Virchows Arch A (1987) 411:515–521

Virchows Archiv A

The distribution of alpha and beta subunits of S-100 protein in malignant Schwannomas arising from neurofibromatosis of von Recklinghausen's disease

Kazuhiko Hayashi [1], Kiyoshi Takahashi [1], Hiroshi Sonobe [1], Yuji Ohtsuki [1], and Kohji Taguchi [2]

[1] Department of Pathology, Kochi Medical School, Nankoku-city, Kochi 781-51, Japan
[2] Department of Pathology, Okayama University Hospital, Okayama-city, Okayama 700, Japan

Summary. The immunohistochemical localization of the alpha and beta subunits of S-100 protein in 4 cases of the malignant Schwannomas arising from von Recklinghausen's disease was investigated by the indirect immunoperoxidase method. S-100 alpha-positive cells were few in presumably normal peripheral nerves, moderate in numbers in plexiform neurofibromas and numerous in malignant Schwannomas. In contrast, S-100 beta immunoreactivity, abundantly detected in normal peripheral nerves and plexiform neurofibromas, was completely negative in all of 4 cases of malignant Schwannoma. In addition, double immunostaining method for both subunits revealed their simultaneous existence in cells in the normal nerves and neurofibroma. These results suggest that malignant change of Schwann cells convert their subunit composition of S-100 protein from beta to alpha in these malignant cells. Although the mechanisms for the proportional conversion of the subunits are as yet undetermined, the immunoreactivity of S-100 alpha subunit may be a useful marker for Schwannoma in malignancy.

Key words: S-100 protein – Alpha and beta subunits – Immunohistochemistry – Malignant Schwannoma – von Recklinghausen's disease

Introduction

S-100 protein, first isolated from bovine brain extract by Moore (1965), is a acidic Ca^{2+}-binding protein which is a mixture of several similar proteins, namely S-100ao, S-100a, and S-100b, which are dimers with a subunit composition of alpha-alpha, alpha-beta, and beta-beta, respectively (Isobe and Okuyama 1978; Isobe et al. 1981a, b; Isobe and Okuyama 1984). The amino acid sequences of the alpha and beta subunits of S-100 protein (S-100alpha, S-100beta) are very similar and about 58% of the residues are identical (Isobe and Okuyama 1981b).

S-100 protein is widely distributed in the central and peripheral nervous systems of many vertebrates (Moore 1972) and was regarded as a nervous system – specific protein. However, it is not restricted to the nervous system but has been demonstrated immunohistochemically outside of the nervous system, in chondrocytes (Stefansson et al. 1982b), folliculostellate cells of the adenohypophysis (Nakajima et al. 1980) interstitial cells of the pineal body (Møller et al. 1978) melanocyte and Langerhans cells of the skin (Cocchia et al. 1981), interdigitating reticulum cells of the lymph node (Takahashi et al. 1981). The distribution and localization of the alpha and beta subunits of S-100 protein in human normal and neoplastic tissues were investigated by Takahashi et al. (1984a, b) who showed that human S-100 positive cells are divided into three groups, namely S-100 beta positive, both S-100 alpha and beta positive, and only S-100 alpha positive.

S-100 protein has not been detected consistently in cases of malignant Schwannoma (Nakajima et al. 1982; Stefansson et al. 1982b; Weiss et al. 1983). Therefore, in order to clarify the localization of S-100 protein subunits in malignant Schwannoma, we investigated in detail the presence and distribution of S-100alpha and S-100beta immunoreactivity in definite cases of this tumour arising from neurofibromatosis. An indirect immunoperoxidase method was used including double immunostaining of both subunits.

