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S-100 protein distribution in liposarcoma An immunoperoxidase study with special reference to the distinction of liposarcoma from myxoid malignant fibrous histiocytoma

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Abstract. The presence and distribution of S-100 protein were studied in 63 cases of liposarcoma and 20 cases of myxoid malignant fibrous histiocytoma (MFH), using the immunoperoxidase technique. Normal adipose tissue and benign lipomatous tumours were also studied by the same technique, for purposes of comparison. In all liposarcomas, most of the adipocytes and vacuolated lipoblasts were positive for S-100 protein, although the tumour cells in non-lipogenic areas of dedifferentiated liposarcoma and the non-vacuolated giant cells with a deeply eosinophilic cytoplasm in the pleomorphic liposarcomas were devoid of S-100 protein immunoreaction products. One third of the myxoid type liposarcomas contained numerous immunoreactive, immature-appearing spindle or oval cells, reminiscent of the primitive fat organs of white adipose tissue. Conversely, none of the myxoid MFHs contained S-100 protein in the tumour cells, including the irregularly vacuolated ones. These results suggests that the immunohistochemical demonstration of S-100 protein is a useful diagnostic tool, particularly for the assessment of vacuolated tumour cells and for the diagnosis of myxoid tumours.

Key words: Liposarcoma – Malignant fibrous histiocytoma – Soft tissue neoplasms – S-100 protein – Immunohistochemistry

Introduction

S-100 protein was first isolated as a soluble nervous tissue-specific protein by Moore in 1965. In the nervous system, this protein is located mainly in astrocytes, oligodendrocytes, Schwann cells, satellite cells in sympathetic ganglia and some neurons (Bradshaw and Schneider 1980). Recently, S-100 protein has been identified in adipocytes (Nakajima et al. 1982b; Suzuki et al. 1982; Hidaka et al. 1983; Kato et al. 1983; Michetti et al. 1983), as

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well as in various cells outside the nervous system; melanocytes and Langerhans cells in the skin (Cocchia et al. 1981; Nakajima et al. 1982a), interdigitating reticulum cells in the lymph nodes (Takahashi et al. 1981), T lymphocytes (Kanamori et al. 1982), folliculo-stellate cells in the pituitary gland (Nakajima et al. 1980), chondrocytes (Stefansson et al. 1982), myoepithelial cells (Nakajima et al. 1982b; Hara et al. 1983). Moreover, Cocchia et al. (1983) and Weiss et al. (1983) confirmed the presence of S-100 protein positive tumour cells in liposarcoma, using an immunoperoxidase method.

We used immunohistochemical approaches to study S-100 protein in a larger number of liposarcomas and myxoid malignant fibrous histiocytomas and compared our findings with observations made in normal adipose tissue and benign lipomatous tumours.

Materials and methods

From files in the Second Department of Pathology, Kyushu University Faculty of Medicine, from 1956 to 1982, we selected for immunohistochemical study 63 cases of liposarcoma (31 myxoid, 16 well-differentiated, 6 dedifferentiated (Evans 1979), 4 pleomorphic, 3 round cell, 3 mixed) and 20 cases of myxoid malignant fibrous histiocytoma. The diagnostic criterion of liposarcoma was the finding of convincing histological evidence of lipogenic differentiation in the tumour cells on conventional sections stained with haematoxylin and eosin (Hashimoto and Enjoji 1982; Enzinger and Weiss 1983).

In addition, white adipose tissue in the subcutis from two aborted humen fetuses of 18 weeks' and 20 weeks' of gestation, human brown adipose tissue from the mediastinum and the perirenal region from a full-term baby with anencephaly and adjacent to a haemangioma in the neck of 15-year-old girl, and various benign lipomatous tumours (6 lipomas, 8 spindle cell lipomas, 2 pleomorphic lipomas, 2 benign lipoblastomatoses, and 1 hibernoma) were used as control material.

