BMC Cancer

RESEARCH

Open Access

Overdiagnosis of atypical lipomatous tumors/well-differentiated liposarcomas by morphological diagnosis using only HE stained specimens: a case–control study with MDM2/CDK4 immunostaining and *MDM2/CDK4* fluorescence in situ hybridization

Kentaro Nomura[1], Masato Tomita[1*], Kishio Kuroda[2], Masakazu Souda[3], Ko Chiba[1], Akihiko Yonekura[1] and Makoto Osaki[1]

Abstract

Background Lipomatous tumors represent the most common type of soft tissue neoplasms. Mouse double minute 2 homolog (MDM2)/cyclin-dependent kinase 4 (CDK4) immunostaining is considered effective in differentiating between benign lipomas and intermediate malignant atypical lipomatous tumors/well-differentiated liposarcomas (ALT/WDLPSs). However, these tumors have traditionally been diagnosed histopathologically using hematoxylin and eosin-stained specimens, which is referred to here as morphological diagnosis. In this study, the accuracy of morphological diagnoses that had been made before MDM2/CDK4 immunostaining became available for distinguishing between lipoma and ALT/WDLPS was examined.

Methods The study participants were 109 patients with a morphological diagnosis of lipoma (68 patients) or ALT/WDLPS (41 patients) who had undergone surgical resection of the tumor in our hospital between 2009 and 2012. Tissue samples from all patients were used for MDM2/CDK4 immunostaining and the confirmation of *MDM2/CDK4* amplification by fluorescence in situ hybridization (FISH).

Results Of the 41 patients with a morphological diagnosis of ALT/WDLPS, only 17 were positive for *MDM2* FISH. In addition, one of the 68 patients with a morphological diagnosis of lipoma showed *MDM2* amplification by FISH. When the definitive diagnosis of ALT/WDLPS was made by the positive results of *MDM2* FISH, the sensitivity and specificity of morphological diagnosis were 41.5% and 98.5%, respectively. The sensitivity of MDM2 and CDK4 immunostaining was 55.6% and 40.0%, respectively, and their specificity was 87.0% and 84.6%, respectively. This indicates that the diagnostic accuracy of these immunostaining assays was not particularly high. The clinical features suggesting ALT/WDLPS were: patient age (older), maximum tumor diameter (large, cut-off value of 125 mm), tumor location (lower limb), and tumor depth (deep-seated).

*Correspondence:
Masato Tomita
mtomita3856@gmail.com
Full list of author information is available at the end of the article

Conclusions Morphological diagnosis alone can accurately diagnose lipomas. However, it has a propensity to overdiagnose ALT/WDLPS. Thus, *MDM2* FISH should be used more proactively, not only for lesions with obvious morphological abnormalities, but also for lipomatous tumors that are clinically suggestive of ALT/WDLPS.

Keywords Atypical lipomatous tumor, Lipoma, FISH, Immunohistochemistry, MDM2, CDK4

Background

Lipomatous tumors represent the most common type of soft tissue neoplasms and are therefore encountered relatively often in routine clinical practice. According to the fifth edition of the WHO Classification of Tumors [1], lipomatous tumors are categorized as benign, intermediate (locally aggressive), or malignant. The most typical examples in the benign and intermediate categories are lipoma and atypical lipomatous tumor/well-differentiated liposarcoma (ALT/WDLPS), respectively [1]. Malignant lipomatous tumors are divided into the following four subclasses: dedifferentiated liposarcoma (DDLPS), myxoid liposarcoma, pleomorphic liposarcoma, and myxoid pleomorphic liposarcoma [1]. ALT/WDLPS is classified into sclerosing, inflammatory, and lipoma-like subtypes. Histological/morphological differentiation of lipoma and lipoma-like ALT/WDLPS is particularly difficult [2]. Since lipomas are benign tumors, they do not always require surgical excision. However, ALT/WDLPS is a soft tissue tumor of intermediate malignant potential with a high risk of local recurrence. In addition, ALT/WDLPS may undergo dedifferentiation. Thus, it often requires surgical excision. Consequently, differential diagnosis between these two tumors is clinically important [3, 4].

In recent years, there have been significant changes in the processes of distinguishing between various types of lipomatous tumors, particularly in the methods of differentiating ALT/WDLPS from lipomas. Traditionally, ALT/WDLPS was diagnosed morphologically, based on the presence of lipoblasts in hematoxylin and eosin (HE)-stained tumor tissue specimens [5]; this histopathology-based diagnosis is referred to as morphological diagnosis in this paper. However, there are many cells that are histologically similar to lipoblasts [6]. Furthermore, lipoblasts can be observed in benign lipomatous tumors as well [6]. Due to these diagnostic challenges, ALT/WDLPS diagnosis has gradually started to rely on different microscopic features, such as the presence of atypical stromal cells and the display of variation in adipocyte size [4]. The key feature is the presence of enlarged, hyperchromatic stromal cells within irregular fibrous bands, usually with a distinctive, finely fibrillar appearance. Alternatively, in tumor molecular biology, studies showed that ALT/WDLPS and DDLPS contain amplified sequences of the mouse double minute 2 homolog (*MDM2*) and cyclin-dependent kinase 4 (*CDK4*) genes, both of which are located on the chromosomal region 12q13-15 [7, 8]. In 2005, Binh et al. [9] reported that immunostaining [10] for MDM2 and CDK4 has high sensitivity and specificity for the identification of ALT/WDLPS and DDLPS, demonstrating the usefulness of this technique for diagnosing these lipomatous tumors. Following the publication of this report, immunostaining for these proteins has become a common ancillary tool for the diagnosis of ALT/WDLPS and DDLPS. Since 2013, our facility has also been performing immunostaining for MDM2 and CDK4, for cases where distinguishing ALT/WDLPS from lipomas is difficult. An alternative when a definitive diagnosis cannot be made using MDM2/CDK4 immunostaining -is fluorescence in situ hybridization (FISH) [11], which can detect the amplification of their corresponding genes.

