The stem cell quiescence and niche signaling is disturbed in the hair follicle of the hairpoor mouse, an MUHH model mouse

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Abstract

Background: Hair follicle stem cells (HFSC) play an essential role in the maintenance of hair homeostasis; during the hair cycle, HFSC remain quiescent for most of its duration. The hairpoor mouse (+/HrHp), an animal model of Marie-Unna hypotrichosis (MUHH), overexpresses hairless in the bulge, inner root sheath, and outer root sheath of HF and shows the same phenotype as in MUHH patients manifesting sparse hair with progression to alopecia with age. The aim of this study was to gain an understanding of the hair cycle and the status of HFSC during the hair cycle of the hairpoor mouse in order to delineate the pathogenesis of MUHH.

Methods: H&E staining was performed in order to define the state of the hair follicle. FACS analysis and immunostaining were performed at the 1st and 2nd telogen stages for observation of the HFSC. A label retaining assay was performed to determine the quiescent state of hair follicles. qRT-PCR was performed to determine expression of factors involved in niche signaling and Wnt signaling.

Results: We observed a drastic decrease in the number of hair follicles after the 1st telogen, followed by an intensified disturbance in the hair cycle with shorter anagen as well as 2nd telogen in the hairpoor mouse. A dramatic reduction in the number of CD34 expressing bulges as well as cells was observed at the telogen of the HFs, with prominent high proliferation of bulge cells, suggesting the loss of HFSC quiescence in the hairpoor mouse. The increased cell proliferation in HF was reiterated following the synchronization of the hair cycle, leading to acceleration of HF cycling. Reduced expression of Fgf18 and Bmp6, the factors involved in HFSC quiescence, was observed in the HFSC niche of the hairpoor mouse. In addition, disturbed expression of Wnt signaling molecules including Wnt7b, Wnt10b, and Sfrp1 was observed, which induced the telogen-to-anagen transition of HFs in the hairpoor mouse.

Conclusions: These results indicate that the quiescent state of HFSC is not properly maintained in the hairpoor mouse, consequently leading HFs to the completely disarrayed hair cycle. These findings may provide an understanding of an underlying mechanism for development of alopecia with age in MUHH patients.

Keywords: Hairpoor mouse, MUHH, Hair follicle stem cell, Wnt signaling, Alopecia

Background

Hair follicle stem cells (HFSC), located in the bulge of the hair follicle (HF), play an important role in the development of hair and maintenance of the HF cycle [1, 2]. During its lifetime, HF continuously undergoes a cycle of growth (anagen), degeneration (catagen), and rest phases (telogen) [3]. Regulation of HF cycling occurs through a precise interplay of several specific signaling activities [4].

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Wnt signaling is a central regulator of hair morphogenesis and hair growth [5]. During the anagen phase, activation of Wnt signaling induces the HF cycle via the proliferation and differentiation of HFSC [6] and Wnt7b plays a role as a critical regulator of the HF cycle [7]. Increased expression of β-catenin, an intracellular signal transducer of the Wnt signaling pathway, occurs at the telogen–anagen transition and remains at the same level throughout the anagen phase [8, 9]. While Wnt signaling induces activation of HFSC proliferation, bone morphogenetic protein (BMP) signaling plays an important role in maintaining quiescence of HFSC [10, 11]. BMP is highly expressed in the HFSC niche during the telogen phase. Bone morphogenetic protein 6 (Bmp6) and fibroblast growth factor 18 (Fgf18), which have been shown to be essential in maintaining quiescence of HFSC, are highly expressed in Keratin 6 (K6) positive cells in the inner layer of the bulge [12]. Fgf18 conditional knockout mice display the shortened telogen phase [13], and similarly, overexpression of Noggin, a BMP antagonist, in mouse skin results in a shortened telogen phase and activation of HF growth [14]. Thus, homeostasis and cyclic activation of HFSC are controlled by the balance between Wnt, BMP, and Fgf18 signaling.

