Miz1 is required for hair follicle structure and hair morphogenesis

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Summary

Previous work has implicated the Myc-binding transcription factor Miz1 in the control of keratinocyte proliferation and in the cellular response to TGFβ. Miz1 is expressed in basal keratinocytes of the interfollicular epidermis and in hair follicles. Here we have conditionally knocked out the POZ/BTB transactivation domain of Miz1 in keratinocytes using a keratin 14 (K14)-Cre mouse deleter strain. K14Cre+/Miz1lox/lox mice have rough fur as a result of altered hair follicle orientation, irregular hair pigmentation and disturbed hair fiber structure. A regional thickening of the epidermis at the hair funnel orifice was accompanied by suprabasal proliferation, indicating a delayed exit of keratinocytes from the cell cycle. In addition, the catagen of the hair cycle was delayed in K14Cre+/Miz1lox/lox mice and intrafollicular keratinocyte proliferation was increased. In aged K14Cre+/Miz1lox/lox animals, the number of hair follicles remained unchanged but the number of visible hairs, especially of zigzag hairs, was reduced and a pigmentary incontinence into the dermis developed. Our data show that Miz1 is involved in controlling proliferation and differentiation in hair follicles and in hair fiber morphogenesis.

Supplementary material available online at http://jcs.biologists.org/cgi/content/full/120/15/2586/DC1

Key words: Myc, Miz1, Transforming growth factor β1, Epidermis, Hair follicle, Catagen

Introduction

Miz1 is a zinc-finger transcription factor, which has been identified as a Myc-binding protein by a yeast two-hybrid approach (Peukert et al., 1997). The N-terminus of Miz1 bears a POZ/BTB domain (Bardwell and Treisman, 1994), which is essential for both transactivation and Miz1-mediated cell cycle arrest (Peukert et al., 1997; Herold et al., 2002; Adhikary et al., 2005). Several proteins bind to and regulate Miz1 activity, including Bcl-6 (Phan et al., 2005), the topoisomerase II binding protein TopBP1 (Herold et al., 2002), the ubiquitin ligase HectH9 (Adhikary et al., 2005), Smad protein complexes containing Smad3 and Smad4 (Seoane et al., 2001) and Myc (Peukert et al., 1997).

A number of observations suggest that Miz1 is involved in restricting proliferation in response to antimitogenic signals; for example, Miz1 is required for upregulation of the cell cycle inhibitor p21cip1 in response to DNA damage and in models of cellular differentiation (Herold et al., 2002; Phan et al., 2005; van de Wetering et al., 2002; Wu et al., 2003; Herold et al., 2002). A second target of Miz1-dependent transactivation is the promoter of the gene encoding the cyclin-dependent kinase inhibitor p15ink4b. Since p15ink4b is regulated by transforming growth factor beta (TGFβ), these findings suggested that Miz1 might have a role in TGFβ-dependent gene regulation (Seoane et al., 2001; Staller et al., 2001).

Inhibition of epithelial cell proliferation by TGFβ is paralleled by a decrease in Myc expression (Pietenpol et al., 1990) that is required for the activation of the p15ink4b gene and exit from the cell cycle (Warner et al., 1999). Myc represses transactivation by Miz1, suggesting that downregulation of Myc allows activation of p15ink4b by Miz1, Smad3 and Smad4 (Seoane et al., 2001; Staller et al., 2001). This model is supported by the finding that Myc but not a mutant, MycV394D, which cannot bind to Miz1 (Herold et al., 2002), represses p15ink4b in primary murine keratinocytes (Gebhardt et al., 2006). In addition, several other genes are regulated in response to TGFβ in the same manner as p15ink4b, suggesting that TGFβ induces a complex genetic program through the relief of Myc-mediated gene repression (Gebhardt et al., 2006). Most of the identified genes are involved in cell-cell or cell-matrix adhesion and some of them, such as integrin receptors, play a role in the maintenance of the epidermal stem cell compartment (Watt, 2002; Alonso and Fuchs, 2003; Moore and Lemischka, 2006).

