Keratin 71 Mutations: From Water Dogs to Woolly Hair

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The study of rare genetic disorders of the hair follicle has resulted in the identification of many causative genes, leading to the potential for the development of novel therapeutic approaches for both inherited and acquired hair disorders. In this issue, Fujimoto *et al.* identify a missense mutation within the keratin 71 (*KRT71*) gene as the cause for autosomal dominant woolly hair/hypotrichosis in a Japanese family. This represents the first human mutation in *KRT71* to be linked to a hair disorder, establishing this gene as an important determinant of mammalian hair texture. Moreover, this finding provides new insight into the relationship between similar phenotypes resulting from mutations in distinct regulatory pathways and underscores the role of the inner root sheath in human hair growth.

Hair follicle development

Along with the sebaceous gland, the sweat gland and the arrector pili muscle, the hair follicle constitutes the pilosebaceous unit, a complex appendage of the skin. Featuring a highly organized structure, the hair follicle is composed of multiple epithelial layers, creating concentric circles of differentiated cell types. The highly keratinized hair shaft at the center of this cylinder consists of three layers of cells: the medulla, cortex, and cuticle. It is surrounded by the inner root sheath (IRS), composed of the Henle's, Huxley's, and IRS cuticle layers. The IRS is enclosed by the companion layer and the outer root sheath, which is continuous with the basal layer of the epidermis. The entire epithelial structure is surrounded by the sebaceous gland, which secretes lipids into the hair canal (Langbein and Schweizer, 2005). Development of the hair follicle occurs during embryogenesis and involves three main steps: hair follicle induction, organogenesis, and differentiation. Remarkably, this developmental program is repeated to a certain extent throughout adult life, as the hair follicle undergoes phases of growth and regression. Reciprocal and coordinated signals between epithelial and mesenchymal stem cells lead to the differentiation of proliferating matrix cells in the anagen hair bulb along the lineages of the IRS and the hair shaft (Fuchs, 2007).

Hair follicle keratins

Keratin proteins have an important role in the mechanical support of hair development. These evolutionarily conserved proteins are classified into type I (acidic) and type II (neutral basic) based on their gene structure, chromosomal location, and ability to form obligate heterodimers with the other keratin type. Acidic and basic keratins bind each other to generate the intermediate filaments, which provide the hair follicle with its structural integrity. All keratin proteins share a structural organization composed of the N-terminal head domain, a central rod domain, and the C-terminal tail domain. Specifically, the N and C termini of the central α-helix contain helix initiation motif and helix termination motif, respectively, which are essential for dimerization of type I and type II keratins (Coulombe and Omary, 2002). About half of the keratins are restricted to various compartments of the hair follicle. Hair keratins, in contrast to epithelial keratins, possess a highly sulfur-rich head and tail domain responsible for the tough, filamentous structure of the hair and nails (Langbein *et al.*, 1999; Schweizer *et al.*, 2006).

Structural disorders of the hair follicle

Mutations in genes encoding keratins, desmosomes, and lipids signaling lead to a variety of hair disorders, highlighting the important role these proteins have in hair follicle development. Monilethrix, a nonsyndromic hair disorder diagnosed by period changes in hair shaft diameter ("beaded hair"), is characterized by fragile scalp hair and nail abnormalities. The autosomal dominant form of this disease is caused by mutations in *KRT81*, *KRT83*, and *KRT86*, all of which are expressed in the hair cortex (Winter *et al.*, 1997; van Steensel *et al.*, 2005). Autosomal recessive pure hair and nail ectodermal dysplasia, a disorder manifested as complete alopecia and nail dystrophy, is linked to mutations in *KRT85*. The KRT85 protein is abundantly expressed in the matrix, precortex, and cuticle of the hair shaft (Naeem *et al.*, 2006; Shimomura *et al.*, 2010b). Mutations in desmoglein 4, the predominant desmoglein of the hair follicle, cause localized autosomal recessive hypotrichosis (Kljuic *et al.*, 2003). In addition, some patients with mutations in *DSG4* display beaded hair morphology, suggesting that mutations in the gene account for a recessive form of Monilethrix (Schaffer *et al.*, 2006; Shimomura *et al.*, 2006). KRT75 is specifically expressed in the companion layer of the hair follicle. Mutations in *KRT75* predispose to the common hair disorder characterized by ingrown beard hairs with inflammation (Winter *et al.*, 2004) and to loose anagen syndrome (Chapalain *et al.*, 2002).

