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Histological Characteristics of the Pelage Skin of Rough fur Mice (C3H/HeJ- *ruf/ruf*)

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Abstract: Pelage skin of C3H/HeJ mice homozygous at an autosomal recessive mutant locus, rough fur (*ruf*) which is located on chromosome 9, was histologically analyzed. Sebaceous glands synthesizing lipids were larger in the mutant mice than in controls in an examination by Sudan IV staining. Electron microscopic analysis of the sebaceous gland showed that lipid droplets were denser in mutant mice than in control mice, and that they were irregular in shape in *ruf* mice while those of controls were round. Our results suggested that rough fur (*ruf*) mice might be an animal model for hyperlipogenesis of the pelage skin.

Key words: animal model, hyperlipogenesis in sebaceous glands, rough fur (*ruf*) mice

A number of mutants that possess skin and hair abnormalities are known in laboratory animals, such as ferrochelatase deficiency (*Fech^{m1Pas}*) mice [1] with erythropoietic protoporphyria [2], ichthyosis (*ic*) mice with trichothiodystrophy [3], waved-1 (*Tgfa^{wal}*) mice with wavy hair [4, 5], SHL mice with apolipoprotein E deficiency [6], ragged (*rg*) rats with hyperlipogenesis [7] and musk shrews [8].

An autosomal recessive mutation was detected on C3H/HeJ inbred mice in 1985. This mutant strain has been maintained by forced heterozygosity as C3H/HeJ-*nm* 1304 and shows phenotypically abnormal hair. After mating experiments, this mutation was named rough

fur (*ruf*) and has been mapped to chromosome 9 [9]. The homozygous mice of *ruf* have typical abnormal appearance: the fur looks unkempt, wet, and hairs break off at 13 to 50 days after birth (Fig. 1). The above mentioned external appearances of *ruf* mice are supposed to be caused by abnormalities of skin structure and/or secretory glands, and the purpose of the present study was to clarify the histological features of the skin of *ruf* mice.

Three pairs of rough fur mutant mice were introduced from the Jackson Laboratory (Maine, USA) in 1990 and have since been maintained at the Research Institute of Environmental Medicine, Nagoya Univer-

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Fig. 1. C3H/HeJ-*ruf/ruf* (upper) and control (lower) mice at 30 days of age. The *ruf* mouse looks wet.

sity, Japan. The subject mice of this experiment were housed at $21.0 \pm 1^\circ\text{C}$, humidity $55 \pm 5\%$ under a 12 hr light-dark schedule. The mice were given a commercial diet (CE2, CLEA Co., Japan) and tap water *ad libitum*. Iodine-starch reaction test was performed according to the protocol described previously [10–12]. For light microscopic examination, mid-dorsal skin was fixed in Bouin's fixative solution for 12 hr, dehydrated with a graded alcohol series, and embedded in paraffin. Five-micrometer sections were stained with hematoxylin and eosin. Frozen sections were also prepared to examine the lipogenesis of the sebaceous glands. The skin tissue was fixed in 10% formaldehyde solution over two days, and hair was removed with scissors. The tissue was embedded in Tissue-tek (4583, Miles Inc., USA), quickly frozen, cut into 15- μm sections using a cryostat-microtome, and stained with Sudan IV. For electron microscopic examination, the skin was fixed in a mixture of 2.5% glutaraldehyde and 2% paraformaldehyde in 0.1 M phosphate buffer for 12 hr at 4°C . After rinsing in 0.1 M phosphate buffer (4% sucrose added, pH 7.4) for 1 hr, the tissue was postfixed in 2% OsO_4 in the same buffer for 2 hr, and finally stained with 2% uranyl acetate. The tissue blocks were then embedded in either Epon 812 or Quetol 812 after dehydration. Ultra-thin sections were further stained with uranyl acetate and lead citrate and observed using a transmission electron microscope (JEM-1210, JEOL Ltd., Japan) at 80kV.

From the external appearances, *ruf* mice were sup-

posed to have abnormalities of the skin structure and/or secretory cells in pelage skin. The appearance of the plucked hair from the mid-dorsal skin of *ruf* mice was compared to that of control mice at 30 days of age. A large number of lipid-like droplets were found to be stuck to the hairs of *ruf* mice under a stereomicroscope (Fig. 2B) while no such droplets were observed on the control hair. The hair of *ruf* mice was occasionally broken. Iodine-starch reaction test showed that either the sweating of both *ruf* and control mice was below the detection level or sweat glands were less on pelage skin (data not shown). Sweat glands also were not found in the pelage skin of either *ruf* or control mice. These results are consistent with Calabrese' report [13] that rodents and rabbits do not possess sweat glands in the pelage skin. Accordingly, the abnormalities of the skin structure in *ruf* mice are supposed to be in the sebaceous glands. Sudan IV-positive large masses of lipid were detected on the surface of the epidermis and in bigger sebaceous glands of *ruf* mice, while no epidermal lipid and smaller lipid masses in the sebaceous gland were seen in control mice (Fig. 2D). It clearly shows that hyper-sebum production in *ruf* mice is a new characteristic. Electron microscopic observation of the sebocytes showed that the density of lipid droplet in the cells was higher in *ruf* mice than in control mice. The lipid droplets in the sebaceous glands were irregular in shape in all ultra-thin sections of the *ruf* mice while those of controls were round (Fig. 2F). The structures of the lipid droplets, as shown in Fig. 2, were not altered when the lipid droplets were rinsed during the processing of thin sections with several solutions.

Animal models with inheritable hyperlipogenesis are known in ragged (*rg*) rats [7], Syrian hamsters [14, 15] and dogs [16]. However, Syrian hamsters and dogs reported in previous papers were not so suitable for genetic studies of animal disease models because they were not inbred and this problem was easily explained by John P. Sundberg [17, 18]. Although the external appearances of homozygous ragged rats are histologically very similar to those of *ruf* mice [7], there have been no further reports since that of Noguchi *et al.* On the other hand, opposite animal models, e.g., asebia (*Scd1^{ab}*), rhino (*hr^{rh}*), and downless (*Edar^{dl}*) mutations, were reported to be characterized by inherited small or absent sebaceous and meibomian glands, hyperkeratosis and alopecia [19–22]. These mutants might have

Fig. 2. Hairs from control (A) or *ruf* (B) mice at 30 days of age. Lipid-like drops can be seen on *ruf* hairs. Sudan IV staining revealed that sebaceous glands are larger in *ruf* mice (D) than in controls (C) at 30 days of age ($\times 50$, scale bar=0.1 mm). Electron micrographs ($\times 10k$, scale bar=1 μm) of sebaceous cells show that lipid granules in the cytoplasm of *ruf* mice (F) are irregular in shape; those of controls are round (E).

similarities to various human diseases in which there is a failure to metabolize branched-chain fatty acid, such as Refsum's disease, X-linked ichthyosis, psoriasis, and eyelid margin [23, 24]. Our study suggested that rough fur (*ruf*) mice might be an animal model for hyperlipogenesis of the pelage skin.

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