Six-micron-thick sections of 10% formalin-fixed, paraffin embedded materials were prepared for the immunohistochemical study. After the tissue sections were deparaffinized and endogenous peroxidase activity was blocked by employing a short 10-min oxidation in 0.01 M aqueous periodic acid followed by treatment with a freshly prepared 0.02% aqueous sodium borohydrate for 30 min, a peroxidase-antiperoxidase (PAP) method (Sternberger 1979) was used to determine the localization of S-100 protein. Dilution and time in 0.01 M phosphate buffered saline, pH 7.4 were as follows: normal swine serum (DAKO-immunoglobulin Co. Ltd., Denmark) (1:10) for 30 min; anti-bovine S-100 protein rabbit IgG (kindly provided by Dr. Takashi Nakajima, National Cancer Center Research Institute, Tokyo. The details of the method for preparation of the anti-S-100 protein antibody were as reported elsewhere) (Nakajima et al. 1982b), diluted 1:300, overnight at 4° C; anti-rabbit IgG swine serum (DAKO-immunoglobulin Co. Ltd., Denmark) (1:30) for 1 h at room temperature; PAP complex (DAKO-immunoglobulin Co. Ltd., Denmark) (1:50) for 1 h at room temperature. The location of antigen was visualized by incubating slides for 5 min with 0.01% hydrogen peroxidase and 0.05% diaminobenzidine in 0.05 M tris buffer, pH 7.6. These sections were counterstained with methyl green. Normal rabbit serum was used instead of the first antibody for the controls, and cells positive for S-100 protein were never observed in any of the controls.

Results

Normal adipose tissues

In the primitive organs of white adipose tissue in the subcutis of the two aborted fetuses, brown benzidine products in the immunoperoxidase reaction for S-100 protein were observed in the cytoplasmic rim and the nuclei

of vacuolated lipoblasts. Some of immature mesenchymal cells with oval, spindle or stellate nuclei in the developing fat lobules also showed strong immunoreactivity for S-100 protein (Fig. 1). Almost all adipocytes of the normal mature fat tissue adjacent to some tumours had immunoreaction products in the narrow rim of the cytoplasm. S-100 immunoreactivity was positive in brown adipose tissue, where the reaction products were located in the cytoplasm or in both the cytoplasm and the nuclei of all kinds of brown fat cells.

Benign lipomatous tumours

All benign lipomatous tumours examined were positive for S-100 protein. The immunoreaction in 6 cases of ordinary lipoma and one case of hibernoma was comparable with that in the normal mature adipose tissue and in the brown adipose tissue, respectively (Fig. 2). Although in 8 cases of spindle cell lipoma and in 2 cases of pleomorphic lipoma, elemental adipocytes and lipoblasts were positive for S-100 protein, the majority of spindle cells or floret-like multinucleated giant cells characterizing these tumours were devoid of S-100 protein immunoreaction products. The immunoreactive findings in benign lipoblastomatosis were reminiscent of those of white adipose tissue in a developing stage.

Liposarcomas

In all 63 cases of liposarcoma, S-100 protein immunoreactive neoplastic lipoblasts or adipocytes were recognized, although the number of positive cells varied from case to case.

1. Myxoid type (31 cases). In almost all cases, the majority of scattered univacuolated and multivacuolated lipoblasts and more mature larger adipocytes in the myxoid stroma had immunoreaction products both in the cytoplasm displaced by vacuoles, and in the nuclei (Fig. 3). Although in two thirds of the cases, most of non-vacuolated oval, stellate or spindle cells with no pleomorphism were devoid of S-100 protein immunoreaction products, one third had many immunoreactive, immature tumour cells, where the reaction procucts were located in both the cytoplasm and the nuclei (Fig. 4). In 8 cases, rounded granular or finely vacuolated tumour cells were frequently present with centrally located small nuclei, resembling cells of brown fat and stained with S-100 protein immunohistochemistry. S-100 immunoreactive lipoblasts closely attached to capillaries were numerous.

