Histol Histopathol (2023) 38: 47-51

http://www.hh.um.es

Histology and Histopathology
From Cell Biology to Tissue Engineering

ORIGINAL ARTICLE **Open Access**

GLUT-1 expression is helpful to distinguish myxofibrosarcoma from nodular fasciitis

Shizuhide Nakayama[1], Jun Nishio[2], Mikiko Aoki[3], Kaori Koga[3], Kazuki Nabeshima[4] and Takuaki Yamamoto[1]

[1]Department of Orthopaedic Surgery, Faculty of Medicine, Fukuoka University, [2]Section of Orthopaedic Surgery, Department of Medicine, Fukuoka Dental College, [3]Department of Pathology, Faculty of Medicine, Fukuoka University and [4]Department of Pathology, Pathological Diagnosis Center, Fukuoka Tokushukai Hospital, Fukuoka, Japan

Summary. Myxofibrosarcoma (MFS) is a fibroblastic/myofibroblastic neoplasm with a variably myxoid stroma. Histologically, MFS shows a wide spectrum of cellularity, pleomorphism and proliferative activity. Because of its variable morphology and lack of discriminatory markers, MFS can be difficult to distinguish from some benign soft-tissue tumors, especially nodular fasciitis (NF). Glucose transporter 1 (GLUT-1) is expressed in a variety of malignant mesenchymal tumors. In the current study, we evaluated GLUT-1 expression to determine its value in distinguishing MFS from NF. Tissue specimens from 14 MFS cases and 16 NF cases were sectioned and stained for GLUT-1 using immunohistochemistry. The percentage of GLUT-1-positive cells was scored as follows: 0 (no staining), 1+ (1-19%), 2+ (20-50%) and 3+ (>50%). Samples with a score of 1+ were defined as GLUT1-expressing samples. GLUT-1 expression was seen in all 14 MFS cases, whereas only 6 NF cases (37.5%) were positive for GLUT-1 and were scored 1+. Notably, 2-3+ GLUT-1 expression was found in 86% of MFS cases and 0% of NF cases. Our results indicate that GLUT-1 is a highly sensitive immunohistochemical marker for MFS and may be useful for the differential diagnosis of MFS and NF.

Key words: GLUT-1, Myxofibrosarcoma, Nodular fasciitis, Immunohistochemistry

Corresponding Author: Dr. Shizuhide Nakayama, Department of Orthopaedic Surgery, Faculty of Medicine, Fukuoka University, 7-45-1 Nanakuma, Jonan-ku, Fukuoka 814-0180, Japan. e-mail: n.shizuhide@gmail.com

DOI: 10.14670/HH-18-490

Introduction

Myxofibrosarcoma (MFS) is a malignant fibroblastic/myofibroblastic neoplasm characterized by an infiltrative growth pattern and a high propensity for local recurrence. MFS usually arises in the subcutaneous tissue of the extremities in older adults. Histologically, MFS shows a broad spectrum of cellularity, pleomorphism and proliferative activity (Huang et al., 2020). Immunohistochemically, smooth muscle action (SMA) reactivity is occasionally found in MFS (Huang et al., 2020). Because of its variable morphology and lack of well-characterized immunohistochemical markers, MFS can be difficult to distinguish from certain benign mesenchymal neoplasms, particularly in the case of low-grade MFS.

Nodular fasciitis (NF) is a benign, self-limited fibroblastic/myofibroblastic neoplasm of unknown etiology (Oliveira et al., 2020). NF mainly occurs in the subcutaneous tissue and underlying fascia of the upper extremities in young to middle-aged adults. NF is often misdiagnosed as a malignancy because of its infiltrative growth pattern, increased mitotic activity and high cellularity (Erber and Agaimy, 2018), which leads to unnecessary, aggressive treatment. Immunohistochemical stainings for SMA and muscle-specific actin are usually positive in NF (Oliveira et al., 2020).