Offprint requests to: K. Hayashi

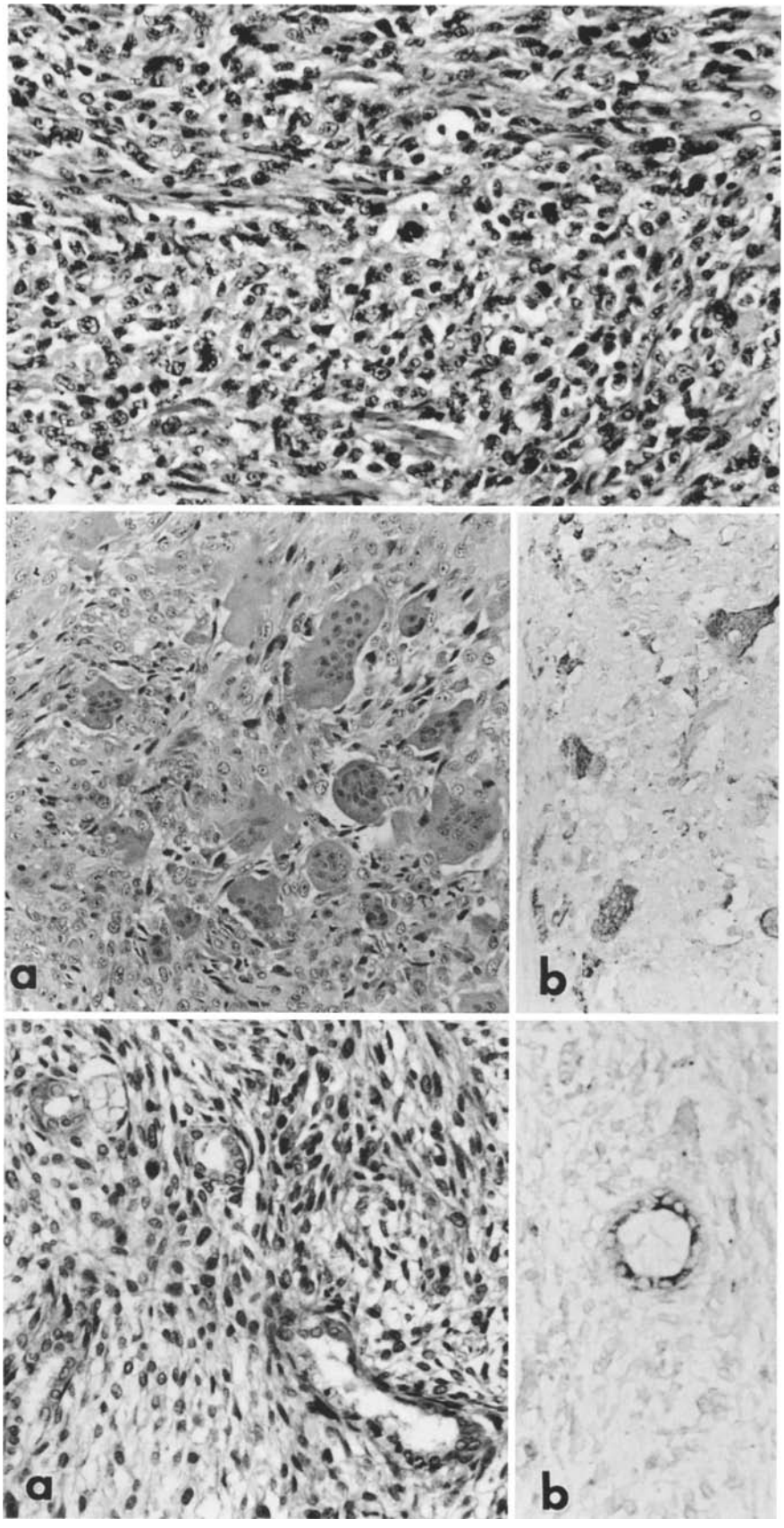

Fig. 1. Plump spindle and polygonal cells in a case of malignant Schwannoma, showing marked pleomorphism and mitotic figures. Case 1. ×220

Fig. 2. a One of the nests composed of epithelioid and multinucleate giant cells. **b** S-100alpha immunoreactivity detected in the giant cells. Case 2. **a** ×175; **b** indirect method, ×140

Fig. 3. a Glandular elements found as evidence for the focal divergent differentiation. **b** Glandular components showing strong S-100alpha immunopositivity. Case 3. **a** × 175; **b** indirect method, ×250

Table 1a. Immunoreactivity for the α and β subunits of S-100 protein in malignant Schwannomas associated with neurofibromatosis

