Original Article
NY-ESO-1 as a diagnostic and prognostic marker for myxoid liposarcoma

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Abstract: Introduction: Myxoid liposarcoma (MLS) is a common lipogenic sarcoma, which is difficult to diagnose in small specimens. New York oesophageal squamous cell carcinoma 1 (NY-ESO-1) is a cancer-testis antigen expressed in neoplastic tissue. In this study, NY-ESO-1 expression was assessed in various soft tissue tumors (STTs), and we also evaluated its diagnostic utility. Methods: We included 434 cases of STTs for collection of clinicopathological data. Tissue microarrays were designed, and immunostaining for NY-ESO-1 was examined. We investigated the correlation between NY-ESO-1 expression and various clinicopathological parameters. We also evaluated the role of NY-ESO-1 as a diagnostic marker for MLS and its possible use in prognostication. Results: Sixty-four of the 434 STTs (14.75%) were immunoreactive for NY-ESO-1, and the most frequent type of tumor in the NY-ESO-1 positive group was MLS (70.3%, 45/64), followed by synovial sarcoma (17.2%, 11/64). MLS showed 72.6% (45/62) immunopositivity for NY-ESO-1. The sensitivity and specificity of NY-ESO-1 expression for the diagnosis of MLS were 84.4% and 100%, respectively, compared to *DDIT3* fluorescence in situ hybridization. When restricting analysis to the MLS (n=62), the NY-ESO-1 positive group had a poor overall survival (OS) rate (P=0.039). Conclusion: NY-ESO-1 was substantially and widely expressed in the majority of MLS cases. NY-ESO-1 positivity by IHC staining was also a predictor of a poor OS in patients with MLS. It is possible to use NY-ESO-1 for diagnosis and for predicting a prognosis in patients with MLS, and it may be used as a therapeutic target.

Keywords: Myxoid liposarcoma, NY-ESO-1, DDIT3, immunohistochemistry, soft tissue tumors

Introduction

Liposarcomas account for the largest proportion of soft tissue tumors (STTs) and can occur in any organ in the human body. Liposarcomas are categorized into several types. In young people, myxoid liposarcomas (MLSs) mainly occur in the lower extremities of the body and have a tendency to metastasize to the intraperitoneal cavity and bone [1]. MLSs are composed of myxoid stroma, fibroblasts, and a few adipocytes with arborizing vasculature; these features are helpful to distinguish them from other STTs. However, there are many kinds of myxoid mesenchymal tumors, ranging from benign types (such as myxoma) to malignant types (such as myxofibrosarcoma), and all of these share similar histo-morphologic features, thus making the accurate diagnosis of an MLS a challenge in clinical practice.

New York oesophageal squamous cell carcinoma 1 (NY-ESO-1, or CTAG1B) is a cancer-testis antigen that was reported in 1997 from a case of oesophageal squamous cell carcinoma [2], and it is currently the focus of several targeted immunotherapeutic strategies [3, 4]. NY-ESO-1 is normally presented only by adult testis germ cells and is uncommonly re-expressed in many malignancies, including melanomas, sarcomas, and carcinomas [3, 5-7]. Although the biological function of this cancer-testis antigen is not known, it is believed to be an immunogenic protein as many members of its family have been shown to elicit spontaneous cellular and humoral immune responses in cancer patients [4].

Recently, a few studies have reported the applicability of NY-ESO-1 as a diagnostic marker to differentiate MLS from other myxoid tumors [8-11]. Herein, we evaluated the expression of NY-ESO-1 in a large cohort of several types of STTs. We analysed the correlation between NY-ESO-1 and clinicopathological parameters, and investigated its diagnostic and prognostic utility for MLS.

Materials and methods

Study population and data collection

This study was performed according to the protocol approved by the Institutional Review Board of the Asan Medical Center (2019-1432). After searching the anonymized research database at the Asan Medical Center, 471 cases of STTs were identified. All cases had undergone surgical resection from 2005 through 2016 at the Asan Medical Center. Thirty-seven cases were excluded due to lack of available clinical data or tissue blocks, and 434 cases were finally retrieved. All cases were histologically reviewed by two pathologists (JSS and UJ) using typical immunohistochemistry and molecular tests for diagnosis. The coordinated cases consisted of 419 sarcomas and 15 benign lipogenic tumors listed as follows: MLSs (n=62), synovial sarcomas (SSs, n=40), osteosarcomas (n=3), Ewing sarcomas/primitive neuroectodermal tumors (n=18), alveolar rhabdomyosarcomas (n=19), fibrosarcomas (n=10), dedifferentiated liposarcomas (DDLPSs, n=33), malignant peripheral nerve sheath tumors (MPNSTs, n=35), myxofibrosarcomas (n=12), pleomorphic liposarcomas (n=11), well-differentiated liposarcomas (n=56), undifferentiated pleomorphic sarcomas (UPSs, n=30), gastrointestinal stromal tumors (GISTs, n=3), perivascular epithelioid cell tumors (PECOMAs, n=5), leiomyosarcomas (n=82), angiomyolipomas (n=2), lipomas (n=10), myxomas (n=2), and lipoblastoma (n=1). All molecular analyses were performed at the time of diagnosis for SS (translocation of *SYT-SSX*), DDLPS (*MDM2* amplification) and Ewing sarcomas/PNET using *EWSR1* Break Apart FISH (fluorescence in situ hybridization).

Clinicopathological data were collected from electronic medical records, including age at diagnosis, sex, location of tumor, tumor size (cm), the FNCLCC grade, treatment history, recurrence, and survival time. Grading was performed only for sarcomas and excluded the following tumors: lipoma, myxoid lipoma, angiomyolipoma, lipoblastoma, PECOMA, GIST, and MPNST. The round cell component, known to be associated with the prognosis of MLS, was evaluated. The criteria for round cell component applied were as follows: ≤5% and >5% [12]. Recurrence data were calculated from the date of initial histologic diagnosis to the date of recurrence, confirmed by biopsy/surgical excision or radiologic evaluation.

