Morphologic Characteristics of a Transplantable Tumor Derived from a Spontaneous Malignant Fibrous Histiocytoma in the Rat

Jyoji YAMATE, Masanori TAJIMA, Kazumoto SHIBUYA, Miheko IHARA, and Satoru KUDOW

Nippon Institute for Biological Science, 2221-1, Shin-machi, Ome, Tokyo 198, Japan

(Received 12 September 1988/Accepted 13 February 1989)

Abstract. Malignant fibrous histiocytoma (MFH) developed spontaneously in subcutaneous tissue of the head of a 15-month-old male Fischer 344 rat. The tumor was serially transplanted into syngeneic rats up to the 45th generation and was designated MFH-MT. Light and electron microscopic examinations revealed that the original and serially transplanted tumors were composed of an admixture of fibroblast-like and histiocyte-like cells arranged in a storiform pattern. Neoplastic cells gave positive reactions for acid phosphatase, alkaline phosphatase, nonspecific esterase, alpha-1 antitrypsin and lysozyme. The tumors transplanted into the lungs and cutaneous tissue of the tail had a mixed histologic appearance of storiform, pleomorphic, myxoid and giant cell types. Moreover sclerosing hemangioma-like and osteosarcoma-like structures were also found. MFH-MT grew well in athymic nude mice showing neoplastic proliferation of pleomorphic cells strongly positive for alpha-1 antitrypsin. Development of MFH-MT was significantly retarded by the two antitumor drugs tested. The retarded tumors consisted predominantly of fibroblast-like cells and abundant collagenic fibers, whereas histiocytic cells decreased in number.—Key words: F344 rat, histochemistry, malignant fibrous histiocytoma, nude mouse, transplantation.


Malignant fibrous histiocytoma (MFH) is composed histologically of spindle-shaped fibroblast-like cells and neoplastic histiocytic cells and often mixed with pleomorphic giant cells, xanthoma cells and varying amounts of inflammatory cells. Depending on the predominating cell types and the amount of intercellular material, human MFH has been classified into storiform, pleomorphic, myxoid, xanthogranulomatous (or inflammatory) and giant cell types [2].

The histogenesis of MFH has long been a matter of controversy. Ultrastructurally, primitive mesenchymal cells with poorly developed cytoplasmic organelles were occasionally found in MFH, suggesting that both histiocyte- and fibroblast-like cells constituting the tumors may be derived from a common undifferentiated stem cell [8, 12, 14]. On the basis of functional and immunohistochemical examinations on neoplastic cells, it has been considered that the neoplastic histiocytes were able to behave as facultative fibroblasts under appropriate conditions [9, 16]. Yumoto and Morimoto [22] succeeded in experimental induction of a tumor resembling MFH in mice by transplanting mouse bone marrow macrophages transformed with SV 40. Moreover, proliferating cells of MFH were found to react with monoclonal antibodies against determinants expressed on monocyte-macrophages, supporting the view that MFH is a tumor of the mononuclear phagocyte system [19].

MFH in humans arose mainly in the limbs, especially in the leg and thigh, and sometimes in the soft tissues of other parts of the body [2, 18]. Spontaneous MFH in animals has rarely been detected in cats [4, 6], dogs [6], pig [20], baboon [5], fox [3], and rats [7, 17], although MFH was experimentally induced by a variety of agents in rats [8, 12].
Recently, we succeeded in serial transplantation of a spontaneous MFH in a rat. The transplantable tumor, designated MFH-MT, had been serially passaged up to the 45th generation in syngeneic rats. The present paper describes morphologic characteristics of the original tumor and MFH-MT transplanted into syngeneic rats and nude mice.

MATERIALS AND METHODS

Animals and environment: Specific-pathogen-free male and female Fischer 344/ DuCrj (F344) rats, which had been purchased from Charles River Japan, Co., Ltd. or produced in the authors' laboratory, were used throughout the experiments. For heterotransplantation, athymic nude mice of 3 strains, BALB/c-nu/nu slc (Shizuoka Agricultural Corporation Association), BALB/cA-Jcl-nu/nu (Clea Japan Inc.) and Crj: CD-1 (ICR)-nu/nu (Charles River Japan Co., Ltd.), were used.

