NIH Public Access
Author Manuscript
J Invest Dermatol. Author manuscript; available in PMC 2014 June 09.

Published in final edited form as:
J Invest Dermatol. 2011 May ; 131(5): 1067–1076. doi:10.1038/jid.2010.400.

Conditional Activin Receptor Type IB (*Acvr1b*) Knockout Mice Reveal Hair Loss Abnormality

Wanglong Qiu[1], **Xiaojun Li**[1], **Hongyan Tang**[2], **Alicia S. Huang**[1], **Andrey A. Panteleyev**[3], **David M. Owens**[4,5], and **Gloria H. Su**[1,5]

[1]Department of Otolaryngology and Head and Neck Surgery, Columbia University College of Physicians and Surgeons, New York, New York, USA

[2]Department of Obstetrics and Gynecology, Columbia University College of Physicians and Surgeons, New York, New York, USA

[3]Division of Medical Sciences, University of Dundee, Dundee, UK

[4]Department of Dermatology, Columbia University College of Physicians and Surgeons, New York, New York, USA

[5]Department of Pathology, Columbia University College of Physicians and Surgeons, New York, New York, USA

Abstract

The *in vivo* functions of the activin A receptor type 1b (Acvr1b) have been difficult to study because *Acvr1b*$^{-/-}$ mice die during embryogenesis. To investigate the roles of *Acvr1b* in the epithelial tissues, we created mice with a conditional disruption of *Acvr1b* (*Acvr1b*$^{flox/flox}$) and crossed them with *K14-Cre* mice. *Acvr1b*$^{flox/flox}$; *K14-Cre* mice displayed various degrees of hairlessness at postnatal day 5, and the phenotype is exacerbated by age. Histological analyses showed that those hair follicles that developed during morphogenesis were later disrupted by delays in hair cycle reentry. Failure in cycling of the hair follicles and regrowth of the hair shaft and the inner root sheath resulted in subsequent severe hair loss. Apart from previous reports of other members of the transforming growth factor-β/activin/bone morphogenic protein pathways, we demonstrate a specialized role for Acvr1b in hair cycling in addition to hair follicle development. *Acvr1b*$^{flox/flox}$; *K14-Cre* mice also had a thicker epidermis than did wild-type mice, which resulted from persistent proliferation of skin epithelial cells; however, no tumor formation was observed by 18 months of age. Our analysis of this *Acvr1b* knockout mouse line provides direct genetic evidence that Acvr1b signaling is required for both hair follicle development and cycling.

© 2010 The Society for Investigative Dermatology

Correspondence: Gloria H. Su, Department of Otolaryngology/Head and Neck Surgery, Columbia University College of Physicians and Surgeons, 1130 St Nicholas Avenue ICRC 1004, New York, New York 10032, USA. gs2157@columbia.edu.

CONFLICT OF INTEREST

The authors state no conflict of interest.

SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at http://www.nature.com/jid

NIH-PA Author Manuscript
NIH-PA Author Manuscript
NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

INTRODUCTION

The activin signaling transduction pathway has multiple roles in mammalian development and tumorigenesis. Activins were originally isolated as regulators of the follicle-stimulating hormone (Ling *et al*., 1986). Additional functions of activins include roles in bone growth and bone morphogenesis (Merino *et al*., 1999), mesoderm induction, nerve cell survival, wound healing, tissue differentiation, inflammation, determination of left–right axes branching morphogenesis of glandular organs (Smith *et al*., 1990; Thomsen *et al*., 1990; Mathews *et al*., 1992; Hubner *et al*., 1997; Gu *et al*., 1998; Woodruff, 1998; Song *et al*., 1999; Kim *et al*., 2000; Ball and Risbridger, 2001; Chen *et al*., 2004; Thompson *et al*., 2004), and tumor suppression (Matzuk *et al*., 1992; Su *et al*., 2001; Burdette *et al*., 2005). Activins have also been described as critical regulators of skin morphogenesis (Matzuk *et al*., 1995b, c; Munz *et al*., 1999). Activins are dimers, comprising two β-subunits linked by a single, covalent disulphide bond; five different β-subunits (A–E) have been identified. Activins A (βAβA) and B (βBβB) are the most common types expressed in various tissues (McDowall *et al*., 2008).

Activin A receptor type 1b (*Acvr1b*), also termed “activin-like kinase 4 (*Alk4*),” is a type I transmembrane serine/threonine kinase receptor that is part of the transforming growth factor-β (TGF-β) receptor superfamily (Massague, 1996, 1998; Piek *et al*., 1999). Activin binds to a type II activin receptor (Acvr2 or Acvr2b) and then recruits Acvr1b (Attisano *et al*., 1993). Receptor heterodimerization activates the type II receptor kinase to phosphorylate the type I receptor, which recruits and phosphorylates regulated Smads2 and 3. Phosphorylated regulated Smads are released and form a heteromeric complex with the Co-Smad, Smad4. The regulated Smad and Co-Smad complex then translocates to the nucleus where it regulates the expression of many genes (Attisano *et al*., 1996; Heldin *et al*., 1997; Derynck *et al*., 1998; Massague, 1996, 1998; Piek *et al*., 1999). In mammals, Acvr1b is expressed by various types of epithelial cells, including interfollicular epidermis, and the outer root sheath (ORS) and the inner root sheath (IRS) of the hair follicles (Roberts and Barth, 1994). Activin signaling through Acvr1b acts on skin epithelial cells in a paracrine manner. Other than activin, only nodal and myostatin are known to signal through Acvr1b, and they are not invoveld in hair growth (Yeo and Whitman, 2001; Zimmers *et al*., 2002).

The roles of activins and other TGF-β family members in mammalian development have been extensively analyzed by gene targeting studies in mice. Activin βA-deficient mice lack whiskers, and incisor and mandibular molar teeth, and have defective secondary palates, resulting in their death within 24 hours after birth because of impaired suckling (Matzuk *et al*., 1995b; Ferguson *et al*., 1998). Mice deficient in activin βB are viable, with normal mesoderm formation and neurulation, but have defects in eyelid development and female reproduction (Vassalli *et al*., 1994). Acvr2a and Acvr2b are functionally redundant; hence, only a few *Acvr2a*$^{-/-}$ or *Acvr2b*$^{-/-}$ mice have skeletal and facial abnormalities; most lack these defects and develop into adults (Matzuk *et al*., 1995a; Song *et al*., 1999). However, *Acvr2a*$^{-/-}$; *Acvr2b*$^{-/-}$ (double mutant) mice fail to form the primitive streak during embryogenesis, and *Acvr2a*$^{-/-}$; *Acvr2b*$^{+/-}$ mice have truncated brain development by embryonic day 9.5 (Song *et al*., 1999). Activin receptor II and activin βA mutants have similar defects in tooth development (Ferguson *et al*., 2001). *Acvr1b*$^{-/-}$ mice do not survive

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

beyond embryonic day 9.5 because of developmental arrest of the egg cylinder before gastrulation (Gu *et al*., 1998). To bypass the requirement of activin signaling in embryonic development, transgenic mice that overexpress activin A (Munz *et al*., 1999), the activin inhibitor follistatin (Wang *et al*., 2004), or a dominant-negative form of Acvr1b (Bamberger *et al*., 2005) were generated and revealed additional roles for activin in skin morphogenesis and/or wound healing.