Hairless (Hr), a transcriptional corepressor, downregulates target genes in conjunction with the thyroid hormone receptor, retinoic acid receptor, and vitamin D receptor [15–17]. The Hr gene, which is expressed in the outer root sheath of HF, is involved in the development of HF through regulation of Wnt signaling [18, 19]. Associations of mutations in the Hr gene with hair loss disorders in humans and mice have been reported [20, 21]. Among them, mutations in the 5′UTR of HR are known to cause Marie-Unna hypotrichosis (MUHH), a genetic disorder of alopecia [22]. We recently reported that the mutant mouse known as “hairpoor” (HrHP) with T-to-A transition substitution at position 403 in the second uORF of 5′UTR of the Hr gene (NM_021877) [23]. This mouse displays overexpression of HR and the characteristics of the human MUHH, which manifests sparse hair in early age and gradual hair loss with age [24]. Shortened hair follicles and abnormal differentiation of keratinocytes was observed in the hairpoor heterozygotes (+/HrHP). In addition, unusually complicated Wnt signaling was demonstrated in this mouse [24, 25]. Although the characteristics of hairpoor mice have been described in these studies, the status of the HFSC in the HR overexpressing hairpoor mouse has not been fully examined.

In the current study, we examined the hair cycle of the hairpoor mouse and investigated the status of the HFSC. Our findings clearly demonstrated that the quiescence of HFSC was not maintained in the hairpoor mouse, exhibiting the shorter telogen phase and premature induction of anagen phase with proliferation of HFSC, consequently leading to a completely disarrayed HF cycle. These phenomena concurred with the downregulation of Fgf18, Bmp6, and Sfrp1 and upregulation of Wnt7b and Wnt10b expression, presumably leading to failure of appropriate signaling activity in the HFSC niche.

Methods

Animals

Housing conditions of animals were as previously reported [21]. All procedures were conducted in accordance with the Laboratory Animals Welfare Act, the Guide for the Care and Use of Laboratory Animals, and the Guidelines and Policies for Rodent Experiments provided by the IACUC (Institutional Animal Care and Use Committee) in the School of Medicine, The Catholic University of Korea. Depilation was achieved by manual plucking of dorsal skin followed by wax strip depilation at 2nd telogen [3].

Histology, immunofluorescence, and immunocytochemistry

Skin samples were taken at the 1st telogen (wild type: P21, hairpoor: P19-20), 2nd telogen (wild type: P49, hairpoor: P35), or indicated date. Tissues were fixed in 4% formaldehyde solution overnight at 4 °C and embedded in paraffin wax. Paraffin sections (6 μm) were prepared, and hematoxylin and eosin (H&E) staining was carried out following a standard protocol [24]. For immunofluorescence or immunohistochemical staining, antigen retrieval was performed in 10 mM sodium citrate (pH 6.0). Skin sections were blocked with 5% BSA in PBS for 1 h at room temperature and incubated with primary antibodies overnight at 4 °C. The primary antibodies used were anti-CD34 (1:200, Abcam, MA, USA), anti-K15 (1:200, Abcam, MA, USA), anti-NFATc1 (1:100, Santacruz, CA, USA), anti-LEF1 (1:100, Cell Signaling Technology, MA, USA), anti-SOX9 (1:100, Santacruz, CA, USA), anti-TCF4 (1:100, Cell Signaling Technology, MA, USA), anti-Ki67 (1:100, NeoMarkers, CA, USA), anti-BrdU (1:200, Abcam, MA, USA), anti-K6 (1:100, Abcam, MA, USA), anti-Bmp6 (1:100, Cell Signaling Technology, MA, USA), and anti-Fgf18 (1:100, Cell Signaling Technology, MA, USA). Alexa Fluor 488 or 594 goat anti-mouse, goat anti-rabbit, goat anti-rodent secondary antibody (1:500, Invitrogen, MA, USA) were used for immunofluorescence. Counterstaining was done with DAPI. Fluorescence signal was observed with a fluorescent microscope (Olympus, Tokyo, Japan) or confocal microscope (Zeiss, Oberkochen, Germany). For immunohistochemical experiments, anti-mouse, anti-rabbit, and anti-rodent HRP-conjugated antibodies (1:500, Santacruz, CA, USA) were utilized. Secondary antibodies
were incubated for 2 h at room temperature. DAB chromogen was used for color reaction (Dako Glostrup, CA, USA), and counterstaining was done with hematoxylin. Image signal was observed with an optical microscope (Olympus, Tokyo, Japan).

