Hair Growth and Skin in the Kinky-Coat (*kc*) Musk Shrew, *Suncus murinus*

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Abstract: Hair growth and skin structure were examined in kinky coat (*kc*) musk shrews, *Suncus murinus*. The first hair growth cycle and the development of hair bulbs were normal. Histological characteristics of hair follicles and bulbs were similar in *kc/kc* and normal *+/kc* shrews. The mean thickness of epidermis and dermis did not differ significantly between the *kc/kc* and *+/kc* shrews. And no histological abnormalities were observed in the epidermis, dermis and hypodermis of the *kc/kc* shrews. Consequently, it is clear that the *kc* mutation had no effects on the hair growth and skin structure, but caused hair shaft modifications as previously observed. These characteristics of the *kc* mutation were quite distinct from those of the previously reported rodent mutations causing complicated abnormalities in the hair and skin.

Key words: hair growth, kinky coat, skin, *Suncus murinus*

Introduction

A number of mutants that affect hair and skin are known in laboratory rodents, such as mice and rats [4, 7]. Most of these mutants show complicated phenotypes that are attributed to the pleiotropic effects of the mutant genes [4, 7]. Relationships between the expression of the genes and the phenotypes have not been revealed. Recently a kinky coat mutation controlled by a single autosomal recessive gene (symbol *kc*) has been found in the musk shrew, *Suncus murinus* [8]. In external appearance, *kc/kc* homozygotes are characterized by curly vibrissae, somewhat unkempt coat hair and wavy tail hair. Under the light microscope and scanning electron microscope, shafts of the vibrissae, coat hair and tail

hair have structural abnormalities such as swellings, longitudinal fissures, twists and hollows. The hair structure of the *kc* mutation does not appear to resemble any of the rodent mutations mentioned above [4, 7], but histological differences or similarities between the shrew and the rodent mutations have not been examined.

In the present study, we observe the hair growth and skin structure of *kc/kc* musk shrews to characterize the *kc* phenotype in more detail, because a previous study has been restricted to the study of the hair shaft as mentioned above [8]. We also show that the *kc/kc* shrew may be useful as an animal model for studying the mechanisms of genetic control of hair growth, because the shrew histologically has a simpler phenotype than rodent mutations of this kind.

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Materials and Methods

Shrews used were 54 *kc/kc* homozygotes and 54 normal littermates from crosses between *+kc* and *kc/kc* shrews or crosses between two *kc/kc* shrews of the BAN-*kc* strain. Under ether anesthesia, samples of skin (5 to 10 mm²) were taken at 2-day intervals from birth to 10 days, at 3-day intervals from 13 to 25 days and at about 4 months. The skin was excised from the lateral sides of the snout, the middorsal region of the back and the proximal dorsal region of the tail of each shrew. Paraffin sections (8 μ m) were stained with hematoxylin and eosin (HE) according to standard procedures.

The first hair growth cycle of shrews was determined according to the criteria used for mice [1–3]. The first hair growth cycle consists of three stages: Anagen, the growing phase; Catagen, the transitional phase from growing to resting; and Telogen, the resting phase during which the fully formed hair is retained in the follicle. The Anagen is further subdivided into six substages: Anagen I, the period when cells of the germ plate go into cell division; Anagen II, the period when the germ plate grows down around the dermal papilla, and the first keratinization and the internal root sheath occurs; Anagen III, the period when the follicle attains its maximum length due to continued proliferation in the external root sheath and the germ region, the bulb is completely formed, and the first melanocytes appear; Anagen IV, the period when the hair is being formed and the internal root sheath is at about the level of the sebaceous gland; Anagen V, the period when the tip of the hair has broken through the tip of the internal root sheath and is at about the level of the epidermis; and Anagen VI, the period when the hair emerges beyond the surface of the skin and the hair growth continues. Detailed explanation of these stages is given elsewhere [1–3].

Maximum length and width of the bulbs of vibrissae, coat hairs and tail hairs were measured on 5 HE-stained sections per shrew with a micrometer. Thickness of epidermis and dermis was also recorded on 10 sections per shrew in the same way. The means of these values were used as measurement data for a shrew.

