Virchows Arch A (1987) 411:509–514

Virchows Archiv A

The existance of rhabdoid cells in specified soft tissue sarcomas

Histopathological, ultrastructural and immunohistochemical evidence

Masazumi Tsuneyoshi, Yutaka Daimaru, Hiroshi Hashimoto, and Munetomo Enjoji

Second Department of Pathology, Faculty of Medicine, Kyushu University 60, 3-1-1 Maidashi, Higashi-ku, Fukuoka 812, Japan

Summary. We report the occurrence of rhabdoid cells in several specified soft tissue sarcomas of round cell variety. The rhabdoid cells had an acidophilic cytoplasm containing a globular perinuclear inclusion and were characterised ultrastructurally by the presence of aggregates of 10 nm intermediate filaments. These filaments contained both cytokeratin and vimentin, as demonstrated immunohistochemically. Extensive sampling of soft tissue sarcomas revealed the presence of such cells in different types of soft tissue round cell sarcomas as follows: 12 of 13 cases of epithelioid sarcomas, 8 of 13 synovial sarcomas (composed predominantly of round cells), 6 of 20 extraskeletal myxoid chondrosarcomas and 4 of 4 round celled malignant mesotheliomas. We wish to stress that the appearance of rhabdoid cells is not a monopoly of one particular type of tumour.

Key words: Soft tissue neoplasm – Sarcoma – Rhabdoid cell – Rhabdoid tumor – Ultrastructure – Immunohistochemistry

Introduction

Malignant rhabdoid tumour (MRT) of the kidney has achieved wide recognition because of its unique light microscopic features and aggressive clinical character (Haas et al. 1981; Vogel et al. 1984). The tumour cells are characterised by an acidophilic cytoplasm and contain a large hyalin-like globular perinuclear inclusions and notched vesicular nuclei with a large prominent nucleolus resembling rhabdomyoblasts, yet neither the ultrastructure nor the immunoperoxidase reaction of these cells suggests a myogenic origin. They are defined as "rhabdoid cells". Extra-renal malignant neoplasms resembling renal rhabdoid tumour have been reported (Gonzalez-Crussi et al. 1982; Lynch et al. 1983; Frierson et al. 1985; Ekfors et al. 1985; Small et al. 1985; Tsuneyoshi et al. 1985; Sotelo-Avila et al. 1986; Biggs et al. 1987).

It has been our experience that some soft tissue sarcomas other than MRT of soft parts also contain rhabdoid cells. We have now analysed round cell sarcomas with rhabdoid cells such as epithelioid sarcoma, monophasic synovial sarcoma, extraskeletal myxoid chondrosarcoma and malignant mesothelioma. Light microscopic, ultrastructural and immunohistochemical comparisons were made.

Offprint requests to: M. Enjoji

Materials and methods

Materials was selected from more than 1000 cases of soft tissue sarcomas registered in the Second Department of Pathology, Kyushu University, Japan, during the period from 1965–1985. There were 30 cases of specified round cell sarcomas with rhabdoid cells (Table 1). The sarcomas included epithelioid sarcoma (12 cases), synovial sarcoma composed predominantly of round tumour cells (8 cases), extraskeletal myxoid chondrosarcoma (6 cases) and malignant mesothelioma of round cell type (4 cases). In addition, 6 cases of MRT of soft parts including 5 cases, as described previously (Tsuneyoshi et al. 1985) and two cases of MRT of the kidney served as controls.

For light microscopy, all paraffin blocks were recut and stained with haematoxylin and eosin, periodic acid-Schiff (PAS), with and without digestion by diastase, Masson's trichrome, alcian blue and colloidal iron for mucin, and silver impregnation for reticulin and Fontana-Masson for melanin.

For electron microscopy, the fresh samples were fixed in 3% glutaraldehyde solution (buffered pH 7.4) and were post-fixed in 1% phosphate-buffered osmium tetroxide. Following dehydration, the tissue blocks were embedded in Epon 812 and cut on a Reichert ultramicrotome. Ultrathin sections were stained with uranyl acetate and lead citrate, and examined under a JEM 100 C electron microscope.

