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Long Term Prognostic Implications of Expression of Glucose Transporter-1 and Hexokinase II in Patients with Stage I Uterine Leiomyosarcoma

Hitomi Tsukada[1], Toshinari Muramatsu[1], Masaki Miyazawa[1], Tetsuji Iida[1], Masae Ikeda[1], Masako Shida[1], Takeshi Hirasawa[1], Hiroshi Kajiwara[2], Masaru Murakami[3], Masanori Yasuda[4] and Mikio Mikami[1]

[1]Department of Obstetrics and Gynecology, Tokai University School of Medicine, [2]Department of Pathology, Tokai University School of Medicine, Shimokasuya 143, Isehara, Kanagawa 259–1193, Japan, [3]Department of Obstetrics and Gynecology, Tokai University Oiso Hospital, Kanagawa 259–0198, Japan and [4]Department of Pathology, Saitama Medical University International Medical Center, Saitama 350–1298, Japan

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Many malignant epithelial tumors show increased expression of glucose transporter-1 (GLUT-1) and hexokinase II (HK-II), both of which are involved in glucose metabolism. GLUT-1 levels are often correlated with prognosis in these tumors. The current retrospective study was conducted to evaluate the importance of GLUT-1 and HK-II expression in leiomyosarcoma (LMS), a malignant uterine non-epithelial tumor with a poor prognosis. The subjects were 23 patients with stage I LMS. Expression of GLUT-1 and HK-II was evaluated immunohistochemically in samples removed surgically, and the MIB-1 index was evaluated as a measure of cell proliferation. The association of these results with prognosis was examined. Twenty samples of leiomyoma (LOM), a benign non-epithelial tumor, were used as controls. Immunohistochemical expression was defined as negative staining (–), weak to sporadic staining (1+), and strong staining (2+) per microscopic field, respectively. Malignancy was evaluated in 2000 cells and the MIB-1 index was calculated. Overall survival for LMS was estimated using the Kaplan-Meier method. Of the LMS cases, 12 were GLUT-1-positive (52.2%; 2+: 2, 1+: 10) and 15 were HK-II-positive (65.2%; 2+: 1, 1+: 14). GLUT-1 expression in LMS was significantly correlated with the MIB1 index. The 10-year survival rates were 90.9% and 58.3% in GLUT-1-negative and GLUT-1-positive cases, respectively, and 75.0% and 73.3% in HK-II-positive and HK-II-negative cases, respectively. GLUT-1 expression was significantly correlated with prognosis. Cases of stage I LMS showed a significant correlation between the expression level of GLUT-1 and the MIB-1 index, an indicator of malignancy. GLUT-1-negative cases had a better prognosis than GLUT-1-positive cases, suggesting that GLUT-1 expression is an effective prognostic marker.

Key words: uterine leiomyosarcoma, immunoexpression, glucose transporter-1, hexokinase II

I. Introduction

Leiomyosarcoma (LMS) is a malignant uterine non-epithelial tumor that accounts for 1% to 3% of all malignant tumors in women. LMS has a poor prognosis, since the primary tumor is likely to undergo recurrence and metastasis [7]. Tissue necrosis and higher mitotic rates are important indicators for malignancy and prognosis [18, 29, 40]. More than 80% of cases of stage III LMS show recurrence and metastasis and the 5-year survival rate in cases of stages II–IV is approximately 8%, indicating an extremely poor prognosis [9, 33]. Surgery is the first option for LMS treatment; however, even if LMS is in the early stage and

Correspondence to: Toshinari Muramatsu, M.D., Department of Obstetrics and Gynecology, Tokai University School of Medicine, Shimokasuya 143, Isehara, Kanagawa 259–1193, Japan.
E-mail: mura-to@is.icc.u-tokai.ac.jp

can be completely removed, distant metastasis to the lung often occurs and results in a poor long-term prognosis [22]. Radiotherapy and combination chemotherapy with doxorubicin have also been used for LMS, but treatment outcomes remain poor [22, 34].