In addition to the importance of activin signaling in mammalian development (Verschueren *et al*., 1995; Gu *et al*., 1998; Chen *et al*., 2004), Acvr1b can also function as a tumor suppressor (Su *et al*., 2001; Danila *et al*., 2002). To circumvent embryonic lethality and to explore the functions of Acvr1b signaling in development and tumorigenesis, we created mice with conditional disruption of *Acvr1b* using *Cre-loxP* and *Flp-FRT* recombination technologies. To investigate the roles of Acvr1b in the surface epithelial tissues, we bred these mice with *K14-Cre* transgenic mice, to generate *Acvr1b* $^{flox/flox}$; *K14-Cre mice*.

RESULTS

Inactivation of *Acvr1b* in the surface epithelial tissues leads to hair loss

In mice, *Acvr1b* consists of 9 exons, maps to chromosome 15 (60.4 cM), and encodes a 4.4-kb mRNA. We generated conditional *Acvr1b* knockout mice using the Cre and Flp recombinase systems (Supplementary Figure S1 online and Figure 1). *Acvr1b* $^{flox/flox}$ mice appeared normal and fertile.

To investigate the role of Acvr1b in the epithelial tissues such as the oral cavity, esophagus, and skin, we generated *Acvr1b* $^{flox/flox}$; *K14-Cre* mice (Figure 1a–c). Epithelial-specific genomic recombination of Acvr1b also occurred in the tongue, esophagus, eye, forestomach, and thymus, but not in the liver, stomach, lung, kidney, small bowel, large bowel, heart, spleen, or brain of adult *Acvr1b* $^{flox/flox}$; K14-Cre mice (Figure 1d). This pattern of tissue-specific *Acvr1b* recombination was confirmed by reverse transcriptase-PCR (Figure 1e). In the skin, *Acvr1b* expression and recombination were restricted to the epidermis and not observed in the dermis (Figure 1f). Acvr1b protein expression in the epidermis and hair follicle epithelium of mutant mice was significantly reduced as shown by immunohistochemical analysis with anti-Acvr1b (Figure 1g). The reduced Acvr1b level was sufficient to result in detectable downregulation of p-Smad2 expression (Figure 1h).

Newborn *Acvr1b*$^{flox/flox}$; *K14-Cre* mice exhibited a wide range of hair loss; ~25% displayed visible hairlessness and stunted growth at postnatal day 5 (P5), whereas 50% were indistinguishable from wild-type mice (controls) (Figure 2). However, as they aged, all *Acvr1b*$^{flox/flox}$; *K14-Cre* mutant mice exhibited increased hair loss (Figure 3, Supplementary Figure S2 online). These phenotypes (diminutive size and alopecia) were exacerbated in mice with two copies of *K14-Cre* (*Acvr1b*$^{flox/flox}$*; K14-Cre*$^{tg/tg}$) (Supplementary Figure S2b–d online), indicating that the severity of the phenotypes was associated with increased Cre activity and more efficient deletion of *Acvr1b* in their hair follicles. All of the following experiments were performed on mice with one copy of *K14-Cre*. Our data indicate that downregulation of Acvr1b expression has severe negative impacts on hair development.

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

Although keratin 14 expression has been reported in mammary glands, *Acvr1b*$^{flox/flox}$; *K14-Cre* adult female mice were able to reproduce and nurse pups. Among *Acvr1b*$^{flox/flox}$; *K14-Cre* mutant mice, those with the severe hair loss phenotype also had abnormalities in number, size, and color of incisor teeth (Supplementary Figure S2 online).

Disruption of the hair follicle cycle in *Acvr1b* $^{flox/flox}$; *K14-Cre* mice

To investigate the hair loss observed in *Acvr1b*$^{flox/flox}$; *K14-Cre* mice in relation to the hair follicle cycle, we examined the histology of the dorsal skin sample obtained from *Acvr1b*$^{flox/flox}$; *K14-Cre* mice at different developmental stages, including follicular morphogenesis (P5), anagen (P11), catagen (P17), telogen (P24), and anagen (P32) stages, compared with littermate controls. As expected, those *Acvr1b*$^{flox/flox}$; *K14-Cre* mice with gross alopecia by P5 displayed corresponding abnormal hair follicles microscopically at morphogenesis (Figure 2c and f). Histological analyses of the skin samples obtained from the least affected *Acvr1b*$^{flox/flox}$; *K14-Cre* mice showed only slight differences from controls at P5, P11, and P17, but striking defects in hair follicle morphology at later time points (at P24 and P32; Figures 2b, e and 3). This result indicates that *Acvr1b*-dependent signaling is essential for hair cycling in addition to its involvement in hair development. At P5 and P11, the hair follicles of these mutant mice were in an early and fully developed anagen phase as controls, respectively; hair bulbs had descended deep into the fat layer of the skin, and well-differentiated hair shafts that had undergone terminal differentiation and lacked nuclei were observed in control and mutant mice (Figure 3a–d). At P17, the dorsal follicles of control mice were in the catagen phase, whereas some of the hair follicles in mutant mice were delayed in entering this phase (Figure 3e and f). At P24, the follicles in control mice started to enter the anagen phase, from quiescence (telogen phase), whereas the hair follicles of mutant mice stayed at the regression (catagen) phase (Figure 3g and h). The skin of *Acvr1b*$^{flox/flox}$; *K14-Cre* mutant mice also started to exhibit enlarged sebaceous glands. At P32, the skin follicles of control mice were entering the full anagen phase; in contrast, the follicles of mutant mice appeared to be completely deregulated (Figure 3i and j). At P32, some mutant follicles enter abnormal anagen, whereas the majority remains in telogen (Supplementary Figure S3 online). Counting the hair follicles at each stage of follicle growth revealed significant differences between control and mutant mice, starting at day 22 (Supplementary Figure S3 online). These data indicated that in mutant mice, the remnant hair follicles faced detrimental cycling disruption starting at the first postnatal hair cycle.

Epithelial *Acvr1b* signaling is required for the keratinized cortex and IRS in hair differentiation

We then investigated whether the defective cycling phenotype observed in the hair follicles of mutant mice resulted from abnormal follicle differentiation. In normal hair follicles, AE13, which is an antibody against type I low-sulfur hair-shaft cortex keratin (Lynch *et al*., 1986), stains the keratins of the cortex and precortex of hair follicles. The cortex makes up the majority of the hair shaft, whereas AE15, which recognizes proteins in the medulla of the hair shaft and in the IRS (O'Guin *et al*., 1992), stains all three layers of the IRS and medulla of the hair follicles (Figure 4a and c, Supplementary Figures S4a, c, and e, and S5a, c, and e online). In *Acvr1b*$^{flox/flox}$; *K14-Cre* mice, AE13 staining was reduced as the hair follicles became disorganized (Supplementary Figure S4b, d, and f online) and was absent

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

from most follicles by the anagen phase (P32; Figure 4b). With AE15, normal staining patterns were observed during morphogenesis, but were visibly reduced as the hair cycle became deregulated (Figure 4d, Supplementary Figure S5b, d, and f online). These findings indicate the absence of the keratinized cortex and defective differentiation of the IRS and hair-shaft medulla in these follicles (Figure 4b and d). They also indicated that matrix cells failed to differentiate toward the IRS and hair shaft in the follicles of mutant mice in preparation for hair cycle reentry. These events might have contributed to the subsequent failure in hair cycling and demonstrate a role for Acvr1b signaling in hair differentiation.

Keratin 5 proteins are specifically expressed in the epidermal basal layer and ORS of the normal hair follicle (Figure 4e) (Byrne *et al*., 1994). Keratin 6 proteins are specific for the companion cell layer that separates the ORS from the IRS (Figure 4g) (Wang *et al*., 2003). High expression levels of keratin 5 and keratin 6 markers were maintained in the hair follicles of *Acvr1b*$^{flox/flox}$; *K14-Cre* mice (Figure 4f and h and Supplementary Figure S6 online). This observation demonstrates that differentiation of the epidermal basal layer, ORS, and companion cell layer was unaffected by deletion of *Acvr1b*.