Morphological diagnosis is undoubtedly the standard method for differentiating ALT/WDLPS from lipomas. However, until immunostaining became available for the differential diagnosis of lipomatous tumors, certain lesions could have been overdiagnosed as ALT/WDLPS, based on clinical findings and magnetic resonance imaging (MRI) results. This could have happened even when these lesions were morphologically regarded as lipomas. Conversely, although immunostaining is now available, this technique (or FISH) is not usually used to confirm the diagnosis of lipoma, when the diagnosis appears to be clear based on morphological and clinical assessments. Consequently, certain patients with ALT/WDLPS may be underdiagnosed as having a lipoma. To help eliminate such overdiagnosis and underdiagnosis, the use of immunostaining and FISH is recommended for differentiating between benign and malignant lipomatous tumors [12, 13]. For lipoma and ALT/WDLPS, there has been no published study evaluating the accuracy of morphological diagnoses that had been made before immunostaining was used to distinguish between these two tumors. Moreover, the accuracy of immunostaining in diagnosing these tumors has been called into question [12]. Therefore, all patients who had been diagnosed in our hospital with a lipoma or ALT/WDLPS, solely on the basis of morphological features (without the use of immunostaining) were first retrospectively identified. Both immunostaining and FISH were then performed on samples collected from these patients.

The objective of this research was to evaluate the accuracy of morphological diagnosis and MDM2/CDK4 immunostaining in differentiating ALT/WDLPS from lipomas. A further aim was to establish the indications for the use of immunostaining and FISH in distinguishing between these two types of tumors.

Materials and methods

Background characteristics of study participants

This study was approved by the Clinical Research Ethics Committee of Nagasaki University Hospital (Approval Number: 17061909–3). Written informed consent was waived by this committee for this retrospective analysis of routinely acquired imaging and clinical data.

The records of all patients with a lipomatous tumor who had undergone surgical resection of the tumor at our institution between 2009 and 2012 were retrospectively reviewed. Of these patients, those who had been diagnosed with a lipoma or ALT/WDLPS on the basis of morphological features were enrolled in this study. The total number of patients was 109, consisting of 68 lipoma and 41 ALT/WDLPS cases. Biopsy samples were excluded from the study. At the time of diagnosis, all patients' specimens were morphologically evaluated by pathologists using only HE-stained tissue sections. They were not examined by FISH or immunostaining. Patient characteristics, including age, sex, maximum tumor diameter, tumor location (upper limb, lower limb, or trunk), and tumor depth (subcutaneous or subfascial), were collected. No cases involving the deep trunk, such as the mediastinum or the retroperitoneum, were identified. The maximum tumor diameter and tumor depth were determined from MRI scans.

Immunostaining and FISH

In all cases, amplification of the *MDM2* and *CDK4* genes was detected by FISH, and the overexpression of their corresponding proteins was analyzed by immunostaining. Paraffin-embedded tissue sections prepared from surgically resected tumor specimens were used for both assays. The results were reviewed by two individuals (a pathologist and a clinical laboratory technologist).

Immunostaining

For MDM2 and CDK4 immunostaining, 4-μm-thick paraffin sections were cut and mounted on silanized glass slides. Sections were dried for 1 h at 58 °C, then overnight at 37 °C. They were subsequently deparaffinized with xylene, rehydrated with ethanol, and pretreated with 10 mM citrate buffer (pH 6.0). Sections were next incubated with hydrogen peroxide to block endogenous peroxidase and stained using a streptavidin–biotin-peroxidase method (Fig. 1). The following primary monoclonal antibodies were purchased from Cell Marque (Rocklin, CA, USA): anti-MDM2 (clone IF2) and anti-CDK4 (clone DCS-31).

FISH

For FISH, 5-μm-thick paraffin sections were cut, baked at 60 °C, and heated at 95 °C in citrate buffer. Sections were then treated with a protease at 37 °C. The following probes were used: Vysis LSI MDM2 SpectrumOrange Probe, Vysis CEP 12 (D12Z3) SpectrumGreen Probe (Abbott, Abbott Park, IL, USA), and ZytoLight SPEC CDK4/CEN 12 Dual Color Probe (ZytoVision GmbH, Bremerhaven, Germany). Evaluation of *MDM2* and *CDK4* gene amplification was carried out as described previously [13, 14]. The genes were considered amplified when the ratio of gene-specific signals to centromeric signals was equal to or greater than 2.0 (i.e., MDM2/CEP12 ≥ 2.0 and CDK4/CEN12 ≥ 2.0, Fig. 2).

