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Disruption of the Hedgehog Signaling Pathway Contributes to the Hair Follicle Cycling Deficiency in Vdr Knockout Mice

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Abstract

Mice null for the Vitamin D receptor (VdrKO) have a disrupted first hair follicle cycle and aborted subsequent hair follicle cycling. We examined the expression of different markers and mediators of hair follicle cycling in the hair follicle of the VdrKO mouse during days 13–22 when the hair follicle normally initiates and completes the first catagen. We compared the expression of those genes in mice with a nonsense mutation in hairless (Rhino), which have a similar alopecia phenotype, and to Cyp27b1 null mice which are deficient in the production of 1,25(OH)2D3, the Vdr ligand, but display normal hair follicle cycling. Our results demonstrate the down regulation of hair follicle markers and the alteration of expression of hedgehog (Hh), Wnt, Fgf, and Tgfβ pathways in VdrKO and Rhino mice, but not in Cyp27b1KO mice. Treatment of VdrKO mice with an agonist to the Hh pathway partially restored hair follicle cycling, suggesting a role of this pathway in the regulation of hair follicle cycling by VDR. These results suggest that Vdr regulates directly or indirectly the expression of genes required for hair follicle cycling, including Hh signaling, independent of 1,25(OH)2D3.

The hair follicle cycles through periods of growth (anagen), apoptosis-driven involution (catagen), and resting (telogen) with hair follicle morphogenesis, initiated during embryogenesis, lasting until about 3 weeks after birth (Cotsarelis, 1997; Soma et al., 1998; Paus and Cotsarelis, 1999; Stenn and Paus, 2001). During anagen, about 15–16 days long in mice, the follicle grows through the dermis, and proliferation, differentiation, and survival predominates. In catagen, about 3–5 days long in mice, proliferation and differentiation of hair follicle keratinocytes are reduced while proximal (dermal portion) hair follicle length is shortened through apoptosis. Telogen is characterized by minimal signaling between dermal papilla (DP) fibroblasts and follicular keratinocytes and by the absence of follicular keratinocyte proliferation. Initiation of a new anagen is characterized by cell proliferation in the proximal follicular epithelium containing hair follicle stem cells (Cotsarelis et al., 1999; Oshima et al., 2001). The control of hair follicle cycling involves multiple pathways, such as fibroblast growth factor (Fgf; Hebert et al., 1994), Transforming growth factor beta (Tgf-β; Philpott et al., 1994; Soma et al., 1998; Foitzik et al., 2000), Hedgehog (Hh; St-Jacques et al., 1998; Sato et al., 1999; Wang et al., 2000), and Wnt (Reddy et al., 2001).

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Mice lacking the Vitamin D receptor (VdrKO mouse) develop their first coat of hair normally, but initiation of subsequent anagen is impaired, leading to alopecia (Li et al., 1997; Yoshizawa et al., 1997). Vdr action in hair follicle cycling is not dependent on its ligand $1,25(OH)_2D_3$, as demonstrated by the lack of alopecia in mice lacking Cyp27b1, the enzyme essential for $1,25(OH)_2D_3$ production (Bikle et al., 2004), and in mice with a Vdr mutated to prevent $1,25(OH)_2D_3$ binding (Skorija et al., 2005). Mice lacking functional hairless (Hr) (Panteleyev et al., 1999; Sundberg et al., 1999) develop a hair follicle phenotype comparable to that seen in the VdrKO mouse. Hairless, which is a known corepressor to some nuclear receptors (Potter et al., 2001, 2002; Moraitis et al., 2002; Moraitis and Giguere, 2003), interacts directly with Vdr (Hsieh et al., 2003; Xie et al., 2006; Wang et al., 2007) and inhibits ligand dependent Vdr mediated transcription. Because of the parallel between VdrKO and hairless mice phenotypes, it has been hypothesized that Hr and Vdr converge to control hair cycling but the role of Hr in modulating ligand independent actions of Vdr as in hair follicle cycling is not known.

The dissociation of the DP from the hair bulb by the end of catagen is thought to account for the failure to initiate the subsequent anagen in Hr and Vdr null animals (Panteleyev et al., 1999; Bikle et al., 2006). The dermal cysts that develop contain markers of the differentiated interfollicular epidermis suggesting their origin from disintegrating outer root sheath, bulge derived cells or epithelial cells (Panteleyev et al., 1999; Xie et al., 2002), although alteration of the differentiation program in dermal cyst epithelium and sebaceous glands has been suggested (Panteleyev et al., 1998; Skorija et al., 2005). At least in the VdrKO mice the defect responsible for the hair follicle cycling abnormality lay in the keratinocytes, not in the mesenchymal cells of the DP (Sakai et al., 2001).

