Biochemical and Biophysical Research Communications 403 (2010) 18–23

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Gsdma3 is required for hair follicle differentiation in mice

Jin Li [a], Yue Zhou [b], Tian Yang [c], Ning Wang [c,1], Xiaohua Lian [c,*], Li Yang [a,*]

[a] *111 Project Laboratory of Biomechanics and Tissue Repair, Bioengineering College, Chongqing University, Chongqing 400044, People's Republic of China*
[b] *Model Animal Research Center, Nanjing University, 12 Xuefu Road, Pukou District, Nanjing 210061, People's Republic of China*
[c] *Department of Cell Biology, College of Basic Medicine, The Third Military Medical University, Chongqing 400038, People's Republic of China*

ARTICLE INFO

Article history:
Received 14 October 2010
Available online 25 October 2010

Keywords:
Gsdma3
Cyclic alopecia
Hair differentiation
Msx2
Hair keratin

ABSTRACT

Hair follicle differentiation is regulated by multiple signaling pathways. However, the known cellular and molecular mechanisms are limited. *Gsdma3* is a novel murine gene and considered to be a mutation hotspot. Six mutants have been reported in Gsdma3 and all these mutants exhibit hair loss and hyperkeratosis phenotypes. In order to verify how the lack of *Gsdma3* affects the hair defects, we use alopecia and excoriation mice, a new mouse mutation in this gene, as our research model. This mutation exhibits progressive hair loss, from head to the whole back, and followed by hair regrowth. We test that Gsdma3 is expressed in matrix, inner root sheath, and hair shaft. Ultrastructural and histological analyses show abnormal hair structures and reduced hair keratins in AE mice. The loss of interlocking structures and abnormal constitutive protein indicate defects in anchoring hair shaft in the hair follicle and resisting external forces. Molecular analysis of *Gsdma3* deficiency and overexpression shows an Msx2/Foxn1/acidic hair keratin genetic pathway is involved. Thus, Gsdma3 is necessary for normal hair follicle differentiation.

1. Introduction

Hair follicle is a complex mini-organ that undergoes cyclic process. During embryonic development, hair follicle is initiated as a result of interactions between the primitive epithelium and the underlying mesenchymal. As the epithelium starting a downward growth into the dermis, the key signal centre dermal papilla (DP) cells are enclosed by matrix keratinocytes, which proliferate and differentiate into the multilayered inner root sheath (IRS), composed of Henle's, Huxley's and cuticle layers, and then the hair. The terminal hair shaft is made up of three layers: cuticle, cortex and medulla. The outermost layer hair shaft cuticle (Cuh) interlocks with IRS cuticle to form an interlocking structure [1], which is necessary for protecting the hair shaft from shedding [2–4].

The hair follicle differentiation program is controlled by a complex network of several pathways, which involves Bmp, homeobox genes, and Wnt. Their specific expression patterns in DP or matrix cells determinate their functions in differentiation. Bmp signaling is key regulator in hair follicle differentiation [5,6]. Inhibition of Bmp signaling by ectopic expression of its inhibitor, *Noggin*, under the control of *Msx2* promoter impairs hair shaft differentiation, accompanied by down-regulation of *Foxn1* and *Hoxc13* [7]. Conditional ablation of BMP receptor 1A induces hair shaft and IRS defects [8,9].

Homeobox genes containing a conserved 180-bp segment (the "homeobox") that encodes a protein domain involved in binding to DNA. They function as transcription factors to play a role in controlling the development of multicellular organisms [10,11]. *Msx2* knockout mice show defects in cyclic alopecia and hair shaft differentiation, which resulting from the altered Foxn1 regulatory pathway [12]. *Msx2* and *Foxn1* double mutants cause more severe hair defects [13]. The *Hoxc13*-deficient mice exhibit severe hair and skin defects that result in alopecia [14]. The hairlessness phenotype is also observed in *Hoxc13* overexpression mice, for its differentiation defects [15,16].

The differentiation program is also dependent on Wnt signaling. Ectopically expressing *Wnt 3* or *DVL2* cause short hair phenotype and cyclical balding, due to the hair shaft structural defects [17]. Lef-1 is the key transcription factor in this pathway. While its mRNA is detected in matrix cells, Lef-1 accumulates in the hair shaft precursor cells, where the activated Wnt signaling can be detected by the Wnt dependent reporter gene TOPGAL [18].

Gsdma3 is a member of a novel murine gene family gasdermin. In previous studies, it was reported as a mutation hotspot and that *Gsdma3* mutations showed alopecia and hyperkeratosis phenotypes in *Bsk*, *Dfl*, *Rco2*, *Fgn*, *Re* (*den*), and *Rim3* mice [19–23]. All these studies indicated an important role *Gsdma3* played in the

* Corresponding authors.
E-mail addresses: 129hx@163.com (X. Lian), yangli@cqu.edu.cn (L. Yang).
[1] Present address: Department of Oncology, Southwest Hospital, Third Military Medical University, Chongqing 400038, People's Republic of China.