Statistical analysis

The *t*-test or Fisher's exact test was used for each variable. The level of significance was set at $p < 0.05$. A receiver-operating characteristic (ROC) curve was used to assess

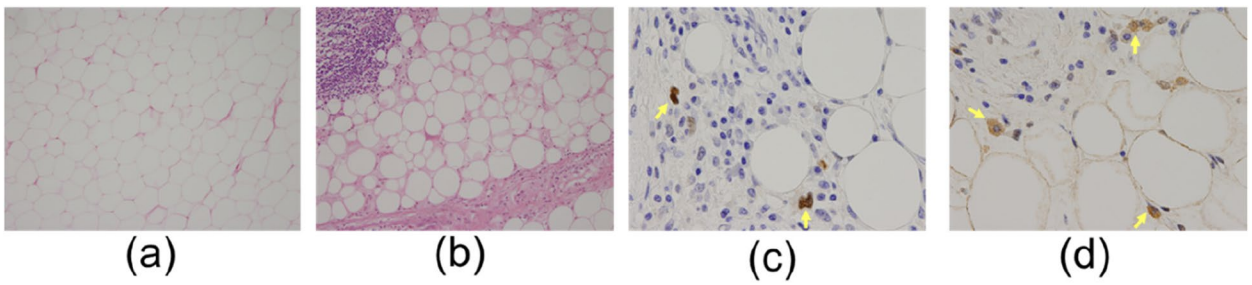

Fig. 1 HE staining and immunostaining. **a** HE staining of lipomas. The tumors are composed of mature adipocytes that show no obvious difference in size. **b** HE staining of ALT/WDLPSs. Adipocytes show variation in cell size. Inflammatory infiltrates and fibrous septa can also be observed. **c** Immunostaining of ALT/WDLPSs for MDM2. Positive nuclear staining for MDM2 is indicated by arrows. **d** Immunostaining of ALT/WDLPSs for CDK4. Positive nuclear staining for CDK4 is indicated by arrows

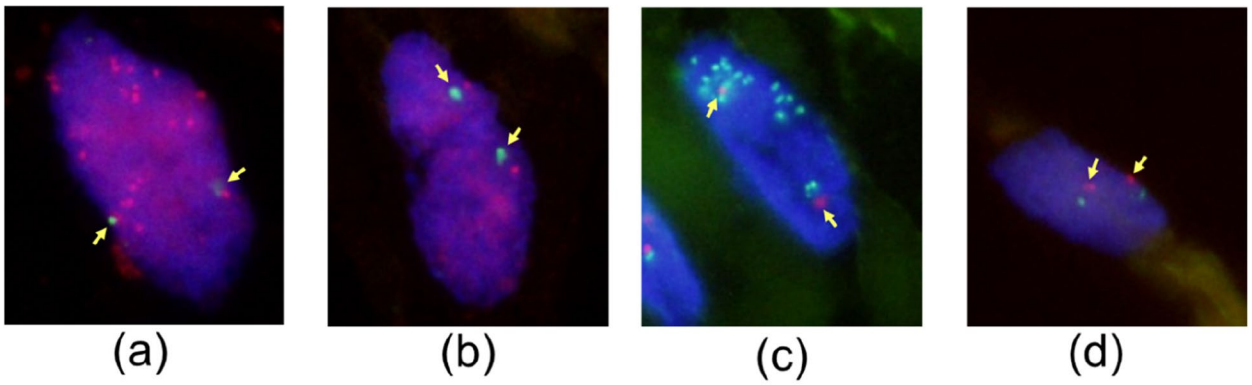

Fig. 2 FISH. **a** Representative image from an *MDM2*-positive case. The intensity of *MDM2* signals (red) is higher than that of centromeric signals (green, denoted by arrows). b Representative image from an *MDM2*-negative case. The intensity of *MDM2* signals (red) is not higher than that of centromeric signals (green, denoted by arrows). **c** Representative image from a *CDK4*-positive case. The intensity of *CDK4* signals (green) is higher than that of centromeric signals (red, denoted by arrows). **d** Representative image from a *CDK4*-negative case. The intensity of *CDK4* signals (green) is not higher than that of centromeric signals (red, denoted by arrows)

the performance of the parameter "maximum tumor diameter" for the diagnosis of ALT/WDLPS. Statistical software JMP 2.0 (Discovery LLC, Cary, NC, USA) was used for all analyses.

Results

Patients' background characteristics

All of 109 patients were Asian. The diagnosis of a lipomatous tumor had made on the basis of morphological features. There was no significant difference in age between the groups with lipoma (mean 59.4 years, range 8–86 years) and ALT/WDLPS (mean 63.5 years, range 33–83 years) (Table 1).

The sex distribution showed no significant difference between these two groups (lipoma, 26 men and 42 women; ALT/WDLPS, 26 men and 19 women). The mean maximum diameter of ALT/WDLPS (120.1 mm, range 36–272 mm) was significantly larger than that of lipoma (73.9 mm, range 10–196 mm). There was no significant difference in tumor location. Lipomas were located in the lower limb and the upper limb/trunk in 19 (27.9%) and 49 (72.1%) patients. ALT/WDLPSs were located at these sites in 18 (43.9%) and 23 (56.1%) patients, respectively. ALT/WDLPSs were found in deep-seated regions significantly more often than lipomas: superficial and deep-seated lipomas were detected in 39 (57.4%) and 29 (42.6%) patients, respectively. On the other hand, superficial and deep-seated ALT/WDLPSs were detected in 11 (26.8%) and 30 (73.1%) patients, respectively. No cases were identified involving the deep trunk, such as the mediastinum or retroperitoneum.