Case No.	Age	Sex	Location	α subunit	β subunit	Comments
Case 1(B)	38yr	F	left lower leg	+++	−	
Case 2(B)	31yr	M	left thigh	++	−	α(+++) and β(−) in giant cell tumour
Case 3(A)	40yr	M	lung and skin meta	+~++	−	α(+++) and β(−) in glandular elements
Case 4(B)	35yr	F	left buttock	++	−	
Case 4(A)	35yr	F	diaphragma meta	−	−	

B: biopsy; A: autopsy; F: female; M: male; yr: years; meta: metastasis; −: absent; +: mild; ++: moderate; +++: marked

Materials and methods

The tissues examined were 4 cases of malignant Schwannoma from patients with neurofibromatosis of von Recklinghausen's disease including one case of glandular type and a case of malignant Schwannoma with giant cell tumours. These were compaired with plexiform neurofibromas and presumably normal peripheral nerves obtained from the cases of malignant Schwannoma. This material consisted of 2 autopsy cases and 3 from biopsies. Controls were composed of 10 neurofibromas, 2 Schwannomas, and 3 normal peripheral nerves, all obtained by biopsy. All tissues were fixed in formalin and embedded in paraffin.

The sera used in this study were rabbit antisera against S-100alpha or S-100beta which were prepared as reported previously (Takahashi et al. 1984a) by the inoculation of bovine S-100ao (alpha-alpha) or S-100b (beta-beta) as immunogens. Their specificities have been characterized elsewhere (Takahashi et al. 1984a).

Deparaffinized 5 μm thick sections were preincubated with 10% normal goat serum for 20 min after blockage of the endogenous peroxidase activity with methanol containing 0.3% hydrogen peroxide for 30 min. Then immunohistochemical staining for the alpha or beta subunit of S-100 protein was performed employing the indirect immunoperoxidase method; sections were reacted with monospecific rabbit antisera against each subunit (10 ng/ml) for 2 h at room temperature. After washing three times with 0.01 M phosphate-buffered saline (PBS) (pH 7.4), the sections were treated with peroxidase conjugated Fab fraction of goat anti-rabbit IgG serum (gamma-chains L-chain specific, Medical Biological Lab., Japan) at 1:40 dilution for 30 min at room temperature. These antisera contained 1% bovine serum albumin and 1% normal human serum to diminish nonspecific immunostaining. Finally sections were soaked in 0.01 M PBS (pH 7.4) containing 3.3′-diaminobenzidine tetrahydrochloride (20 mg/100 ml, Dotite, Japan) and 0.005% hydrogen peroxide for 3–5 min and counterstained lightly with methyl green or haematoxylin solution.

A double immunostaining method was also performed using the method of Nakane (1968) for simultaneous recognition of both subunits in the same section. In the present study, both antigens defined by the anti-S-100alpha antibody (applied first) and the anti-S-100 beta antibody (applied second) were investigated. 3.3′-diaminobenzidine tetrahydrochloride (see above) and 4-chloro-1-naphtol (20 mg in 100 ml of 0.05 M tris-buffered saline containing 2% absolute ethanol and 0.005% hydrogen peroxide, Tokyo Kasei Co., Tokyo, Japan) were used as chromogens for the first and the second antibody reactions, respectively. Non-immunized rabbit serum and PBS were used as controls instead of the specific antibody.

The degrees of immunoreactivity for S-100alpha or S-100beta were determined by semiquantitative estimates of each number of positive cells stained by the indirect immunoperoxidase method as absent (0%), mild (less than 10%), moderate (less than 50%), and marked (more than 50%).

Table 1b. Immunoreactivity for the α and β subunits of S-100 protein in presumably normal peripheral nerves, plexiform neurofibromas and malignant tumours within the same specimens of case 1, 2 and 4 of malignant Schwannomas

Materials	α subunit	β subunit
Presumably normal peripheral nerves	−~+	+++
Plexiform neurofibromas	+~++	++~+++
Malignant tumours	++~+++	−

−: absent; +: mild; ++: moderate; +++: marked

Results

Each malignant tumour originating from a peripheral nerve was confirmed by gross or microscopic examination either at surgery or at autopsy in all 4 cases. The malignant Schwannoma, contiguous plexiform neurofibromas, and presumably normal peripheral nerves were observed in the same sections in cases 1, 2 and 4. The malignant Schwannomas showed variability in cytology and histological pattern. Most malignant tumours were composed of spindle cells and/or polygonal cells which showed a marked pleomorphism and numerous mitoses (Fig. 1). Fascicles of tumour cells and geographic necrosis were observed on occasion. Many clusters of epithelioid tumour cells and multinucleated giant cells were found in some parts of case 2 (Fig. 2a). Some glandular patterns were observed in case 3 (Fig. 3a).