For the immunohistochemical study, paraffin sections were used. In addition, fresh samples from seven tumours were

Table 1. Cases with rhabdoid cells in round cell sarcomas

	No. Case	RC (+)	RC (++)	RC (+++)
Epithelioid sarcoma	12/13	8	4	0
Synovial sarcoma of predominantly round cell type	8/13	6	2	0
Extraskeletal myxoid chondrosarcoma	6/20	3	1	2
Malignant mesothelioma of epithelioid round cell type	4/ 4	4	0	0
Malignant rhabdoid tumour of soft parts	6/ 6	0	0	6
Malignant rhabdoid tumour of the kidney	2/ 2	0	0	2

RC: Rhabdoid cell. (+): occasionally, (++): infrequently, (+++): frequently

quickly frozen in dry ice-acetone and cut in a cryostat. Immunoperoxidase studies were performed on tissue sections using the avidin-biotin peroxidase complex (ABC) method described by Hsu et al. (1981). The sources of reagents, dilutions of antisera used for immunohistochemistry were as follows: monoclonal antibodies anti-cytokeratin (54 kd) (Labsystem, Helsinki, Finland), anti-cytokeratin (66 kd) (ENZO, Helsinki, Finland), anti-vimentin (Labsystem, Finland; ENZO, Helsinki, Finland), and anti-neurofilament (ENZO, Helsinki, Finland) and antisera rabbit anti-myoglobin (Behringer Diagnostics, Somerville, New Jersey, USA), rabbit anti-desmin, anti-carcinoembryonic antigen (CEA), rabbit anti-alpha-antitrypsin, rabbit anti-alpha-antichymotrypsin (Dakopatts, Denmark) and anti-bovine S-100 protein (provided by Dr. T. Nakajima, Tokyo, Japan. The sections were incubated in turn in primary antibodies, biotinylated anti-rabbit (mouse or goat) immunoglobulins (Vector Laboratories, Inc., Burlingame, CA, USA) and avidin-biotin peroxidase complex solution (Vector Laboratories, Inc., USA). Finally, the sections were developed with 3,3′-diaminobenzidine with 0.03% H_2O_2 for colour and counterstained with methyl green.

Results

Light microscopic findings

Twelve examples of epithelioid sarcoma (92%) in our files exhibited frequent aggregates of so-called rhabdoid cells. In 4 cases there were focal aggregates of such cells, in many areas, while in 8 others there were scattered rhabdoid cells in limited areas (Table 1). The tumours were situated in the dermis and subcutaneous tissue and appeared multifocal, often with central necrosis in each focus. The shape of epithelioid cells varied from ovoid, plump and polyhedral to spindle, and the size from moderate to large. There was a type termed "myogenous cells" by Dabska and Koszarowski (1982) having a markedly eosinophilic cytoplasm, resembling the cells of rhabdomyosarcoma. These cells were compatible with so-called rhabdoid cells and were essentially similar to those seen in MRT of soft parts, although the cytoplasm was more abundant and glassy than that of the cells in the former. There was some variation in the shape and size among these particular cells. Another variant of the epithelioid cells resembled ganglion cells, or the cells of melanoma or carcinoma. A state of transition between epithelioid cells and spindle cells was evident (Fig. 1).

Of the 68 synovial sarcomas in our file, 13 were composed of predominantly round tumour cells. In eight (62%) of the 13, rhabdoid cells were only occasionally evident (Fig. 2). These eight tumours with rhabdoid cells, showed a focal glandular differentiation (Fig. 2) or a focal biphasic differentiation with areas of both fusiform cells and of epithelioid or clear cells. Although six of the eight tumours contained only tiny aggregates of rhabdoid cells in limited areas, the remaining two had larger foci of such cells (Table 1). The nuclei of rhabdoid cells in the tumours were, however, more uniform in size and shape than those in the MRT and contained a more finely dispersed chromatin.

Six (30%) of the 20 cases of extraskeletal myxoid chondrosarcoma had rhabdoid cells. Two exhibited a large number of rhabdoid cells, over wide areas (Fig. 3) although another contained focal widely distributed aggregates of these cells and the remaining three had tiny aggregates (Table 1). The cytoplasm was as distinctively eosinophilic and glassy as in the MRT of soft parts and contained a globular inclusion (Fig. 3). These tumours, as in other examples, were characterised by the presence of a myxoid matrix and multinodular or lobulated configuration.

In the 4 malignant mesotheliomas rhabdoid cells were evident only occasionally (Table 1). These cells had a more irregularly shaped, vesicular nuclei and less conspicuous nucleoli than those seen in MRT of soft parts. The cytoplasmic inclusions, however, appeared larger, less eosinophilic and less distinct in outline than that of MRT. Intracytoplasmic vacuoles were detected in these mesothelial tumours. The vacuoles stained positively with alcian blue and colloidal iron and the stainability was diminished or lost after treatment with hyaluronidase.

