Myxoid Liposarcoma with *EWS–CHOP* Type 1 Fusion Gene

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Abstract. *Background: In myxoid liposarcoma (MLS), the t(12;16)(q13;p11) chromosomal translocation and its resultant fusion transcript, the human translocation liposarcoma (TLS)–CCAAT/enhancer binding protein (C/EBP) homologous protein (CHOP), are found in the majority of cases. On the other hand, the variant translocation, t(12;22)(q13;q12) creating the Ewing sarcoma (EWS)–CHOP fusion transcript, is detectable in a limited number of cases. Patients and Methods: Tissue from MLS arising in the left thigh of a 19-year-old female was analyzed for possible detection of chromosome translocation and fusion transcript. Fluorescent in situ hybridization (FISH) and reverse transcription-polymerase chain reaction (RT-PCR) methods were used. Results: FISH analysis demonstrated a rearrangement in the CHOP gene. RT-PCR analysis confirmed the presence of EWS–CHOP chimeric transcript type 1, in which exon 7 of EWS was in-frame fused to exon 2 of CHOP with a serine (AGT) to methionine (ATG) transition at the junction. The patient underwent a radical segmental resection including a left vastus medialis musclectomy. Sixty months following the surgical resection, the patient was alive with no evidence of disease. Conclusion: Analysis of MLS with EWS–CHOP variant transcripts, type 1 through type 4, including this case together with 15 cases in the literature, indicated that MLS with type 1 fusion transcript may show a more favorable clinical behavior than MLS with other types of fusion transcript.*

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Key Words: CCAAT/enhancer binding protein (C/EBP) homologous protein (CHOP) gene, chromosomal translocation, Ewing sarcoma (EWS) gene, fluorescence *in situ* hybridization, FISH, fusion gene, myxoid liposarcoma, MLS, reverse transcription-polymerase chain reaction, RT-PCR.

Liposarcoma is the most common malignant soft tissue tumor. Myxoid liposarcoma (MLS) is one of the distinct subtypes of liposarcoma, and is the second most common after well-differentiated liposarcoma (1, 2). The reciprocal translocation of t(12;16)(q13;p11) and the resultant fusion transcript, the human translocation liposarcoma (*TLS*)–*CCAAT*/enhancer binding protein (C/EBP) homologous protein (*CHOP*), are commonly associated with MLS. On the other hand, the variant t(12;22)(q13;q12) translocation, creating the Ewing sarcoma (*EWS*)–*CHOP* fusion transcript, is detectable in a limited number of MLS cases (3-12). Because of its rarity, the clinical picture of MLS with *EWS–CHOP* has been poorly documented. Therefore, this case study presents a detailed clinical report of a case with MLS carrying the *EWS–CHOP* type 1 fusion transcript. Analysis of the 15 cases in the literature together with the current case indicates that MLS with *EWS–CHOP* type 1 transcript may show more favorable clinical behavior than MLS with other variant types of *EWS–CHOP*.

Patients and Methods

Case material. Tumor tissue adjacent to the part used for histological examination at open biopsy was frozen and stored at –80˚C. Procurement of frozen tissues and retrospective clinical data collection was approved by the University of Tokushima Review Board.

Fluorescence in situ hybridization (FISH) analysis. An interphase FISH analysis was performed on formalin-fixed paraffin-embedded tissue sections of the tumor using a commercially available CHOP (12q13) dual color, break-apart rearrangement probe (Abbott Molecular Inc., Des Plaines, IL, USA). The FISH analysis was performed according to the manufacturer's instructions, as described in a previous study (13). Hybridization signals were visualized with an epifluorescence microscope, and images were captured on a charge-coupled device (CCD) camera. A total of 50 nuclei showing both green and orange signals were counted and the percentages of the fused signals were calculated. In cases where nuclei overlapped and the complete area of each nucleus was not visible, or the nuclei were too close together to determine boundaries, the signals were not counted.