The staining results are tabulated in Tables 1a, 1b and 2. All of 4 cases of malignant Schwannomas associated with neurofibromatosis except for one obtained from autopsy material were markedly stained with the antibody to S-100alpha, but none of them showed immunoreactivity for S-100beta. S-100alpha immunoreactivity was demonstrated especially in large malignant tumour cells possess-

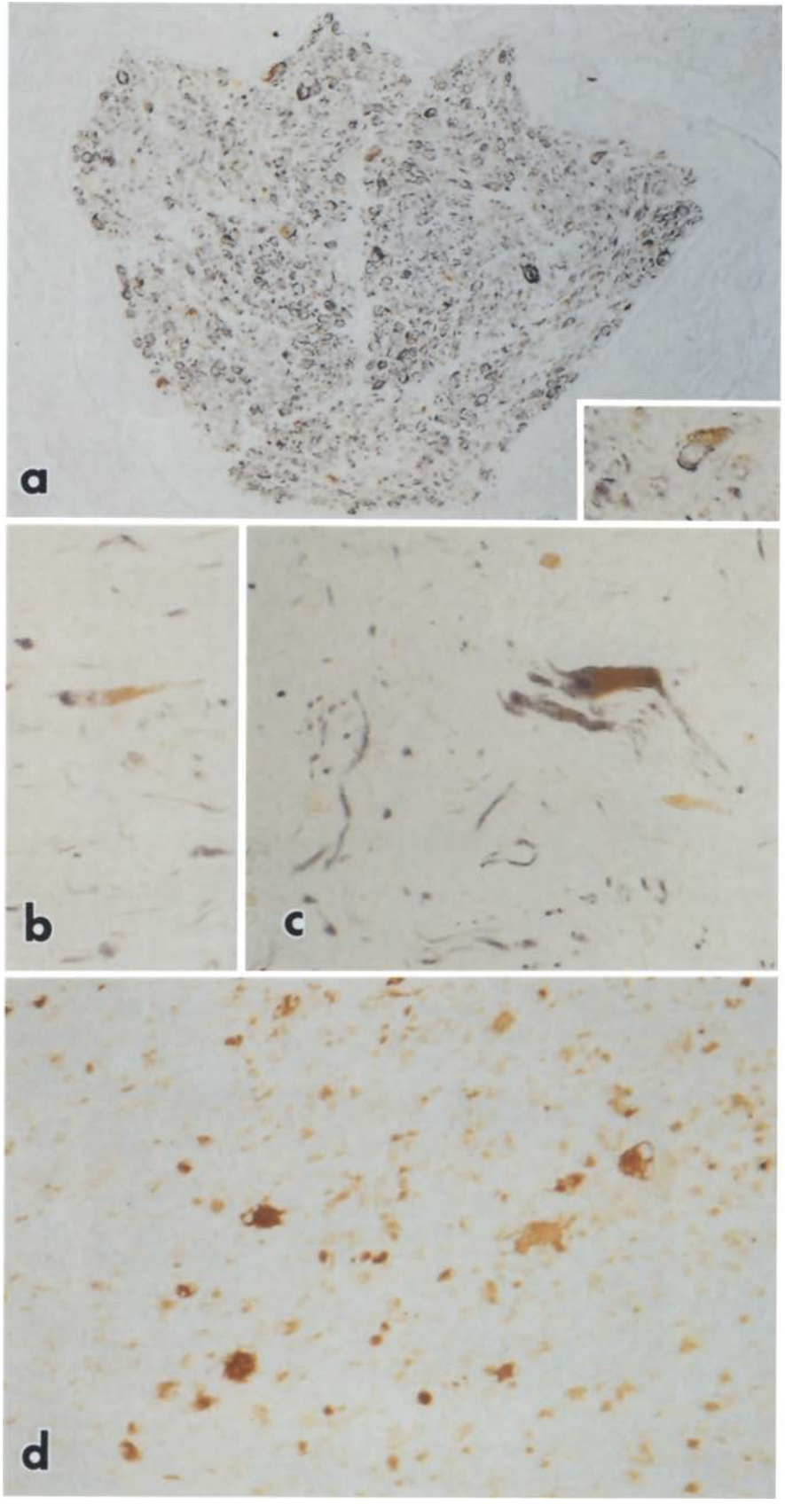

Fig. 4a. S-100 alpha immunoreactivity (*brown*) was detected in several Schwann cells in a presumably normal peripheral nerve fascicle near malignant Schwannoma in neurofibromatosis. S-100 beta immunoreactivity (*dark purple*) was found in most of Schwann cells. Coexistence of both S-100 alpha and S-100 beta was observed in some Schwann cells (*inset*). Case 1. Double immunostaining, ×160; **b, c** S-100 alpha-positive cells (*brown*) was observed moderate in numbers in the plexiform neurofibromas being contiguous to malignant Schwannoma, while most of the tumour cells showed S-100 beta positivity (*dark purple*). Case 1. Double immunostaining, ×400; **d** Tumour cells in a case of malignant Schwannoma arising from neurofibromatosis were strongly positive for S-100 alpha (*brown*) but completely negative for S-100 beta (*dark purple*). Case 1. Double immunostaining, ×200

Table 2. Immunoreactivity for the α and β subunits of S-100 protein in control materials, non-associated with neurofibromatosis

Control materials	No. of cases	α subunit (+)	β subunit (+)
Normal peripheral nerve	3	1/ 3	3/ 3
Neurofibroma	10	8/10	10/10
Schwannoma	2	0/ 2	2/ 2

ing rich cytoplasm (Fig. 4d), epithelioid tumour cells and multinucleated giant cells in case 2 (Fig. 2b) and glandular elements in case 3 (Fig. 3b). Positive immunostaining for S-100beta was not detected in the malignant tumour cells (Fig. 4d), but was observed in the surrounding plexiform neurofibromas and presumably normal peripheral nerves in the same section (Fig. 4a, b, c). However, immunoreactivity for S-100alpha