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Characteristics of a rat fibrosarcoma-derived transplantable tumour line (SS) and cultured cell lines (SS-P and SS-A3-1) showing myofibroblastic and histiocytic phenotypes

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Abstract A transplantable tumour line (SS) was established in syngeneic rats from a spontaneous fibrosarcoma that had arisen in the submandibular salivary gland of a 24-month-old male F344 rat. A cell line (SS-P) was induced from SS, and a cloned cell line (SS-A3-1) was isolated from SS-P. The primary tumour consisted of oval to spindle-shaped cells arranged in bundles with abundant collagen fibres; ultrastructurally, neoplastic cells exhibited fusiform morphology with prominent rough endoplasmic reticulum. SS tumours showed marked interlacing fascicle and herring-bone growth patterns. SS-P and SS-A3-1 were simmilar morphologically to each other, consisting of oval, spindle or polygonal cells and occasional multinucleated giant cells. Tumours induced by SS-P and SS-A3-1 were histologically similar to SS tumours. Immunohistochemically, all cells in the primary tumour, SS tumours and tumours induced both by SS-P and SS-A3-1 and by SS-P and SS-A3-1 cultures gave a positive reaction to vimentin. Interestingly, neoplastic cells reacting to ED1 (rat macrophage/histiocyte-specific antibody) and α-smooth muscle actin (α-SMA) appeared in SS tumours and tumours induced by SS-P and SS-A3-1 and by SS-P and SS-A3-1 cultures. Cells with histiocytic fine structures and myofibroblastic cells with cytoplasmic actin-like microfilaments were also observed by electron microscopy. The present rat fibrosarcoma-derived transplantable tumour line (SS) and cell lines (SS-P and SS-A3-1) might express myofibroblastic and histiocytic phenotypes, probably depending on the surrounding conditions. These cell lines may prove useful for studying the mechanisms of phenotypic plasticity in neoplastic fibroblasts.

Key words Rat-fibrosarcoma-derived transplantable tumour · Rat fibrosarcoma-derived cell line · Myofibroblastic cell · Histiocytic cell · Phenotypic modulation

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Introduction

Fibrosarcomas are well-defined soft-tissue tumours, which are derived from fibroblasts [8]. These are the most common cells of connective tissue, and fibrosarcomas thus arise at various sites and in many organs [8, 18, 20]. Although fibroblastic cells have traditionally been considered to be relatively uniform in morphology and function, they appear to be endowed with multiple functional and morphological properties [15, 23]. Myofibroblastic differentiation has been observed in human fibrosarcomas by electron microscopy and immunohistochemistry [8, 10, 18, 26], and these cells found in granulation tissues and fibrotic lesions have been considered to be derived from pre-existing fibroblasts [3, 4, 6, 19, 23, 25, 34, 40]. Some studies have revealed that fibroblastic cells have the capacity to undergo histiocytic and adipocytic differentiation or epithelial conversion, depending on genetic and/or microenvironmental factors [2, 16, 29, 31]. Fibroblasts are mysterious cells, and the plasticity of their phenotype remains to be investigated [12, 15, 23, 42].

Recently, we established a transplantable tumour line (SS) in syngeneic rats from a spontaneous fibrosarcoma that had arisen in the submandibular salivary gland of an aged F344 rat. An in vitro-passaged cell line (SS-P) was induced from SS, and a cloned cell line (SS-A3-1) was further isolated from SS-P. In this study, the morphologi-

cal characteristics of the primary tumour, SS, and of SS-P and SS-A3-1 were investigated by light/electron microscopy and enzyme/immunohistochemistry. Interestingly, apart from fibroblastic morphology, cells with myofibroblastic and histiocytic phenotypes were seen in the derivatives.

Materials and methods

Specific-pathogen-free male and female F3-44/DuCrj rats, aged 8–24 weeks and weighing 135–368 g, were used throughout the experiments. After purchase from Charles River Japan (Shiga, Japan), they were housed in an animal room at a controlled temperature of 22±3° C and with a 12-h light dark-cycle and fed a standard commercial laboratory diet for rats ad libitum. All rats used were killed by ether anaesthesia.

The primary tumour was found in the right submandibular gland of a male F344 rat sacrificed at the age of 24 months; the rat was in a life-span study designed to yield pathological background data with advancing age. A tissue fragment 2 mm in diameter was aseptically cut from the primary tumour, which was later diagnosed as fibrosarcoma by pathological examinations. The tissue fragment was transplanted subcutaneously into a syngeneic male rat through a trochar under ether anaesthesia [38, 39]. Five weeks after the implantation, a subcutaneous tumour developed into a nodule weighing 3 g. A portion of the subcutaneous tumour was used for transplantation in the second generation. Serial transplantations were then made at 4- to 7-week intervals for each generation until the 10th using the same method as described above; in each passage, one to four male and female rats were used. The transplantable tumour line was designated SS. The primary tumour and the SS tumours that developed at all passages were examined morphologically.

To establish the cell lines, tumour tissues removed aseptically from SS at passage 3 were minced with scissors and dispersed with 0.1% trypsin and 0.02% ethylenediaminetetraacetic acid (EDTA) in phosphate-buffered saline (PBS) for 3 h. The dispersed cells were washed twice in PBS and seeded in plastic tissue culture flasks (25 cm^2). The growth medium was Eagle's minimum essential medium (MEM: Nissui, Japan) containing 10% fetal bovine serum (Bioserum, UBC, Japan), 0.03% L-glutamine (Nissui), penicillin (100 U/ml) and streptomycin (100 µg/ml). The cultures were incubated at 37° C in a humidified 5% CO_2 atmosphere. Confluent cell sheets were treated with a mixture of 0.1% trypsin and 0.02% EDTA in PBS, and cells were subcultured at 7- to 10-day intervals [30, 38]. The cell line, named SS-P, was serially passaged until the 35th generation.

SS-P cells at passage 33 were cloned twice consecutively by a limiting dilution technique as described elsewhere [36]. Five cloned cell lines, named SS-A3-1 to SS-A3-5, were isolated. Morphological observations with a phase-contrast microscope showed that these cloned cell lines were very similar to each other. A clone (SS-A3-1) was successively subcultured in MEM until the 20th passage by the same methods as described in SS-P culture.

