Transcriptome analysis reveals the genetic basis underlying the development of skin appendages and immunity in hedgehog (*Atelerix albiventris*)

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Abstract

Background: The expression of hair features is an evolutionary adaptation resulting from interactions between many organisms and their environment. Elucidation of the mechanisms that underlie the expression of such traits is a topic in evolutionary biology research; however, the genetic basis of skin appendage development and differentiation remains poorly understood. Therefore, we assessed the *de novo* transcriptome of the hedgehog (*Atelerix albiventris*) at three developmental stages and compared gene expression profiles between abdomen hair and dorsal spine tissues.

Results: We identified 328,576 unigenes in our transcriptome, among which 3,598 were differentially expressed between hair- and spine-type tissues. We identified 3 keratin genes related to hair and spine development through comparative analysis of tissues before and after growth of skin appendages. Dorsal and abdomen skin tissues 5 days after birth were compared and the resulting differentially expressed genes (DEGs) were mainly enriched in keratin

filament, intermediate filament, epithelium cell differentiation, and epidermis development based on GO enrichment analysis, and tight junction, p53, and cell cycle signaling pathways based on KEGG enrichment analysis. Expression variations of *MBP8, SFN, Wnt10, KRT1*, and *KRT2* may be the main factors regulating hair and spine differentiation for the hedgehog. Strikingly, DEGs in hair-type tissues were also significantly enriched in immune-related terms and pathways with hair-type tissues exhibiting more upregulated immune genes than spine-type tissues. Thus, we propose that spine development was an adaptation that provided protection against injuries or stress and reduced hedgehog vulnerability to infection.

Conclusion: Our study provided a list of potential genes involved in the regulation of skin appendage development and differentiation in *A. albiventris*. This is the first transcriptome survey of hair traits for a non-model mammal species, and the candidate genes provided here may provide valuable information for further studies of skin appendages and skin disorder treatments.

Keywords: hedgehog, skin appendage, adaptive evolution, RNA-Seq, molecular biology

Background

Perceiving and responding to life-threatening signals and regulating their own morphological characteristics constitute a fundamental challenge for all mammals. Evolution has shaped the ability of organisms to adapt skin appendages to increase their chances of survival. Accordingly, a variety of appendages have evolved on the skin of mammals, either to assist organisms in carrying out specific behaviors or to protect them from predators and pathogenic bacteria [1,2].

Compared with animals that possess passive defense adaptations such as feathers, scales, etc., the hedgehog has evolved into an unusual, nocturnal, spine-covered mammal [3]. Spines

50 potentiate attacking power and enhance the role of skin appendages as a defense mechanism.
51 The hedgehog progresses through a series of well-defined stages during its life cycle, from
52 embryonic, to non-spine, to with-spine stages. These transitions are governed by tightly
53 regulated gene expression at pre-transcriptional, epigenetic, and translational levels. Spine- and
54 hair-type skin tissues from the hedgehog thus offer a natural model to analyze the genomic
55 basis for the evolution of epidermal appendage formation.
56
57 Development of hair follicles on a skin appendage with a complex growth cycle is
58 characterized by anagen, catagen, and telogen stages, and many key signaling pathways and
59 genes are involved in their regulation [4–6]. According to many studies, the WNT/β-catenin
60 signaling pathway in the dermis may be the first dermal signal [7–9]. Sonic hedgehog (SHH)
61 is another secreted protein in the follicular placode that plays a major part in epithelial-
62 mesenchymal signaling [10,11]. SHH depends on WNT signaling and is required for the
63 proliferation of follicular epithelium and development of dermal condensate into dermal papilla
64 [4]. In addition, many key genes related to skin appendage development have been identified;
65 *TGFaR* (epidermal growth factor receptor) and transcription factor *ETS2* regulate hair follicle
66 shape and are responsible for hair follicle architecture and wavy hair [12,13], and *HOXC13*
67 and *KRT75* control hair shaft differentiation [14–17]. Further, *EDA* and *EDAR* interact with
68 members of the bone morphogenetic protein (BMP) family, some of which inhibit follicle
69 development to establish follicle patterning [2,18–20].
70
71 The abovementioned studies primarily focused on mammalian hairs and scales; little is known
72 about changes in gene expression during the development of spine appendages in mammals.
73 Therefore, we aimed to perform a detailed analysis of the transcriptome of skin from abdomen
74 hair and dorsal spines of the hedgehog (*Atelerix albiventris*) by comparing groups of transcripts