2. Well-differentiated type (16 cases). In all cases, the majority of adipocytes, showing a greater variation in size than those of the normal mature fat or ordinary lipoma, and scattered univacuolated and multivacuolated lipoblasts, were both positive for S-100 protein in the cytoplasm. The reaction was pushed away by one or more vacuoles and frequently displaced together with the nuclei, although the staining intensity varied from strong to weak (Fig. 5a). In contrast, in most non-vacuolated cells with atypical hyperchro-

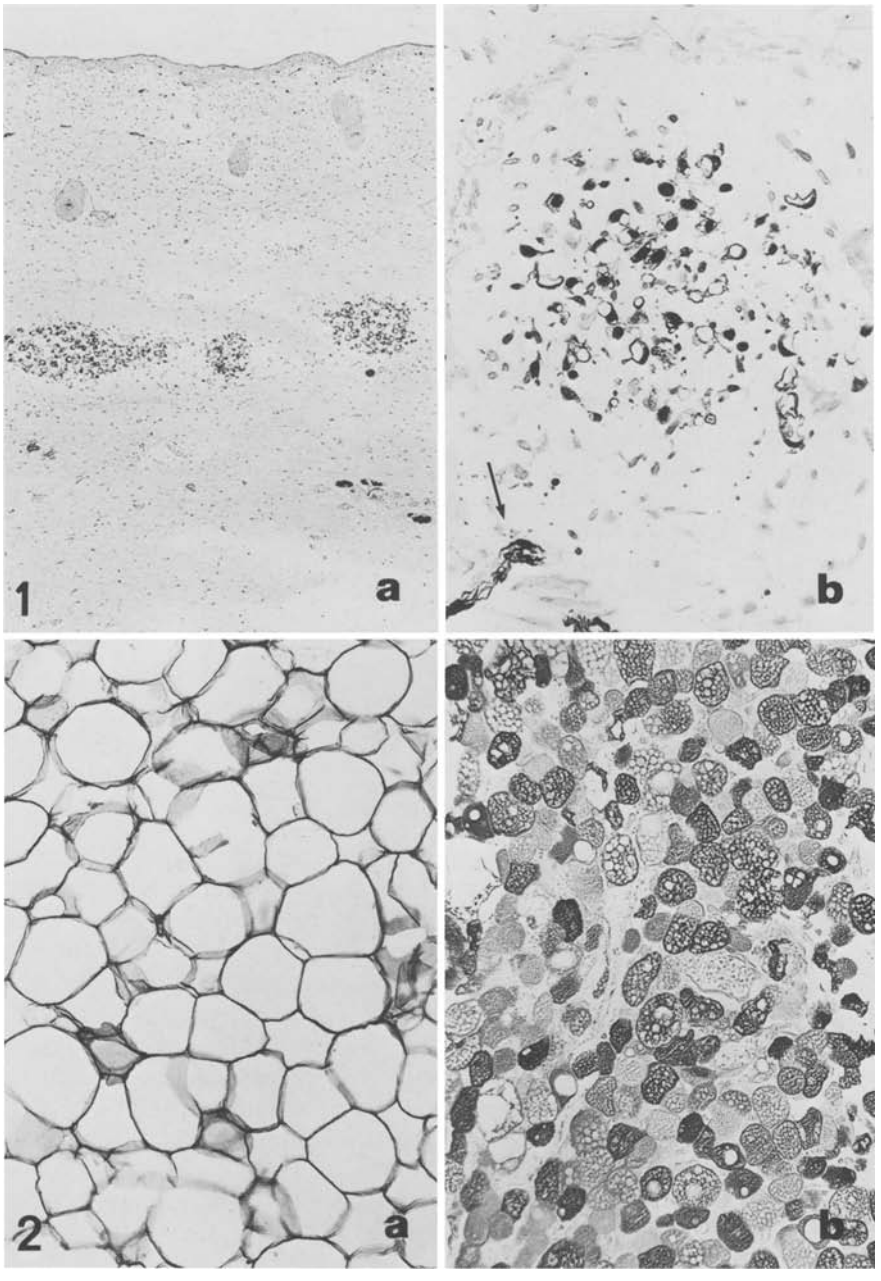


Fig. 1 a, b. Immunohistochemistry for S-100 protein in a 20-week-old human aborted fetus. **a** Subcutaneous developing fat lobules are strongly positive for S-100 protein (× 48). **b** Reaction product is present in the cytoplasm and the nuclei of vacuolated lipoblasts, immature-appearing spindle or oval cells and Schwann cells of peripheral nerve fibers (*arrow*) (× 250)

