

Figure 4 Histological review. (a) The border between pleomorphic area and myxoid area observed in the NCCS099 sample ($D_{\min} = 0.46$ to myxofibrosarcoma) (hematoxylin and eosin stain). (b-d) Histology of the NCCS102 sample ($D_{\min} = 0.50$ to leiomyosarcoma). This tumor showed marked pleomorphism (b) hematoxylin and eosin stain, but tumor cells were positive for desmin (c) and α SMA (d). (e) Fibrosarcomatous fascicular area seen in the NCCS104 sample ($D_{\min} = 0.39$ to fibrosarcoma) (hematoxylin and eosin stain). (f) Epithelioid structure observed in the NCCS097 sample ($D_{\min} = 0.56$ to MPNST) (hematoxylin and eosin stain).

fibrosarcoma-like herringbone and fascicular patterns by microscopic analysis (Figure 4e).

Among twelve samples showing moderate similarity to other types of sarcomas ($0.50 < D_{\min} \leq 0.75$),

the NCCS096 sample close to the dedifferentiated liposarcoma centroid ($D_{\min} = 0.64$) was obtained from a recurrent sarcoma in the retroperitoneum. Although microscopic findings did not show

evidence of adipocytic differentiation or features of well-differentiated liposarcoma in regions adjacent to the tumor, the site of involvement suggested the possibility that the tumor originated from dedifferentiated liposarcoma. All five samples close to myxofibrosarcoma ($0.50 < D_{\min} \leq 0.75$) showed scattered myxoid areas, but these findings were not sufficient to reclassify them as myxofibrosarcoma histologically. The NCCS097 sample, another pleomorphic sarcoma close to the MPNST centroid ($D_{\min} = 0.56$), exhibited scattered whorled and epithelioid structures (Figure 4f) as well as tumor cells positive for cytokeratin, neurofilament and α SMA, indicating that this tumor had neuroectodermal differentiation. Its similarity to leiomyosarcoma ($D = 0.58$) would be reflected in α SMA positivity. For the NCCS091 sample close to MPNST and the other four close to fibrosarcoma, we did not observe any significant histological similarity to MPNST or fibrosarcoma, respectively. In summary, although more than half of the MFH samples ($n = 12$) were moderately similar in terms of gene expression to other sarcomas ($0.50 < D_{\min} \leq 0.75$), only little resemblance was detectable by histological examination. Finally, the remaining six samples with high D_{\min} values ($D_{\min} > 0.75$) showed no identifiable histological similarity to the five sarcoma types (dedifferentiated liposarcoma, myxoid/round cell liposarcoma, leiomyosarcoma, MPNST and fibrosarcoma).

Genes Overexpressed in Myxofibrosarcoma

Diagnostically useful markers for myxofibrosarcoma are not well known. To search for candidate markers that genetically characterize myxofibrosarcoma, we selected upregulated genes by comparing myxofibrosarcoma samples ($n = 15$) with other spindle cell and pleomorphic sarcoma samples ($n = 25$). Three samples excluded from the previous analysis and 21 samples of MFH were not used for the marker search. From 11 300 probe sets, we selected 10 probe sets (five genes) with P -values < 0.001 based on the Student's t -test and more than five-fold greater

expression (Table 1). Among them, expression of four probe sets (four genes) in respective spindle cell and pleomorphic sarcomas are shown in Figure 5a by the box-and-whisker plots. Since *ANK1* expression in MFH was much higher than that seen in myxofibrosarcoma (data not shown), its upregulation was not considered to be specific to myxofibrosarcoma. We performed quantitative RT-PCR with three other genes, *WISP2*, *GPR64* and *TNXB*, to verify the microarray findings (Figure 5b). Quantitative RT-PCR data confirmed consistent high expression of *GPR64* and *TNXB* in myxofibrosarcoma samples and in some MFH samples showing similarity to myxofibrosarcoma in terms of gene expression.

Discussion

An important aim of this study was to obtain new insights to classify a diverse group of soft tissue sarcomas. Our data showed that soft tissue sarcomas examined roughly fell into four groups (Figure 1a) (1) synovial sarcoma; (2) myxoid/round cell liposarcoma; (3) lipoma, well-differentiated liposarcoma with part of dedifferentiated liposarcoma and (4) spindle cell and pleomorphic sarcomas. Six histological types of spindle cell and pleomorphic sarcomas (dedifferentiated liposarcoma, myxofibrosarcoma, leiomyosarcoma, MPNST, fibrosarcoma and MFH) did not display distinct profiles but they shared a similar gene expression profile, forming a loose cluster in the hierarchical clustering analysis (Figure 1b). These results were broadly consistent with previous reports,^{7,10} and histological similarity among spindle cell and pleomorphic sarcomas could be explained by similarities in gene expression. We could find some MPNST samples were located adjacent to the robust synovial sarcoma cluster in the hierarchical clustering analysis (Figure 1b), indicating that those MPNST samples shared similar expression patterns with synovial sarcoma as reported by Nagayama *et al*.⁸ Our data also showed a common gene expression signature in synovial sarcoma and myxoid/round cell liposarco-

Table 1 Genes highly expressed in myxofibrosarcoma

Gene symbol	Fold change	P-value	Description	Probe set ID
<i>WISP2</i>	10.99	1.6×10^{-5}	WNT1 inducible signaling pathway protein 2	205792_at
<i>GPR64</i>	10.56	7.7×10^{-5}	G protein-coupled receptor 64	206002_at
<i>TNXB</i>	8.30	3.7×10^{-5}	Tenascin XB	208609_s_at
<i>ANK1</i>	7.03	1.8×10^{-5}	Ankyrin 1, erythrocytic	208352_x_at
<i>S100A3</i>	6.41	3.0×10^{-7}	S100 calcium binding protein A3	206027_at
<i>ANK1</i>	5.99	5.5×10^{-5}	Ankyrin 1, erythrocytic	205391_x_at
<i>TNXB</i>	5.96	2.9×10^{-5}	Tenascin XB	213451_x_at
<i>TNXB</i>	5.86	6.5×10^{-4}	Tenascin XB	216339_s_at
<i>TNXB</i>	5.74	1.3×10^{-4}	Tenascin XB	206093_x_at
<i>TNXB</i>	5.74	4.7×10^{-5}	Tenascin XB	216333_x_at

The top 10 probe sets with high fold changes were selected from 321 probe sets differentially expressed ($P < 0.001$ by Student's t -test) between myxofibrosarcoma samples ($n = 15$) and other spindle cell and pleomorphic sarcoma samples ($n = 25$) analyzed in Figure 3b.

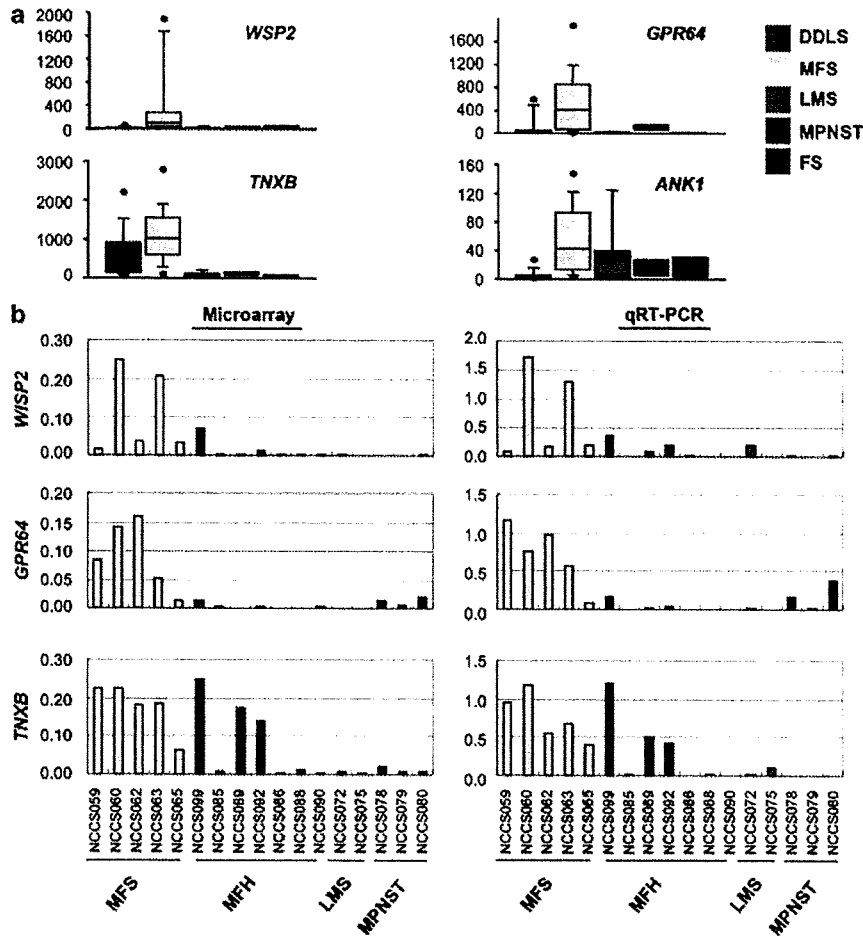


Figure 5 Genes highly expressed in myxofibrosarcoma. (a) Box-and-whisker plots indicating expression values for each histological type of spindle cell and pleomorphic sarcomas. A total of 40 control samples were analyzed. (b) Comparison between microarray analysis and quantitative RT-PCR of *WSP2*, *GPR64* and *TNXB* expression. Expression levels were normalized to that of *ACTB* in both microarray and RT-PCR data. NCCS099 ($D_{\min}=0.46$), NCCS085 ($D_{\min}=0.66$), NCCS089 ($D_{\min}=0.75$) and NCCS092 ($D_{\min}=0.66$) were similar to myxofibrosarcoma in terms of gene expression (see Figure 3c). DDLS, dedifferentiated liposarcoma; MFS, myxofibrosarcoma; LMS, leiomyosarcoma and FS, fibrosarcoma.