Tissue microarray (TMA) formation

TMAs were formed from 2 mm cores of the respective tumor areas from paraffin embedded blocks and they were made into 11 TMA blocks in total. The TMAs were used in immunohistochemistry (IHC) for NY-ESO-1 and in FISH for *DDIT3*.

Immunohistochemistry

IHC staining was performed with an antibody against NY-ESO-1 (1:100, clone E978, monoclonal, Santa Cruz Biotechnology, Santa Cruz, CA, USA) using a Ventana BenchMark XT autostainer (Ventana Medical Systems, Tucson, AZ, USA) according to the manufacturer's instructions.

Expression of NY-ESO-1 was interpreted as positive or negative. Positivity was designated as diffuse homogeneous cytoplasmic/nuclear immunoreactivity in more than 50% of the tumor cells [10, 11]. The positive control was tissue from the testis.

FISH assay

We performed the FISH assay using a commercially available *DDIT3* probe (ZytoLight® SPEC *DDIT3* dual color break-apart probe, catalogue #Z-2100-50) in the 62 patients with MLS. The *DDIT3* probe is a mixture of two directly labelled probes hybridizing to the 12q13.3-q14.1 band. The orange fluorochrome directly labelled probe hybridizes proximal to the *DDIT3* gene, and the green fluorochrome directly labelled probe hybridizes distal to that gene.

The FISH analyses were interpreted by two experienced pathologists (JSS and UJ) who were unaware of the clinical data. Because no standard criteria defines *DDIT3* FISH positivity, we interpreted the presence of a *DDIT3* rearrangement based on previously published criteria [13], i.e., at least 100 cells must have been
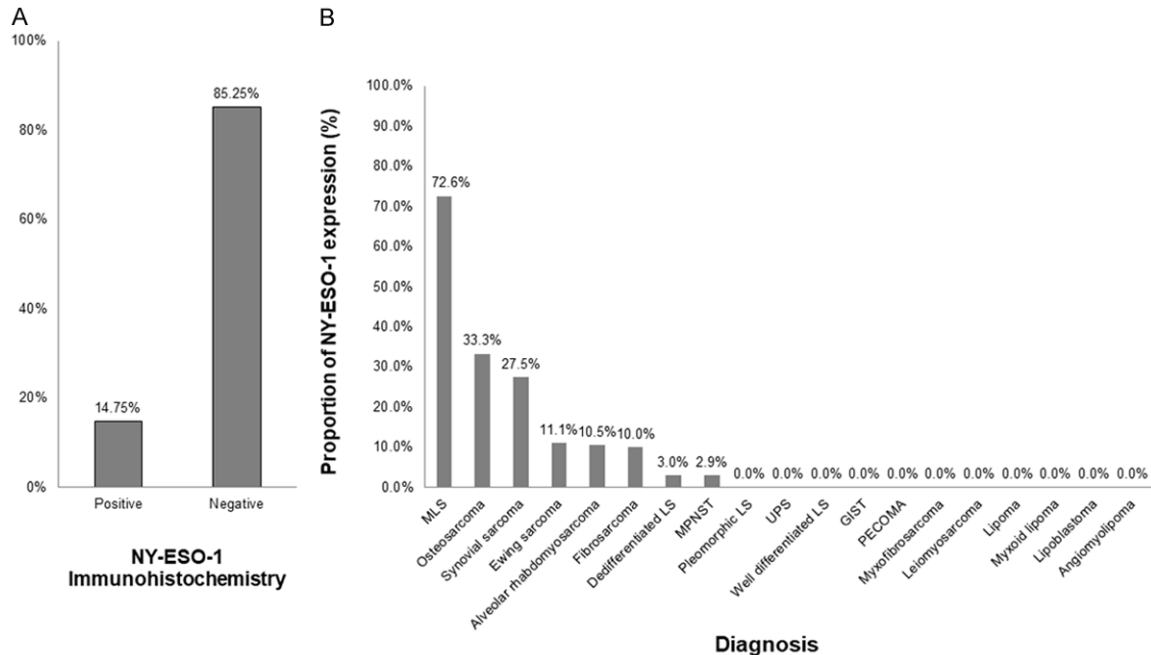
Figure 1. Results of NY-ESO-1 expression. A. Immunopositivity for NY-ESO-1 was 14.75% (64/434). B. NY-ESO-1 was expressed in several soft tissue tumors. The most frequent NY-ESO-1 expression was observed in myxoid liposarcoma (72.6%). Abbreviations: MLS, Myxoid liposarcoma; MPNST, Malignant peripheral nerve sheath tumors; UPS, Undifferentiated pleomorphic sarcoma; LS, Liposarcoma; GIST, Gastrointestinal stromal tumor; PECOMA, Perivascular epithelioid cell tumor.

counted, and at least 10% of the counted cells must demonstrate green and orange signals separated by at least two signal diameters.

Statistical analyses

Statistical analyses were performed using SPSS version 18 (IBM, Chicago, IL). Ages and tumor sizes were described with mean ± standard deviation. Tumor sizes were compared using unpaired t-tests. For the analyses between other clinicopathological parameters and the IHC results, Chi-square tests were used. The Kaplan-Meier method with log-rank tests was used for the analysis of overall survival (OS) and disease-free survival (DFS) rates. Multivariate analysis using Cox regression was performed to assess clinicopathological variables as independent factors for survival. All *P*-values less than 0.05 were considered to be statistically significant.

