Case Report

PRIMARY HISTIOCYTIC SARCOMA OF THE EPIDIDYMIS IN B6C3F1 MOUSE

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Abstract: A case of histiocytic sarcoma developed primarily in the epididymis of B6C3F1 mouse at 109 weeks old was reported. Metastases were observed in the liver and adipose tissue of the abdominal cavity. Tumor cells showed atypism, pleomorphism and active erythrophagocytosis. Touton type giant cells, which were PAS positive after diastase digestion, were seen in the tumor. Electronmicroscopically, the tumor cells had many slender processes forming interdigitation with the adjacent cells. However, no junctional complex or basal lamina was observed in the tumor cells. Tubular autolysosomes in the cytoplasm were characteristic feature of tumor cells of the present case. The vacuoles containing finger print membranes similar to the structures observed in human eosinophilic granuloma of the bone were found in some of the tumor cells. The structures similar to Birbeck’s granule of the T-zone histiocyte were also observed.

This case seems to be the first report on histiocytic sarcoma developed in the epididymis of B6C3F1 mice. Since fibrohistiocytic proliferative lesion developed in the epididymis of B6C3F1 mice. Since fibrohistiocytic proliferative lesions were known to develop occasionally in the epididymis of B6C3F1 mice, differential diagnosis from this proliferative lesions is important. Further immunohistological analysis for elucidating the origin and pathogenesis of this neoplastic lesion will be needed. (J Toxicol Pathol 7: 95-102, 1994)

Key words: Histiocytic sarcoma, B6C3F1 mouse, Epididymis

Introduction

Histiocytic sarcoma in mice is known to develop, generally, in the liver of males and the uterus or liver of females. This tumor has been referred to a variety of synonym, such as reticulum cell sarcoma; type A (Dunn, T.B., 1954)1, endometrial sarcoma (Chouroulinkov et al., 1969)2, malignant schwannoma (Stewart, H.L., 1974)3, and histiocytic lymphoma (Frith, C.H., 1989)4. However, this tumor has been classified as histiocytic sarcoma by Pattengale, P.K. and Frith, C.H. in 19835. The characteristic clinical sign was an extremely large abdomen. According to literature, the mice bearing histiocytic sarcoma died so suddenly that the moribund mice were seldom obtained6. Gross findings of histiocytic sarcoma in the mice varied depending on the affected organs. The liver was usually enlarged 2 or 3 times more than normal size and often mottled. The spleen was also enlarged by increased hematopoiesis. The uterus showed a single, firm, whitish nodule in the lumen and often associated with hydrometra involving either one or both sides of the uterine horn. The reported primary sites of histiocytic sarcoma in mice were either of the liver, uterus, mesenteric lymph node, spleen or Peyer’s patch (Porta et al., 1979)7. Metastasis was observed frequently in the lung and liver, and less frequently, in the other organs including the epididymis. Histologically, the typical tumor cell had a dark nucleus rich in chromatin and abundant eosinophilic cytoplasm. Usually, there were
great variations in size or shape of the cell and nucleus and nuclear–cytoplasmic ratio. However, the tumor cells in the uterus were spindle in shape like the fibroblasts, arranged occasionally in fibrous fashion. Erythrophagocytosis occurred occasionally in both uterus and liver, but it was more common in the liver. Frith, C.H. (1980) studied in detail on occurrence, distribution, and morphology of histiocytic sarcoma of both C57BL/6 and BALB/C strain mice and on transplantation and tissue culture of them. He concluded that the tumor cell may be originated from a poorly differentiated mesenchymal cell, but should be classified as a histiocytic cell based on the cytological characteristics of containing the lysozomes and phagocytized cellular particles in their cytoplasm. This paper is probably the first case report on primary histiocytic sarcoma developing in the epididymis of B6C3F1 strain mouse and with metastases. The epididymis of this strain of mouse was known to show the development of a variety of tumors such as interstitial cell tumor, sarcoma, lipoma, hemangiosarcoma, malignant schwannoma, hemangioma, leiomyosarcoma and histiocigenic tumor.

**Case Report**

The present case was found in a B6C3F1 mouse of the medium-dose treated group in a 2-year-oncogenicity test. The mouse was purchased from SLC (Shizuoka, Japan) at 4 weeks old. Mice were housed individually in the environment that temperature (23±1°C) and humidity (55±5%) were controlled. Fluorescent lighting provided illumination 12 hours per day. Foods and tap water were supplied *ad libitum*. Commercial modified NIH Open Formula for Rat and Mouse was used as the basal diet. The mouse was sacrificed at 109 weeks of age. Although this case belonged to the treated group, no evident carcinogenicity of the test substance was observed in this study for 2 years.