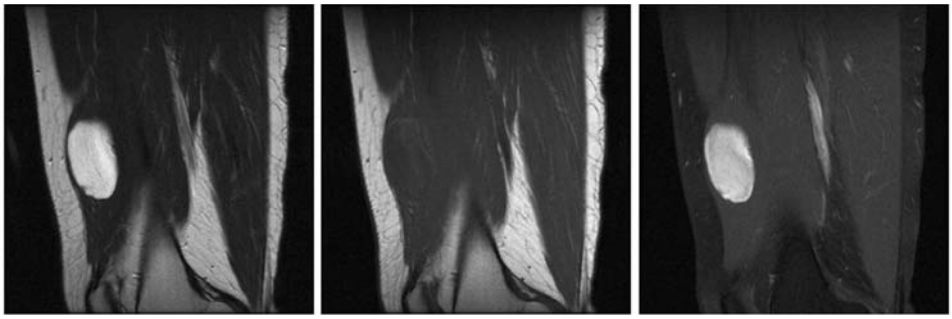

Figure 1. *Magnetic resonance imaging findings. Sagittal T2-weighted (left) image demonstrating an oval soft tissue mass with non-homogeneous high signal intensity at the anterior aspect of the left thigh, located within the vastus medialis muscle. T1-weighted image revealing an iso-intensity (center). After gadolinium diethylenetriaminepentaacetic acid injection, the fat-suppressed T1-weighted image showed non-homogeneous enhancement (right).*

Reverse transcription-polymerase chain reaction (RT-PCR) and DNA sequencing analysis. Total RNA was extracted using RNeasy Lipid Tissue Mini Kit (Qiagen, Hilden, Germany), according to the manufacturer's instructions. After DNase (Invitrogen, Carlsbad, CA, USA) treatment, cDNA was synthesized using 1 μg of total RNA, 50 ng of random hexamers, and 50 U Superscript II Reverse Transcriptase (Invitrogen) in a total volume of 20 μl. PCR was performed using 1 μl cDNA as a template, 18 μl PCR SuperMix (Invitrogen), and 10 pM each of *EWS* 501F (forward) 5'-CCA GCC CAG CCT AGG ATA TGG ACA-3' and *CHOP* 194R (reverse) 5'-CTG GAC AGT GTC CCG AAG GAG AAA-3' primers (7) in a total volume of 20 μl. Denaturation for 2 min at 95˚C was followed by 35 cycles of 30 s at 95˚C, 30 s at 60˚C, and 60 s at 72˚C.

The PCR products were separated by 1% agarose gel electrophoresis and were visualized by ethidium bromide staining. After purification, the PCR products were directly sequenced using the PRISM BigDye Terminator Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA) and either of the primers used for the PCR amplification.

Results

Clinical report. A 19-year-old female visited the host hospital for a medical evaluation of a painless mass on her left thigh shortly after she noticed it. Physical examination revealed an elastic hard, non-tender mass that was approximately 4 cm in the longitudinal dimension. No neurological or vascular compromise was identified. Magnetic resonance imaging (MRI) demonstrated a non-homogeneous mass, arising in the vastus medialis muscle (Figure 1). An open biopsy was performed. Macroscopically, lobulated gelatinous material was encapsulated (Figure 2). Histological examination revealed that the biopsy specimen was composed of vacuolar lipoblasts embedded in a myxoid matrix with a delicate plexiform capillary vascular network consistent with a diagnosis of myxoid liposarcoma (Figure 3). Subsequently, a radical segmental resection including a left vastus medialis musclectomy was performed. Grossly, the lobulated gelatinous mass measured 3.5×2.5×2.0 cm (Figure 4). There was no histological evidence of focal round cell differentiation in the resection specimen. The patient was alive 60 months following the surgical resection, with no evidence of disease.

FISH analysis. An interphase FISH analysis showed that at least 10% of the cells from the tumor showed a split signal pattern of one green and one orange, demonstrating a rearrangement in the *CHOP* gene (Figure 5).

RT-PCR analysis. One-step RT-PCR analysis using two primers, *EWS* 501F and *CHOP* 194R, amplified a 0.45-kbp fragment from cDNA of the MLS. No amplified products were obtained when cDNAs prepared from lipoblastoma, well-differentiated liposarcoma and myxoid malignant fibrous histiocytoma were used (Figure 6). To verify the presence of *EWS–CHOP* chimeric transcript, the 0.45-kbp cDNA fragment was analyzed by direct sequencing, showing that it corresponded to *EWS–CHOP* chimeric transcript type 1, in which exon 7 of *EWS* was in frame fused to exon 2 of *CHOP* with a serine (AGT) to methionine (ATG) transition at the junction (Figure 7).