Cultured cells of SS-P and SS-A3-1 at various passage levels were examined, and tumours that developed in syngeneic rats following inoculation of 10^6 cells of SS-P and SS-A3-1 at different passages were also examined by light/electron microscopy (Table 1).

For microscopic examination, tumours were weighed and fixed in 10% neutral buffered formalin. They were embedded in paraffin, sectioned and stained with haematoxylin-eosin (HE), periodic acid–Schiff (PAS), Watanabe's silver impregnation for reticulin, azan-Mallory for collagen, phosphotungstic acid haematoxylin (PTAH), and alcian blue (pH 2.5). Frozen sections from the formalin-fixed tissues were stained with oil red 0.

Formalin-fixed, deparaffinized sections were also stained by the indirect immunoperoxidase method. Primary antibodies used are shown in Table 1. The detailed procedures have been described elsewhere [39, 41]. Briefly, after treatments with 0.1% trypsin solution and then with 3% H_2O_2, the sections were incubated for 14 h at 4° C with the primary antibodies listed in Table 1. Subsequently, they were incubated for 1.5 h at 37°C with secondary antibody. The secondary antibody used was 400-fold-diluted peroxidase-conjugated affinity-purified goat anti-mouse IgG Fc fragment antibody (Jackson Immunoresearch Laboratories (JIL). Pa., USA) for the monoclonal primary antibodies or 400-fold-diluted peroxidase-conjugated affinity-purified goat anti-rabbit IgG (JIL) for the polyclonal primary antibodies. Positive reactions were visualized

Table 1 Primary antibodies used and results in the enzyme/immunohistochemical examinations of the primary fibrosarcoma, its transplantable tumour line (SS), cultured cell lines (SS-P and SS-A3-1) and the tumours induced by SS-P and SS-A3-1 cells.[a] (*α-SMA* α-smooth muscle actin, *ED1* rat macrophage/histiocyte-specific antibody, *ACP* acid phosphatase, *NSE* nonspecific esterase, *ALP* alkalin phosphatase, *SMT* smooth muscle tumour [38], *MFH* a transplantable rat malignant fibrous histiocytoma [36], *3+* all cells were positive, *2 +* a number of cells but not all were positive, *+* occasional cells were positive, ± only a few cells positive, – no cells positive, *NE* not examined)

Antibody (source)[b]	Monoclonal /polyclonal	Dilution	Rat tissue used as positive control[c]	Staining results					
				Primary tumour	SS tumours	Cultured cells		Induced tumours	
						SS-P	SS-A3-1	SS-P	SS-A3-1
Vimentin (DAKO)	Mono	×400	MFH	3+	3+	3+	3+	3+	3+
Desmin (DAKO)	Poly	×400	SMT	–	+	–	–	+	+
α-SMA (DAKO)	Mono	×400	SMT	±	+~2+	+	+	+~2+	+
Keratin (DAKO)	Poly	×400	Epidermis	–	–	–	–	–	–
S-100 protein (DAKO)	Poly	×400	Schwannoma	–	–	–	–	–	–
ED1 (CHEMICON)	Mono	×400	Histiocytic cells in MFH	–	+~2+	2+	2+	+~2+	+~2+
Enzymatic staining (method)									
ACP (Gomori's method, pH 5.0)		MFH		N E	+	2+	2+	+	+
NSE (Alpha-naphthyl acetate, pH 7.4)		MFH		N E	+	2+	2+	+	+
ALP (Naphthol AS, pH 9.0)		MFH		N E	–	–	–	–	–

[a] SS tumours were examined at each passage to the 10th generation. Cultured SS-P cells at passages 5, 7, 11, 19, 25 and 30, and cultured SS-A3-1 cells at passages 3, 8, 10 and 15 were examined. Tumours induced in syngeneic rats by inoculating SS-P cells at passages 6, 18, 25 and 30, and tumours by SS-A3-1 cells at passages 3, 10 and 15 were examined

[b] DAKO Corp., Santa Barbara, Calif., CHEMICON International, USA

[c] Described in earlier papers [38, 39, 41]

with diaminobenzidine tetrahydrochloride, and sections were counterstained with haematoxylin. Rat tissues were used as positive controls [38, 39, 41], and sections treated with nonimmune mouse or rabbit serum instead of primary antibody, as negative controls.

Frozen sections from fresh tumour tissues were stained for acid phosphatase (ACP; Gomori's method, pH 5.0), nonspecific esterase (NSE; alpha-naphtyl acetate method, pH 7.4) and alkaline phosphatase (ALP; naphthol AS method, pH 9.0) by the same enzyme-histochemical methods as described previously [38, 39, 41].

For electron microscopy, small blocks from tumours were fixed in 2.5% buffered glutaraldehyde and postfixed in 1% buffered osmium tetroxide. They were embedded in epoxy resin and sectioned. The ultra-thin sections were stained with uranyl acetate and lead citrate and examined in an electron microscope (Hitachi, H-600) at 75 kV.

Doubling time was determined from viable cell numbers at 2 and 3 days after 2.5×10^5 cells had been seeded in each glass flask (5 cm in diameter) [36, 41]. The viable cells, assessed by trypan blue dye exclusion, were counted in triplicate cultures using a haemocytometer. For chromosome number, subconfluent cell sheets were exposed to colchicine in a concentration of 0.5 μg/ml for 3 h. After being suspended in a hypotonic solution, cells were fixed in a mixture of methanol 3:acetic acid 1, and stained with Giemsa [30]. Chromosome numbers in 50 cells were counted.

For enzyme/immunohistochemistry, cells grown for 24–48 h on tissue culture glass slides were fixed in cold acetone [36, 41]. The enzyme-histochemical staining included ACP, NSE and ALP, and the methods were as described for in vivo observations. After treatment with 3% H_2O_2, the fixed cells were also stained by the indirect immunoperoxidase method as described above, using the primary antibodies listed in Table 1. Cultured cells fixed in Bouin's solution were stained with HE for morphology. For electron microscopy, pelleted cells were processed and examined as described above.

