Malignant peripheral nerve sheath tumour arising within neurofibroma. An immunohistochemical analysis in the comparison between benign and malignant components

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Abstract

Aims—To compare the expression of immunohistochemical variables between benign and malignant components of malignant peripheral nerve sheath tumour (MPNST) arising within neurofibroma.

Methods—Eight cases of MPNST arising within a neurofibroma, associated with neurofibromatosis type 1 (NF1), were studied. The areas of MPNST and neurofibroma were compared immunohistochemically with regard to the expression of proliferative activity (MIB-1), growth factors, p53, bcl-2, neural cell adhesion molecule (N-CAM), and CD34.

Results—The expression of transforming growth factor β 1 (TGF- β 1), TGF- β receptor type II, hepatocyte growth factor α (HGF- α), c-met, p53, and N-CAM was higher in the areas of MPNST than in the neurofibromatous areas in four, five, five, eight, five, and three of the eight cases. CD34 expression was lower in the areas of MPNST than in the neurofibroma areas in three of the eight cases.

Conclusions—On the basis of these findings, TGF- β 1, HGF- α , and p53 might be involved in the malignant transformation of neurofibroma to MPNST.

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Keywords: malignant peripheral nerve sheath tumour; neurofibroma; immunohistochemistry; transforming growth factor β ; hepatocyte growth factor; p53

Malignant peripheral nerve sheath tumour (MPNST) is a malignant neurogenic tumour that occurs with high frequency in association with neurofibromatosis type 1, arising either de novo or in transition from neurofibroma.¹ There have been many studies of MPNST and neurofibroma²⁻¹⁷; however, only a few have

dealt with the immunohistochemical comparison between the sarcomatous area and the adjacent neurofibromatous area in MPNST arising within a neurofibroma.⁵⁻⁷

It has been reported that cultured rat Schwann cells respond to some mitogens, including transforming growth factor β (TGF- β), hepatocyte growth factor (HGF), and platelet derived growth factor (PDGF).¹⁸⁻²¹ In addition, accumulation of some mitogens, such as HGF and basic fibroblast growth factor (FGF), has also been found in neurofibromatosis.²²⁻²³ TGF- β isoforms have been reported to mediate their activity by high affinity binding to the type II receptor.²⁴ They inhibit the proliferation of epithelial cells or epithelial tumour cells, but promote the synthesis of extracellular matrix.²⁵ However, their role in the tumorigenesis of sarcomas, including neurogenic tumour, has not yet been reported.

HGF/c-met autocrine signalling systems have been suggested to contribute to tumour progression in several carcinomas and sarcomas, including MPNST.²⁶⁻²⁸ However, differences in the expression of HGF/c-met between areas of neurofibroma and MPNST have not yet been clarified.

Mutations of the p53 gene have also been found in some neurogenic tumours.²⁹⁻³¹ More recently, neural cell adhesion molecule (N-CAM) and CD34, which is a myeloid progenitor cell antigen found in some fibroblast subsets, have been studied in relation to tumour behaviour.³²⁻³⁴

In our study, we examined immunohistochemically the expression of these factors, which are considered to be responsible for the development and progression of MPNST, using paraffin wax embedded tissue specimens. In addition, we compared the topological difference in expression of the above factors between the sarcomatous and neurofibromatous areas.

Materials and methods

SPECIMENS

Eight cases of MPNST arising within a neurofibroma were selected from 69 cases of MPNST registered in the files of the second department of pathology, Kyushu University, Fukuoka, Japan, from 1980 to 1997. We defined MPNST within a neurofibroma as a tumour in which a sarcomatous area was surrounded by a neurofibromatous area. In the sarcomatous area, the tumour was characterised by a proliferation of wavy and buckled

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Table 1 Clinical summary

Case	Age (years)	Sex	Site	Size (cm)	Treatment	Follow up
1	60	M	Forearm	1.6	Surgery	Alive (147 mo)
2	37	M	Lower leg	4	Surgery	Dead (32 mo)
3	52	F	Chest	4.3	Surgery	Alive (58 mo)
4	62	F	Abdomen	11	Surgery	Alive (65 mo)
5	17	M	Retroperitoneum	15	Surgery	Dead (16 mo)
6	27	M	Thigh	27	Surgery	Dead (11 mo)
7	32	F	Thigh	5	Surgery	Alive (15 mo)
8	38	M	Neck	6	Surgery	Dead (7 mo)

mo, months.