In previous experiments, we showed that constitutive Miz1 (Zbtb17)-knockout mice die at E7.5, demonstrating that Miz1 is essential for early embryonic development (Adhikary et al., 2003). Here we have explored the function of Miz1 in the skin with the help of mice that carry a conditional allele of Miz1 (C. Kosan, N. Fang, M. Godmann et al., unpublished). We removed the Miz1 POZ/BTB transactivation domain in
keratinocytes, using a mouse strain that expresses Cre recombinase under the control of the keratin 14 (K14) promoter (Huelsken et al., 2001). Our data show that Miz1 has an essential role in controlling exit from the cell cycle during the hair cycle and during epithelial morphogenesis, as well as in hair morphogenesis.

Results
Keratinocyte-specific ablation of the Miz1 POZ/BTB domain in mice
Miz1 is expressed in basal keratinocytes of the interfollicular epidermis (Gebhardt et al., 2006) and of the hair follicle, during all stages of the hair cycle (supplementary material Fig. S1A-C). We generated mice that carry a conditional allele of Miz1 in which the first two coding exons, which encode a POZ/BTB domain (Bardwell and Treisman, 1994), are flanked by loxP sites (C. Kosan, N. Fang, M. Godmann et al., unpublished) (supplementary material Fig. S1D). Mice that are homozygous for the Miz1 lox allele and that express Cre recombinase under control of the keratin 14 promoter (K14Cre) (Huelsken et al., 2001; Wang et al., 1997), lack the POZ/BTB domain of Miz1 in keratinocytes of the interfollicular epidermis, the hair follicle and the sebaceous glands. Recombination starts at about E15 in K14Cre+ mice (Huelsken et al., 2001). Allele-specific PCR using genomic DNA from keratinocytes that were isolated from back skin of 1-day-old mice, without subsequent culture, showed that recombination was essentially complete in these cells (Fig. 1A; for primer position see supplementary material Fig. S1D). This result was confirmed by RT-PCR analysis using RNA from keratinocytes taken from 1-day-old mice and cultured for 3-4 days (Fig. 1B). In western blots of immunoprecipitated proteins from keratinocyte lysates a truncated Miz1 translation product was detected using a monoclonal antibody raised against the C-terminal part of Miz1, showing that the recombined allele results in the production of an N-terminally truncated version of Miz1 lacking specifically the POZ/BTB domain (Fig. 1C). We conclude that the Miz1 POZ/BTB domain was specifically ablated by K14Cre in keratinocytes from day E15 onwards, when the K14 promoter is usually activated (Huelsken et al., 2001).

Deletion of the Miz1 POZ/BTB domain in keratinocytes impairs late hair follicle and hair morphogenesis
Both K14Cre+/Miz1<sup>lox/lox</sup> and K14Cre+/Miz1<sup>lox/ΔPOZ</sup> mice exhibited rough fur with 100 % penetrance (Fig. 2A; 115 mice analyzed). This became obvious as soon as the first hairs were visible (at about P5; supplementary material Fig. S2). Initially, the rough areas occurred in a striped pattern, which may reflect the initial expression pattern of the Cre recombinase (Huelsken et al., 2001). The striped pattern disappeared when mice grew older. By contrast, there were no obvious morphological abnormalities in heterozygous K14Cre+/Miz1<sup>lox/+</sup> and K14Cre+/Miz1<sup>lox/ΔPOZ</sup> mice (36 animals), or in K14Cre+/Miz1<sup>lox/lox</sup> and K14Cre+/Miz1<sup>lox/+</sup> control mice (106 animals).

Histology revealed that the length of hair follicles showed a higher variation in K14Cre+/Miz1<sup>lox/lox</sup> mice compared with control littermates (Fig. 2B-K), and that the orientation of some follicles in respect to the skin surface was altered (Fig. 2K). In addition, in some areas, cyst-like hair follicles were observed containing hair remnants (Fig. 2H). These cystic alterations occurred predominantly in the upper part of the follicles and the epithelium of the cyst-like structures was continuous with the interfollicular epidermis as proved by serial sections (data not shown). Similar structures called pseudocysts have been described in nude mice (Meier et al., 1999). In addition to a variation in follicle orientation and length, the expression of keratin 14 (K14) was altered compared with wild-type skin. Usually, cells from the basal cell layer of the outer root sheet express K14 with a decreasing expression level towards the bottom of the follicle. In K14Cre+/Miz1<sup>lox/lox</sup> mice, we also observed a strong expression of K14 in the lower part of some hair follicles (Fig. 2D,E,I-K). This ectopic expression was especially abundant in follicles delayed in catagen (Fig. 2I-K).