To further investigate aberrations in hair differentiation, using immunohistochemistry, we examined the expression of the transcription factor Lef1, which regulates hair-specific keratin genes (Zhou *et al*., 1995). Surprisingly, although follicles of *Acvr1b*$^{flox/flox}$; *K14-Cre* mice lost immunoreactivity with AE13 as they became deregulated, *Acvr1b*-null follicles had increased numbers of Lef1-positive cells per hair follicle (Supplementary Figure S7 online). At the catagen phase (P17), increased levels of Lef1 expression were observed in the matrix cells of mutant hair follicles (Figure 4j), compared with littermate controls (Figure 4i).

Increased proliferation in the epidermis and follicles of aging *Acvr1b*$^{flox/flox}$; *K14-Cre* mice

A total of 20 *Acvr1b*$^{flox/flox}$; *K14-Cre* mice were observed for 18 months to examine the long-term effects of *Acvr1b* deletion in the skin epidermis. The severity of the hair loss phenotype increased with aging. At 5 months, *Acvr1b*$^{flox/flox}$; *K14-Cre* mice were completely alopecic. The few remaining hair follicles were abnormally enlarged, without shaft formation (Figure 5b–f); the defective, dilated hair follicles resulted in large cysts that contained keratinaceous debris (Figure 5c, e, and f). These mice also had increased size and numbers of sebaceous glands in their skin (Figure 5d). Increased proliferation was also observed in the few remaining hair follicles of mutant mice (Figure 5g and i). BrdU-positive cells could be observed in the upper ORS of the remaining hair follicles with or without cyst association (Figure 5i). The percentage of BrdU-positive cells per hair follicle in *Acvr1b*$^{flox/flox}$; *K14-Cre* mice (11.2±3.5%) was significantly greater than that in controls (6.2±3.6%) (P<0.01). Those hair follicles that had completely disintegrated into cysts did not display increased BrdU positivity.

By 5 months of age, the epidermis of *Acvr1b*$^{flox/flox}$; *K14-Cre* mice was also thicker than that of controls (Figure 5). The epidermis of *Acvr1b*$^{flox/flox}$; *K14-Cre* mice (Figure 5b–f, h, and i) had 3–5 more basal layers of epithelial cells than did littermate controls (Figure 5a and g). BrdU incorporation was 10-fold greater in the basal layer of the epidermis of mutant mice (22.9±13.6) (Figure 5h and i) than in that of controls (2.5±1.5; P<0.0001) (Figure 5g).

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

No differences in epidermal proliferation were apparent between *Acvr1b*$^{flox/flox}$; *K14-Cre* mice and controls at young ages (from P11 to P32; Figure 3a–h). The numbers of BrdU-positive cells per hair follicle were also not significantly higher in mutants than in controls before P32 (Supplementary Figure S8 online). The persistent proliferation of epidermal and follicular epithelial cells only becomes apparent as mice age, possibly resulting from accumulative deregulation of activin signaling over time.

Samples collected from the skin, tongue, and esophagus of mice were also examined at 5-, 6-, 8-, 10-, 12-, 15, and 18-month time points in *Acvr1b*$^{flox/flox}$; *K14-Cre* mice and controls. No gross anatomical difference and no tumor formation were observed in the samples obtained from mutant mice.

DISCUSSION

The *Acvr1b*$^{flox/flox}$; *K14-Cre* mutant mice displayed a high level of *Acvr1b* recombination in the skin and tongue and a low level in the esophagus and forestomach (Figure 1d and e). These results are consistent with previous studies that showed that *K14-Cre* mice had high levels of Cre activity in the epidermis, hair follicles, oral epithelium, and developing teeth and low levels in the esophagus and forestomach at embryonic day 14.5 and P1 (Vassar and Fuchs, 1991; Byrne *et al*., 1994; Wang *et al*., 1997). Despite the frequent recombination events observed in the tongue and skin, the most prominent phenotypes of *Acvr1b*$^{flox/flox}$; *K14-Cre* mice were observed in the skin. The skin might require greater levels of *Acvr1b*-dependent activin signaling than the tongue, or the high level of *K14* expression in the skin might cause more severe phenotypes. The abnormalities observed in *Acvr1b*$^{flox/flox}$; *K14-Cre* mice are presumably due to the lack of Acv1b-dependent signaling through Smad2, because p-Smad2 expression was absent in response to the loss of Acv1b expression (Figure 1).

The severity of the hair loss phenotype observed in *Acvr1b*$^{flox/flox}$; *K14-Cre* mice depended on the efficiency of Cre-recombination and increased with age (Figure 2, Supplementary Figure S2, 3, and 5 online). Mutant mice also had abnormal tooth morphogenesis (missing, shortening, or overextending incisors) (Supplementary Figure S2 online). These phenotypes are distinct from those observed in activin bA-deficient mice, which lacked vibrissae, vibrissae follicles, incisor, and mandibular molar teeth; these mice died within 24 hours of birth (Matzuk *et al*., 1995b; Ferguson *et al*., 1998). The differences in phenotypes might arise because activin signaling has many roles in embryonic development and in *Acvr1b*$^{flox/flox}$; *K14-Cre* mice, Acvr1b is inactivated later in development (Matzuk *et al*., 1995a; Gu *et al*., 1998; Song *et al*., 1999). The differences are not likely to arise from inefficient Cre recombination in the oral cavity because transgenic mice that express a dominant-negative form of Acvr1b have mild delays in postnatal hair follicle morphogenesis, but not incisor abnormality or shorten lifespan (Bamberger *et al*., 2005). A closer examination of *Acvr1b*$^{flox/flox}$; *K14-Cre* revealed an important role for *Acvr1b* in both hair follicle development and hair cycle reentry. Disruption in the re-development of the IRS and hair shaft during hair regrowth might have contributed to the defective hair cycling reentry and continual hair loss beyond morphogenesis. Our studies of *Acvr1b*$^{flox/flox}$; *K14-Cre* mice reveal a previously unreported role for activin signaling in hair cycling and incisor phenotypes.

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

Other studies in which TGF-β/bone morphogenic protein family members were inhibited demonstrated their involvement in hair follicle morphogenesis. Mice deficient in TGF-β2, Bmpr1a, noggin, Smad4, or Smad7 have a profound delay in hair follicle development and differentiation (Botchkarev *et al*., 1999; Foitzik *et al*., 1999; Kobielak *et al*., 2003; Han *et al*., 2006; Qiao *et al*., 2006; Owens *et al*., 2008). Acvr1b signaling differs from that of other TGF-β receptors in that it has an additional role in hair follicle cycling. Lef1 expression was lost in *Smad4*$^{flox/flox}$; *MMTV-Cre* mice, whereas overexpression of Left1 was observed in *Bmpr1a*$^{flox/flox}$; *K14-Cre* and *Acvr1b*$^{flox/flox}$; *K14-Cre* mice (Figure 4, Supplementary Figure S6 online) (Kobielak *et al*., 2003; Qiao *et al*., 2006). Our data, together with previous studies, indicate that although the ligands, receptors, and mediators of the TGF-β, activin, bone morphogenic protein signaling pathways all contribute to hair follicle development and differentiation, yet each has non-overlapping function that cannot be compensated by the expressions of the others, because the absence of one gene would significantly impact the normalcy of hair follicles.

