Amelanotic Melanocytic Tumors of the Pinna in Six F344 Rats

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ABSTRACT. Spontaneous amelanotic melanocytic tumors of the pinna were found in six females of 960 male and 960 female albino (F344/DuCrj) rats which had been used in three different 24-month chronic toxicity studies. The age when the pinna tumors were detected ranged from 37 to 59 weeks. The tumors were located unilaterally in the pinna and observed as subcutaneous spherical to irregular, solid white masses measuring 7 to 25 mm in diameter. The pinna tumors were histologically classified into spindle cell and pleomorphic cell types. The spindle cell type was observed in four rats and composed of fusiform cells arranged in interlacing bundles. The pleomorphic cell type was observed in the remaining two rats and composed of pleomorphic large cells arranged in sheets. One tumor of the latter type metastasized to the submaxillary lymph node and lung. Melanin pigments were not demonstrated in any of the tumors. In immunohistochemistry, nuclei and cytoplasm of tumor cells in all the tumors were slightly positive for S-100 protein. Ultrastructurally, tumor cells contained a considerable number of premelanosomes in the cytoplasm. Desmosomes were occasionally observed between the cell membranes of the adjacent tumor cells. No distinct basal lamina was seen around tumor cells.—**KEY WORDS:** F344 rat, melanocytic tumor, premelanosome.

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Melanocytic tumors usually are cutaneous tumors originating from epidermal or dermal melanocytes, but can occur wherever melanocyte clusters are found. Spontaneous melanocytic tumors are observed in various animal species [6] and human beings [9]. They are common in dogs, gray horses, and some lines of miniature pigs, but are uncommon in cats and sheep [6]. In rats, spontaneous melanocytic tumors are also uncommon [2, 13]. The majority of these tumors have been reported to occur in pigment-forming rat strains such as ACI, BN/Bi, and (WAG × BN)F1. On the other hand, in albino rats, the spontaneous occurrence of melanocytic tumors is extremely rare [8, 14]. This is probably due to the difficulty in diagnosing tumors without melanin pigments as melanocytic tumors in light microscopy, since melanin pigment production is absent in amelanotic melanomas.

One of the predilection sites in melanocytic tumors in rats with colored hair have been described to be the skin of the pinna aurium [2, 13, 14]. In albino rats, there has been only one report on the spontaneous occurrence of melanocytic tumors in the pinna aurium [14]. We found amelanotic melanocytic tumors in the pinna aurium of six albino rats used for long-term toxicity studies. The present report describes the histological, immunohistochemical and ultrastructural features of these tumors.

MATERIALS AND METHODS

Amelanotic melanocytic tumors of the pinna occurred in six females of 960 male and 960 female F344/DuCrj rats supplied by Charles River Japan, Inc. (Kanagawa). These rats had been used as treated or untreated animals in three different 24-month chronic toxicity studies (A, B, C). In these toxicity studies, rats were housed in groups of 5 in wire mesh stainless cages (310W × 400D × 230H) which were placed in barrier-sustained animal rooms controlled at: temperature, 24 ± 1°C; relative humidity, 55 ± 5%; and illumination, 12 hrs per day. They were given free access to a standard laboratory diet (HF Mash, Oriental Yeast Co., Ltd.) and tap water. Out of the six pinna tumors detected, one (0.6%) was found in 180 untreated control female rats and the remaining 5 (0.6%) in 780 treated ones. The incidences of the tumors in treated groups were comparable to those in untreated control groups and extremely low (0/80 to 1/80). The age when the pinna tumors were first detected in clinical observations varied from 37 to 59 weeks. Of these tumor bearers, two (one control and one treated rats) were subjected to interim kill based on the protocol of the toxicity studies, and the remainders were euthanatized at 108 weeks of age when the toxicity studies were terminated (Table 1). These rats in life showed no evidences of deterioration of

Table 1. History of F344 female rats with melanocytic tumor in the pinna

Case no.	Toxicity study	Treated/control	Age of death (weeks)	Age of tumor detection (weeks)	Tumor size (mm in diameter)	Metastasis	Cell type
1	C	Control	82	59	10	No	Spindle
2	B	Treated	82	55	8	No	Spindle
3	A	Treated	108	46	7	No	Spindle
4	A	Treated	108	42	10	No	Spindle
5	A	Treated	108	48	15	No	Pleomorphic
6	C	Treated	108	37	28	SLN ^{a)} , Lung	Pleomorphic

a) Submaxillary lymph node.

their health condition due to the tumor growth.

Various organs and tissues as well as macroscopic lesions of these rats were fixed in 10% neutral buffered formalin, routinely processed into paraffin sections, and microscopically examined on the hematoxylin and eosin-stained sections. For microscopic examinations, additional sections of the pinnal tumors and metastatic foci of the submaxillary lymph node and lung were stained with periodic acid-Schiff (PAS), periodic acid-methenamine-silver (PAM), Prussian blue, and a modified Warthin-Starry method for melanin pigment. A standard avidin-biotin technique was used to demonstrate immunohistochemically S-100 protein and desmin in the formalin-fixed, paraffin-embedded sections. Rabbit-derived polyclonal antibodies, anti-S-100 protein (Advance Co., Ltd., Japan) and anti-desmin (Dakopatts, Denmark) serums, were used and diluted 1:100 to apply. For electron microscopy, blocks (1 mm³) of the formalin-fixed tissue from each pinnal tumor were post-fixed in 1% phosphate buffered osmium tetroxide and embedded in Quetol 812. Ultrathin sections were stained with uranyl acetate and lead citrate, and were examined with a Hitachi H-600 transmission electron microscope.