Results of immunostaining

Table 2 shows the results of MDM2 and CDK4 immunostaining of specimens from 109 patients with a lipomatous tumor. Of the 41 patients who had been diagnosed with ALT/WDLPS based on morphological characteristics, seven (17%) were positive on MDM2

Table 1 Patients' background characteristics and clinical data

		LIPOMA ($N=68$)	ALT/WDLPS ($N=41$)	*P*-VALUE
Age (y)	Mean (range)	59.4 (8–86)	63.5 (33–83)	0.123
Sex	Male:Female	26: 42	26: 19	0.054
Maximum tumor diameter (mm)	Mean (range)	73.9 (10–196)	120.1 (36–272)	**< 0.001**
Tumor site	Upper limb, Trunk	49 (72.1%)	23 (56.1%)	0.099
	Lower limb	19 (27.9%)	18 (43.9%)	
Tissue plane	Subcutaneous	39 (57.4%)	11 (26.8%)	**0.002**
	Subfascial	29 (42.6%)	30 (73.1%)	

Data are presented as mean (min–max) or number (%)

ALT/WDLPS atypical lipomatous tumor/well-differentiated liposarcoma

Bold font indicates significant difference with $p < 0.05$ by t-test or Fisher's exact test

Table 2 Results of MDM2 and CDK4 immunostaining according to morphological diagnosis

	MDM2(+) CDK4(+)	MDM2(+) CDK4(-)	MDM2(-) CDK4(+)	MDM2(-) CDK4(-)
Lipoma *n* = 68	0	2 (2.9%)	1 (1.5%)	65 (95.6%)
ALT/WDLPS *n* = 41	1 (2.4%)	6 (14.6%)	3 (7.3%)	31 (75.6%)

Data are presented as number (%)

ALT/WDLPS atypical lipomatous tumor/well-differentiated liposarcoma, *MDM2* Mouse double minute 2 homolog, *CDK4* Cyclin-dependent kinase 4

immunostaining. There were two (2.9%) MDM2-positive cases among the 68 patients who had been diagnosed with a lipoma. As for CDK4, staining was positive in four (9.7%) of the 41 ALT/WDLPS patients, and one (1.5%) of the 68 lipoma patients was also positive for this protein. Thus, the rate of concordance (i.e., both positive or both negative) between MDM2 and CDK4 immunostaining was 88.9%.

Results of FISH (accuracy of morphological diagnosis)

The results of *MDM2* and *CDK4* FISH assays are shown in Table 3. Of the 41 patients who had been found to have ALT/WDLPS based on morphological diagnosis, 17 (41.5%) and 15 (36.6%) were positive for *MDM2* and *CDK4* amplification, respectively. Conversely, of the 68 patients who had been diagnosed with a lipoma, one was positive for both *MDM2* and *CDK4*. Individuals who were negative for *MDM2* and positive for *CDK4* were not found in either of these two patient groups. Two patients were positive only for *MDM2*, and 16 and 91 patients were positive and negative for both genes, respectively. Therefore, *MDM2* and *CDK4* FISH assays had a high concordance rate of 95.1%. Assuming that the definitive diagnosis of ALT/WDLPS can be made based on positive *MDM2* FISH, according to the present results, the sensitivity and specificity of morphological diagnosis were 41.5% and 98.5%, respectively.

Table 3 Results of *MDM2* and *CDK4* FISH according to morphological diagnosis

	***MDM2*(+) *CDK4*(+)**	***MDM2*(+) *CDK4*(-)**	***MDM2*(-) *CDK4*(+)**	***MDM2*(-) *CDK4*(-)**
Lipoma *n* = 68	1 (1.5%)	0	0	67 (98.5%)
ALT/WDLPS *n* = 41	15 (36.6%)	2 (4.9%)	0	24 (58.5%)

Data are presented as number (%)

ALT/WDLPS atypical lipomatous tumor/well-differentiated liposarcoma

MDM2 Mouse double minute 2 homolog, CDK4, Cyclin-dependent kinase 4

Diagnostic accuracy of immunostaining

The accuracy of MDM2 and CDK4 immunostaining in diagnosing lipoma and ALT/WDLPS was examined using the above assumption regarding the definitive diagnosis of ALT/WDLPS (Table 4).

The sensitivity and specificity of MDM2 immunostaining were 55.6% and 87.0%, respectively, and those of CDK4 immunostaining were 40.0% and 84.6%, respectively. Thus, both MDM2 and CDK4 immunostaining have lower specificity than morphological diagnosis. However, the sensitivity of CDK4 immunostaining is comparable to that of morphological diagnosis, and MDM2 immunostaining is more sensitive than morphological diagnosis.

Results of *MDM2* FISH and patient characteristics

Table 5 compared the background characteristics and clinical data between lipoma and ALT/WDLPS patients, based on the assumption that the definitive diagnosis of ALT/WDLPS can be made by positive *MDM2* FISH results.

There were 91 lipoma and 18 ALT/WDLPS patients. The mean age of ALT/WDLPS patients (71.2 years, range 55–83 years) was significantly older than that of lipoma patients (59.0 years, range 8–86 years). The sex distribution showed no significant difference between the lipoma (44 men and 47 women) and ALT/WDLPS (5 men and 13 women) patient groups. The mean maximum diameter was significantly greater for ALT/WDLPS (150.1 mm, range 43–270 mm) than for lipoma (76.1 mm, range 10–226 mm). On ROC curve analysis of maximum tumor diameter, the area under the curve (AUC) was 0.854 (95% confidence interval 0.737–0.972), with the optimal cut-off value of 125 mm (Fig. 3).