Results

NY-ESO-1 expression in STTs by immunohistochemistry

NY-ESO-1 IHC staining was performed on 434 STTs, and 64 of the 434 STTs (14.75%) were immunoreactive for NY-ESO-1, while 370 of 434 cases (85.25%) were negative (**Figure 1A**). NY-ESO-1 expression differed among different tumor subtypes (**Figure 1B**). The tumor type that expressed it most frequently was MLS (72.6%, 45/62), followed by osteosarcoma (33.3%, 1/3) and SS (27.5%, 11/40). In addition, small numbers of other STTs, including Ewing sarcoma, alveolar rhabdomyosarcoma, fibrosarcoma, DDLPS, and MPNST exhibited NY-ESO-1 expression. The test results for all benign STTs were negative for NY-ESO-1. Representative images are shown in **Figure 2**. Interestingly, one case of DDLPS showed immunoreactivity for NY-ESO-1.

*Frequency of DDIT3 rearrangement and correlation between DDIT3 FISH and IHC for NY-ESO-1 in MLSs*

Thirty-eight cases of MLSs were retrieved for *DDIT3* rearrangement assessment by FISH. The presence of the diagnostic *DDIT3* breakapart was monitored with a break-up signal (**Figure 3A**). The results were available for 57 cases, and of these, 55 (96.5%) cases of MLSs were observed to have a *DDIT3* rearrangement. When the positive cut-off value
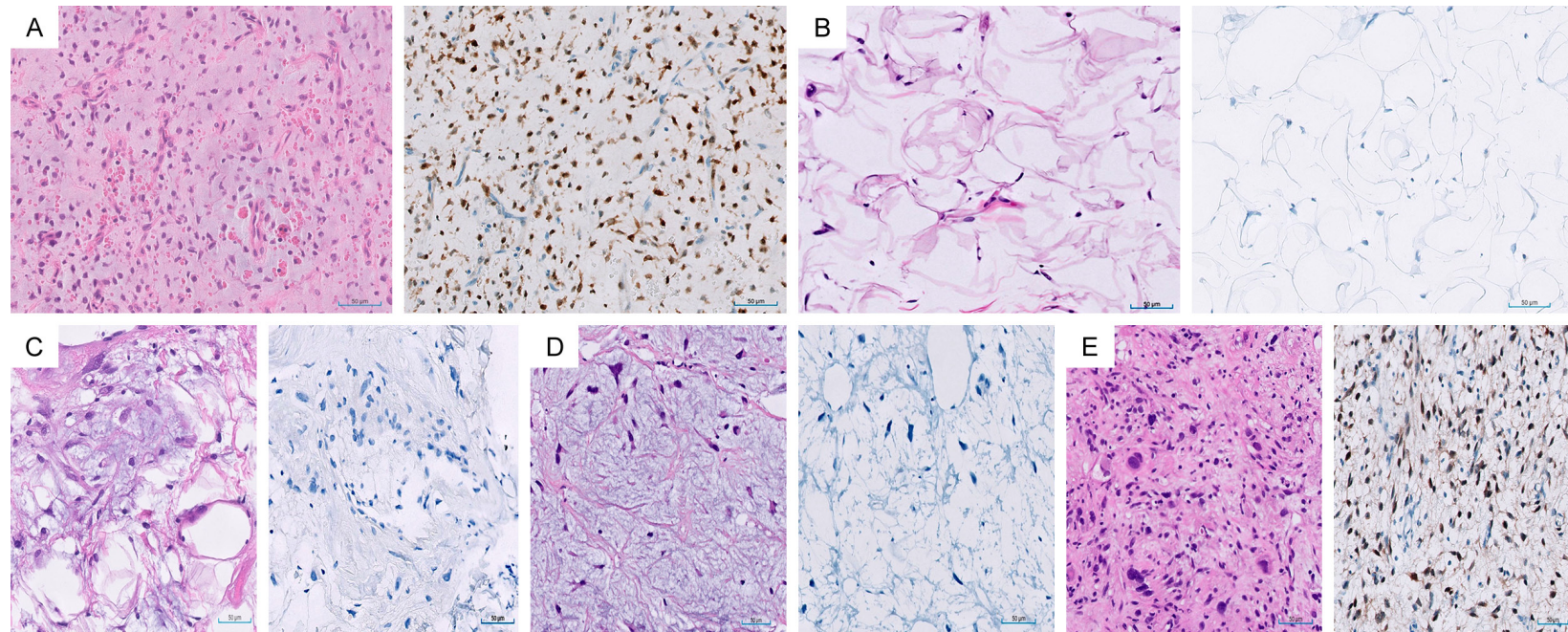
Figure 2. Representative images of NY-ESO-1 expression in variable soft tissue tumors. Myxoid liposarcoma (A, 200×) and dedifferentiated liposarcoma (E, 200×) exhibited strong and diffuse positivity for NY-ESO-1 in the cytoplasm and nuclei. Well-differentiated liposarcoma (B, 200×), pleomorphic liposarcoma (C, 200×), and myxofibrosarcoma (D, 200×) exhibited immuno-negativity for NY-ESO-1.
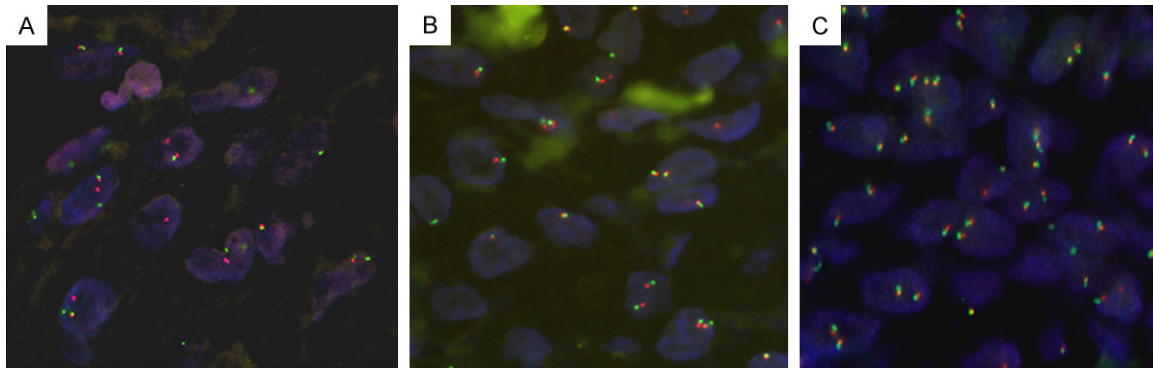
Figure 3. Fluorescence *in situ* hybridization for the *DD1T3* gene shows the break-apart signals in soft tissue tumors. Arrows indicate split red and green signals in Myxoid liposarcoma (A), thus indicating a rearrangement of *DDIT3* gene. Negative FISH analysis of DDIT3 rearrangement was observed in osteosarcoma (B) and synovial sarcoma (C).