Results

The primary tumour was a demarcated nodule involving the submandibular gland. It was grey and measured 2×1×1 cm. Neither infiltrative growth into surrounding subcutis nor metastasis to distant organs was seen. Histologically, the tumour consisted mainly of oval to spindle-shaped cells, with a small round or fusiform nucleus (Fig. 1). These neoplastic cells were arranged in bundles. Acini and ducts of the gland were entrapped by infiltrating neoplastic cells. Mitotic figures were occasionally seen. The tumour cells were separated by abundant collagen fibres stained blue by the azan-Mallory stain. Silver stain showed well-developed, thick reticular fibres surrounding each neoplastic cell. PAS-positive basement membrane-like material was not detected. PTAH stains failed to demonstrate intracytoplasmic cross-striations or longitudinal striations. Oil red O-positive cells were not seen. The intercellular material was negative for alcian blue (pH 2.5). All neoplastic cells were strongly immunoreactive for vimentin. Only a few cells gave a positive reaction to α-SMA, and no cells reacting to desmin, ED1, keratin, or S-100 protein were found in tumour tissue (Table 1).

Ultrastructurally, most neoplastic cells examined showed a spindle-shaped configuration with an elongated nucleus, and their cytoplasm possessed some mitochondria and well-developed rough endoplasmic reticulum with slightly dilated cisternae (Fig. 2).

Serial transplantations to the 10th passage were made in both sexes, with a 100% take rate. Except for the first generation, there were no marked differences in tumour growth between different passages; transplanted tissue fragments grew into nodules 5–6 cm in diameter and 70–90 g in weight by 7 weeks after subcutaneous implantation.

Macroscopically, subcutaneous tumours were well demarcated by their fibrous capsule, with haemorrhagic and necrotic areas on the cut surface. No invasive growth or metastasis was present in any rat bearing a tumour.

Although the histological findings in SS tumours were generally similar to those in the original tumour, there were some differences. In SS tumour at all passage levels, cell pleomorphism and growth fashions such as interlacing fascicle and herring-bone pattern (Fig. 3) were evident; occasional multinucleated giant cells were also seen, and mitotic figures were more frequent. As in the original tumour, all neoplastic cells in SS were immunopositive for vimentin and negative for keratin and S-100 protein (Table 1). However, in SS tumours, neoplastic cells with abundant, eosinophilic cytoplasm, in which intracytoplasmic fibrils were demonstrable by PTAH staining, were seen frequently. These cells were reactive to α-SMA, and some were desmin positive. ED1-positive cells were often seen throughout SS tumours. There were also cells reactive for ACP and NSE in high cellularity areas, but no cells reacting to ALP were present.

Apart from elongated fibroblastic cells with well or moderately developed rough endoplasmic reticulum, as seen in the primary tumour, cells with microfilament bundles running parallel to the plasma membrane (Fig. 4) or cells with diffusely distributed microfilaments with occasional dense bodies in the cytoplasm were observed by electron microscopy.

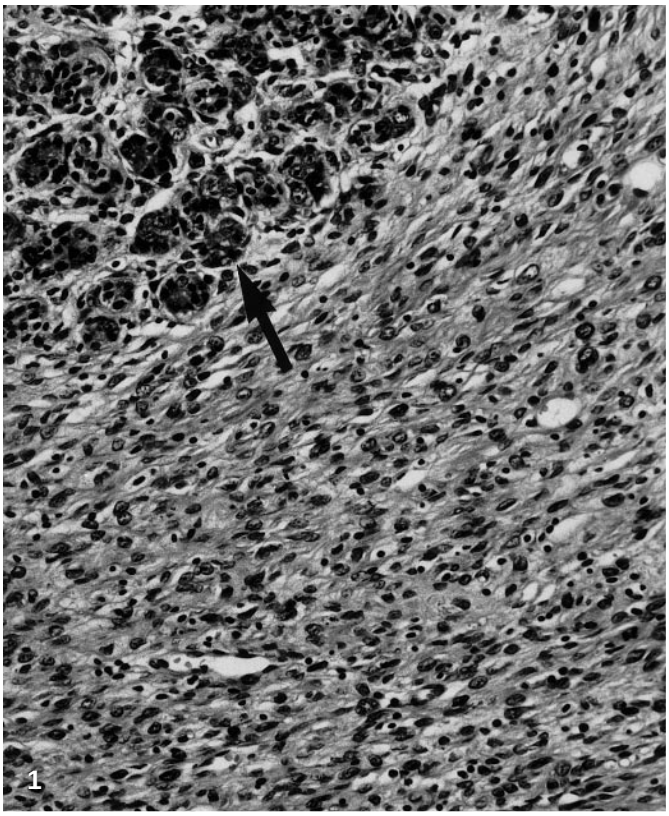
The doubling times of SS-P at passage 23 and SS-A3-1 at passage 5 were 36.5 h and 19.6 h, respectively. Chromosome numbers in SS-P at passage 25 were distributed between 58 and 71 with a peak at 65, whereas those in SS-A3-1 at passage 5 were mostly between 56 and 58 with a peak at 57. Since the chromosome number in normal rat cells is 42 [30], those of SS-P and SS-A3-1 showed abnormal distribution, indicating numerical aberration.

Fig. 1 The primary tumour arising in the submandibular salivary gland of an F344 rat. It consists of oval to spindle-shaped cells with small round or fusiform nuclei; they are arranged in bundles with abundant collagen fibres between them. *Arrow* indicates acini entrapped by infiltrating neoplastic cells. HE, ×350 ▶

Fig. 2 Electron micrograph of a neoplastic cell in the primary tumour. The cell has some mitochondria and well-developed rough endoplasmic reticulum (*N* nucleus). ×18,000

Fig. 3 Rat fibrosarcoma-derived transplantable tumour (SS) at passage 5, showing a herring-bone growth pattern. HE, ×450

Fig. 4 Electron micrograph of a neoplastic cell in the SS tumour. The cell has well-developed rough endoplasmic reticulum (*rER*) and microfilament bundles (*arrowheads*) running parallel to the plasma membrane; *arrows* indicate dense bodies in the microfilament bundle. ×20,000