Table 4 Results of MDM2 and CDK4 immunostaining according to the outcomes of *MDM2* FISH

	Immunohistochemistry			
	MDM2(+) CDK4(+)	**MDM2(+) CDK4(-)**	**MDM2(-) CDK4(+)**	**MDM2(-) CDK4(-)**
FISH *MDM2*-negative (lipoma) *n* = 91	0	4 (4.4%)	3 (3.3%)	84 (92.3%)
FISH *MDM2*-positive (ALT/WDLPS) *n* = 18	1 (5.6%)	4 (22.2%)	1 (5.6%)	12 (66.7%)

Data are presented as number (%)

ALT/WDLPS atypical lipomatous tumor/well-differentiated liposarcoma

MDM2 Mouse double minute 2 homolog, *CDK4* Cyclin-dependent kinase 4, *FISH* Fluorescence in situ hybridization

Table 5 Patients' characteristics and clinical data when the definitive diagnosis of ALT/WDLPS was made by positive MDM2 FISH

		FISH *MDM2*-negative (Lipoma, $n=91$)	FISH *MDM2*-positive (ALT/WDLPS, $n=18$)	*P*-value
Age (y)	Mean (range)	59.0 (8–86)	71.2 (55–83)	**< 0.001**
Sex	Male:Female	44:47	5:13	0.126
Maximum tumor diameter (mm)	Mean (range)	76.1 (10–226)	150.1 (43–270)	**< 0.001**
Tumor site	Upper limb, Trunk	66 (72.5%)	4(22.2%)	**< 0.001**
	Lower limb	25 (27.5%)	14 (77.8%)	
Tissue plane	Subcutaneous	49 (53.8%)	1 (5.6%)	**< 0.001**
	Subfascial	42 (46.2%)	17 (94.4.%)	

Data are presented as mean (min–max) or number (%)

FISH Fluorescence in situ hybridization, *MDM2* Mouse double minute protein 2, *ALT/WDLPS* atypical lipomatous tumor/well-differentiated liposarcoma

Bold font indicates significant difference with $p < 0.05$ by t-test or Fisher's exact test

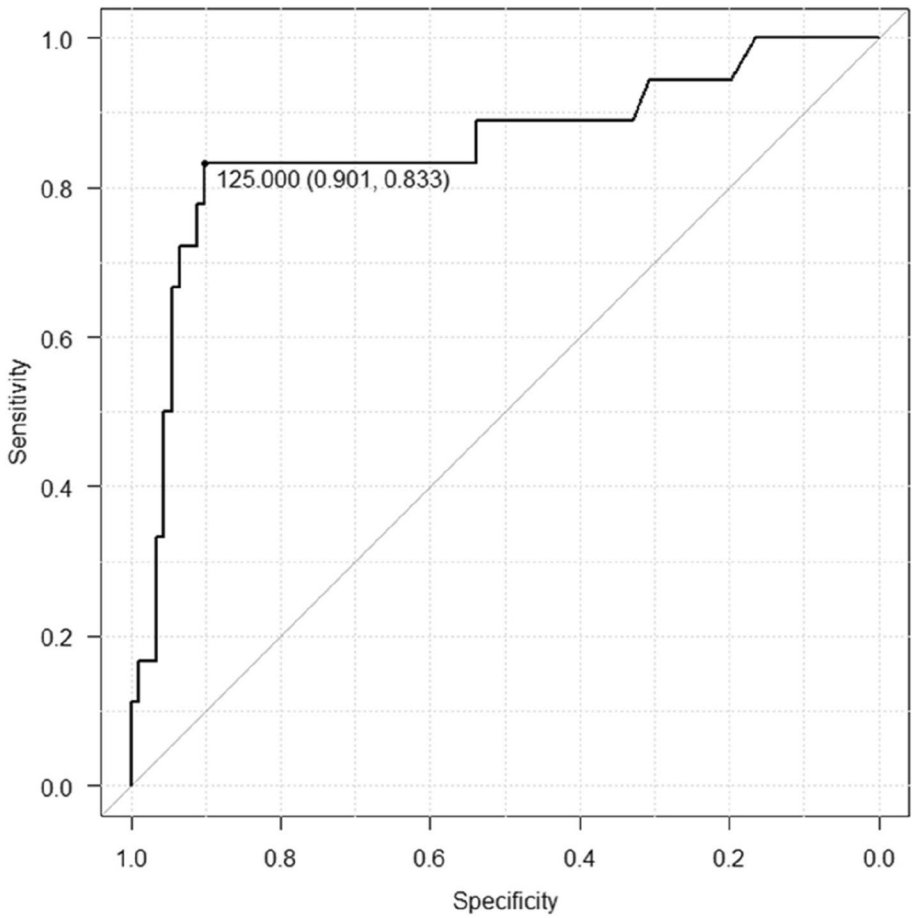

Fig. 3 ROC curve demonstrating the association between the maximum tumor diameter and ALT/WDLPS diagnosis. The AUC is 0.854 (95% confidence interval 0.737–0.972), and the optimal cut-off value is 125 mm

As for tumor location, ALT/WDLPSs were found in the lower limb significantly more often than lipomas. Lipomas were located in the lower limb and the upper limb/trunk in 25 (27.5%) and 66 (72.5%) patients, respectively, and ALT/WDLPSs were located at these sites in 14 (77.8%) and 4 (22.2%) patients, respectively. As for tissue

plane, ALT/WDLPSs arose in deep-seated locations significantly more frequently than lipomas. Superficial and deep-seated lipomas were detected in 49 (53.8%) and 42 (46.2%) patients, respectively, whereas superficial and deep-seated ALT/WDLPSs were detected in 1 (5.6%) and 17 (94.4%) patients, respectively.