Hair follicle morphogenesis and hair cycling are controlled by complex, bidirectional epithelial–mesenchymal interactions (Fuchs and Raghavan, 2002; Millar, 2002). We show that Acvr1b is expressed in epithelial compartments, but not in dermal cells, which are consistent with previous studies (Nakamura *et al*., 2003). Activin was mainly expressed in the mesenchyme. Therefore, it was proposed that activin-βA, produced by cells of the mesenchymal compartment, binds to activin receptors in the epithelial compartment of the hair follicle to inhibit hair follicle development (Nakamura *et al*., 2003). Hair follicular morphogenesis initiates from an epithelial downgrowth, which proliferates and differentiates to first form the IRS and then the hair shaft itself. In this study, we show that Acvr1b signaling is required for the differentiation and regrowth of IRS and hair shaft in adult mice (Figure 4, Supplementary Figures S4 and S5 online). It remains unknown whether the defective IRS and hair shaft result directly from *Acvr1b* loss or from a lack of proper progenitor cells that give rise to these structures. Further studies are required to determine whether Acvr1b signaling requirements in the IRS and hair-shaft development differ between the embryonic and the first postnatal anagen phase. It is possible that the secondary hair germ cells and bulge stem cells are directly affected by *Acvr1b* inactivation and that damage to these cells reduces the progenitor population required for anagen-phase hair follicle regrowth (Ito *et al*., 2004). The lower-most portion of the telogen follicle consists of the bulge and secondary hair germ, and the progeny of stem cells in the bulge form the lower follicle and hair (Wilson *et al*., 1994). In *Acvr1b*$^{flox/flox}$; *K14-Cre* mice, secondary hair germ cells might not be able to survive an extended, aberrant catagen phase, and bulge stem cells might also become impaired. Fibroblast growth factor, activin/bone morphogenic protein, and Wnt signaling have been implicated in regulating hair germ cell and bulge cells in hair regeneration (Wilson *et al*., 1994; Ohyama *et al*., 2006). It will be important to examine the role of *Acvr1b* in regulating hair germ and bulge stem cells.

Although *Acvr1b*$^{flox/flox}$; *K14-Cre* mice had abnormal incisors, the mechanisms of this phenotype are unknown. The chalky white color of the incisors indicates severe enamel defects in *Acvr1b*$^{flox/flox}$; *K14-Cre* mice. The normal maintenance of the sharp, cutting edge of the continuously growing rodent incisor depends on the asymmetric deposition of enamel,

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

and the physical contacts of the upper and lower incisors keep each other sharp and to prevent overgrowth. Therefore, overextending incisors observed in mutant mice also suggest enamel problems and/or compromised growth of the incisor epithelium. The epithelial stem cell niche is located at the apical end of the tooth, and its progeny give rise to ameloblasts that form the hard enamel (Jernvall and Thesleff, 2000; Thesleff *et al*., 2001). Recent studies have shown that activin A regulates the proliferation of incisor epithelial stem/progenitor cells (Thesleff *et al*., 2007; Wang *et al*., 2007). Our data suggest a possible role of Acvr1b in regulating enamel development and will be further investigated in future studies.

Activin inhibits growth in many types of cells and acts as a tumor suppressor in tumorigenesis. Activins have been shown to have tumor-suppressive properties in endocrine, breast, liver, kidney, and brain cells (Risbridger *et al*., 2001; Su *et al*., 2001; Danila *et al*., 2002; Burdette *et al*., 2005; Panopoulou *et al*., 2005). Somatic mutations of activin receptors, including ACVR1B, have been reported in many tumor types (D'Abronzo *et al*., 1999; Su *et al*., 2001; Hempen *et al*., 2003; Rossi *et al*., 2005). In most tissues, activins therefore inhibit the development of cancer. In this study, disruption of Avcr1b-dependent activin signaling resulted in hyperplasia of the epidermis and in persistent proliferation of skin epithelial cells at 5 months of age (Figure 5). These data support a tumor-suppressive role of Acvr1b, consistent with previous data that indicate that activin signaling mediates proliferation and differentiation of skin epithelial cells (Matzuk *et al*., 1995c; Munz *et al*., 1999; Cruise *et al*., 2004). However, unlike the spontaneous tumor formation observed in *Smad4*, *Bmpr1a*, and *TbetaRII* conditional knockout mice (Kobielak *et al*., 2003; Qiao *et al*., 2006), conditional deletion of *Acvr1b* was not sufficient to initiate tumor formation in the skin.

Our study of the first conditional *Acvr1b* knockout mouse line provides direct evidence that Acvr1b signaling has a required and a specific role in the cycling and differentiation of hair follicle and tooth morphogenesis. The mechanisms behind these phenotypes remain to be elucidated. Similar to those mice with condition disruptions in *activin βA*, *Smad*, or *Bmpr1a*, loss of *Acvr1b* affected hair follicle morphogenesis. However, our data also reveal an additional role for *Acvr1b* in hair follicle cycling and the differentiation of IRS and hair shaft, indicating that TGF-β receptor family, its ligands, and mediators each has a specific role in hair follicle development, differentiation, and cycling. As $Acvr1b^{-/-}$ mice die during embryogenesis, mice with conditional disruption of *Acvr1b* are a useful tool for investigating activin signaling *in vivo*.

MATERIALS AND METHODS

Mouse strains

To our knowledge, a previously unreported conditional *Acvr1b* knockout mouse model was generated with Cre and Flp recombinase systems. In brief, Cre-induced recombination would delete exons 2 and 3 of the *Acvr1b* gene and result in a frameshift mutation that eliminates Acvr1b expression. Details on the generation of the targeting construct, selection of embryonic stem cell clones, generation of chimeric mice, and germline transmission are as described in Supplementary Data online ("Materials and Methods" and Supplementary Figure S1 online). *CMV-Flp* transgenic mouse was a gift from Dr Thomas Ludwig at the

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

Columbia University Medical Center. *K14-Cre* transgenic mouse (FVB-Tg(K14-Cre)8Brn) was obtained from the Mouse Repository of the Mouse Models of Human Cancers Consortium (Jonkers *et al*., 2001). All mouse lines, including *Acvr1b*-floxed mice, were maintained by backcrossing to C57BL/6J.

DNA and RNA isolations and analyses

The *Acvr1b*-floxed mice were genotyped by detection of the *loxP* loci, and Cre-mediated recombination was determined by PCR and reverse transcriptase-PCR strategies that used genomic DNA or RNA, respectively (Figure 1 and Supplementary Data online). In brief, the PCR strategy is as depicted in Figure 1a. The primers for reverse transcriptase-PCR are located within exons 1 and 5, flanking exons 2 and 3 of the *Acvr1b* cDNA sequences.

Histology

Tissue samples were fixed in 10% formalin (containing 4% paraformaldehyde), embedded in paraffin, and cut into 5-μm sections. Frozen tissues were embedded in optimal cutting temperature compound and stored at −80 °C. The samples that contained bone were decalcified with 4% formic acid and 4% hydrochloric acid for 4 hours after fixation. For histological analysis, sections were stained with hematoxylin and eosin. Detailed protocols on immunohistochemistry are listed in the “Materials and Methods” section in Supplementary Data online.

Detection of proliferating cells by labeling with BrdU

Mice were administered intraperitoneal injections of BrdU (100 mg BrdU per 1 kg body weight, Sigma-Aldrich, St Louis, MO) and killed 2 hours after injection. Skin samples from wild-type and mutant mice were fixed in 10% formalin overnight and embedded in paraffin. Deparaffinized 5-μm sections were rehydrated, and subjected to the detection of BrdU incorporation with biotinylated mouse anti-BrdU (Zymed Laboratories/Invitrogen, Carlsbad, CA). The detailed procedure was performed according to the recommendations of the manufacturer.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We sincerely thank Dr Richard L. Huganir and Dr Kogo Takamiya at The Johns Hopkins University for their generosity in sharing their designed vectors and cloning strategies. This project was funded by the Michael Rolfe Foundation, NCI Temin Award CA95434, NCI R01 CA109525, NCI R21 CA127701, and P30 AR044535.

