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 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 fusion 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 EWS–CHOP type 1 fusion transcript may show a more favorable clinical behavior than MLS with other types of fusion transcript.

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, the nuclei were too close together to determine boundaries, the signals were not counted.
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 \textit{EWS} 501F (forward) 5'-CCA GCC CAG CCT AGG ATA TGG ACA-3' and \textit{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 musculctomy 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 \textit{CHOP} gene (Figure 5).

**RT-PCR analysis.** One-step RT-PCR analysis using two primers, \textit{EWS} 501F and \textit{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 \textit{EWS–CHOP} chimeric transcript, the 0.45-kbp cDNA fragment was analyzed by direct sequencing, showing that it corresponded to \textit{EWS–CHOP} chimeric transcript type 1, in which exon 7 of \textit{EWS} was in frame fused to exon 2 of \textit{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 \textit{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. Vacular 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).
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, and two cases of undescribed type. Mean age, proportion of male patients (% male), proportion of RC component (%), 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. 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


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