Many malignant epithelial tumors show increased glucose uptake [42]. Expression of glucose transporter-1 (GLUT-1) is often increased in malignant hypoxic cells and hexokinase II (HK-II) expression also increases. This causes resistance to radiotherapy and chemotherapy and enhanced recurrence and metastasis, which underlie the close relationship of GLUT-1 expression with prognosis [2, 10, 15, 28, 37]. In a clinicopathologic study of epithelial ovarian cancer, we found increased GLUT-1 expression and strong expression of hypoxia inducible factor-1 (HIF-1α), with a clear increase in glucose uptake. Similarly, high expression levels of HIF-1α and GLUT-1 have been shown in clear cell carcinoma, which also has a poor prognosis and is common in Japanese patients [16, 43]. Thus, the current study was performed to examine expression of GLUT-1 and HK-II and the relationship of these data with the long term prognosis of LMS, which has not been examined in previous studies.

II. Materials and Methods

Patients and treatments

The subjects were 23 patients (mean age: 51.5 years old; range: 35–70 years old) with clinical stage I LMS who underwent hysterectomy between March 1987 and May 2005 in our hospital. Adjuvant chemotherapy were performed in 14 patients (61%) (CYVADIC, n=12, 86%; cyclophosphamide 500 mg/m^2 and doxorubicin 50 mg/m^2 on day 1, vincristine sulfate 1.5 mg/m^2 on days 1 and 5, and dacarbazine 250 mg/m^2 on days 1 through 5 for three to five monthly cycles); IAP, n=1, 7%; doxorubicin 50 mg/m^2 and cisplatin 50 mg/m^2 on day 1 and ifosfamide 1000 mg/m^2 on days 1 through 5 for three monthly cycles; and weekly TC, n=1, 7%; paclitaxel 60 mg/m^2 and carboplatin AUC 1.7 on day 1 for 16 weekly cycles). The benign controls were 20 specimens of uterine leiomyomas that were removed surgically in the same period. At least 2 sections were isolated from each tumor and a tumor with at least one stained section was judged to be positive. The study was approved by the institutional review board and informed consent was obtained from all patients.

Immunohistochemistry and histological examination

Formalin-fixed and paraffin-embedded tissue blocks were cut into 4-μm sections for immunohistochemistry and hematoxylin and eosin staining.

The presence of a malignant mesenchymal tumors was examined based on positive staining for SMA (Sigma 1A4, Sigma Chemical Co., St. Louis, MO), vimentin (clone V9, DakoCytomation, Glostrup, Denmark), desmin (clone D33, DakoCytomation, Glostrup, Denmark) and MIB-1 (Ki-67/clonal MIB1, DakoCytomation, Glostrup, Denmark), and negative staining for CD34 (QBEnd/10, Novocastra, Newcastle, UK) in an initial histological examination.

GLUT-1 expression was evaluated immunohistochemically using rabbit polyclonal anti-human GLUT-1 antibody (DAKO, Carpinteria, CA, USA) at a dilution of 1:50. HK-II expression was evaluated with a polyclonal rabbit anti-HK-II antibody (Chemicon International, Inc., Temecula, CA) diluted at 1:500. The sections were washed and then incubated with anti-rabbit IgG conjugated to horseradish peroxidase-labeled-dextran polymer (EnVision Kit, DAKO) for 60 min at room temperature. The extent of GLUT-1 and HK-II expression was assessed semi-quantitatively according to the following scoring scheme: negative staining (–), weak to sporadic staining (1+), and strong staining (2+). In judging the staining level, erythrocytes and pancreatic tissue were used as positive controls for GLUT-1 and HK-II, respectively.

The histological grade of LMS was evaluated by two expert pathologists by counting of 2000 cells, with >20 mitosis events in 10 microscopic fields judged to be positive. These data were used to calculate the MIB-1 index for cell proliferation.

Statistical analysis

The relationship between immunohistochemical scores and MIB-1 index was determined by linear regression for the GLUT-1 and HK-II data. Survival curves related to immunoreactivity were constructed using the Kaplan-Meier method and assessed by log-rank test. $P<0.05$ was considered to indicate significance in all analyses.