Abbreviations

Acvr1b	*activin A receptor type 1b*
IRS	*inner root sheath*
ORS	*outer root sheath*

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

TGF-β *transforming growth factor-β*

REFERENCES

Attisano L, Carcamo J, Ventura F, et al. Identification of human activin and TGF beta type I receptors that form heteromeric kinase complexes with type II receptors. Cell. 1993; 75:671–80. [PubMed: 8242742]

Attisano L, Wrana JL, Montalvo E, et al. Activation of signalling by the activin receptor complex. Mol Cell Biol. 1996; 16:1066–73. [PubMed: 8622651]

Ball EM, Risbridger GP. Activins as regulators of branching morphogenesis. Dev Biol. 2001; 238:1–12. [PubMed: 11783989]

Bamberger C, Scharer A, Antsiferova M, et al. Activin controls skin morphogenesis and wound repair predominantly via stromal cells and in a concentration-dependent manner via keratinocytes. Am J Pathol. 2005; 167:733–47. [PubMed: 16127153]

Botchkarev VA, Botchkareva NV, Roth W, et al. Noggin is a mesenchymally derived stimulator of hair-follicle induction. Nat Cell Biol. 1999; 1:158–64. [PubMed: 10559902]

Burdette JE, Jeruss JS, Kurley SJ, et al. Activin A mediates growth inhibition and cell cycle arrest through Smads in human breast cancer cells. Cancer Res. 2005; 65:7968–75. [PubMed: 16140969]

Byrne C, Tainsky M, Fuchs E. Programming gene expression in developing epidermis. Development. 1994; 120:2369–83. [PubMed: 7525178]

Chen Y, Mironova E, Whitaker LL, et al. ALK4 functions as a receptor for multiple TGF beta-related ligands to regulate left-right axis determination and mesoderm induction in Xenopus. Dev Biol. 2004; 268:280–94. [PubMed: 15063168]

Cruise BA, Xu P, Hall AK. Wounds increase activin in skin and a vasoactive neuropeptide in sensory ganglia. Dev Biol. 2004; 271:1–10. [PubMed: 15196945]

D'Abronzo FH, Swearingen B, Klibanski A, et al. Mutational analysis of activin/transforming growth factor-beta type I and type II receptor kinases in human pituitary tumors. J Clin Endocrinol Metab. 1999; 84:1716–21. [PubMed: 10323406]

Danila DC, Zhang X, Zhou Y, et al. Overexpression of wild-type activin receptor alk4-1 restores activin antiproliferative effects in human pituitary tumor cells. J Clin Endocrinol Metab. 2002; 87:4741–6. [PubMed: 12364468]

Derynck R, Zhang Y, Feng XH. Smads: transcriptional activators of TGF-beta responses. Cell. 1998; 95:737–40. [PubMed: 9865691]

Ferguson CA, Tucker AS, Christensen L, et al. Activin is an essential early mesenchymal signal in tooth development that is required for patterning of the murine dentition. Genes Dev. 1998; 12:2636–49. [PubMed: 9716414]

Ferguson CA, Tucker AS, Heikinheimo K, et al. The role of effectors of the activin signalling pathway, activin receptors IIA and IIB, and Smad2, in patterning of tooth development. Development. 2001; 128:4605–13. [PubMed: 11714685]

Foitzik K, Paus R, Doetschman T, et al. The TGF-beta2 isoform is both a required and sufficient inducer of murine hair follicle morphogenesis. Dev Biol. 1999; 212:278–89. [PubMed: 10433821]

Fuchs E, Raghavan S. Getting under the skin of epidermal morphogenesis. Nat Rev Genet. 2002; 3:199–209. [PubMed: 11972157]

Gu Z, Nomura M, Simpson BB, et al. The type I activin receptor ActRIB is required for egg cylinder organization and gastrulation in the mouse. Genes Dev. 1998; 12:844–57. [PubMed: 9512518]

Han G, Li AG, Liang YY, et al. Smad7-induced beta-catenin degradation alters epidermal appendage development. Develop Cell. 2006; 11:301–12. see comment.

Heldin CH, Miyazono K, Ten Dijke P. TGF-beta signalling from cell membrane to nucleus through SMAD proteins. Nature. 1997; 390:465–71. [PubMed: 9393997]

Hempen PM, Zhang L, Bansal RK, et al. Evidence of selection for clones having genetic inactivation of the activin A type II receptor (ACVR2) gene in gastrointestinal cancers. Cancer Res. 2003; 63:994–9. [PubMed: 12615714]

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

Hubner G, Brauchle M, Gregor M, et al. Activin A: a novel player and inflammatory marker in inflammatory bowel disease? Lab Invest. 1997; 77:311–8. [PubMed: 9354766]

Ito M, Kizawa K, Hamada K, et al. Hair follicle stem cells in the lower bulge form the secondary germ, a biochemically distinct but functionally equivalent progenitor cell population, at the termination of catagen. Differentiation. 2004; 72:548–57. [PubMed: 15617565]

Jernvall J, Thesleff I. Reiterative signaling and patterning during mammalian tooth morphogenesis. Mech Dev. 2000; 92:19–29. [PubMed: 10704885]

Jonkers J, Meuwissen R, van der Gulden H, et al. Synergistic tumor suppressor activity of BRCA2 and p53 in a conditional mouse model for breast cancer. Nat Genet. 2001; 29:418–25. [PubMed: 11694875]

Kim SK, Hebrok M, Li E, et al. Activin receptor patterning of foregut organogenesis. Genes Dev. 2000; 14:1866–71. [PubMed: 10921901]

Kobielak K, Pasolli HA, Alonso L, et al. Defining BMP functions in the hair follicle by conditional ablation of BMP receptor IA. J Cell Biol. 2003; 163:609–23. [PubMed: 14610062]

Ling N, Ying SY, Ueno N, et al. Pituitary FSH is released by a heterodimer of the beta-subunits from the two forms of inhibin. Nature. 1986; 321:779–82. [PubMed: 3086749]

Lynch MH, O'Guin WM, Hardy C, et al. Acidic and basic hair/nail ("hard") keratins: their colocalization in upper cortical and cuticle cells of the human hair follicle and their relationship to "soft" keratins. J Cell Biol. 1986; 103:2593–606. [PubMed: 2432071]

Massague J. TGFbeta signaling: receptors, transducers, and Mad proteins. Cell. 1996; 85:947–50. [PubMed: 8674122]

Massague J. TGF-beta signal transduction. Annu Rev Biochem. 1998; 67:753–91. [PubMed: 9759503]

Mathews LS, Vale WW, Kintner CR. Cloning of a second type of activin receptor and functional characterization in Xenopus embryos. Science. 1992; 255:1702–5. [PubMed: 1313188]

Matzuk MM, Finegold MJ, Su JG, et al. Alpha-inhibin is a tumour-suppressor gene with gonadal specificity in mice. Nature. 1992; 360:313–9. [PubMed: 1448148]

Matzuk MM, Kumar TR, Bradley A. Different phenotypes for mice deficient in either activins or activin receptor type II. Nature. 1995a; 374:356–60. [PubMed: 7885474]

Matzuk MM, Kumar TR, Vassalli A, et al. Functional analysis of activins during mammalian development. Nature. 1995b; 374:354–6. [PubMed: 7885473]