0006-291X/$ - see front matter © 2010 Published by Elsevier Inc.
doi:10.1016/j.bbrc.2010.10.094

maintenance of hair follicles. However, little is known about what leads to hair loss in *Gsdma3* mutants, and what potential regulatory mechanisms are involved. Earlier studies have shown the *Gsdma3* mRNA and protein expression by in situ hybridization and immunohistochemistry, respectively, but the results remains inconclusive [19,21,23].

In this study, we have defined hair shaft differentiation defects in *Gsdma3* mutants, account for its hairless phenotype. We further investigated that expression of *Msx2* and its downstreams, *Foxn1*, *Hoxc13*, and *notch1*, is dependent of *Gsdm 3*. These results suggest that *Gsdma3* is necessary in hair shaft differentiation, and involved in *Msx2* regulatory pathway.

2. Materials and methods

2.1. AE mice

AE (alopecia & excoriation) mice on a C57BL/6 background was a gift from Model Animal Research Center (MARC) of Nanjing University. The mice were generated by substitution of nucleotide 1112 (T–C), changes tyrosine 344 to histidine (TAC–CAC). AE mice were heterozygous and dominant mutation, the chimeras were backcrossed with C57BL/6 to generate the mice.

2.2. Scanning electron microscopy (SEM)

Hairs from the dorsal skin of mice were attached to electric conduction paste and were analyzed by using a SEM (Amray, USA) at 20 kV.

2.3. Transmission electron microscopy (TEM)

Back skins of P14 WT and AE mice were trimmed into 1 mm × 2 mm pieces and fixed in 2.5% glutaraldehyde in 0.1 M phosphate buffer (PH7.4), followed by postfixation in 1% osmium tetroxide and dehydration with a series of increasing concentrations of acetone. Samples were embedded in Epon 618. Ultrathin sections were stained with lead citrate solution and observed under a TECNAI-10 (Philips, Netherlands).

2.4. Immunofluorescence and immunohistochemistry

Skin tissue was obtained from the midback region of age-matched AE and WT mouse littermates. For immunohistochemistry, skins were fixed in 4% paraformaldehyde (PFA) in PBS, embedded in paraffin and sectioned at 5 μm thickness. Paraffin sections were rehydrated in decreasing concentrations of ethanol and subjected to antigen unmasking in boiling citric acid buffer. Two-step IHC detection reagent was used after primary antibody Gsdma3. For immunofluorescence, tissues were rehydrated in 30% sucrose solution overnight after 4% PFA, till sinking. Tissues embedded in OCT and sections were immunostained by using the primary antibodies, followed by secondary antibodies and DAPI counterstaining, and observed by confocal microscopy (Zeiss, Germany). The following primary antibodies were used: Gsdma3 (rabbit polyclonal, 1:100; GL Biochem, China); Msx2 (goat polyclonal, 1:100; Santa, USA); AE15 (1:2; gift); AE13 (1:10; gift); Lef1 (goat polyclonal, 1:100; Santa, USA).

2.5. Total RNA extraction and semi-quantitative RT-PCR

Back skins were dissected from P9, P12, P15 WT and AE mice and flash-frozen with liquid nitrogen. Homogenize tissue samples using Trizol (Invitrogen, USA) reagent, and total RNA was isolated according to manufacturer's protocol. About 60 ng of total RNA was reverse transcribed to cDNA using RT kit (Toyobo, Japan) according to manufacturer's protocol. Amplify the cDNA with PCR mix (Tiangen, China), and examine the PCR product by running 1% agarose gels.

2.6. Nake plasmid injection

Recombinant mouse Gsdma3 expression plasmid (pEGFP-N1-Gsdma3) and empty expression plasmid vector pEGFP-N1 were purchased from Open Biosystems Company. The plasmid was extracted and concentrated by plasmid max kit (Omega, USA). Dilute 60 μg of plasmid DNA in 80 μl of saline and inject subcutaneously. The control mice were injected with the same amount of empty plasmid vector [24]. The mice hairs were synchronized by depilation in their second telogen [4], because the cells were more susceptive after depilation. The skin samples were harvested two days after injection for RT-PCR analysis.

3. Results

3.1. AE mice display cyclic alopecia

The newborn heterozygous AE mice could not be distinguished from wild-type littermates until postnatal day 9 (P9), by their thickened and folded skin (Fig. 1A). Then the external hairs exhibited a short and curly phenotype in AE mice. At P25, the mice began to lose their hairs from the head region, and progressing toward the whole back. At P35, they lost nearly all of their body hair. However, when a new hair cycle was initiated, the hairs regrew. This process was followed by progressive hair loss and regrowth (Fig. 1B). No obvious gender effects were observed.