Table 1. Correlation between *DDIT3* FISH and NY-ESO-1

Variables	*DDIT3* FISH (N=57)		*P*-value
	Positive	Negative	
NY-ESO-1			0.001*
Positive	42 (79.4%)	0 (0.0%)	
Negative	13 (20.6%)	2 (100%)	

*This was analysed by a McNemar test.

exceeded 10%, the mean value of the positive *DDIT3* rearranged group was 42.9%, ranging from 38.6 to 47.3%.

Other than MLS, other STTs which remarkably expressed NY-ESO-1 were also evaluated for *DDIT3* rearrangement by FISH (**Figure 3B**, **3C**). Osteosarcoma and SS groups presented an average 3% DDIT3 break-apart proportion.

Based on the results of *DDIT3* FISH, the sensitivity and specificity of NY-ESO-1 IHC were 84.4% and 100%, respectively (P=0.016). These results are shown in **Table 1**.

Correlation between NY-ESO-1 expression and clinicopathological features

The age of patients with NY-ESO-1 expressing tumors ranged from 16 to 71 years (median, 45 y), and the age of patients with tumors not expressing NY-ESO-1 ranged from 1 to 88 years (median, 51 y). The tumor size of the NY-ESO-1 expressing cases ranged from 1.0 to 22.0 cm (median, 9.9 cm), and the tumor size of the negative cases ranged from 1.0 to 45.0 cm (median, 9.3 cm). These parameters, along with age (P=0.051), sex (P=0.224), and tumor size (P=0.267), were not correlated with NY-ESO-1 expression.

NY-ESO-1 positivity was associated with a higher FNCLCC grade (P=0.001) and with location of the tumor in a lower extremity (P=0.001). When restricting the analysis to the MLSs (n=62), the proportion of the round cell component was not correlated with NY-ESO-1 expression (P=0.515). These findings are summarized in **Table 2**.

Diagnostic utility of NY-ESO-1 expression between MLS and non-MLS

The expression of NY-ESO-1 in MLS was compared with other STTs. NY-ESO-1 exhibited significantly higher immunoreactivity in MLSs than non-MLSs (72.6% *versus* 5.1%, P<0.001), supporting its use as a diagnostic marker of MLSs. These findings are summarized in **Table 3**.

Survival analyses

The OS and DFS rates were analysed for all STTs in regard to NY-ESO-1 status (**Figure 4**). The NY-ESO-1 positive group showed significant differences compared to the NY-ESO-1 negative group for OS (P=0.043, log-rank, **Figure 4A**) and had a tendency towards a shorter DFS (P=0.093, log-rank, **Figure 4B**) in all STTs (N=434). When restricting the analysis to the MLSs only (n=62), the NY-ESO-1 positive group had a shorter DFS (P=0.083, log-rank, n=45, **Figure 4D**) and a significant correlation with shorter OS (P=0.039, log-rank, n=45, **Figure 4C**).

Table 2. Clinicopathological parameters by NY-ESO-1 expression

Parameter	NY-ESO-1 (N=434)		P value
	Positive (N=64, %)	Negative (N=370, %)	
Patient age (years)	44.9±13.6	51.3±18.1	0.051[†]
Sex			0.224
Male	36 (17.0)	176 (83.0)	
Female	28 (12.6)	194 (87.4)	
FNCLCC Grade[*]			0.009
1	5 (8.9)	60 (19.1)	
2	17 (30.4)	47 (15.0)	
3	34 (60.7)	207 (65.9)	
Location			0.001
Upper extremity	3 (4.7)	28 (7.6)	
Lower extremity	39 (60.9)	64 (17.3)	
Abdomen	8 (12.5)	112 (30.3)	
Retroperitoneum	1 (1.6)	79 (21.4)	
Head and neck	4 (6.3)	42 (11.4)	
Thorax	7 (10.9)	43 (11.6)	
Back and spine	2 (3.1)	2 (0.5)	
Tumor size (cm)	9.9±5.7	9.3±7.2	0.267[†]
Round cell component[г]			0.515
≤5%	28 (62.2)	10 (58.8)	
>5%	17 (37.8)	7 (41.2)	

[†]This was analysed by a t-test. [*]Total submitted cases of soft tissue tumors numbered 370 due to the exclusion of 77 benign soft tissue tumor cases. [г]This was analysed in 62 cases of myxoid liposarcoma.

Table 3. Diagnostic utility of NY-ESO-1 between MLS and non-MLS

	MLS (n=62)	Other soft tissue tumors (n=372)	P-value
NY-ESO-1 (+)	45 (72.6%)	19 (5.1%)	<0.001[†]
NY-ESO-1 (-)	17 (27.4%)	353 (94.9%)	

MLS, myxoid liposarcoma; [†]This was analysed by a Chi-square test.

The prognostic significance of the presence of NY-ESO-1 was analysed as a function of the round cell component of MLSs. In MLSs with less than 5% round cell component, NY-ESO-1 expression was related to a shorter DFS (P=0.037, log-rank, Figure S1B, Supplemental Digital Content 1), but it had no significance for OS (P=0.465, log-rank, Figure S1A, Supplemental Digital Content 1). In MLSs with more than 5% round cell component, NY-ESO-1 presence had no significant correlation with OS (P=0.294, log-rank, Figure S1C, Supplemental Digital Content 1) or DFS (P=0.428, log-rank, Figure S1D, Supplemental Digital Content 1).