The animals were maintained in barrier rooms conditioned to a temperature of 23±2°C and a relative humidity of 50±20%, and with a 12-hr light-dark cycle. A standard commercial laboratory diet for rats and mice (CRF-1: Charles River Japan Co., Ltd.) and tap water were available ad libitum.

Serial transplantation in syngeneic rats and heterotransplantation in athymic nude mice: Tumor masses were minced into small pieces, <2 mm in diameter, with scissors and then transplanted subcutaneously at the midline of the interscapular region through a trochar with a diameter of 2 mm. The long (a) and short (b) diameters in millimeters of tumors developing in subcutaneous tissue were measured once weekly with calipers, and tumor volume was estimated by the following formula: $a \times b^2/2$.

The tumors were serially transplanted using two to four male and female F344 rats, 10 to 25 weeks of age, in each generation. The passage was repeated every 5 to 6 weeks after transplantation when tumor reached size of >5 cm in diameter. The growth curve of tumors in syngeneic rats was determined using ten 6-week-old male F344 rats, which were transplanted subcutaneously with a piece of tumor tissue from the 9th generation. Six of them were killed 6 weeks after transplantation, and the remainder were observed until death.

A histologic comparison was made between tumors transplanted at various sites of rat's body, since MFH has generally been known to be composed of different cell types and of variable histologic patterns depending on different portions of tumors examined. Tumor tissues at the 4th and 11th passage levels were minced and trypsinized to disperse cells. After being washed, 0.1 to 0.5 ml of a cell suspension containing $10^2$ to $10^7$ cells/ml were inoculated subcutaneously in the back and head, intraperitoneally, intradermally at the root of the tail, or intravenously.

Tumor growths in heterotransplantation were observed for 5 weeks in 5 male and 5 female nude mice for each of the 3 strains. These mice were transplanted subcutaneously with a piece of tumor tissue from the 21st generation at the age of 5 weeks.

Susceptibility of MFH-MT to antitumor drugs: Adriamycin (ADR) obtained from Kyowa Hakko Kogyo Co., Ltd. (Tokyo, Japan) and cis-Diaminedichloroplatinum (CDDP) from Nippon Kayaku Co., Ltd. (Tokyo, Japan) were used as antitumor drugs. Experiments were performed on eighteen 6-week-old male rats which had been transplanted subcutaneously with a piece of tumor tissue from the 10th generation. The animals were allotted to 3 groups of 6 each; two groups were treated with the drugs and one group received distilled water via peritoneal route and served as controls.
The antitumor drugs were dissolved in distilled water and injected intraperitoneally at dosage levels of 4 mg/kg for ADR and 5 mg/kg for CDDP, equivalent to 1/4 and 2/3 of the 50% lethal doses by peritoneal route, respectively. The drugs were first injected on the following day after transplantation and thereafter, once weekly for 5 weeks.

Light microscopy: Necropsies were performed on all the animals which were killed under anesthesia or found dead during the experiments. After being weighed, tumor tissues were fixed in 10% neutral buffered formalin. They were embedded in paraffin, sectioned and stained with hematoxylin and eosin (H & E). Selected sections were also stained with the periodic acid-Schiff (PAS) with or without diastase digestion, Watanabe's silver impregnation for reticulin, azan-Mallory, alcian blue (pH 2.5) and by von Kossa's method. Frozen sections from the formalin-fixed tissues were stained with Sudan III and oil red 0.

For enzyme histochemistry, fresh specimens were fixed in 4% formal calcium for 12 hr at 4°C, and stored in Holt's hypertonic gum sucrose solution for 12 to 24 hr. The tissues were frozen, and sections were cut at 10 μm thick and stained by the Gomori's method for acid phosphatase (pH 5.0), by the alpha-naphthyl acetate method for nonspecific esterase (pH 7.4), and by the naphthol AS method for alkaline phosphatase (pH 9.0).

Paraffin-embedded sections were immunohistochemically stained by the peroxidase antiperoxidase (PAP) technique using a commercial kit (Cambridge Research Laboratory Universal Immunoperoxidase Staining Kit, CRL, Mass.). Rabbit antiserum to alpha-1 antitrypsin (CRL), S-100 protein (CRL), factor 8-related antigen (CRL), myoglobin (CRL), lysozyme (CRL), keratin (CRL) and desmin (Miles Laboratory, Naperville, IL.) were used as primary antibodies. Normal rabbit sera were used as negative controls.