3.2. Gsdma3. is required for IRS and hair shaft differentiation

The cyclic alopecia can be the result of defects in hair shaft and IRS structures. Such defects were defined in *Sox21*-null and *Msx2* KO mice [12,25]. To investigate the cause of phenotype observed in AE mice, we first examined the hair shaft by SEM. The analyses showed that the diameter of hair shaft in AE mice became thinner. What is more, the smooth surface of WT hairs were covered by the scaly tile pattern of the outer cuticle (Fig. 2A and B), while the hairs of AE mice was covered by flattened cuticles with wrinkled surfaces (Fig. 2C and D). To verify this result, we further tested hair shaft by TEM. The result showed that in WT mice hairs, the Cuh was made from keratinized cuticle cells that overlap each other in a roof tile effect (Fig. 2E and F). Such structure was likely to interlock with Cui to anchor the hair shaft in the hair follicle. However, the interlocking structure could not be seen in AE cuticles (Fig. 2G and H), suggesting an anchorage failure in *Gsdma3* mutants. Further, we identified that Krt32 and Krt82, expressed in Cuh [26], were downregulated in AE mice by RT-PCR (Fig. 2I). The results were consistent with the Cuh defects observed in TEM.

Next, we tested hair shaft and IRS differentiation markers by immunofluorescence. AE15 is a monoclonal antibody that reacts with intermediate filament-associated protein in trichohyalin granules [27]. The AE15-positive cells were restricted to three concentric layers of IRS and medulla of hair shaft in both WT and AE mice, but the arrangement was disorganized in *Gsdma3* mutants (Fig. 2J and K). AE13, characterizing acidic keratins in the upper cortex and hair cuticle cells [28], was visibly decreased in AE mice (Fig. 2L and M), suggesting a keratinization failure. Altogether, functional defect of *Gsdma3* disrupts IRS and hair shaft differentiation.

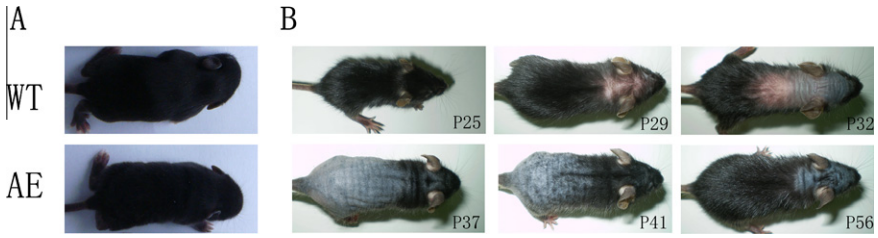

Fig. 1. Cyclic alopecia in Gsdma3 mutant mice. (A) Comparation of skin phenotypes in AE and WT mice at P9. Gsdma3 mutants exhibit a thickened and folded neck region. (B) A cyclic balding pattern in AE mice. It took us more than 8 weeks to follow up the balding pattern. Given images showed major events in the hair loss and regrowth process.