ma samples, distinguishing them from other tumors. Overexpression of genes encoding ribosomal proteins in myxoid/round cell liposarcoma was reported previously.¹¹ Another report showed that *SOX11*, *CTAG1*, *CTAG2* and *PRAME* were overexpressed in liposarcomas and absent or minimally expressed in all other tumors examined.¹⁵ Among those genes, *CTAG1* and *PRAME* are both categorized as cancer testis antigens, and their expression in synovial sarcoma has also been reported.¹⁶ Consistent with those reports, we found that *SOX11*, *CTAG1*, *CTAG2* and *PRAME* are highly expressed in both synovial sarcoma and myxoid/round cell liposarcoma. These similarities in gene expression may correlate with biological characteristics of synovial sarcoma and myxoid/round cell liposarcoma and suggest that these two sarcomas may share a common oncogenic pathway.

The so-called MFH was thought to be the most common soft tissue sarcoma in adults, and cur-

rently, it is widely accepted as a common morphological manifestation of a variety of poorly differentiated sarcomas. Re-evaluation of 'MFH' by different methods has been undertaken. Fletcher *et al*⁵ reclassified 100 tumors primarily diagnosed as 'MFH' by histological methods and showed that the most common diagnosis was myxofibrosarcoma ($n=29$), followed by leiomyosarcoma ($n=20$). Hasegawa *et al*¹⁴ examined immunoreactivity for smooth muscle markers from 100 samples of 'MFH' and reported that a large subset showed poorly differentiated smooth muscle or myofibroblastic features and should be regarded as pleomorphic leiomyosarcoma or pleomorphic myofibrosarcomas. Using comparative genomic hybridization, Derre *et al*¹⁷ showed similar recurrent genomic imbalances in 'MFH' and leiomyosarcoma, and Coindre *et al*¹⁸ reported that most inflammatory types of MFH developing in the retroperitoneum are identical to dedifferentiated liposarcoma. Here, we discussed

the possibility that 21 MFH samples could be reclassified into other types of spindle cell and pleomorphic sarcomas based on similarities in gene expression. For convenience of evaluation, we separated MFH samples into three groups according to the level of similarity to other sarcoma types. MFH with marked similarity ($D_{\min} \leq 0.5$), MFH with moderate similarity ($0.50 < D_{\min} \leq 0.75$) and MFH with no similarity ($D_{\min} > 0.75$). Three samples very similar in gene expression to other sarcoma types ($D_{\min} \leq 0.5$) resembled the corresponding histological types of spindle cell and pleomorphic sarcomas, and we concluded that these samples could probably be diagnosed as pleomorphic subtypes of those respective sarcomas based on current histological criteria. We then found that despite only marginal histological resemblance, more than half of the MFH samples (12/21) showed gene expression profiles similar to other sarcoma types ($0.50 < D_{\min} \leq 0.75$). We considered that these moderate similarities in gene expression could correspond with pleomorphic change in each sarcoma type. Thus, although the samples cannot be diagnosed based on current histological criteria, it is possible to reclassify them as a pleomorphic subtype of those sarcomas based on gene expression. In this study, 40% (6/15) of reclassified MFH samples ($D_{\min} \leq 0.75$) were similar to myxofibrosarcoma and 33% (5/15) were similar to fibrosarcoma, suggesting that a large subset of 'MFH' represents pleomorphic subtypes of fibroblastic sarcomas. Among the six cases of MFH similar to myxofibrosarcoma, five other than NCCS089 had deep-seated lesions, four (NCCS085, NCCS092, NCCS094 and NCCS101) had distant metastasis, and one (NCCS094) suffered local recurrence after surgery. Although the local recurrence rate (1/6) was unexpectedly low and distant metastasis rate (4/6) was high compared to canonical myxofibrosarcoma, these data could be consistent with the report showing that deep-seated lesions of myxofibrosarcoma were higher-grade, pleomorphic and large and increased the incidence of distant metastases.¹⁹ About 30% of the MFH samples (6/21) did not show similarities to other sarcoma types ($D_{\min} > 0.75$). One possibility is that 'de novo undifferentiated pleomorphic sarcomas' truly exist. It is also possible that these samples represent advanced stage of dedifferentiation, which is beyond the analytical power of our study design. Another possibility is that the samples were derived from sarcomas of other differentiation not examined in this study. Extraskelatal osteosarcoma, rhabdomyosarcoma and other sarcomas could be the candidate. Reclassification accuracy should be improved by examining additional histological types of spindle cell and pleomorphic sarcomas.

Given that almost one third of MFH samples shared similar gene expression patterns ($D_{\min} \leq 0.75$) with myxofibrosarcoma, we hypothesize that a large subset of 'MFH' may be pleomorphic subtype of myxofibrosarcoma. Myxofibrosarcoma is one of the

most frequent sarcomas seen in late adults. However, little is known about its normal tissue counterparts, or factors underlying its extremely high local recurrence rate,¹⁹ nor are there any good markers available for histological diagnosis. Identification of genes highly expressed in myxofibrosarcoma would offer an important clue to address these problems. Here, we found *WISP2*, *GPR64* and *TNXB* were upregulated in myxofibrosarcoma compared with other spindle cell and pleomorphic sarcomas. *WISP2* is a member of the WNT1 inducible signaling pathway (WISP) protein subfamily, which belongs to the connective tissue growth factor family. WISP family members are secreted, cell- and matrix-associated proteins that play critical roles in cell differentiation and survival, wound repair, vascular disease, fibrosis and progression of certain cancers.²⁰⁻²² *GPR64* is a highly conserved, tissue-specific heptahelical receptor of the human epididymis,²³⁻²⁵ and there are no reports on the relationship of *GPR64* to any type of cancer. *TNXB* is the largest member of the tenascin family of extracellular matrix proteins, which have anti-adhesive effects as opposed to the adhesion activity of fibronectin. It is expressed in musculoskeletal, cardiac and dermis tissue, and its deficiency is associated with the connective tissue disorder Ehlers-Danlos syndrome.²⁶⁻²⁸ Although it is not clear if these genes play a role in myxofibrosarcoma, they may serve as novel diagnostic markers.

In this study, we primarily analyzed gene expression of MFH and other types of spindle cell and pleomorphic sarcomas (dedifferentiated liposarcoma, myxofibrosarcoma, leiomyosarcoma, MPNST and fibrosarcoma). Although these sarcomas showed a similar gene expression pattern and formed a relatively loose cluster, samples from five types of spindle cell and pleomorphic sarcomas were classified into respective histological types by excluding MFH samples. We identified genes that were differentially expressed among the five sarcoma types and could reclassify more than 70% of MFH samples into the five sarcoma types based on their similarities in gene expression using a combination of simple statistical analysis. These results suggest that gene expression profiling will be a useful tool to reclassify MFH and to aid histological diagnosis of a diverse group of soft tissue sarcomas. Although we cannot currently predict differences in clinical behavior of reclassified MFH due to the limited number of samples analyzed, accumulation of gene expression data should improve prediction of clinically important events, such as local recurrence, metastasis or therapeutic responses.

Acknowledgement

We are grateful to Ms Rie Ito and Ms Sachiyo Mitani for technical assistance.

Disclosure/conflict of interest

This work was supported by the program for promotion of Fundamental Studies in Health Sciences of the National Institute of Biomedical Innovation (NiBio) and by Grants-in-Aid from the Ministry of Education, Culture, Sports, Science and Technology, Japan. There is no conflict of interest to declare.

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Supplementary Information accompanies the paper on Modern Pathology website (<http://www.nature.com/modpathol>)

New approach for assessing vascular distribution within bone tumors using dynamic contrast-enhanced MRI

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Received: 15 September 2006 / Accepted: 11 December 2006 / Published online: 30 May 2007
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Abstract

Purpose To differentiate benign from malignant bone tumors by analyzing the vascular distribution within bone tumors with dynamic contrast-enhanced MRI.

Methods We studied dynamic contrast-enhanced MRI for 49 bone tumors (22 malignant and 27 benign tumors). Seven small regions of interest (ROI) were set inside the largest portion of each tumor. Four ROI were placed evenly on the periphery and three ROI were placed evenly on the line of the longest breadth within the tumor. The slope of the curve (%Slope) was calculated on the time–intensity curves of the whole tumor and of each ROI. The variance values for the %Slope of the ROI were calculated to assess the dispersion of the intensity change at each ROI within the tumor.