was observed in numerous malignant tumour cells, moderate numbers of tumour cells in the neurofibromas and in small numbers of Schwann cells of the presumably normal peripheral nerves in the same material. It was not seen in endoneurial and perineurial cells (Fig. 4a–d). Co-existence of both S-100alpha and S-100beta was demonstrated in Schwann cells of the presumably normal peripheral nerves and the tumour cells of neurofibromas (Fig. 4a–c) by the double immunostaining method.

The results of the immunostaining for S-100alpha and S-100beta in the control materials are summarized in Table 2. S-100alpha positive cells in presumably normal parts of peripheral nerves in neurofibromatosis were more prevalent and showed stronger positivity than those observed in normal peripheral nerves.

Discussion

S-100 protein is known to be present in Schwann cells and satellite cells, but not in perineurial and endoneurial cells (Nakajima et al. 1982). The immunoreactivity for S-100 protein can be detected in all benign nerve sheath tumours such as Schwannoma, neurofibroma, granular cell tumour and so on (Nakajima et al. 1982; Stefansson et al. 1982a; Weiss et al. 1983), but is still controversial in malignant Schwannomas. For example, the reactivity was demonstrated in 10 out of 14 cases of malignant Schwannomas (Nakajima et al. 1982), in 18 out of 36 cases (Weiss et al. 1983), in none of 4 cases (Stefansson et al. 1982a) and in 9 out of 13 cases (Herrera et al. 1984). It was also noticed that even in S-100 protein positive cases, malignant tumour cells showed only rare to occasional positive immunoreactivity for S-100 protein, and their intensity was less than that observed in the cases of benign Schwannoma and neurofibroma (Nakajima et al. 1982; Weiss et al. 1983).

The plexiform neurofibromas and normal nerve fascicles were positive for S-100 protein, even while the malignant parts of Schwannomas remained negative in the same sections (Nakajima et al. 1982; Stefansson et al. 1982a).

