Ultrastructure of the Dorsal Skins of Hairless Descendants Derived from Mexican Hairless Dogs

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Abstract: Ultrastructural characteristics of the dorsal skin of hairless descendants derived from Mexican hairless dogs (3-month-, 1-year- and 5-year-old) were investigated and compared with those of age-matched beagles. Except that beagles had no melanocytes in epidermis, there was no essential difference in ultrastructural characteristics of epidermis between hairless dogs and beagles. In the Stratum basale of epidermis of hairless dogs, a small number of melanocytes and a few Langerhans cell (LC)-like cells were observed among keratinocytes. Melanocytes contained many melanosomes and a few premelanosomes. LC-like cells were characterized by electron-lucent cytoplasm and lobulated nucleus and lacked melanin granules, tonofilaments and desmosomal attachments. Unlike LC in humans and rodents, these LC-like cells did not contain Birbeck granules. Although age-related ultrastructural changes of dorsal skin were generally not clear both in hairless dogs and in beagles, cells containing melanin granules were not infrequently observed among dermal collagen bundles in 5-year-old hairless dogs.

Key words: dorsal skin, hairless dog, ultrastructure

Kimura *et al.* [5] have shown that an autosomal dominant gene (*Hm*: hairless, Mexican type) is responsible for the hairless characteristics of Mexican hairless dogs (MHDs), and they have established a colony of experimental hairless dogs by backcrossing (BC) male hairless F_1 hybrids between a male MHD and female beagles to female beagles. Now, a colony consisting of F_1 and BCF_1 to BCF_5 hybrids is maintained in the Research Center, Nihon Nosan Kogyo Co., Ltd., Tsukuba, Japan. The hairless dogs possess melanocytes in their epidermis and develop melanin deposition in response to solar

ultraviolet irradiation [6], and therefore this dog seems to be useful in the field of dermatological science such as photobiology. Recently, light microscopic characteristics of the dorsal skin of hairless dogs have been reported by Kimura and Doi [7]. As one of a series of studies to analyze the morphological characteristics of the skin of this hairless dog, this report mainly describes the ultrastructural findings of the skin.

Male hairless descendants of MHD and male beagles were examined. According to their ages, they were divided into 3 groups; 3 months old (3 hairless dogs,

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BCF₄, and 3 beagles), 1 year old (3 hairless dogs, BCF₃), and 5 years old (3 hairless dogs, F₁, and 3 beagles). Under local anesthesia with 2% lidocaine, skin specimens were obtained from the dorsal skin of each dog using a 6-mm biopsy punch (Nagatoishi Co., Ltd., Tokyo). For light microscopic study, a part of each skin specimen was fixed in 10% neutral buffered formalin, and 4 μ m paraffin sections were stained with hematoxylin and eosin and by Fontana-Masson's method. For electron microscopic study, the remaining specimens were cut into small pieces, fixed in 2.5% glutaraldehyde and 2.0% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4), postfixed in 1.0% osmium tetroxide in the same buffer, and embedded in epoxy resin (Quetol 812, Nisshin EM Co., Ltd., Tokyo). Semithin sections were stained with toluidine blue. Ultrathin sections were double stained with uranyl acetate and lead citrate and observed under a JEM-1200EX electron microscope (JEOL Co., Ltd.,

Tokyo).

Light microscopic findings of the dorsal skin of hairless dogs were almost similar to those reported by Kimura and Doi [7]. Briefly, the epidermis of 3-month-old hairless dog was thick, and the border between epidermis and dermis was wavy. In addition, epidermal ingrowths, which are considered to be hair germ or hair plug, were prominent. The epidermis became thinner and flatter, and epidermal ingrowths almost disappeared at 1 or 5 years old. A small number of hair follicles and sebaceous glands were detected regardless of the age.

In this study, in addition to these findings, an age-related change in the distribution of melanin granules in the epidermis of hairless dogs was observed. Namely, melanin granules were sporadically distributed mainly in the *Stratum (St.) basale* and epidermal ingrowths at 3 months old (Fig. 1A). Melanin granules tended to be spread over the whole length of the *St. basale* and *St.*

Fig. 1. Dorsal skin of a 3-month-old (A) and a 5-year-old hairless dog (B) and of a 5-year-old beagle (C). Melanin granules are seen in the epidermal ingrowth (A), in the whole length of *St. basale* and *St. spinosum* (B), and in no layer of epidermis (C). A and B: Fontana-Masson's method, $\times 150$, C: HE $\times 60$.

spinosum with advancing age (Fig. 1B). This finding is interesting because the number of DOPA-positive melanocytes in the epidermis of hairless dogs rather decreased with advancing age [7], and, as described later, there were no remarkable age-related changes in melanocyte ultrastructure. It has been reported that lysosomes in keratinocytes play an important role in melanin degradation [4]. From this point of view, it suggests that a decrease in lysosomal activity and a delay in turnover of keratinocytes have involved in this age-related melanin deposition.