75 differentially expressed at different hedgehog developmental stages. We identified key
76 candidate genes related to the development and differentiation of skin appendages whose
77 importance should be verified in future studies. Nonetheless, the presented novel information
78 will be widely applicable in many fields, such as skin disease and skin immune genes, and
79 provide insight into the molecular mechanisms of skin appendage development in general.
80

81 **Results**

82 **Illumina sequencing and *de novo* assembly**

83 To identify the transcriptome and molecular mechanisms governing skin appendage
84 development and differentiation, we analyzed temporal changes in transcript abundance of *A.*
85 *albiventris* (Fig. 1a). RNA sequencing generated 82.2 ± 6.0 G (mean ± SD) read pairs for 16
86 skin tissue samples (Supplementary Table 1). After quality trimming, 96.8 ± 0.4% of reads
87 were retained, indicating a high-quality dataset (>90% reads with ≥Q30). The *de novo* assembly
88 using Trinity revealed 328,576 unigenes, which were used for subsequent analysis. We
89 evaluated biological reproducibility by individually comparing biological replicates through
90 principle component analysis (PCA) (Fig. 1b). As expected, similar gene expression patterns
91 in both hair- and spine-type samples were observed. PCA analysis also demonstrated more
92 separation between the two skin appendage types. A neural network graph based on self-
93 organizing feature map (SOM) analysis revealed dynamic transcriptional changes at the
94 individual stages of appendage development in *A. albiventris* (Fig. 1c).
95

96 **Functional annotation and classification of unigenes**

97 Use of the NR and Swiss-Prot databases yielded reliable protein annotations for approximately
98 42% of unigenes. Absolute unigenes were annotated using seven major public protein
99 databases (Table 1), of which the greatest number of matches (68.3%) was obtained using the

NT database. Of 328,576 unigenes assembled, 243,444 genes (74.1%) exhibited a positive match against at least one database. Thus, our gene annotation results from *A. albiventris* transcriptome were considered high quality.

In our study, 70,833 unigenes were assigned to 56 sub-categories of GO terms belonging to the following three main categories: biological process (BP), cellular component (CC), and molecular function (MF). These main categories included 26, 20, and 10 sub-term categories, respectively (Fig. 2). The most enriched GO terms were related to cellular and metabolic processes (BP), cell and organelle (CC), and binding and catalytic activity (MF). Importantly, we found some GO terms (level 4) in the BP category related to skin appendage development and differentiation, including (positive/negative) regulation of cell differentiation, regulation of cell proliferation, reproductive structure development, cell development, and ovarian follicle cell development.

In addition, we annotated all unigenes using KEGG pathway analysis to understand their top-level function and role in the hedgehog biological system. Overall, 36,283 unigenes were mapped to 232 biological pathways clustered in 5 KO hierarchies in the functional ortholog system. Among these KO hierarchies, 15 unique KEGG pathways represented cellular processes (A) and 30, 22, 98, and 72 pathways represented environmental information processing (B), genetic information processing (C), metabolism (D), and organismal systems (E), respectively (Fig. 3). The largest KO group was translation (5743 unigenes, 13%), followed by signal transduction (5551 unigenes, 12.6%), and endocrine system (3223 unigenes, 7.3%). Further, a small proportion of unigenes were annotated in the B category of signal transduction related to skin appendage development and differentiation, including Rap1 (814 unigenes), Wnt (355 unigenes), Hippo (626 unigenes), TGF-β (206 unigenes), and Notch (171

125 unigenes) signaling pathways.

126

127 **Analysis of differentially expressed genes in skin appendage tissues**

128 Analysis of DEGs in skin appendage tissues finally identified 3,598 DEGs in the three
129 developmental stages, and 816 DEGs shared by the two types of tissues (Fig. 4a-c). As shown
130 in the Venn diagram (Fig. 4d), more DEGs were found in tissues with spines (HH1Y and HS1Y)
131 than in tissues without spines (HH1N and HS1N).