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Disorders of the hair shaft

The IRS is a critical structural element for supporting and molding the hair shaft. Heterozygous, nonsense mutations in *CDSN* (corneodesmosin), expressed in the IRS (Mils *et al.*, 1992), cause hereditary hypotrichosis simplex restricted to the scalp. Patients with this disorder display disruption of the IRS and aggregates of abnormal CDSN around the hair follicle and the papillary dermis, implying that the mutant CDSN protein is acting in a dominant negative manner (Levy-Nissenbaum *et al.*, 2003). Nonsyndromic forms of hereditary woolly hair (WH) are hair shaft anomalies characterized by tightly curled, slow growing hair, and they are sometimes associated with sparse or depigmented hair. WH can show either as autosomal dominant (ADWH) or autosomal recessive (ARWH). Dominant forms of WH have been linked to mutations in the helix initiation motif of KRT74. KRT74 is a type II keratin highly expressed in the Huxley's layer of the IRS. *KRT74* mutations have been shown to interfere with the proper formation of intermediate filaments, resulting in collapse of the hair follicle (Shimomura *et al.*, 2010c). Recessive forms of WH, whose features slowed or arrested hair growth resulting in shortened hair shaft length, have been linked to the PA-PLA$_1\alpha$/LPA/LPA6 signaling pathway. Mutations in two components of this pathway, the *LIPH* gene (Kazantseva *et al.*, 2006) and the *LPAR6/P2RY5* gene (Shimomura *et al.*, 2008), both expressed in the IRS of the hair follicle, have been found in patients with ARWH.

KRT71: a new determinant of human hair texture

Mutations in *Krt71*, known to be expressed throughout the IRS, have been identified in mice, rats, and dogs, and are linked to a wavy-coat phenotype. *Krt71* mutant mice (''*Caracul* mice'') display wavy pelage and curvy vibrissae, which are inherited in an autosomal dominant manner (Kikkawa *et al.*, 2003). *Rex* rats, which possess a *Krt71* mutation resulting in the deletion of six amino acids, display curly hair in heterozygous mice and hair loss in homozygous mice (Kuramoto *et al.*,

2010). Portuguese water dogs were found to display curly versus straight hair, depending on whether they carried a particular allele of *Krt71* (Cadieu *et al.*, 2009). However, until now, hair disorders linked to human *KRT71* have not been reported. In this issue, Fujimoto *et al.* (2012) describe a mutation within the IRS-specific *KRT71* gene in a Japanese family affected with ADWH. To reveal the genetic basis of the disease, the investigators first excluded mutations in genes known to cause WH, such as *KRT74*, *LPAR6*, *LIPH*, and *CDSN*, as well as in genes involved in other forms of hereditary hypotrichosis, such as *APCDD1* (Shimomura *et al.*, 2010a). On the basis of prior evidence implicating *KRT71* in wavy/curly coat in animals, and on the known relationship between genes expressed in the hair follicle IRS and WH phenotypes, the investigators then examined whether mutations in *KRT71* are responsible for this phenotype.

> The K71–K74 gene cluster regulates hair texture across species.