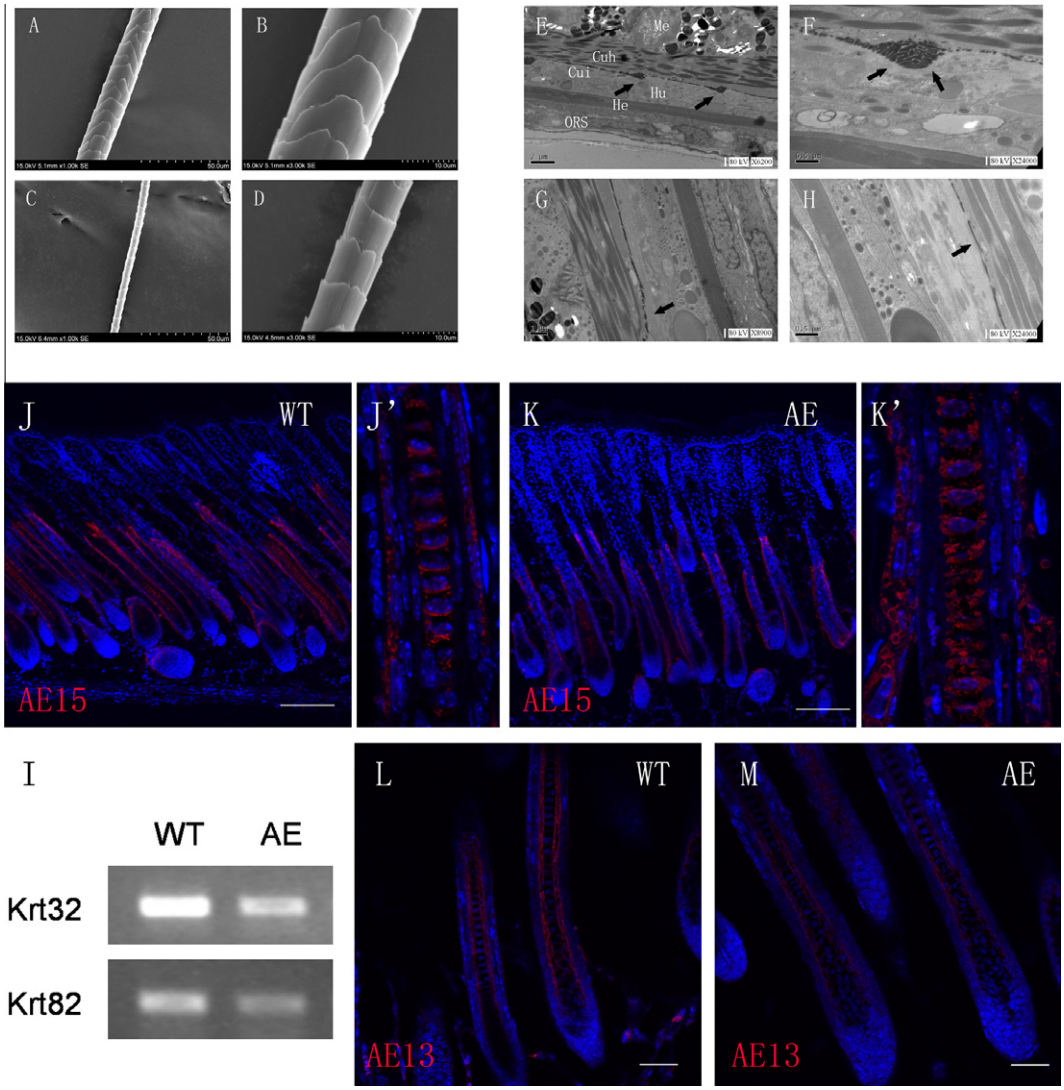

Fig. 2. Differentiation defects in Gsdma3 mutants. (A–D) Comparation of plucked dorsal hairs in WT (A and B) and AE (C and D) mice by SEM analyses. Hairs in Gsdma3 mutants were shown thinner diameter. Unlike normal scaly hairs, the outer cuticle in AE mice became flattened. (E–H) TEM images showing fine structure of hair follicles of P15 WT (E and F) and AE (G and H) mice. The cuticle cells (arrows) no longer overlapped each other like roof tiles as normal in Gsmda 3-deficient mice. (I) Semi-quantitative RT-PCR analyses of Cuh markers at P9 skins from WT and AE mice. Both Krt32 and Krt82 were downregulated in the mutants. (J–M) Immunohistological detection of AE 15 (J and K) and AE13 (L and M) in WT (J and L) and AE (K and M) mice. J′ and K′ are enlarged images for J and K. The arrangement of AE 15 was disorganized and AE13 expression wad reduced in AE mice. ORS: outer root sheath; He: Henle's layer; Hu: Huxley's layer; Me: medulla. Scale bars: 200 μm in J and K, 100 μm in L and M.

3.3. The expression of Gsdma3 is responsible for the hair follicle differentiation defects

To investigate whether the Gsdma3 expression pattern is associated with the hair differentiation defects in AE mice, we examined the protein expression in early and mid-anagen in WT mice skin. At P3, Gsdma3 was expressed in matrix and pre-cortical cells (Fig. 3A). At P9, Gsdma3 expression expanded into IRS and hair shaft (Fig. 3B), where was likely to be responsible for the differentiation defects in AE mice. Next, we identified whether the Gsdma3 expressing cells overlapped the AE15 and AE13-positive cells. Gsdma3 was found to be co-localized with AE15 in the three concentric layers of the IRS, but not in the medulla (Fig. 3C). Cells positive for Gsdma3 were also partly labeled by AE13 in outer cuticle

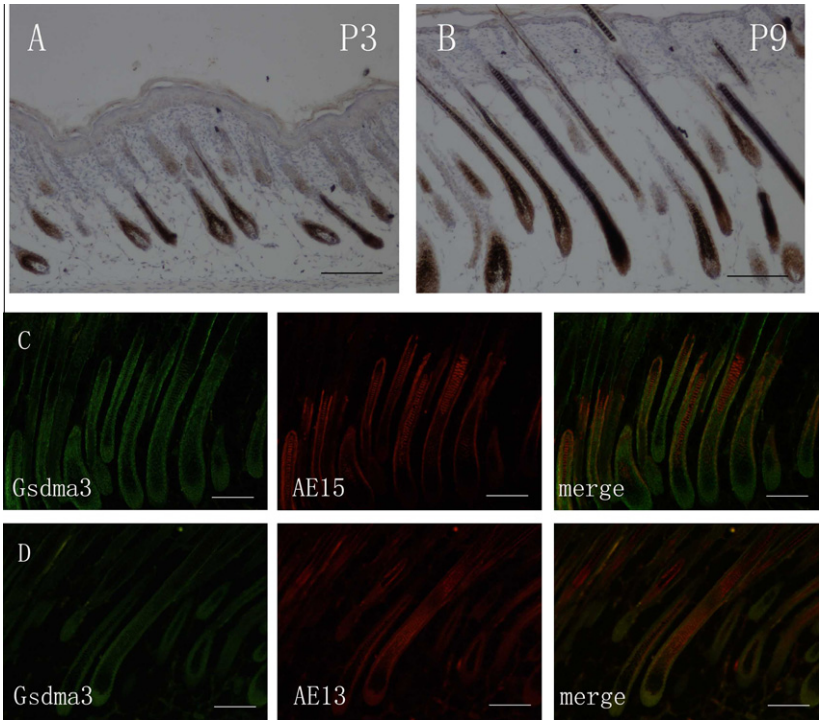

Fig. 3. Expression of Gsdma3. (A and B) Gsdma3 expression was detected in matrix and pre-cortical cells in early anagen at P3 (A) by immunochemistry, and then expanded into IRS and hair shaft region at P9 (B). (C and D) Confocal images of Gsdma3 expression. (C) Gsdma3 is co-expressed with AE15 in the IRS at P9. (D) Gsdma3 is expressed in the outer cuticle of hair shaft labeled with the AE13 antibody. Scale bar: 100 μm.