Results Mean value of the %Slopes of whole tumor regions for malignant bone tumors ($70.4 \pm 60.3\%$) was significantly higher than that for benign bone tumors ($37.6 \pm 52.9\%$) ($P = 0.015$), although giant cell tumor

(GCT), a locally aggressive tumor, had a relatively higher %Slope. Mean value of the variance of %Slopes for malignant bone tumors (3485.9 ± 5942.5) was significantly higher than that for all benign tumors (470.4 ± 583.9) ($P = 0.012$), indicating that the %Slope values of seven ROI within malignant bone tumors varied more widely compared with the ROI inside benign bone tumors. GCT also demonstrated a lower value.

Conclusion Our method of analyzing the signal intensity change at seven separate regions that evaluates the vascular distribution within a tumor could be a useful tool for differentiating between benign and malignant bone tumors.

Keywords Bone tumors · Dynamic contrast-enhanced MRI · Differential diagnosis

Introduction

Magnetic resonance imaging (MRI) has an important role in the diagnosis and management of patients with bone tumors (Zimmer et al. 1985; Pettersson et al. 1987; Bloem et al. 1988; Hoffer et al. 2000). MRI directly demonstrates a lesion relative to the surrounding normal structures with accurate anatomical detail. Although various reports have been designed to characterize bone tumors through MRI, results have been controversial (Lang et al. 1998). The ability of static clinical MRI to depict tumor viability is limited by several factors: (1) T1-weighted MRI alone cannot differentiate viable tumor from non-viable tissue or edema (Holscher et al. 1990), (2) T2-weighted MRI cannot adequately distinguish tumor from necrosis, and lesion boundaries are frequently overestimated because of the presence of edema and hemorrhage (Pan et al. 1990; Sanchez et al. 1990; Brisse et al. 2004), and (3) static

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T1-weighted contrast media-enhanced MRI can differentiate necrosis and hemorrhage from viable tumor but has difficulty separating viable tumor from surrounding inflammation (Erlemann et al. 1990; Verstraete and Lang 2000).

Dynamic contrast-enhanced MRI is a method of physiologic imaging that evaluates the early enhancement kinetics of water-soluble MR contrast media and shows the signal intensity of a region in real time (Erlemann et al. 1989). Faster contrast enhancement is suitable for visualizing increased vascularity such as capillary permeability and viable tumor, whereas slower enhancement demonstrates less vascular areas such as non-viable tumor, tissue necrosis and inflammation (Kormano and Dean 1976; Verstraete et al. 1995). Dynamic contrast-enhanced MRI is expected to be excellent for assessing response to chemotherapy compared with conventional static MRI because it can assess tumor vascularity that may indicate viability of malignant bone tumors (Erlemann et al. 1990; Fletcher et al. 1992; Kawai et al. 1997; Egmont-Petersen et al. 2000). Despite its usefulness for assessing the therapeutic effect on bone sarcomas, it is still controversial as to whether dynamic contrast-enhanced MRI can differentiate malignant bone tumors from benign bone tumors.

Previous papers demonstrated that the time-course change of intensity can suggest the malignancy of bone tumors with a 60–80% sensitivity (Verstraete et al. 1994b; Bloem et al. 1997; Geirnaerd et al. 2000). This may be the reason why these papers set the region of interest (ROI) over the entire tumor area imaged in order to examine the intensity change. There are various tissues and fluids within malignant bone tumors, such as high-grade tumor tissue, necrotic and edematous tissue, hemorrhage, and inflammation. One ROI set covering the entire area of a tumor may not be appropriate for evaluating vascularity within a tumor since the intensity change over the whole tumor can be averaged. For studying vascularity of bone tumors, it would be more reliable to designate several ROI sets inside the tumor as seen on MRI and to evaluate each of them. In the present study, we examined the intensity change of seven ROI sets within the tumor boundaries as determined with dynamic MRI and assessed their values for differentiating the malignancy of bone tumors.

Patients and methods

Patients

Forty-nine patients with primary bone tumors were studied using dynamic contrast-enhanced MRI during the preoperative routine examination before initial treatment (Table 1). Diagnosis of all patients was made by histological

examination. The patients consisted of 32 males and 17 females with a mean age of 30 years (4–74 years). Twenty-four tumors were of the femur, seven of the tibia, four of the humerus, three of the pelvis, two of the fibula, and nine were from other locations. There were 22 malignant bone tumors, consisting of 15 osteosarcoma, 3 chondrosarcoma, 2 Ewing's sarcoma, 1 malignant fibrous histiocytoma (MFH) and 1 chordoma. There were also 27 benign bone tumors consisting of 5 giant cell tumors (GCT), 5 enchondroma, 4 chondroblastoma, 2 osteochondroma, 2 non ossifying fibroma, 2 aneurysmal bone cysts, 2 eosinophilic granuloma, 2 fibrous dysplasia, 2 solitary bone cysts and 1 osteofibrous dysplasia.

Dynamic contrast-enhanced MRI study

All MR examinations were performed using a 0.5 T (Flexart Hyper; Toshiba, Tokyo, Japan) clinical imager. A quadrature detection (QD) body coil was used for pelvic and femoral imaging, and a 20 cm circular surface coil was adapted for imaging the other locations.

Prior to dynamic contrast-enhanced MRI study, we performed the following pulse sequences: axial, coronal, and sagittal T1-weighted spin echo imaging: repetition time (TR), 550 ms; echo time (TE), 15 ms; number of excitations (NEX), 1.7; slice thickness, 5–10 mm, field of view (FOV), 20–35 cm; matrix size, 192 × 256; acquisition time, 6 min, axial, coronal, and sagittal T2-weighted fast spin echo imaging: TR, 4,000 ms; TE, 102 ms; echo train length, 13; NEX, 3; slice thickness, 5–10 mm; FOV, 20–35 cm; matrix size, 192 × 256; acquisition time, 6 min 4 s.

For a dynamic contrast-enhanced MRI, imaging plane was selected based on obtaining the largest and most representative area of the tumor. Immediately after the first

Table 1 Pathological diagnosis in 49 patients with bone tumors

Benign tumor	No. (n = 27)	Malignant tumor	No. (n = 22)
Giant cell tumor	5	Osteosarcoma	15
Enchondroma	5	Chondrosarcoma	3
Chondroblastoma	4	Ewing sarcoma	2
Osteochondroma	2	Malignant fibrous histiocytoma	1
Non-ossifying fibroma	2	Chordoma	1
Aneurysmal bone cyst	2		
Eosinophilic granuloma	2		
Fibrous dysplasia	2		
Solitary bone cyst	2		
Osteofibrous dysplasia	1		

sequence, a bolus injection of gadolinium diethylene triamine pentaacetic acid (Gd-DTPA) (0.1 mmol/kg) was administered intravenously followed by ultra-fast sequences using a field echo sequence (TR, 70 ms; TE, 5 ms; flip angle, 70°; NEX, 1; slice thickness, 10 mm; FOV, 20–35 cm; matrix size, 128–192 × 256; echo delay time, 20 ms) at intervals of 13 s for 5 min. The signal intensity of the largest portion of the tumor was measured and plotted against time. The progression of enhancement was subjectively classified into three groups according to the shape of the time–signal intensity curve by modified classification reported previously (van der Woude et al. 1998b) and described in Fig. 1. In the analysis of dynamic MRI, we hypothesized that Type I (rapid pattern) was indicative of malignant lesions, and type II (slow pattern) and type III (flat pattern) were indicative of benign lesions.

Seven regions of interest (ROI) were set within the largest portion of the tumor. Four ROI were placed evenly on the periphery of the largest portion of the tumor, and three ROI were placed evenly on the line of the longest breadth within the tumor (Fig. 2). The signal intensity of each of the seven ROI set inside the tumor were also plotted against time on the time–intensity curve.

The slope of the curve (%Slope), which shows the percent increase in signal intensity per minute over the

baseline value, was derived by using the following formula (Erlemann et al. 1990; Hanna et al. 1992) (Fig. 3):

$$\%Slope = (SI_{max} - SI_{base}) \times 100 / \{SI_{base} \times (T_{max} - T_{base})\}$$

SI_{max} : signal intensity demonstrated at a timepoint of T_{max} ,

SI_{base} : signal intensity before the injection of Gd-DTPA, T_{max} : first timepoint at which the sum of the next two consecutive %Slope values becomes less than 10% per minute,

T_{base} : timepoint immediately preceding signal intensity increase.

The variance values for %Slope of the seven ROI set inside the tumor were calculated to assess the dispersion of the intensity change at each of these ROI, which could indicate the heterogeneity of the vascularity within the tumor.