5-bromodeoxy-uridine (BrdU) labeling
For label-retaining cell experiments, 10-day-old mice were injected with 5-bromodeoxy-uridine (BrdU, 50 μg/g) intraperitoneally (i.p) every 12 h for 48 h [26]. After a 60-day chase period, the dorsal skin was isolated. For proliferation analysis, BrdU (150 μg/g) was i.p injected to mice 1 h prior to kill at the indicated days.

qRT-PCR
Total RNA was extracted from either dorsal skin or epidermis using Trizol reagent (Invitrogen, Carlsbad, CA, USA) following the manufacturer’s instructions. The single-stranded cDNAs were synthesized using the Prime Script 1st strand cDNA Synthesis kit (Takara, Tokyo, Japan) and used for real-time PCR using a CFX96 Touch™ Real-Time PCR Detection System (Bio-Rad Laboratories, Hercules, CA, USA). Primer sequences for each gene are listed in Additional file 1: Table S1.

FACS analysis
Mouse epidermis were isolated from back skin of 1st and 2nd telogen mice as described previously [27]. Cells were labeled with anti-integrin α6 antibody conjugated to phycoerythrin (BD Bioscience, NJ, USA) and anti-CD34 conjugated to FITC (eBioscience, Waltham, MA, USA) for 30 min on ice. Cells were then analyzed using BD FACS CantoII sorter and FACSDiva™ software analysis system.

Statistical analysis
P values were determined using Student’s t tests. Statistical significance was set at p < 0.05.

Results
Alteration in hair cycling of the hairpoor mouse
The hairpoor mouse exhibited increased proliferation and differentiation of epidermal cells [24] and premature catagen followed by the early telogen at the first hair cycle [25], indicating aberrant HF development. Because the abnormal hair loss with aging in hairpoor mice was similar to that of MUHH patients, we hypothesized that HFSC might be affected in this disorder leading to an abnormal HF cycle, resulting in acceleration of hair loss.

To examine this hypothesis, we first observed and compared the hair cycle between hairpoor and littermate wild-type mice, including the second telogen stage (Additional file 2: Fig. S1 and Fig. 1a). Results of H&E staining showed a much shortened anagen stage following the premature catagen and earlier onset of the 1st telogen in the hairpoor mouse compared to that of the wild-type mouse. At P19-20, the HF of +/+HrHp was at the 1st telogen stage, while HF of the wild-type mouse was still at the 1st catagen, showing the earlier onset of telogen in the hairpoor mouse. Due to the shortened cycle, the onset of the 2nd telogen was much earlier and its duration was remarkably shorter in the hairpoor mouse compared to the wild-type mouse. The 2nd telogen started at P35 and lasted for approximately one day (P35-P36) in hairpoor HF, while it started at P42 and lasted for more than 2 weeks in wild-type HF (Fig. 1a).

Among the stage of telogen HFs of the hairpoor mouse, the clear morphological abnormality was not observed compared to the wild-type HF as shown in Fig. 1b. However, stages of the hairpoor mouse differed significantly from that of the wild-type mouse. At the 1st telogen, the proportion of telogen HF was 91.5% in the wild-type mouse, whereas it was only 58.3% in the hairpoor mouse. In the case of the 2nd telogen, all the wild-type HF was at telogen phase, but only about 30% of the hairpoor HF were at telogen phase and 70% were already into the anagen phase (Fig. 1c). Following the short 2nd telogen, the HFs were a mixture of mostly anagen, a few catagen, and telogen HFs in the hairpoor mouse (data not shown).

Of particular interest, no significant difference in the number of HFs was observed between the wild-type and the hairpoor mouse until P21. However, at P28, the number of HFs in +/+HrHp was drastically lower than that of the wild-type mouse and the difference was maintained throughout the observed period (Fig. 1d), suggesting that the capability of HFs for regeneration appeared to have been affected by the first telogen and those defects persisted in the hairpoor mouse. These results clearly indicated that the transition of the stages of the HF cycle was abnormal in the hairpoor mouse and raised a possibility of the impairment of HFSC that controls the HF cycle.