Glucose transporter 1 (GLUT-1), also known as solute carrier family 2 member 1 (SLC2A1), is an erythrocyte-type glucose transporter protein and a member of the mammalian facilitative glucose transporter family (Olson and Pessin, 1996). GLUT-1 is normally expressed in erythrocytes, perineurium of peripheral nerves, blood–brain barrier and placenta (Harik et al., 1990; Pardridge et al., 1990; Olson and Pessin, 1996; Illsley, 2000; García-Álvarez et al., 2007). Recently, increased expression of GLUT-1 has been reported in a wide variety of mesenchymal neoplasms (Endo et al., 2007; Ahrens et al., 2008; Smeland et al., 2012). Therefore, the purpose of this study was to evaluate the utility of GLUT-1 expression for

differentially diagnosing MFS and NF.

Materials and methods

Patients and tissue specimens

We retrospectively reviewed 14 cases of MFS and 16 cases of NF diagnosed between 1999 and 2021 at Fukuoka University Hospital. The diagnoses for all cases were confirmed after rereview of available histological and immunostained sections in accordance with the most recent World Health Organization classification (Huang et al., 2020; Oliveira et al., 2020). Only two cases of NF that were previously confirmed to have ubiquitin-specific peptidase 6 (USP6) rearrangements by fluorescence in situ hybridization (FISH) were included. In the present study, MFS was defined as a sarcoma with a myxoid area that was greater than 10%. The study protocol was approved by the Institutional Review Board of Fukuoka University Hospital (approval number: U20-08-003) and the requirement for written informed consent from each patient was waived. Clinical data were retrieved from electronic medical records and included sex and age of each patient and the anatomical site of the neoplasm.

Immunohistochemistry

Biopsied or surgically resected specimens were fixed in 10% formalin and embedded in paraffin blocks. Tissues were sectioned at 4-μm thickness, and sections were deparaffinized and immersed in 0.3% hydrogen peroxide in methanol for 15 min at room temperature (RT) to block endogenous peroxidase activity. The sections were heated in 10 mM citrate buffer (pH 6.0) in a microwave oven (700W) for 10 min to retrieve epitopes. After non-specific sites were blocked with 5% non-fat dry milk for 1h at RT, the sections were incubated with a polyclonal antibody against GLUT-1 (1:1,000; Millipore, Temecula, CA, USA) for 2h at 37°C. Sections were then washed and incubated with Dako ChemMate EnVision (Dako, Carpinteria, CA, USA). Immunoreactive proteins were visualized with 3,3′-diaminobenzidine (Dako), followed by counter-staining with hematoxylin. Appropriate positive and negative controls were used throughout the study. GLUT-1 expression was scored by categorizing the percentage of stained cells as follows: 0 (no staining), 1+ (1%–19%), 2+ (20%–50%) and 3+ (>50%). Samples with a score of 1+ were defined as GLUT1-expressing samples. Both membranous and cytoplasmic staining patterns were considered positive. Immunohistochemical staining was scored independently by three observers (SN, MA and KK).

Statistical analysis

Patient characteristics and expression of GLUT-1 were compared between the MFS and NF groups. Continuous and categorical variables were analyzed by the Mann–Whitney U test and chi-squared test, respectively. A p-value of <0.05 was considered significant. Statistical evaluations were performed using BellCurve for Excel (Social Survey Research Information Co., Ltd.; Tokyo, Japan).

Results

The clinical characteristics of patients are summarized in Table 1. There were 10 men and 4 women in the MFS group and 7 men and 9 women in the NF group. The median age was 73.5 years in the MFS group and 35 years in the NF group ($p<0.001$). There was a tendency for more tumors to occur in the head in the NF group (5/16 cases, 31%) compared with those in the MFS group (0/14 cases, 0%); however, there was no significant difference in the anatomical site between the two groups ($p=0.052$).

Representative histological and immunohisto-chemical features of MFS and NF are shown in Figs. 1, 2, respectively. Immunohistochemical results are summarized in Table 2. GLUT1 staining intensity scores in the MFS group were 0 in 0/14 cases (0%), 1+ in 2/14 (14%), 2+ in 5/14 (36%) and 3+ in 7/14 (50%); in the NF group, the scores were 0 in 10/16 cases (62.5%), 1+ in 6/16 (37.5%) and 2+ or 3+ in 0/16 (0%). GLUT-1 expression was observed in all 14 MFS cases. In contrast, only 6 NF cases (37.5%) were positive for GLUT-1immunostaining with erythrocytes serving as an internal control. Furthermore, 2–3+ GLUT-1 expression was found in 86% of MFS cases but was not observed in the NF cases ($p<0.001$) (Table 3).