of hair shaft, but not the inner cortex. So it was confirmed that Gsdma3 was expressed in cuticle and cortex layers of hair shaft (Fig. 3D). We conclude that the exact location of Gsdma3 is restricted to the matrix, IRS, and Cuh. These cell types are associated with hair follicle differentiation.

3.4. Gsdma3 is involved in Msx2 regulatory pathway

To gain further insight into the molecular mechanisms that may be affected by *Gsdma3* deficiency, we tested master regulators of signaling pathways that involved in hair follicle differentiation. We first tested Lef1 expression by IF, and no obvious difference was observed in WT and AE mice. We also examined *Msx2* regulatory pathway, the deficiency of which cause similar cyclic alopecia [12]. Interestingly, the master regulators displayed a visible down-regulation in AE mice at P9 (Fig. 4A). We speculated that *Gsdma3* might be a member of *Msx2* pathway. Similarly with *Gsdma3*, *Msx2* is known to be expressed in matrix and IRS. So we detected whether Msx2 expression overlapped with Gsdma3. The result showed that Msx2 was co-expression with *Gsdma3* in matrix and IRS (Fig. 4B). When overexpressing *Gsdma3* by nake plasmid DNA injection into the skin, all of these genes expression were up-regulated (Fig. 4C). These results demonstrated that *Gsdma3* might regulate *Msx2* pathway directly or indirectly.

4. Discussion

In our study, we have analyzed hair follicle differentiation defects in *Gsdma3* mutants. These defects are reflected in its flattened Cuh, reduced keratins in cortex, and abnormal arrangement of trichohyalin in IRS in AE mice. Through the detection of molecular defects in AE mice, we suppose that *Gsdma3* may be involved in the regulation of Msx2/Foxn1/acidic hair keratin genetic pathway.

The hair loss phenotypes in AE mice are similar to other six *Gsdma3* mutants. The role of *Gsdma3* in hair follicle differentiation has been mentioned in recent studies, but the speculations are only based on the expression of Gsdma3 [21,23]. Our study provides direct evidence of Gsdma3 affect hair shaft and IRS differentiation.

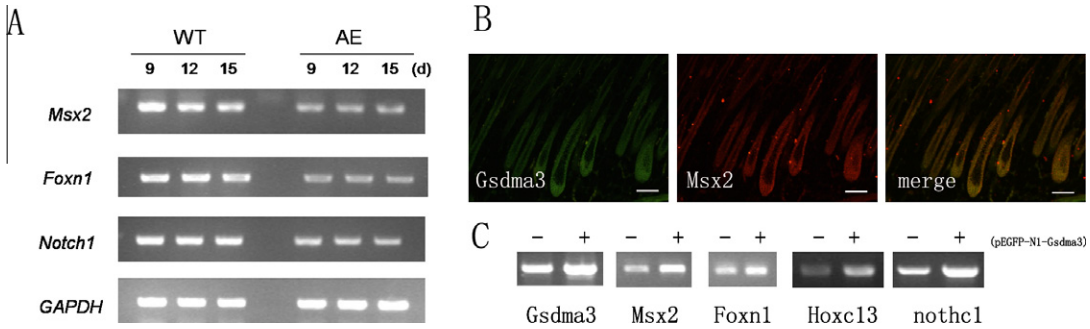

Fig. 4. Gsdma3 is involved in Msx2 regulatory pathway. (A) Master regulators of Msx2 pathway were down-regulated in AE mice. (B) Gsdma3 was co-localized with Msx2 in matrix and IRS. (C) Msx2/Foxn1/acidic hair keratin genetic pathway was activated by overexpressing Gsdma3. Scale bar: 100 μm.

Outer cuticles of healthy hair are made from keratinized cuticle cells, which overlap each other like roof tiles. The outer cuticle is the strongest part of the hair fiber and is responsible for protecting the inner cortex. Once its structure damaged, the hair fiber cannot resist much of the mechanical strength. AE15-positive trichohyalin functions as cross-bridging protein, which cross-links to several barrier proteins to make IRS mechanically strong [29]. AE13 detects type-keratins in hair cortex and cuticle cells [28]. Interestingly, in this study, decreased AE13 is observed in AE mice, consistent with the observed defects by SEM and TEM in cuticle cell differentiation. These results suggest that the cyclic alopecia in *Gsdma3*-deficient mouse is due to weakness in resisting external forces and anchoring hairs in the hair follicle.