**Material and Method**

The tissue specimens were fixed in 10% neutral buffered formalin and processed routinely and embedded in paraffin. The paraffin blocks were sectioned in 3μm. The sections obtained were stained with hematoxylin and eosin (HE) and a variety of special staining methods such as periodic-acid–Schiff (PAS) reaction, silver impregnation stain, Pearls method for iron pigment, Schmorl reaction, Gomori’s one step trichrome, Gmeline reaction and Iodine reaction for bile pigment. Moreover, immunohistochemical examination was performed with anti-PCNA antibody (Dako Corp.) and anti-bovine S-100 antibody (Dako Corp.). For electron-microscopical exam-

![Fig. 1. Monotonous proliferation of tumor cells. Nuclear pleomorphism and atypism were evident. Mitoses were frequent (arrowheads). HE (original magnification: ×400)](image)
The tissue specimen was prefixed in 2% glutaraldehyde 2.5% paraformaldehyde and postfixed in 1% osmium tetroxide in 0.1 M cacodylate buffer solution for 2 hours at 4°C. This material was processed routinely and embedded in the mixture of Epon 812 and Araldyte. The ultrathin section was made using an ultra-microtome and stained with 4% uranyl acetate and lead citrate. This section was examined with transmission electron microscopy Hitachi H-1000 at 75 kV.

**Gross findings**

A yellowish nodule measuring $22 \times 10$ mm in size was observed at the left cauda of the epididymis and white patch measuring 3 mm in size was also seen at the caput of that. There were a lot of white patches (each 1 mm in size) in the liver and also many red or brown patches in the peritoneum.

**Microscopic findings**

The nodule of the epididymis was his...
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Histologically composed mostly of monotonous proliferation of the histiocytic tumor cells (Fig. 1). The tumor cells had usually the pleomorphic nuclei having scant heterochromatin and were varied in size and shape, markedly. The nucleoli were indistinct. Mitotic figures were frequent. A variety of pigmentation such as deposits of hemosiderin, hematoidin and lipofuscin were observed depending on the area of the nodule. There were also observed erythrophagocytosis by tumor cells (Fig. 2), appearance of foamy cells and Touton type giant cells and proliferation of fibroblast-like cell (Fig. 3).

However, production of either reticulin fibers or collagen fibers in the tumor was generally scant, except the area, where the reticulin fibers show a boxing appearance as seen in the mesenchymal origin tumor. The giant cells reacted with a PAS reaction stain positively and this reaction was resistant to diastase digestion (Fig. 4). Multiple metastases were observed in both the liver and peritoneum. The tumor cells in the liver proliferated in a crawling fashion along sinusoid (Fig. 5).

The white patch observed at the caput of the
epididymis was a spermatic granuloma. Many of the histiocytic sarcoma cells in the epididymis and the metastatic areas were PCNA positive. S-100 protein was negative in the histiocytic sarcoma cells.

**Electron-microscopic findings**

Abundant lysozomes, many processes of the plasma membrane forming interdigitation with the adjacent cells and various vacuoles were the characteristic features of many of the tumor cells (Fig. 6). Tubular autolysosomes were prominent in some of the tumor cells (Fig. 7). Cytoplasmic filaments like actin filaments were also observed adjacent to the lysosomes. The width of the filaments were about 6 nm (Fig. 8). Most of
Fig. 8. Various secondary lysozomes and vacuoles. Some of the vacuoles contained finger print membrane (arrowhead). Stained by uranyl acetate and lead citrate. Bar=0.5 μm

vacuoles were without membrane. Moreover, the vacuoles containing finger print membrane were observed in some tumor cells (Fig. 8). In a part of solid proliferation, tumor cells lacked these findings described above, but contained a moderate number of mitochondria, ribosomes and rough endoplasmic reticulum. The basal lamina or intercellular junctional complex was not seen.

Discussion and Conclusion

Histiocytic sarcoma in mice occurring in the liver and uterus was confirmed that both tumor is identical cytologically (Frith, C.H., 1980)\(^4\). Histiocytic sarcoma occurring primarily in the liver of mice is considered to be derived from the Kupffer’s cell as those in the liver of rats and that of the uterus is from undifferentiated mesenchymal cell. This case of the epididymis in origin is considered to be derived also from the undifferentiated mesenchymal cell. Fibroblast–like component of fibrous xanthoma in human is generally considered to be derived from the facultative fibroblast of histiocytic origin as pointed out by Kauffman, S.L. and Stout, A.P. (1961)\(^{18}\). However, it may be derived from fibroblast or undifferentiated mesenchymal cell. Histiocytic sarcoma in human is likely to be associated with fixed macrophage\(^{14}\). Pure proliferation of histiocyte is thought to be rather rare in the field of human dermatology since the proliferating histiocytes are usually accompanied with the proliferation of other mesenchymal cell.