132

133 To validate expression patterns indicated by the transcriptome data, 11 differentiation-related
134 DEGs were selected for RT-qPCR analysis including *KRT2*, *LEF1*, *RSPO2*, *ARRB*, *TGFB2*,
135 *GRB2*, *SFN*, *TCF7*, *CTNNB1*, *HOXC13*, and *WIF1* (Supplementary Table 2). Expression trends
136 determined by RT-qPCR significantly correlated with the RNA-Seq data (Fig. 5a). Expression
137 of these 11 genes at the three developmental stages was also analyzed, revealing expression
138 patterns similar to those determined by RNA-sequencing (Fig. 5b). Expression of *LEF1*,
139 *TGFB2*, *SFN*, and *WIF1* at stages I–III increased significantly. However, expression of *KRT1*
140 and *RSPO2* gradually decreased. Overall, both approaches confirmed the observed DEG trend
141 patterns, indicating the accuracy of the transcriptome data and *de novo* RNA-Seq data.

142

143 **Differentially expressed genes related to skin appendage development**

144 In order to further understand the spine development mechanism of the hedgehog, we
145 conducted DEG analysis before and after spine development. In total, 28 DEGs were identified
146 between HS1N and HS1Y, among them, 11 genes were downregulated, and the remaining 17
147 genes were upregulated (Supplementary Table 3). Based on GO and KEGG annotation, we
148 found one downregulated gene of unknown open reading frame (LOC103122410) related to
149 cell differentiation and multi-cellular organismal development in the BP category, and one

150 downregulated keratin-associated protein like gene (LOC103118355) related to keratin
151 filament.
152
153 In order to identify genes that activate the development of hedgehog abdominal hair, we
154 analyzed DEGs before and after the occurrence of abdominal hair. A total 82 DEGs were
155 identified between HH1N and HH1Y, of which 62 genes were downregulated, and the
156 remaining 20 genes were upregulated (Supplementary Table 4). Notably, LOC103122410 was
157 also present in these DEGs; in addition, keratin gene *KRT2* was highly expressed in HH1Y. We
158 speculate that these genes play an important role in activating the development of hedgehog
159 abdominal hair and spines.
160

161 **Differentially expressed genes related to skin appendage differentiation**

162 We identified 1,517 DEGs by comparing the gene expression profiles of spine- (HS5) and hair-
163 type (HH5) tissues in *A. albiventris* five days after birth. We screened 2 genes related to cell
164 differentiation (*NOTCH* and keratin-associated protein (*KRTAP*) 9-2), 18 genes related to cell
165 proliferation (*TGFB1, FGF, KRT2, TCHH, BMP3, BMP8, APOA1,CCDC85A, INHBB,*
166 *MICAL2, VSIG8, FCN, GDF5, DMD, GDNF, FAT1, CSF*, and *GDF15*), and many keratin-
167 related genes (*KRT1, KRT2*, *KRTAP*, etc). These genes might be indicative of hedgehog
168 transcriptome involvement in spine and hair differentiation.
169
170 Based on annotation of the *A. albiventirs* transcripts with the GO database, the DEGs were
171 significantly enriched in keratin-related terms. Keratin filament is an important component of
172 skin appendages; the main DEGs involved in this process included *KRT1, KRT2, MYH, DES,*
173 *BMP8, SHH*, and several *KRTAP* genes (Fig. 6a, b). In addition, *CSTA* was involved in the
174 biological process of keratinocyte differentiation. In a directed acyclic graph diagram