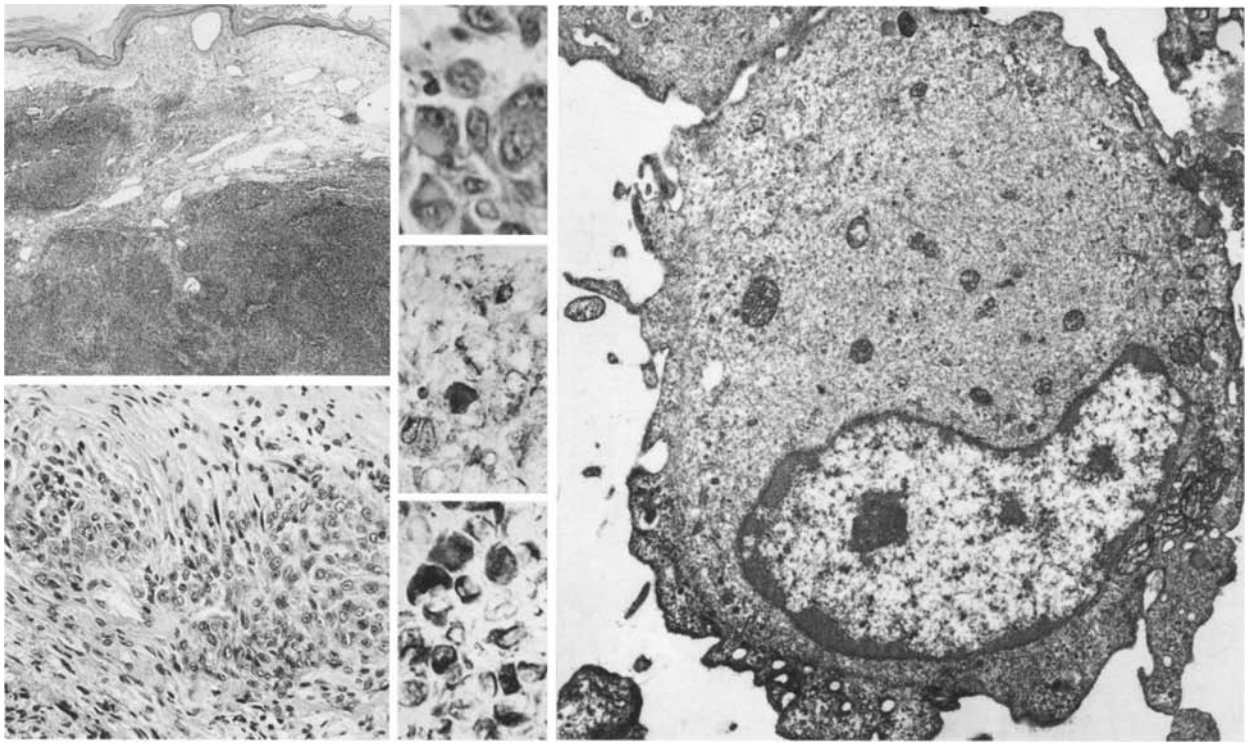

Fig. 1. Epithelioid sarcoma on the forearm in a 21 year old man. (*Left*) *Top*: Subcutaneous multinodular and granulomatous growth (8×). *Bottom*: Epithelioid elements of polygonal cells merging with spindle cells (200×). (*Middle*) *Top*: So-called "rhabdoid cells" with abundant glassy eosinophilic cytoplasm, eccentric nuclei and prominent nucleoli (600×). Cytokeratin (*center*) and vimentin (*bottom*) immunoreactive cytoplasm of rhabdoid cells (cryostat section, ABC technique, 390×). (*Right*) Electron micrograph of a rhabdoid cell containing compact masses of intermediate filaments (5600×)

For electron microscopy, five epithelioid sarcomas, six synovial sarcomas predominantly composed of round tumour cells, three extraskeletal myxoid chondrosarcomas and two round-celled malignant mesotheliomas, were processed. Three MRTs of soft parts served as controls. A variable number of tumour cells resembling rhabdoid cells seen in MRT of soft parts were detected in each type of tumour examined. The characteristic feature of these cells was the presence of bundles of cytoplasmic 10 nm intermediate filaments usually occupying the paranuclear zone (Figs. 1–3).