Matzuk MM, Lu N, Vogel H, et al. Multiple defects and perinatal death in mice deficient in follistatin. Nature. 1995c; 374:360–3. [PubMed: 7885475]

McDowall M, Edwards NM, Jahoda CA, et al. The role of activins and follistatins in skin and hair follicle development and function. Cytokine Growth Factor Rev. 2008; 19:415–26. [PubMed: 18922734]

Merino R, Macias D, Ganan Y, et al. Control of digit formation by activin signalling. Development. 1999; 126:2161–70. [PubMed: 10207141]

Millar SE. Molecular mechanisms regulating hair follicle development. J Invest Dermatol. 2002; 118:216–25. [PubMed: 11841536]

Munz B, Smola H, Engelhardt F, et al. Overexpression of activin A in the skin of transgenic mice reveals new activities of activin in epidermal morphogenesis, dermal fibrosis and wound repair. EMBO J. 1999; 18:5205–15. [PubMed: 10508154]

Nakamura M, Matzuk MM, Gerstmayer B, et al. Control of pelage hair follicle development and cycling by complex interactions between follistatin and activin. FASEB J. 2003; 17:497–9. [PubMed: 12514121]

O'Guin WM, Sun TT, Manabe M. Interaction of trichohyalin with intermediate filaments: three immunologically defined stages of trichohyalin maturation. J Invest Dermatol. 1992; 98:24–32. [PubMed: 1728637]

Ohyama M, Terunuma A, Tock CL, et al. Characterization and isolation of stem cell-enriched human hair follicle bulge cells. J Clin Invest. 2006; 116:249–60. [PubMed: 16395407]

Owens P, Han G, Li AG, et al. The role of Smads in skin development. J Invest Dermatol. 2008; 128:783–90. [PubMed: 18337711]

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript

Panopoulou E, Murphy C, Rasmussen H, et al. Activin A suppresses neuroblastoma xenograft tumor growth via antimitotic and antiangiogenic mechanisms. Cancer Res. 2005; 65:1877–86. [PubMed: 15753386]

Piek E, Heldin CH, Ten Dijke P. Specificity, diversity, and regulation in TGF-beta superfamily signaling. FASEB J. 1999; 13:2105–24. [PubMed: 10593858]

Qiao W, Li AG, Owens P, et al. Hair follicle defects and squamous cell carcinoma formation in Smad4 conditional knockout mouse skin. Oncogene. 2006; 25:207–17. [PubMed: 16170355]

Risbridger GP, Schmitt JF, Robertson DM. Activins and inhibins in endocrine and other tumors. Endocr Rev. 2001; 22:836–58. [PubMed: 11739336]

Roberts VJ, Barth SL. Expression of messenger ribonucleic acids encoding the inhibin/activin system during mid- and late-gestation rat embryogenesis. Endocrinology. 1994; 134:914–23. [PubMed: 8299586]

Rossi MR, Ionov Y, Bakin AV, et al. Truncating mutations in the ACVR2 gene attenuates activin signaling in prostate cancer cells. Cancer Genet Cytogenet. 2005; 163:123–9. [PubMed: 16337854]

Smith JC, Price BM, Van Nimmen K, et al. Identification of a potent Xenopus mesoderm-inducing factor as a homologue of activin A. Nature. 1990; 345:729–31. [PubMed: 2113615]

Song J, Oh SP, Schrewe H, et al. The type II activin receptors are essential for egg cylinder growth, gastrulation, and rostral head development in mice. Dev Biol. 1999; 213:157–69. [PubMed: 10452853]

Su GH, Bansal R, Murphy KM, et al. ACVR1B (ALK4, activin receptor type 1B) gene mutations in pancreatic carcinoma. Proc Natl Acad Sci USA. 2001; 98:3254–7. [PubMed: 11248065]

Thesleff I, Keranen S, Jernvall J. Enamel knots as signaling centers linking tooth morphogenesis and odontoblast differentiation. Adv Dent Res. 2001; 15:14–8. [PubMed: 12640732]

Thesleff I, Wang XP, Suomalainen M. Regulation of epithelial stem cells in tooth regeneration. C R Biol. 2007; 330:561–4. [PubMed: 17631454]

Thompson TB, Cook RW, Chapman SC, et al. Beta A versus beta B: is it merely a matter of expression? Mol Cell Endocrinol. 2004; 225:9–17. [PubMed: 15451562]

Thomsen G, Woolf T, Whitman M, et al. Activins are expressed early in Xenopus embryogenesis and can induce axial mesoderm and anterior structures. Cell. 1990; 63:485–93. [PubMed: 2225062]

Vassalli A, Matzuk MM, Gardner HA, et al. Activin/inhibin beta B subunit gene disruption leads to defects in eyelid development and female reproduction. Genes Dev. 1994; 8:414–27. [PubMed: 8125256]

Vassar R, Fuchs E. Transgenic mice provide new insights into the role of TGF-alpha during epidermal development and differentiation. Genes Dev. 1991; 5:714–27. [PubMed: 1709129]

Verschueren K, Dewulf N, Goumans MJ, et al. Expression of type I and type IB receptors for activin in midgestation mouse embryos suggests distinct functions in organogenesis. Mech Dev. 1995; 52:109–23. [PubMed: 7577669]

Wang X, Zinkel S, Polonsky K, et al. Transgenic studies with a keratin promoter-driven growth hormone transgene: prospects for gene therapy. Proc Natl Acad Sci USA. 1997; 94:219–26. [PubMed: 8990189]

Wang XP, Suomalainen M, Felszeghy S, et al. An integrated gene regulatory network controls stem cell proliferation in teeth. PLoS Biol. 2007; 5:e159. [PubMed: 17564495]

Wang Z, Wong P, Langbein L, et al. Type II epithelial keratin 6hf (K6hf) is expressed in the companion layer, matrix, and medulla in anagen-stage hair follicles. J Invest Dermatol. 2003; 121:1276–82. [PubMed: 14675170]

Wang XP, Suomalainen M, Jorgez CJ, et al. Follistatin regulates enamel patterning in mouse incisors by asymmetrically inhibiting BMP signaling and ameloblast differentiation. Dev Cell. 2004; 7:719–30. [PubMed: 15525533]

Wilson C, Cotsarelis G, Wei ZG, et al. Cells within the bulge region of mouse hair follicle transiently proliferate during early anagen: heterogeneity and functional differences of various hair cycles. Differentiation. 1994; 55:127–36. [PubMed: 8143930]

Woodruff TK. Regulation of cellular and system function by activin. Biochem Pharmacol. 1998; 55:953–63. [PubMed: 9605419]

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

Yeo C, Whitman M. Nodal signals to Smads through Cripto-dependent and Cripto-independent mechanisms. Mol Cell. 2001; 7:949–57. [PubMed: 11389842]

Zhou P, Byrne C, Jacobs J, et al. Lymphoid enhancer factor 1 directs hair follicle patterning and epithelial cell fate. Genes Dev. 1995; 9:700–13. [PubMed: 7537238]

Zimmers TA, Davies MV, Koniaris LG, et al. Induction of cachexia in mice by systemically administered myostatin. Science. 2002; 296:1486–8. [PubMed: 12029139]