RESULTS

The tumors were located unilaterally in the pinna aurium and observed as subcutaneous spherical to irregular masses measuring 7 to 25 mm in diameter, which were covered by the hairless skin. Ulceration and crust formation were common in the large masses. The cut surface of the masses was whitish, fine and firm. Only one (Case 6) of the six tumors metastasized and formed a well-demarcated white nodule of 25 mm in diameter in the submaxillary lymph node and a few white nodules less than 1 mm in diameter in the lung.

Histologically, each of the pinnal tumors was observed as a solid mass in the subcutis (Fig. 1) and grew in direct contact with the epidermis which sometimes became hyperplastic (Fig. 2). PAM stain often showed the partial destruction of the basement membranes in the epidermal junction by tumor cell invasion. All the tumors were sharply demarcated but devoid of capsules. The auricular cartilage was usually intact, even when it was involved within tumor tissues (Fig. 1).

The tumors were subclassified into two types: spindle cell type and pleomorphic cell type (Table 1). The former type was observed in four of the six cases and composed of fusiform cells arranged in interlacing bundles (Fig. 3). The fusiform cells had slightly pleomorphic oval nuclei with sparse chromatin and inconspicuous nucleoli. Their cytoplasm was eosinophilic, but cell margins were indistinct. Mitotic figures were uncommon and necrosis was not seen. In stroma, relatively abundant collagen and reticulin were found especially at the peripheries of

Fig. 1. Solid tumor mass in the pinnal subcutis (Case 4). H-E stain. $\times 34$.

Fig. 2. Spindle cell type of the pinna tumor (Case 4). Tumor cells grow in direct contact with the epidermis which shows partial hyperplasia. H-E stain. $\times 85$.

Fig. 4. Pleomorphic cell type of amelanotic melanocytic tumor of the pinna (Case 6). Fairly large, but varying-sized pleomorphic cells are arranged in sheets. H-E stain. $\times 340$.

Fig. 3. Spindle cell type of amelanotic melanocytic tumor of the pinna (Case 1). The tumor is composed of fusiform cells arranged in interlacing bundles. Tumor cells have slightly pleomorphic oval nuclei and eosinophilic cytoplasm, but cell margins are indistinct. H-E stain. $\times 340$.

Fig. 5. Electron micrograph of the cytoplasm of tumor cells (Case 3). Many premelanosomes, mitochondria with tubulo-vesicular cristae, well-formed Golgi apparatus, and a desmosome (arrow head) are seen. $\times 28,000$.

masses. In some areas of this type, the tumor cells with slender-oval nuclei were arranged in looser bundles with a minimal amount of stromal tissue. Focal necroses accompanied by macrophages laden with hemosiderin, focal nuclear palisading, and occasional mitoses were also present in these areas.

The pleomorphic cell type was observed in the remaining two cases including the case (Case 6) which had metastases. This type was composed of pleomorphic cells arranged in sheets or much looser

bundles with scant stromal tissue. The tumor cells were generally large and had abundant eosinophilic cytoplasm and fairly distinct cytoplasmic boundaries with irregular contours (Fig. 4). Nuclei were oval to irregular and had one to three conspicuous nucleoli.

Other histological characteristics of this type included massive necroses and frequent mitoses. The metastatic foci of the submaxillary lymph node and lung in Case 6 had the morphologic resemblance to the primary tumor.

Melanin pigments were not demonstrated in any of the tumors stained with a modified Warthin-Starry method. Immunohistochemistry revealed that nuclei and cytoplasm of tumor cells were slightly positive for S-100 protein in all the tumors but negative for desmin.

Ultrastructurally, both of the spindle cell and pleomorphic cell tumors were composed of cells with abundant cytoplasm and cytoplasmic protrusions. The cells were closely apposed to each other and there were occasional desmosomes between the cell membranes of the adjacent tumor cells (Fig. 5). Cytoplasmic protrusions of tumor cells were frequently seen in one of the six tumors (Case 1). In tumors of the spindle cell type, nuclei of the tumor cells were usually round or oval but often showed prominent invaginations, resulting in irregular profiles. In the pleomorphic cell type, majority of the tumor cells had larger cytoplasm and conspicuous

nucleoli, and their cytoplasmic protrusions were less distinct than those of the spindle cell type. The tumor cells of both cell types contained a considerable number of single membrane-bound premelanosomes which were spread or accumulated in the cytoplasm (Figs. 5 and 6). The premelanosomes were round or ellipsoid and contained internal structures such as longitudinally paralleled long filaments with or without transverse striations and randomly arranged short filaments (Fig. 6). There were also spherical, membrane-delineated vesicles considered to be immature type of premelanosomes in tumor cells. No melanized melanosomes were found in any of the tumor cells. Other characteristics of intracytoplasmic structures included the presence of well-formed Golgi apparatus and rough endoplasmic reticulum, many small vesicles and free ribosomes, and mitochondria with tubulo-vesicular cristae. In intercellular spaces, a small number of collagen fibrils were observed. No distinct basal lamina was seen around tumor cells.