Table 2 Antibodies used in our study

Antibody/antigen	Type	Source	Dilution	Positive control
MIB-1	Mouse	Immunotech	1/100	Normal skin
p53 (Pab1801)	Mouse	Calbiochem	1/100	Adenocarcinoma of the stomach
bcl-2	Mouse	Calbiochem	1/100	Normal lymph node
TGF β 1	Rabbit	Santa Cruz	1/250	Normal colon
TGF β receptor II	Rabbit	Santa Cruz	1/250	Normal colon
HGF α	Rabbit	IBL	1/50	Fibroblasts of the inflamed urinary bladder
c-met	Rabbit	Santa Cruz	1/500	Normal skin
PDGF	Rabbit	Calbiochem	1/250	Normal skin
PDGF receptor β	Mouse	Oncogene Science	1/50	Normal skin
N-CAM	Mouse	Novo Castra	1/50	Neuroblastoma
CD34	Mouse	Novo Castra	1/50	Haemangioma

HGF, hepatocyte growth factor; N-CAM, neural cell adhesion molecule; PDGF, platelet derived growth factor; TGF, transforming growth factor.

spindle cells. Densely cellular fascicles alternated with hypocellular zones. Mitotic activity was present. The patients, comprising five men and three women, ranged in age from 17 to 62 years (mean, 40.6). All the patients had von Recklinghausen's disease (neurofibromatosis type 1). Table 1 summarises the clinical data of these patients. The histological sections were stained with haematoxylin and eosin, and for each of the tumours the sections with the most characteristic features of both neurofibroma and MPNST were selected for immunohistochemical studies. All of the light microscopic and immunohistochemical studies were performed on formalin fixed, paraffin wax embedded materials.

IMMUNOHISTOCHEMICAL STUDY

Formalin fixed paraffin wax embedded tissue sections (4 μ m thick) were mounted on to glass slides coated with 3-aminopropyltriethoxy silane, before being air dried overnight at room

temperature. The sections were dewaxed in xylene and rehydrated in ethanol, and endogenous peroxidase was blocked by methanol containing 0.3% hydrogen peroxide for 30 minutes. For the purpose of antigen retrieval, the sections were placed in plastic jars containing 0.1% Tween 20 in citrate buffer and then heated in a microwave oven (H2800 microwave processor; Energy Beam Science, Massachusetts, USA) at 99°C for 20 minutes. Non-specific protein binding was inhibited by treatment with normal rabbit serum (for mouse monoclonal antibodies) or goat serum (for rabbit polyclonal antibodies) for 10 minutes. Sections were incubated at 4°C overnight with primary antibodies, before being stained with the streptavidin-biotin complex/horseradish peroxidase (SAB/HRP) method using a Histofine SAB-PO(M) immunohistochemical staining kit (Nichirei, Tokyo, Japan). The sections were then reacted with a 3,3'-diaminobenzidine, peroxytrichloride substrate solution, counterstained with haematoxylin, and mounted. Table 2 summarises the primary antibodies used in our study. The degree of

Figure 1 (A) Cut surface of the resected tumour in case 3. Section shows a multinodular solid and white mass. The central firm portion blends with the surrounding subcutaneous fat and muscular tissue. (B) Topographical distribution of histological features of both malignant peripheral nerve sheath tumour and neurofibroma in case 3.

Figure 2 (A) Cut surface of the resected tumour in case 4. The section shows a well circumscribed solid and greyish yellow mass with nodular growth pattern and interlacing fascicles. (B) Topographical distribution of both sarcomatous and neurofibromatous components in case 4.

Figure 3 (A–D) Histological picture exhibiting the features of neurofibroma and malignant peripheral nerve sheath tumour (MPNST) (case 4). (A) The MPNST area is surrounded by a neurofibromatous area. Densely cellular fascicles alternate with hypocellular zones in the MPNST area (haematoxylin and eosin stained; original magnification, $\times 12$). (B) The tumour is composed of interlacing bundles of elongated cells with wavy nuclei, without atypism in the neurofibroma (top), and with atypism in the MPNST (bottom) (haematoxylin and eosin stained; original magnification, $\times 20$). (C) The tumour is composed of wavy spindle cells arranged in fascicles and with wavy nuclei. Densely cellular fascicles alternate with hypocellular zones (haematoxylin and eosin stained; original magnification, $\times 25$). (D) The nuclei of some tumour cells are wavy or buckled. Mitotic figures are seen frequently (haematoxylin and eosin stained; original magnification, $\times 570$). (E) Histological picture exhibiting the features of neurofibroma (case 4). The tumour is made up of interlacing bundles of elongated cells with wavy nuclei, without atypism, associated with wire-like strands of collagen (haematoxylin and eosin stained; original magnification, $\times 125$).

staining was classified as: negative ($< 3\%$ of the cells), 1+ (positive; 3–50% of the cells), and 2+ (strongly positive; $> 50\%$ of the cells).

The MIB-1 labelling index (LI) was estimated by counting the number of positive cells/1000 tumour cells.