175 associated with this term, cell differentiation, epithelial cell differentiation, and epidermis
176 development were enriched, and the main DEGs involved in this process included *KRT1, KRT2,*
177 *MBP8, FGF, Wnt10*, and *MYH* (Fig. 6b). The only significantly enriched KEGG pathways
178 among all DEGs comparing HH5 and HS5 tissues were tight junction and p53 signaling
179 pathways (Fig. 6c; Table 2). In addition, eight pathways related to skin appendage development
180 and differentiation were enriched, including cell cycle, MAPK, Rap1, Hippo, VEGF, TGF-beta,
181 and PPAR signaling pathways. It is noteworthy that *SFN, BMP8, Wnt3, Wnt10, MYH*, and *SFN*
182 were found in both annotation results, suggesting they are closely related to differentiation of
183 keratinocytes and epidermal cells.
184
185 In our data, keratin-related genes were highly abundant and also significantly overexpressed in
186 the dorsal spine-type tissues. In the hedgehog transcriptome, more than 3,000 transcripts were
187 annotated to 6 keratin genes (*KRT1, KRT2, KRT5, KRT10, TCHP*, and *CHRNA1*) and
188 approximately 20 keratin-associated proteins. Through alignment of *KRT1* gene sequences
189 from 20 species, we found that the hedgehog *KRT1* gene sequence has a deletion of 45 bp
190 compared with other species (Fig. 6d). The two hedgehog species were isolated into one clade
191 in the bayesian phylogenetic tree (Fig. 6e), suggesting that *KRT1* may be one of the important
192 regulatory genes for the development and differentiation of hedgehog skin appendages.
193
194 **Immune-related genes in epidermis of hedgehog**
195 In this study, we also found many immune-related genes comparing HH5 and HS5. After
196 KEGG enrichment analysis, we found no significant enrichment of immune-related pathways
197 in spine-type tissues, and fewer immune-related pathways and genes than in hair-type tissues.
198 Hair-type tissues exhibited 19 enriched immune-related pathways, three of which were
199 significantly enriched, and 47 transcripts annotated with 32 immune-related genes (Fig. 7a,b).

200 In addition, we observed similar GO enrichment analysis results. The number of terms and
201 genes enriched in hair-type tissues was significantly higher than in spine-type tissues, of which
202 two of the seven terms were significantly enriched, and 25 transcripts were annotated with 11
203 immune-related genes (Fig. 7c,d).
204
205 **Discussion**
206 The genetic basis of morphological variation, both within and between species, provides a
207 major topic in evolutionary biology. In mammals, the development of skin appendages such as
208 hair, tooth, and scale involves complex interactions between the epidermis and the underlying
209 mesenchyme as part of an established hierarchical morphogenetic process [21]. Specifically,
210 mammals develop a coat containing many distinct types of hair. Such diversity is associated
211 with molecular and signaling pathways that drive formation and induction in a specific spatial
212 and temporal manner [22]. Reciprocal interactions between epithelial and mesenchymal tissues
213 constitute a central mechanism that determines the location, size, and shape of organs [23].
214
215 In the current study, we aimed to explore the genetic basis for hedgehog skin appendage
216 differentiation and development and the resulting expression of the spine trait. We identified
217 328,576 unigenes in our transcriptome, all of which were annotated in 7 databases (Table 1).
218 Taken together, our *de novo* assemblies revealed higher quality compared with previous studies
219 [24,25] that formed the foundation of all our subsequent analyses. According to GO and KEGG
220 annotation analysis, a total of 70,833 and 36,238 unigenes were mapped to 56 sub-categories
221 and 232 biological pathways, respectively (Fig. 2,3). We identified some of the key pathways
222 involved in skin development; these annotations may provide a valuable resource for further
223 understanding the specific functions and pathways in *A. albiventris*.
224

225 Newborn hedgehogs begin to develop hair/spines approximately two hours after birth. We
226 found 6 shared genes (*APOE, COX2, COX3, FCN, RP-L11e, SH3GL*) through analysis of
227 DEGs before and after hair/spine development, indicating that these genes are essential
228 regulatory genes in the development of skin appendages, whether hair or spine. Further, we
229 compared the gene expression profiles of spine- and hair-type tissues to systematically assess
230 key regulatory genes for spine and hair differentiation in *A. albiventris*. *SFN*, upregulated in
231 spine-type tissues, is a regulator of mitotic translation that interacts with a variety of translation
232 and initiation factors [26], is enriched in the p53 signaling pathway, and has a role in
233 keratinocyte differentiation and skin barrier establishment in the BP category (GO). *SFN* plays
234 an important role in maintaining hair follicle development, especially affecting the formation
235 of hair shaft structure [27]. Bu *et al*. (2014) determined that SFN gene and protein were
236 significantly highly expressed in the thicker, longer, and harder skin of wool, indicating that
237 *SFN* was involved in the regulation of wool character development [28]. In addition, we
238 identified *FGF* as the upregulated DEG enriched in the MAPK and Rap1 signaling pathways.
239 These pathways often act together by forming signaling loops during organogenesis [29] and
240 induce the most fundamental biological processes, such as the formation of periodic patterns.
241 Hebert *et al.* (1994) demonstrated that *FGF* plays an important role in regulation of the hair
242 cycle growth, and functions as an inhibitor of hair elongation by promoting progression from
243 anagen stage, the growth phase of the hair follicle, to catagen stage, the apoptosis-induced
244 regression phase [16]. Hence, we believe that *SFN* and *FGF* may be important regulators that
245 affect spine development and differentiation in *A. albiventris*.
246
247 We also compared *KRT1* gene sequences of 20 mammals with that of the hedgehog, in which
248 we identified a 45-bp deletion in the latter. Keratins, the major structural proteins of epithelia,
249 are a diverse group of cytoskeletal scaffolding proteins that form intermediate filament