Statistical analysis

The Mann–Whitney U test was used to evaluate the difference in %Slope and the variance value for %Slope. The

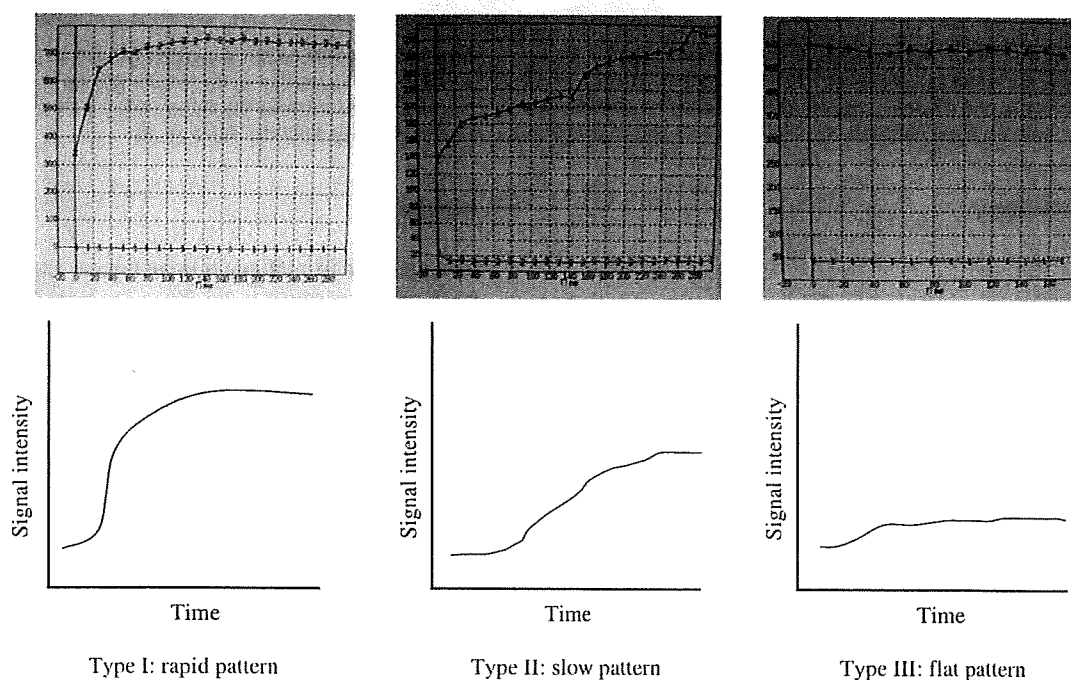


Fig. 1 Three types of time-signal intensity curves reported by van der Woude et al. (1998b). Type I curve (*rapid pattern*): early onset of enhancement followed by rapidly progressive linear increase of signal intensity (represented by a steep slope, parallel to the arterial curve) to an early maximum followed by a distinct transition to a stable level of signal intensity. Type II curve (*slow pattern*): steady increase or

flattening of the curve after the steepest part of the time-signal intensity curve. Type III curve (*flat pattern*): linear enhancement with a very low slope, representing slow or absent enhancement, similar to a curve representing normal muscle. In the analysis of dynamic MRI, we hypothesized that Type I curve was indicative of malignant lesions, and Type II and III curves were indicative of benign lesions

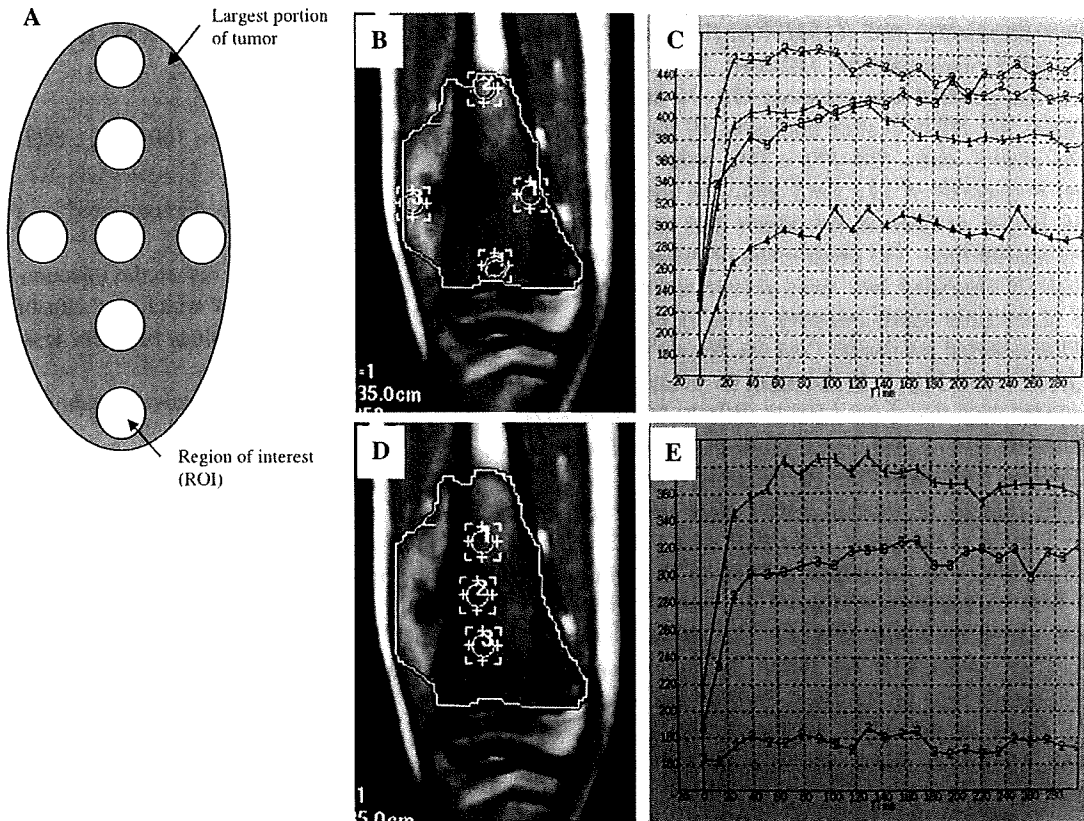


Fig. 2 A large ROI and seven small ROI are set within the largest portion of the tumor (a: scheme of 7 ROI set within the tumor). Seven ROI placed on the dynamic contrast-enhanced MRI of osteosarcoma arising from the distal femur (b, d). Four ROI were placed evenly on

the periphery of the largest portion of the tumor (b), and 3 ROI were placed evenly on the line of the longest breadth within the tumor (d). The time–signal intensity curves of each 7 ROI were plotted against time on the time–intensity curve (c, e)

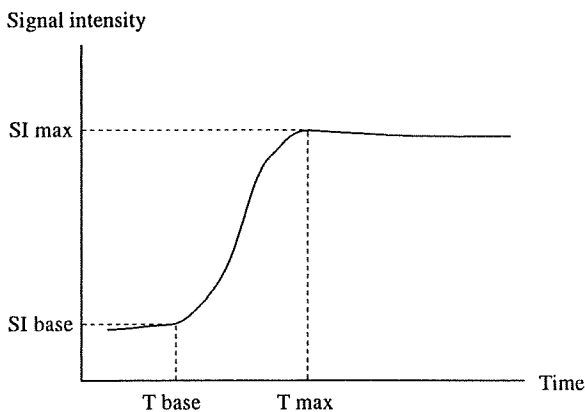


Fig. 3 %Slope was calculated to show the percent increase in signal intensity per minute over the baseline value: $\%Slope = (SI_{max} - SI_{base}) \times 100 / (SI_{base} \times (T_{max} - T_{base}))$

level of significance was chosen at $P < 0.05$. All data was calculated and evaluated using Statview 5.0J software (SAS Institute Inc., Cary, NC, USA).

Results

Seventeen of 22 malignant bone tumors and 6 of 27 benign bone tumors resulted in the Type I pattern (Table 2). Those 6 benign tumors with Type I pattern consisted of four GCT and two chondroblastoma. Type II pattern was found in 17 benign bone tumors and 4 of 22 malignant bone tumors, which included 2 osteosarcoma and 2 chondrosarcoma. There were four benign bone tumors and one chordoma demonstrating the Type III pattern. Seventeen of 22 malignant bone tumors revealed rapid pattern (Type I curve), and 21 of 27 benign bone tumors demonstrated slow or flat pattern (Type II or III curve). Differentiation of benign from malignant bone tumors based on the time–intensity curve was possible with a sensitivity of 77% and a specificity of 78%.

Table 3 demonstrates each %Slope value representing a whole tumor area with dynamic contrast-enhanced MRI. The mean value of %Slopes for malignant bone tumors ($70.4 \pm 60.3\%$) was significantly higher than that for benign bone tumors ($37.6 \pm 52.9\%$) ($P = 0.015$, Mann–

Table 2 The pattern of the progression of enhancement on dynamic MRI

Pattern	Benign (n = 27)	Malignant (n = 22)
Type I: rapid pattern	6	17
Type II: slow pattern	17	4
Type III: flat pattern	4	1

Whitney *U* test). Moreover, this significant difference was even more pronounced ($15.8 \pm 14.3\%$) ($P < 0.0001$) when the mean value of %Slopes for GCT ($133.4 \pm 4.7\%$) was not counted among the benign bone tumors since the %Slopes for GCT was comparatively higher than the other benign bone tumors. Chordoma, one of the malignant bone tumors, had a lower mean value of %Slope than most of benign tumors.

