Dermal Histology of Hairless Rat Derived from Wistar Strain

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Abstract: The dermal histology of 9-week-old hairless rats derived from Wistar rats was investigated and compared with that of age-matched Wistar rats. Skin samples were taken from 4 portions: the head, dorsal and ventral skin, and foot pad. The epidermis of the 3 portions other than the foot pad was thicker in hairless rats than in Wistar rats. The hair canals of hairless rats contained lamellar cornified tissues instead of hair, and some of the hair follicles deep in the dermis exhibited cyst formation. S-100-immunoreactive cells were detected in the basal layer of epidermis and their number in hairless rats was greater than that in Wistar rats. Ultrastructurally, electron-lucent cells, i.e. Langerhans cells, which contained Birbeck granules and/or cored tubules, were observed more frequently in the epidermis of hairless rats than in that of Wistar rats. Some cells in one hairless rat, which had similar characteristics to Langerhans cells without Birbeck granules, contained confronting cisternae.

Key words: hairless, histology, Langerhans cells, skin, Wistar rats

Introduction

In rats, mutant recessive genes at many different loci such as *at*, *ba*, *fz*, *hr*, *n*, *nznu* and *rnu*, have been reported to influence hair follicle development and finally cause hypotrichosis or hairlessness [4, 6, 11, 13]. Nishimura *et al.* have reported the dominant mutant gene responsible for hairlessness which is fatal in the case of homozygosity [11]. Kondo's colleagues found a hairless mutant in a colony of rats of Wistar strain (Crj: Wistar) and their descendants (Fig. 1) have been maintained as an inbred strain in Japan SLC Inc., Shizuoka. An autosomal incomplete dominant gene

(*HI*) responsible for the hairless characteristics has been identified [12]. In this study, histological features of the skin of this animal were investigated and compared with those of age-matched Wistar rats, as a first step in a series of studies to analyze the characteristics of this rat strain.

Materials and Methods

Two 9-week-old hairless rats and two age-matched rats of Slc: Wistar strain (Wistar rats) were obtained from Japan SLC, Inc., Shizuoka, and used in this study. The animals were sacrificed by exsanguination under

(Received 28 February 1995 / Accepted 8 June 1995)

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Fig. 1. Appearance of a hairless rat derived from the Wistar rat.

ether anesthesia. Immediately after necropsy, skin samples were excised from the following 4 portions: head, dorsal and ventral skin, and foot pad. They were fixed in 10% neutral buffered formalin, and 4 μ m-paraffin sections were cut and stained with hematoxylin and eosin (HE). For immunohistochemical detection of S-100-immunoreactive cells [1], some sections of the dorsal skin of each animal were also stained by the labelled streptavidin-biotin (LSAB) method with an LSAB kit (DAKO Japan Co., Ltd., Kyoto). Anti-bovine S-100 rabbit serum was used as the first antibody, which reacted with A and B units of S-100 (Seikagaku Corporation Co., Ltd., Tokyo). Sections treated with normal rabbit serum instead of the above-mentioned first antibody served as controls. Small pieces of the dorsal skin of each animal were fixed with 2.5% glutaraldehyde, 2.0% paraformaldehyde in 0.05 M phosphate buffer (pH 7.4), postfixed with 1% osmium tetroxide in the same buffer and processed for electron microscopy. Ultrathin sections were double-stained with uranyl acetate and lead citrate, and observed under a JEOL 1200EX electron microscope (JEOL Co., Ltd., Tokyo).

Results

Light microscopic findings: The epidermis of the foot pad of both strains was similar, being composed of 6 to 10 cell layers (Figs. 2a and b). The epidermis on the other three portions of the hairless rats was composed of 4 to 6 cell layers and was thicker than that of Wistar rats (2 to 4 layers) (Figs. 2c and d). Stratum basale, spinosum and granulosum were clearly identified in

hairless rats. The hair canals of hairless rats contained lamellar cornified tissues instead of hair and were associated well-developed sebaceous glands (Fig. 2e). Some of the hair follicles deep in the dermis exhibited cyst formation with parakeratosis of the external root sheath cells (Fig. 2f). No melanin granules were observed in any part of the epidermis of either strain. S-100-immunoreactive cells were detected in the basal layer of the epidermis and their number in hairless rats was larger than that in Wistar rats (Figs. 2g and h).

Electron microscopical findings: In both strains, keratinocytes with prominent tonofilaments in cytoplasm were connected to each other with desmosomes, and granular cells which contained many keratohyalin granules were observed (Figs. 3a and b). Polyhedral stratum spinosum cells were identified more frequently in hairless rats (Fig. 3a). Compared with Wistar rats (Fig. 3d), external root sheath cells had keratohyalin granules in the apical cytoplasm (Fig. 3c) and showed signs of keratinization as suggested by the results of light microscopy. In the epidermis, Langerhans cells were detected. These cells were electron-lucent without tonofilaments and contained a lobulated nucleus and cytoplasmic process. They did not build up desmosomes between adjacent cells (Fig. 4a). Some of them had racket-shaped organella, i.e. Birbeck granules, and/or rod-shaped organella, i.e. cored tubules (Figs. 4b, c and d.). They were detected more frequently in hairless rats than in Wistar rats. Furthermore, in one hairless rat, some cells, which had similar characteristics to the above-mentioned Langerhans cells but lacked Birbeck granules, contained confronting cisternae. They comprised a pair of closely apposed cisternae and ribosome attached to the outer membrane (Figs. 4e and f). Some with a round terminal and containing an electron-dense core were also observed (Fig. 4f).