The rhabdoid cells seen in all four tumour types showed a close resemblance, but cardinal differences were sometimes evident. The rhabdoid cells seen in epithelioid sarcomas and synovial sarcomas rarely had a cytoplasmic filopodia or microvilli (Figs. 1, 2). The rhabdoid cells appearing in the extraskeletal myxoid chondrosarcomas, though having filamentous masses, were characteristic of chondroblasts in the presence of cytoplasmic processes forming microvilli and patches of dense, finely fibrillar material attached to the surface of the tumour cells, with pinocytotic vesicles (Fig. 3). In the malignant mesotheliomas, however, the rhabdoid cells were characterised by the presence of apical microvilli.

Intracytoplasmic inclusions of the rhabdoid cells in the four type tumors studied were postive, without exception, for cytokeratin and vimentin, and the stainability was strikingly similar (Figs. 1–3). Stains for desmin, myoglobin, neurofilament, $alpha_1$-antitrypsin, $alpha_1$-antichymotrypsin and CEA were negative. In the case of extraskeletal myxoid chondrosarcoma, cytoplasm of the rhabdoid cells stained positively for S-100 protein and its perinuclear inclusion was positive for both cytokeratin and vimentin by double labeling technique. Cytokeratin was also displayed in the tumour cells, except for rhabdoid cells of epithelioid sarcoma, synovial sarcoma and malignant mesothelioma.

Discussion

The unique cells termed "rhabdoid cells", are characterised by a large hyalin-like globular perinuclear inclusion. Ultrastructurally, the inclusion of 10 nm intermediate filaments lack differentia-