Results

Figures 1 (case 3) and 2 (case 4) demonstrate the topographic distinctions between the sarcomatous areas and the neurofibromatous areas. In these cases, the sarcomatous components were located within the central region of the entire tumour. Microscopically, in case 4, the sarcomatous area was composed of spindle cells arranged in fascicles adjacent to the neurofibromatous area (fig 3A and B). Densely cellular fascicles alternated with hypocellular

zones (fig 3C). The nuclei of some tumour cells were wavy or buckled (fig 3D). Mitotic figures were seen frequently. The surrounding less cellular area of case 4 showed interlacing bundles of elongated cells, which had wavy nuclei without atypism associated with wire-like strands of collagen (fig 3E). The other tumour (case 5) showed a whorled structure, which was reminiscent of tactoid differentiation (fig 4).

Tables 3–5 summarise the results of our immunohistochemical analyses.

The MIB-1 LI for both the neurofibroma and the MPNST areas ranged from 3.3 to 49.8, with a mean (SD) of 19.0 (15.7). The MIB-1 LI was found to be higher in the MPNST areas than in the neurofibroma areas (mean, 31.9; SD 11.7 *v* mean, 6.1; SD, 2.4; $p = 0.0001$) (table 3).

Figure 4 Histological picture exhibiting the features of malignant peripheral nerve sheath tumour (case 5). The tumour is composed of spindle cells arranged in fascicles reminiscent of tactoid differentiation. Nuclei are wavy and twisted (haematoxylin and eosin stained; original magnification, $\times 200$).

Table 3 MIB-1 staining

Case	MIB-1 LI	
	NF	MPNST
1	5.5	21.3
2	6.5	37.3
3	6.9	23.3
4	3.5	14.5
5	4.3	41.8
6	9.7	49.8
7	8.7	36.7
8	3.3	30.8

LI, labelling index; MPNST, malignant peripheral nerve sheath tumour; NF, neurofibroma.

Positive reactions with TGF- β 1, TGF- β receptor type II, HGF- α , and c-met were observed more frequently in the MPNST areas than in the neurofibroma areas in four, five, five, and four of the eight cases, respectively (fig 5). A positive reaction with PDGF was found more frequently in the MPNST areas than in the neurofibroma areas in two of the eight

Figure 5 Immunohistochemical staining patterns of the transforming growth factor β receptor type II. The degree of staining in the neurofibroma (top) is graded as 1+, whereas that in the MPNST (bottom) is graded as 2+ (case 5) (original magnification, $\times 200$).

cases. However, positive reactions with PDGF receptor β were seen in only one case (case 7).

Immunoreactivity for bcl-2 was similar in both the MPNST and neurofibroma areas.

In contrast, five of the eight cases showed immunoreactivity for p53 in the MPNST areas, whereas three of the eight cases showed no immunoreactivity in either the MPNST or neurofibroma areas (fig 6).

Furthermore, N-CAM positivity was seen more frequently in the MPNST areas than in neurofibroma areas in three of the eight cases. In addition, CD34 positive cells were present in the neurofibroma areas, but not in the MPNST areas in three of the eight cases (fig 7).

Discussion

We speculated that many factors were involved in the malignant transformation from neurofibroma to MPNST. To clarify the mechanisms and factors underlying this transformation, we selected a total of eight cases of MPNST arising within a neurofibroma and undertook immunohistochemical studies.

Table 4 Staining in neurofibroma (NF) and malignant peripheral nerve sheath tumour (MPNST) areas

Case	TGF- β 1		TGF- β receptor II		HGF- α		c-met		PDGF		PDGF receptor β	
	NF	MPNST	NF	MPNST	NF	MPNST	NF	MPNST	NF	MPNST	NF	MPNST
1	1+	2+	1+	2+	1+	2+	1+	2+	1+	2+	—	—
2	2+	2+	2+	2+	1+	2+	1+	2+	1+	2+	—	—
3	2+	2+	2+	2+	2+	2+	2+	2+	1+	1+	—	—
4	1+	2+	1+	2+	1+	2+	1+	2+	1+	1+	—	—
5	1+	2+	1+	2+	2+	2+	2+	2+	1+	1+	—	—
6	2+	2+	1+	2+	1+	2+	2+	2+	1+	1+	—	—
7	1+	2+	1+	2+	1+	2+	1+	2+	1+	1+	—	1+
8	2+	2+	2+	2+	2+	2+	2+	2+	1+	1+	—	—

—, <3% of cells positive; 1+, 3–50% of cells positive; 2+, >50% of cells positive.

HGF, hepatocyte growth factor; PDGF, platelet derived growth factor; TGF, transforming growth factor.