Electron microscopy: Small blocks of tumor tissues were fixed in 2.5% glutaraldehyde for 2 hr and postfixed with 1% osmium tetroxide in 0.2 M cacodylate buffer for 1 hr. The blocks were embedded in epoxy resin and sectioned. Thin sections were stained with uranyl acetate and lead citrate, and examined in a JEM-100B electron microscope at 80 kV.

RESULTS

Original tumor: The original tumor arose from subcutaneous tissue of the head of a 15-month-old male F344 rat, which was under the observation of a life-span study. When discovered, the tumor was a solid nodule, one cm in diameter. During the subsequent 2 months, it grew to a size of 4 cm in diameter accompanying a bloody surface. The animal lost body-weight from 494 g to 443 g and became depressed. Therefore, the animal was subjected to a complete necropsy at the age of 17 months. The tumor was a nonencapsulated, firm mass weighing 25 g, and partially invaded the surrounding tissues, but no metastases were found. The cut surface of the tumor was white in color and was multilobular with a fascicular structure. Microscopically, the tumor was rich in cell component and contained irregularly defined areas of necrosis and hemorrhage. The cell dense areas were composed of a mixture of fibroblast-like cells and histiocyte-like cells arranged in a storiform or cartwheel pattern (Fig. 3). Histiocytic cells had abundant, eosinophilic cytoplasm and their nuclei appeared dark and oval or round in shape. All cells were surrounded by reticulin fibers demonstrable by the Watanabe's silver impregnation. A moderate amount of collagen fibers was present throughout tumor tissue. The cell less dense areas were composed of cells containing Sudan III- and oil red 0-positive
lipid droplets and diastase-resistant PAS-positive material in their cytoplasm as well as fibroblastic and histiocytic cells. Bizarre giant cells were rarely found. Mitotic figures were frequent. Thus, the original tumor was diagnosed as storiform type of MFH.

Serial transplantation of MFH in syngeneic rats: In the 1st and 2nd passages, one half of the recipients developed MFH, whereas, transplantability reached 100% positive from the 3rd passage onwards. There were no noticeable differences between tumor growths in different passage levels, except the 1st passage in which the tumor grew into a nodule of 2 cm in diameter 12 weeks after transplantation. The growth curve of tumors determined in the 9th generation is shown in Fig. 1. A piece of tumor tissue developed into a nodule of 6 cm in diameter, weighing 101 g on the average, 6 weeks after transplantation. Recipients bearing large tumors died 8 to 9 weeks after transplantation, showing depression and emaciation. The weights of tumors removed from dead animals ranged from 244 g to 320 g, and tumor:body-weight ratio was 65% on the average.

Some nodules which developed during the serial passages had cysts varying in size, containing transparent, sticky fluid. No metastatic lesions were found. The serially transplanted tumors were histologically indistinguishable from the original tumor.

Two predominant cell types, fibroblast-like cells and histiocyte-like cells, were also observed by electron microscopy. The former was characterized by spindle-shaped, elongated cytoplasm, rich in dilated rough-
surfaced endoplasmic reticulum and an elongated nucleus with prominent nucleoli. This type of cells was distributed in close association with bundles of collagenic fibers (Fig. 4). The latter had abundant cytoplasm with a profusion of surface folds and indented or horseshoe-shaped nuclei. The cytoplasm contained lysosomes, well-developed Golgi apparatus and rough-surfaced endoplasmic reticulum (Fig. 5).

Neoplastic cells gave faintly to moderately positive reactions for acid-phosphatase (Fig. 6), nonspecific esterase and alpha-1 antitrypsin (Fig. 7) and lysozyme, and strongly positive reaction for alkaline phosphatase (Fig. 8). Positive reactions for S-100 protein, myoglobin, desmin, keratin and factor 8-related antigen were not demonstrable in proliferating tumor cells, although

Fig. 4. Electron micrograph of fibroblast-like cells in MFH-MT. ×3,300.

Fig. 5. Electron micrograph of histiocyte-like cells in MFH-MT. ×4,000.

Fig. 7. Neoplastic cells in storiform type of MFH-MT. A faintly positive reaction for alpha-1 antitrypsin is represented as coarse intracytoplasmic granules (arrows). PAP method, counterstained with hematoxylin, ×300.

Fig. 6. A moderate reaction for acid phosphatase is expressed as coarse intracytoplasmic granules in neoplastic cells. Gomori’s method, ×220.