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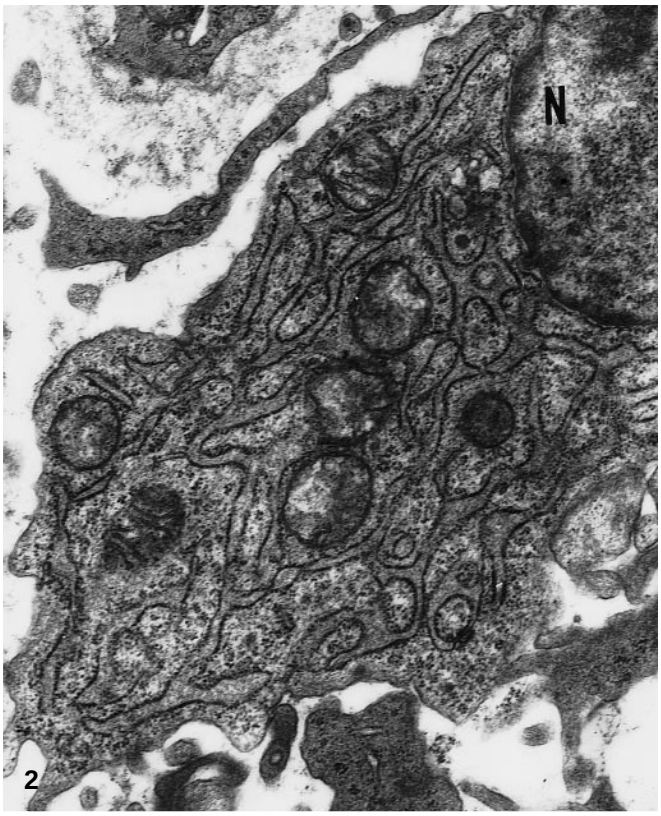

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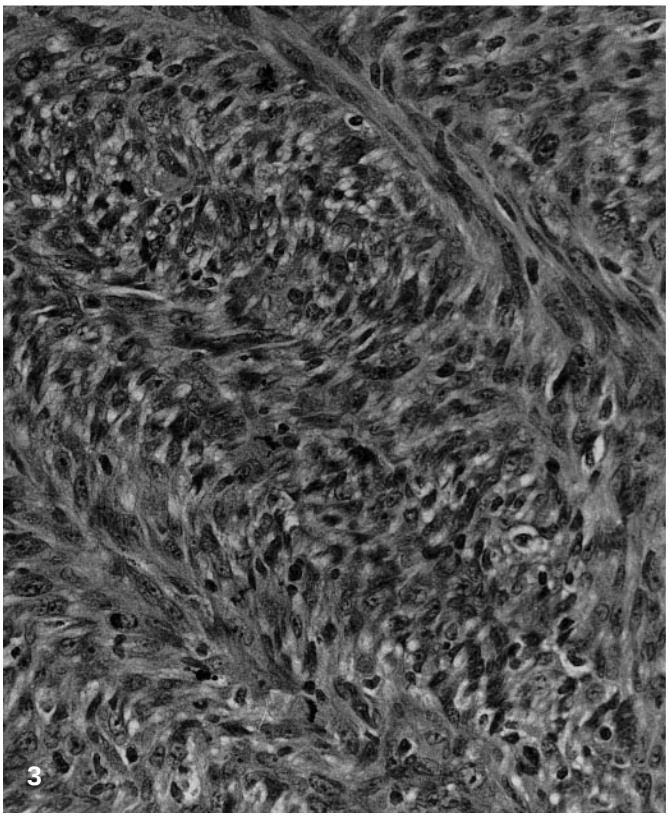

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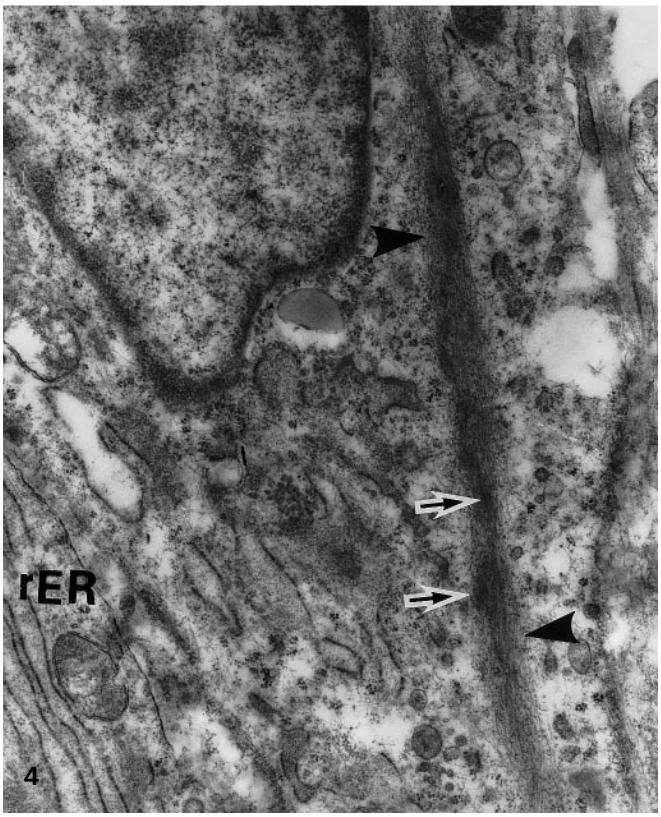

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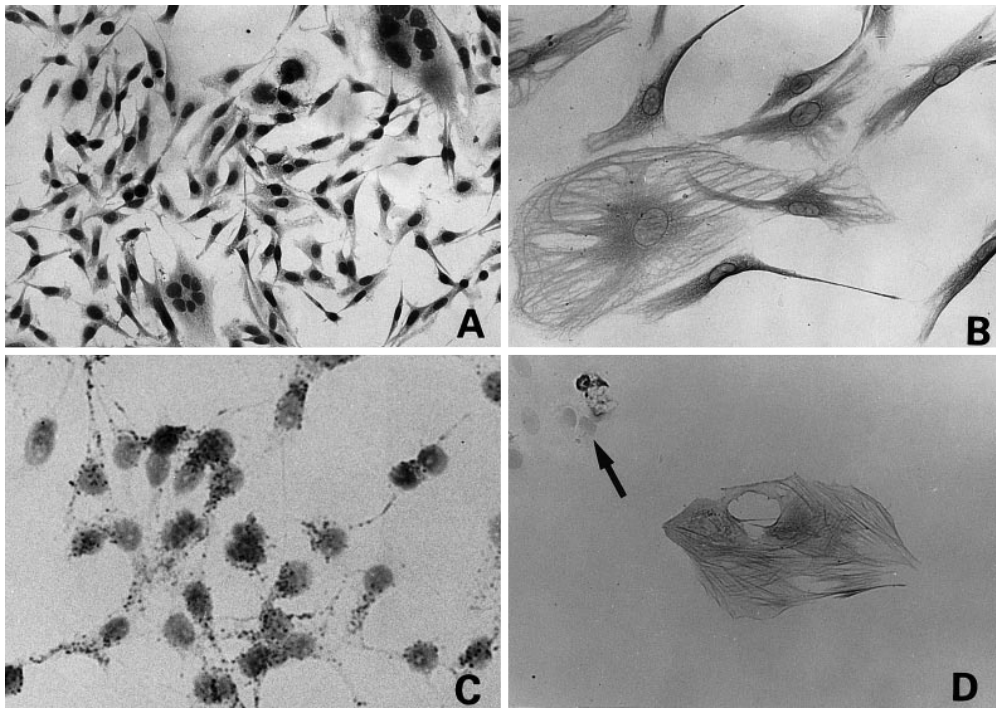


