation of *Dlx1*, *Dlx2*, *Dlx1/2*, or *Dlx5* results in craniofacial, bone, and vestibular defects, but with the limbs lacking any overt abnormalities, probably due to overlapping function of other *Dlx* family member(s). However, the targeted disruption of the *Dlx5/6* pair led to severe craniofacial, limb, and inner ear defects (Robledo et al., 2002; Merlo et al., 2002), demonstrating the requirement of these *Dlx* genes in limb development. In *Dlx5/Dlx6*^{-/-} embryos, there is also a homeotic transformation of the proximal structures of the jaw, indicating that these genes play crucial roles in establishing the proximal-distal identities in the pharyngeal arches (Depew et al., 2002).

The *Dlx3* gene is somewhat distinct in that, as of yet, it has not been detected in the central nervous system and has an essential role in epidermal and placental development (Morasso et al., 1996, 1999; Beanan and Sargent, 2000). Furthermore, although the linked gene, *Dlx4*, is also expressed in placenta (Quinn et al., 1997, 1998), the functional role of each of these genes is specific and cannot be compensated by the other, as *Dlx3*-null mice die midgestation due to deficiency in placental development. Likewise to *Dlx3*, *Dlx4* has not been detected in the nervous system. *Dlx3* is expressed in the ectoplacental cone, chorionic plate, and labyrinthine layer of the placenta (Morasso et al., 1999), and functions as a transcriptional activator in placental trophoblasts (Roberson et al., 2001). The *Dlx3* gene is also broadly expressed in the embryonic ectoderm, as well as in the tooth, hair follicle, and mammary gland, and later has a role in the interfollicular epidermis (Morasso et al., 1995, 1996). A role for *Dlx3* in differentiation was shown by transgenic overexpression in the basal proliferative layer of the stratified epidermis. This led to cessation of proliferation of the basal cells and premature differentiation, with precocious induction of terminal differentiation marker expression (Morasso et al., 1996).

A DNA region necessary for the ectodermal-specific transcriptional expression of *Dlx3* was identified 5' upstream of the mouse and the *Xenopus* *Dlx3* homolog, as assayed in cultured keratinocytes and by LacZ reporter in transgenic mice (Morasso et al., 1995; Park and Morasso, 1999). Recently, a transcriptional enhancer that regulates the visceral arch mesenchyme-specific expression of *Dlx3* was also identified to a highly conserved sequence in the intergenic region between *Dlx3* and *Dlx4* (Sumiyama and Ruddle, 2003). These results support the hypothesis that cis-elements responsible for epithelial and mesenchymal expression are

independent and located separately, 5' for ectodermal and intergenic for mesenchymal expression. Interestingly, *Dlx1/2* and *Dlx5/6* genes also have intergenic enhancers that control their nested expression in the mesenchyme of the branchial arches (Park et al., 2004).

p63

p63, in addition to p53 and p73, belongs to the p53 transcription factor family (Yang et al., 2002). p53 is an important tumor suppressor gene, with loss of function identified in more than 50% of human cancers (Levine, 1997). Mice engineered to have deletions or mutations in the p53 gene resembling human cancers develop spontaneous tumors at high rates (Malkin et al., 1990; Srivastava et al., 1990; Donehower et al., 1992; Armstrong et al., 1995).

Although all p53 family members have substantial homology and conservation at the amino acid level in certain domains, i.e., DNA binding region, transactivation and oligomerization domains, the genomic organization for p63 is quite distinct. The p63 gene contains two promoters and is transcribed to different isoforms with dissimilar transcriptional activities. These two promoters are responsible for giving rise by alternative splicing to six isoforms, with either full N-terminus containing transactivating domain (TAp63) or deleted N-terminus domain (Δ Np63) (Yang et al., 1998b). The TAp63 and Δ Np63 isoforms possess DNA binding and oligomerization domains, but have three possible variants of carboxyl terminus, termed alpha (α), beta (β), and gamma (γ) isoforms. TAp63 and Δ N α isoforms contain a sterile alpha motif (SAM), a domain with reputed importance in protein-protein interactions (Qiao and Bowie, 2005).

p63 is expressed in the nuclei of basal cells of the skin, esophagus, mammary glands, tongue, cervix, urothelium, prostate, limb buds, branchial arches, and oral epithelium. The essential role of p63 in epithelial development was demonstrated with the analysis of p63-null mice, which display perinatal lethality with dramatic defects in the limbs and skin, lack of ectodermal appendages such as teeth and mammary gland, and craniofacial malformations where the maxilla and mandible are truncated and the secondary palate fails to close (Yang et al., 1999; Mills et al., 1999).