Table 1. Patient characteristics.

	MFS (N=14)	NF (N=16)	*P*-value
Sex male / female	10 / 4	7 / 9	0.13
Median age (range)	73.5 (44-86)	35 (23-56)	<0.001
Anatomical site			0.052
head	0 (0%)	5 (31%)	
trunk	3 (21%)	4 (25%)	
limbs	11 (79%)	7 (44%)	

MFS, myxofibrosarcoma; NF, nodular fasciitis.

Table 2. Staining intensity scores for GLUT-1 in MFS and NF tissues.

Tumor	Staining score			
	0	1+	2+	3+
MFS (N=14)	0 (0%)	2 (14%)	5 (36%)	7 (50%)
NF (N=16)	10 (62.5%)	6 (37.5%)	0 (0%)	0 (0%)

GLUT-1, glucose transporter 1; MFS, myxofibrosarcoma; NF, nodular fasciitis.

Discussion

There are currently no reliable immunohistochemical markers for discriminating between MFS and NF. In the present study, we showed that GLUT-1 was a sensitive immunohistochemical marker that differentiated MFS from NF. To the best of our knowledge, this is the first report to demonstrate the usefulness of GLUT-1 immunostaining for distinguishing between MFS and NF.

Table 3. 2–3+ of GLUT-1 in MFS and NF tissues.

Tumor	2–3+ expression of GLUT-1		*P*-value
	(-)	(+)	
MFS (N=14)	2 (14%)	12 (86%)	<0.001
NF (N=16)	16 (100%)	0 (0%)	

GLUT-1, glucose transporter 1; MFS, myxofibrosarcoma; NF, nodular fasciitis.