Discussion

In this study, the aim was to elucidate the accuracy of the morphological diagnosis of lipoma and ALT/WDLPS. To this end, 109 patients who had been diagnosed with a lipomatous tumor before immunostaining became available for differentiating between lipoma and ALT/WDLPS were first identified retrospectively. Using tumor tissue specimens collected from these patients, immunostaining for MDM2 and CDK4, as well as FISH for their corresponding genes, was then performed. The results indicated that morphological diagnosis had low sensitivity and high specificity. In addition, the sensitivity of immunostaining was not particularly high, and its specificity was lower than that of morphological diagnosis. In addition, the present results showed the specific features of tumors that were definitively diagnosed as ALT/WDLPS based on the positive outcome of *MDM2* FISH. According to these features, ALT/WDLPSs are: (1) often found in elderly patients, (2) larger in size, (3) located in the lower limb, and (4) frequently found in deep-seated regions.

Generally, *MDM2* amplification identified by FISH is considered the gold standard for ALT/WDLPS diagnosis [15, 16]. When this standard was applied to the present data, the sensitivity and specificity of morphological diagnosis were found to be 41.5% and 98.5%, respectively. To date, no studies have investigated the accuracy of the morphological diagnoses of lipoma and ALT/WDLPS that were made prior to the application of immunostaining in the differentiation between these two tumors. In the present study, of the 68 patients who had been morphologically diagnosed as having a lipoma, only one (1.5%) was positive for *MDM2* amplification. This indicates that, in general, lipomas can be accurately diagnosed based on morphology, as long as the tumors exhibit their typical pathological characteristics. In contrast, of the 41 patients who had been morphologically diagnosed with ALT/WDLPS, 17 were positive for *MDM2* FISH, and more than half (24) of the patients did not show *MDM2* amplification. Based on this result, these 24 patients were re-diagnosed as having a lipoma. ALT/WDLPS is a soft tissue tumor of intermediate malignant behavior with a potential for dedifferentiation, and its recurrence rate is higher than that of lipoma. Therefore, it is imperative to avoid underdiagnosing ALT/WDLPSs as lipomas. In an attempt to circumvent this problem of underdiagnosis, certain benign tumors were presumably overdiagnosed as ALT/WDLPSs in our hospital during the period selected for this study. These benign tumors likely include: lipomas harboring lipoblast-like cells (including lipomas with degenerative changes); large lesions with the clinical appearance of malignant tumors; and deep-seated tumors. Thus, unless tumors can be clearly diagnosed as lipomas on the basis of morphology, the use of FISH for the confirmation of *MDM2* amplification would be beneficial for preventing these overdiagnosis cases.

When the definitive diagnosis of ALT/WDLPS was made based on the positive results of *MDM2* FISH, the sensitivity and specificity of MDM2 immunostaining were 55.6% and 87.0%, respectively. These values were both lower than those reported by Binh et al. [9]. Previous studies have found a high concordance rate between MDM2/CDK4 immunostaining and *MDM2* FISH. However, according to Clay et al. [17], the reason for this high concordance rate is that these studies preferentially investigated cases where the diagnosis could be readily established histologically. They reported that, when they analyzed cases of lipomatous tumors with ambiguous histological features, immunostaining for MDM2 and CDK4 had a sensitivity of 45% and 41%, respectively, and a specificity of 98% and 92%, respectively. In the present study, the sensitivity of MDM2 immunostaining was low (55.6%), although it was higher than that of morphological diagnosis. The specificity of the staining was also low (87.0%), resulting in the underdiagnosis of ALT/WDLPS. Thus, due to their relatively low sensitivity, immunostaining for MDM2 and CDK4 is not a reliable tool for diagnosing ALT/WDLPS in routine clinical settings. Although MDM2/CDK4 immunostaining is a simple and easy technique, the use of FISH is strongly recommended in cases where the immunostaining cannot completely rule out the possibility of ALT/WDLPS.

When *MDM2* amplification detected by FISH was used for definitively diagnosing ALT/WDLPS, the present study participants were found to include 91 lipoma and 18 ALT/WDLPS patients. Previous studies demonstrated that the predictive factors for ALT/WDLPS were: patient age (older) [18, 19], depth (deep-seated) [19–21], and tumor location (lower limb) [18, 21–23]. The present results are in line with these conclusions. It has also been shown that a large (≥10–15 cm) tumor is another indicator of ALT/WDLPS [12, 18, 21, 22]. Consistent with this finding, the mean maximum diameter of ALT/WDLPSs in the present study was 150.1 mm, which was significantly larger than that of lipomas. On ROC curve analysis of the maximum tumor diameter, the optimal cut-off value for differentiating ALT/WDLPS from a lipoma was 125 mm. A similar cut-off