250 networks and provide structural support to keratinocytes that maintain skin integrity [30]. In
251 general, *KRT1* is highly conserved in mammals, however, molecular defects in keratin
252 intermediate filament-related genes can cause keratinocyte and tissue-specific fragility,
253 accounting for a large number of genetic disorders in skin and its appendages [31,32].
254 Therefore, whether the deletion phenomenon in hedgehog *KRT1* has special significance for
255 spine development and differentiation in the hedgehog warrants further research and
256 verification.
257
258 Lastly, we analyzed immune-related genes in the skin transcriptome of *A. albiventris*. We found
259 that hair-type skin had more immune-related genes than spine-type skin. Choo *et al.* (2016)
260 found that interferon epsilon (*IFNE*), which was exclusively expressed in epithelial cells and
261 was important to mucosal immunity, was pseudogenized in pangolins. They proposed that scale
262 development provided protection against injuries or stress and reduced pangolin vulnerability
263 to infection, thus protection by scales on the pangolin body compensated for the low immunity
264 of this species to a certain extent [33]. From our current data, we speculate that there may be
265 significant differences in the immune function of skin with different appendage types, and that
266 the occurrence of spines may be an innovative physical protection for dorsal skin with
267 relatively low immunity.
268
269 **Conclusions**
270 In the current study, we conducted a comprehensive transcriptome analysis of *A. albiventris* to
271 explore the genetic basis for the growth of skin appendages. Transcriptome analysis provided
272 a rich list of unigenes expressed in hair- and spine-type tissues at three different developmental
273 stages, of which 328,576 unigenes and 3,598 DEGs were identified. Candidate genes were
274 identified that are likely involved in the regulation of hair and spine growth and differentiation.

275 The knowledge acquired in this study of the molecular and signaling pathways related to hair
276 and spine expression greatly contributes to the current genetic resources for the hedgehog and
277 mammalian species that harbor shaggy appendages, as well as traits that could be potentially
278 exploited for curing skin diseases of other animals, even humans.

279

280 **Methods**

281 **Ethics statement**

282 All animal procedures in the study were approved by the ethics committee for animal
283 experiments at the Guangdong Institute of Applied Biological Resources (reference number
284 G2ABR20170523) and followed basic principles. We confirm that all methods were performed
285 in accordance with relevant guidelines and regulations.

286

287 **Biological samples**

288 According to observations of the growth characteristics of hedgehogs, hairs and spines on the
289 back and abdomen begin to appear approximately two hours after birth; therefore we obtained
290 8 hedgehogs at 3 different stages of appendage development from a commercial animal farm
291 (Dongguan City, China). Hedgehog hair (HH) and spine (HS) tissues were collected from 2
292 specimens within 2 h of birth (Stage I, HH1N/HS1N), 3 specimens after 2 h but within the first
293 birth day (Stage II, HH1Y/HS1Y), and 3 specimens 5 days after birth (Stage III, HH5/HS5).
294 For collection of skin appendages, animals were first anesthetized with diethyl ether and then
295 killed via cervical dislocation. Sixteen skin tissue samples representing the two types of
296 appendages (abdomen hair-type and dorsal spine-type) were rapidly excised, immediately
297 snap-frozen on dry ice, and stored at –80 °C until RNA extraction.