Malignant Schwannomas are often associated with von Recklinghausen's disease (neurofibromatosis) and may be diagnosed when undifferentiated sarcoma arises from a peripheral nerve or is contiguous to neurofibroma (Stefansson et al. 1982a; Ducatman et al. 1986). Malignant Schwannoma is one of those tumours which are difficult to diagnose correctly, especially when not related to neurofibromatosis or peripheral nerves. The reason why S-100 protein was not consistently demonstrated within the malignant cases of Schwannoma as reported previously is probably that precise diagnosis of malignant Schwannomas is sometimes very difficult, and that the antibodies used are from different sources. In fact, S-100 protein is known to be a mixture of S-100ao, S-100a, and S-100b. S-100b, a dimer with a subunit composition of beta-beta is a dominant component of S-100 protein. But conventional antibodies against S-100 protein have small but varying amounts of anti-S-100alpha antibody (Isobe and Okuyama 1978; Isobe et al. 1981; Isobe and Okuyama 1984) and therefore antibodies from different sources containing varied amounts of antibodies to S-100alpha give different immunoreactivity for S-100 protein in malignant Schwannoma. The present study on the distribution of subunits of S-100 protein in malignant Schwannomas in neurofibromatosis shows that S-100beta immunoreactivity detected strongly in the presumably normal peripheral nerves and neurofibromas, disappeared completely in the malignant parts of the tumours. S-100alpha immunoreactivity present occasionally in the presumably normal peripheral nerves and moderately in neurofibromas increased dramatically in the malignant areas of tumours. Moreover, both S-100alpha and S-100beta immunoreactivity were simultaneously observed in some normal Schwann cells and also in tumour cells in neurofibromas. These findings suggest that dominance for subunits of S-100 protein changes from S-100beta in normal Schwann cells and their benign tumours to S-100alpha in malignant Schwannomas accompanied by their malignant conversion. The absence of S-100beta in 4 cases of malignant Schwannoma

is very interesting but not unexpected, because a decreasing tendency of S-100 protein expression with increasing malignancy had been noted not only in central nervous system tumours (Jacque et al. 1979; Yamaguchi 1980), but also in peripheral nervous system tumours (Weiss et al. 1983). Therefore, we considered that S-100alpha immunoreactivity might be a useful marker for the malignant Schwannomas in diagnosis. However, further studies on large numbers of malignant Schwannomas are necessary to confirm these interesting findings. Relatively increased numbers of S-100alpha positive Schwann cells even in presumably normal peripheral nerves might suggest that these nerves already showed a tendency toward neoplastic direction. In addition, as for the presence of S-100alpha immunoreactivity, special attention should be paid to the fixation and freshness of the tissues examined, since materials from autopsy cases in our study showed no, or relatively weak immunoreactivity for S-100alpha, while those from biopsy were strongly positive for S-100alpha. This may be one of the reasons for the different data from the present study on the presence of S-100alpha immunoreactivity in the normal Schwann cells and neurofibromas (Takahashi et al. 1984a).

Other interesting findings in the present study are strong immunoreactivity for S-100alpha only in focal glandular components, epithelioid, and multinucleated giant cells in the malignant Schwannomas. In epithelioid malignant Schwannomas, S-100 protein was detected in 2 out of 7 cases (Weiss et al. 1983). In general, nearly 20% of 120 cases of malignant Schwannomas showed focal divergent differentiation to mesenchymal elements such as rhabdomyosarcoma, chondrosarcoma, osteosarcoma and angiosarcoma, but epithelial components such as glandular, squamous and undifferentiated epithelia were observed only in one case (Ducatman et al. 1986). The occurrence of divergent mesenchymal elements in malignant Schwannomas has been explained by ectomesenchymal theory but that of epithelial elements in malignant Schwannomas is less easily explained (Ducatman and Scheithauer 1984; Ducatman et al. 1986).