Fig. 2 a, b. Both mature adipocytes in lipoma (**a**) and brown fat cells in hibernoma (**b**) are stained by S-100 protein immunohistochemistry (× 124)

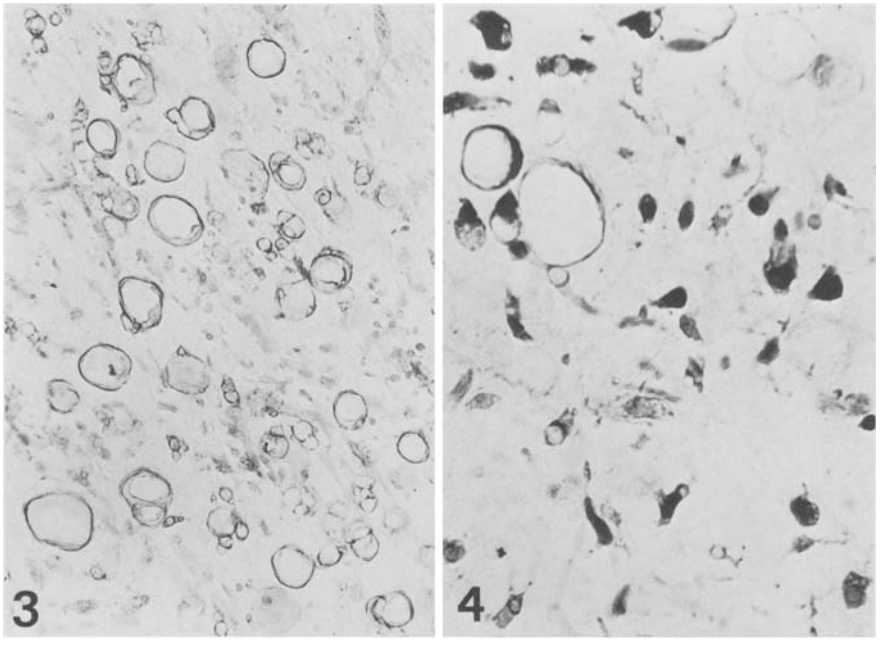


Fig. 3. Immunohistochemistry for S-100 protein in myxoid liposarcoma. Many S-100 protein positive univacuolated and multivacuolated lipoblasts are seen (× 250)

Fig. 4. Note S-100 protein positive immature-appearing oval or spindle cells in myxoid liposarcoma, in addition to positive vacuolated lipoblasts (× 470)

matic nuclei situated along or within fibrous septa of lipoma-like liposarcoma (9 cases) or embedded in fibrosclerotic matrix of the sclerosing one (7 cases), immunoreaction products were not observed (Fig. 5b).

3. Dedifferentiated type (6 cases). S-100 protein immunoreactivity in the well-differentiated area was essentially the same as that of well-differentiated liposarcoma, whereas in the non-lipogenic poorly differentiated area reminiscent of malignant fibrous histiocytoma or fibrosarcoma, tumour cells were negative for S-100 protein. There were a few S-100 protein positive non-neoplastic cells with dendritic or rounded cytoplasm and small twisted nuclei probably consistent with T-zone histiocytes, described by Watanabe et al. (1983), and thought to be on the same cell lineage as Langerhans cells of the skin and interdigitating reticulum cells of the lymph node.