Decrease in the number of CD34+ HFSC in hairpoor mice
In order to dissect the abnormal hair cycle in the hairpoor mouse, we attempted to determine whether there were any changes in HFSC. First, the number of bulges expressing K15, a bulge marker, and CD34, a HFSC marker [28], was determined at telogen stages using immunohistochemistry. At both telogen stages, all K15+ bulges had similar intensities for both wild-type and hairpoor mice (Fig. 2a). In contrast to K15, CD34 expression displayed a different pattern between two lines. Almost all the K15+ bulges were CD34+ at the first telogen (7.25/7.5) and the second telogen (6.8/6.8) stages of the wild-type HFs. In contrast, on average, only 45% (3.5/7.75) and 70% (3.8/5.4) of the K15+ bulges showed CD34+ expression at the first and second telogen of the
hairpoor HFs, respectively. In determining the number of CD34+ bulges, the bulge showing a detectable expression level of CD34 was regarded as a CD34+ bulge (Fig. 2b). In addition to the reduced number of CD34+ bulges, much weaker expression was observed in the CD34+ bulges, of hairpoor HFs compared to those of wild-type HFs (Fig. 2a, b). Of particular interest, a higher proportion of CD34+/K15+ bulges was observed at the 2nd telogen than at the 1st telogen of hairpoor HFs, although the expression level was much lower than that of wild-type HF.

qRT-PCR was performed to determine the expression level of Cd34 mRNA in order to assess the extent of the reduced expression of CD34 in hairpoor HFs. A significant reduction in the relative expression level of Cd34 mRNA was observed in the epidermis of hairpoor compared to that of wild type (Fig. 2c), with less than 20% of that of the wild-type mouse for both telogen stages.

Next, using FACS analysis, we determined the proportion of HFSC (ITGA6+/CD34+) in the bulge at both telogen stages (Fig. 2d, e). In wild-type mice, ITGA6+/CD34+ cells composed 5.2% and 9.6% of the bulge cells at the 1st and 2nd telogen stages, respectively. In contrast, the bulges in hairpoor mice contained only 0.2% and 1.8% ITGA6+/CD34+ cells of the bulge cells at the 1st and the 2nd telogen stages, respectively. This result clearly indicates that the number of HFSC expressing CD34 was significantly reduced in the hairpoor mouse. Taken together, significantly fewer HFSC present in the HFs were observed in the hairpoor mouse compared to
the wild type, suggesting aberrance in the maintenance of HFSC in the hairpoor mouse.

Loss of HFSC quiescence and increased cell proliferation in the hairpoor HF
In order to determine whether the quiescence of HFSC was disturbed in the hairpoor mouse, we first determined the number of label-retaining cells (LRC) in HF using the 5-bromodeoxy-uridine (BrdU) incorporation method at 60 days post an injection of BrdU at P10. Less than one BrdU⁺ LRC was detected in the bulge of hairpoor HF, while the wild-type HF contained three BrdU⁺ LRCs on average (Fig. 3a, b). Cell proliferation in HFs was then determined in order to delineate whether HFSC lost quiescence. Incorporation of BrdU in cells 1 h after BrdU injection was measured for assessment of cell proliferation. The experiments were performed at the anagen I–II period for both wild-type and hairpoor mice and revealed that the hairpoor mouse exhibited 15 BrdU⁺ cells/HF on average, while the wild-type mouse showed 0.48 BrdU⁺ cells/HF (Fig. 3c, d), showing excessive cell proliferation in hairpoor HFs.

To further characterize HFSC in the hairpoor mouse, a histological study was performed on skin sections prepared after synchronization of HF cycling via depilation at the 2nd telogen phase. As shown in Fig. 4a, the HF cycle of the hairpoor mouse was much shorter than that of the wild-type mouse. At 14 days post-depilation, while the wild-type HFs were still in the full anagen phase, the hairpoor HFs were into the catagen phase (Fig. 4a). This deviation in the hair cycle of the hairpoor mouse was more prominent in subsequent days. Specifically, at 21 days post-depilation, the hairpoor HFs were in the anagen phase again, whereas the wild-type HFs were in the late catagen/early telogen phase, indicating that the telogen stage of the hairpoor HFs passed between 14 and 21 days post-depilation. In contrast, the wild-type HFs began the telogen stage at 21 days and remained in the same stage at 28 days post-depilation, while the hairpoor HFs were in anagen stage again.