III. Results

Hematoxylin & eosin and immunohistochemical staining

Malignant mesenchymal tumors identified from positive staining for SMA, vimentin, desmin and MIB-1 and negative staining for CD34 were excluded from the study (Fig. 1).

GLUT-1 and HKII expression, and MIB-1 index

Of the 23 cases of stage I LMS, 12 (52.2%) were GLUT-1-positive, including 2 2+ and 10 1+ cases; and 15 (65.2%) were HK-II-positive, including 1 2+ and 14 1+ cases (Table 1, Figs. 2, 3). The MIB-1 index was ≥5% in 10 cases and <5% in 13 (Fig. 4). Of the 20 benign controls, 2 (10%) were GLUT-1-positive, 4 (20%) were HK-II-positive, and all showed 1+ staining. MIB-1 was negative in all control specimens (Table 2).

Relationships of GLUT-1 and HK-II expression with the MIB-1 index

GLUT-1 expression levels showed a significant correlation with the MIB-1 index ($R^2=0.403$, $p=0.0011$, Fig. 4). In contrast, there was no correlation between HK-II expression and the MIB-1 index ($p=0.703$, Fig. 5).

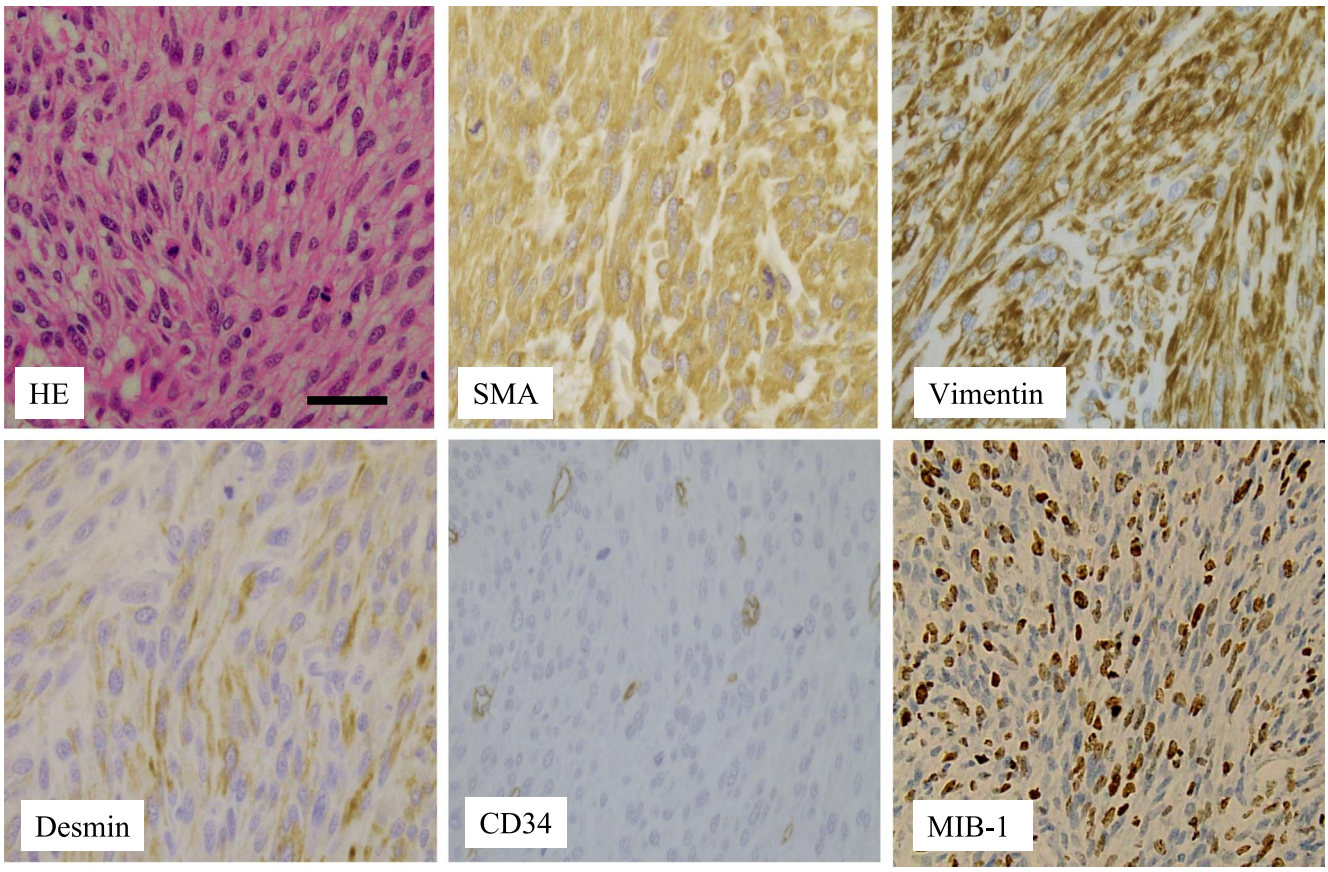

Fig. 1. Malignant mesenchymal tumors detected with hematoxylin-eosin (HE) staining, positive immunostaining for SMA, vimentin, desmin and MIB-1, and negative immunostaining for CD34. Bar=100 μm.

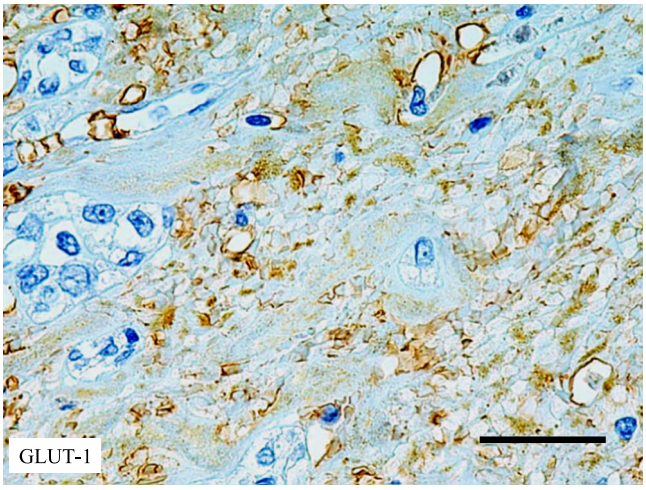

Fig. 2. Immunohistochemical staining showed strong GLUT-1 expression in sarcoma cell membranes. Bar=100 μm.

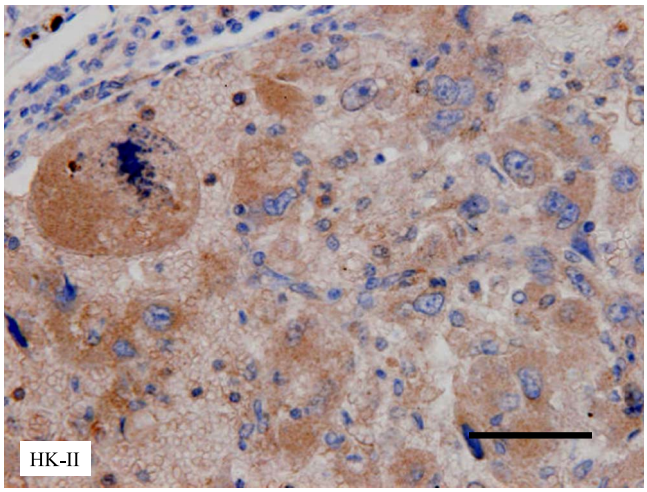

Fig. 3. Immunohistochemical staining was strongly positive for hexokinase (HK)-II in sarcoma cells. Bar=100 μm.

Survival curves based on GLUT-1 expression and HK-II expression

The Kaplan-Meier estimates of overall survival (OS) at 10 years for LMS were 90.9% in the GLUT-1-negative group and 58.3% in the GLUT-1-positive group, showing a significant difference in prognosis (Fig. 6). The Kaplan-Meier estimates of OS at 10 years were 75.0% and 73.3% in the HK-II-negative and HK-II-positive groups, with no significant difference between these groups (Fig. 7).