In this article, we examined in Hr and Vdr mutant mice the expression of pathways involved in hair follicle cycling during the end of anagen, catagen and telogen phases of the first hair follicle cycle. We identified multiple pathways whose expression is reduced in both VdrKO and Rhino mice, particularly the Hh signaling pathway, whose stimulation in VdrKO mice with an agonist temporarily restored partial hair follicle cycling, suggesting this pathway as one of the targets of Vdr signaling. Moreover, our results suggest parallels in disrupted signaling mechanisms during hair follicle cycling in both VdrKO and Rhino mice, which could at least partially explain the transformation of their hair follicle structures into similar abnormal structures.

Materials and Methods

Animals

All animal experimentation has been approved by the SFVAMC Animal Review Committee.

Mice heterozygous for the Vdr null mutation, outbred to C57BL/6, were provided by Dr. Shigeaki Kato (University of Tokyo, Japan) and fed a special diet to prevent the rickets phenotype (Li et al., 1998). Mice heterozygous for the Cyp27b1 null mutation, outbred to C57BL/6 (Dardenne et al., 2001), were provided by Pr René St Arnaud (Shriners Hospital,

Montreal, Canada). Mice heterozygous for the RHJ/Le mutation (Cachon-Gonzalez et al., 1994) were obtained from the Jackson Laboratory (Bar Harbor, ME).

Genotyping was performed by PCR using primers designed to amplify the mutant or wild type Vdr DNA, primers encompassing Cyp27b1 deleted exon 8 and primers from A. Christiano (Personal communication; Supplementary Table 1).

Hh pathway agonist treatment

Five to 10 weeks old mice were shaved and treated daily for 8 consecutive days with 25 μl of smoothened agonist (SAG, 566660, Calbiochem, Germany, at 0.18 μg/ml in 5% DMSO 95% acetone) on the shaved back of at least three mice for each time point.

Immunohistochemistry

Antibodies (Covance, Berkeley, CA) were used at 4 μg/ml in 10 mM Tris buffer, pH 7.6, 4% bovine serum albumin, 1% teleostean skin gelatin, 0.1% Tween 20, and 500 mM NaCl. The binding of the antibodies was detected by biotinylated goat anti-rabbit IgG, followed by ABC-peroxidase reagent (Vector, Burlingame, CA) and revealed with diaminobenzidine substrate (QualTek Laboratories, Santa Barbara, CA) followed by hematoxylin counterstaining. Omitting the first antibody resulted in no signal, indicating the specificity of the immunodetection.

Alkaline phosphatase staining

Skin cryo-sections were stained for alkaline phosphatase activity using Leukocyte Alkaline phosphatase kit (Sigma–Aldrich, St Louis, CA) following the manufacturer's instructions and subsequently counterstained with hematoxylin.

Quantitative RT-PCR

RNA was extracted using RNA-STAT 60 (Tel-Test, Friendwood, TX) from multiple plucked hair follicles from the back skin of mice using a hair epilator (Braun, Boston, MA) and reversed transcribed using the TaqMan reverse transcription kit (ABI, Foster City, CA). cDNA amounts were quantified by quantitative real time PCR (PE Biosystems 7900 HT) using TaqMan Universal PCR Master Mix (ABI) and TaqMan Gene Expression Assays (ABI) or SYBRGreen Universal PCR Master Mix (ABI) and primers from PrimerBank (Wang and Seed, 2003; Supplementary Table 1) and normalized to mitochondrial ribosomal protein L19 expression.

Results

Alopecia development in the VdrKO, Rhino, Cyp27b1KO, and VdrKO/Cyp27b1KO mice

At day 13, compared to their wild-type littermates, hair follicles from VdrKO mice are normal whereas hair follicles from Rhino mice appear ectatic with inclusion of cornified material (Fig. 1A,C,E). By day 19 both mutant hair follicles have a similar abnormal morphology with follicular dystrophy, scattered dilated areas around the sebaceous gland, twisted fibers and hyperplasia of the outer root sheath. (Fig. 1B,D,F). The hair follicles from Cyp27b1KO mice are normal at both days (Fig. 1G,H).

To determine whether the lack of the Vdr ligand, $1,25(OH)_2D_3$, would contribute to the VdrKO phenotype, we analyzed hair follicle histomorphology in double mutants for Vdr/Cyp27b1. These mice have a phenotype little different from the VdrKO mice, with the formation of utricles and dermal cysts (Fig. 1I).

Differentiation marker expression during the first hair follicle cycle in VdrKO, Rhino, and Cyp27b1KO mice

Since Rhino mice lose their hair completely by 3–4 weeks, whereas VdrKO mice lose their hair over the subsequent 15– 20 weeks, we compared the expression of epithelial markers from day 13 to 22 after birth in VdrKO and Rhino mice and in VdrKO mice at 11 weeks to provide a more comparable time of near total alopecia to the Rhino mouse at 3 weeks. Rhino mice hair follicle could not be analyzed at 11 weeks since no hair was available for plucking. Plucked hair follicles, used as a source of mRNA, from wild-type, VdrKO and Rhino mice had comparable histomorphology (Supplementary Fig. 1A,B) and expression of hair follicle markers K17 and K31 at similar levels (Supplementary Fig. 1C).