Yang et al. (1999) reported that p63^{-/-} surface epithelia presented areas with partial stratification and presence of the differentiation-specific markers loricrin, involucrin, and filaggrin. They observed that the ectoderm developed normally until embryonic day 13.5 (E13.5), at which point the ectoderm responds to mesodermal signals triggering cell division and differentiation. They proposed that at E13.5, the single-layered ectodermal cells, instead of undergoing the asymmetric division to generate a stem cell population, began to differentiate in unison, with no stem cells left behind; by birth, the fetuses are missing a completely stratified epidermis and a wide range of other organs. These results led the authors to suggest that p63 is essential for the maintenance of the epidermal stem cell population necessary for epithelial morphogenesis and renewal (Yang et al., 1999).

Simultaneously, analysis of the p63-null mice by Mills et al. (1999) indicated that p63 is required for commitment of the immature ectoderm to epidermal lineages. Koster et al. (2004) reported that p63^{-/-}embryos contained a layer of simple epithelial cells expressing K18, which probably represents ectoderm prior to stratification. They showed that TA isoforms are the first isoforms expressed in the single-layered surface ectoderm and are required for initiation of epithelial stratification during embryogenesis. Furthermore, ectopic K14-driven TAp63 α expression in the basal cells induced stratification. These authors proposed that during later stages of embryonic development, a switch from TAp63 to Δ Np63 isoforms occurs gradually and is required for epidermal maturation (Koster et al., 2004; Koster and Roop, 2004a, 2004b). Δ Np63 is also required for the maintenance of the proliferative potential of basal keratinocytes (Parsa et al., 1999; King et al., 2003; Westfall et al., 2003; Koster et al.,

2004). The role of p63 in initiating stratification is not only restricted to the epidermis, but is a generalized requirement for other stratified epithelia, such as Mullerian duct cells, the common precursor for uterine and vaginal epithelia (Kurita et al., 2005).

Independent of the role of specific p63 isoforms during epidermal differentiation, the importance of p63 in the development of epithelial-mesenchymal interactions during embryogenesis is reflected by the dramatic lack of ectodermal appendage formation, absence of limbs, and alteration in craniofacial structures in the p63^{-/-} mice. The function and targets of the specific p63 isoforms in the formation of ectodermal structures, such as hair and tooth, are the focus of much interest. p63 regulates morphogenesis of surface ectoderm and its derivatives via multiple signaling pathways, i.e., BMP, FGF, Notch, β -catenin, and Edar, with BMP-7 and Notch1 as direct transcriptional targets of p63 (J. Laurikkala, M.L. Mikkola, M. James, M. Tummers, A. Mills, I. Thesleff, personal communication).

Increasing evidence also shows that p63 plays an important role in the regulation of cell cycle and development of human cancers. The MDM2/ARF (murine double mutant 2; alternative reading frame of Ink4A gene) pathway primarily controls p53 protein level. Ratovitski et al. (2001) demonstrated that p53 associates with and targets Δ Np63 into a protein degradation pathway. This interaction occurs through the DNA binding domains of both proteins and may balance the capacity of Δ Np63 to accelerate tumorigenesis or to induce epithelial proliferation. Furthermore, Calabro et al. (2002) showed that MDM2 interacts with Δ Np63, preventing p63 proteasome-dependent degradation, in contrast with the role of MDM2 in targeting p53 for degradation. An extended study demonstrated that transcriptional activity of p63 could be inhibited by direct interaction with ARF, through the N-terminus of both proteins (Calabro et al., 2004).

Another pathway with an important role in epithelial-mesenchymal development is the inhibitor of kappa B kinase α (IKK α regulation pathway). As with p63^{-/-} mice, IKK α ^{-/-} mice display defects in appendage development, such as limbs, craniofacial structures, and hair follicles. However, development of these structures is arrested at a later stage in IKK α ^{-/-} than in p63^{-/-} embryos (Hu et al., 1999; Li et al., 1999; Takeda et al., 1999). Sil et al. (2004) have rescued the IKK α neonatal lethality by reintroducing the expression of IKK α under control of K14 promoter in the IKK α ^{-/-} background. This resulted in a complete rescue of the epidermal phenotype. Interestingly, defects in limb and craniofacial structures were completely rescued as well, indicating that epithelial IKK α expression is required for patterning of the underlying mesenchyme.

Mutations in Dlx and p63 Genes: EDs

EDs have been defined as a group of pathological conditions that share common anomalies in epithelial- and mesenchymal-derived organs such as hair, tooth, nails, and sweat glands and have been associated with abnormalities in other organs (Priolo et al., 2000; Priolo and Lagana, 2001). Mutations in the DLX3 gene are responsible for developmental abnormalities in humans, causing syndromes such as tricho-dento osseous syndrome (TDO) and amelogenesis imperfecta hypoplastic-hypomaturation with taurodontism (AIHHT), while mutations in the p63 gene have been associated with ectodactyly-ectodermal dysplasia-cleft lip/palate (EEC), ankyloblepharon-ectodermal dysplasia clefting syndrome (AEC), and split hand/foot malformation (SHFM) (Fig. 2B). The position and type of the mutation, i.e., deletion, frameshift, missense, are in causative relation with particular syndromes.