The differentiation of hair follicle is the product of a series of reciprocal and sequential interactions between DP and matrix cells. Once the hair follicle induction process is completed, epidermal placode invigilates into dermis. During differentiation, transient amplifying (TA) cells in hair bulb proliferate rapidly, and produce transient progenitor cells. These cells are arranged in concentric layers, which give rise to different layers of hair shaft and IRS [30,31]. Our result shows that Gsdma3 protein is expressed in matrix, IRS and hair shaft, suggesting a role in cell type decision and subsequent differentiation.

Several signaling molecules are reported to be involved in this process. Inhibition of Bmp signaling by ectopic expression of *noggin* under *Msx2* promoter leads to an absence of trichohyalin in medulla and acidic hair keratins in the cortex and cuticle. In addition, several transcription factors, like *Msx2*, *Foxn1*, and *Hoxc13*, are downregulated significantly [7]. This indicates that they lie in the downstream of Bmp genetic pathway. Dynamic expression of *Msx2* first is in matrix and pre-cortical cells, and then expanded into the upper region of hair follicle, including IRS and hair shaft [12,32,33]. Strong nuclear Gsdma3 is detected to merge with cells expressing Msx2 suggesting they may play overlapping roles. This may be a possible explanation for why the AE mice have the similar cyclic alopecia phenotype to $Msx2^{-/-}$ [12,34]. Foxn1 is known to be expressed in the hair cortex [35,36]. As its expression is much reduced in Msx2 knockout mice, Foxn1 had been considered to be downstream of *Msx2* [12]. Recently, it has been shown that *Msx2* and *Foxn1* function downstream of BMP in parallel pathways [13]. A loss of function mutation in *Foxn1* cause hairless in nude mice resulting from the downregulation of hair keratins, which are essential for normal hair structure [37–39]. Interestingly, *Foxn1* is downregulated in AE mice, consistent with the observed reduced acidic hair keratins in cortex cells. *Notch1* expression in matrix can be regulated by Foxn1 directly [13]. Its overexpression in cortex can cause differentiation defects in neighboring medulla and cuticle cells by a non-autonomous effect [40,41]. The observed reduced *Nothc1* in AE mice may be downregulated by decreased *Msx2* and *Foxn1*. *Hoxc13* is also required in hair shaft differentiation and is involved in hair keratin expression directly [42]. However, the expression of *Hoxc13* is not altered in AE mice. *Sox21* is another gene responsible for normal Cuh differentiation [25], but its expression is unaltered either. In our study, when overexpressing *Gsdma3* by plasmid injection, all of *Msx2*, *Foxn1* and their target genes, including *Hoxc13*, are upregulated. The present evidence provides clues that *Gsdma3* functions upstream or in parallel with the *Msx2* cascade in the hair follicle.

It should be noted that this study has discussed only one possible explanation for the cyclic alopecia. Another possible cause, the abnormal hair cycling in AE mice, cannot be ruled out. Club hairs in telogen are eventually shed from the follicle, so prolonged telogen and increased percentage of follicles in telogen can lead to excessive shedding [43]. Telogen in AE mice starts at P25, distinguished from the red skin, and lasts 7–8 days (Fig. 1B). It is 5 days longer than WT mice. So the alopecia phenotype may be the result of the loss of club hairs.

In conclusion, our results show that *Gsdma3* is essential in normal hair follicle differentiation and functions by regulating Msx2/Foxn1/acidic hair keratin cascade.

Acknowledgments

This work was supported by Grants 30872270 from National Nature Science Foundation of China and CSTC, 2009AA5045 from Key Science and Technology Program of CQ. We thank Dr. Tung-Tien Sun for AE13 and AE15 antibodies.