As for the primary site of histiocytic sarcoma, hepatic origin of histiocytic sarcoma is usually not so difficult to identify, because histiocytic sarcoma occurring in the liver shows a frequent metastasis within the liver, but seldom in the uterus even in females. Frith, C.H. (1990) emphasized that histiocytic sarcoma of the uterine–origin should be differentiated from stromal cell sarcoma, fibrosarcoma or leiomyosarcoma\(^6\). The preferential organ for its metastasis is the liver suggesting a specific affinity to the liver of the tumor cells.

Histiocyte contains generally both microfilaments and microtubules in the cytoplasm, although there was no description on their presence in the tumor cells of histiocytic sarcoma of rats and mice\(^{4,15}\). The tumor cells of our case, however, demonstrated to contain microfilaments and tubular autolysozomes or nematolysozomes of Sakai et al. (1988)\(^{16}\). These lysozomes were closely related to the actin–like filaments and suggesting an active phagocytosis of the tumor cells. And the tumor cell showed also the structures similar to vacuoles containing the finger print...
membrane that was known to be observed in the cells of eosinophilic granuloma of the bone belonging to a group of human histiocytes X (Fig. 8). Birbeck’s granule-like structures were also observed in a part of tumor cells (Fig. 9). Birbeck’s granules are well known to exist in the T zone histiocytes, showing a positive reaction with anti-S-100 protein antibody17, but this reaction was negative in the tumor cells of the present case. Both rats and mice bearing histiocytic sarcoma, especially rats, are known to show hyaline droplets containing lysozymes derived from the tumor cells in the epithelium of the renal tubules18,19, though they were not observed in the kidney of this case. The reason may be of less grade of tumor burden19.

Yellowish nodular lesions consisting of fibrous–histiocytic components were infrequently observed in the epididymis and subcutaneous tissue and a few in the uterus and preputial gland of mice. Most of these lesions were diagnosed xanthofibroma or xanthogranuloma, having a characteristics of containing deposits of cholesterin cleft, foamy cells and Touton type giant cells, histologically. Giant cells seen in histiocytic sarcoma are usually of foreign body type or Langerhans’s type6,15. Histiocytic sarcoma were recently observed in the subcutaneous tissue and the seminal vesicles other than epididymis in B6C3F1 mice. Both xanthofibroma and histiocytic sarcoma are likely to be derived from the common cell in origin as suggested by the similar primary region of the subcutaneous or reproductive organ/tissue. Macrophage is known to play a specific role in metabolic process of lipoprotein in the living body and to have the specific intracellular cholesterol metabolism differing from the other cells. Multinucleated giant cells show positive PAS reaction, that was resistant to diastase digestion, as the diagnostic characteristics of reticulo-histiocytic granuloma in human dermatology. The similar giant cells were also seen in xanthofibroma of mouse.

Both histiocytic sarcoma and xanthofibroma are often accompanied by lymphocytic infiltration. The former is known to produce cytokines20. Lymphocytic infiltration suggests local antibody-production against sperm following tissue destruction by tumor invasion21. Xanthofibroma in human fits the category of the benign fibrous histiocyтомa. The tumor that was diagnosed benign fibrous histiocytoma is reported to show metastasis22, local recurrence22,23 and invasive infiltration22,24. Now there seems to be little doubt that most of human retroperitoneal xanthogranulomas are malignant22. Since atypical features of histiogenic proliferative lesions were observed in the present case in addition to metastases to the liver and the adipose tissue of the abdominal cavity, this case should be diagnosed histiocytic sarcoma.

As for etiology of xanthofibroma in mice, Itagaki et al. suggested its histiogenic origin. This kind of tumors seems to develop in the subcutaneous tissue of mouse where outer power is added easily, like xanthoma tuberosum occurring in the subcutaneous tissue of human25. Human xanthomatous lesion is classified in two categories depending on with or without hyperlipoproteinemia. Currently, we have no evidence of the correlation of murine xanthomatous lesions with abnormal cholesterol metabolism.

In conclusion, the present case appears to be the first case of primary histiocytic sarcoma arising from the epididymis of B6C3F1 strain mouse. Although this mouse belonged to the medium-dose treated group, no evident carcinogenicity was observed on the test substance. So this tumor may be of spontaneous origin.

As for the origin of the tumor cells, it is likely to be derived from the immature mesenchymal cells, similar to the cells of histiocytic sarcoma originating from the other organs. Lysozymes and MAC-2 seem to be appropriate immunohistological markers in the identifying its histiogenic origin26. Further development of suitable monoclonal antibodies against histiocytes and mesenchymal cells and the increase in number of these cases in analysis may lead to the exact classification and elucidation of their morphogenesis. Macrophages may modify histological features by their heterogeneity and release of a variety of cytokines. Consequently, proliferative lesions composed of fibro–histiocytic components as described above should be comprehended by a wide category. In case of without showing
metastasis, it may be difficult to distinguish this tumor from xanthofibroma, probably common in origin.

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References