4. Pleomorphic type (4 cases). Most multivacuolated giant cells with bizarre or scalloped nuclei had immunoreaction products in the cytoplasm, or in both the cytoplasm and the nuclei (Fig. 6). However, non-vacuolated giant cells with deeply eosinophilic cytoplasm (haematoxylin and eosin stain) were negative for S-100 protein and resembled those seen in cases of malignant fibrous histiocytoma.

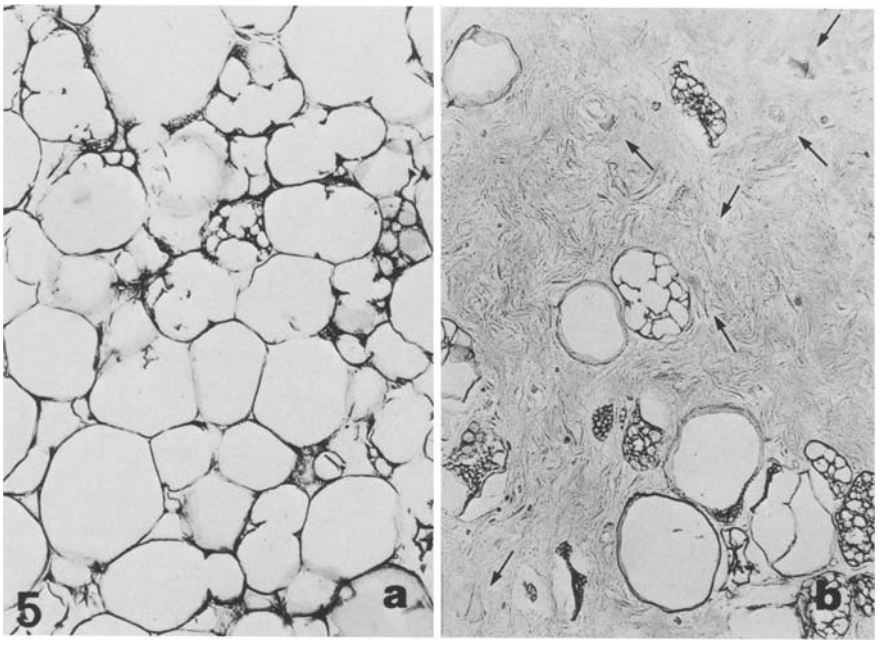


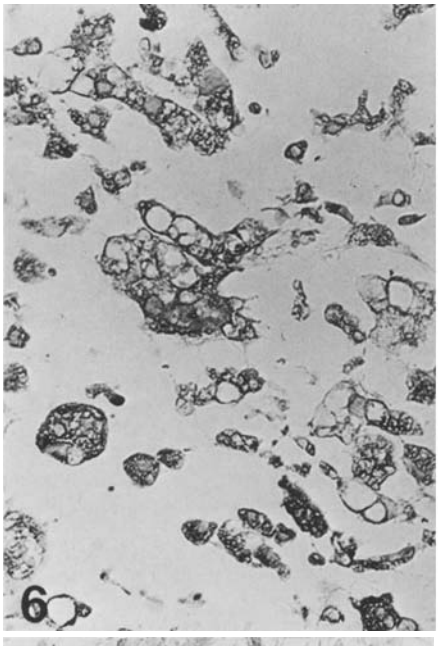
Fig. 5a, b. Immunohistochemistry for S-100 protein in well-differentiated liposarcoma. **a** In lipoma-like form, variable-sized adipocytes and multivacuolated lipoblasts have S-100 protein in a rim of the cytoplasm (×220). **b** Non-vacuolated atypical cells in sclerosing form (*arrows*) are devoid of S-100 protein (×116)