Using direct sequencing, in this issue Fujimoto *et al.* (2012) show that the patients carry a heterozygous nucleotide change in exon 1 of the *KRT71* gene, which was predicted to result in substitution of phenylalanine with cysteine within the helix initiation motif of the KRT71 protein. To determine whether this mutation affects KRT71 function, the investigators transfected expression vectors for either wild-type or mutated *KRT71* into human cell lines. They showed that the mutation does not affect the expression levels of KRT71, but instead causes severe mislocalization of the protein, resulting in disruption of the keratin intermediate filament network owing to the inability of KRT71 to heterodimerize with KRT14 and KRT18. Although both KRT71 and KRT74 are implicated in WH phenotypes, the Japanese family with *KRT71* mutations also displayed defects in facial hair, whereas the previously reported *KRT74* mutation carriers showed a

phenotype in scalp hair only. The increased severity of this phenotype is likely attributable to the expression of KRT71 in all three layers of the IRS, whereas KRT74 is restricted to Huxley's layer. By conducting immunofluorescence studies on normal human scalp skin, the investigators then examined a possible relationship between the PA-PLA$_1\alpha$/LPA/LPA6 pathway and KRT71. In human hair follicles, KRT71 is expressed in all the three layers of the IRS, and it overlaps with the PA-PLA$_1\alpha$ protein. Moreover, a separate study (Inoue *et al.*, 2011) demonstrated that Krt71 expression was significantly reduced in PA-PLA$_1\alpha$ knockout mice, which have a wavy-coat phenotype. This suggests that the PA-PLA$_1\alpha$/LPA/LPA6 pathway is involved in regulation of KRT71 expression in the hair follicle.

These lines of evidence, together with genetic data from mouse models and canine studies, highlight the importance of *KRT71* as well as the *KRT71–KRT74* gene cluster in the regulation of hair texture across mammalian species. This study also provides new information on molecular interactions among genes involved in hair-shaft maintenance, allowing for the development of improved diagnosis and treatment. Interestingly, a comparison of people of African and European ancestry, with divergent distribution of hair texture, revealed several single-nucleotide polymorphisms within *KRT71* that showed significant differences in allele frequencies (Shimomura *et al.*, 2010c). Further investigation of allelic variations and mutations in these genes will likely elucidate the genetic factors that control the shaping of hair fibers.

CONFLICT OF INTEREST
The authors state no conflict of interest.

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Beyond ABC: Another Mechanism of Drug Resistance in Melanoma Side Population

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It has been shown that a side population (SP), which is characterized by high chemical efflux capacity, is present in human melanoma cell lines. However, it was not clear whether patients' samples contain the same subpopulation. In this issue, Luo *et al.* (2012) report that they have isolated SP cells directly from patients' melanomas. SP cells are resistant to paclitaxel because of the upregulation of ABCB1 and ABCB5. Notably, these cells are also resistant to temozolomide, which is not a substrate for ATP-Binding Cassette (ABC) transporters, in an interleukin (IL)-8-dependent manner. This study provides novel clues for understanding how a small, but critical, subpopulation within melanomas is resistant to therapies.

Melanomas are often resistant to pharmacological therapies. Conventional chemotherapy drugs currently used in clinics are of limited value in treating advanced melanomas. Tumor shrinkage induced by these drugs is often temporary, and most tumors progress or relapse after short periods of time. Both intrinsic and acquired mechanisms have roles in the chemoresistance of melanoma cells. One of the key properties for intrinsic resistance is the expression of certain ABC superfamily proteins, which function as ATP-dependent efflux transporters. The ABC family comprises nearly 50 members

that are evolutionary highly conserved and have high sequence homology among its members. These transporters regulate tissue protection against endogenous and exogenous cellular toxins. Some of the ABC transporters are expressed ubiquitously among diverse cancer cell lines (NCI-60), whereas others are expressed selectively in cancer cells derived from particular tissue types (Szakacs *et al.*, 2004). Melanoma cells express a group of ABC transporters, including ABCA9, ABCB1, ABCB5, ABCB8, ABCC2, and ABCD1 (Chen *et al.*, 2009). Although it has been reported that ABCB5 and ABCB8

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