Fig. 8. Tumor tissue with a storiform pattern showing diffuse, strongly positive reaction for alkaline phosphatase. Naphthol AS method, ×200.
the endothelial cells and peripheral nerve included in the tumor tissues were positive for factor 8-related antigen and S-100 protein, respectively.

Histologic diversity of tumors transplanted in different sites: Lung tumors, which developed 12 months after intravenous inoculation, and cutaneous tumors, which developed at the root of the tail 8 months after intradermal inoculation, were composed of a mixture of pleomorphic, myxoid, giant cell and storiform types. The pleomorphic type consisted of pleomorphic round cells and occasional giant cells. The myxoid type was composed of loosely arranged round, polygonal cells and giant cells (Fig. 9), with intercellular material being strongly positive for alcian blue. Cells constituting the giant cell type had a large, lobulated nucleus with dense chromatin (Fig. 10). Osteosarcoma-like structures consisting of osteoblasts, osteoids and calcifying areas were found in parts of the cutaneous tumor developed in the tail (Fig. 11). In the lung tumors, there were areas composed of elongated cells arranged in an interlocking pattern (Fig. 12) or round cells arranged in a compact sheet and sclerosing hemangioma-like structures (Fig. 13). Occasional elongated cells gave a positive reaction for S-100 protein.

Tumors, which developed in the subcutaneous tissue of the head and back 3
months after subcutaneous inoculation, and tumors, which developed in the parietal peritoneum and mesentery 3 months after intraperitoneal inoculation, possessed histologic characteristics of storiform type similar to that of the original tumor.

Transplantation of MFH-MT into athymic nude mice: Tumor grew in all nude mice of the 3 strains after subcutaneous transplantation with tumor tissues from the 21st generation in rats. No significant differences were observed in tumor growth between both sexes and between the strains. At the end of the observation period of 5 weeks, tumors developed into nodules ranging in diameter from 1 cm to 3 cm, and in weight from 1 g to 3 g. All the tumors were well-circumscribed and had a watery appearance, and there was neither infiltrative growth nor metastasis. They were composed mostly of pleomorphic cells, and gave a strongly positive reaction for alpha-1 antitrypsin (Fig. 14). They were classified as pleomorphic type of MFH.

Effects of antitumor drugs on MFH-MT: As shown in Fig. 2, both CDDP and ADR caused a significant retardation of tumor development from 2 weeks after transplantation (P<0.05; determined by the student's t test). The inhibitory effect appeared to be greater in the CDDP-treated group than in the ADR-treated group. At the end of the observation period of 5 weeks, tumor weights in the control, CDDP and ADR groups were 31.2, 1.2 and 7.0 g, respectively. Tumors in rats which were treated with the antitumor drugs were composed predominantly of fibroblast-like cells and plenty of collagenic fibers (Fig. 15). On the other hand, histiocytic cells appeared to decrease in number.

DISCUSSION

The original rat MFH and serially transplanted MFH-MT showed a similar histological appearance to the original tumor. These results suggest that MFH-MT is a suitable model for the study of malignant fibrous histiocytoma. The effects of antitumor drugs on MFH-MT have been evaluated, and the results indicate that CDDP is more effective than ADR. The mechanism of the inhibitory effect of CDDP on tumor growth remains to be elucidated. Further studies are needed to understand the role of CDDP in the treatment of MFH.
planted MFH reported here were composed mainly of fibroblastic and histiocytic cells arranged in a storiform pattern. These tumors were similar morphologically to human MFH, particularly the storiform type [2]. The MFH induced in rats by 4-(hydroxyamino)-quinoline 1-oxide were classified into fibrous, giant cell and myxoid types [12]. The cell populations in the experimentally induced MFH have been shown to consist of following three major cell types: fibroblast-like cells, histiocyte-like cells, and undifferentiated cells possibly representing primitive mesenchymal cells [12]. The predominant cell types in MFH-MT were fibroblastic and histiocytic cells, whereas no undifferentiated cells were identified by both light and electron microscopy. Iwasaki et al. questioned on the presence of undifferentiated cells in MFH because these cells were indistinguishable from immature lymphoid cells scattered in tumors [10]. In MFH-MT, lipid-laden cells (xanthoma cells) were often seen in areas containing loosely arranged neoplastic cells as observed in human MFH, whereas giant cells were not so common as in human MFH [2]. It has been suggested that xanthoma cells, giant cells and cells possessing diastase-resistant PAS-positive material in their cytoplasm may originate from histiocyte-like cells [12, 13].