Filaggrin expression was strongly increased in the VdrKO hair follicles from day 19 to 77 and was even more increased in Rhino mice (Supplementary Fig. 2 and Supplementary Table 1). Caspase 14 expression was also increased in Rhino, at day 22, but not before 11 weeks in VdrKO mice. The expression of both genes was not affected in Cyp27b1KO. Immunohistochemistry of filaggrin showed restricted expression at day 13 to the interfollicular epidermis in all mice and reduced expression in Rhino mice. In VdrKO and Rhino mice, as the hair follicle abnormality progresses in days 19 and 22, filaggrin expression was increased in the interfollicular epidermis and utricle (Fig. 2A) but not in Cyp27b1KO mice (Fig. 2B). Expression of the epithelial marker transglutaminase 1 was increased in Rhino mice at day 22, and expression of K1 and K16 was increased at 11 weeks in VdrKO mice (Table 1).

We then extended the analysis to the expression of hair specific markers, to further describe the phenotype of these mutants. Expression of krt2-16 was decreased at day 22 and at 11 weeks in VdrKO mice and at day 19 in Rhino mice (Supplementary Fig. 3 and Supplementary Table 1). Expression of K17 was also decreased in VdrKO and Rhino mice at days 19 and 22 (Sup Fig. 1C). Expression of K31, K32, K33 and K34 was decreased at 11 weeks in both VdrKO and Rhino mice (Table 1). Versican expression was decreased in VdrKO and Rhino mice, at day 22 and day 13, respectively (Table 1). None of these gene expressions was affected in Cyp27b1KO mice (data not shown).

Alterations in cell cycle and apoptosis markers in VdrKO and Rhino mice

Since hair follicle transition from anagen to catagen is associated with reduced proliferation and induction of apoptosis, we analyzed the expression of some markers of cell cycling and apoptosis. p27 expression, a cell cycle inhibitor, was decreased in VdrKO mice at days 17 and 22 and in Rhino mice at days 17 and 19 (Supplementary Fig. 2 and Supplementary Table 1). Bcl2 expression, an apoptosis inhibitor, was reduced as early as day 13 in both VdrKO and Rhino mice with a more pronounced reduction at day 17 in VdrKO mice. In contrast, caspase 3 expression, an apoptosis marker, was reduced in VdrKO mice at day 22

and in Rhino mice at day 17. None of these gene expressions was affected in Cyp27b1KO mice (data not shown).

Alterations in signaling pathways in VdrKO and Rhino mice

Since both VdrKO and Rhino mice present comparable morphological alterations in their hair follicle structure, we analyzed the expression of signal transduction pathways thought to be important for hair follicle cycling.

The Hh pathway was the most affected by the lack of either Vdr or Hr. In VdrKO mice, Shh mRNA and protein expression was decreased at day 15 (Fig. 2C), but most profoundly at day 77 (Supplementary Fig. 3 and Supplementary Table 1). Expression of Patched, Gli1 and Gli2 was reduced at day 22 in both mutants, but more strongly in Rhino mice. Both Vdr and Shh were expressed in the outer root sheath of 11-week-old wild-type mice (Fig. 2D), but Shh expression was absent from VdrKO utricles while over-expressed in the epidermis.

Tgfb pathway members Tgfβr1 and Tgfβr2 expression was decreased in VdrKO at day 22 and the decrease started at day 17 and was more pronounced in Rhino mice (Supplementary Fig. 3 and Supplementary Table 1). The Fgf pathway was impacted in that Fgfr2 expression was reduced in both VdrKO and Rhino mice at day 17 (Table 1). The Wnt pathway was affected with Wnt4 expression decreased in VdrKO mice at day 17 and day 22. Furthermore, at day 77 lef-1 expression was markedly reduced in VdrKO mice. Expression of Bmp pathway members Bmp4 and Noggin was decreased at day 77 by the lack of Vdr.

Hr expression was increased in the VdrKO mice, although Vdr expression was not altered in Rhino mice. None of these gene expressions was affected in Cyp27b1KO mice (data not shown).

We found no difference in the expression of these genes between VdrKO and wild-type neonatal mouse epidermal keratinocytes (data not shown), consistent with the observations that these changes only develop subsequent to the initial hair follicle (and epidermal) development.

Restoration of hair follicle cycling following Hh pathway agonist treatment

To assess the role of decreased Hh signaling pathway expression in VdrKO and Rhino mice, we stimulated this pathway with a smoothened agonist (SAG; Chen et al., 2002, 2004).

By day 9, blackening of the skin was visible in 8–10 weeks old wild-type mice at the site of the application (Fig. 3). By day 13 hair growth was visible, and by day 22 the area with new hair reached its maximal size. In VdrKO mice, blackening of the skin was visible 15 days after treatment and hair growth by day 22, although not to the extent seen in wild-type mice. SAG did not stimulate hair growth in 5-week-old Rhino mice.