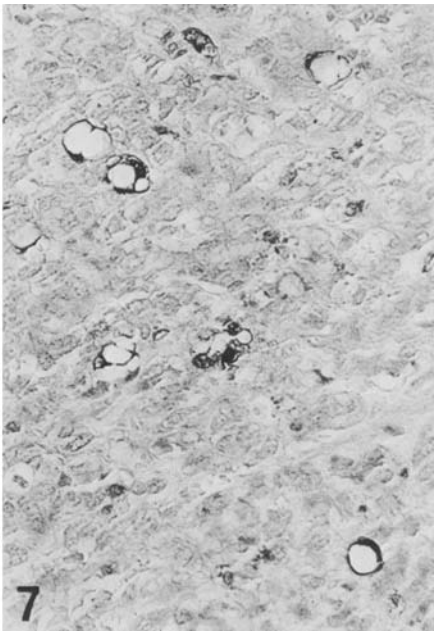
5. Round cell type (3 cases). In two of the 3 cases, almost all scattered univacuolated lipoblasts with a signet-ring appearance were positive for S-100 protein (Fig. 7). In addition, a few, compactly arranged, non-vacuolated round or oval tumour cells had immunoreaction products in both the cytoplasm and the nuclei, although most of these cells were negative. In the remaining tumour located in the retroauricular region and composed of a proliferation of tumour cells with ample, eosinophilic, granular or finely vacuolated cytoplasm and closely resembling hibernoma cells, these cells were almost wholly stained for S-100 protein.

6. Mixed type (3 cases). The three mixed forms of liposarcoma consisted of a combination of well-differentiated and pleomorphic types, well-differentiated and myxoid types, and round cell and myxoid types, respectively. The immunoreactive finding in these cases were akin to those of each type described above.

Myxoid variant of malignant fibrous histiocytoma (MFH)

In all 20 cases of myxoid MFH, neither irregularly vacuolated cells nor other histiocyte-like oval or round and fibroblast-like spindle tumour cells were stained for S-100 protein (Fig. 8), although, in some cases, scattered S-100 protein positive non-neoplastic T-zone histiocytes were demonstrated, particularly in the presence of a prominent lymphocytic infiltrate.





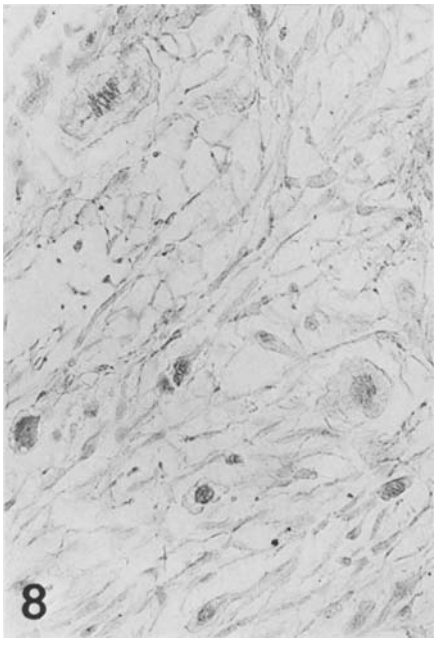


Fig. 6. S-100 protein positive multivacuolated giant cells in pleomorphic liposarcoma are numerous (× 320)

Fig. 7. S-100 protein immunoreactive vacuolated cells are scattered among compactly arranged round or oval tumour cells in round cell liposarcoma (× 260)

Fig. 8. Immunohistochemistry for S-100 protein in myxoid variant of malignant fibrous histiocytoma. Tumour cells including irregularly vacuolated cells are devoid of S-100 protein reaction product (× 210)

Discussion

Isobe et al. (1978) showed that bovine brain S-100 protein is a mixture of two predominant components; S-100a and S-100b. Both of these proteins are dimers with a subunit composition of $\alpha\beta$ (S-100a) and $\beta\beta$ (S-100b). Hidaka et al. (1983) used an immunohistochemical method to identify S-

100b protein in adipose tissue. The function of S-100 protein in adipose tissue nervous system and other tissues remains unknown. Suzuki et al. (1984) found that S-100 protein levels in adipose tissue are markedly decreased by serial injection of epinephrine into rats. They speculated a role for S-100 protein in the cellular processes of lipid metabolism in adipocytes.