In about 30-40% of the hair follicles from K14Cre+/Miz1<sup>lox/lox</sup> mice, the number of interfollicular epidermal cell layers was increased and cornified layers were

Fig. 1. Conditional knockout of Miz1 POZ/BTB domain in keratinocytes. (A) K14cre mediates efficient recombination of the Miz1 lox allele in primary keratinocytes. For genotyping, PCR was performed on genomic DNA from keratinocytes that were isolated from back skin of 1-day-old mice without subsequent culture, using primers indicated in supplementary material Fig. S1. Note that in K14Cre+/Miz1<sup>lox/+</sup> keratinocytes, the wild-type fragment using primers 1 and 5 is inefficiently amplified because of competition in the PCR by the shorter fragment. The wild-type and loxP allele amplified with primers 1 and 5 (about 1400 bp) differed only in 70 bp and could not be distinguished. (B) Levels of Miz1 mRNA in keratinocytes K14Cre+/Miz1<sup>lox/lox</sup> and control mice. Miz1 mRNA was almost undetectable in K14Cre+/Miz1<sup>lox/lox</sup> keratinocytes using primers 3 and 4, which are located in the region coding for the POZ/BTB domain. By contrast, when primers 6 and 7, which are located outside the POZ/BTB domain coding region, were used, a signal was obtained as in control mice, indicating the presence of a truncated Miz1 transcript lacking the POZ/BTB coding region. (C) Western blot analysis, using the anti-Miz1 antibody 10E2, of proteins immunoprecipitated from keratinocyte lysates using the anti-Miz1 antibody H-190. A truncated Miz1 protein could be detected in K14Cre+/Miz1<sup>lox/lox</sup> and K14Cre+/Miz1<sup>lox/ΔPOZ</sup> keratinocytes (arrow). Arrowhead indicates Miz1.
thickened (Fig. 3A,B). These lesions were often located at the transition between interfollicular and intrafollicular epidermis at the edge of the hair funnel. In these regions, the expression of keratin 1 was seen throughout the suprabasal layers (Fig. 3F) and the expression of filaggrin was seen throughout the cornified layer (Fig. 3H; red). Although the expression pattern of K1 and filaggrin matched the normal distribution in suprabasal cells and in cells of the cornified layer, respectively, the expression of these proteins in the epidermis was increased owing to the increased thickness of those layers (Fig. 3F,H). In addition, it is remarkable that K1 expression extended from the interfollicular epidermis to the cyst-like structures, which seemed to be derived from the hair follicles. Furthermore, expression of K14 (Fig. 3G,H; green) and of Ki67 (Fig. 3C,D; red), a marker of cell proliferation, was not restricted to the basal cell layer in K14Cre+/Miz1lox/lox mice, but extended to the suprabasal cells. These observations show that keratinocytes located at the orifice of the hair funnel have a delayed exit from the cell cycle. They may reflect an increased proliferative compartment, because proliferating cells are exclusively located in the basal epidermal layer of normal skin (Fuchs, 1995). Furthermore, the thickened epidermis and cornified layer indicate an enlarged compartment of differentiation.

Sebaceous glands were larger in mice overexpressing Myc (Arnold and Watt, 2001), and showed a 129-fold upregulation of the sebaceous cell marker brain and skin serine protease (BSSP) in the skin (Frye et al., 2003). In order to test whether the structure of sebaceous glands is affected in K14Cre+/Miz1lox/lox mice we morphometrically measured the area of sebaceous glands per hair follicle. We found a 1.5-fold increase of sebaceous glands in K14Cre+/Miz1lox/lox mice (742±238 μm²/follicle in control mice versus 1093±206 μm²/follicle in K14Cre+/Miz1lox/lox mice), but this difference was not statistically significant. RT-PCR using primers against BSSP did not reveal a different expression of this protein between control and K14Cre+/Miz1lox/lox mice (data not shown).