The variance values for %Slopes of the seven ROI located within a tumor were estimated as the dispersion of intensity change (%Slope) at each ROI of a tumor. Table 4 shows each variance value for the %Slopes of the seven ROI within each tumor. The mean value of the variance of %Slopes for malignant bone tumors (3485.9 ± 5942.5) was significantly higher than that for all benign tumors (470.4 ± 583.9) ($P = 0.012$), indicating that %Slope values of the seven ROIs inside a malignant bone tumor varied more widely than the seven ROI within a benign bone tumor. The vascularity of malignant bone tumors was more heterogeneous than that of benign bone tumors. In particular, the mean variance value of %Slope for GCT (1223.7 ± 727.4) was lower than that of high-grade malignant bone tumors such as MFH (4481.4), osteosarcoma (3953.2 ± 6979.6), and Ewing sarcoma (2720.4 ± 1813.9), indicating that the vascularity within a high-grade malignant tumor is more heterogeneous than that of a GCT. Chordoma had the lowest mean value of the variance of %Slope (0.0) among all the bone tumors.

Table 3 %Slope of the whole tumor area at the largest portion

Malignant tumor	Mean %Slope	Benign tumor	Mean %Slope
Ewing sarcoma	113.1	Giant cell tumor	133.4
Malignant fibrous histiocytoma	82.4	Chondroblastoma	32.8
Osteosarcoma	73.6	Fibrous dysplasia	23.0
Chondrosarcoma	43.3	Eosinophilic granuloma	21.1
Chordoma	7.4	Solitary bone cyst	16.9
		Non-ossifying fibroma	15.9
		Aneurysmal bone cyst	13.0
		Enchondroma	5.7
		Osteofibrous dysplasia	0.0
Average	70.4		37.6

Table 4 The variance value for %Slope of 7ROI located inside the tumor

Malignant tumor	Mean variance value for %Slope	Benign tumor	Mean variance value for %Slope
Malignant fibrous histiocytoma	4481.4	Giant cell tumor	1223.7
Osteosarcoma	3953.2	Aneurysmal bone cyst	726.5
Ewing sarcoma	2720.4	Solitary bone cyst	570.7
Chondrosarcoma	2490.1	Chondroblastoma	523.1
Chordoma	0.0	Non-ossifying fibroma	456.9
		Enchondroma	125.6
		Eosinophilic granuloma	95.7
		Fibrous dysplasia	52.6
		Osteofibrous dysplasia	0.0
Average	3485.9		470.4

Discussion

Dynamic contrast-enhanced MRI, which is performed by ultra fast MRI after a bolus injection of contrast media, can be used to evaluate blood flow by calculating the signal intensity of a region in real time (Erlemann 1992), thus indicating the vascularity of a tumor. By analyzing tumor vascularity using dynamic MRI, many authors have shown that the slope value can be useful for indicating bone tumor viability and predicting tumor necrosis in bone sarcomas after chemotherapy (Erlemann et al. 1990; Bonnerot et al. 1992; Fletcher et al. 1992). Moreover, dynamic contrast-enhanced MRI is excellent for depicting anatomical position like a conventional static MRI and has merit in the evaluation of vascularity in an arbitrary section demonstrating the most representative area of the tumor (Lang et al. 1995; Verstraete and Lang 2000).

van der Woude et al. (1998a) performed dynamic enhanced MRI in 175 patients with a musculoskeletal tumor and assessed the interval between arterial enhancement and onset of tumor enhancement, the pattern (peripheral or diffuse) of initial enhancement, and the shape of the time-signal intensity curve. They concluded that benign bone tumors could not be accurately differentiated from malignant bone tumors on the basis of their defined parameters (sensitivity, 63–76%; specificity, 50–76%). Our study also showed that it was possible, with a sensitivity of 77% and specificity of 78%, to differentiate benign from malignant bone tumors based on the time-intensity curve at one section of a tumor. Bloem et al. (1997) have reported in a

review article concerning MRI for patients with musculoskeletal tumors that not only viable malignant tumors, but also biologically active benign lesions such as eosinophilic granuloma, osteoid osteoma, osteoblastoma, chondroblastoma and GCT displayed rapid-enhancement time-intensity curves of dynamic contrast-enhanced MRI. Furthermore, they reported that not only most benign lesions, but also low-grade chondrosarcoma and sclerotic osteosarcoma, displayed low-enhancement time-intensity curves. We obtained similar results in our series.

Our results demonstrated that the mean value of the %Slopes for malignant bone tumors ($70.4 \pm 60.3\%$) was significantly higher than that for benign bone tumors ($37.6 \pm 52.9\%$). Verstraete et al. (1994b) reported that a significant difference was found in the slope values of dynamic contrast-enhanced MRI between malignant and benign musculoskeletal tumors. However, they indicated that dynamic contrast-enhanced MRI could depict tissue vascularization and perfusion rather than actual benignity or malignancy since there was an overlap in the slope values of highly vascular benign lesions and malignant lesions.

GCT, the most common benign tumor in our series, had the highest mean value of %Slope (133.4) among all the bone tumors. Verstraete et al. (1994a) also demonstrated same result that GCT showed the highest mean value of %Slope among all musculoskeletal tumors. Chordoma, one of low-grade malignant bone tumors, had much lower mean value of %Slope (7.4) than the average of benign bone tumors (37.6). van der Woude et al. (1998b) showed that only 6 of 26 low-grade malignant tumors showed a Type I curve. There was also a considerable overlap of %Slope value between aggressive benign bone tumors and low-grade malignant tumors.

As mentioned above, the overall rate of enhancement is not a reliable indicator of a benign versus a malignant bone lesion. As a method for evaluating any difference in enhancement within a tumor, Ma et al. have reported that the rim-to-center differential enhancement ratio can potentially be used to differentiate benign from malignant bone tumors (Ma et al. 1997). The increased rim enhancement with delayed center enhancement, which indicated a solid, malignant mass of bone, might be due, in part, to the high internal, interstitial pressure exhibited by malignant neoplasms but not benign tumors. There are generally some necrotic areas and/or undifferentiated lesions with aggressive tumor tissue inside a malignant tumor mass, indicating that the inside of a malignant tumor can be vascularly heterogeneous. The rim-to-center enhancement cannot be enough to reflect the vascular heterogeneity inside the tumor.

In order to study the vascular heterogeneity inside a tumor, we set up seven small ROI within each tumor and

calculated the intensity change of each ROI. We considered the variance value for slopes of the seven ROI as the dispersion of the blood flow at each ROI, which demonstrated the heterogeneity of vascularity inside the tumor. The distribution of slope values in malignant bone tumors was significantly higher than that in benign tumors. This suggested that the malignant bone tumors were composed of regions having various changes of signal intensity, indicating that the viability inside malignant tumors was heterogeneous. Therefore, seven ROI could also be useful for determining the optimum site for biopsy.

Chordoma, one of low-grade malignant tumors, had the lowest mean value of the variance of %Slopes among all the bone tumors in the present study. This might be the reason why chordoma was only one case in our series and showed very low vascularity within the tumor. Because the number of each tumor type is small ($n = 1-5$), except for osteosarcoma ($n = 15$) in the present study, prospective studies that include larger numbers of patients are needed to validate the use of our analyzing method.

In conclusion, the signal intensity change observed with dynamic contrast-enhanced MRI can be used to assess the vascularity of a tumor. Previous papers showed that the intensity change over a whole tumor section can predict the viability of the tumor, but this method was not enough to determine the malignancy of bone tumors. Our method of analysis looking at the intensity change at seven separate regions can indicate the heterogeneity of vascularity within a tumor, which could make our method useful for differentiating between benign and malignant bone tumors.

Acknowledgments This work was supported in part by a Grant-in-aid for Young Scientists (B) from the Ministry of Education, Culture, Sports, Science and Technology (No.18791040, No.15790792), by a grant from Japan Orthopaedics and Traumatology Foundation Inc. (No.0158), by Grants-in-Aid for Clinical Cancer Research and Grants-in-Aid for Cancer Research (14S-4 and -5) from the Ministry of Health, Labor and Welfare, and by a grant from JSPS Fujita Memorial Fund for Medical Research.

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Prognostic Implications of Glucose Transporter Protein-1 (Glut-1) Overexpression in Bone and Soft-Tissue Sarcomas

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Received June 28, 2007; accepted August 13, 2007

Background: The glucose transporter protein 1 (Glut-1) overexpression is associated with poor overall survival (OS) in various malignant tumors. The aim of this study was to investigate prognostic significance of Glut-1 overexpression in patients with bone and soft-tissue sarcomas.

Methods: A total of 67 patients (mean age, 43 years; range, 8–79 years) with bone and soft tissue sarcomas were analyzed. Pathologic confirmation was observed from surgical specimens in all patients. Pathologic variables including tumor differentiation, necrosis, mitotic index, MIB-1 (Ki-67) grade and Glut-1 expression were assessed. Clinical characteristics and pathologic variables were determined by Kaplan–Meyer curve of OS after treatment.