value of 130 mm was previously obtained in a study that performed FISH on specimens from 113 patients with a lipomatous tumor [19]. Taken together, the present and previous studies show that *MDM2* FISH should be conducted when lipomatous tumors display the clinical characteristics described above [i.e., older age, tumor location (deep-seated, lower limb), and large tumor size]. As for morphological diagnosis, clinical features suggesting ALT/WDLPS have been reported to be the presence of cytologic atypia [8]. The key feature is the presence of enlarged, hyperchromatic stromal cells within irregular fibrous bands, usually with a distinctive, finely fibrillar appearance. The present study also showed that the morphological diagnosis of lipoma is largely reliable. Previously, Zhang et al. [20] suggested indications for performing FISH assays in diagnosing lipomatous tumors located in the trunk and extremities. They were: cytologic atypia, recurrent lipomas, retroperitoneal tumors, and deep-seated tumors without cytologic atypia larger than 15 cm. In the present study, only one ALT/WDLPS patient had been underdiagnosed as having a lipoma. Morphologically, the tumor in this patient contained multivacuolated lipoblast-like cells. However, there were no clear findings suggestive of malignancy. Since lipomas with degenerative changes are known to show characteristics similar to those of ALT/WDLPSs, the final diagnosis of this patient was an intramuscular lipoma. However, the patient was a 64-year-old woman, and the tumor was located in the femoral muscle. Furthermore, the maximum tumor diameter was 154 mm. Thus, there were clinical findings that suggested a diagnosis of ALT/WDLPS. The present study indicates that, when tumors are clearly diagnosed based on morphological features, they do not usually need to be investigated by FISH. However, as in the above case, certain clinical features [such as age (older), tumor location (lower limb), depth (deep-seated), and size (larger)] increase the likelihood of ALT/WDLPS. Preventing the underdiagnosis of ALT/WDLPS is critical in clinical practice. Thus, in the presence of these clinical features, lipomatous tumors should be examined by FISH, even when they do not exhibit cytologic atypia.

Limitations

The MRI findings analyzed in this study were limited to the maximum diameter and depth of tumors. There were no detailed investigations regarding other MRI characteristics, such as contrast effects and the presence or absence of septation and nonadipose components. No cases involving the deep trunk were identified. Therefore, we were unable to examine WDLPS arising in deep sites, such as the mediastinum or retroperitoneum. Although a history of rapid growth is important to distinguish lipoma from ALT/WDLPS, this could not be examined because we were unable to collect detailed information for all cases.

Conclusions

The accuracy of morphological diagnosis was examined by performing immunostaining and FISH on specimens from 109 patients with a lipomatous tumor. These patients had been diagnosed solely by morphology before immunostaining was applied to the diagnosis of lipomatous tumors. The results demonstrated that lipomas can be accurately diagnosed by morphology alone. On the other hand, ALT/WDLPSs were overdiagnosed. They indicated that, if a morphology-based diagnosis of a lipomatous tumor is not straightforward, it must be confirmed by *MDM2* FISH. In addition, based on the present results, we strongly recommend the use of *MDM2* FISH for definitive diagnosis when ALT/WDLPS is suspected by certain clinical findings, such as tumor location (lower limb), depth (deep-seated), size (larger), and patient age (older).

Abbreviations

FISH	Fluorescence in situ hybridization
MRI	Magnetic resonance imaging
ALT/WDLPS	Atypical lipomatous tumor/well-differentiated liposarcoma
DDLPS	Dedifferentiated liposarcoma
MDM2	Mouse double minute 2 homolog
CDK4	Cyclin-dependent kinase 4
CEP12	Centromeric probe for chromosome 12
HE	Hematoxylin and eosin

Acknowledgements

The authors are grateful to Prof. Junya Fukuoka of the Department of Pathology Informatics, Nagasaki University Graduate School of Biomedical Sciences for useful discussion and help with editing the manuscript. The authors thank FORTE Science Communications (https://www.forte-science.co.jp/) for English language editing.

Authors' contributions

KN and MT designed the study, collected patients' information, and analyzed data. KK and MS performed immunostaining and FISH, respectively. KN, KC, AY, and MO analyzed data and wrote the paper. All authors read and approved the final manuscript.

Funding

Not applicable.

Data availability

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

This study was approved by the Clinical Research Ethics Committee of Nagasaki University Hospital (Approval Number: 17061909–3). Written informed consent was waived by this committee for this retrospective analysis of routinely acquired imaging and clinical data. The outline of this research was published on the homepage of the Clinical Research Center of Nagasaki University Hospital. This guaranteed the patients and any other individuals the opportunity to refuse participation in the study. We did not receive any refusion of participation. Each author certifies that all investigations were conducted in conformity with the ethical principles of research.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

Author details
[1]Department of Orthopaedic Surgery, Nagasaki University Graduate School of Biomedical Sciences, 1-7-1 Sakamoto, Nagasaki 852-8501, Japan. [2]Department of Pathology Informatics, Nagasaki University Graduate School of Biomedical Sciences, 1-7-1 Sakamoto, Nagasaki 852-8501, Japan. [3]Cancer Genomic Unit Clinical Genome Center, Nagasaki University Hospital, 1-7-1 Sakamoto, Nagasaki 852-8501, Japan.

Received: 19 March 2024 Accepted: 18 November 2024
Published online: 21 November 2024