Discussion

Follow-up at 60 months revealed that the 19-year-old female MLS case with *EWS–CHOP* type 1 fusion transcript showed favorable clinical course, alive with no evidence of disease (ANED). In general, MLS is a disease of young adults with no gender predilection, and is prone to recur locally (1, 2). Its clinical course remains unpredictable, and the most commonly used prognostic factor is the high histological grade, assessed by histopathological evaluation of the round cell (RC) proportion, although visual quantification and sampling errors inevitably cause biases. In accordance with the favorable clinical course, histopathological assessment of the current case identified no RC component.

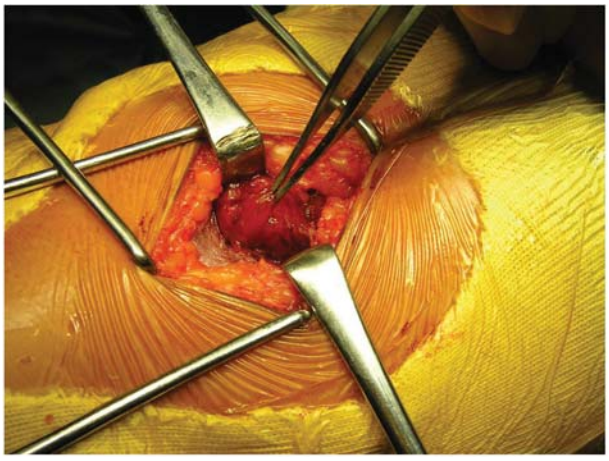

Figure 2. *Macroscopic findings at open biopsy. Gelatinous material was encapsulated.*

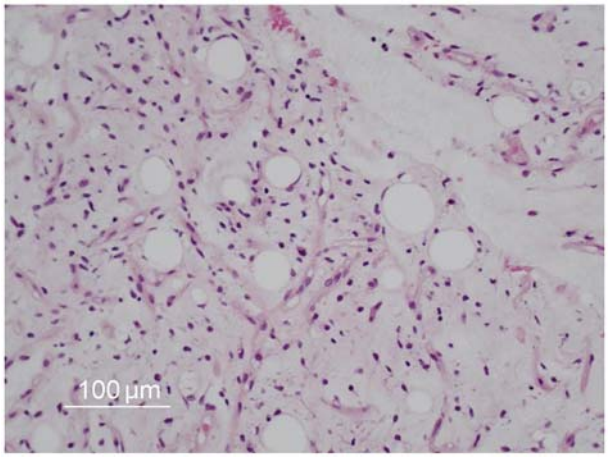

Figure 3. *Histological findings of biopsy specimen. Vacuolar lipoblasts were embedded in a myxoid matrix with a delicate plexiform capillary vascular network. No obvious round cell component was identified. Hematoxylin and eosin staining.*

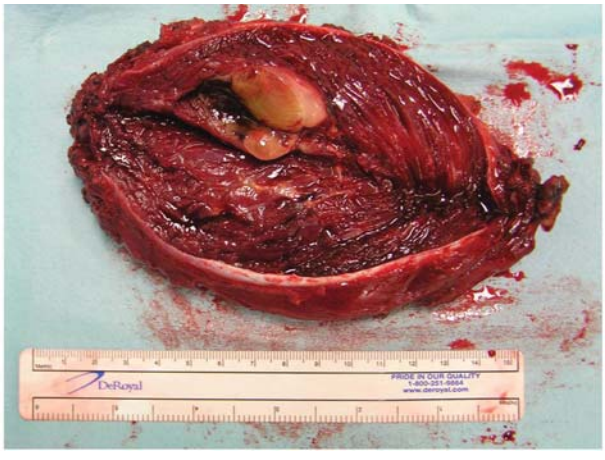

Figure 4. *Resection specimen. A capsulated yellowish gelatinous material comprised the tumor.*

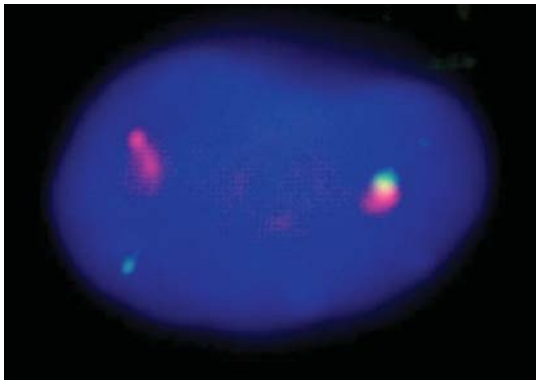

Figure 5. *Dual color fluorescence in situ hybridization (FISH) analysis demonstrating chromosomal rearrangements of the CHOP gene region on chromosome 12q13. Note the abnormal signal pattern, one green, one orange, and one fusion (yellow), in the cell with t(12q13).*

