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Desmoglein 4 is regulated by transcription factors implicated in hair shaft differentiation

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ABSTRACT

The hair fiber is made of specialized keratinocytes, known as trichocytes, that primarily express hair keratins, which are cemented by a multitude of keratin-associated proteins (KAPs). The hair keratins form the intermediate filament cytoskeleton of the trichocytes, which are linked to abundant cell–cell adhesion junctions, called desmosomes. Desmoglein 4 (DSG4) is the major desmosomal cadherin expressed in the hair shaft cortex where the hair keratins are highly expressed. In humans, mutations affecting either the hair keratins or *DSG4* lead to beaded hair phenotypes with features of monilethrix. In this work, we postulated that the regulatory pathways governing the expression of hair shaft components, such as hair keratins and *DSG4*, are shared. Therefore, we studied the transcriptional regulation of *DSG4* by transcription factors/pathways that are known regulators of hair keratin or KAP expression. We show that HOXC13, LEF1 and FOXN1 repress *DSG4* transcription and provide *in vitro* and *in vivo* evidence correlating the Notch pathway with the activation and/or maintenance of *DSG4* expression in the hair follicle.

1. Introduction

In the mammalian hair follicle (HF), the hair shaft is produced during the anagen or growth phase of the hair cycle, which continues repeatedly throughout the life of the animal (Hardy, 1992). The hair shaft or hair fiber is the only part of the HF that protrudes above the skin surface and plays various physiological roles including protection of the skin and thermal insulation. The hair shaft consists of three concentric layers with a cuticle on the outside, a cortex, and a medulla on the inside (Fig. 1A). In humans, the cortex makes up the bulk of the hair shaft, with the size and shape of the medulla varying greatly depending on hair type and ethnic background (Jave-Suarez et al., 2002). The hair shaft is surrounded by the inner root sheath (IRS) whose innermost layer is in direct contact with hair shaft cuticle cells, and is called the IRS cuticle. The IRS plays a pivotal and supportive role during hair shaft growth (Rogers, 2004). The outermost layers of the HF consist of the companion layer and the outer root sheath (ORS), which is produced during the downgrowth of the HF (Fuchs et al., 2001).

The production and assembly of the hair shaft layers are highly coordinated processes that involve the very rapid proliferation of the matrix cells in the HF bulb, followed by a gradual cellular differentiation program that takes place within the funnel-shaped precortex region (Fig. 1A). It has been shown that the physical position of the matrix cells along the proximo-distal axis of the basement membrane overlying the dermal papilla cells determines, in part, their final differentiation fate in the concentric layers of the HF (Hardy, 1992; Langbein and Schweizer, 2005; Legue and Nicolas, 2005).

The major structural proteins found in the hair shaft are the hair keratins and keratin-associated proteins (KAPs) (Lee et al., 2006). Keratins constitute the intermediate filament cytoskeleton of both epidermal keratinocytes and the specialized hair shaft trichocytes, which is indispensable since these cells must withstand substantial and continuous mechanical stress (Rogers, 2004). To achieve this, the intermediate filaments or keratins form an intracellular network that links to the plaque proteins of

Abbreviations: HF, hair follicle; *DSG4*/DSG4, human desmoglein 4 gene/protein; *Dsg4*/Dsg4, mouse or rat desmoglein 4 gene/protein; LAH, localized autosomal recessive hypotrichosis; *lah*, *lanceolate hair* rat or mouse; ORS, outer root sheath; IRS, inner root sheath; PSDCKO, *Presenilin 1* and *2* double conditional knockout

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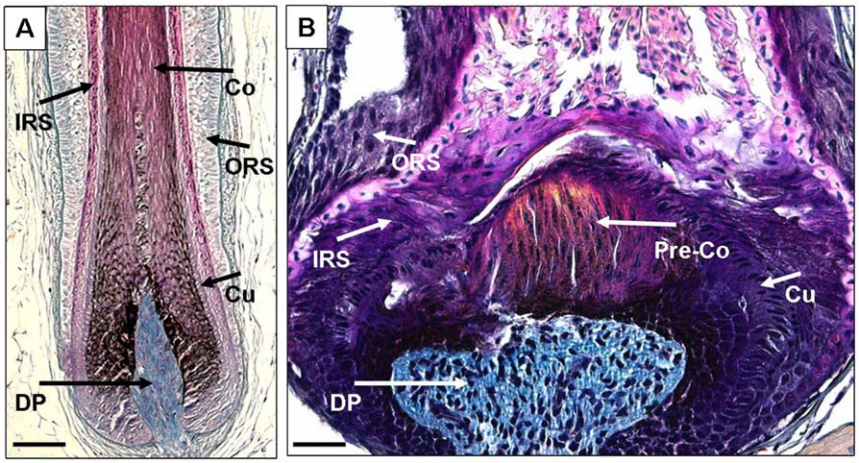

Fig. 1. Dane and Herman staining of a normal human HF (A) and a Dsg4 −/− rat vibrissae follicle (B). The dermal papilla is light blue. Note the abnormal keratinized mass at the tip of the abnormal precortex in (B) which is a hallmark of Dsg4 mutations in rodents. Abbreviations: DP, dermal papilla; ORS, outer root sheath; IRS, inner root sheath; Co, cortex; pre-Co, pre-cortex; Cu, cuticle. Scale bars: (A) 100 μm; (B) 40 μm.

cell–cell adhesion complexes called desmosomes. These intra- and inter-cellular networks of keratins and desmosomes provide the hair fiber with its properties of relatively high tensile strength as well as elasticity.