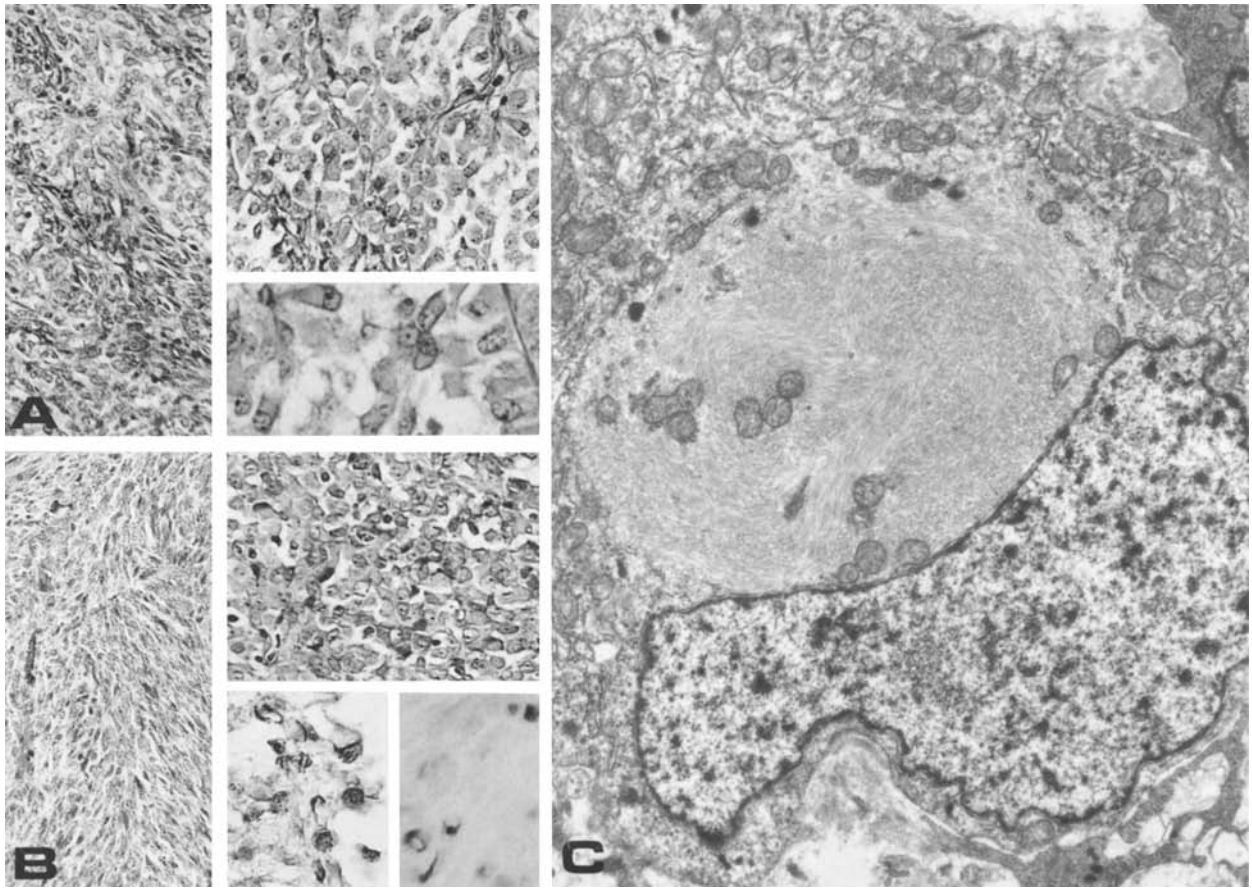

Fig. 2A. Synovial sarcoma of the predominantly round cell type of the leg in a 50 year old woman. *Left*: Only focal biphasic differentiation (180×). *Right*: Nests of round or polygonol cells (*top*; 200×) containing rhabdoid cells (*bottom*; 400×). **B** Synovial sarcoma of predominantly round cell type of the axilla in a 56 year old woman. *Left*: Some areas showing smoothly interlacing fascicles of spindle cells (128×). *Right top*: Solid nests of round or polygonal cells containing rhabdoid cells (240×). *Right bottom*: Vimentin (*left*) and cytokeratin (*right*) immunoreactive cytoplasm of rhabdoid cells (cryostat section, ABC technique, 390×). **C** Electron micrograph showing a rhabdoid cell with intermediate filaments packed in concentric whorls (8000×)

tion toward any specific derivation. This was true in our cases of MRT of soft parts (Tsuneyoshi et al. 1985) and in those documented elsewhere. The inclusions stained positively for both cytokeratin and vimentin, with the immunoperoxidase technique.

A histological review of soft tissue sarcomas in our files, particularly of round cell varieties, revealed that the rhabdoid cells are not monopolized by the MRT of soft parts and that other types of soft tissue sarcoma occasionally contain such cells. While the presence of rhabdoid cells in different types of tumours has apparently not been reported heretofore, several authors have used other expressions to describe similar findings in several types of tumours. In epithelioid sarcomas, Dabska and Koszarowski (1982) described myogenous cells resembling malignant rhabdomyoblasts among several cell variants of epithelioid cells. Sajjad and Mackay (1982) noted intracytoplasmic hyalin-like inclusions in a synovial sarcoma following intra-arterial chemotherapy. In malignant mesothelioma, a hyalin-appearing cytoplasm with intermediate filaments was reported by Dardick et al. (1984).

The rhabdoid cells appearing in the different tumour types in addition to MRT of soft parts resembled one another, though these cells often showed cytodifferentiation toward their respective tumour types. Epithelioid sarcoma, frequently containing these cells in varying numbers, could be distinguished in most cases from MRT of soft parts by its occurrence in the subcutaneous or superficial soft tissue and its growth pattern of nodularity or granuloma-like structures (Chase et al. 1984). Synovial sarcoma, occasionally with rhabdoid cells in limited areas, contained immunoreactiveproducts of keratin proteins, as did epithelioid sar-