Results: Glut-1 overexpression was found in 56 patients (83%). The patients with Glut-1 overexpression showed significantly poor OS compared with those without Glut-1 overexpression ($P = 0.029$). The presence of metastasis, treatment without surgical resection, tumor differentiation, necrosis, mitotic index and MIB-1 grade were also significantly negative prognostic factors. The presence of metastasis was independently associated with poor OS ($P = 0.031$).

Conclusions: Assessment of Glut-1 expression prior to treatment has a predictive potential effect in patients with bone and soft-tissue sarcomas.

Key words: sarcoma – glucose transporter protein – prognosis

INTRODUCTION

Bone and soft-tissue sarcomas are classified according to their grade, which represents the most important prognostic factors. The presence of necrosis has been shown to be an independent parameter for predicting prognosis (1). The size and the location of the tumor are other important prognostic factors (2).

The glucose transporter protein-1 (Glut-1) is one of the proteins upregulated in hypoxic conditions. The presence of hypoxia in tumors is leading to resistance to radiotherapy and chemotherapy and is associated with an increased potential for metastases (3–5). This latter finding is thought to be related to the promotion of genomic instability associated with an carcinogenesis and malignant progression (6). Glut-1

is also associated with an increased expression of some proteins that can change tumor cells to survive the severe micro-environment. Glut-1 also promotes glucose metabolism and is overexpressed in several tumors (7–9). The level of Glut-1 expression might be a suitable marker of hypoxia and glucose metabolism, which could be measured simply and inexpensively as part of the routine histologic assessment of tumors (10,11). Increased expression of Glut-1 has been shown to be correlated with a poor prognosis in a variety of tumors (12–14). However, little is known about its expression in bone and soft-tissue sarcomas.

In the present study, immunohistochemical staining of Glut-1 was performed prospectively in the patients with bone and soft-tissue sarcomas. The aim of the present study was to test the hypothesis that Glut-1 overexpression is related to the clinical outcome in patients with bone and soft-tissue sarcomas.

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PATIENTS AND METHODS

PATIENTS

Sixty-seven patients aged 8–79 years and having histologically proven sarcomas arising from bone ($n = 22$, 33%) or soft tissue ($n = 55$, 67%) since July 2001 until July 2006 were included in this study. All patients underwent initial staging based on a review of the medical history, physical examinations and imaging studies. This study was conducted in accordance with the Helsinki declaration, and all the patients had provided their informed consent for the review of their records.

TREATMENT AND FOLLOW-UP

Surgical resection was performed in 54 patients (81%). Surgical procedures were wide resection ($n = 33$, 61%), marginal resection ($n = 15$, 22%), radical resection ($n = 3$, 4%) and intralesional resection ($n = 3$, 4%). Adjuvant chemotherapy was performed in 46 patients (69%). Radiotherapy was performed in 24 patients (36%), mean dose was 46.5 Gy (range, 30–70.4 Gy). The follow-up period was dated from the time of diagnosis, and the mean follow-up period was 25 months (range, 6–60 months). During the follow-up, 39 patients (58%) developed metastases. Metastatic sites included lung ($n = 26$, 39%), bone ($n = 7$, 10%), lymph node ($n = 6$, 9%), soft tissue ($n = 4$, 6%), adrenal gland ($n = 2$, 3%), liver ($n = 1$, 1%), heart ($n = 1$, 1%) and pancreas ($n = 1$, 1%). Seventeen patients (25%) died with disease, 19 patients (28%) were alive with disease and 31 patients (46%) were no evidence of disease.

HISTOLOGICAL EXAMINATION

Pathology specimens of all the patients' tumors were obtained by incisional biopsy or surgical resection after the imaging studies, and histologic slides were prepared by two expert pathologists for diagnosis. Each tumor was staged according to the TNM classification of UICC and AJCC staging protocol for bone and soft-tissue sarcomas (15,16). Whenever necessary, immunohistochemical staining was carried out to confirm the diagnosis or tumor type according to the WHO classification system (17). The histologic grade of the tumor was determined using a three-grade system in which tumor differentiation, tumor necrosis and MIB-1 LI were given a score of 0, 1, 2 or 3, respectively, and the scores were added together (18). Lesions with MIB-1 LI of 0–9, 10–29 and >30% were assigned MIB-1 scores of 1, 2 and 3, respectively. The three separate scores were added together to produce a combined grade: lesions whose total score was 2 or 3 were classified as Grade 1, those whose total score was 4 or 5 were clarified as Grade 2 and those whose total score was 6, 7 or 8 were clarified as Grade 3. According to this MIB-1 system, tumors were assigned Grades 1–3.

IMMUNOHISTOCHEMICAL ANALYSIS OF GLUT-1

Immunohistochemical analysis was performed using the labeled streptavidin–biotin method and tissue sections from paraffin blocks. The sections were dewaxed, rehydrated and moistened, then pretreated in an autoclave before being incubated with an affinity-purified goat polyclonal anti-Glut-1 antibody (A3536; diluted 1:500; DakoCytomation). The intensity of Glut-1 staining was quantified with regard to the percentage of cells stained. It was scored as 0 (0%), 1 (1–9%), 2 (10–29%) or 3 (>30%). The results were evaluated by an expert pathologist who was unaware of the clinical status of the patients. The sections were examined using a multi-head microscope, and a consensus judgment was adopted based on Glut-1 staining score of the tumor. The staining scores of 2 and 3 were regarded as indicators of the overexpression of Glut-1.

STATISTICAL ANALYSIS

For the study of the prognostic value, overall survival (OS) was chosen as an end point. OS was defined as the time from diagnosis to death from any cause. Univariate regression analysis was performed to assess the value of all the prognostic factors for the prediction of OS by comparing Kaplan–Meier OS curves and carrying out log-rank tests. Kruskal–Wallis test was performed to compare the relations between MIB-1 grade and Glut-1 intensity. Multivariate proportional hazards (Cox) regression analysis was used to test the independency of established prognostic factors for the prediction of OS. Differences and correlations at a P -value of <0.05 was considered statistically significant. All data analyses were performed using SPSS 12.0J (SPSS, Chicago, IL, USA).

RESULTS

The characteristics of 67 patients (mean age, 43 years; range, 8–79 years) are shown in Table 1. Most frequent histologic types were osteosarcoma ($n = 16$, 24%) followed by pleomorphic malignant fibrous histiocytoma ($n = 12$, 18%). The tumors were located in the trunk in 37 patients (55%) and in the extremity in 30 patients (45%). The largest diameter of the tumor is 9.4 cm (range, 1.2–24.0 cm). Twenty-two bone sarcomas (33%) and 45 soft-tissue sarcomas (67%) were also included in Table 1. Tumor stage was IA ($n = 2$, 3%), IB ($n = 4$, 6%), IIA ($n = 14$, 21%), IIB ($n = 9$, 13%), III ($n = 12$, 18%), IV ($n = 18$, 27%), IVA ($n = 4$, 6%) and IVB ($n = 4$, 6%).

The univariate analysis results are summarized in Table 2. The presence of metastasis ($P = 0.0030$) and the treatment without surgical resection ($P = 0.0010$) were significantly associated with poor OS. Age, gender, tumor type, anatomical site (trunk or extremity), tumor size and treatment modality had no prognostic value.

Table 1. Patient characteristics

Parameter	Value (%)
Age (year)	
Mean ± SD	43 ± 22
Median	38
Range	8–79
Gender	
Male	42 (63)
Female	25 (37)
Histologic diagnosis	
Osteosarcoma	16 (24)
Pleomorphic MFH	12 (18)
Liposarcoma	6 (9)
Ewing sarcoma	6 (9)
Chondrosarcoma	5 (7)
Synovial sarcoma	5 (7)
Myxofibrosarcoma	4 (6)
Leiomyosarcoma	3 (4)
Rhabdomyosarcoma	3 (4)
Angiosarcoma	2 (3)
Alveolar soft-tissue sarcoma	1 (1)
Epithelioid sarcoma	1 (1)
Fibrosarcoma	1 (1)
MPNST	1 (1)
Unclassified	1 (1)

The numbers of the parentheses are percentages.
SD, standard deviation; MFH, malignant fibrous histiocytoma; MPNST, malignant peripheral nerve sheath tumor.

Mitotic grades were Grade 1 (*n* = 8, 12%), Grade 2 (*n* = 11, 16%) and Grade 3 (*n* = 48, 72%). The median MIB-1 index of tumor was 32.5%. MIB-1 grades were Grade 1 (*n* = 7, 10%), Grade 2 (*n* = 10, 15%) and Grade 3 (*n* = 50, 75%). On the basis of pathologic examinations, it was found that tumor differentiation (*P* = 0.0165), necrosis (*P* = 0.0399), mitotic index (*P* = 0.0071) and MIB-1 grade (*P* = 0.0311) were associated with a trend toward poor OS.