Cell proliferation in HFs was examined at 7 days after depilation when both the wild-type and hairpoor HFs were in anagen phase. Using immunohistochemical detection of Ki67, there is a higher proliferation of the cells in the bulge and bulb of the hairpoor HFs compared
to those of wild-type HFs (Fig. 4b). In addition, expression of Lymphoid enhancer-binding factor 1 (LEF1), transcription activator in the presence of β-catenin, was observed at both the bulges of 1st and 2nd telogen staged HFs of the hairpoor mouse (Fig. 4c, d). Taken together, quiescence could not be maintained by HFSC in the hairpoor mouse, resulting in proliferation of cells in HF.

Expression of HFSC markers in hairpoor mice

Expression of various HFSC markers including LIM homeobox 2 (LHX2), SRY-box 9 (SOX9), nuclear factor of activated T cell 1 (NFATc1), and transcription factor 4 (TCF4) [29–33] was observed using immunohistochemistry and qRT-PCR in order to dissect the status of the HFSC of hairpoor mice. LHX2, which is expressed in the bulge, plays an essential role in morphogenesis of HF as well as in maintenance of quiescence of HFSC. SOX9, which is known to have an association with Shh signaling, is expressed in the bulge and outer root sheath. As shown in Fig. 5a, b, no significant difference in expression of LHX2 and SOX9 was observed in the 1st and 2nd telogen phases of HF of both the wild-type and the hairpoor mouse HFs. Expression of other bulge markers, NFATc1 and TCF4, was also detected in the junctional zone and interfollicular epidermis, and the bulges, respectively. TCF4, which has been shown to suppress HFSC quiescence and thus inhibit transition of HFs from telogen to anagen, was also expressed in the bulge. As with LHX2 and SOX9, no noticeable difference in expression of NFATc1 and TCF4 was observed between hairpoor and wild-type HFs (Fig. 5c). Thus, no difference in expression of all the HFSC markers tested in this study with exception of CD34 was observed between the hairpoor and the wild type.

Disruption of HFSC quiescence signaling in the hairpoor mouse

Next, we addressed the question of whether HFSC niche signaling was involved in the abnormal hair cycling of the hairpoor mouse. Using IHC, we observed the expression of K6, a HFSC niche marker; according to the results, all the K15+ bulges showed K6+ in the hairpoor HFs as the same, in the wild type (Additional file 3: Fig. S2). Because regulation of the quiescence of HFSC through expression of Bmp6 and Fgf18 by K6+ has been demonstrated, we also observed expression of Bmp6 and Fgf18 in the epidermis of mice [12]. The pattern of expression of Bmp6 differed from that of Fgf18. Significantly decreased expression of Bmp6 was observed only in the 2nd telogen phase in hairpoor HFs compared to that of wild-type HFs. In contrast to Bmp6, significantly decreased expression of Fgf18 was observed in both the 1st and 2nd telogen phases in the hairpoor HFs compared to the wild-type ones (Fig. 6a, b and c). These findings demonstrate that reduced expression of Fgf18 and Bmp6 concurred with disturbance of HFSC quiescence in hairpoor mice, suggesting that the lack of expression of the quiescence maintaining regulators may cause disarrayed HF cycling. Next, we attempted to determine whether the HF in the telogen phase of the hairpoor mouse was in the competent telogen state or the telogen–anagen transition state [4] by determining the expression of Wnt signaling molecules in the skin. Expression of Sfrp1, a Wnt inhibitor, was reduced in the 2nd telogen of the hairpoor HFs to 40% of that of the wild type (Fig. 6d), and expression of Wnt7b was increased in both 1st and 2nd telogen in the hairpoor compared to the wild type (Fig. 6e). In addition, remarkably increased expression