Table 1. *Characteristics of patients with stage I uterine leiomyosarcoma*

Case	Age	Operation	Chemotherapy	Expression		MIB-1 Index	Tumor size maximum (cm)
				GLUT-1	HK II		
1.	48	ATH+RSO	N	(–)	(–)	0.2	3
2.	36	Myomec → ATH+BSO	CYVADIC	(–)	(1+)	0.4	5
3.	38	Myomec → ATH+BSO+PLN	CYVADIC	(–)	(1+)	0.8	10
4.	59	ATH+BSO	CYVADIC	(–)	(1+)	1.1	5
5.	35	ATH+BSO	N	(–)	(1+)	0.1	5
6.	62	ATH+BSO	CYVADIC	(–)	(–)	2.7	6
7.	52	ATH+BSO	CYVADIC	(–)	(–)	3.3	10
8.	42	ATH	N	(–)	(1+)	2.8	6
9.	52	ATH+BSO	N	(–)	(–)	1.6	13
10.	61	ATH+BSO	N	(1+)	(1+)	0.3	8
11.	57	ATH+BSO+PLN	CYVADIC	(1+)	(–)	5.2	11
12.	64	ATH+BSO+PLN	N	(1+)	(–)	37.8	7
13.	58	ATH+BSO	CYVADIC	(2+)	(1+)	23.7	8
14.	70	ATH+BSO	N	(1+)	(1+)	1.9	8
15.	44	ATH+BSO	CYVADIC	(2+)	(1+)	20.0	6
16.	66	ATH+BSO	IAP	(1+)	(1+)	15.0	12
17.	48	ATH+BSO	CYVADIC	(1+)	(1+)	31.9	7
18.	56	ATH+BSO+PLN+OMT	Weekly TC	(1+)	(2+)	3.1	4
19.	45	ATH+BSO	N	(–)	(–)	0.4	5
20.	54	ATH+BSO	N	(–)	(1+)	6.3	8
21.	44	ATH+BSO+PLN	CYVADIC	(1+)	(1+)	30.3	11
22.	45	ATH+BSO	CYVADIC	(1+)	(–)	9.7	7
23.	53	ATH+BSO	CYVADIC	(1+)	(1+)	29.2	10

Myomec, myomectomy; ATH, abdominal total hysterectomy; BSO, bilateral salpingo-oophorectomy; RSO, right salpingo-oophorectomy; PLN, pelvic lymph adenectomy; OMT, omentectomy; N, no treatment; CYVADIC, cyclophosphamide, vincristine, doxorubicin and dacarbazine; TC, paclitaxel and carboplatin.

Table 2. *Characteristics of patients with uterine leiomyoma*

Case	Age	Expression		MIB-1 Index	Tumor size maximum (cm)
		GLUT-1	HK II		
1.	56	(–)	(1+)	0.0	8 (mu)
2.	39	(–)	(–)	0.0	8 (mu)
3.	49	(–)	(–)	0.0	6 (mu)
4.	38	(–)	(–)	0.0	14 (mu)
5.	46	(–)	(–)	0.0	3 (mu)
6.	47	(1+)	(1+)	0.0	14 (mu)
7.	73	(–)	(–)	0.0	4 (mu)
8.	36	(–)	(–)	0.0	25 (mu)
9.	69	(–)	(1+)	0.0	5
10.	66	(–)	(–)	0.0	4 (mu)
11.	27	(–)	(–)	0.0	10 (mu)
12.	61	(1+)	(–)	0.0	15 (mu)
13.	44	(–)	(1+)	0.0	3 (mu)
14.	48	(–)	(–)	0.0	6 (mu)
15.	52	(–)	(–)	0.0	6
16.	50	(–)	(–)	0.0	11 (mu)
17.	47	(–)	(–)	0.0	25 (mu)
18.	46	(–)	(–)	0.0	8 (mu)
19.	46	(–)	(–)	0.0	8 (mu)
20.	47	(–)	(–)	0.0	17

(mu): multiple myomas.