It is very important to understand that not only S-100beta but also S-100alpha are demonstrated in various kinds of cells and their tumours (Takahashi et al. 1984a, 1984b). S-100alpha immunoreactivity is detected in neurons, glia, blood monocytes, and the macrophages of lymph node and lung. It is also detected in epithelioid cells, Langhans giant cells, and foreign body giant cells, and considered to be one of the characteristic features of cells in the human mononuclear phagocyte system (Takahashi et al. 1984b). Therefore, some of the S-100alpha positive cells in the present study might be macrophages. However, most of S-100alpha positive malignant cells in malignant Schwannomas should not be considered to be macrophage-derived cells but might better be assumed to possess a kind of histiocytic character. Schwann cells are facultative fibroblasts and histiocytes, and their histiocytic role was revealed in a precess such as wallerian degeneration (Reed et al. 1983).

In conclusion, subunits of S-100 protein might be useful markers for malignant Schwannoma in diagnosis and for better understanding of the biological characteristics of malignant Schwannoma associated with neurofibromatosis.

Acknowledgement: The authors are grateful to Mr. Y. Okada and Mr. S. Awai for their skillful technical assistance and to Miss M. Ohkochi for preparing the manuscript.

References

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

Ducatman BS, Scheithauer BW (1984) Malignant peripheral nerve sheath tumors with divergent differentiation. Cancer 54:1049–1057

Ducatman BS, Scheithauer BW, Piepgras DG, Reiman HM, Ilstrup DM (1986) Malignant peripheral nerve sheath tumors. A clinicopathological study of 120 cases. Cancer 57:2006–2021

Herrera GA, Pinto dae Moraes H (1984) Neurogenic sarcomas in patients with neurofibromatosis (von Recklinghausen's disease). Light, electron microscopy and immunohistochemistry study. Virchows Arch [A] 403:361–376

Isobe T, Okuyama T (1978) The amino-acid sequence of S-100 protein (PAP I-b protein) and its relation to the calcium-binding proteins. Eur J Biochem 89:379–388

Isobe T, Ishioka N, Okuyama T (1981a) Structural relation of two S-100 proteins in bovine brain; Subunit composition of S-100a protein. Eur J Biochem 115:469–474

Isobe T, Okuyama T (1981b) The amino-acid sequence of the alpha subunit in bovine brain S-100a protein. Eur J Biochem 116:79–86

Isobe T, Okuyama T (1984) S 100 protein. Protein Nuclei Acid Enzyme 29:1085–1100

Jacque CM, Kujas M, Poreau A, Raoul M, Collier P, Racadot J, Baumann N (1979) GFA and S-100 protein levels as an index for malignancy in human gliomas and in neurinomas. J Natl Cancer Inst 62:479–483

Møller M, Ingrid A, Bock E (1978) Immunohistochemical demonstration of S 100 protein and GFA protein in interstitial cells of rat pineal gland. Brain Res 140:1–13

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

Moore BW (1972) Chemistry and biology of two proteins S-100 and 14-3-2, specific to the nervous system. In International Review of Neurobiology, Pfeiffer CC, Smythies JR (eds.) Academic Press, New York London, pp 215–225

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

Nakane PK (1968) Simultaneous localization of multiple tissue antigens using the peroxidase-labelled antibody method; A study on pituitary glands of the rat. J Histochem Cytochem 16:557–560

Reed RJ, Harkin JC (1983) The tumors of the peripheral nervous system. Supplement 2nd ed. Armed Forces Institute of Pathology, Washington, D.C., pp 1–2

Stefansson K, Wollmann R, Jerkovic M (1982a) S-100 protein in soft-tissue tumors derived from Schwann cells and melanocytes. Am J Pathol 106:261–268

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

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

Takahashi K, Isobe T, Ohtsuki Y, Akagi T, Sonobe H, Okuyama T (1984a) Immunohistochemical study on the distribution of alpha and beta subunits of S-100 protein in human neoplasm and normal tissues. Virchows Arch [Cell Pathol] 45:385–396

Takahashi K, Isobe T, Ohtsuki Y, Sonobe H, Takeda I, Akagi T (1984b) Immunohistochemical localization and distribution of S-100 proteins in the human lymphoreticular system. Am J Pathol 116:497–503

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

Yamaguchi H (1980) Studies on the immunohistochemical localization of S-100 and glial fibrillary acidic protein in the rat nervous system and in human brain tumors. Brain Nerve 32:1055–1064

Accepted June 15, 1987