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

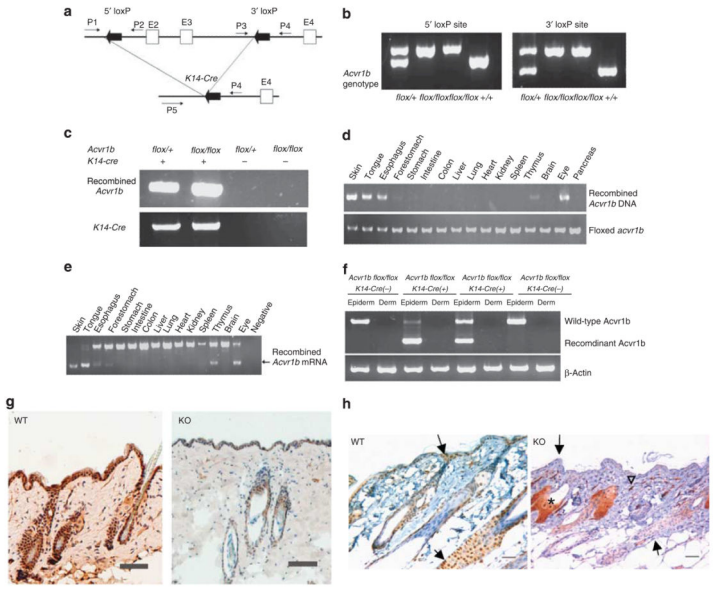

Figure 1. Genotyping and tissue-specific recombination in *Acvr1b* $^{flox/flox}$; K14-Cre mice

(**a**) PCR strategies and primers (P) for *Acrv1b* genotyping and Cre-mediated recombination. (**b**) *Acvr1b* genotyping using P1/P2 or P3/P4. The upper bands include a loxP site and are therefore bigger than the wild-type allele (lower bands). (**c**) The recombined *Acvr1b* fragment was detected only in mice with the *K14-Cre* gene (using P4/P5). Tissue-specific recombination of *Acvr1b* was detected at (**d**) the genomic DNA level and (**e**) confirmed by RT-PCR. (**f**) *Acvr1b* recombination was observed only in the epidermis (not the dermis) by RT-PCR. (**g**) Acvr1b protein expression was dramatically decreased in the skin epidermis and hair follicle epithelia of an adult *Acvr1b* $^{flox/flox}$; *K14-Cre* knockout (KO) mouse compared with that of an adult wild-type (WT) mouse by anti-Acvr1b immunohistochemistry, which is supported by the downregulation of p-Smad2 expression in epithelial cells of the mutant skin (P17) (arrows). P-Smad2 expression is maintained in fibroblast cells (triangle) in the mutant skin, whereas non-specific expression is also observed in the sebaceous glands (*) (**h**). *Acvr1b*, activin A receptor type 1b; RT-PCR, reverse transcriptase-PCR. All scale bars = 50 μm.

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

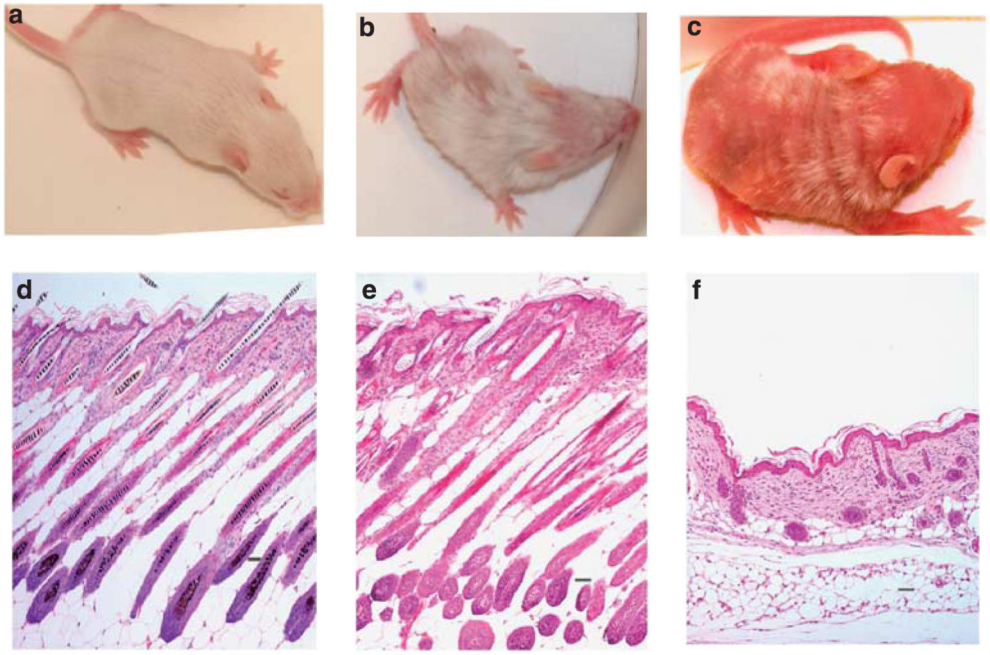

Figure 2. Distinct severity of hair loss displayed among *Acvr1b*$^{flox/flox}$; *K14-Cre* conditional knockout mice

Three 10-day-old mice of (**a**) the wild-type genotype or the (**b, c**) *Acvr1b*$^{flox/flox}$; *K14-Cre* genotype. *Acvr1b*$^{flox/flox}$; *K14-Cre* mutant mice displayed a gradient of abnormal hair morphogenesis. Here, we show representatives of mutant mice with relative unaffected hair development (panel **b**) or grossly hairless phenotype (panel **c**). The corresponding histological H&E sections for (**d**) wild-type and (**e, f**) two mutant mice are also shown here. Histology analyses are consistent with the gross hair phenotypes. *Acvr1b*, activin A receptor type 1b; H&E, hematoxylin and eosin. All scale bars = 50 μm.

NIH-PA Author Manuscript
NIH-PA Author Manuscript
NIH-PA Author Manuscript

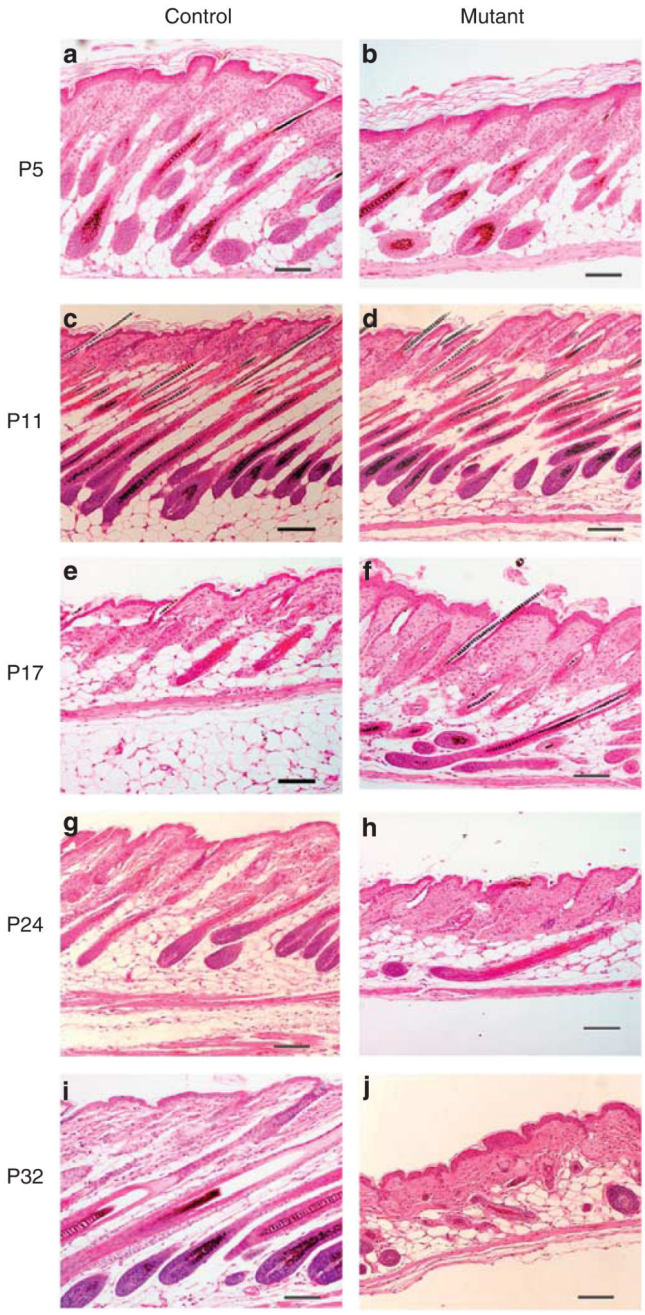

Figure 3. Histological analyses of postnatal hair follicle development in *Acvr1b* $^{flox/flox}$; *K14-Cre* mice

