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.

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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 allows activation of 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

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 Miz1-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 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. Kusan, N. Fang, M. Godmann et al., unpublished) (supplementary material Fig. S1D). Mice that are homozygous for the Miz1lox 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 K14Crelox 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 using the anti-Miz1 antibody H-190. A truncated Miz1 protein could be detected in K14Crelox/+ transcript lacking the POZ/BTB coding region. (C) Western blot analysis, using the anti-Miz1 antibody 10E2, of proteins immunoprecipitated from keratinocyte lysates a truncated 3-4 days (Fig. 1B). In western blots of immunoprecipitated proteins from keratinocytes taken from 1-day-old mice and cultured for 3–4 days, a truncated Miz1 protein was detected with a monoclonal antibody raised against the C-terminal part of Miz1, showing that the recombinant allele results in the production of an N-terminally truncated version of Miz1 lacking 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 K14Crelox+/Miz1flloxflo and K14Crelox+/Miz1flOmapox 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 K14Crelox+/Miz1fllox+ and K14Crelox+/Miz1flloxOmapox mice (36 animals), or in K14Crelox+/Miz1flloxflo and K14Crelox+/Miz1fllox+ control mice (106 animals).

Histology revealed that the length of hair follicles showed a higher variation in K14Crelox+/Miz1flloxflo 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 K14Crelox+/Miz1flloxflo 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 K14Crelox+/Miz1flloxflo mice, the number of interfollicular epidermal cell layers was increased and cornified layers were...
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+/Miz1lox/lox 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+/Miz1lox/lox 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+/Miz1lox/lox 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) (Botchkarev 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+/Miz1lox/lox 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+/Miz1lox/lox mice, indicating that anagen is not impaired (Fig. 4I; P26/27). In addition, K14Cre+/Miz1lox/lox 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+/Miz1lox/lox 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+/Miz1lox/lox 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+/Miz1lox/lox 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+/Miz1lox/lox mice exhibited focal areas in the dermis and subcutis around hair follicles with a high content of melanin, which was, at least partly, phagocytosed 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+/Miz1lox/lox 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+/Miz1lox/lox 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+/Miz1lox/lox mice.

**Discussion**

The morphological phenotype of K14Cre+/Miz1lox/lox 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, depending on the chosen time point and the hair follicle morphology. (I) Quantitative analysis of the percentage of subcutaneous follicles at different ages as indicated. The bars represent the medians. The differences of the medians for P18/19 and for one year were statistically significant (for both $P<0.0001$). (J) Proliferation of keratinocytes measured by the BrdU-labeling index. Mice received BrdU 1.5 hours before being sacrificed and sections were stained with an anti-BrdU antibody to count the percentage of S-phase cells, either in the interfollicular skin or in the hair follicle. The twofold difference found in the hair follicle epithelium was statistically significant ($P<0.0001$). The bars represent the medians. n, number of animals analyzed.

Fig. 4. Hair cycle alteration in K14Cre$^+$/Miz1$^{lox/lox}$ mice. (A-H) Representative sections of comparable areas of back skin from P19 (A,B), P23 (C,D), P26 (E,F) and 1-year-old (G,H) control (A,C,E,G) and K14Cre$^+$/Miz1$^{lox/lox}$ mice (B,D,F,H). Note the sharp boundary between dermis and subcutis (arrow). Such sections were used to count the number of all hair follicle and those which are extending into the subcutis, representing hair follicle either in catagen or anagen, depending on the chosen time point and the hair follicle morphology. (I) Quantitative analysis of the percentage of subcutaneous follicles at different ages as indicated. The bars represent the medians. The differences of the medians for P18/19 and for one year were statistically significant (for both $P<0.0001$). (J) Proliferation of keratinocytes measured by the BrdU-labeling index. Mice received BrdU 1.5 hours before being sacrificed and sections were stained with an anti-BrdU antibody to count the percentage of S-phase cells, either in the interfollicular skin or in the hair follicle. The twofold difference found in the hair follicle epithelium was statistically significant ($P<0.0001$). The bars represent the medians. n, number of animals analyzed.
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 TGFβ and Miz1 gene regulation in keratinocytes (Gebhardt et al., 2006), was reduced in response to TGFβ 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 pigmentary incontinence described in inflammatory skin diseases (Schulberg-Lever and Lever, 1973; Nagao and Iijima, 1974; Masu and Seiji, 1983) and in senile lentigo (Ünver et al., 2006).
aberrant pigmentation of the epidermis in the upper third of hair follicles was occasionally observed in K14Cre+/Miz1^lox/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%) (Sundler 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 lympho toxin-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 TGFβ.

Materials and Methods

Transgenic mice

Miz1^lox/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 Miz1^+/lox ES cells leading to a Miz1^flx/flx genotype. Miz1^lox/lox mice were generated by crossing Miz1^+/lox mice with Miz1^lox/lox mice. Miz1^+/lox or Miz1^lox/lox mice were crossed with K14Creneo mice (Huelsken et al., 2001), here designated K14Cre mice. These mice express Cre recombinase under the control of the keratin 14 promoter, inducing Miz1 ablation in basal keratinocytes of the epidermal layer of the skin. These mice express Cre recombinase under the control of the keratin 14 promoter, inducing Miz1 ablation in basal keratinocytes of the epidermal layer of the skin (Fuchs, 1995; Wang et al., 1997). Mice were genotyped by standard PCR, using the primers 1, 2 and 5 listed in supplementary material Table S1, with an addition, the Fontana-Masson silver method for melanin staining was applied (1:3000; Amersham Biosciences). Proteins were visualized by using ECL plus blot was incubated with the monoclonal anti-Miz1 antibody 10E2 (1:400).

RNA isolation and preamplification

Total cellular RNA was isolated with the RNeasy reagent (Qiagen) and a subsequent DNA 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 amplification. Primer sequences are shown in supplementary material S1.

Real-time quantitative PCR (RQ-PCR)

cDNA was synthesized as described above. Real-time PCRs were performed with the light cycler ABIprism 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 available 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 separated by 10% reducing SDS polyacrylamide gel electrophoresis and transferred to Immobilon-P transfer membrane (Millipore) by standard procedures. The western blot was incubated with the monoclonal anti-Miz1 antibody 10E2 (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 precut in 3% bovine serum albumin (Roth), 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).

Pictures were taken either with the BX61 Olympus microscope equipped with a F-View digital camera (Soft Imaging System, Münster, Germany) or with the Axiosvert100 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+/Miz1^lox/lox mice using a 20× lens and the program analysis5 (Soft Imaging System, Münster, Germany). 50-60 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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References


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

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<td>Clusterin (clu):</td>
<td>CCGATCTCCAGAGCTTGATT</td>
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<td>TGAGCATGGTCCAGGTGAAG</td>
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<td>Galectin-1 (Lgals1):</td>
<td>GAATGTCTCAAAGTTCGAGGGAG</td>
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<td>AGTTGATGCGGCTCCAGTGGAAG</td>
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<td>Integrin α6 (itga6):</td>
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<td>Integrin β4 (itgb4):</td>
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<td>Procollagen Vα2 (col5a1):</td>
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<td>Moesin (msn):</td>
<td>AGCAAGAGCTGAGAACAGCAG</td>
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<td>Clusterin (Real time):</td>
<td>AAGTACTACCTCGGTTCTCCA</td>
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<td>Galectin-1 (Real time):</td>
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<td>rps16 (Real time):</td>
<td>GCTACCCAGGGCCTTTGAGAT</td>
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