Glut-1 expression (Figs 1–3) was found in 64 patients (96%) and their intensity was 1 (*n* = 8, 12%), 2 (*n* = 15, 22%) and 3 (*n* = 41, 61%). Glut-1 immunostaining was absent in three tumors (4%): two well-differentiated liposarcomas and one clear cell chondrosarcoma. Along with Glut-1 expression in cytoplasm of tumor cells, specific Glut-1 expression was also seen in erythrocytes, perineurium of the peripheral nerves and lymphocytes in the germinal zone. Glut-1 overexpression was significantly associated with the histologic grade by Kruskal–Wallis test (*P* < 0.0001) (Table 3). No significant difference in Glut-1 staining intensity was found among the other variables, including age, gender, anatomical site (trunk or extremity), tumor size and

Table 2. Univariate analysis of overall survival (OS)

Variables	<i>n</i>	1-year survival (%)	2-year survival (%)	3-year survival (%)	<i>P</i> value
Age (years)					
<38	33	90.2	81.5	73.4	0.7592
≥38	34	88.0	76.7	68.2	
Gender					
Female	25	95.8	91.5	74.9	0.4413
Male	42	85.0	69.9	69.9	
Tumor type					
Bone sarcoma	22	90.4	90.4	81.4	0.8504
Soft-tissue sarcoma	45	88.5	73.5	65.4	
Anatomical site					
Extremities	30	92.7	81.8	81.8	0.1316
Trunk	37	86.2	76.8	59.7	
Metastasis					
Negative	28	96.4	96.4	96.4	0.0030
Positive	39	84.3	68.2	57.2	
Size (cm)					
0–5	16	100.0	100.0	100.0	0.0506
5–10	22	80.0	56.3	42.2	
>10	29	89.7	80.4	71.5	
SR					
(–)	13	61.5	52.8	52.8	0.0010
(+)	54	96.0	86.1	76.8	
AC					
(–)	20	89.7	89.7	89.7	0.0740
(+)	47	88.9	75.1	63.0	
RT					
(–)	43	90.3	87.5	81.3	0.0909
(+)	24	87.1	67.0	53.6	
Therapeutic combination					
AC alone	5	40.0	40.0	40.0	<0.0001
RT alone	1	0.0	0.0	0.0	
AC + RT	7	85.7	68.6	68.6	
SR alone	17	100.0	100.0	100.0	
SR + AC	22	94.4	87.7	73.1	
SR + AC + RT	15	93.3	70.6	56.6	
Tumor differentiation					
1 or 2	15	100.0	100.0	100.0	0.0165
3	52	85.9	73.8	64.3	
Necrosis					
0	41	92.6	85.4	72.3	0.0399
1	11	90.9	80.8	80.8	
2	15	78.6	62.5	62.5	

Continued

Table 2. Continued

Variables	n	1-year survival (%)	2-year survival (%)	3-year survival (%)	P value
Mitotic index					
0 or 1 or 2	32	96.9	96.9	87.2	0.0006
3	35	81.7	62.2	56.0	
Glut-1 overexpression					
(-)	19	94.1	94.1	94.1	0.0292
(+)	48	87.2	74.7	65.0	
MIB-1 grade					
1 or 2	17	100.0	100.0	83.3	0.0311
3	50	85.2	72.1	67.0	
Mitotic grade					
1 or 2	19	94.7	94.7	94.7	0.0198
3	48	86.7	72.9	62.1	

SR, surgical resection; AC, adjuvant chemotherapy; RT, radiotherapy; Glut-1, glucose transporter protein 1.

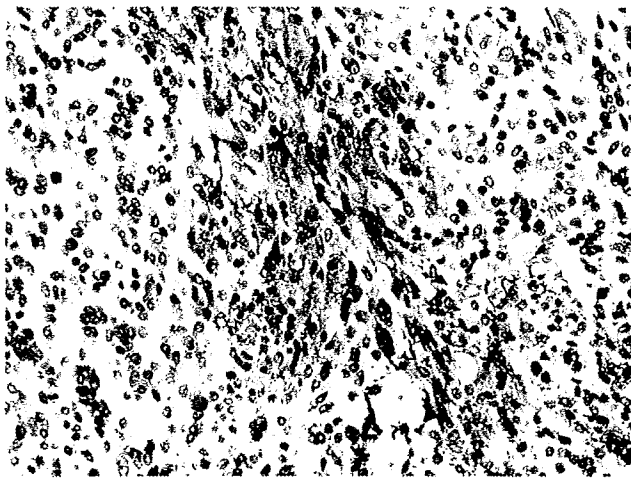


Figure 1. Immunohistochemical analysis of Glut-1. Intensity of Glut-1 staining score: 0 (0%), 1 (1–9%). Histologic diagnosis was pleomorphic malignant fibrous histiocytoma. Glut-1, glucose transporter protein 1.

the presence of local recurrence or metastasis and treatment modality. On the basis of univariate analysis, it was found that Glut-1 overexpression was significantly associated with poor OS ($P = 0.029$, Fig. 4).

Multivariate analysis shows that the presence of metastasis is associated with significantly poor OS ($P = 0.031$, Table 4) than those without metastasis. It also shows that the anatomical site (trunk or extremity), the presence of metastasis, treatment without surgical resection, tumor size, treatment modality, tumor differentiation, necrosis, mitotic index, MIB-1 grade and Glut-1 overexpression were not independently associated with OS. When we excluded the presence of metastasis from variables, mitotic index was significant



Figure 2. Immunohistochemical analysis of Glut-1. Intensity of Glut-1 staining score: 2 (10–29%). Histologic diagnosis was pleomorphic malignant fibrous histiocytoma.

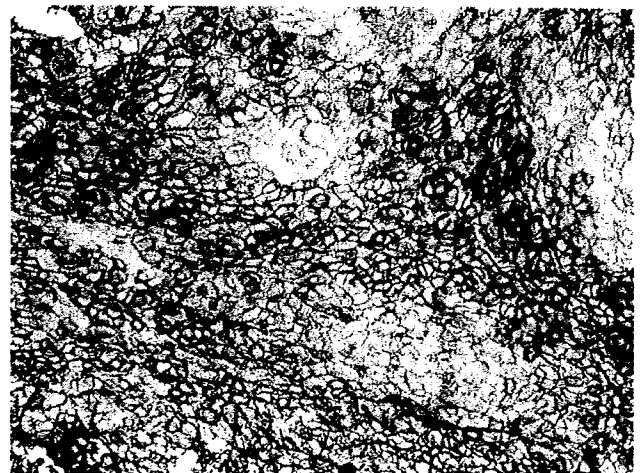


Figure 3. Immunohistochemical analysis of Glut-1. Intensity of Glut-1 staining score: 3 (>30%). Histologic diagnosis was synovial sarcoma.

indicator of poor OS [hazard ratio, 8.709; 95% confidence interval 1.980–38.319; $P = 0.004$].

DISCUSSION

This study suggests three notable features. First, Glut-1 overexpression is a possible adverse prognostic factor similar to the presence of metastasis, treatment without surgical resection, tumor differentiation, necrosis, mitotic index and MIB-1 grade. Secondly, the presence of metastasis is independently associated with poor prognosis in multivariate analysis. Thirdly, there is significant correlation between Glut-1 intensity and MIB-1 grade. Our study is the first that focusing on the relations between Glut-1 expression and prognosis in patients with bone and soft-tissue sarcomas.

Glut-1 expression has been investigated in a variety of tumors (12–14), however, its expression in bone and

Table 3. MIB-1 grade and Glut-1 intensity

MIB-1 grade	Glut-1 intensity			
	0	1	2	3
1	3 (4)	4 (6)	0	0
2	0	4 (6)	1 (1)	5 (7)
3	0	8 (12)	7 (10)	35 (52)

The numbers of the parentheses are percentages.

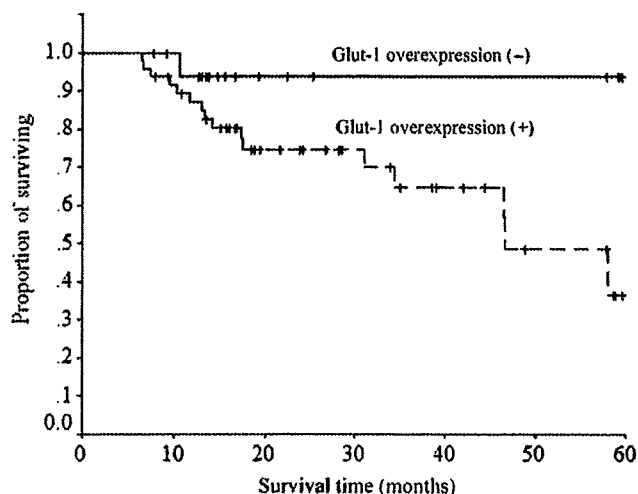


Figure 4. Kaplan–Meier estimated overall survival by Glut-1 overexpression. Patients with Glut-1 overexpression were associated with poor overall survival compared with patients without Glut-1 overexpression.

soft-tissue sarcomas has rarely studied. The aim of this study was to determine whether the intensity of Glut-1 expression in a tumor could serve as a surrogate marker of the survival. On the basis of this study, it was found that Glut-1 overexpression is associated with OS in the univariate analysis. However, it is unknown whether Glut-1 overexpression is more predictive of OS than other variables. Therefore, the immunohistochemical results support the significance of Glut-1 expression as a biomarker of poor prognosis in patients with bone and soft-tissue sarcomas. Glut-1 antibody is achieved easily and inexpensively, and Glut-1 staining can be done as a part of routine pathologic procedure.