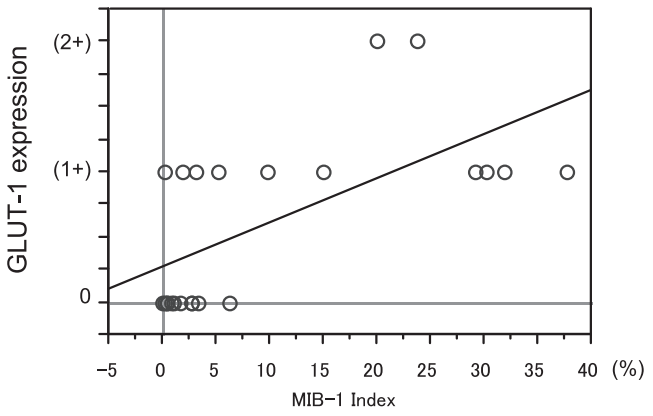

Fig. 4. Simple regression analysis of GLUT-1 expression and the MIB-1 index.

IV. Discussion

Uterine LMS is likely to show recurrence and metastasis, even in the early stage, and effective treatment has not been established. In 1985, Omura *et al.* evaluated 48 cases of stage I and II uterine LMS in a randomized comparative study, and found recurrence rates of 44% in

patients given adjuvant chemotherapy of 8 cycles of doxorubicin after resection and 61% in those who underwent observation only, with no significant difference between the groups [27]. A more recent randomized phase III trial of adjuvant pelvic radiotherapy versus observation for stage I and II uterine sarcomas (carcinosarcoma, leiomyosarcoma or endometrial stromal sarcoma) indicated that radiotherapy did not contribute to control of local metastasis or survival rate [32]. Hensley *et al.* conducted a prospective study in 23 cases (stage I: 15, II: 3, III: 1, and IV: 4) of high grade uterine LMS for a mean period of 49 months after complete resection, and found that progression free survival (PFS) at 2 years was 45% after treatment with gemcitabine 900 mg/m^2 (on days 1 and 8 i.v.) plus docetaxel 75 mg/m^2 (on day 8 i.v.) for 4 cycles at 3-week intervals. The PFS in stage I and II cases at 2–3 years was 59%, which suggested that adjuvant chemotherapy with gemcitabine plus docetaxel after complete resection may improve the prognosis of early stage LMS [13]. Several pilot studies of adjuvant therapies, including CYVADIC (cyclophosphamide, vincristine, doxorubicin, and dacarbazine) therapy, ifosfamide single therapy, and API (doxorubicin, cisplatin and ifosfamide) plus radiotherapy have been conducted for early stage LMS [21, 26, 30], with 3- and 5-year survival rates ranging from 67% to 89% (one study with CYVADIC therapy had a 15-year survival rate of 69%).

There is currently no established surgical procedure or anticancer treatment for uterine sarcoma. This may be because of the relatively small number of cases of uterine sarcoma and because the disease is often not diagnosed before surgery. Cases 1 and 2 were young patients who underwent myomectomy and were diagnosed with uterine sarcoma in a postoperative pathologic examination. Consequently, these patients underwent hysterectomy in an

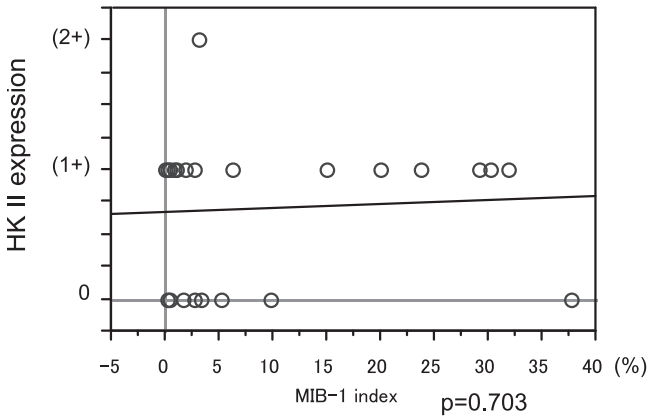

Fig. 5. Simple regression analysis of HK-II expression and the MIB-1 index.