Deletion of the Miz1 POZ/BTB domain in keratinocytes impairs cycling of hair follicles
On about day 15 after birth (P15) murine hair follicles synchronously undergo a phase of regression called catagen (Botchkarev and Paus, 2003; Foitzik et al., 2000). During this process, which is finished by about P19, the length of the hair follicle is shortened and the remnant follicle lies completely in the dermis. Catagen is delayed in TGFβ-null mice (Foitzik et al., 2000) and almost completely blocked in Smad4-knockout mice (Yang et al., 2005). To assess whether the hair cycle in
K14Cre+/Miz1<sup>lox/lox</sup> mice is altered, we counted the number of hair follicles extending into the subcutis at P19 (Fig. 4A,B). The percentage of such follicles was significantly (P<0.0001) increased in K14Cre+/Miz1<sup>lox/lox</sup> mice (up to 58%; median value 35.7%) compared with control mice (up to 8%; median value 3.4%) (Fig. 4I; P18/19). Follicular keratinocytes proliferate in anagen, but not in catagen of the hair cycle. Consistent with this notion, the BrdU-labeling index was elevated twofold in the hair follicle epidermis at P18-19 in K14Cre+/Miz1<sup>lox/lox</sup> mice (Fig. 4I; P<0.0001). By contrast, no difference in proliferation was seen in the interfollicular epidermis (Fig. 4J).

After a short period of resting (3-4 days; telogen), hair follicles again start to grow out into the subcutis (anagen) (Botchkarév and Paus, 2003). To test further whether the hair cycle is delayed, we counted the number of hair follicles in the subcutis at P22-23 (Fig. 4C,D), which approximately matches the time point of the telogen-anagen transition. Even at this late time point, there was a considerable number of follicles in K14Cre+/Miz1<sup>lox/lox</sup> mice reaching the subcutis without being in the anagen of the next hair cycle, whereas in control mice, only anagenic follicles were detectable in the subcutis (Fig. 4I; P22/23). At P26-27 most hair follicles are expected to be in anagen, extending into the subcutis (Fig. 4E,F). Quantification of the percentage of late anagen hair follicles did not show a difference between control and K14Cre+/Miz1<sup>lox/lox</sup> mice, indicating that anagen is not impaired (Fig. 4I; P26/27). In addition, K14Cre+/Miz1<sup>lox/lox</sup> mice at 1 year of age showed a significantly elevated number of follicles extending into the subcutis, arguing that the delay in catagen was not restricted to the first hair cycle (Fig. 4G-I). We conclude that the ablation of the POZ/BTB domain in the Miz1 protein results in a delay of catagen during the hair cycle.

**Long-term skin alterations after deletion of the Miz1 POZ/BTB domain**

We observed K14Cre+/Miz1<sup>lox/lox</sup> mice up to an age of 18 months. At this advanced age the fur was still rough, but not in a striped pattern (data not shown). In addition, K14Cre+/Miz1<sup>lox/lox</sup> mice exhibited a significant loss of hairs on several areas of the back skin, compared with age-matched control mice (Fig. 5K), although the number of hair follicles did not change (data not shown). No other lesions such as ulcerations, complete alopecia or spontaneous tumors have occurred in these mice.

The fur and skin color of 1-year-old K14Cre+/Miz1<sup>lox/lox</sup> mice were usually darker than that of age-matched wild-type mice. Histology of the back skin showed numerous thickened follicles with hair remnants and abnormal content distribution of melanin (Fig. 5A-H). In areas of substantial decrease of hair density, stereo microscopy revealed black dot-like areas (Fig. 5D), which are probably the cyst-like structures (Fig. 2H), and hyperpigmentation of the upper third of the hair follicle (Fig. 5G). Compared with wild-type animals, K14Cre+/Miz1<sup>lox/lox</sup> mice exhibited focal areas in the dermis and subcutis around hair follicles with a high content of melanin, which was, at least partly, phagocyted by macrophage-like cells (Fig. 5E-H).

Pigmentation of the different hair types (zigzag, awl, guard) (Dry, 1926) was characterized by larger melanin packages and an irregular arrangement of melanin in K14Cre+/Miz1<sup>lox/lox</sup> mice (Fig. 5J). Usually the zigzag hairs are the most abundant hair type, making up about 70% of all hairs (Sundberg and Hogan, 1994). In K14Cre+/Miz1<sup>lox/lox</sup> mice, only 24-54% (mean value 41.2%) of all hairs counted, were zigzag hairs indicating that this hair type is mostly affected (Fig. 5I). Moreover, the zigzag hairs found lacked the characteristic bending (Fig. 5J, arrow). The significant reduction in zigzag hairs, whose tips usually show lighter pigmentation than other hair types (Fig. 5K), together with pigment accumulation in the dermis, account for the darker appearance of the fur and the skin of K14Cre+/Miz1<sup>lox/lox</sup> mice.