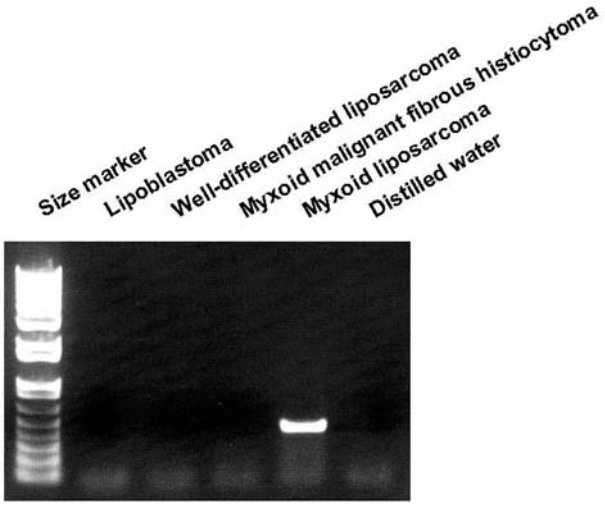

Figure 6. *Detection of EWS–CHOP chimeric transcripts by RT-PCR. cDNAs were amplified by primers EWS 501F and CHOP 194R. Lane 1, molecular size marker; lane 2, lipoblastoma; lane 3, well-differentiated liposarcoma with extensive myxoid change; lane 4, myxoid malignant fibrous histiocytoma; lane 5, myxoid liposarcoma with EWS–CHOP chimeric transcript type 1 (0.45-kbp); lane 6, distilled water (as a negative control).*

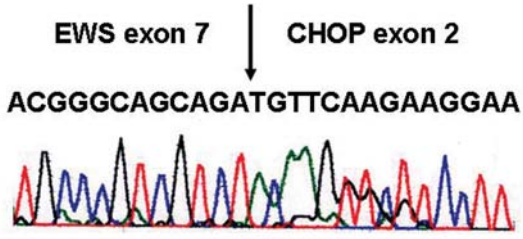

Figure 7. *The type 1 EWS–CHOP chimeric transcript. Partial nucleotide sequence of the 0.45-kbp fragment cDNA (top) and sequence chromatogram (bottom) showing the junction of EWS exon 7 and CHOP exon 2 (arrow).*

Table I. *Myxoid liposarcoma with EWS–CHOP fusion gene.*

No.	Age (years)/ Gender	Location	Size (cm)	Round cell component (%)	Follow-up	Fusion type	Reference
1	45/M	Buttock	11	5%	19 mo ANED	Type 1 (7:2)	(3)
2	40/M	Thigh	5	No	68 mo ANED	Type 1 (7:2)	(3)
3	23/F	Knee	ND	15%	12 mo ANED	Type 1 (7:2)	(4)
4	25/M	ND	ND	ND (high grade)	Met lung	Type 1 (7:2)	(4)
5	33/F	Thigh	11.5	30%	51 mo DOD; mets at presentation	Type 1 (7:2)	(5, 6)
6	33/F	Thigh	19	20%	37 mo ANED	Type 1 (7:2)	(6)
7	26/ND	Thigh	13	ND	10 mo ANED	Type 1 (7:2)	(7)
8	48/ND	Thigh	8	ND	49 mo rec; 54 mo ANED	Type 1 (7:2)	(7)
9	36/M	Calf	18	20%	4 mo ANED	Type 1 (7:2)	(8)
10	19/F	Thigh	4	No	60 mo ANED	Type 1 (7;2)	This report
11	26/M	Thigh	35	Unusual	10 mo mets; 12 mo rec; 16 mo AWD	Type 2 (10:2)	(7)
12	16/M	Retroperitoneum	30	Unusual	8 mo rec; 15 mo AWD	Type 2 (10:2)	(7)
13	43/F	Thigh	ND	ND	24 mo rec; 54 mo met bone, DOD	Type 3 (13:2)	(9)
14	16/M	Thigh	9	No	18 mo bone sarcoma	Type 4 (13:3)	(10)
15	18/M	Lower leg	ND	No	36 mo ANED	ND	(11)
16	19/M	Lower leg	ND	No	ND	ND	(11)

ANED: Alive no evidence of disease; AWD: alive with disease; DOD: dead of disease; F: female; M: male; met: metastasis; mo: months; ND: no data; rec: local recurrence.