Survival analysis by FNCLCC grade and NY-ESO-1 status in MLSs was also conducted (Figure S2, Supplemental Digital Content 2). NY-ESO-1 presence tended to be related to a shorter DFS in the FNCLCC grade 2 group, but the difference was not considerable for either OS (P=0.411, log-rank, Figure S2A, Supplemental Digital Content 2) or DFS (P=0.053, log-rank, n=23, Figure S2B, Supplemental Digital Content 2). NY-ESO-1 expression was also not significantly associated with OS (P=0.173, log-rank, n=20, Figure S2C, Supplemental Digital Content 2) or DFS (P=0.491, log-rank, n=16, Figure S2D, Supplemental Digital Content 2) in the grade 3 group.

In the univariate analysis, NY-ESO-1 expression, round cell component, and FNCLCC grade of MLSs all tended to be correlated with disease-free survival. In multivariate analysis, after inclusion of only MLSs during stepwise forward selection, round cell component exceeding 5% remained an independent prognostic factor for a shorter disease-free survival (P=0.005), irrespective of the status of NY-ESO-1 (P=0.084) and FNCLCC grade (P=0.609) (**Table 4**).

Discussion

We evaluated the presence of NY-ESO-1 in a large STT cohort (n=434) and demonstrated that the most frequent NY-ESO-1 positive type of STT was MLS (70.3%, i.e., 45 out of 64 cases). Moreover, 45 out of 62 cases of MLS (72.6%) showed immunoreactivity for NYESO-1. Compared to *DDIT3* gene rearrangement, NY-ESO-1 showed good sensitivity (84.4%) and specificity (100%), allowing a robust diagnosis of MLS. In addition, the presence of NY-ESO-1 was related to a higher tumor grade and shorter OS in MLSs, suggesting it may also have a prognostic role.
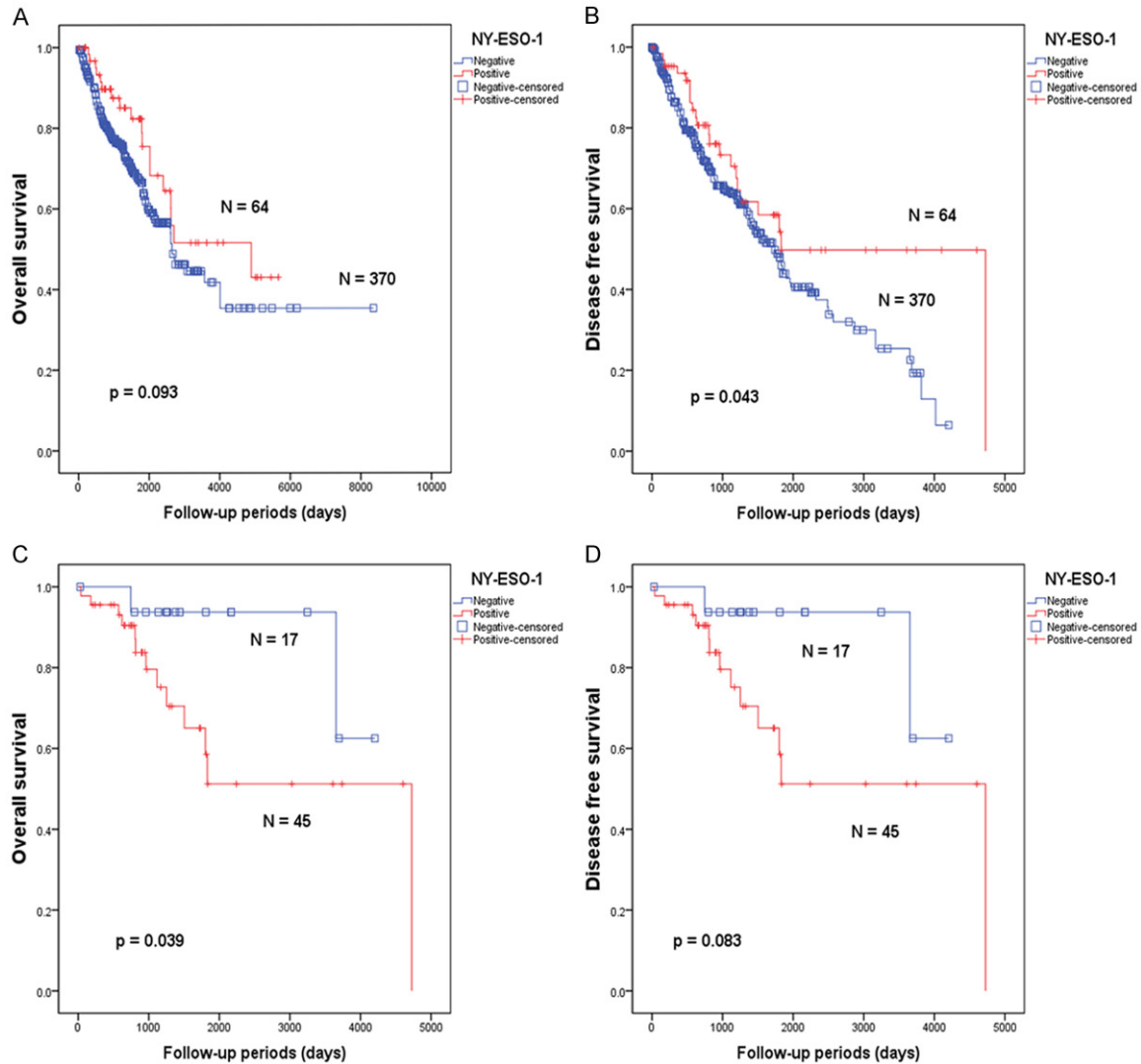
Figure 4. Survival analysis. Kaplan-Meier estimation of overall survival (OS, A) and disease-free survival (DFS, B) by NY-ESO-1 expression in all soft tissue tumors (n=434). The NY-ESO-1 positive group exhibited a significant correlation with shorter DFS (P=0.043, log-rank, B) but did not show differences compared to the NY-ESO-1 negative group for OS (P=0.093, log-rank, A). (C, D) When restricting the analysis to the myxoid liposarcoma group only (n=62), the NY-ESO-1 positive group exhibited a shorter DFS (P=0.083, log-rank, n=45, D) and a significant tendency towards a poor OS (P=0.039, log-rank, n=45, C).