**Discussion**

The morphological phenotype of K14Cre+/Miz1<sup>lox/lox</sup> mice comprises an irregular order of hair follicles, alterations of hair follicle and hair fiber morphology, a decrease of hair density in aged animals and a delay in the catagen of the hair cycle. A
1.5-fold increase in the number of sebaceous glands was statistically not significant and sebaceous cell markers, such as BSSP, were not elevated.

In mice, hairs undergo a synchronized hair cycle during the first 25 days after birth. Completely developed hairs (anagen) enter a phase of regression (catagen) at about P15. Catagen is complete by about P19, and after a short resting stage (telogen) of 2-3 days, hairs again enter anagen (Botchkarev and Paus, 2003). In skin of K14Cre+/Miz1<sup>lox/lox</sup> mice, the onset and progression of catagen between P15 and P19 was delayed in a high proportion of the hair follicles (Fig. 4). TGFβ1 is a main player in the induction and progression of catagen. This was originally surmised by the observation that the expression of TGFβ receptors and TGFβ correlated with the onset of catagen and that the local application of TGFβ induced hair follicle regression (Welker et al., 1997; Paus et al., 1997). Stronger evidence came from TGFβ-null mice, which revealed a delay of catagen (Foitzik et al., 2000). Moreover, an almost complete block of catagen occurs in mice deficient in Smad4, which is an essential component of the TGFβ-signaling pathway (Yang et al., 2005).

TGFβ1 is thought to activate the promoter of several genes through two pathways: activation of a Smad3/4 protein complex and downregulation of Myc with a subsequent relief of Miz1 repression, allowing a synergistic activation of the promoter by Miz1 and the Smad complex (Seoane et al., 2001; Gebhardt et al., 2006). This dual-input model has been confirmed for the p15<sub>ink4b</sub> gene, which is involved in the inhibition of keratinocyte proliferation by TGFβ (Pietenpol et al., 1990; Warner et al., 1999). Consistent with a role for Miz1 in TGFβ1-mediated gene regulation, we found that the induction of a number of TGFβ target genes was reduced in keratinocytes isolated from K14Cre+/Miz1<sup>lox/lox</sup> mice relative to control keratinocytes (supplementary material Fig. S3). In vivo, K14Cre+/Miz1<sup>lox/lox</sup> follicular keratinocytes show a twofold increase in the BrdU-labeling index, demonstrating a requirement for Miz1 in controlling cell cycle exit during the anagen-catagen transition of the hair cycle. By contrast,
p15ink4b-knockout mice had no obvious skin phenotype (Latre et al., 2000), indicating that the catagen delay in K14Cre+/Miz1lox/lox mice is not mediated by p15ink4b.

During catagen, epidermal cells of the follicles are removed by apoptosis (Botchkareva et al., 2006) and several genes have been suggested to cause involution (Botchkarev and Paus, 2003; Randall et al., 2003). The expression of the proapoptotic protein clusterin (Reddy et al., 1996; Yang et al., 2000; Leskov et al., 2003), which is a target of TGFβ1 and Miz1 gene regulation in keratinocytes (Gebhardt et al., 2006), was reduced in response to TGFβ1 in K14Cre+/Miz1lox/lox mice (supplementary material Fig. S3B,D). An impaired induction of clusterin during hair follicle regression could complement the delay of cell cycle exit and differentiation by a simultaneous decrease in apoptosis. Taken together the data are consistent with the view that the deletion of the Miz1 POZ domain interferes with the TGFβ pathway in this stage of the hair cycle.

In addition to the alterations in the hair cycle, we observed K14- and Ki67-positive cells in suprabasal layers of the hair funnel, indicating a delayed differentiation in K14Cre+/Miz1lox/lox keratinocytes. This phenomenon has not been reported in TGFβ1-deficient mice. Previous work has shown that the switch between proliferation and differentiation in epidermal keratinocytes is accompanied by a loss of Myc expression and an increase in expression of the Max-network proteins Mxd1 and Mxd3, suggesting that Myc-activated target genes are repressed during differentiation (Hurlin et al., 1995).