Skin sections of 9-week-old VdrKO mice SAG treated showed utricles present by day 9 (Fig. 4A), but by day 15, utricles had regressed and hair follicles with a hair shaft were visible. At day 22 and 29 hair growth was visible and all hair follicles displayed a hair shaft. However, hair follicles at day 15, 22, or 29 retained an abnormal structure, with multiple

sebaceous structures per follicle and a poorly formed distal portion. By day 70, when hair growth had stopped, hair follicles displayed an enlarged hair follicle structure, short dermal invasion and lack of hair shaft.

VdrKO mice treated with SAG did not grow as much hair, nor as fast as wild-type mice and did not respond to a second SAG treatment (Fig. 4B, data not shown). While only VdrKO mice treated before or by 11 weeks of age showed hair growth, wild-type mice grew hair at various ages, tested up to 24 weeks (data not shown).

Since the DP plays a key role in the induction of anagen from telogen, we determined DP localization through its alkaline phosphatase activity. Alkaline phosphatase activity was observed in the DP region of each wild-type hair follicle (Fig. 4B) but not in VdrKO mice even after SAG treatment.

Discussion

VdrKO and Rhino mice show an arrest in hair follicle cycling and the development of utricles by day 19. The normal histological structure of hair follicle in Cyp27b1KO mice and the similar histology between VdrKO/Cyp27b1KO and VdrKO mice indicate the lack of a role for $1,25(OH)_2D_3$ in the control of this cycle. Previous studies (Hsieh et al., 2003; Xie et al., 2006; Wang et al., 2007) indicate that Hr functions as a corepressor for $1,25(OH)_2D_3$ dependent Vdr transcriptional activity in epidermal keratinocytes. Our observations of comparably impacted signaling mechanisms in the absence of either Vdr or Hr suggests similar regulation of these pathways by both Vdr and Hr. Such results are consistent with but do not prove their interaction in the control of hair follicle cycling.

Expression of epithelial markers was increased in VdrKO and Rhino hair follicles, whereas the expression of hair specific markers was reduced. Rhino mice have reduced filaggrin expression at day 13, although its expression increases more rapidly than in VdrKO mice subsequently. Moreover, the follicles of Rhino mice appear to be ectatic with inclusion of some cornified material suggesting alteration in their differentiation several days before that seen in VdrKO mice. In VdrKO mice at day 13, filaggrin expression is restricted to the interfollicular epidermis, but with time filaggrin expression increases in the interfollicular epidermis and subsequently in the hair follicle as the utricle develops. This suggests a dissociation between the changes in epidermal and hair follicle keratinocytes, as observed in HrKO where filaggrin expression is increased as early as day 9 in the epidermis, and only by day 19 in the hair follicle (Zarach et al., 2004). At 11 weeks, VdrKO mice hair follicles showed a further decrease in hair follicle marker expression and an increase in epithelial marker expression suggesting a transition in differentiation of the hair follicle keratinocytes to those with more epidermal features much as observed in HrKO mouse skin (Zarach et al., 2004). The more rapid changes in hair follicle structure and gene expression in Rhino compared to VdrKO mice suggest that Hr plays an additional role relative to Vdr in the control of hair follicle cycling, as discussed below.

Hair follicle transition from anagen to catagen is accompanied by apoptosis with changes in expression of markers, such as p27 (Mitsui et al., 1997), bcl2 (Lindner et al., 1997;

Botchkarev et al., 2001), and caspase 3 (Lindner et al., 1997; Soma et al., 1998). In both mutants, bcl2 expression was reduced early (d13), whereas p27 and caspase 3 expression were reduced later, suggesting that apoptosis was accelerated early in catagen, but proliferation dominated the later stages, consistent with our previous observations showing increased proliferation in the later stages of catagen in VdrKO (Bikle et al., 2006). Reduced expression of Tgfβr1 and Tgfβr2 in the latter stages of catagen suggests Vdr control of hair follicle proliferation and apoptosis is in part mediated through the Tgfβ pathway (Philpott et al., 1994; Soma et al., 1998, 2002; Foitzik et al., 2000).

Versican, a key player in the DP initiation of anagen (Kishimoto et al., 1999), showed decreased expression in both VdrKO and Rhino mice consistent with the dissociation of the DP from their hair follicles by the end of catagen (Panteleyev et al., 1999; Bikle et al., 2006). Since VdrKO alopecia is due to the lack of Vdr in keratinocytes, not in cells from the DP (Sakai et al., 2001), decreased Versican expression could be an indirect effect of Vdr regulation, or simply represents fewer DP in the plucked VdrKO and Rhino hair follicles by day 22.