In MLS cases with *TLS–CHOP* fusion gene, up to nine structural variants of fusion proteins are generated according to the locations of the gene breakpoints (14). It is possible that such variation modulates the oncogenic activity of the fusion protein and thus influences the clinical course. In Ewing sarcoma and synovial sarcoma, correlations between fusion type and clinical outcome have been indicated (15, 16). For MLS with *TLS–CHOP* fusion gene, Bode-Lesniewska *et al*. showed that the median disease-free survival time in patients with type 2 variant was five times longer than that in patients with type 1 variant (10). However, to the Authors' knowledge, correlation between the known *EWS–CHOP* fusion types, type 1 through type 4, and clinical course has not been explored to date.

According to the fusion type, Table I summarizes the 15 MLS cases with *EWS–CHOP* fusion gene in the literature (case no. 1-9, 11-16), together with the current case (case no. 10). There were ten cases of type 1 fusion, two type 2, one type 3, one type 4 and two cases of undescribed type. Mean age, proportion of male patients (% male), proportion of RC component (% RC) and proportion of ANED at the final follow-up (% ANED) were 29.1 years (n=16), 64.3% (n=14), 9% (n=10) and 60% (n=15), respectively. These were compatible with literature values on the *TLS–CHOP* fusion gene, showing mean age of 44.1 years (n=163), 57.5% males (n=146), 11.5% RC (n=124) and 63.9% ANED (n=133) (6, 7, 10-12).

The *EWS–CHOP* type 1 fusion transcript differs from other variant types of *EWS–CHOP* by lacking RNA-binding domain (7, 9, 10). Table II compares the age, % males, size and % ANED, between MLS with *EWS–CHOP* type 1 fusion gene and MLS with other types of *EWS–CHOP* fusion.

Table II. *Comparison between MLS with EWS–CHOP type 1 fusion gene and MLS with other types of EWS–CHOP.*

Fusion type	Type 1	Other (types 2, 3 and 4)	*p*-Value
Mean age	32.8 (n=10)	25.3 (n=4)	0.247*
% male	50 (n=8)	75 (n=4)	0.576**
Mean size (cm)	11.2 (n=8)	24.7 (n=3)	0.036*
% ANED	80 (n=10)	0 (n=4)	0.015**

*Unpaired two-tailed *t*-test; **two-tailed Fisher exact probability test. n: Number of cases with available information; % male: population of male patients; % ANED: proportion of patients alive with no evidence of disease at the final follow-up.

While age and % males were not statistically significant, size and % ANED were when a value of $p<0.05$ was considered significant. These data indicate that MLS with *EWS–CHOP* type 1 fusion gene is related to smaller tumor size and higher proportion of ANED at the final follow-up. At this time, it is not clear whether lacking an RNA-binding domain in the EWS–CHOP fusion protein attenuates its oncogenic activity and/or affects the clinical course of MLS.

In summary, this report documented a detailed clinical picture of MLS with *EWS-CHOP* type 1 fusion gene. Analysis of MLS with *EWS-CHOP* indicated that MLS with type 1 fusion gene may show more favorable clinical behavior than MLS with other types of fusion gene. Further molecular and clinicopathological correlation studies in more cases of MLS, as well as functional analysis of EWS–CHOP proteins, may establish the role of RNA-binding domain in MLS biology.