Fig. 5A–D Cultured cells of SS-P established from the SS tumour. **A** Monolayer culture consisting of oval, spindle-shaped or polygonal cells with hyperchromatic nuclei and occasional multinucleated giant cells. HE, ×200. **B** All cultured cells are reactive for vimentin. Immunohistochemistry, counterstained with haematoxylin, ×350. **C** Many cells react to ED1 (rat macrophage/histiocyte-specific antibody), and the positive products appear as fine granules in the cytoplasm. Immunohistochemistry, counterstained with haematoxylin, ×250. **D** Two cells have developed filamentous reactions to α-smooth muscle actin (α-SMA) in their cytoplasm. *Arrow* points out cells negative for α-SMA. Immunohistochemistry, counterstained with haematoxylin, ×300

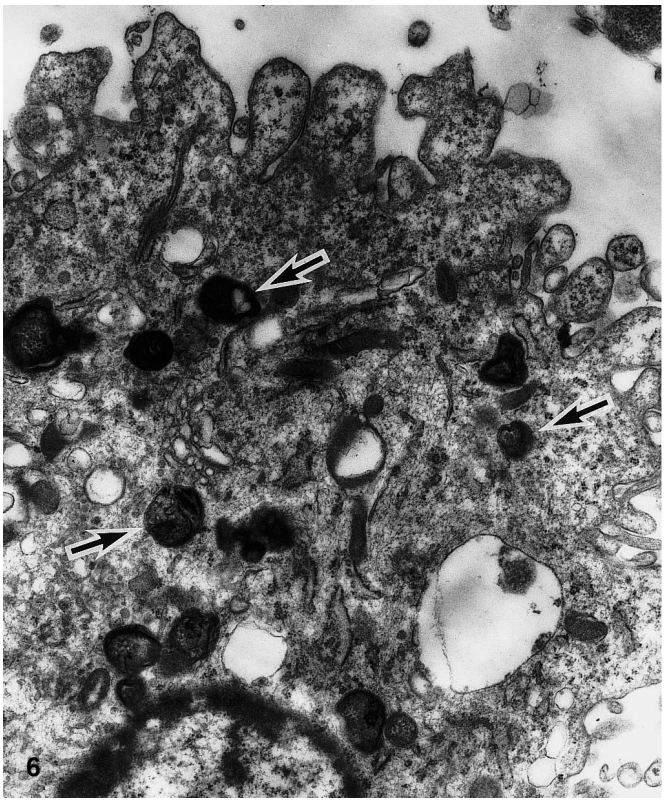


Fig. 6 Electron micrograph of a SS-P cell having many lysosomes (*arrows*) and some surface folds, giving an appearance of histiocytic cells. ×15,000

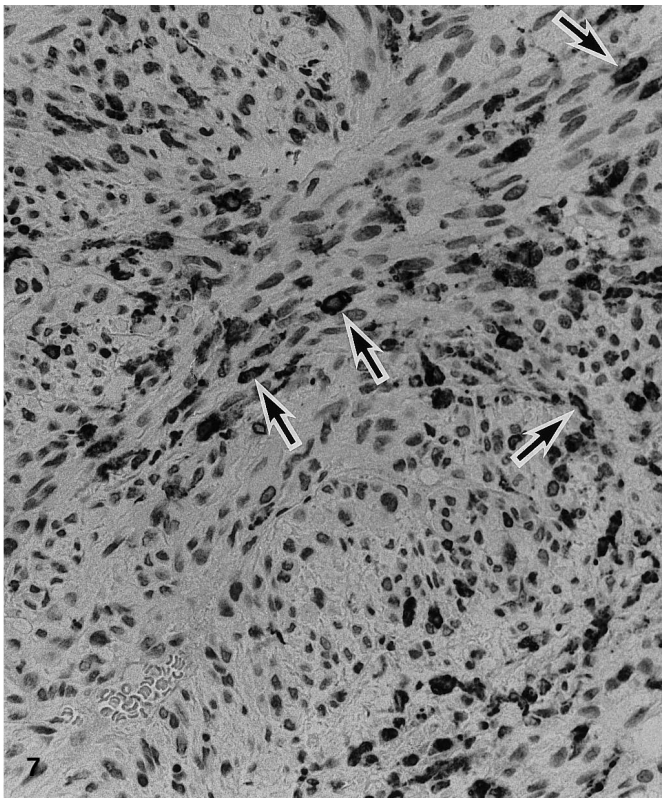
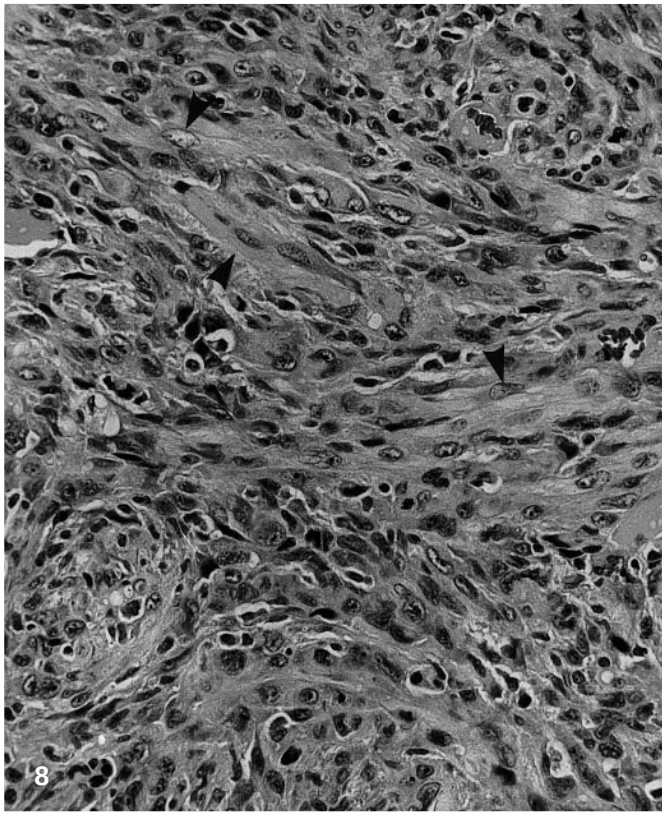