Mutations in the DLX3 gene are linked to human TDO, which is characterized by defects in the development of hair and teeth, increased bone density in the cranium, and absence of overt limb malformations (Wright et al., 1997; Price et al., 1998). The mutation is due to a 4-bp deletion immediately downstream of the homeobox region (DNA binding domain), resulting

in a truncated DLX3 protein C-terminus that can potentially still bind DNA but is functionally altered. In humans, the TDO mutation results in a dominant phenotype. Analysis of mice heterozygous for the *Dlx3* mutation (Morasso et al., 1999) did not present any of the abnormalities characteristic of TDO, suggesting that in humans, the dominant pattern of inheritance is due to the formation of nonfunctional complexes involving the frame-shifted or -truncated DLX3, which act through either a dominant-negative or a gain-of-function mechanism, as opposed to haploinsufficiency. Recently, evaluation of TDO patients has shown that, in addition to altered intramembranous bone formation in the skull, this mutation also causes alteration in endochondral bone development, suggesting that DLX3 is important in bone formation and homeostasis of the appendicular skeleton (Haldeman et al., 2004; Hassan et al., 2004).

Another autosomal dominant disorder linked to DLX3 mutations is AIHHT, characterized by dental enamel defects and enlarged pulp chambers with no alterations in hair or bone (Dong et al., 2005). Mutations in this disorder have been linked to a 2-bp deletion, causing a frameshift in the sequence that leads to the alteration of the last two amino acids of the DNA binding homeodomain and addition of missense residues, as well as introducing a premature stop codon, truncating the protein by 88 amino acids. In addition to TDO and AIHHT, the location for a recessive factor essential for cleft palate risk, *clf1*, encompassed a region containing the murine DLX3 and DLX4 in chromosome 11 (Juriloff et al., 2001).

Transcriptional activation by *Dlx3* protein depends on two regions located on either side of the DNA binding homeodomain (Feledy et al., 1999; Bryan and Morasso, 2000). Furthermore, the activity of the *Dlx* proteins might be modulated by another homologous family of homeodomain proteins, *Msx* (Zhang et al., 1997; Bryan and Morasso, 2000). It will be of great interest to determine how the TDO and AIHHT mutations affect the transcriptional activity of DLX3 or its ability to interact with other proteins, and how this relates to the phenotype detected in the specific ectodermal derivatives.

SHFM type I (SHFM-1) disease locus maps to chromosome 7q21.3-q22, a region of chromosome 7 that contains the DLX5-6 gene cluster (Scherer et al., 1994; Crackower et al., 1996). SHFM has also been linked to chromosomes Xq26 and 10q24 (Faiyaz ul Haque et al., 1993; Nunes et al., 1995). Targeted inactivation of both *Dlx5* and *Dlx6* genes in mice causes, in homozygous mutants, bilateral ectrodactyly with a severe defect of the central ray of the hindlimbs (Merlo et al., 2002; Robledo et al., 2002). In the targeted allele, *Dlx5*, *Dlx6*, and the intervening sequences were removed, generating a 17-kb deletion. Important regulatory sequences are contained in the intergenic regions of the *Dlx* pair genes (Zerucha et al., 2000; Ghanem et al., 2003). Although, as of yet, no mutations in the coding regions of the DLX5 and DLX6 genes have been linked to SHFM-1, the phenotype raises the possibility that DLX5/DLX6 gene misregulation by disruption of cis-acting regulatory elements might be involved in the pathogenesis of this syndrome.

In terms of the relation of p63 to EDs, linkage mapping has identified the p63 locus on the 3q27 chromosome as causative for EEC syndrome (Celli et al., 1999; Wessagowit et al., 2000; van Bokhoven et al., 2001). EEC is characterized by ED, ectodactyly, and cleft lip with or without cleft palate. Other symptoms, such as chronic respiratory infection, mental retardation, conductive hearing loss, urogenital problems, and facial dysmorphism, are variable. The majority of missense mutations causing EEC syndrome are located in the DNA binding domain of the p63 protein, affecting all six isoforms. Mutations are targeted to specific amino acids, with high predisposition for arginine codons in positions R204W/Q, R279C/H/Q, R280C/H/S, and R304W/Q. The prediction that p63 mutant proteins in EEC syndrome disrupt the DNA binding capacity was supported by data that showed the inability of mutated

p63 γ to promote expression of a reporter gene, and the inability of Δ Np63 α to compete with p53 for binding to the specific site (Celli et al., 1999).