Fig. 3 Hairpoor mouse displays the loss of label-retaining HFSC and increased cell proliferation in HF. a Immunofluorescence staining of BrdU-LRC in the CD34+ bulge at P70. White arrowhead indicates LRC in bulge. Bulge, Bu; scale bar = 25 μm. b Average number of LRC in bulge at P70. Data are mean ± SD. *P < 0.05. Mouse number n = 3 per genotype. Observed follicle number per each mouse n = 10. c Immunofluorescence staining of BrdU (1 h chase) at anagen II HFs (the wild-type mouse at P23 and the hairpoor mouse at P21). Scale bar = 100 μm. a,c Nuclei were counterstained with DAPI. d Quantification of BrdU positive cells in follicle. Data are mean ± SD. **P < 0.01, ***P < 0.001. n = 3 per genotype. Number of HFs counted in each mouse ≥ 10
of Axin2 and Wnt10b mRNA was observed in the 2nd telogen of the hairpoor mouse compared to the wild-type mouse (Fig. 6f, g). These results were the same even when normalized to Sdha (Additional file 4: Fig. S3). These observations suggest that overexpression of HR in the hairpoor mouse led to the reduction of HFSC quiescence signaling molecules in the HFSC niche and concomitantly activated the Wnt signaling pathway, resulting in transition of the hairpoor HFs from telogen-to-telogen–anagen transition state.

**Discussion**

HR is expressed in HF and regulates downstream gene expression as a transcriptional cofactor [17, 34]. In skin, HR regulates the HF cycle by regulating Wise expression through involvement in Wnt signaling [34]. However, association of HR with HFSC has not been examined. Here, we report that overexpression of HR resulted in complete disruption of the HF cycle which may be caused by abnormal expression of molecules involved in regulation of HFSC in the HF niche using the hairpoor mouse.

The hairpoor mouse (+/HrHp) is a human MUHH disease model, and this heterozygote displays scarce hair.
during young age and then gradual progression to baldness with aging as manifested by MUHH patients [23, 24]. We previously reported that the length of HF was shorter, with a premature catagen stage followed by earlier onset of telogen stage in the first cycle of the hair-poor mouse compared to that of the wild-type mouse [25]. This abnormal hair cycle was further explored in the current study and found that the abnormality of the early HF cycle is accentuated in the 2nd cycle. The 2nd cycle of the hair-poor HF displayed a much shorter anagen stage and a lack of stable telogen stage which was composed of a mixed population of anagen and stage of...
telogen HFs, whereas the wild-type HFs showed the stable and long 2nd telogen stage with all HFs at telogen phase (Fig. 1, Additional file 2: Fig. S1). In addition, the reduced numbers of HFs were observed in the 4-week-old mice, which persisted until at least P56 of hairpoor mice. This phenomenon continued, and the decrease in number of HFs became greater in mice older than 1 year (data not shown). These results indicate that overexpression of Hr disturbed not only the first cycle but also the following cycles, suggesting involvement of Hr in the status of HFSC.

Expression of stem cell-specific markers provides valuable information in determination of the status of stem cells. Of the HFSC associated markers examined in this study, all the markers including NFATc1, SOX9, LHX2, and TCF4 with exception of CD34 expressed in all the K15+ bulges of hairpoor HFs. Expression of these HFSC markers has been shown to determine the quiescent state or in transition from telogen to anagen in HF [12, 13].
CD34, the most widely used HFSC marker, is known to provide the evidence that with Fgf18 dissection of Sfrp1 in the hairpoor mouse, markedly reduced expression of Axin2 expression in maintaining HFSC quiescence [44]. In the hairpoor mouse, HR contributes in part to loss of HFSC quiescence. The relationship between Bmp6 and Sfrp1 was recently shown to maintain HFSC quiescence through Fgf18 without altering expression of Bmp6 [43]. Our results clearly show the evidence that Bmp6 and Fgf18 expressed in the K6+ inner bulge play different roles in maintaining HFSC quiescence. The relationship between Hr and Foxp1 remains to be explored.