298

299 **RNA extraction and RNA-seq**

300 Total RNA from each tissue sample was extracted using the RNeasy Kit (Qiagen, Hilden,
301 Germany). RNA purity was determined using a NanoPhotometer® spectrophotometer (Implen,
302 Inc., Westlake Village, CA, USA). All unigenes were annotated using the basic local alignment
303 search tool (BLASTX), considering hits with e-values of 1E−5 against seven databases: NCBI
304 non-redundant protein sequences (Nr); NCBI non-redundant nucleotide sequences (Nt);
305 Protein family (Pfam) database; Clusters of Orthologous Groups of Proteins (KOG/COG)
306 database; Swiss-Prot (a manually annotated and reviewed protein sequence database); KEGG
307 Ortholog (KO) database; Gene Ontology (GO) database. Differentially expressed genes (DEGs)
308 were identified by comparing gene expression levels between samples (or sample groups).
309 Bowtie2 [34] was used to align clean reads with all unigenes and RSEM [35] was used to
310 calculated gene expression levels for each sample. Differential gene expression was analyzed
311 using DESeq270 V1.16.1 in RStudio71 V1.0.143 running R72 V3.4.1. All sequencing was
312 conducted by Novogene (Beijing, China).
313

314 **Quantitative real-time reverse-transcription PCR (RT-qPCR)**

315 RNA was extracted from skin appendage tissue samples using TRIzol reagent (Invitrogen,
316 Carslbad, CA, USA). Then, cDNA was synthesized using the Toyobo reverse-transcription kit
317 (Toyobo, Osaka, Japan). Eleven genes related to hair and/or spine development were selected
318 for analysis based on functional annotation data, and fluorescent qPCR primers were designed
319 accordingly (Supplementary Table 2). RT-qPCR was performed in a 20-μL reaction volume,
320 with four technical replicates for each sample, using the TransStart® Top Green qPCR
321 SuperMix kit (TransGen Biotech, Beijing, China). Relative gene expression levels were
322 analyzed using the $2^{(-\Delta\Delta CT)}$ method (Bustin *et al*. 2010). PCR conditions were as follows:
323 pre-denaturation at 95 °C for 10 min; 40 cycles of 15 s at 95 °C (denaturation), 30 s at 58 °C
324 (annealing), and 20 s at 72 °C (extension); and a final melting curve stage from 60-95 °C to

325 verify the specificity of the amplicons.

326

327 **Phylogenetic analysis**

328 *KRT1* sequence authenticity was verified by BLAST search in GenBank (Supplementary Table
329 5). Sequences were edited using MAFFT v 6.81b [36] and Mesquite [37]. MRMODELTEST
330 v.2.3 [38] was used to select the best-fit model of nucleotide substitution under the Akaike
331 information criterion (AIC) [39]. Bayesian inference of phylogeny was performed using
332 BEAST v 1.6.1 [40] with default settings except for GRT+I+G model. An uncorrelated relaxed
333 clock fixed to lognormal distribution was employed as the site model using a Yule speciation
334 tree prior sampled every 10,000th generation for 100 million generations. Effective sample
335 seizes (ESS) were verified using Tracer v1.5 and a consensus tree was constructed in
336 TreeAnnotator v1.6.1 with 20% burn-in. For the concatenated dataset, all parameters were
337 estimated independently for each partition and displayed using FigTree v1.4.2.
338 (http://www.geospiza.com/finchtv).

339

340 **Abbreviations**

341 *A. albiventris, Atelerix albiventris*; Hiseq, high-throughput sequencing; PCA, principal
342 component analysis; KRT, keratin; FGF, fibroblast growth factor; SHH, sonic hedgehog; BMP,
343 bone morphogenetic protein; DEGs, differentially expressed genes; RT-qPCR, quantitative real
344 time polymerase chain reaction; SOM, self-organizing feature map

345

346 **Declarations**

347 **Ethics approval and consent to participate**

348 All animal procedures were approved by the ethics committee for animal experiments at the
349 Guangdong Institute of Applied Biological Resources (reference number G2ABR20170523)

and followed basic principles.