Table 4. Multivariate analysis of disease-free survival in myxoid liposarcoma

Parameter		P value	HR	95% CI
Round cell component	[<5%] vs >5%	0.005	20.732	2.524-170.313
FNCLCC grade	[2] vs 3	0.609	1.393	0.392-4.954
NY-ESO-1	[Negative] vs positive	0.084	6.858	0.773-60.869

[ ] represents the reference parameters. HR represents hazard ratio. CI represents confidence interval.

Six studies [9-11, 14-16] examining NY-ESO-1 expression in MLS have been published in English, and their results are summarized in **Table 5**. In brief, 234 cases of MLS, including our cases, were stained for NY-ESO-1, and the positive immunoreactivity for NY-ESO-1 ranged from 55.6% to 100% (average 87.5%). One previous study [14] and the present study have reported outcomes in terms of NY-ESO-1

Table 5. Summary of published series of NY-ESO-1 expression in myxoid liposarcoma

Studies	# of total soft tissue	# of MLS	NY-ESO-1 positivity (IHC)	*FUS/DDIT3* FISH	Outcomes
Hemminger *et al.* (2013) [11]	138	38	95% (36/38)	NA	NA
Hemminger *et al.* (2013) [10]	44	18	89% (16/18)	NA	NA
Pollack *et al.* (2012) [9]	25	25	100% (25/25)	NA	NA
Iura *et al.* (2015) [14]	185	93	89.7% (83/93)	NA	Correlated with tumor grade, poor prognosis
Shurell *et al.* (2016) [16]	161	13	100% (13/13)	NA	NA
Kakimoto *et al.* (2019) [15]	82	9	55.6% (5/9)	NA	NA
Present study	434	62	72.6% (45/62)	*DDIT3* rearranged (55/57, 96.5%)	Correlated with tumor grade poor disease-free survival
Total	1069	258	223 (86.4%)	55 (96.5%)	

#numbers. MLS, myxoid liposarcoma; IHC, immunohistochemistry; FISH, fluorescence in situ hybridization; NA, not available.

expression, and both studies show that NY-ESO-1 expression in MLS is correlated with tumor grade and also with a poor prognosis.

The expression of NY-ESO-1 in MLS was observed in 55.6%-100% (**Table 5**) of cases in prior studies; our study observed a 71.1% expression. Although this is an acceptable result, it has a slightly lower positivity level compared to other studies. Several reasons for this can be considered, including the possibilities of tumor heterogeneity of MLS, poor quality of the tissues in the paraffin blocks, and misdiagnoses. The possibility of misdiagnoses in this study was excluded because the retrieved cases were confirmed by *DDIT3* Break Apart FISH. In addition, we performed a pilot study of immunohistochemistry using whole-tissue sections to exclude the possibility of tumor heterogeneity. We found that the expression pattern of NY-ESO-1 was diffuse and homogeneous. Some of the tumor blocks we used may have been too old, and degeneration of the protein may have occurred, which in turn might have affected the evaluation of NY-ESO-1 expression. Similarly, our study encountered lower expression of NY-ESO-1 in synovial sarcomas (27.5%) than other studies, which have reported immunoreactivity for NY-ESO-1 ranging from 49% [17] to 80% [18] of synovial sarcomas.

The present study showed the immunoreactivity for NY-ESO-1 in 434 cases of STTs. MLS in this study comprised 14.3% (62/434) of the cases, and a positive response for NY-ESO-1 was encountered in 72.6% of the MLSs. Thus, NY-ESO-1 seems to be a tumor-associated antigen of MLS. Similar to the results of a previous study of Hemminger et al. [11], NY-ESO-1 IHC

staining of well-differentiated liposarcomas and myxofibrosarcomas was negative. Finally, no expression of NY-ESO-1 was observed in pleomorphic liposarcomas, leiomyosarcomas, lipomas, myxoid lipomas, or angiomyolipomas. The use of NY-ESO-1 expression as a diagnostic tool is meaningful, but the reasons for immunonegative responses for NY-ESO-1 in some MLS cases need to be established.

Kakimoto et al. reported that OS was substantially better among patients with NY-ESO-1-positive tumors than in those with NY-ESO-1-negative tumors for high-grade sarcomas [15]. However, other studies showed that high-level expression of NY-ESO-1 was correlated with a poor prognosis. Increased IHC expression of NY-ESO-1 is fairly strongly correlated with tumor size, the presence of tumoral necrosis, pleomorphism, and an increased round-cell component, as well as an advanced stage at diagnosis and a poor overall prognosis [19]. From a prognostic point of view, high NY-ESO-1 expression is also a significant risk factor associated with poor DFS and OS, consistent with our results [19]. Although few studies have evaluated NY-ESO-1 expression and the prognosis of MLS, most patients with NY-ESO-1 expression are found to have a poor prognosis [19]. Therefore, we need to investigate the mechanism of action of the NY-ESO-1 protein in tumors. It remains unclear whether NY-ESO-1 is involved in the tumorigenesis of MLS and what mechanisms underlie the expression of this antigen in this particular tumor type.