Wnt signaling was affected in VdrKO mice with reduced Wnt4, believed to signal hair shaft precursor differentiation (Reddy et al., 2001), and lef-1 expression. Fgfr2 expression, a promoter of hair follicle induction (Petiot et al., 2003), was reduced in VdrKO and Rhino mice as well as the expression of Tgfβr I/II. These findings are consistent with extended anagen or delayed catagen when Fgf or Tgfβ signaling is impaired and abbreviated anagen when Tgfβ is ectopically expressed (Hebert et al., 1994; Philpott et al., 1994; Soma et al., 1998; Foitzik et al., 2000).

The Hh pathway is required for anagen induction, and ShhKO skin grafts in nude mice generate hairless skin phenotypically similar to VdrKO skin (St-Jacques et al., 1998; Sato et al., 1999; Wang et al., 2000). Shh and Vdr are expressed in the same cells of the interfollicular epidermis and outer root sheath of the hair follicle, but expression of Shh is decreased toward the end of catagen and is lost in VdrKO utricles suggesting a dependence on Vdr at least in hair follicle keratinocytes. Our immunostaining results contrast with previous work showing very localized Shh mRNA expression in the hair follicle (Sato et al., 1999) and could reflect a difference in the timing at which the observations were made. Patched expression is affected at only certain time points of the cycle, illustrating the activation of this pathway at specific phases of the cycle. Temporarily partial restoration of hair growth in VdrKO mice following SAG application further suggests that the Hh pathway is a key target of Vdr signaling, but that other pathways remain involved. The limited time frame for SAG action in the VdrKO mouse suggests a continued loss of the pathways critical for hair follicle cycling, supported by the further decrease in expression of such pathway elements at 11 weeks compared to 3 weeks. Separation of the DP from the hair follicle in VdrKO, which also fails to reconnect after SAG treatment, could also be a limiting event in SAG action. The absence of response to SAG treatment in Rhino mice, suggests that they have a more rapid loss of such pathways leading to alopecia earlier than do VdrKO mice (Fig. 5).

The expression of these genes in cultured epidermal keratinocytes was not affected by the lack of Vdr, indicating that these genes are not affected by loss of VDR prior to or at birth, but no doubt also reflecting the inability of neonatal keratinocytes to differentiate into hair follicle structures in vitro. Lacking a good in vitro model to study the direct actions of Vdr and Hr on hair follicle cycling we cannot rule out that at least some of the changes seen in vivo are not the cause but a consequence of the Hr and Vdr null phenotypes.

The phenotypes of Hr, Vdr, and Rxr mutants are similar as hair follicle cycling fails after initial hair follicle development, and all mutants develop utricles and dermal cysts, suggesting that Hr could act at least in part through Vdr:Rxr hetero-dimers (Li et al., 1997, 2001; Yoshizawa et al., 1997; Miller et al., 2001; Hsieh et al., 2003). However, hair loss in Vdr and Rxr mutants is delayed relative to HrKO mice and for multiple markers indicating that the lack of functional Hr has an earlier and more striking effect on the genes involved with hair follicle cycling than the lack of Vdr. These differences suggest that Hr acts through other nuclear receptors (e.g., Rxr partners) or has an additional role not born by Vdr (Fig. 5). Regardless of the differences in timing and severity, the same pathways are affected, suggesting an interdependent role of both Vdr and Hr in regulating hair follicle cycling.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Abbreviations

Vdr	Vitamin D receptor
Hr	hairless
$1,25(OH)_2D_3$	1,25 dihydroxyvitamin D3
Cyp27b1	25 hydroxyvitamin D 1- hydroxylase
SAG	smoothened agonist

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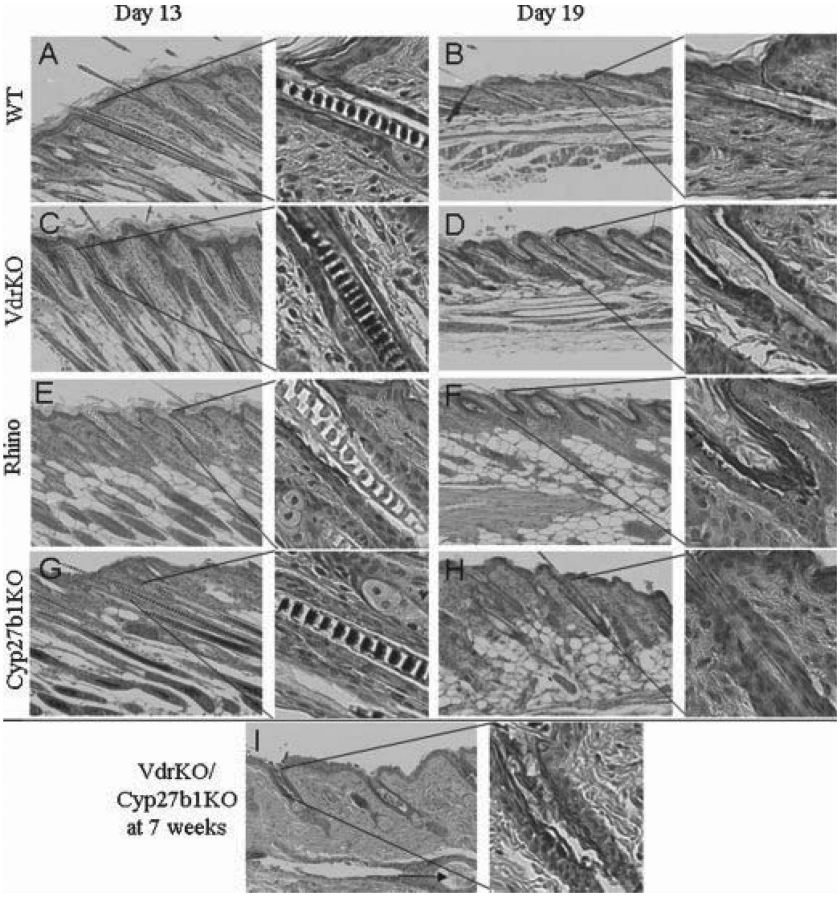