Reference

[1] M. Ito, Z. Yang, T. Andl, C. Cui, N. Kim, S.E. Millar, G. Cotsarelis, Wnt-dependent de novo hair follicle regeneration in adult mouse skin after wounding, Nature 447 (2007) 316–320.
[2] M.H. Hardy, The secret life of the hair follicle, Trends Genet. 8 (1992) 55–61.
[3] R. Paus, S. Muller-Rover, C. Van Der Veen, M. Maurer, S. Eichmuller, G. Ling, U. Hofmann, K. Foitzik, L. Mecklenburg, B. Handjiski, A comprehensive guide for the recognition and classification of distinct stages of hair follicle morphogenesis, J. Invest. Dermatol. 113 (1999) 523–532.
[4] K.S. Stenn, R. Paus, Controls of hair follicle cycling, Physiol. Rev. 81 (2001) 449–494.
[5] V.A. Botchkarev, Bone morphogenetic proteins and their antagonists in skin and hair follicle biology, J. Invest. Dermatol. 120 (2003) 36–47.
[6] V.A. Botchkarev, A.A. Sharov, BMP signaling in the control of skin development and hair follicle growth, Differentiation 72 (2004) 512–526.
[7] H. Kulessa, G. Turk, B.L. Hogan, Inhibition of Bmp signaling affects growth and differentiation in the anagen hair follicle, EMBO J. 19 (2000) 6664–6674.
[8] K. Kobielak, H.A. Pasolli, L. Alonso, L. Polak, E. Fuchs, Defining BMP functions in the hair follicle by conditional ablation of BMP receptor IA, J. Cell Biol. 163 (2003) 609–623.
[9] T. Andl, K. Ahn, A. Kairo, E.Y. Chu, L. Wine-Lee, S.T. Reddy, N.J. Croft, J.A. Cebra-Thomas, D. Metzger, P. Chambon, K.M. Lyons, Y. Mishina, J.T. Seykora, E.B. Crenshaw 3rd, S.E. Millar, Epithelial Bmpr1a regulates differentiation and proliferation in postnatal hair follicles and is essential for tooth development, Development 131 (2004) 2257–2268.
[10] W. McGinnis, R. Krumlauf, Homeobox genes and axial patterning, Cell 68 (1992) 283–302.
[11] O. Duverger, M.I. Morasso, Role of homeobox genes in the patterning, specification, and differentiation of ectodermal appendages in mammals, J. Cell Physiol. 216 (2008) 337–346.
[12] L. Ma, J. Liu, T. Wu, M. Plikus, T.X. Jiang, Q. Bi, Y.H. Liu, S. Muller-Rover, H. Peters, J.P. Sundberg, R. Maxson, R.L. Maas, C.M. Chuong, 'Cyclic alopecia' in Msx2 mutants: defects in hair cycling and hair shaft differentiation, Development 130 (2003) 379–389.
[13] J. Cai, J. Lee, R. Kopan, L. Ma, Genetic interplays between Msx2 and Foxn1 are required for Notch1 expression and hair shaft differentiation, Dev. Biol. 326 (2009) 420–430.
[14] A.R. Godwin, M.R. Capecchi, Hoxc13 mutant mice lack external hair, Genes Dev. 12 (1998) 11–20.
[15] A.V. Tkatchenko, R.P. Visconti, L. Shang, T. Papenbrock, N.D. Pruett, T. Ito, M. Ogawa, A. Awgulewitsch, Overexpression of Hoxc13 in differentiating keratinocytes results in downregulation of a novel hair keratin gene cluster and alopecia, Development 128 (2001) 1547–1558.
[16] R.L. Peterson, T.V. Tkatchenko, N.D. Pruett, C.S. Potter, D.F. Jacobs, A. Awgulewitsch, Epididymal cysteine-rich secretory protein 1 encoding gene is expressed in murine hair follicles and downregulated in mice overexpressing Hoxc13, J. Investig. Dermatol. Symp. Proc. 10 (2005) 238–242.
[17] S.E. Millar, K. Willert, P.C. Salinas, H. Roelink, R. Nusse, D.J. Sussman, G.S. Barsh, WNT signaling in the control of hair growth and structure, Dev. Biol. 207 (1999) 133–149.
[18] R. DasGupta, E. Fuchs, Multiple roles for activated LEF/TCF transcription complexes during hair follicle development and differentiation, Development 126 (1999) 4557–4568.
[19] F. Runkel, A. Marquardt, C. Stoeger, E. Kochmann, D. Simon, B. Kohnke, D. Korthaus, F. Wattler, H. Fuchs, M. Hrabe de Angelis, G. Stumm, M. Nehls, S. Wattler, T. Franz, M. Augustin, The dominant alopecia phenotypes Bareskin, Rex-denuded, and reduced coat 2 are caused by mutations in gasdermin 3, Genomics 84 (2004) 824–835.
[20] R.M. Porter, C.A. Jahoda, D.P. Lunny, G. Henderson, J. Ross, W.H. McLean, N.V. Whittock, N.J. Wilson, J. Reichelt, T.M. Magin, E.B. Lane, Defolliculated (dfl): a dominant mouse mutation leading to poor sebaceous gland differentiation and total elimination of pelage follicles, J. Invest. Dermatol. 119 (2002) 32–37.
[21] D.P. Lunny, E. Weed, P.M. Nolan, A. Marquardt, M. Augustin, R.M. Porter, Mutations in gasdermin 3 cause aberrant differentiation of the hair follicle and sebaceous gland, J. Invest. Dermatol. 124 (2005) 615–621.
[22] M.F. Lyon, P.H. Glenister, Bareskin (Bsk), Mouse News Lett. 71 (1984).