DISCUSSION

Most spontaneous melanocytic tumors in rats with colored hair have been described to occur as tiny black spots or raised firm black masses of the skin [14]. Histologically, most of the tumor cells closely packed and were filled with dark-brown pigment granules which stained positively by the Fontana reaction for melanin. In immunohistochemistry, nuclei and cytoplasm of the tumor cells were slightly positive for S-100 protein. In large melanocytic tumors or in later stage of the tumor development, epithelial, spindle cell, schwannoma-type, and anaplastic areas were recognized even within the same tumor, and melanin producing activities were decreased in these areas [14]. There have been two papers reporting amelanotic melanocytic tumors occurring spontaneously in albino rats [8, 14]. Magnusson *et al.* [8] reported that 8 cases of intraocular melanomas were observed in treated and untreated albino rats (2310 males and 2310 females) from 6 different toxicity studies for a period of 2 years and two of them was diagnosed as amelanotic melanoma. However, the morphology of this amelanotic tumor was not described in detail. In the other report, an amelanotic melanocytic tumor was found in the pinna of a 37-month-old untreated female WAG/Rij albino rat [14]. This tumor was composed of spindle cells with faintly eosinophilic

Fig. 6. Electron micrograph of accumulated premelanosomes in the cytoplasm of a tumor cell (Case 4). They have various internal structures. $\times 28,000$. Inset: A premelanosome showing typical internal structures: paralleled long filaments with distinct transverse striations. Case 4. $\times 57,000$.

cytoplasm arranged in small bundles and showed predominant Schwann cell growth pattern. Regrettably, no ultrastructural examination was performed on the tumor. The histological and immunohistochemical findings of the spindle cell type in the present tumors are similar to those in the spindle cell areas of the melanocytic tumors in rats with colored hair or those in the amelanotic melanocytic tumor of a WAG/Rij albino rat.

Electron microscopic examinations on our cases revealed many premelanosomes within the cytoplasm of tumor cells. In melanocytes of mammalian species, four stages of melanosome development can be recognized in melanin production: stages I and II (unmelanized), stage III (partly melanized), and stage IV (completely melanized) [4, 11]. The melanosomes observed in the present study correspond to stage I or II melanosomes and melanin was not deposited in them. In electron microscopic studies performed on a pigment-containing melanocytic tumor in the skin of a (WAG/BN)F1 rat [14], unmelanized premelanosomes as well as many partly or completely melanized melanosomes have been observed in the cytoplasm of tumor cells. The ultrastructure of premelanosomes in our cases is quite identical to those in the pigment-containing melanocytic tumor. The findings of the present ultrastructural study suggest that tumors in our cases do not have an ability to synthesize melanin but produce stage I and II melanosomes. This might be related to the fact that melanin in the melanosomes is absent in melanocytes of the skin and hair follicles in albino animals [4].

The spindle cell type tumors in our cases had the morphological resemblance to well-differentiated schwannomas with Antoni type A pattern in rats [12]. In addition, melanosomes was also demonstrated in Schwann cells of normal human and rat skin [3], and melanotic schwannomas have been induced in Long-Evans rats by ethylnitrosourea [10]. The question may arise as to whether neoplastic melanosome-containing cells in the present cases originated from melanocytes or Schwann cells. In rats, normal Schwann cells [3] and neoplastic cells of well-differentiated schwannomas [1, 5, 7] are usually surrounded by a basal lamina. In addition, premelanosomes have not been confirmed ultrastructurally in spontaneous schwannomas in rats [1, 5, 7]. We did not find a distinct basal lamina around tumor cells in the present cases, and a considerable number of premelanosomes enough to be suggestive of the

characteristic feature of the tumor cells were observed in their cytoplasm.

Based on the findings in this study, the term "primary amelanotic melanocytic tumor" is considered to be appropriate for the six pinna tumors. These tumors were considered to be spontaneous since the incidences of them in chemical-treated groups were comparable to those in untreated control groups. The incidence of spontaneous melanocytic tumors in rats with colored hair such as BN/Bi [2] and ACI [13] rats has been described to be 2.3–4.1%. On the other hand, the incidence of pinna melanocytic tumors in albino (F344) rats examined in the present study is 0.3% (6/1920). The predilection sites in melanocytic tumors in pigment-forming rats have been described to be the skin of the genitalia, tail, and eye as well as the skin of the pinna [14]. In routine long-term toxicity studies, we occasionally encounter the spindle cell tumors resembling the present cases in these anatomical sites other than the pinna of albino rats. If these tumors are also examined electron microscopically, the incidence of melanocytic tumors in albino rats may be increased.

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