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References

1 Weiss SW and Goldblum JR: Liposarcoma. *In*: Enzinger and Weiss's Soft Tissue Tumors, 4th ed. Weiss SW and Goldblum JR (eds.). St. Louis, Mosby, pp. 641-93, 2001.
2 Antonescu CR and Ladanyi M: Myxoid liposarcoma. *In*: World Health Organization Classification Tumours: Tumours of Soft Tissue and Bone. Fletcher CDM, Unni KK and Mertens F (eds.). Lyon, IARC Press, pp. 40-43, 2002.
3 Panagopoulos I, Hoglund M, Mertrns F, Mandahl N, Mitelman F and Aman P: Fusion of the *EWS* and *CHOP* genes in myxoid liposarcoma. Oncogene *12*: 489-494, 1996.
4 Dal Cin P, Sciot R, Panagopoulos I, Aman P, Samson I, Mandahl N, Mittelman F, van den Berghe H and Fletcher CDM: Additional evidence of a variant translocation t(12;22) with *EWS/CHOP* fusion in myxoid liposarcoma: Clinicopathological features. J Pathol *182*: 437-441, 1997.
5 Antonescu CR, Elahi A, Healey JH, Brennan MF, Lui MY, Lewis J, Jhanwar SC, Woodruff JM and Ladanyi M: Monoclonality of multifocal myxoid liposarcoma: Confirmation by analysis of *TLS–CHOP* or *EWS–CHOP* rearrangements. Clin Cancer Res *6*: 2788-2793, 2000.
6 Antonescu CR, Tschernyavsky SJ, Decuseara R, Leung DH, Woodruff JM, Brennan MF, Bridge JA, Neff JR, Goldblum JR and Ladanyi M: Prognostic impact of P53 status, *TLS–CHOP* fusion transcript structure, and histological grade in myxoid liposarcoma: A molecular and clinicopathologic study of 82 cases. Clin Cancer Res *7*: 3977-3987, 2001.
7 Hosaka T, Nakashima Y, Kusuzaki K, Murata H, Nakayama T, Nakamata T, Aoyama T, Okamoto T, Nishijo K, Araki N, Tsuboyama T, Nakamura T and Toguchida J: A novel type of EWS-CHOP fusion gene in two cases of myxoid liposarcoma. J Mol Diagn *4*: 164-171, 2002.
8 Birch NC, Antonescu CR, Nelson M, Sarran L, Neff JR, Seemayer T and Bridge JA: Inconspicuous insertion 22;12 in myxoid/round cell liposarcoma accompanied by the secondary structural abnormality der(16)t(1;16). J Mol Diagn *5*: 191-194, 2003.
9 Matsui Y, Ueda T, Kubo T, Hasegawa T, Tomita Y, Okamoto M, Myoui A, Kakunaga S, Yasui N and Yoshikawa H: A novel type of *EWS–CHOP* fusion gene in myxoid liposarcoma. Biochem Biophys Res Commun *348*: 437-440, 2006.
10 Bode-Lesniewska B, Frigerio S, Exner U, Abdou MT, Moch H and Zimmermann DR: Relevance of translocation type in myxoid liposarcoma and identification of a novel *EWSR1–DDIT3* fusion. Genes Chromosomes Cancer *46*: 961-971, 2007.
11 Alaggio R, Coffin CM, Weiss SW, Bridge JA, Issakov J, Oliveira AM and Folpe AL: Liposarcomas in young patients: A study of 82 cases occurring in patients younger than 22 years of age. Am J Surg Pathol *33*: 645-658, 2009.
12 Kubo T, Matsui Y, Naka N, Araki N, Myoui A, Endo K, Ohtani O, Suzuki K, Kimura T, Yoshikawa H and Ueda T: Specificity of fusion genes in adipocytic tumors. Anticancer Res *30*: 661-664, 2010.
13 Yamaguchi U, Hasegawa T, Morimoto Y, Tateishi U, Endo M, Nakatani F, Kawai A, Chuman H, Beppu Y, Kurotaki H and Furuta K: A practical approach to the clinical diagnosis of Ewing's sarcoma/primitive neuroectodermal tumour and other small round cell tumours sharing *EWS* rearrangement using new fluorescence *in situ* hybridization probes for EWSR1 on formalin-fixed, paraffin wax-embedded tissue. J Clin Pathol *58*: 1051-1056, 2005.
14 Panagopoulos I, Mertrns F, Isaksson M and Mandahl N: A novel *FUS/CHOP* chimera in myxoid liposarcoma. Biochem Biophys Res Commun *279*: 838-845, 2000.
15 Lin PP, Brody RI, Hamelin AC, Bradner JE, Healey JH and Ladanyi M: Differential transactivation by alternative EWS–FLI fusion proteins correlates with clinical heterogeneity in Ewing's sarcoma. Cancer Res *59*: 1428-1432, 1999.
16 Ladanyi M, Antonescu CR, Leung DH, Woodruff JM, Kawai A, Healey JH, Brennan MF, Bridge JA, Neff JR, Barr FG, Goldsmith JD, Brooks JSJ, Goldblum JR, Ali SZ, Shipley J, Cooper CS, Fisher C, Skytting B and Larsson O: Impact of *SYT–SSX* fusion type on the clinical behavior of synovial sarcoma: a multi-institutional retrospective study of 243 patients. Cancer Res *62*: 135-140, 2002.

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