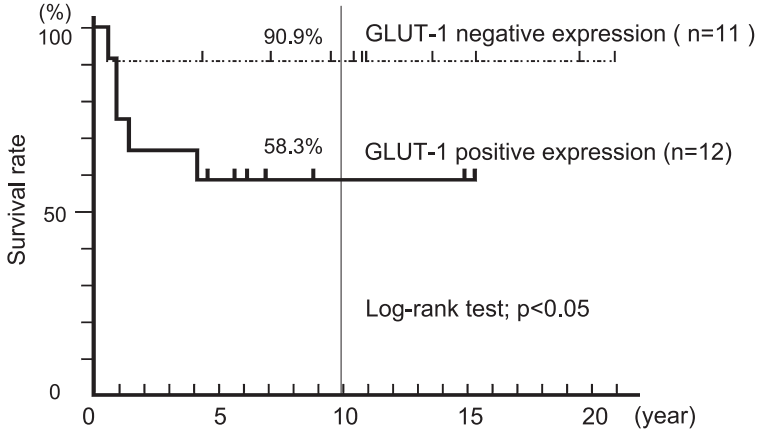

Fig. 6. Survival curves for GLUT-1-positive and GLUT-1-negative cases of LMS.

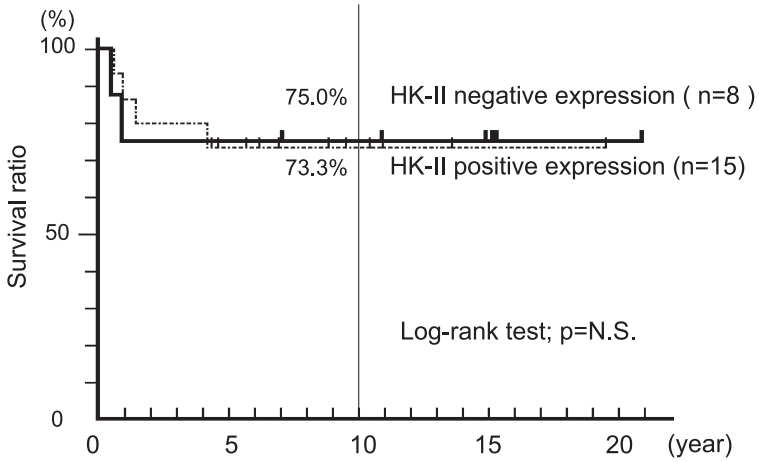

Fig. 7. Survival curves of HK-II-positive and HK-II-negative cases of LMS.

abdominal reoperation. Several small-scale studies have indicated that CYVADIC chemotherapy improves prognosis after total hysterectomy and adnexectomy [12, 31, 41]. In our study, no gross residual tumor was found during lymph node dissection. Twelve patients (52%) were treated with CYVADIC chemotherapy and 9 (39%) did not receive this chemotherapy. Three patients died in each of these groups. Most previous studies and the current study were performed at single centers and with a limited number of patients. Therefore, multicenter randomized clinical trials are required to establish more reliable evidence of the efficacy of treatment.

In our previous investigation of different histological types (serous, mucous, endometrioid and clear cell) of epithelial ovarian cancer, we found that expression levels of GLUT-1 and HIF1α were correlated in the respective histological types. Expression of both proteins was especially high in serous adenocarcinoma, which is frequently found in epithelial ovary cancer, and clear cell adenocarcinoma, which is chemoresistant and associated with recurrence and metastasis. Histopathologically, these two tumors have fewer vascular vessels, but have papillary proliferation and a stratified structure, and cause extensive necrosis in progression. Therefore, hypoxia is induced as the cancer progresses, and this leads to strong expression of GLUT-1 and HIF-1α [16, 43].