Fig. 7 Tumour induced by SS-P cells, showing an interlacing fascicle growth pattern. ED1-positive cells (*arrows*) are often intermingled in the area. Immunohistochemistry, counterstained with haematoxylin, ×450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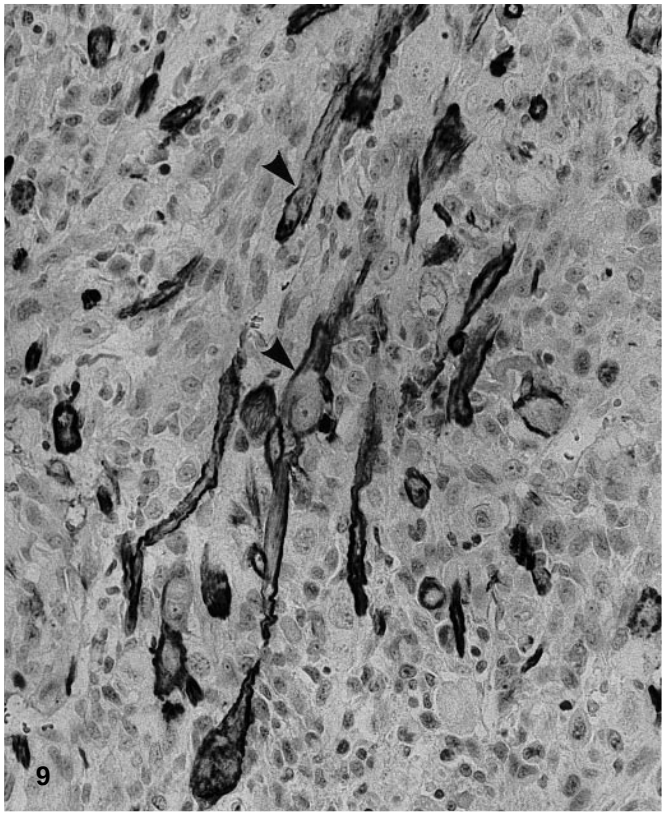
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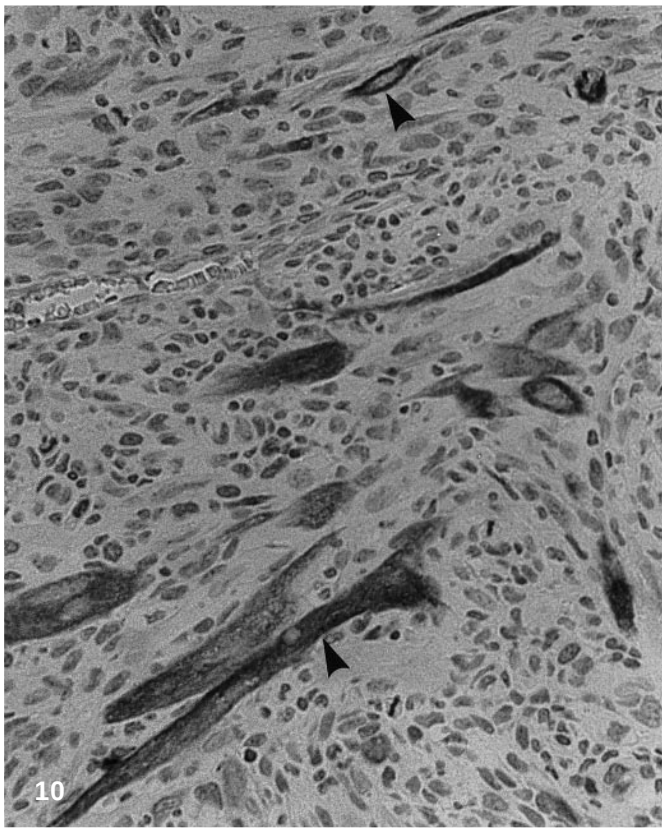
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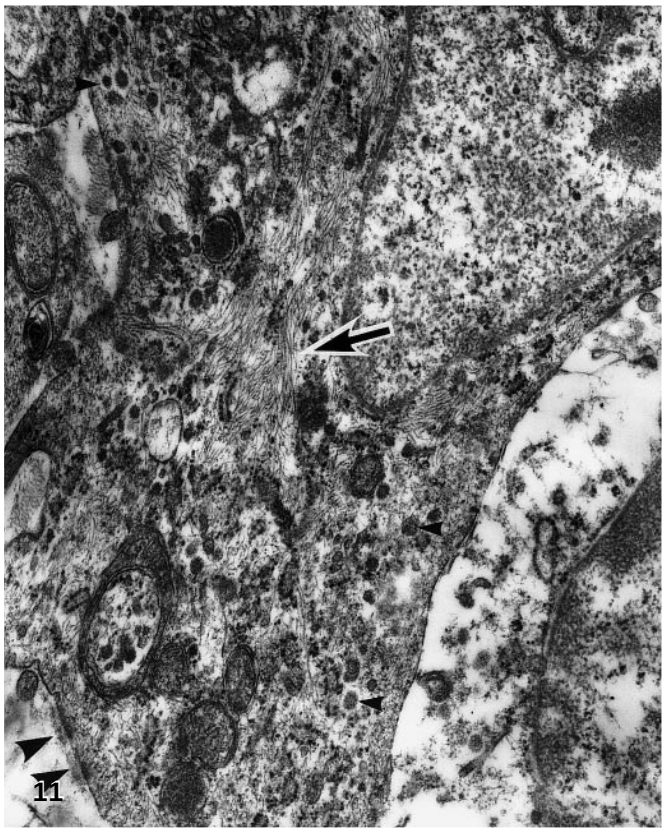
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Monolayer cultures of SS-P and SS-A3-1 at different passage levels were similar morphologically and immunohistochemically (Table 1); the cultures consisted of oval, spindle-shaped or polygonal cells with hyperchromatic nuclei, and occasional multinucleated giant cells (Fig. 5A). All cultured cells were reactive for vimentin (Fig. 5B), but negative for desmin, keratin, S-100 protein and ALP. ACP-, NSE- and ED1-positive cells were frequently seen. The positive reactions to ED1 appeared as fine granules in the cytoplasm (Fig. 5C). Occasional cells with relatively abundant cytoplasm developed filamentous reactions to α-SMA (Fig. 5D), but were negative for desmin.