Our findings suggest that activation of Miz1-dependent transactivation causally contributes to the transition from proliferation to differentiation in K14Cre+/Miz1lox/lox keratinocytes.

In addition to the described morphological changes of the hair follicle, the hair fiber structure was altered. The melanin inclusions were coarse and irregularly arranged in the hair fibers of K14Cre+/Miz1lox/lox mice compared with hair fibers from control mice, indicating impairment of the hair pigmentation process in the hair bulb. This is confirmed by the observation that melanin accumulates around hair follicles in the dermis with aging (Fig. 5F), which is probably due to a poorly defined process known as pigmented incontinence described in inflammatory skin diseases (Schaumberg-Lever and Lever, 1973; Nagao and Iijima, 1974; Masu and Seiji, 1983) and in senile lentigo (Ünver et al., 2006). In addition,
aberrant pigmentation of the epidermis in the upper third of hair follicles was occasionally observed in K14Cre+/Miz1lox/lox mice. These ectopic melanin locations suggest that the transfer of melanosomes from melanocytes to keratinocytes is impaired.

The fur of mice comprises at least three different hair types: zigzag hairs (about 70%), guard hairs (about 28%) and awl hairs (about 2%) (Sundberg and Hogan, 1994). The relative number of zigzag hairs was reduced to about 40-50% and the characteristic bending of this hair type, usually leading to three kinks per hair, were absent in most hairs of this type. Recently, a similar hair phenotype has been described in lymphotoxin-β (LT-β) but not in LT-α-knockout mice (Cui et al., 2006). It remains to be seen whether Miz1 is also linked to the LT-β signaling pathway regulating gene expression independently of TGFB.

Materials and Methods

Transgenic mouse

Miz1lox/lox mice were generated according to standard procedures (Nagy et al., 2003) (C. Kosan, N. Fang, M. Godmann et al., unpublished). In brief, exon 3 and exon 1 of Miz1, encoding the POZ/BTB domain, were flanked by loxP sites. A further mouse strain was generated by transient expression of Cre recombinase in Miz1crossing material Table S1. An internal control for normalization. Primer sequences are shown in supplementary material Table S1. 

Cell culture

1-day old partum mice were exposed to decapitation, keratinocytes were isolated and cells were cultivated in Eagle’s minimum essential medium with Earle’s BSS and without CaCl2 (Cambrex) as described (Geibhardt et al., 2006).

RNA isolation and preamplification

Total cellular RNA was isolated with the RNeasy reagent (Qiagen) and a subsequent DNase digestion was included. RNA was amplified with MessageAmp RNA amplification kit (Ambion). All procedures were performed according to the manufacturer’s instructions. RNA quality was controlled by agarose gel electrophoresis.

Reverse-transcriptase PCR (RT-PCR)

First-strand cDNAs of amplified RNA were synthesized with M-MLV Reverse Transcriptase (Invitrogen) and random hexamer primers (Promega). For each PCR amplification, aliquots were taken after different cycles to determine the linear range of the amplification. Primer sequences are available in supplementary material Table S1.

Real-time quantitative PCR (RQ-PCR)

cDNA was synthesized as described above. Real-time PCRs were performed with the light cycle ABI Prism 7000, using the qPCR™ Core Kit for Sybr Green I (Eurogentec) according to the manufacturer’s protocol. rps16 was used as an internal control for normalization. Primer sequences are shown in supplementary material Table S1.

Immunoprecipitation and western blot analysis

Lysates from keratinocytes (300 µg protein) were immunoprecipitated using the monoclonal anti-Miz1 antibody H-190 (Santa Cruz). Precipitated proteins were transferred to Immobilon-P transfer membrane (Millipore) by standard procedures. The western blot was incubated with the monoclonal anti-Miz1 antibody 1E2E (1:400). Peroxidase-conjugated anti-mouse immunoglobulin was used as secondary antibody (1:3000; Amersham Biosciences). Proteins were visualized by using ECL plus Western Blotting Detection System (Amersham Biosciences).