Many studies have evaluated the relationship between the expression level of GLUT-1 and progression of epithelial and gynecologic cancers, with the general finding that strong GLUT-1 expression is associated with a poorer prognosis [1, 6, 11, 17, 19, 44, 45]. In a study of 67 patients with bone and soft tissue sarcoma or non-epithelial carcinoma (stage IA–IVB, 15 different histological types), Endo *et al.* found a correlation of prognosis with therapy including surgical resection and adjuvant chemotherapy ($p<0.0001$), tumor differentiation ($p=0.017$), necrotic grade ($p=0.04$), mitotic grade ($p=0.0198$), MIB-1 grade ($p=0.031$), and GLUT-1 expression ($p=0.029$) in univariate analysis of 3-year survival; but found that metastasis ($p=0.031$) was the only significant prognostic factor in multivariate analysis of overall survival. However, Kaplan-Meier estimates of overall survival at 5 years were <40% in the GLUT-1-positive group and 90% in the GLUT-1-negative group, showing a significant difference in prognosis [8].

In the present study, all the cases of non-epithelial carcinoma were in clinical stage I, involved uterine LMS with a single histological type, and were completely resectable in surgery. Thus, there was less variation in the subjects in this study in comparison with Endo *et al.*, which included cases of different histological types and progression. The 5-year OS of all LMS patients was approximately 74%, and the MIB-1 index and expression level of GLUT-1 had a significant positive correlation. The 5-year survival rates were 90.9% and 58.3% in GLUT-1-negative and GLUT-1-positive cases, respectively. Thus, GLUT-1 expression was significantly correlated with prognosis in uterine LSM, as found in previous studies of malignant epithelial tumors. In contrast, there was no relationship of HK-II expression with the MIB-1 index or prognosis, and no significant difference in survival between HK-II-positive and HK-II-negative cases.

Previous studies have shown that expression of GLUT-1 and HK-II in epithelial cancer cells, including breast, esophageal, and lung cancer cells, plays a pivotal role in glucose metabolism and that the expression levels of GLUT-1 and HK-II are correlated with malignancy [5, 14, 17, 23, 28, 38]. The subjects of the current study were 23 patients with LMS associated with non-epithelial malignancy and the prognosis correlated with the presence or absence of expression of GLUT-1 in malignant cells, but not with expression of HK-II. Therefore, it is possible that the occurrence of malignant cells depends on glucose metabolism, glucose enzyme activity and phosphorylation, but not on epithelial cell malignancy.

The recent development of [^{18}F]-fluorodeoxyglucose positron emission tomography (FDG-PET) allows imaging based on the difference in glucose metabolism between malignant and normal cells. Thus, FDG-PET is effective for detection of early stage malignant tumors, and has high sensitivity for detection of recurrence and metastasis in malignant gynecological epithelial tumors [3, 4, 20, 35]. In the first study of non-epithelial bone and soft tissue sarcomas using FDG-PET, Tateishi *et al.* showed an association between higher GLUT-1 expression and a higher standardized uptake value (SUV) of [^{18}F]fluorodeoxyglucose, thereby suggesting the efficacy of FDG-PET diagnostic imaging for non-epithelial tumors, as well as MIB-1 grade, mitotic grade, and tumor differentiation [36].

We have also evaluated FDG-PET for early detection of recurrence and metastasis of advanced ovary cancer after treatment. The rates of detection of intraperitoneal and retroperitoneal metastasis by FDG-PET were 93.9% and 92.9%, respectively, and metastasis was detected in 14 (50%) of 28 metastatic lymph nodes of normal size. FDG-PET detected recurrence in 87.5% of CA125-positive patients with no symptoms and negative results in conventional CT and ultrasonography [24]. The efficacy of follow-up FDG-PET was evaluated in patients with uterine LMS, and early-stage minimal recurrent lesions were detected in patients in whom conventional CT and ultrasonography did not show intraperitoneal recurrence. Two of 5 patients underwent reoperation for a recurrent tumor and survived for one year or more after surgery.

Benign non-epithelial tumors such as uterine leiomyomas are rarely positive in PET, but such cases should be followed up carefully because some may be false-positives [39]. However, FDG-PET is effective for detection of early-stage intraperitoneal recurrence that may be overlooked in conventional diagnostic imaging [25]. The clinicopathological results reported here show that malignant non-epithelial tumors have high glucose metabolic activity and high GLUT-1 expression, and these findings support the use of FDG-PET for detection of malignant lesions.

V. Acknowledgments

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VI. References

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