The presence of S-100 protein positive tumour cells in liposarcoma was confirmed using an immunoperoxidase method (Cocchia et al. 1983; Weiss et al. 1983). We attempted to elucidate a more precise distribution of S-100 protein in a fairly large number of liposarcomas, using the PAP method, and to determine whether this protein would be a useful marker for liposarcoma. In immunohistochemical studies on normal adipose tissues, various benign lipomatous tumours and all types of liposarcomas, both white and brown fat cells and almost all neoplastic adipocytes and lipoblasts contained S-100 protein in the cytoplasm and sometimes in the nuclei. Furthermore, in myxoid and round cell type liposarcoma, immature non-vacuolated spindle, stellate or oval cells not infrequently accumulated immunoreaction products for S-100 protein. Such immunohistochemical features of myxoid liposarcoma often bear a close resemblance to those seen in the primitive fat organs of white adipose tissue, according to Wassermann's detailed description (1965) concerning the development of adipose tissue, and also in benign lipoblastomatosis.

On the other hand, the tumour cells of non-lipogenic areas resembling malignant fibrous histiocytoma or fibrosarcoma in dedifferentiated liposarcoma, and non-vacuolated giant cells with deeply eosinophilic cytoplasm in pleomorphic liposarcoma were devoid of S-100 protein immunoreaction products. The electron microscopic study by Bolen and Thorning (1984) revealed that cells in the dedifferentiated regions of well-differentiated liposarcomas had ultrastructural features of mesenchymal cells with no specific evidence of lipogenic differentiation ranging from poorly differentiated forms to more differentiated fibroblast-like or histiocyte-like forms. Plausible explanations for S-100 protein-negative cells such as spindle cells in spindle cell lipoma, floret-like giant cells in pleomorphic lipoma (Shmookler and Enzinger 1981) and non-vacuolated atypical cells in the fibrous septa of lipoma-like well-differentiated liposarcoma or in fibrosclerotic matrix of sclerosing variety of the same tumour cannot be given from our study.

It is sometimes difficult to distinguish liposarcoma from tumours with vacuolated cells or with a myxoid matrix in conventional histological slides, particularly from the myxoid variant of malignant fibrous histiocytoma (Weiss and Enzinger 1977). The complete lack of S-100 protein in irregularly vacuolated cells of myxoid MFH in our immunohistochemical study aids in a differential diagnosis between myxoid liposarcoma and MFH. Of other myxoid tumours, extraskeletal myxoid chondrosarcoma (chordoid sarcoma) (Enzinger and Shiraki 1972) shows a strong immunoreactivity for S-100 protein in almost all tumour cells (unpublished data) as well as in normal cartilage (Stefansson et al. 1982).

Thus, the immunohistochemical technique for S-100 protein is a useful

tool for the diagnosis of soft tissue tumours especially with regard to aspects of assessment of vacuolated cells and the differentiation of myxoid tumours.

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References

Bolen JW, Thorning D (1984) Liposarcomas. A histogenetic approach to the classification of adipose tissue neoplasms. Am J Surg Pathol 8:3–17

Bradshaw RA, Schneider DM (1980) Proteins of the nervous system. 2nd ed, Raven Press, New York, pp 1–57

Cocchia D, Michetti F, Donato R (1981) Immunochemical and immunocytochemical localization of S-100 antigen in normal human skin. Nature 294:85–87

Cocchia D, Lauriola L, Stolfi VM, Tallini G, Michetti F (1983) S-100 antigen labels neoplastic cells in liposarcoma and cartilaginous tumours. Virchows Arch [Pathol Anat] 402:139–145

Enzinger FM, Shiraki M (1972) Extraskeletal myxoid chondrosarcoma. An analysis of 34 cases. Hum Pathol 3:421–435

Enzinger FM, Weiss SW (1983) Liposarcoma. In: Soft Tissue Tumors. Mosby, St. Louis, pp 242–280