Ultrastructurally, cells of SS-P and SS-A3-1 were analogous to each other; many cells examined had various numbers of lysosomes and some surface folds, giving an appearance of histiocytic cells (Fig. 6). Occasional cells possessed microfilaments and moderately developed rough endoplasmic reticulum in their cytoplasm.

SS-P and SS-A3-1 cells were tumorigenic in syngeneic rats. Tumours induced by SS-P and SS-A3-1 cells grew into nodules 3–5 cm in diameter by 6–8 weeks after subcutaneous inoculation. The tumours were also well demarcated; no invasion into surrounding tissues and no metastasis were found. Histologically, the induced tumours bore a close resemblance to SS tumours. Immunohistochemically, as shown in Table 1, all tumour cells were reactive for vimentin, and ED1-positive cells were often intermingled. The ED1-positive cells seen in necrotic areas were large, round, and had abundant, occasionally foamy cytoplasm and an indented nucleus with prominent nucleoli, indicating infiltrating macrophages. In contrast, ED1-positive cells in areas of high cellularity, together with ED1-negative neoplastic cells, were arranged in interlacing fascicles and both ED1-positive and ED1-negative cells were oval to fusiform in shape with a hyperchromatic nucleus (Fig. 7): the ED1-positive cells appeared to be proliferating neoplastic cells. There were also areas consisting of oval to elongated cells with abundant cytoplasm and a round or fusiform nucleus (Fig. 8); they gave a strongly positive reaction to α-SMA (Fig. 9). In these areas, some cells also reacted to desmin (Fig. 10). Ultrastructurally, cells with abundant cytoplasm were characterized by numerous cytoplasmic microfilaments, prominent plasmalemmal pinocytosis and interrupted basal lamina (Fig. 11).

Fig. 8 Tumour induced by SS-P cells, showing an interlacing fascicle arrangement. Some cells are oval or fusiform in shape, with abundant cytoplasm (*arrowheads*). HE, ×450

Fig. 9 Tumour induced by SS-P cells. There are cells reacting strongly to α-SMA (*arrowheads*). Immunohistochemistry, counterstained with haematoxylin, ×450

Fig. 10 Tumour induced by SS-P cells. Some cells in the area showing an interlacing fascicle arrangement give a positive reaction to desmin (*arrowheads*). Immunohistochemistry, counterstained with heamatoxylin, ×450

Fig. 11 Tumour induced by SS-P cells. Fine structures of a neoplastic cell in the area showing an interlacing fascicle arrangement. The cell has many diffusely distributed microfilaments (*arrow*), plasmalemmal pinocytosis (*small arrowheads*) and interrupted basal lamina (*large arrowheads*). ×16,000

Discussion

In F344 rats used in carcinogenesis tests as controls or in life-span studies, the incidence of spontaneously occurring neoplasms in the salivary gland is less than 0.1% [20]. The salivary gland tumours reported hitherto have been adenomas, adenocarcinomas and mesenchymal tumours such as malignant schwannoma, fibrosarcoma and undifferentiated sarcoma [20]. Schwannomas consist of S-100 protein-positive, spindle-shaped neoplastic cells with a palisade arrangement of their nuclei [7, 8, 20]. Undifferentiated sarcoma is characterized by marked cell pleomorphism, with fusiform or polygonal cells and multinucleated giant cells arranged haphazardly; the original cell may be at a more primitive stage [20]. In the present primary tumour, no cells reactive to S-100 protein or keratin-positive epithelial cells were found. Oval to spindle-shaped cells were arranged in bundles with abundant collagen fibres among neoplastic cells and revealed significant pleomorphism. Ultrastructurally, neoplastic cells exhibited fusiform morphology with prominent rough endoplasmic reticulum. These characteristics have been reported in fibrosarcoma [8, 9, 18]. There were no demonstrable findings suggestive of smooth and striated muscle origin. The primary tumour was thus diagnosed as a fibrosarcoma with perivascular or interlobular connective tissue cells as a possible origin.

Compared with the primary tumour, SS tumours and tumours induced by SS-P cells showed a histology more typical of fibrosarcomas [8, 20]: in particular, interlacing fascicles or classic herring-bone patterns. More interestingly, many cells reacting to α-SMA were intermingled in SS tumours and tumours induced by SS-P cells, although such cell types were rarely found in the primary tumour, α-SMA-positive cells were also observed in SS-P cultures. In contrast to chromosome numbers of the parent SS-P, showing a wide distribution, those of SS-A3-1 were distributed within a restricted range, indicating high clonality. Even in SS-A3-1 cultures and tumours induced by SS-A3-1 cells, cells reacting to α-SMA were also seen. This excludes contamination by non-neoplastic cells and suggests phenotypic changes in neoplastic fibroblasts.