As previously described by Kimura and Doi [7], age-related changes of the dorsal skin of beagles were not prominent, and their skin had a thin epidermis and well-developed hair follicles with associated glands (Fig. 1C). Melanin granules were detected only in the hair roots.

Electron microscopically, the epidermis of dorsal skin of hairless dogs was composed of *St. basale*, *St. spinosum*, *St. granulosum* and *St. corneum*, but *St. lucidum* was not detected (Fig. 2). Age-related changes were not clear in the ultrastructure of epidermis.

Keratinocytes had prominent tonofilaments in their cytoplasm, and in *St. basale*, they were bound to the basement membrane by hemidesmosomes (Fig. 2). In *St. basale*, melanocytes were seen among keratinocytes and they were abundant in epidermal ingrowths of 3-month-old hairless dogs. In addition, a few Langerhans

cell (LC)-like cells were observed in *St. basale*.

Melanocytes had almost the same ultrastructural features with those of humans [2]. Namely, they contained many melanosomes and a small number of premelanosomes and had cytoplasmic processes (Fig. 3). But, they were devoid of desmosomes and tonofilaments.

LC-like cells had electron-lucent cytoplasm, lobulated nucleus and cytoplasmic processes (Fig. 4). They lacked melanin granules, desmosomal attachments and tonofilaments. Moore and Mariassy [10] reported that Birbeck granules, which characterize LC of humans [1, 12] and rodents [3, 8, 11], were not observed in mongrel dogs. LC-like cells in the hairless dogs, also, did not contain any Birbeck granules. Similar LC-like cells were reported in the epidermis of hairless micropigs [9].

In *St. spinosum*, keratinocytes were tightly connected with each other by desmosomes, and contained some melanin granules in their cytoplasm in 1- and 5-year-old hairless dogs (Fig. 5). Keratohyalin granules were observed in *St. granulosum*.

In the dermis, cells containing melanin granules were observed beneath the epidermal ingrowths and among collagen bundles (Fig. 6). Most of them appear to be melanophages engorging a large number of melanin granules, and the remaining ones may be melanocytes dropped from epidermal ingrowths. In addition, small

Fig. 2. Dorsal skin epidermis of a 3-month-old hairless dog. Keratinocytes are bound to the basement membrane by hemidesmosomes (arrowheads in Inset). $\times 1,700$, Inset $\times 9,400$

Fig. 3. Melanocyte of a 3-month-old hairless dog. Melanosomes (arrow) and premelanosomes (arrowheads in Inset) are seen in cytoplasm. $\times 2,100$, Inset $\times 11,100$.

Fig. 4. Langerhans cell-like cell of a 1-year-old hairless dog. $\times 4,800$.

Fig. 5. Keratinocytes of a 5-year-old hairless dog. They are tightly connected with each other by desmosomes (arrowheads in Inset), and contained some melanin granules in their cytoplasm. $\times 2,100$, Inset $\times 7,400$.

Fig. 6. Dermal cells containing melanin granules among collagen bundles. $\times 3,400$.

Fig. 7. Cholesterol clefts and small aggregations of ribosome-like granules (arrowheads) in epithelial cell of sebaceous gland in a 5-year-old hairless dog. $\times 3,800$. Inset shows high magnification of a small aggregation of ribosome-like granules. $\times 10,900$.

aggregations of ribosome-like granules (approximately 25 to 30 nm in diameter) making up crystal-like array and cholesterol clefts were sometimes observed in epithelial cells of the sebaceous glands (Fig. 7). This finding was observed only in 5-year-old hairless dogs, and although its meaning is not clear, this may be one of the age-related changes in the sebaceous epithelial cells of hairless dogs.

On the other hand, the ultrastructure of epidermal constituent cells of beagles was almost similar to that

of hairless dogs, except that melanocytes were found only in hair roots in beagles.

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