Immunohistochemistry

Tissue samples were fixed in 4% PBS-buffered formalin and 5 µm paraffin sections were stained with hematoxylin and eosin according to standard procedures. In addition, the Fontana-Masson silver method for melanin staining was applied (Unver et al., 2006). For immunofluorescence, sections were incubated in 3% bovine serum albumin (Rotth). 0.1% Tween 20 in PBS for 30 minutes and the first antibody was applied overnight at 4°C. After incubation with fluorescence-labeled secondary antibodies for 45 minutes at room temperature (Alexa Fluor 546 goat-anti-rabbit IgG, Alexa Fluor 488 goat-anti-mouse IgG or Alexa Fluor 488 goat-anti-rabbit IgG; 1:1000; Molecular Probes), nuclei were counterstained with DAPI (1 ng/ml; 1:2000) and slides were mounted in Mowiol (Hoechst). For BrdU staining, mice received 200 µl of a BrdU solution (10 mg/ml in PBS) by intraperitoneal injection 1.5 hours before being sacrificed. Slides were pretreated in 2 M HCl, 0.1% Triton X-100 for 30 minutes at room temperature. The following antibodies were used at the dilutions indicated: anti-Keratin 1 (1:1000; Covance), anti-Keratin 14 (1:200; Covance), anti-Filaggrin (1:500; Covance) and anti-BrdU (1:50; DakoCytomation).

Patches were taken either with the BX61 Olympus microscope equipped with a F-View digital camera (Soft Imaging System, Münster, Germany) or with the Axiovert100 microscope (Zeiss) equipped with a MC 100 SPOT camera.

Hair analysis

Hairs were pulled out of the skin or were collected by shaving from comparable sites of the back. Hairs were roughly oriented and stuck to a strip of tape that was then stuck to a slide. More than 300 hairs per animal were counted under the microscope, distinguishing between zigzag hairs and hairs exhibiting more than one row of melanin inclusions.

Morphometric analysis

The area of sebaceous glands were measured in three control and three K14Cre+/Miz1lox/lox mice using a 200-lens and the program analysis® (Soft Imaging System, Münster, Germany). 56-60 hair follicles per animal were analyzed. The sebaceous gland area from all glands measured was divided by the number of follicles analyzed and expressed in µm²/follicle.

Statistical analysis

To test the significance of the quantitative data, the unpaired, two-tailed Student’s t-test was applied using the Graph Pad Prism program.

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### Table S1. Sequences of primers used for genotyping and RT-PCR experiments

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<td>AGCCACCTTACACCTTTAGGT</td>
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<tr>
<td>p21 (cdkn1a)</td>
<td>CTGGAGGGCAACTTCTGGT</td>
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<td>TCTGACGTTCAAAGCTTTG</td>
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<td>p15 (cdkn2b, RT-PCR and Real time)</td>
<td>AGATCCCAAGCCCTGGAAC</td>
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<td>TCGCAGTACCTCAGGAATG</td>
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<td>fxyd6</td>
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<td>GGGGCTTCTGATGAAACTG</td>
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<td>cd151</td>
<td>GGCATCTGGCTGTATGTA</td>
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<td>GCTTACCACAGGCCAGCTACC</td>
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<tr>
<td>Clusterin (clu)</td>
<td>CCAGTTCCAGAGCGGGTATG</td>
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<td>TGAGCATGTTCGACTGGAAG</td>
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<td>Galectin-1 (Lgals1)</td>
<td>GAATGTCTCAAAGTTCCGGGGAG</td>
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<td>AGTTGATGGCCTCCATGGT</td>
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<tr>
<td>Integrin α6 (itga6)</td>
<td>ACTCTCAACTGAGAGGTGAACC</td>
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<td>AAGAAGAGGCCAGGAGGATG</td>
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<td>Integrin β4 (itgb4)</td>
<td>CCAGAGCGTGAGGATCAT</td>
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<td>Procollagen Vα2 (col5a1)</td>
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<td>CCAAATCTCTGATGGAATG</td>
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<tr>
<td>Moesin (msn)</td>
<td>AGCAAGAGCTGGAAGAGCAG</td>
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<tr>
<td>Clusterin (Real time)</td>
<td>AAGTACTACCTTCGGGTCTCACA</td>
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<tr>
<td>Galectin-1 (Real time)</td>
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<td>rps16 (Real time)</td>
<td>GCTACCAGGGCCCTTGGAGAT</td>
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