Our results suggested that glucose transport by Glut-1 plays an important role in progression of bone and soft-tissue sarcoma. Besides, glucose transport and metabolism provide crucial prognostic information, they have the potential of being future treatment targets. Inhibition of glucose transport is investigated in some cancer cells, for example, inhibition of glucose transport by cytochalasin-B presented increased gemcitabine-induced apoptosis in hepatoma cells (30). Investigation about the inhibition of glucose transport in bone and soft-tissue sarcomas is challenges for the future.

Table 4. Multivariate analysis of overall survival (OS)

	B	SE	Wald	HR	95% CI	P-value
Metastasis	0.632	0.293	4.671	1.882	1.061–3.340	0.031

SE, standard error; HR, hazard ratio; CI, confidence interval.

Glut-1 expression is the common mediator of glucose uptake in malignant tumors (12–14). However, Glut-1 immunostaining was absent in three tumors (4%) in our study: two well-differentiated liposarcomas and a clear cell chondrosarcoma. Glucose transporter proteins other than Glut-1 exist and expresses in various histologic kinds of malignant tumor (31). It is possible that Glut-1 negative tumors would have been positive for other glucose transporters.

The present study had limitations. Follow-up duration of our study is relatively short to calculate patient OS. Whether Glut-1 overexpression adds original information to several prognostic variables requires a further evaluation in an ongoing long-term study. Glut-1 overexpression has a poor prognostic significance in the univariate analysis. This finding to some extent validates our study population because Glut-1 is an excellent indicator of tumor grade and one of the most important prognostic factors in patients with soft-tissue sarcomas treated with combination therapy. However, Glut-1 overexpression was not independently associated with poor prognosis in our study. Short duration of follow-up in our study may affect the results of multivariate analysis. Treatment regimens and duration were not the same for all the patients. Since combination therapy in our study is eligible for patients with bone and soft-tissue sarcomas, this might be biased with the study analysis.

In conclusion, Glut-1 overexpression could be a negative prognostic factor in patients with bone and soft-tissue sarcomas. These findings support the concept of pretherapeutic stratification with Glut-1 immunostaining to identify high-risk patients and propose a more risk-adapted approach of treatment in patients with bone and soft-tissue sarcomas.

FUNDING

This work was supported in part by grants from Scientific Research Expenses for Health and Welfare Programs and the Grant-in-Aid for Cancer Research from the Ministry of Health, Labour and Welfare.

Conflict of interest statement

None declared.

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chapter

IV

思春期貧血

Key Words

- 思春期貧血
- 鉄欠乏性貧血
- ヘモグロビン
- 異食症
- *Helicobacter pylori*

POINT

思春期は、身体の発育に伴う血液量の増加による鉄の需要の増加、女子における月経の開始などにより体内の鉄は減少するが、それに見合う供給が不足することが多く、鉄欠乏に陥りやすい。鉄欠乏がこうじると鉄欠乏性貧血となるが、鉄欠乏は貧血を呈する以前より、鉄依存性酵素の活性低下がおこり、言語学習能力や記憶力の低下が報告されている。



はじめに

思春期は、身体の発育が著しい時期である。血液量は体重に比例するといわれており、思春期は血液量の増大が著しい時期ともいえる。さらに運動量の増加、そして女子においては月経の発来などがみられる時期である。これらを鉄という観点からみると、思春期は鉄の需要と喪失の両者の増大がおこる時期ということになり、鉄欠乏が非常におこりやすい時期であるということになる。

鉄はヘモグロビンの構成要素であるため、欠乏が進むと鉄欠乏性貧血となることが知られている。ところが、鉄欠乏性貧血は緩徐に進行する貧血であるために、生体は貧血状態に対する適応が成立し、自覚症状が出現するまでにはかなりの時間がかかることが多く、気がつかれないうちに、鉄欠乏性貧血が進行していることが少なくない。学校保健法が改正される平成6年以前は、採血による貧血検診が多くの学校で施

行され、自覚症状がない中学生・高校生の貧血も、検診で発見されることが少なくなかったが、近年そのような検診も減少傾向にあり、妊婦の貧血の頻度が上昇したり、献血時の血液検査ではじめて貧血を指摘される頻度が上昇しているといわれている。さらに女子のダイエット志向もあり、近年、貧血を呈する思春期女子の割合は、明らかに増加している¹⁾。



鉄欠乏と思春期

体内の鉄量は健常の成人男性では体重1kgあたり約50mg、女性では約40mgといわれている。しかし、体内での鉄の動態はほとんど閉鎖回路の中で行われており、その出納は1日1mgの鉄が吸収され、1mgが排泄されるだけとごくわずかである(図)。ところが体重1kgの増加に対し鉄は35~45mg必要とされ、1mLの出血では0.5mgの鉄が喪失する。1回の月経時の出血は30~60mLといわれているので、

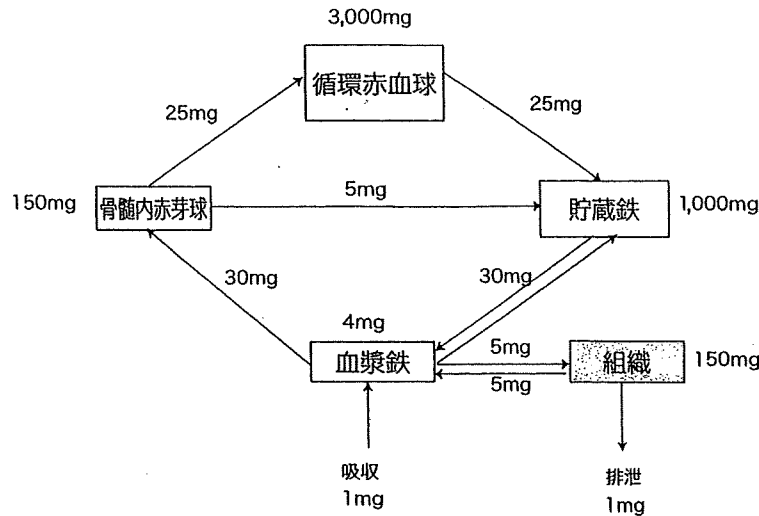


図 体内鉄の動態 (数字は体重 60 kg の男子での 1 日の代謝鉄量)

鉄量としては 15~30 mg が 1 カ月に 1 回喪失していることになる。

思春期はスポーツなども激しく行う時期である。スポーツと鉄欠乏についての因果関係は明確にされているわけではないが、足底への機械的衝撃が血球を破壊し、湧出したヘモグロビン内の鉄が汗などともに体外へ排出されること、スポーツによるストレスが消化管出血を惹起すること、腎血管攣縮や膀胱壁の刺激による血尿、大量の発汗に伴う鉄の体外への排出、腸管の循環血流量低下に伴う鉄吸収の低下などが原因としてあげられている。

すなわち、思春期のような時期には鉄は容易に不足してくるわけである。思春期貧血ということがほとんど鉄欠乏性貧血と同義語になっていることも、このような観点からみれば当然のことである。

鉄欠乏と臨床検査

鉄が欠乏すると、まず肝臓や脾臓などの貯蔵鉄が減少し、その後に血清鉄の減少がおこる。そのときに、鉄が結合しているトランスフェリンという蛋白が増加するという現象がみられる。そして鉄欠乏がさらに進行すると、ヘモグ

ロビン中の鉄が欠乏してヘム合成が低下し、貧血を呈することになる。臨床検査として、これらの病態を把握すると以下のようなになる。

まず、貯蔵鉄の減少は血清フェリチンの低下として表れる。血清フェリチンが 12 ng/mL 以下であれば、貯蔵鉄はかなり減少しているといわれている。なお、血清フェリチン 1 ng/mL は貯蔵鉄約 8 mg に相当する。トランスフェリンの増加は、総鉄結合能の上昇として捉えられる。正常では血清中のトランスフェリンの約 1/3 に鉄が結合されている。これは血清鉄を総鉄結合能で除したトランスフェリン飽和度として表されるが、鉄欠乏時には、この値が 16% 以下になる。感染時には血清鉄の減少が著しいことがあるので、血清鉄の減少だけでは必ずしも鉄欠乏ではないことに留意が必要であり、鉄欠乏の評価には、必ず総鉄結合能と合せて検査を行う必要がある。総鉄結合能が 360 $\mu\text{g/dL}$ 以上に上昇している場合は、ほとんどが鉄欠乏である。鉄欠乏による貧血は赤血球の大きさが平均的に小さく、赤血球中のヘモグロビンが少ないために小球性低色素性貧血である。赤血球像では、非薄赤血球や小赤血球、赤血球サイズの大小不同などがみられる。また鉄欠乏性貧血時には、赤血球の産生に必要な鉄が不足するため、赤血球