[23] S. Tanaka, M. Tamura, A. Aoki, T. Fujii, H. Komiyama, T. Sagai, T. Shiroishi, A new Gsdma3 mutation affecting anagen phase of first hair cycle, Biochem. Biophys. Res. Commun. 359 (2007) 902–907.
[24] S. Chesnoy, P.Y. Lee, L. Huang, Intradermal injection of transforming growth factor-beta1 gene enhances wound healing in genetically diabetic mice, Pharm. Res. 20 (2003) 345–350.
[25] M. Kiso, S. Tanaka, R. Saba, S. Matsuda, A. Shimizu, M. Ohyama, H.J. Okano, T. Shiroishi, H. Okano, Y. Saga, The disruption of Sox21-mediated hair shaft cuticle differentiation causes cyclic alopecia in mice, Proc. Natl. Acad. Sci. USA 106 (2009) 9292–9297.
[26] L. Langbein, M.A. Rogers, H. Winter, S. Praetzel, U. Beckhaus, H.R. Rackwitz, J. Schweizer, The catalog of human hair keratins. I. Expression of the nine type I members in the hair follicle, J. Biol. Chem. 274 (1999) 19874–19884.
[27] W.M. O'Guin, T.T. Sun, M. Manabe, Interaction of trichohyalin with intermediate filaments: three immunologically defined stages of trichohyalin maturation, J. Invest. Dermatol. 98 (1992) 24–32.
[28] M.H. Lynch, W.M. O'Guin, C. Hardy, L. Mak, T.T. Sun, Acidic, basic hair/nail (''hard'') keratins: their colocalization in upper cortical, cuticle cells of the human hair follicle and their relationship to ''Soft'' keratins, J. Cell. Biol. 103 (1986) 2593–2606.
[29] P.M. Steinert, D.A. Parry, L.N. Marekov, Trichohyalin mechanically strengthens the hair follicle: multiple cross-bridging roles in the inner root shealth, J. Biol. Chem. 278 (2003) 41409–41419.
[30] E. Legue, J.F. Nicolas, Hair follicle renewal: organization of stem cells in the matrix and the role of stereotyped lineages and behaviors, Development 132 (2005) 4143–4154.
[31] Y.V. Zhang, J. Cheong, N. Ciapurin, D.J. McDermitt, T. Tumbar, Distinct self-renewal and differentiation phases in the niche of infrequently dividing hair follicle stem cells, Cell Stem Cell 5 (2009) 267–278.
[32] T.X. Jiang, Y.H. Liu, R.B. Widelitz, R.K. Kundu, R.E. Maxson, C.M. Chuong, Epidermal dysplasia and abnormal hair follicles in transgenic mice overexpressing homeobox gene MSX-2, J. Invest. Dermatol. 113 (1999) 230–237.
[33] E.J. Stelnicki, L.G. Komuves, D. Holmes, W. Clavin, M.R. Harrison, N.S. Adzick, C. Largman, The human homeobox genes MSX-1, MSX-2, and MOX-1 are differentially expressed in the dermis and epidermis in fetal and adult skin, Differentiation 62 (1997) 33–41.
[34] I. Satokata, L. Ma, H. Ohshima, M. Bei, I. Woo, K. Nishizawa, T. Maeda, Y. Takano, M. Uchiyama, S. Heaney, H. Peters, Z. Tang, R. Maxson, R. Maas, Msx2 deficiency in mice causes pleiotropic defects in bone growth and ectodermal organ formation, Nat. Genet. 24 (2000) 391–395.
[35] Y. Nakamura, M. Ichinohe, M. Hirata, H. Matsuura, T. Fujiwara, T. Igarashi, M. Nakahara, H. Yamaguchi, S. Yasugi, T. Takenawa, K. Fukami, Phospholipase C-delta1 is an essential molecule downstream of Foxn1, the gene responsible for the nude mutation, in normal hair development, FASEB J. 22 (2008) 841–849.
[36] S.A. Johns, S. Soullier, P. Rashbass, V.T. Cunliffe, Foxn1 is required for tissue assembly and desmosomal cadherin expression in the hair shaft, Dev. Dyn. 232 (2005) 1062–1068.
[37] S.P. Flanagan, 'Nude', a new hairless gene with pleiotropic effects in the mouse, Genet. Res. 8 (1966) 295–309.
[38] L. Mecklenburg, M. Nakamura, J.P. Sundberg, R. Paus, The nude mouse skin phenotype: the role of Foxn1 in hair follicle development and cycling, Exp. Mol. Pathol. 71 (2001) 171–178.
[39] L. Mecklenburg, R. Paus, Z. Halata, L.S. Bechtold, P. Fleckman, J.P. Sundberg, FOXN1 is critical for onycholemmal terminal differentiation in nude (Foxn1) mice, J. Invest. Dermatol. 123 (2004) 1001–1011.
[40] M.H. Lin, C. Leimeister, M. Gessler, R. Kopan, Activation of the Notch pathway in the hair cortex leads to aberrant differentiation of the adjacent hair-shaft layers, Development 127 (2000) 2421–2432.
[41] Y.H. Pan, M.H. Lin, X.L. Tian, H.T. Cheng, T. Gridley, J. Shen, R. Kopan, Gamma-secretase functions through notch signaling to maintain skin appendages but is not required for their patterning or initial morphogenesis, Develop. Cell 7 (2004) 731–743.
[42] L.F. Jave-Suarez, H. Winter, L. Langbein, M.A. Rogers, J. Schweizer, HOXC13 is involved in the regulation of human hair keratin gene expression, J. Biol. Chem. 277 (2002) 3718–3726.
[43] R. Paus, G. Cotsarelis, The biology of hair follicles, N. Engl. J. Med. 341 (1999) 491–497.