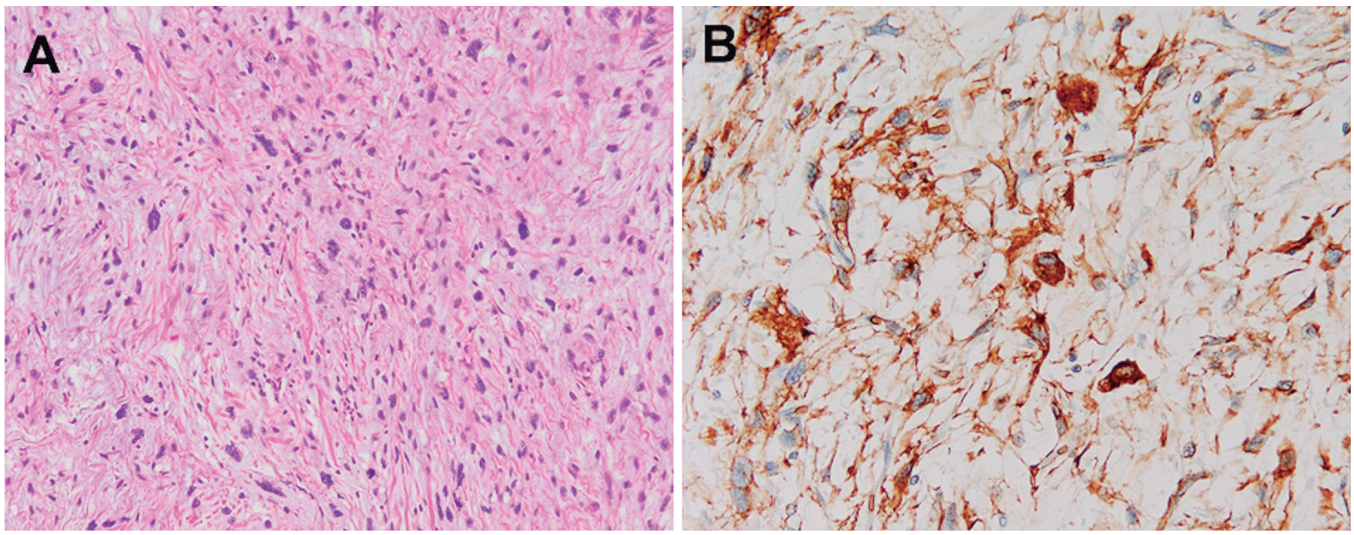

Fig. 1. Histological and immunohistochemical features of myxofibrosarcoma. **A.** Myxofibrosarcoma consists of spindle or stellate-shaped cells with atypical, hyperchromatic nuclei in a myxoid stroma (hematoxylin and eosin staining). **B.** The neoplastic cells show strong cytoplasmic staining for glucose transporter 1. A, x 100; B, x 200.

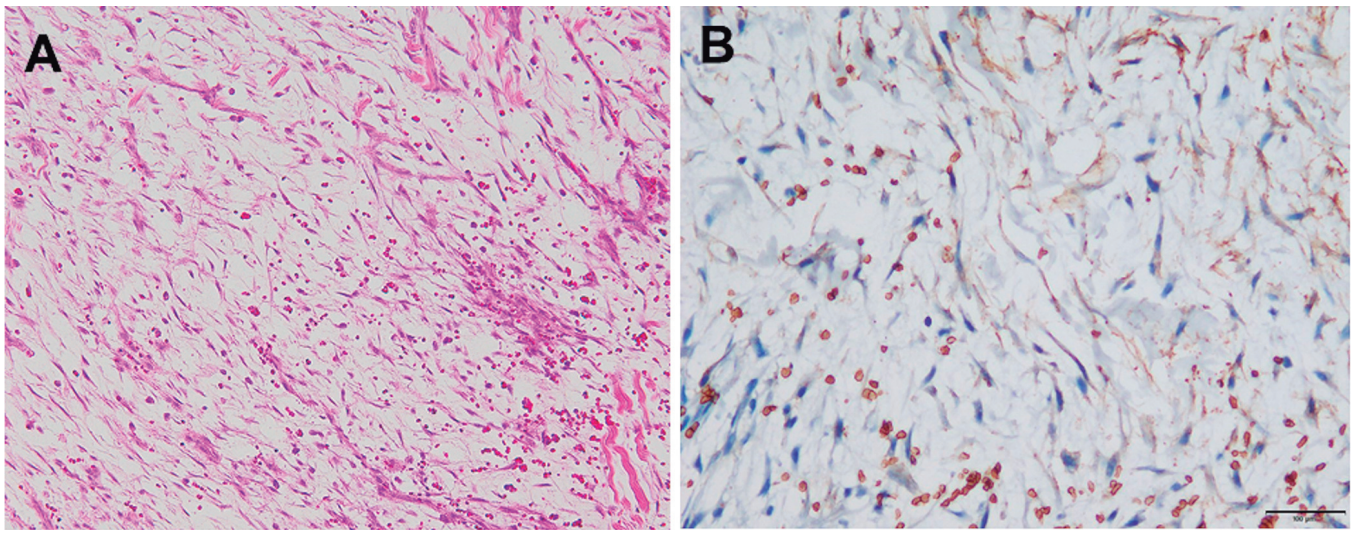

Fig. 2. Histological and immunohistochemical features of nodular fasciitis. **A.** Nodular fasciitis consists of plump spindle-shaped cells with a vague fascicular pattern. Extravasated erythrocytes and lymphocytes can be observed (hematoxylin and eosin staining). **B.** The neoplastic cells show focal and weak expression of glucose transporter 1 (GLUT-1). Note the GLUT-1-positive red blood cells. A, x 100; B, x 200.