Fig. 1.

Histomorphology of VdrKO, Rhino, and Cyp27b1KO mice compared to their wild-type littermates. Hematoxylin staining of VdrKO and Rhino mice at day 19 as well as VdrKO/Cyp27b1KO mice at 7 weeks shows morphologically abnormal hair follicle structures, compared to their wild-type littermates and Cyp27b1KO mice. The arrow in picture I indicates a dermal cyst. The bars denote 50 and 10 μm.

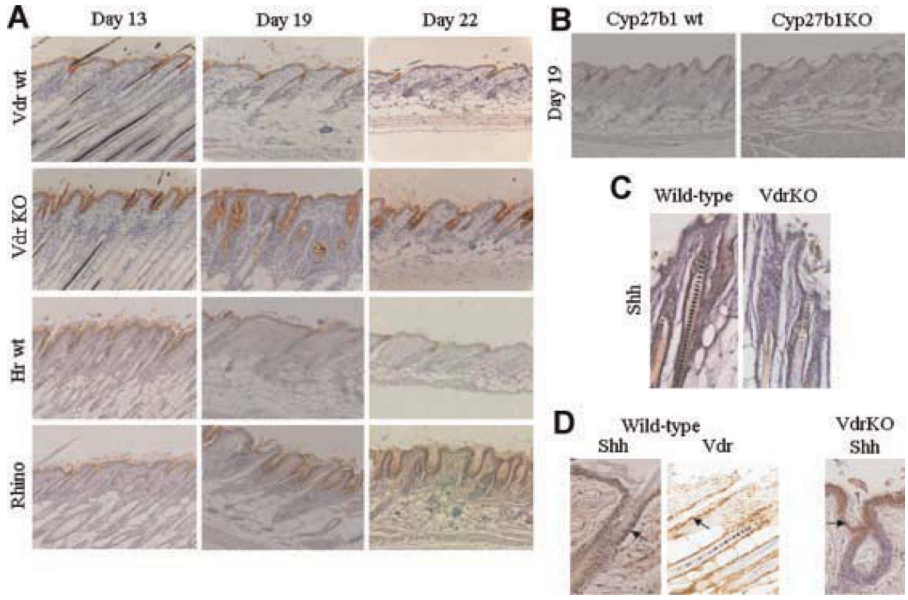

Fig. 2.

Increased protein levels of filaggrin in the epidermis and hair follicles from VdrKO and Rhinomice but not from Cyp27b1KO mice.Filaggrin protein as shown by the brown signal was detected in theepidermis from(A).VdrKO,Rhinomiceand their wild-type littermatesatday13,19,and22 or (B). Cyp27b1KO mice at day 19 after birth by immunohistochemistry. C: Shh expression was decreased in 15d old VdrKO hair follicle compared to their wild-type littermate. D: Shh and Vdr expression was detected in outer root sheath cells of 11-week-old wild-type hair follicle (arrows). Shh expression was absent from 11-week-old VdrKO utricles but was expressed in the epidermis(arrow).Slides were counterstained with hematoxylin (blue stain). The bar denotes 50 μm.