To-date, the FUS-DDIT3 fusion product is known to upregulate the expression of CCAAT/enhancer binding protein (C/EBP), which leads

to the transcription of peroxisome proliferator-activated receptors gamma and other genes involved in adipocyte differentiation. The regulation of cancer-testis antigen expression is unknown, but unmethylated CpG islands in the gene encoding the cancer-testis antigen have been observed in cancer, while methylated CpG islands are present in normal cells. This pathogenesis could affect transcription factors in MLS. Since the NY-ESO-1 protein is expressed only in the presence of unmethylated CpG islands, the FUS-DDIT fusion product may act as an inhibitor of site-specific DNA methyltransferase, or it may be associated with transcription factors that reverse DNA methylation [20-22]. Several molecular pathways are involved in the tumorigenesis of MLS, and NY-ESO-1 expression might be one of them. With respect to the mechanism of development of MLS, it can be hypothesized that NY-ESO-1 may be an intermediate rather than an end product. This hypothesis explains why NY-ESO-1-negative MLS cases were observed in this study and the inconsistent findings with regard to prognosis in several studies. Based on this hypothesis, further research is needed to elucidate the role of NY-ESO-1 in the pathogenesis of MLS. In addition, the end product of the pathogenesis should be identified and used as a diagnostic tool to improve the accuracy of diagnosis and to enhance NY-ESO-1 targeted therapy.

Since the discovery of NY-ESO-1, a large number of studies have documented variable expression of NY-ESO-1 and have studied its targeted therapy [6-8, 18, 23-27]. In cases of immune therapy using NY-ESO-1, anti-tumor responses involving CD4+ and CD8+ T cell responses lead to clinical benefit [26, 28-30]. Several clinical trials targeting the NY-ESO-1 antigen, including the NY-ESO-1 protein vaccine, NY-ESO-1 peptide vaccine, NY-ESO-1 T-cell repertoire, and NY-ESO-1-specific monoclonal antibody are being conducted [31]. Notwithstanding the fact that NY-ESO-1 exhibits the capacity to induce a strong natural anti-NY-ESO-1 antibody, manipulated methods to increase the efficacy of NY-ESO-1 targeted therapy can be suggested as follows: enhancing anti-NY-ESO-1 T cell responses can produce vaccines for blocking checkpoint inhibitors with Tregs depletion, anti-NY-ESO-1 chimeric antigen receptor T cells (CAR T), and combined

radiotherapy or chemotherapy with anti-NY-ESO-1 antibody [31].

Conclusion

NY-ESO-1 was strongly and diffusely expressed in the majority of MLSs. NY-ESO-1 expression showed significant differences between MLS and non-MLS tumors. IHC staining for NY-ESO-1 was not only diagnostically useful for MLS but also showed a relationship with DFS. NY-ESO-1 shows promise for the diagnosis and prognostication of MLS, and it may also be a therapeutic target.

Disclosure of conflict of interest

None.