Results

Vibrissae (sinus hair shown in Figs. 3a and 3b) re-

mained the Anagen VI of the first hair growth cycle (Fig. 1a). Neither Catagen nor Telogen vibrissae were observed in adult shrews (data not shown). Overhair (see Figs. 3c and 3d), which was previously classified as Type A hair [8], and tail hair (long hair in Figs. 3e and 3f) were between Anagen V and Anagen VI at birth, in Anagen VI from 2 to 10 days, in Catagen at about 13 days, and by 25 days in Telogen (Figs. 1b and 1c). Underhair, which was previously classified as Types B and C hairs [8], was between Anagen III and Anagen VI from birth to 4 days, in Anagen VI from 6 to 10 days, in Catagen at around 13 days, and in Telogen by 25 days (Fig. 1d). For adult shrews, the overhair and underhair were roughly in Anagen V, Anagen VI or Telogen of the second or later hair growth cycle, whereas the tail hair was mainly in Telogen (data not shown). There were no clear differences in growth cycles of the respective hair types between *kc/kc* and *+kc* shrews and also between the sexes.

The mean length and mean width of bulbs of vibrissae, overhair, underhair and tail hair did not differ significantly between *kc/kc* and *+kc* shrews (*t*-test or Cochran-Cox test, $P < 0.05$) at each day (Fig. 1). Except for vibrissae, the bulb sizes of the other hairs increased rapidly during Anagen, but decreased rapidly through Catagen to Telogen (Figs. 1b, 1c and 1d). In contrast, the bulb of the vibrissae grew exponentially by around 10 days, and after that its growth reached a plateau (Fig. 1a). These growth patterns of respective hair bulbs were nearly identical in *kc/kc* and *+kc* shrews (Fig. 1).

The bulb of vibrissae was nearly round in shape, whereas those of overhair, underhair and tail hair were oval (Fig. 3). The external root sheath, stained purple by HE, was composed of several cell layers of vibrissae and tail hair, and one or two cell layers of overhair and underhair. The internal root sheath stained pale red by HE reached the middle region of dermis for respective hairs. These and the other histological characteristics of the follicles and bulbs for each hair were not clearly distinguished between *kc/kc* and *+kc* shrews and also between the sexes.

There were no significant differences in the mean thickness of snout, back and tail skin (epidermis and dermis) between *kc/kc* and *+kc* shrews (*t*-test or Cochran-Cox test, $P < 0.05$) each day (Fig. 2). The thickness of epidermis from respective body parts appeared to be constant at all ages, but the thickness of the der-

Fig. 1. Changes in length and width (mean $\pm$ SE) of vibrissa (a), tail hair (b), overhair (c), and underhair (d) bulbs from *+/kc* and *kc/kc* musk shrews during hair development. Hair growth cycles for respective hairs are shown on the horizontal axes (An III to An VI: Anagen III to Anagen VI, Ca: Catagen, Te: Telogen). Open circles and open squares are *+/kc* shrews, closed circles and closed squares *kc/kc* shrews. Sample size is for 5 individuals at each day from zero to 22 days after birth and 3 individuals at 25 days.

mis increased rapidly from birth to about 10 days for snout and from birth to about 6 days for back and tail, and after that the growth of the dermis from each body part ceased. The growth patterns of the epidermis and dermis were quite similar in *kc/kc* and *+/kc* shrews (Fig. 2). The period of the rapid growth phase of the dermises was nearly identical that of the bulbs from respective body parts (Figs. 1 and 2).

No abnormalities were observed in the horny layer (stratum corneum) of epidermis from *kc/kc* and *+/kc* shrews. Also the dermis and hypodermis of both genotypes did not show any abnormalities (Fig. 3). In addition, no sex differences in skin structure were observed for either genotype.

Discussion

The hair growth system is complex [6, 13, 14], but the subsequent process of hair growth has been clarified. The dermal papilla in the hair bulb plays a very important role in hair growth, as Hardy [6] and Trigg [14] have mentioned. In hair follicle initiation, the hair plug formation is induced by the first dermal message from the dermal mesenchymal cells from which the dermal papilla appears to be formed by responding to the epidermal message. The second dermal message is transmitted from the dermal papilla to the hair matrix cells. This message stimulates the matrix cells to differentiate into the internal root sheath, cuticle, cortex and medulla [6]. In addition, during normal hair growth, the internal root sheath has many functions, such as

control of the outward movement of the hair shaft and maintenance of a constant calibre in the developing shaft [14]. Thus an alteration in these aspects causes abnormal hair development. For example, the short and thin hair of a poor coat mouse results from an abnormality in the hair matrix [9]. The thin hair, the lack of hair type characteristics and the short period of the first hair growth cycle in a fuzzy (*fz*) mouse are due to abnormal development of the dermal papilla [14]. The waved phenotypes in waved-1 (*wa-1*), rex (*Re*) and wavy coat (*Re^{wc}*) mice appear to be caused by the defects in the internal root sheath function [14]. Furthermore, the effect of the ichthyosis (*ic*) mouse

mutation on the skin and hair is exerted through action in the epidermis [5].