In enzyme histochemical and immunohistochemical examinations of MFH-MT, neoplastic cells gave faintly to moderately positive reactions for acid-phosphatase, non-specific esterase, alpha-1 antitrypsin and lysozyme, suggesting the neoplastic cells may be of histiocytic origin. Positive reactivities for these stainings have frequently been demonstrated in human MFH, although the degree of reactions varied from case to case; the storiform type was shown to have a weaker reactivity than did the other types [9, 13, 14]. Among these enzymes, alpha-1 antitrypsin was reported to be the most reliable and useful marker for neoplastic cells forming MFH [14]. A strongly positive reaction for alkaline phosphatase was observed in cells of MFH-MT. Neoplastic cells constituting osteosarcomas [21] and MFH [15] were positive for alkaline phosphatase. Since osteosarcoma-like lesions have occasionally been found in rat MFH [8] as seen in MFH-MT, MFH and osteosarcoma may be derived from a common precursor cell.

It has generally been accepted that MFH has a great histologic variability [2, 18]. Particularly, MFH-MT transplanted in the lungs and tail revealed a variety of histologic features. There were storiform, pleomorphic, giant cell and myxoid types as well as sclerosing hemangioma- and osteosarcoma-like structures and areas consisting of elongated cells positive for S-100 protein. These findings seemed to support the hypothesis that MFH may originate from pluripotential mesenchymal stem cells.

MFH-MT grew well in athymic nude mice of three strains. The tumor grafts in these mice appeared to consist mostly of pleomorphic cells, which showed more strongly positive reaction for alpha-1 antitrypsin than did the original tumor with a storiform pattern, suggesting a possible change in property of neoplastic cells. Similar results have been obtained by Shirasuna et al. [16] by inoculating nude mice with cloned cells established from human MFH. On the other hand, xenografts in nude mice of two cell lines derived from human MFH retained histologic features of the original tumor [15].

ADR and CDDP have been demonstrated to have a broad range of antitumor activity against human and animal tumors [1, 11]. The development of MFH-MT was significantly retarded by both ADR and CDDP. It is interesting to note that MFH-MT, which was treated with the drugs, was comprised almost entirely of fibroblast-like
cells and abundant collagenic fibers, whereas histiocyte-like cells decreased in number. These observations provide further evidence that MFH is heterogeneous and composed of cell types differing in susceptibility to antitumor drugs.

ACKNOWLEDGMENTS. This study was supported in part by Grant-in-Aid for Scientific Research (No. 62760263) from the Ministry of Education, Science and Culture of Japan. We are grateful to Messrs. Y. Ogata and H. Watanabe, and Mrs. S. Sannai for their technical assistance.

REFERENCES

要約

ラットにおける自然発生恶性線維性組織球腫由来の可移植性腫瘍の形態学的特徴：山手丈至・田島正典・渋谷一元・伊原三重子・工藤悟（日本生物科学研究所）——15カ月齢雄Fischer 344ラットの頭部の皮下組織に恶性線維性組織球腫(MFH)が自然発生した。この腫瘍は同種ラットにおいて45代まで継続的に移植され、MFH-MTと命名された。光学顕微鏡及び電子顕微鏡検査により起源及び細胞移殖腫瘍はstoriform状に配列する線維芽細胞様細胞及び組織球様細胞の混在から成っていた。腫瘍細胞は酸性フォスファターゼ、アルカリフォスファターゼ、非特異的エステラーゼ、alpha-1 antitrypsin及びlysozymeに対し陽性反応を示した。肺及び尾の皮膚組織に移植されたMFH-MTはstoriform、pleomorphic、myxoid及びgiant cell型の混在した組織様式を有し、さらには硬化性血管様及び骨肉腫様構造も認められた。MFH-MTは無胸腺ヌードマウスにおいて、alpha-1 antitrypsinに染まる多形性細胞の腫瘍性増殖を示し、よく発育した。2種の抗腫瘍剤投与によりMFH-MTの増殖は有意に抑制され、抑制された腫瘍は、主に線維芽細胞様細胞及び豊富なコラーゲン線維から成ったが、組織球様細胞の出現数は減少した。