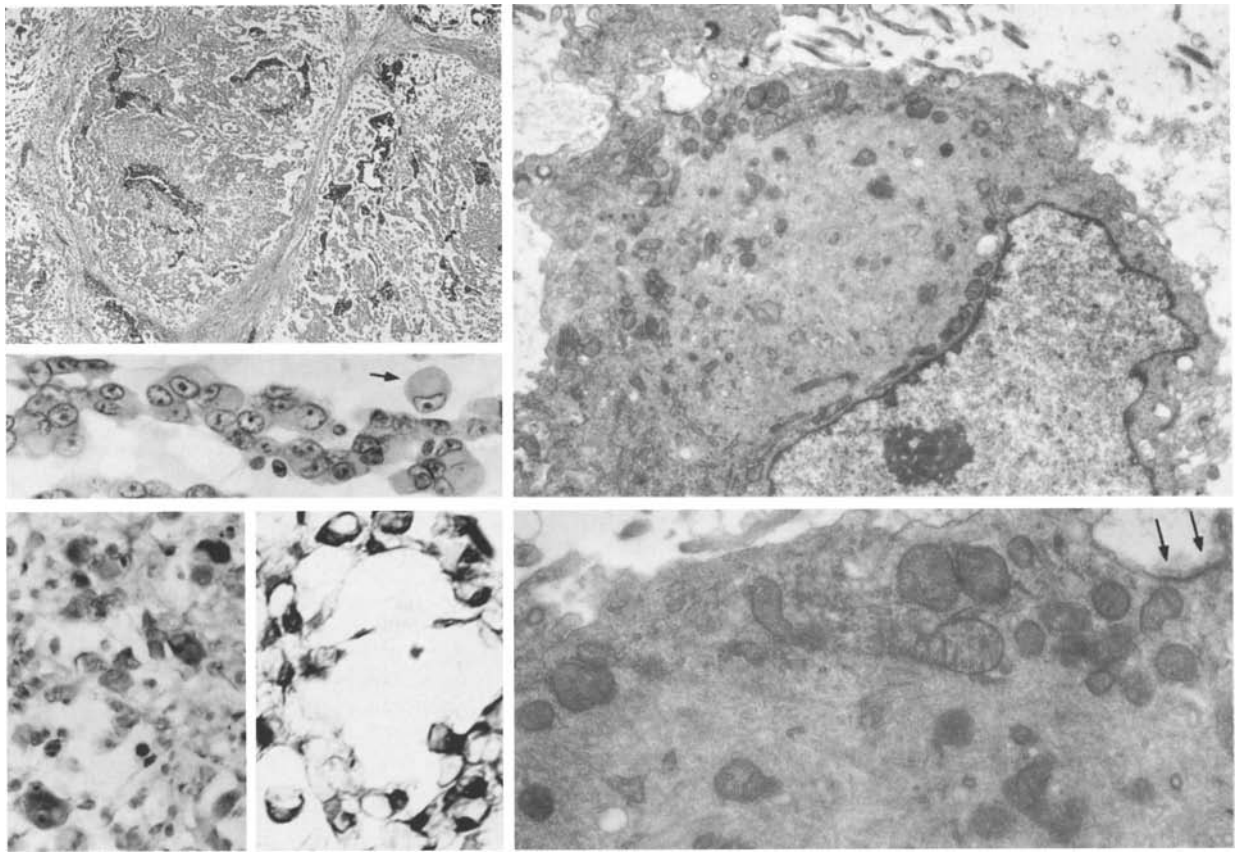

Fig. 3. Extraskeletal myxoid chondrosarcoma of the inguinal area in 53 year old man. (*Left*) *Top*: Multinodular growth with myxoid ground substance (18 ×). *Center*: Round cells showing frequent rhabdoid cells with an eosinophilic cytoplasm containing hyalin-like inclusion and eccentric nuclei. (350x). *Bottom*: Vimentin (*left*) and cytokeratin (*right*) immunoreactive cytoplasm (cryostat section, ABC technique, 370 ×). (*Right*) *Top*: A rhabdoid cell containing intracytoplasmic compact whorls of intermediate filaments admixed with mitochodria (8500 ×). *Bottom*: Cytoplasmic microvillous formation and patches of dense, finely fibrillar material (*arrow*) attached to the surface of the tumour cell (21 200 ×)

coma (Corson et al. 1983). In both epithelioid sarcoma and synovial sarcoma, the rhabdoid cells sometimes possessed filopodia or microvilli under the electron microscope, showing differentiation toward synovial lining cells. Several instances of extraskeletal myxoid chondrosarcoma carried rhabdoid cells only occasionally, though two cases did contain them over wide areas. In addition to the positivity of perinuclear inclusion for cytokeratin and vimentin, the reaction to S-100 protein was positive in the cytoplasm of rhabdoid cells in this chondrosarcoma. These cells were ultrastructurally characteristic of chondroblasts. Rhabdoid cells were scattered infrequently in some malignant mesotheliomas, in which they had apical microvilli, a feature representing mesothelial differentiation.