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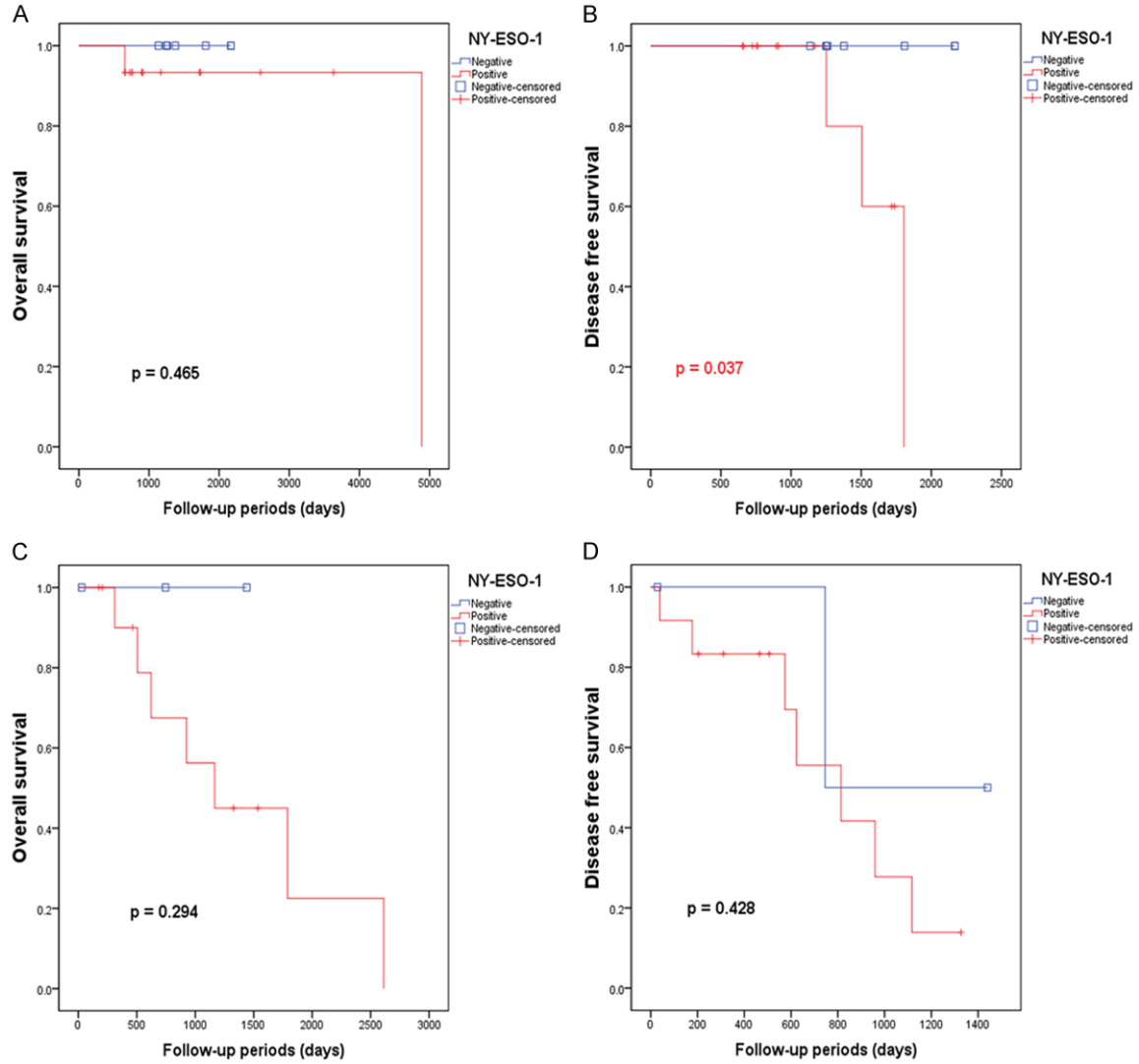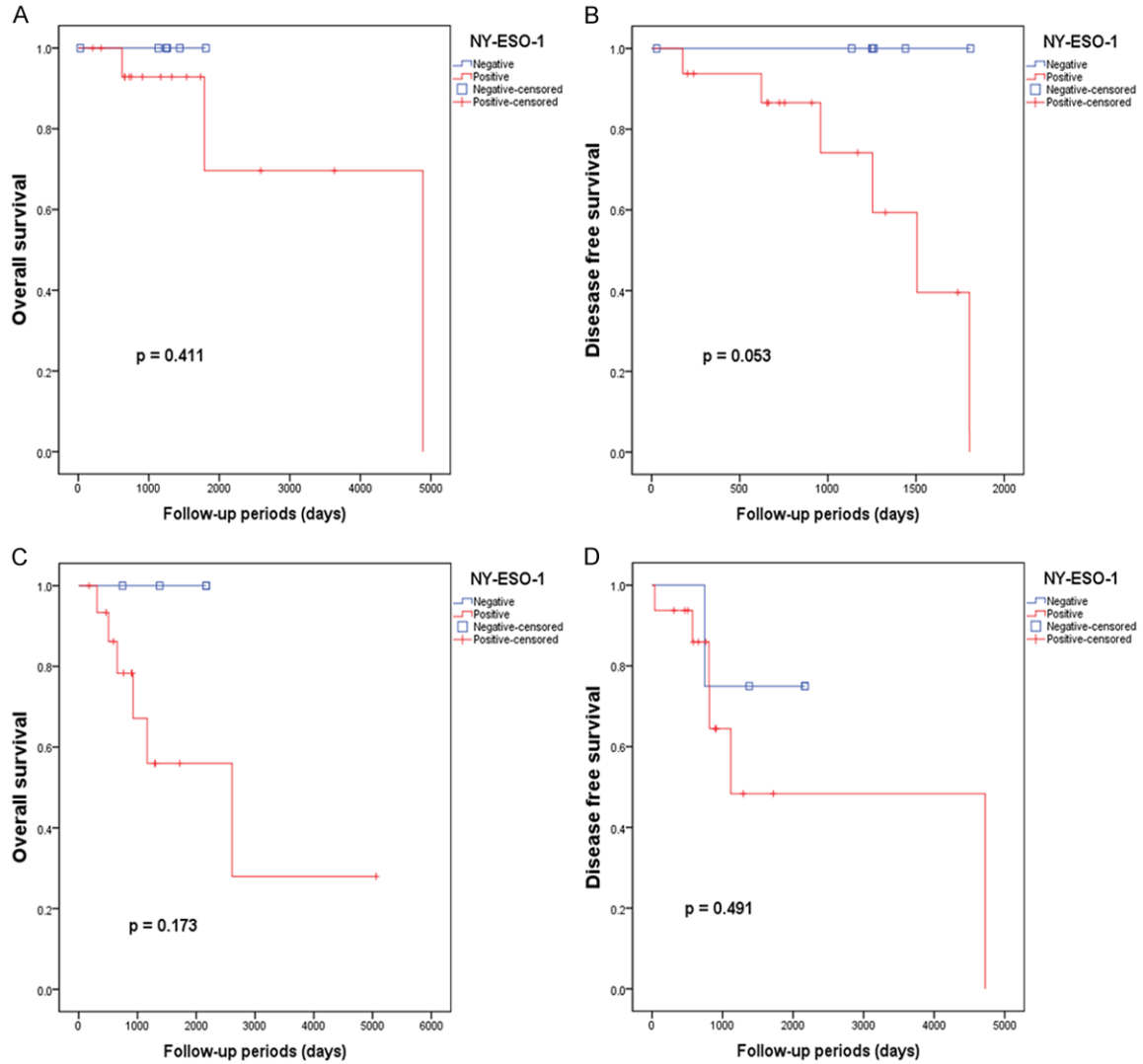
Figure S1. Supplemental digital content 1. Survival analysis by the Kaplan-Meier method of overall survival (OS, A) and disease-free survival (DFS, B) in terms of NY-ESO-1 expression according to round cell component of MLSs (n=62). In MLSs with less than 5% round cell component, the NY-ESO-1 expression was correlated with a shorter DFS (P=0.037, B) but exhibited no correlation with OS (P=0.465, A). In MLSs with more than 5% round cell component, NY-ESO-1 expression exhibited no correlation with OS (P=0.294, C) or DFS (P=0.428, D).

Figure S2. Supplemental digital content 2. Survival analysis by the Kaplan-Meier method of overall survival (OS, A) and disease-free survival (DFS, B) between FNCLCC grade and NY-ESO-1 in MLSs. NY-ESO-1 expression exhibited a tendency to be related to a shorter DFS in the FNCLCC grade 2 group (P=0.053, B) but not with OS (P=0.411, A). In the FNCLCC grade 3 group, NY-ESO-1 expression exhibited no significant association with either OS (P=0.173, C) or DFS (P=0.491, D).