Hematoxylin–eosin staining analysis of tissue sections from the control (**a, c, e, g, i**) and mutant (**b, d, f, h, j**) dorsal skin at P5 (panels **a** and **b**), P11 (panels **c** and **d**), P17 (panels **e** and **f**), P24 (panels **g** and **h**), and P32 (panels **i** and **j**). Hair follicle development was similar between control and mutant mice at P5 and P11. The skin of control mice at P17 (panel **e**) was at the catagen phase, whereas follicles of the matched mutant mice (panel **f**) were at a mixed anagen and catagen phase. The skin of control mice at P24 (panel **g**) was in transition

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

from the telogen to the anagen phases, whereas the mutant follicles were mostly at the catagen phase (panel **h**). At P32, the follicles of control mice were entering the full anagen phase (panel **i**), whereas mutant mice remained at the catagen and telogen phases (panel **j**). *Acvr1b*, activin A receptor type 1b. All scale bars = 50 μm.

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

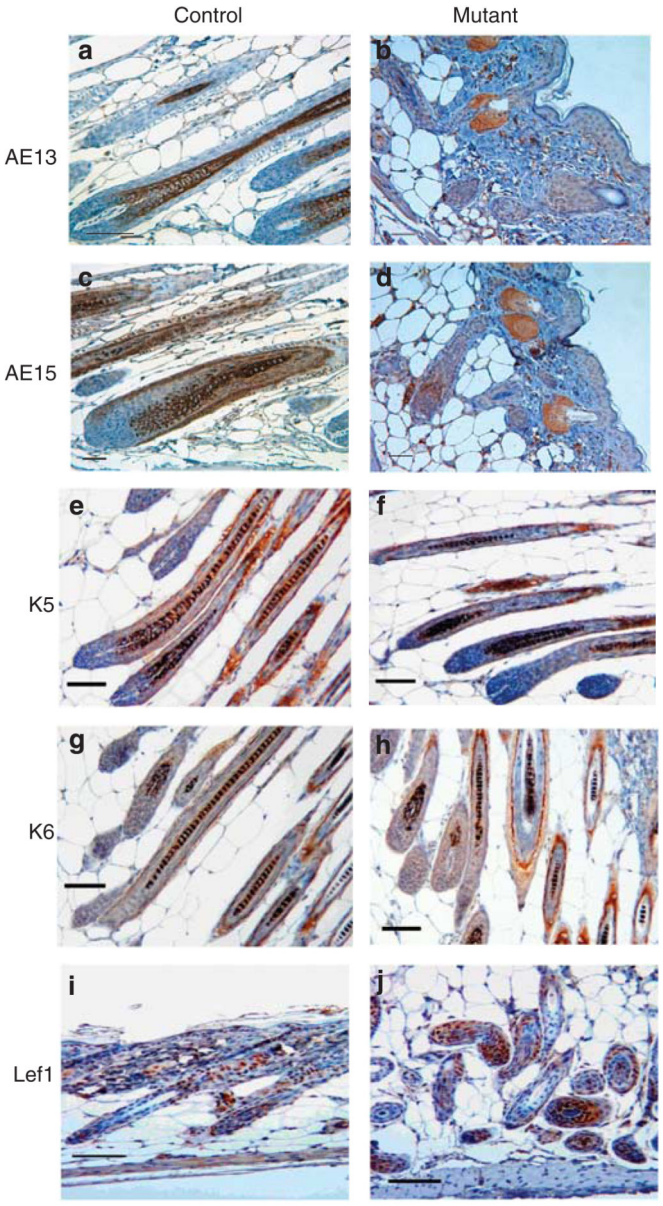

Figure 4. Defects in hair shaft and IRS differentiation in conditional *Acvr1b* $^{flox/flox}$; K14-Cre mutant mice

The antibody AE13 stains the precortex and cortex of the hair keratin in (**a**) wild-type mice, whereas (**c**) AE15 stains the IRS and medulla of hair follicles. (**b**) AE13 and (**d**) AE15 staining were significantly reduced in *Acvr1b* $^{flox/flox}$; K14-Cre mutant mice at anagen (P32). Sebaceous gland cells were non-specifically stained. (**e–h**) K5 and K6 markers appeared to be maintained in the follicles of mutant mice (panels f and h) compared with wild-type littermates at anagen (P11) (panels **e** and **g**). Higher levels of (**i, j**) Lef1 expression were observed in the matrix cells of the hair follicles of mutant mice at catagen (p17). *Acvr1b*, activin A receptor type 1b; IRS, inner root sheath. All scale bars = 50 μm.

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

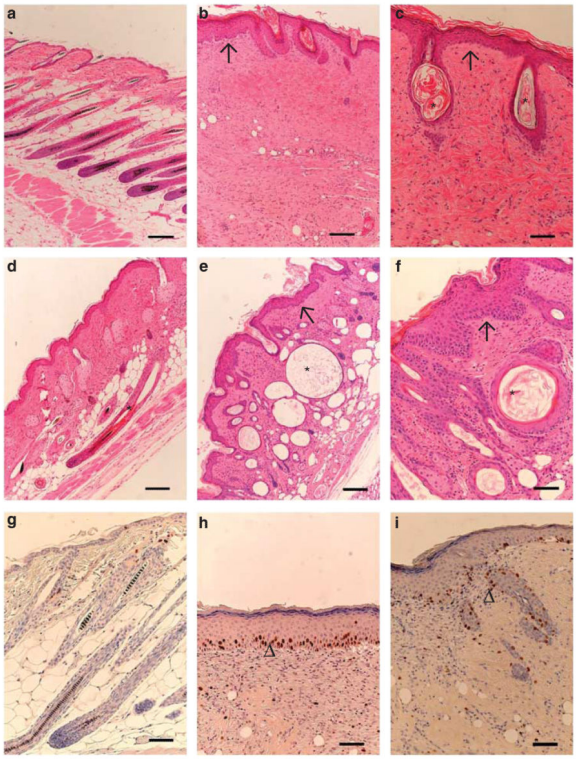

Figure 5. Histological analyses of the dorsal skin in adult wild-type and conditional *Acvr1b* $^{flox/flox}$; *K14-Cre* mice

(**a**) The dorsal skin of 5-month-old wild-type mice was stained with hematoxylin–eosin or (**g**) with anti-BrdU after incorporation (**g**). The dorsal skin of 5-month-old mutant mice was stained with (**b–f**) hematoxylin–eosin or (**h, i**) with anti-BrdU after incorporation. Increased hyperplasia (arrows) and cell proliferation (arrowheads) were observed in the epidermis as hair loss and disintegration of the hair follicles (asterisk) became more apparent in aging mutant mice. Acvr1b, activin A receptor type 1b. All scale bars = 50 μm.