Evans HL (1979) Liposarcoma. A study of 55 cases with a reassessment of its classification. Am J Surg Pathol 3:507–523

Hara K, Ito M, Takeuchi J, Iijima S, Endo T, Hidaka H (1983) Distribution of S-100b protein in normal salivary glands and salivary gland tumors. Virchows Arch [Pathol Anat] 401:237–249

Hashimoto H, Enjoji M (1982) Liposarcoma. A clinicopathologic subtyping of 52 cases. Acta Pathol Jpn 32:933–948

Hidaka H, Endo T, Kawamoto S, Yamada E, Umekawa H, Tanabe K, Hara K (1983) Purification and characterization of adipose tissue S-100b protein. J Biol Chem 258:2705–2709

Isobe T, Tsugita A, Okuyama T (1978) The amino acid sequence and the subunit structure of bovine brain S 100 protein (PAP1-b). J Neurochem 30:921–923

Kanamori M, Endo T, Shirakawa S, Sakurai M, Hidaka H (1982) S-100 antigen in human T lymphocytes. Biochem Biophys Res Commun 108:1447–1453

Kato K, Suzuki F, Nakajima T (1983) S-100 protein in adipose tissue. Int J Biochem 15:609–613

Michetti F, Dell'Anna E, Tiberio G, Cocchia D (1983) Immunochemical and immunocytochemical study of S-100 protein in rat adipocytes. Brain Res 262:352–356

Moore BW (1965) A soluble protein characteristic of the nervous system. Biochem Biophys Res Commun 19:739–744

Nakajima T, Yamaguchi H, Takahashi K (1980) S 100 protein in folliculostellate cells of the rat pituitary anterior lobe. Brain Res 191:523–531

Nakajima T, Sato Y, Watanabe S, Shimosato Y, Ishihara K, Isobe T (1982a) Immunoelectron microscopical demonstration of S-100 protein in epidermal Langerhans cells. Biomed Res 3:226–231

Nakajima T, Watanabe S, Sato Y, Kameya T, Hirota T, Shimosato Y (1982b) An immunoperoxidase study of S-100 protein distribution in normal and neoplastic tissues. Am J Surg Pathol 6:715–727

Shmookler BM, Enzinger FM (1981) Pleomorphic lipoma: An benign tumor simulating liposarcoma. A clinicopathologic analysis of 48 cases. Cancer 47:126–133

Stefansson K, Wollmann RL, Moore BW, Arnason BGW (1982) S-100 protein in human chondrocytes. Nature 295:63–64

Sternberger LA (1979) Immunocytochemistry. 2nd ed, John Wiley and Sons, New York

Suzuki F, Nakajima T, Kato K (1982) Peripheral distribution of nervous system-specific S-100 protein in rat. J Biochem 92:835–838

Suzuki F, Kato K, Nakajima T (1984) Regulation of nervous system-specific S-100 protein and enolase levels in adipose tissue by catecholamines. J Neurochem 42:130–134

Takahashi K, Yamaguchi H, Ishizeki J, Nakajima T, Nakazato Y (1981) Immunohistochemical and electron microscopic localization of S-100 protein in the interdigitating reticulum cells of the human lymph node. Virchows Arch [Cell Pathol] 37:125–135

Wassermann F (1965) The development of adipose tissue. In: Renold AE, Gahill GF, Jr (eds) Handbook of Physiology, Section 5, Adipose Tissue. Waverly Press, Baltimore, pp 87–100

Watanabe S, Nakajima T, Shimosato Y, Shimamura K, Sakuma H (1983) T-zone histiocytes with S 100 protein. Development and distribution in human fetuses. Acta Pathol Jpn 33:15–22

Weiss SW, Enzinger FM (1977) Myxoid variant of malignant fibrous histiocytoma. Cancer 39:1672–1685

Weiss SW, Langloss JM, Enzinger FM (1983) Value of S-100 protein in the diagnosis of soft tissue tumors with particular reference to benign and malignant Schwann cell tumors. Lab Invest 49:299–308

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