References

1. Sbaraglia M, Bellan E, Dei Tos AP. The 2020 WHO Classification of Soft Tissue Tumours: News and perspectives. Pathologica. 2021;113(2):70–84. https://doi.org/10.32074/1591-951X-213.
2. Szymanska J, Virolainen M, Tarkkanen M, Wiklund T, Asko-Seljavaara S, Tukiainen E, Elomaa I, Blomqvist C, Knuutila S. Overrepresentation of 1q21-23 and 12q13-21 in lipoma-like liposarcomas but not in benign lipomas: a comparative genomic hybridization study. Cancer Genet Cytogenet. 1997;99(1):14–8. https://doi.org/10.1016/s0165-4608(96)00436-0. (PMID: 9352790).
3. Kammerer-Jacquet SF, Thierry S, Cabillic F, et al. Differential diagnosis of atypical lipomatous tumor/well-differentiated liposarcoma and dedifferentiated liposarcoma: utility of p16 in combination with MDM2 and CDK4 immunohistochemistry. Hum Pathol. 2017;59:34–40. https://doi.org/10.1016/j.humpath.2016.08.009.
4. Dei Tos AP. Liposarcomas: Diagnostic pitfalls and new insights. Histopathology. 2014;64(1):38–52. https://doi.org/10.1111/his.12311.
5. Stout AP. Liposarcoma-the Malignant Tumor of Lipoblasts. Ann Surg. 1944;119(1):86–107.
6. Hisaoka M. Lipoblast: morphologic features and diagnostic value. J UOEH. 2014;36(2):115–21. https://doi.org/10.7888/juoeh.36.115.
7. Nilbert M, Rydholm A, Mitelman F, Meltzer PS, Mandahl N. Characterization of the 12q13-15 Amplicon in Soft Tissue Tumors. Cancer Genet Cytogenet. 1995;83(1):32–6. https://doi.org/10.1016/s0165-4608(95)00016-x.
8. Weaver J, Downs-Kelly E, Goldblum JR, et al. Fluorescence in situ hybridization for MDM2 gene amplification as a diagnostic tool in lipomatous neoplasms. Mod Pathol. 2008;21(8):943–9. https://doi.org/10.1038/modpathol.2008.84.
9. Binh MBN, Sastre-Garau X, Guillou L, et al. MDM2 and CDK4 immunostainings are useful adjuncts in diagnosing well-differentiated and dedifferentiated liposarcoma subtypes: A comparative analysis of 559 soft tissue neoplasms with genetic data. Am J Surg Pathol. 2005;29(10):1340–7. https://doi.org/10.1097/01.PAS.0000170343.09562.39.
10. Ramos-Vara JA. Technical Aspects of Immunohistochemistry. Vet Pathol. 2005;42:405–26.
11. Langer-Safer PR, Levine M, Ward DC. Immunological Method for Mapping Genes on Drosophila Polytene Chromosomes. Proc Natl Acad Sci U S A. 1982;79(14):4381–5. https://doi.org/10.1073/pnas.79.14.4381.
12. Ryan S, Visgauss J, Kerr D, et al. The Value of MRI in Distinguishing Subtypes of Lipomatous Extremity Tumors Needs Reassessment in the Era of MDM2 and CDK4 Testing. Sarcoma. 2018;2018:1901896. https://doi.org/10.1155/2018/1901896. (eCollection 2018).
13. Wong DD, Low IC, Peverall J, et al. MDM2/CDK4 gene amplification in large/deep-seated "lipomas": Incidence, predictors and clinical significance. Pathology. 2016;48(3):203–9. https://doi.org/10.1016/j.pathol.2016.02.007.
14. Sirvent N, Coindre JM, Maire G, et al. Detection of MDM2-CDK4 Amplification by Fluorescence In Situ Hybridization in 200 Paraffin-embedded Tumor Samples: Utility in Diagnosing Adipocytic Lesions and Comparison With Immunohistochemistry and Real-time PCR. Am J Surg Pathol. 2007;31(10):1476–89. https://doi.org/10.1097/PAS.0b013e3180581fff.
15. Sciot R. Mdm2 amplified sarcomas: A literature review. Diagnostics. 2021;11(3):496. https://doi.org/10.3390/diagnostics11030496.
16. Weaver J, Rao P, Goldblum JR, et al. Can MDM2 analytical tests performed on core needle biopsy be relied upon to diagnose well-differentiated liposarcoma. Mod Pathol. 2010;23(10):1301–6. https://doi.org/10.1038/modpathol.2010.106.
17. Clay MR, Martinez AP, Weiss SW, Edgar MA. MDM2 and CDK4 immunohistochemistry: Should it be used in problematic differentiated lipomatous tumors?: A new perspective. Am J Surg Pathol. 2016;40(12):1647–52. https://doi.org/10.1097/PAS.0000000000000713.
18. Birdn JE, Morse LJ, Feng L, et al. Non-radiographic risk factors differentiating atypical lipomatous tumors from lipomas. Front Oncol. 2016;22(6):197. https://doi.org/10.3389/fonc.2016.00197.
19. Knebel C, Neumann J, Schwaiger BJ, et al. Differentiating atypical lipomatous tumors from lipomas with magnetic resonance imaging: A comparison with MDM2 gene amplification status. BMC Cancer. 2019;19(1):309. https://doi.org/10.1186/s12885-019-5524-5.
20. Zhang H, Erickson-Johnson M, Wang X, et al. Molecular Testing for Lipomatous Tumors: Critical Analysis and Test Recommendations Based on the Analysis of 405 Extremity-Based Tumors. Am J Surg Pathol. 2010;34(9):1304–11. https://doi.org/10.1097/PAS.0b013e3181e92d0b.
21. Brisson M, Kashima T, Delaney D, et al. MRI characteristics of lipoma and atypical lipomatous tumor/well- differentiated liposarcoma: Retrospective comparison with histology and MDM2 gene amplification. Skeletal Radiol. 2013;42(5):635–47. https://doi.org/10.1007/s00256-012-1517-z.
22. Asano Y, Miwa S, Yamamoto N, et al. A scoring system combining clinical, radiological, and histopathological examinations for differential diagnosis between lipoma and atypical lipomatous tumor/well-differentiated liposarcoma. Sci Rep. 2022;12(1):237. https://doi.org/10.1038/s41598-021-04004-1.
23. Kalimuthu SN, Tilley C, Forbes G, et al. Clinical outcome in patients with peripherally-sited atypical lipomatous tumours and dedifferentiated liposarcoma. J Pathol: Clin Res. 2015;1(2):106–12. https://doi.org/10.1002/cjp2.12.

Publisher's Note