Fibroblastic cells with actin-like microfilaments are regarded as myofibroblasts [11, 23, 24]. The filaments are immunoreactive for α-SMA [3, 4, 11, 23]. Myofibroblasts have ultrastructural features intermediate between those of fibroblasts and smooth muscles [6, 23, 24]. By electron microscopy, cells with intermediate filaments, pinocytic vesicles and interrupted basal lamina, some of which were characteristic of smooth muscles [10, 38], and cells with filamentous bundles running parallel to the cell membrane were found in SS tumours and tumours induced by SS-P and SS-A3-1 cells. These fine

structures have also been reported in myofibroblastic cells in fibrosarcomas and myofibrosarcomas [10, 13, 33, 42]. The myofibrosarcomas reported in humans are composed of a uniform cell population of neoplastic myofibroblastic cells [10, 42].

It is well known that cytoskeletal features change in the development of myofibroblasts from stromal fibroblasts [23]. The spectrum is divided into the following four main phenotypes: expressing of vimentin (V cells), co-expressing vimentin and desmin (VD cells), co-expressing vimentin and α-SMA (VA cells) and co-expressing vimentin, desmin and α-SMA (VAD cells). Such phenotypic modulations have been well established in fibroblastic cells appearing in skin wound healing, hypertrophic scars, superficial fibromatosis and scleroderma lesions [6, 23, 34]. In the present study, all neoplastic cells were positive for vimentin (V cells); α-SMA-positive cells (VA cells) were often seen in SS tumours and tumours induced by SS-P and SS-A3-1 cells; some cells showed a positive reaction for desmin (VD or VAD cells). These observations indicate that neoplastic rat fibroblasts could also alter cytoskeletal proteins under different conditions.

ED1 antibody recognizes cytoplasmic antigens of cells in rat monocyte/macrophage lineage [1] and has been widely used for identification of macrophages/histiocytes in experimentally induced rat liver, heart and kidney lesions [14, 19, 28, 40]. It is interesting to note that many cells of SS-P and SS-A3-1 gave a positive reaction to ED1, indicating expression of histiocytic immunophenotype. Electron microscopy revealed that cells of SS-P and SS-A3-1 had histiocytic fine structures including many lysosomes and cell surface folds, and the presence of lysosomal enzymes (ACP and NSE) was confirmed by the enzymatic staining. Positive staining of CD13 and CD68, both of which are specific markers for human monocytes/macrophages, has recently been reported on human malignant fibrous histiocytoma (MFH) and fibrosarcoma cell lines [27]. ED1-positive reactions have been also found in cultured cells derived from rat MFH and malignant meningioma [30, 41]. These phenomena were interpreted as a phenotypic modulation probably attributable to culture conditions. In addition, it was reported that murine fibroblast-like cells grown in the presence of human serum underwent differentiation toward macrophages with many lysosomes in the cytoplasm [16].

A number of cells in the high-cellularity areas of SS tumours and tumours induced by SS-P and SS-A3-1 cells gave a positive reaction for ED1, ACP and NSE. The positive cells in SS tumours were probably due to phenotypic modulation by serial implants, and those in SS-P and SS-A3-1 tumours might be derived from cells expressing histiocytic antigen/enzymes in vitro. However, we could not exclude the possibility that host-derived macrophages might be intermingled in the tumour tissues; the non-neoplastic, infiltrating macrophages might be induced from monocyte chemoattractant protein-1 (MCP-1) produced by neoplastic cells. MCP-1 is well known to induce reactive macrophages in tumour tissues [27, 30, 35]. There were many infiltrating macrophages in tumours produced by inoculating rat glioma cells, a cell line producing much MCP-1 [35]. MCP-1 production should be investigated to clarify the significance of ED1-positive cells in SS tumours and in SS-P- and SS-A3-1-induced tumours, and the relationship between the ED1-positive cells and “true histiocytes” also remains to be investigated. However, positive reactions of SS-P and SS-A3-1 cells to ED1 at least indicate that rat neoplastic fibroblasts might express histiocytic immunophenotypes.

Our observations on a rat fibrosarcoma-derived transplantable tumour line (SS) and cell lines (SS-P and SS-A3-1) raised the possibility that fibrosarcoma cells may have various phenotypes, such as histiocytic cells and myofibroblastic cells with divergent cytoskeletal proteins.

Phenotypic modulation of fibroblasts into myofibroblasts in fibrotic lesions has been thought to be induced by cell growth factors, particularly the transforming growth factor-β and platelet-derived growth factor produced by infiltrating macrophages [14, 19, 23, 25, 28, 32]. In vitro observations have also demonstrated that besides these cytokines, heparin and its nonanticoagulant derivatives influence the expression of α-SMA in fibroblasts [4, 23]. A cell line derived from a mouse glioblastoma expressed multiple phenotypes, depending on conditioned media containing different growth factors [5]. Microenvironmental conditions in vitro or in vivo developed by cell-to-cell and cell-to-matrix interactions might effect cellular modulation [4, 23, 41].

Cell metaplasia and histological modulation in tumours have been considered to be secondary to genetic changes [2, 22, 29, 31]. Different oncogenes, such as *ras* and *myc*, may induce tumours with different histological features when transfected into the same cell line derived from keratinocytes or melanocytes [21, 22]. Activated *ras* oncogene-transfected 3T3 murine mesenchymal stem cells, of which the original cell lines have a predilection for undergoing adipocyte differentiation, lost the ability to differentiate to adipocytes and instead differentiated into macrophage-like cells [31]. Recently, we reported that cisplatin (an anticancer drug)-resistant cells induced from rat MFH cell lines showed diverse differentiations such as myofibroblasts, osteoblasts and lipoblasts when inoculated into syngeneic rats [41], and it was also demonstrated that actinomycin D, an anticancer drug, could induce cell differentiation in human rhabdomyosarcoma-derived cell lines [17]: these anticancer drugs might give rise to genetic alterations in neoplastic cells.

In an attempt to investigate possible factors leading to the phenotypic alterations, we added TGF-β (10 μg/ml) to the culture medium of SS-A3-1. However, the numbers of α-SMA-positive cells were not increased (data not shown). Furthermore, we failed to demonstrate multipotential differentiation of cisplatin-resistant cell lines induced from SS-P when they were inoculated into syngeneic rats (data not shown). Factors provoking phenotypic modulations in SS tumour cells and cultured SS-

P and SS-A3-1 cells remain to elucidated. Our cell lines (SS, SS-P and SS-A3-1) may be useful in investigation of the mechanisms involved in expressions of myofibroblastic and histiocytic phenotypes in fibrosarcoma cells.

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