AEC is characterized by ED, ankyloblepharon, and cleft lip with cleft palate. The ectodermal involvement is much more severe than in EEC, while limb involvement is rare in AEC. The missense mutations causing AEC are located in the SAM domain of p63, affecting the α isoforms of p63 protein (McGrath et al., 2001). These mutations are likely to be involved in disruption of protein-protein interactions, either by substitution of critical amino acids or by destroying the compact globular structure of the SAM domain (van Bokhoven and McKeon, 2002). In addition to mutations in the SAM domain, AEC has recently been linked to deletion of exon 11, which eliminates amino acids in the C-terminus of α and β isoforms (van Bokhoven and McKeon, 2002).

SHFM is primarily characterized by limb malformation involving the central rays of the autopod (hand/foot). Syndactyly, median clefts of the hands and feet, and aplasia and/or hypoplasia of the phalanges, metacarpals, and metatarsals may be specific symptoms of SHFM. Lobster claw-like phenotype of hands and feet are present in severe cases (Duijf et al., 2003). There are five p63 mutations correlated with SHFM: the missense mutations K193E and K194E (within the DNA binding domain), the nonsense mutations Q634X and E639X, and the splice site mutation IVS4-2A>C (Ianakiev et al., 2000; van Bokhoven and Brunner, 2002). R280C and R280H mutations are located at the same codon of the p63 gene, but are common for SHFM and EEC.

In summary, there is a clear correlation between the position where the mutation occurs, the type of mutation, and the observed abnormal phenotype (van Bokhoven et al., 2001). The vast majority of p63 mutations responsible for EEC are missense mutations in the DNA binding domain, while mutations causative of AEC are mainly missense mutations falling within the SAM domain (McGrath et al., 2001).

Recent reports have also demonstrated that in some syndromes, manifestations are caused by defects on target genes of p63. Sidow et al. (1997) and Sasaki et al. (2002) showed that syndactylism in mice is caused by deregulation of the p63-specific target, Jagged2. Furthermore, the functional role of p63-mutated proteins related to human syndromes has been investigated in vitro. Huang et al. (2004) showed that the Q634X mutation involved in SHFM modulates the interaction between p63 and Ubc9, affecting the regulation of the target SUMO-1. Therefore, p63 mutations may affect specific protein complexes, modulate p63-mediated transcriptional regulation, and exert a role in a wide variety of different human syndromes.

FUTURE DIRECTIONS

The existence of malformations due to either p63 or Dlx gene mutations that translate to partially overlapping phenotypes suggests that these genes might be components of common signaling cascades regulating epidermal and ectodermal appendage development. The severity of the phenotype in p63-null mice suggests that it is an upstream crucial regulator of these signaling pathways. Analysis of the regulatory regions of the Dlx genes, as well as the expression patterns throughout embryonic development of each of the Dlx genes in the p63 mutant embryos, may determine if they are direct downstream targets of p63 isoforms regulation, and if Dlx misregulation is involved in the pathogenesis of ectodermal human syndromes associated with p63 molecular lesions.

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Figure 1.

Development of ectodermal organs through epithelial-mesenchymal interaction.

Morphologically distinct organs, such as hair and tooth, develop through embryogenesis from interaction between two adjacent tissues: the epithelium (blue) and the mesenchyme (pink).

An epithelial placode is formed, invaginates into the underlying mesenchyme, and subsequently surrounds the condensed bud of mesenchymal cells to ultimately develop into a hair follicle in the stratified epidermis or tooth in the oral cavity. The signaling molecules regulating aspects of each step from the epithelial and mesenchymal regions are indicated (BMP, FGF, SHH, Wnt). IRS, inner root sheath; ORS, outer root sheath.

Figure 2.

A: Dlx gene family: chromosomal location and intron–exon organization. Dlx family is comprised of six members, organized into three closely linked, convergently transcribed (arrows) loci. Each pair is located near a Hox cluster: DLX1 and DLX2 with HoxD on human chromosome 2, DLX5 and DLX6 with HoxA on human chromosome 7, and DLX3 and DLX4 with HoxB cluster on human chromosome 17. Bottom panel: Schematic representation of the exon–intron organization of the DLX3 gene. The DNA binding homeodomain region is demarcated. The sites of DLX3 mutations identified in the TDO and AIHHT human disorders are indicated. **B:** p63 gene structure and transcripts. Top panel is a schematic representation of the intron–exon structure, identifying the two transcriptional initiation sites (TA and ΔN), and bottom panels represent the six different p63 isoforms generated by alternative splicing (α, β, and γ). The DNA binding, oligomerization, and SAM domains are indicated. p63

mutations identified in different human disorders are indicated for EEC (pink), AEC (blue), and SHFM (black).