The proposed origins of rhabdoid cells in MRT of soft parts include mesenchymal (Frierson et al. 1985; Sotelo-Avila et al. 1986) and epithelial (Vogel et al. 1984; Ekfors et al. 1985). Furthermore, the mechanism of formation of intermediate filaments to make up an intracytoplasmic hyaline inclusion has remained controversial. Sajjad and Mackay (1982) suggested that such rhabdoid cells may appear following chemotherapy. However, no such relationship could be traced in the current study with different types of soft tissue sarcomas. Haas et al. (1981) pointed out that the arrangement of filaments might indicate a particular line of cellular differentiation. While co-expression of cytokeratin and vimentin of the intermediate filaments has been noted in cells of both carcinoma and sarcoma, it is likely that the co-expression indicates primitiveness of a tumour cell. It is of practical importance to recognize the occurrence of rhabdoid cells among a variety of round cell soft tissue sarcomas, in order to differentiate MRT of soft parts from other sarcomas with rhabdoid cells and from a rhabdomyosarcoma.

Acknowledgements. We thank Dr. N. Shinohara, National Fukuoka Central Hospital, Dr. S. Masuda, Kyushu University,

and Dr. T. Kitajima, National Kyushu Cancer Center for making available the fresh materials used in this study. M. Ohara provided comments on the manuscript. This work is presented in part at the 45th Annual Meeting of the Japanese Cancer Association on October 23, 1986, Sapporo, Japan.

References

Biggs PJ, Garen PD, Powers JM, Garvin AJ (1987) Malignant rhabdoid tumor of the central nervous system. Hum Pathol 18:332–337

Chase DR, Enzinger FM, Weiss SW, Longloss JM (1984) Keratin in epithelioid sarcoma. An immunohistochemical study. Am J Surg Pathol 8:435–441

Corson JM, Weiss LM, Banks-Schlegel SP, Pinkus GS (1983) Letters to the editor. Keratin proteins in synovial sarcoma. Am J Surg Pathol 7:107–109

Dabska M, Koszarowski T (1982) Clinical and pathologic study of aponeurotic (epithelioid) sarcoma. Pathol Ann 17:129–153

Dardick I, Al-Jabi M, McCaughey WTE, Srigley JR, Nostrand AWP, Ritchie AC (1984) Ultrastructure of poorly differentiated diffuse epithelial mesotheliomas. Ultrastruc Pathol 7:151–160

Ekfors TO, Aho HJ, Kekomäki M (1985) Malignant rhabdoid tomor of the prostatic region. Immunohistological and ultrastructural evidence for epithelial origin. Virchows Arch [A] 406:381–388

Frierson HF, Mills SE, Innes DJ (1985) Malignant rhabdoid tumor of the perlvis. Cancer 55:1963–1967

Gonzalez-Crussi F, Goldschmidt RA, Hsueh W, Trujillo YP (1982) Infantile sarcoma with intracytoplasmic filamentous inclusions: Distinctive tumor of possible histiocytic origin. Cancer 49:2365–2375

Haas JE, Palmer NF, Weinberg AG, Beckwith JB (1981) Ultrastructure of malignant rhabdoid tumor of the kidney. A distinctive renal tumor of children. Hum Pathol 12:646–657

Hsu SM, Raine L, Fanger H (1981) The use of antiavidin antibody and avidin-biotin-peroxidase complex in immunoperoxidase technics. Am J Clin Pathol 75:816–821

Lynch HT, Shurin SB, Dahms BB, Izant RJ, Lynch J, Danes BS (1983) Paravertebral malignant rhabdoid tumor in infancy. In vitro studies of a familial tumor. Cancer 52:290–296

Sajjad SM, Mackay B (1982) Hyaline inclusions in a synovial sarcoma following intra-arterial chemotherapy. Ultrastruct Pathol 3:313–318

Small EJ, Gordon GJ, Dahms BB (1985) Malignant rhabdoid tumor of the heart in an infant. Cancer 55:2850–2853

Sotelo-Avila C, Gonzalez-Crussi F, deMello D, Vogler C. Gooch WM, Gale G, Pena R (1986) Renal and extrarenal rhabdoid tumors in children: A clinicopathologic study of 14 patients. Semin Diag Pathol 3:151–163

Tsuneyoshi M, Daimaru Y, Hashimoto H, Enjoji M (1985) Malignant soft tissue neoplasms with the histologic features of renal rhabdoid tumors. Hum Pathol 16:1235–1242

Vogel AM, Gown AM, Caughlan J, Haas JE, Beckwith JB (1984) Rhabdoid tumors of the kidney contain mesenchymal specific and epithelial specific intermediate filament proteins. Lab Invest 50:232–238

Accepted July 17, 1987