Desmosomes are calcium-dependent cell junctions that are prevalent in tissues that are subjected to continuous mechanical stress, such as the skin and heart (Bazzi and Christiano, 2007). The desmosomal cadherins, desmogleins (Dsg1-4) and desmocollins (Dsc1-3), are at the core of the adhesive interface of desmosomes. Of the desmogleins, desmoglein 4 (Dsg4) is highly expressed in the cortex region of the hair shaft, and its functional absence leads to localized autosomal recessive hypotrichosis (LAH) in humans and the *lanceolate hair* phenotype in rodents (*lah*), characterized by abnormal hair shaft differentiation and hair loss in these organisms (Fig. 1B) (Kljuic et al., 2003; Bazzi and Christiano, 2007; Messenger et al., 2005; Wajid et al., 2007). Interestingly, some LAH patients present with an additional beaded hair phenotype resembling that of monilethrix patients, who have mutations in hair keratins (Schaffer et al., 2006; Shimomura et al., 2006; Zlotogorski et al., 2006).

The expression of hair shaft components is governed by a large number of transcription factors (e.g. LEF1, β-catenin, SMAD, NICD, FOXN1, MSX2 and HOXC13), many of which operate as downstream effectors of the major morphogenic pathways. For example, earlier studies have shown that the Wnt pathway with its downstream effector, Lef1, is one of the major regulators of the hair shaft differentiation process. Lef1 knockout mice completely lack vibrissae and display a significant reduction in pelage HFs (van Genderen et al., 1994). Another well-established regulator of hair keratin promoters in the precortex is Hoxc13. Similar to Lef1, Hoxc13 knockout mice lack hair fibers (Godwin and Capecchi, 1998). Foxn1 and Msx2 transcription factors have also been shown to regulate hair keratin expression and their knock out phenotypes resemble those of Lef1 and Hoxc13 (Mecklenburg et al., 2005; Ma et al., 2003). In addition, the knockout of Notch1 or γ-secretase, which is a presenilin-like protease that releases the active form of Notch (NICD), results in abnormal hair cortex differentiation and precortical swellings reminiscent of those observed in Dsg4 mutant mice (Pan et al., 2004).

Based on the close functional relationship between hair keratins and DSG4, as well as the similarity of the phenotypes that result when both genes are perturbed, we hypothesized that the transcription factors that regulate the expression of hair keratins and KAPs might also regulate the expression of *DSG4* (Bazzi et al., 2006). Here, we show that HOXC13, LEF1 and FOXN1 repress *DSG4* transcription in HaCaT keratinocytes *in vitro* and provide evidence that the Notch pathway indirectly regulates *DSG4* expression in the HF and epidermis.

2. Materials and methods

2.1. Animals

The rodent models used in this study have been described previously: *Dsg4* −/− mice are known as $lah^{J-/-}$ (Sundberg et al., 2000; Kljuic et al., 2003) and rats are known as $lah^{IC-/-}$ (or *Iffa Credo*) (Bazzi et al., 2004), *presenilin 1* and *2* double conditional knockout (PSDCKO, genotype: Msx2-Cre +/−; *Psn1 flox/flox*; *Psn2* −/−) (Pan et al., 2004).

2.2. Histology

The histological staining procedures used in this study are either standard haematoxylin and eosin staining or Dane and Herman staining where indicated (Dane and Herman, 1963). The latter staining procedure of paraffin-embedded tissue sections is based on haematoxylin, phloxine B, alcian blue, and orange G interspersed with washes in water. The slides are then permanently mounted and photographed with regular light microscopy.

2.3. Immunofluorescence

Fresh frozen sections of human HFs discarded after surgery were fixed in 4% PFA for 10 min at room temperature. Frozen sections of mouse back skin were fixed in methanol at −20 °C for 15 min followed by acetone for 1 min at −20 °C. The sections were washed in 1XPBS, blocked in 10% of the appropriate serum, and incubated with the primary antibody overnight at 4 °C. After washing in 1XPBS, 594 or 688 Alexafluor® conjugated secondary antibodies (Molecular Probes, Invitrogen, Carlsbad, CA, USA) were applied and the signal was visualized using an HRC Axiocam fitted onto an Axioplan2 fluorescence microscope (Carl Zeiss, Thornwood, NY, USA). The antibodies used were guinea pig anti-HOXC13 and anti-Ha1 (1:2000, a kind gift of Dr. Lutz Langbein), rabbit anti-FOXN1 (1:200, Santa Cruz), goat anti-LEF1 (1:100,