Consent for publication

Not applicable

Availability of data and materials

Data analyzed in the current study are included within the article and its supplementary material. All unigene sequences from *A. albiventris* have been deposited in the GenBank Sequence Read Archive (SRA) under accession number PRJNA561241 for SUB6195278. We have uploaded supplemental material to figshare via the GSA Portal. Supplementary Table 1 contains the summary of sequencing statistics for the transcriptomes; Supplementary Table 2 contains the summary of primer information used in real-time PCR analysis; Supplementary Table 3 contains the differentially expressed genes between HS1N and HS1Y; Supplementary Table 4 contains the differentially expressed genes between HH1N and HH1Y; Supplementary Table 5 contains the species name and NCBI serial numbers of 19 additional species for phylogenetic analysis.

Competing interests

The authors declare that they have no competing interests or other interests that might be perceived to influence the results and/or discussion reported in this paper.

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Authors' contributions

H. Li and J. Chen designed research; H. Li analyzed data and wrote the report; B. Yang developed software necessary to perform and record experiments; L. Li, X. Zhang, H. Ahmad, and H. Jiang provided expertise and advice on computational analysis; all authors edited the report.

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490 **Tables, figures, supplementary tables**
491
492 **Figure 1 Phenotypes of skin appendages from 3 developmental stages of *Atelerix*
493 *albiventris***. (a) Different phenotypes from 3 different developmental stages of *A. albiventris*;
494 (b) Principal components analysis (PCA); (c) Gene expression-specific and phenotype-specific
495 gene-trait correlation analysis based on self-organizing feature map module analysis.
496
497 **Figure 2 Gene Ontology classification of assembled unigenes in *Atelerix albiventris*.**
498
499 **Figure 3 KEGG classification of assembled unigenes in *Atelerix albiventris*.** (A) cellular

500 processes; (B) environmental information processing; (C) genetic information processing; (D)
501 metabolism, (E) organismal systems.
502
503 **Figure 4 Differential expression analysis of hair- and spine-type tissues in *Atelerix*
504 *albiventris*.** Volcano plot of DEGs for (a) 'HH1N vs HS1N', (b) 'HH1Y vs HS1Y', (c) 'HH5
505 vs HS5'. (d) Venn diagram showing co-DEGs among different tissues in *Atelerix albiventris.*
506 DEGs, differentially expressed genes.
507
508 **Figure 5 Quantitative real-time PCR analysis**. (a) qPCR confirmation of 10 DEGs identified
509 by RNA-seq in stage III tissues; (b) Relative expression levels of 10 DEGs in tissues at 3
510 developmental stages. Bars represent the relative expression levels of unigenes in stage III
511 tissues normalized with respect to the internal control GAPDH. Error bars represent the
512 standard error of three biological replicates. DEGs, differentially expressed genes.
513
514
515 **Figure 6 KEGG and GO enrichment analysis of DEGs related to differentiation of KRT1**
516 **gene**. (a,b) Directed acyclic graph related to skin appendage development and differentiation
517 in GO enrichment analysis. (c) Dot plots of enriched KEGG pathways. (d) Multiple sequence
518 alignment of *KRT1* gene from 20 species. (e) Bayesian phylogenetic tree with *KRT1* sequences
519 from 20 species. DEGs, differentially expressed genes.
520
521 **Figure 7 KEGG and GO enrichment analysis of DEGs related to immunity**. (a,b) up- and
522 downregulated KEGG signaling pathways and key genes related to immunity. (c,d) up- and
523 downregulated GO terms and key genes related to immunity. DEGs, differentially expressed
524 genes.

525

526 **Table 1 Summary of functional annotations of assembled unigenes with public protein**

527 **databases**

528

529 **Table 2 Candidate pathways and genes related to development and differentiation of**

530 **spine and hair**

531

532 **Supplementary Table 1 Sample IDs for transcriptome sequencing**

533

534 **Supplementary Table 2 Summary of primers for real-time PCR analysis**

535

536 **Supplementary Table 3 Differentially expressed genes between HS1N and HS1Y**

537

538 **Supplementary Table 4 Differentially expressed genes between HH1N and HH1Y**

539

540 **Supplementary Table 5 NCBI accession numbers of *KRT1* sequences from 19 species**