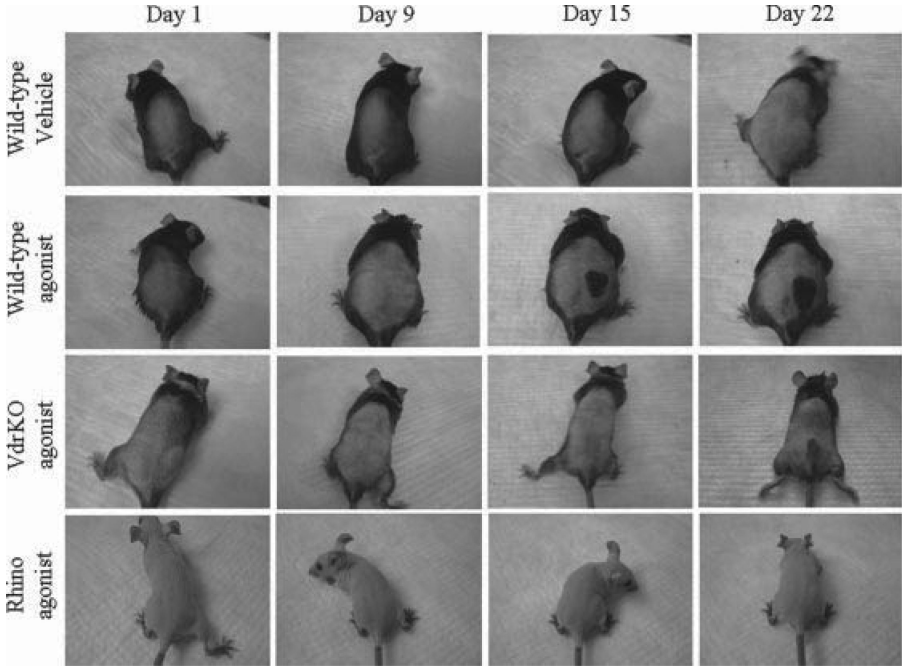

Fig. 3.

Hh signaling pathway agonist stimulates hair growth in wild-type and VdrKO but not in Rhino mice. Five to 11 weeks old mice were shaved and treated daily for 8 consecutive days with either Hh signaling pathway agonist or vehicle solution. Both wild-type and VdrKO mice display hair growth following treatment with the agonist where as vehicle wild-type and agonist treated Rhinomice do not.Pictures were taken at the beginning of the treatment (day 1), after the end of the treatment (day 9) and at subsequent days 15 and 22 to monitor hair growth.

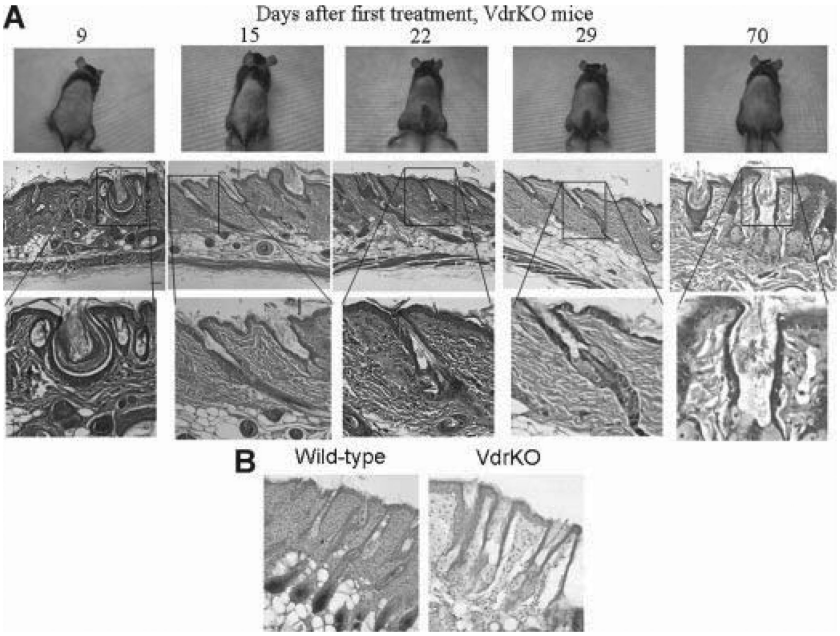

Fig. 4.

Hh signaling pathway agonist partially restores hair follicle in VdrKO mice. A: 9 weeks old VdrKO skin samples collected 9 days after SAG treatment show no hair growth,whereas samples collected at days 15,22,and 29 display hair growth and partial resolution of the utricle phenotype. By day 70 no more hair growt hwas visible and utricles started tore-appear.B:Wild-type and VdrKO mice skin samples were collected 22 days after treatment with SAG and dermal papilla(DP) localization was analyzed by alkaline phosphatase staining. Wild-type mice display DP at the expected location, whereas VdrKO mice treated with SAG do not display alkaline phosphatase staining indicating the absence of DP. The red bar denotes 50 μm.