Table 5 Staining in neurofibroma (NF) and malignant peripheral nerve sheath tumour (MPNST) areas

Case	p53		bcl-2		N-CAM		CD34	
	NF	MPNST	NF	MPNST	NF	MPNST	NF	MPNST
1	—	1+	—	—	—	—	2+	—
2	—	1+	—	—	—	—	—	—
3	—	—	1+	1+	—	—	1+	1+
4	—	—	1+	1+	—	—	2+	2+
5	—	1+	1+	1+	—	2+	2+	—
6	—	1+	1+	1+	2+	2+	1+	—
7	—	—	1+	1+	1+	2+	—	—
8	—	1+	1+	1+	1+	2+	1+	1+

—, <3% of cells positive; 1, 3–50% of cells positive; 2, $\geq$ 50% of cells positive.

N-CAM, neural cell adhesion molecule.

Figure 6 Immunohistochemical staining patterns for p53. Staining in the neurofibroma (B) is negative, whereas that in the MPNST (A) is graded as 1+ (case 8) (original magnification, $\times 400$).

Figure 7 Immunohistochemical staining patterns for CD34. The degree of staining in the neurofibroma (top) is graded as 2+, whereas the MPNST (bottom) is negative (case 5) (original magnification, $\times 200$).

HGF, TGF- β , PDGF, and basic FGF have been reported to be involved in Schwann cell proliferation or growth of neurofibroma.^{18–23} HGF/c-met interaction, in an autocrine manner, has been demonstrated in some tumours, including MPNST.^{26–28} We studied the role of TGF- β , HGF, and PDGF in the malignant transformation from neurofibroma to MPNST. In our series, TGF- β 1/TGF- β receptor type II and HGF- α /c-met were expressed both in the MPNST and neurofibroma areas, but immunoreactivity was greater in MPNST than in neurofibroma areas in almost half the cases. Thus, it seems likely that the HGF- α /c-met and TGF- β 1/TGF- β receptor type II signalling systems play a role in the process of MPNST formation. TGF- β isotypes have been shown to inhibit the proliferation of epithelial tumours, but promote the synthesis of extracellular components.²⁵ In addition, our results showed that TGF- β 1 might help promote the malignant growth of sarcomas, such as neurogenic tumour.

Immunohistochemical staining with the MIB-1 antibody was used to assess the proliferative activity of the tumour cells.³⁵ In our study, the MIB-1 LI was higher in the MPNST areas than in the neurofibroma areas. This result is similar to that of Kindblom *et al*, who reported MIB-1 immunostaining results

when comparing benign and malignant neurogenic tumours.⁷

Moreover, it has also been shown that the anti-apoptotic protein bcl-2 is expressed to some extent in gliomas, and is expressed more often in low grade astrocytomas than in malignant gliomas.^{36–37} We found that bcl-2 was expressed in both the MPNST and neurofibroma areas, and that there was no definite distinction between the two regarding bcl-2 immunoreactivity. Consequently, it seems that bcl-2 does not play an important role in the progression of MPNST.

In contrast, in our study, five of the eight MPNSTs arising within a neurofibroma showed immunoreactivity for p53 in the sarcomatous areas, but not in the adjacent neurofibromatous areas. Furthermore, there was no occurrence of p53 immunoreactivity in either the sarcomatous or neurofibromatous areas in three of the eight cases. Our data differ from the report of Halling *et al* because they reported that all seven MPNSTs arising within a neurofibroma showed immunoreactivity for p53 in the sarcomatous areas.⁶ Differences in the anti-p53 antibody used may underlie the differences seen in these results. However, our study showed that p53 plays an important role in the evolution of MPNST from neurofibroma. Our data were compatible with molecular data, which showed that 17p deletion or mutations of p53 occurred during the malignant transformation from neurofibroma to MPNST.³¹

In our study, N-CAM immunoreactivity in the MPNST areas was higher than that in the neurofibroma areas in three of the eight cases. Our results are similar to those of Miettinen *et al*, who reported that consistent N-CAM immunoreactivity was seen in malignant schwannoma, whereas only variable staining was seen in benign schwannoma.³² The reason for this has yet to be determined. CD34 is a myeloid progenitor cell antigen, which is also present in dermal dendritic cells, endothelial cells, and some subsets of fibroblasts.^{33–34} In our series, the immunoreactivity of neurofibroma was higher than that of MPNST in three of the eight cases. Some fibroblasts in neurofibroma seem to express CD34, but this expression seems to be lost during the process of MPNST formation. There is also the possibility that N-CAM and CD34 play a role in the malignant transformation of neurogenic tumours.

In conclusion, our immunohistochemical study showed that TGF- β 1/TGF- β receptor type II, HGF- α /c-met, and p53 seem to play a role in the malignant transformation of neurofibroma to MPNST. In addition, these factors might be useful in distinguishing clinicopathologically between MPNST and neurofibroma. However, further studies to determine the precise mechanism behind this transformation are necessary.

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