Discussion

The histological characteristics of hairless rats derived from rats of Wistar strain were clarified in this study.

The epidermis from 3 portions of hairless rats examined, but not that from the foot pad, was thicker than that of Wistar rats. In this connection, the thickness of the epidermis of athymic nude mice is similar to that of thymic mice [5]. Moreover, in hairless dogs, the epi-

Fig. 2. a. Epidermis of the foot pad of a hairless rat. HE. $\times 110$.
 b. Epidermis of the foot pad of a Wistar rat. HE. $\times 110$.
 c. Epidermis of the dorsal skin of a hairless rat. HE. $\times 110$.
 d. Epidermis of the dorsal skin of a Wistar rat. HE. $\times 110$.
 e. Epidermis of the dorsal skin of a hairless rat. The hair canal contains lamellar cornified tissue. $\times 110$.
 f. Dermis of the dorsal skin of a hairless rat. Cyst formation with parakeratosis. $\times 110$.
 g. Epidermis of the dorsal skin of a hairless rat. S-100-immunoreactive cells (arrowheads) are seen in the basal layer. LSAB method. $\times 220$.
 h. Epidermis of the dorsal skin of a Wistar rat. Hardly any S-100-immunoreactive cells are seen. LSAB method. $\times 220$.

Fig. 3. a. Keratinocytes in the dorsal skin of a hairless rat. $\times 2,800$.
b. Keratinocytes in the dorsal skin of a Wistar rat. $\times 3,200$.
c. Hair follicle in the dorsal skin of a hairless rat. $\times 2,400$.
d. Hair follicle in the dorsal skin of a Wistar rat. $\times 4,000$.

Fig. 4. a. Epidermal electron-lucent cell (Langerhans cell) in the dorsal skin of a hairless rat. $\times 8,000$.
 b. High magnification of the part indicated by an arrowhead in Fig. 4a. Birbeck granule. $\times 56,000$.
 c. High magnification of the part indicated by an arrow in Fig. 4a. Cored tubule. $\times 56,000$.
 d. High magnification of the part indicated by a double arrow in the same cell of the other serial section in Fig. 4a. Cored tubule fuses to plasma membrane. $\times 46,900$.
 e. and f. Confronting cisternae in electron-lucent cells in the dorsal skin of a hairless rat.
 e. $\times 56,000$. f. $\times 37,500$.

dermis of pups is thick and, as the dogs grow older, it becomes thinner [7].

Abnormal keratinization and cyst-formation of hair follicles have been reported in hypotrichotic or hairless mutants of mice and rats and the features observed in this study are very similar to those reported in athymic nude mice [14] and hypotrichotic Norway rats [13]. The results of this study indicate that the formation of cysts is associated with either the widened/hyperplastic hair canals or with epithelial units of disorganized hair

follicles stranded in dermis, as suggested in rhino mice [10].

Albinism in rodents is characterized by the complete absence of melanin pigments even though melanocytes are present [2]. It was impossible to identify melanocytes without melanin pigment, i.e. amelanotic melanocytes, by ultrastructural observation in this study, because there are no special criteria of this type of cell.

The frequency of S-100-immunoreactive cells seen in light microscopy and of Langerhans cells in electron

microscopy was higher in hairless rats than in Wistar rats, although it is possible that the subpopulation of S-100-immunoreactive cells is composed of both Langerhans cells and the above-mentioned amelanotic melanocytes [1]. Kobayashi and Hoshino have reported that the Langerhans cells containing both Birbeck granules and cored tubules seen in this study are found after the injection of sterile saline into the skin of mice although cored tubule-containing cells occur separately from Langerhans cells with Birbeck granules [8]. In view of these findings, hairless rats may be more sensitive to stimuli such as microorganic or physical ones, resulting in easy activation of Langerhans cells. It is necessary to carry out further histochemical and morphometric studies on Langerhans cells and melanocytes.

Confronting cisternae are multilayered membranous formations derived from the nuclear envelope or rER and these have been seen in mitotic and interphase cells of many types, such as HeLa cells in culture, rat hepatoma, fibromyxosarcoma, and so on [3]. This is the first report stating that these structures have been seen in epidermal cells which have similar characteristics to Langerhans cells without Birbeck granules. This structure has also been reported in normal human lymphocytes incubated with *Staphylococcus aureus*, otherwise known as paired or double-layered cisternae [9]. This may be one indication that these cells are activated.

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