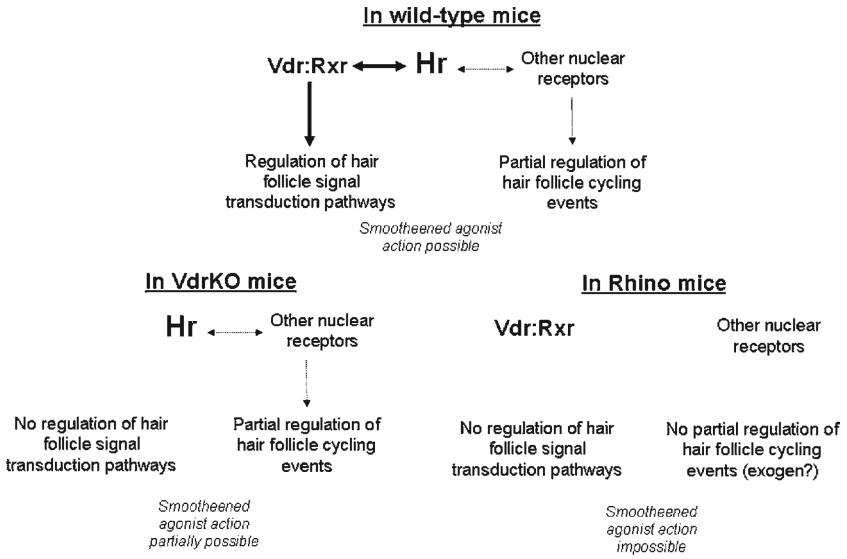

Fig. 5.

Model of Vdr and Hairless action to control the regulation of hair follicle cycling. Vdr and Hairless are each necessary for hair follicle cycling, their interaction likely beinga key controller. In the absence of Vdr, Vdr-Hairless control of hairfollicle cycling is absent, with some residual Hairless actions remaining potentially through interaction with other nuclear receptors. In the absence of Hairless, Vdr-hairless control of hair follicle cycling is completely ineffective.

TABLE 1

Complete results of Q-PCR analysis for VdrKO mice versus wild-type and Rhino mice versus wild-type hair follicles

		13		15		17		19		22		77	
Family	**Gene**	**VdrKO**	**Rhino**	**VdrKO**	**Rhino**	**VdrKO**	**Rhino**	**VdrKO**	**Rhino**	**VdrKO**	**Rhino**	**VdrKO**	**Rhino**
Cell cycle and apoptosis	p27	—	—	—	—	–3.2	–3.2	—	–1.7	–1.9	—	–2	
	Bcl2	–2.8	–2.5	—	—	–37	—	—	–2.3	—	—	–2	
	Caspase 3	3	—	—	—	—	–7	—	—	–2.3	—	—	
Epithelial and hair markers	Caspase 14	—	—	—	—	—	—	—	—	—	8	27	32
	Filaggrin	—	—	—	—	—	—	7.3	8	16	45	23	14
	Krt1-16 (K16)	—		—		—		—		—		5.8	
	Krt2-1 (K1)	—		—		—		—		—		4	—
	K31	—	—	—	—	—	—	—	—	—	—	–97	–63
	K32	—	—	—	—	—	—	—	—	—	—	–7	–12
	K33	—	—	—	—	—	—	—	—	—	—	–73	–85
	K34	—	—	—	—	—	—	—	—	—	—	–57	–72
	Krt2-16	—	—	—	—	—	—	—	—	–6	—	–93	–4
	Tg1	—	—	—	—	—	—	—	—	—	4.4	—	
	Versican	—	–2.8	—	—	—	—	—	—	–2.4	—	—	
Hh Pathway	Shh	—	—	–1.7	—	—	—	—	—	—	—	–164	–500
	Patched	—	—	—	—	—	–5.5	—	—	–1.7	–17	–3	
	Smoothened	—	—	—	—	—	—	—	—	—	—	–2	
	Gli1	—	—	—	—	—	–10	—	—	–2.6	–58	–7	
	Gli2	—	—	—	—	—	–22	–2.2	—	–2.2	–43	–2	
	Tgfbr1	—	—	—	—	—	–2.3	—	—	–1.9	–2.8	—	
	Tgfbr2	—	—	—	—	—	–22	—	–1.7	–1.9	–16	–2	–2
Fgf pathway	Fgfr2	—	—	—	—	–2.7	–2.5	—	—	—	—	—	
Wnt pathway	Beta-catenin	—	—	—	—	—	—	—	—	—	—	—	
	Lef1	—		—		—		—		—		–21	–15
	Wnt3a	—		—		—		—		—		–3	
	Wnt4	—	—	—	—	–4.1	—	—	—	–1.9	—	—	–3
	Wnt10b	—		—		—		—		—		–3	

		13		15		17		19		22		77	
Family	**Gene**	**VdrKO**	**Rhino**	**VdrKO**	**Rhino**	**VdrKO**	**Rhino**	**VdrKO**	**Rhino**	**VdrKO**	**Rhino**	**VdrKO**	**Rhino**
Bmp pathway	Bmp4	—		—		—		—		—		–2	
	Noggin	—		—		—		—		—		–4	
Transcription pathway	Hairless	—		—		—		2		—		2	
	VDR		—		—		—		—		—		—

The numbers reflect fold differences in expression for VdrKO or Rhino mice relative to their wild-type littermates. A positive value represents increased expression in the mutant relative to the wild type, a negative value decreased expression. —, no variation; gray box, not analyzed; black box, the mutated gene.