A previous study reported that *kc/kc* shrews externally have curly or wavy hair on the snout, back and tail and microscopically have structural modifications in the hair shafts, but the length and width of the shafts do not differ significantly between *kc/kc* and *+kc* shrews [8]. The present study showed that the first hair growth cycle and the developmental patterns of hair bulbs are quite normal in the *kc/kc* shrews (Fig. 1). Moreover, it seemed that hair follicles and bulbs, especially the internal root sheath and dermal papilla, in the *kc/kc* shrews do not have any histological abnormalities (Fig. 3). These facts suggest that the follicles and bulbs of the *kc/kc* shrews have the functions essential for normal hair development.

In addition, there were no significant differences in thickness of epidermis and dermis between *kc/kc* and *+kc* shrews (Fig. 2). And no histological abnormalities were observed in the epidermis, dermis and hypodermis of the *kc/kc* shrews (Fig. 3). These indicate that the *kc* gene does not directly act on the skin of these shrews.

Consequently, it is clear that the *kc* gene has no pleiotropic effects on either hair growth or skin structure, but exerts its effect only on the hair shaft as previously observed [8]. This simple phenotype of the *kc* mutation was quite distinct from the complicated phenotypes observed in the hair and skin of the rodent mutations previously reported [4, 7, 14]. Thus, the *kc/kc* shrew may be valuable as an animal model for studying the mechanisms of genetic control of hair growth.

The structural component proteins of hairs are the hard keratins, which are different from the soft keratins of epidermis [10, 13]. The hair keratins are divided into two groups, the intermediate filament proteins (IF) and the intermediate filament associated proteins (IFAP) [10, 13]. In sheep, over 60 genes encoding the IF and IFAP are known [10]. Powell and Rogers reported that in transgenic mice with many copies of the sheep wool keratin IF gene, the hair produced is wavy and tends to break off at the base of the hair [11]. They concluded that these characteristics of the transgenic hair containing increased IF and decreased IFAP are caused by disruption of the normal ratio of IF and IFAP [11]. Moreover, the fragile hair of a naked (*N*) mouse contains a reduced IFAP [12]. Thus, hair abnormalities

Fig. 2. Changes in thickness (mean $\pm$ SE) of epidermis and dermis on the snout (a), back (b), and tail (c) of *+kc* and *kc/kc* musk shrews. Open circles and open squares are *+kc* shrews, closed circles and closed squares *kc/kc* shrews. Sample size is shown in the legend to Fig. 1.

Fig. 3. Light micrographs of skin from $+/kc$ and kc/kc musk shrews. (a) and (b): Snout skin from newborn shrews, showing the follicles of vibrissae (sinus hair) in Anagen VI of hair growth cycle (arrows). (c) and (d): Back skin from 2-day-old shrews, indicating the follicles of overhair in Anagen VI (arrows) and the follicles of underhair. (e) and (f): Tail skin from newborn shrews, showing the follicles of tail hair (long hair) in Anagen VI (arrows) and the small follicles of short hair. B: blood sinus with cavernous tissue, D: dermis, E: epidermis, H: hypodermis, I: inner root sheath, O: outer root sheath, and P: pilomotor muscle. (a) $\times 150$, (b) $\times 120$, (c, d) $\times 180$, (e, f) $\times 120$.

observed in the *kc/kc* shrews may be caused by partial defects or abnormal synthesis of the hair keratins. On the other hand, human nails are composed of about 85% hard keratins and about 15% soft keratins [13]. Although the claws of the *kc/kc* shrews externally appear to be normal, it is unknown whether the hard keratins of the shrew claws are abnormal for synthesis. Analysis of the shrew hair and claw keratins is awaited in order to understand the sites of *kc* gene action.

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