During the telogen, expression of Sfrp1, a Wnt inhibitor, was observed in both inner and outer bulges and was in balance with Wnt7b expression in the niche cells with Axin2 expression in maintaining HFSC quiescence [44]. In the hairpoor mouse, markedly reduced expression of Sfrp1 was observed in the 2nd telogen. We have shown that expression of HR resulted in suppressed expression of Sfrp1 mRNA [45]. Thus, presumably overexpression of HR contributes in part to loss of HFSC quiescence via downregulation of Sfrp1 expression in the hairpoor mouse. Activation of the Wnt signaling pathway during the 2nd telogen was demonstrated by increased expression of Axin2, Wnt7b and Wnt10b, which explains the findings that a large portion of HFs were in the telogen–anagen transition state in hairpoor mice. In addition, we previously documented that another Wnt inhibitor, Dickkopf-related protein 1 (Dkk1), was overexpressed in hairpoor mouse [25]. Expression of Dkk1 in anagen and catagen HFs was demonstrated, and it is known to induce HF from anagen to catagen [46]. Taken together, these findings strongly suggest that the acceleration of HF cycling was led by aberrant Wnt signaling in the hairpoor mouse. This may be related to development of alopecia with aging in the hairpoor mouse, and further study is required in order to better elucidate this disorder. This study provided a basis for understanding the mechanism of hair loss in MUHH patients. Currently, there is no available treatment for hair loss induced by MUHH disease. If the reciprocal mechanism between HFSC and the HR gene in MUHH disease is fully understood, modulation of the signaling pathways such as Wnt might provide a valuable therapeutic opportunity.

Conclusions
In conclusion, in HR overexpressing hairpoor mice, the HFSC quiescence is not maintained due to decreased HFSC niche signals including Fgf18, Bmp6, and Sfrp1. This leads to the telogen-to-anagen transition of HFs at the telogen phase, and proliferation of HF occurs through activation of the Wnt signaling in the hairpoor mouse (Fig. 6h). Consequently, this causes abnormalities of HF cycling, which eventually leads to development of alopecia. Our results indicate that HR plays an important role in HFSC quiescence by regulating the signaling networks in HF. Our study elucidates the molecular genetic etiology of MUHH disease and may provide therapeutic directions.

Abbreviations
HFSC: Hair follicle stem cell; HF: Hair follicle; Hr: Hairless; MUHH: Marie-Unna hypotrichosis; LRC: Label retaining cell; BMP: Bone morphogenetic protein; K6: Keratin 6; BrdU: 5-Bromodeoxy-uridine; LEF1: Lymphoid enhancer-binding factor1; LHX2: LIM homeobox 2; SOX9: SRY-box 9; NFATc1: Nuclear factor of activated T cell 1; TCF4: Transcription factor 4; Dkk1: Dickkopf-related protein 1.

Supplementary Information
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Additional file 1: Table S1. List of gene specific primers.

Additional file 2: Figure S1. Hematoxylin and eosin (H&E) stain of hairpoor mouse (+/HrHp) back skin. Hematoxylin and eosin (H&E) stain of back skin of mice at indicated day. +/HrHp; the hairpoor mouse, +/+; the wild type mouse. Scale bar = 200 μm. P; postnatal day.

Additional file 3: Figure S2. Immunofluorescence analyses of HFSC niche. (a) Immunofluorescence analyses of K6 (red) and K15 (green) in wild type and hairpoor mouse at the telogen phases. Nuclei were counterstained with DAPI. (b) Quantification of K6 positive cells in K15 positive bulge cell. n=3 per genotype. Scale bar =50μm.

Additional file 4: Figure S3. Expression of Fgf18, Bmp6 and Wnt molecules in hairpoor mouse. Expression level of each gene was determined by qRT-PCR analyses and normalized against that of Succinate Dehydrogenase Complex Flavoprotein Subunit A (Sdha). The relative expression
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Author contributions

KC and SKY planned and designed experiments, performed experiments and data analysis, and wrote the manuscript. SHP analyzed FACS data and provision of study material. SY-P analyzed qRT-PCR and generated hairpoor mice. All authors have read and approved the final version of the manuscript.

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Availability of data and materials

The dataset used and/or analyzed during the current study is available from the corresponding author upon reasonable request.

Declarations

Ethics approval and consent to participate

All experiments were performed according to standard protocols, in compliance with the Guidelines and Policies for Rodent Experiments provided by the IACUC (Institutional Animal Care and Use Committee) in the School of Medicine, The Catholic University of Korea. Our study was approved by our IACUC in the Catholic University of Korea, and the approval number is 2017-0072-01.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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