In the field of mesenchymal neoplasms, GLUT-1 was first described by North et al. in 2000 as a useful marker for the distinction between juvenile hemangioma and its histological mimics (North et al., 2000). Subsequently, GLUT-1 expression was demonstrated in various mesenchymal neoplasms (Endo et al., 2007; Ahrens et al., 2008; Smeland et al., 2012). In the present study, GLUT-1 immunoreactivity was observed in all MFS cases. MFS tissue specimens showed cytoplasmic and membranous expression of GLUT-1 with varying intensities. Increased expression of GLUT-1 is a characteristic of malignant epithelial cells. Expression of GLUT-1 is associated with poor overall survival and disease-free survival in a variety of carcinomas (Yu et al., 2017). Endo et al. reported that increased expression of GLUT-1 in bone and soft-tissue sarcomas was related to poor overall survival (Endo et al., 2007). Smeland et al. showed that GLUT-1 was an independent negative prognostic factor for soft-tissue sarcoma (STS) (Smeland et al., 2012). Similarly, Kubo et al. reported that GLUT-1 expression was an independent prognostic factor for survival in patients with osteosarcoma (Kubo et al., 2015). However, little has been known regarding the biological significance of GLUT-1 expression in MFS.

GLUT-1 is also known to play a crucial role in the cellular response to hypoxia. Indeed, GLUT-1 expression was usually most intense in the neoplastic cells adjacent to necrotic areas in the MFS specimens. Previous studies showed that hypoxia is a predictor of metastasis in patients with STS (Brizel et al., 1996; Nordsmark et al., 2001). Recently, Kim et al. demonstrated that the expression of hypoxic markers including GLUT-1 was associated with a higher histological grade and advanced tumor stage of STS (Kim et al., 2012). In the present study, GLUT-1 expression was not seen in any NF cases. Orrock et al. reported that all three examined NF cases were negative for GLUT-1 (Orrock et al., 2009). The authors suggested that the absence of GLUT-1 expression in NF may reflect relative normoxia in these small, well-vascularized lesions that typically lack necrosis. In the present study, necrosis was absent in all NF cases, which may be related to the finding of the weakness of GLUT-1 expression.

MFS generally displays highly complex karyotypes with extensive intratumoral heterogeneity (Nishio et al., 2011). Interestingly, recurrent amplifications of the chromosome 7q region have been identified in MFS (Sambri et al., 2020). This chromosomal region harbors several candidate genes that may be connected with GLUT-1, such as *BRAF* (B-Raf proto-oncogene; at 7q34), *leptin* (at 7q32.1) and *erythropoietin* (at 7q22.1). For instance, the activating mutation of *BRAF* V600E was correlated with GLUT-1 expression in papillary thyroid carcinomas (Grabellus et al., 2012). Recently, *a* SLC37A3 (solute carrier family 37 member 3)-*BRAF* gene fusion was detected in a single case of MFS (Ogura et al., 2018). The expression and membrane recruitment of GLUT-1 was also enhanced by leptin (Han et al., 2018). Erythropoietin expression also showed a positive correlation with GLUT-1 in colorectal adenocarcinomas, particularly in a subgroup of deeper invading groups (Wincewicz et al., 2010). In contrast, NF is genetically characterized by USP6 rearrangements (Erickson-Johnson et al., 2011). Multiple fusion partners for USP6 have been identified in NF (Nakayama et al., 2021). Therefore, molecular tests may be useful to confirm the diagnosis in histologically challenging cases.

The present study has several limitations. First, our study included a small number of cases because of the rarity of soft-tissue neoplasms. Second, this investigation was a retrospective study conducted at a single center and may therefore have ascertainment bias. Third, none of the NF cases in our study showed necrosis or ischemic changes. Unusual cases of NF with necrosis or ischemia may have increased GLUT-1 expression because of the hypoxic environment.

In conclusion, our results suggest that determining GLUT-1 expression levels can aid in distinguishing MFS from NF. Further studies are needed to investigate the role of GLUT-1 as well as its therapeutic relevance in MFS.

Acknowledgements. The authors would like to thank Ms. Miki Onitsuka for her excellent technical assistance. This study was supported in part by the Ogata Foundation and the Japan Society for the Promotion of Science KAKENHI (21K09336). We thank Susan Zunino, PhD, from Edanz (https://jp.edanz.com/ac) for editing a draft of this article.

Conflict of interest. The authors declare that they have no competing interests.

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Accepted July 6, 2022