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MDM2 dual-color in situ hybridization (DISH) aids the diagnosis of intimal sarcomas

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ABSTRACT

Intimal sarcoma is a rare malignant mesenchymal tumor arising from the intima of the great vessels and the heart, and is associated with poor outcomes. As clinico-radiological findings and pathological features are often non-specific, the diagnosis of intimal sarcoma is challenging. Recently, *MDM2* amplification was reported to be a characteristic genetic event in this tumor. In the present study, we examined MDM2 status by immunohistochemistry, and by fluorescence and dual-color in situ hybridization (FISH and DISH) using intimal sarcoma (10 tumors), angiosarcoma (5), pulmonary sarcomatoid carcinoma (p-SC) (14) and chronic pulmonary thrombosis (CPT) (3) to investigate *MDM2* amplification for the diagnosis of intimal sarcoma. MDM2 and CDK4 were immunopositive in all 10 intimal sarcoma tumors, and high-level amplification of *MDM2* was detected in eight tumors by both FISH and DISH. The other two tumors had polysomy of chromosome 12 and overexpression of p53 protein. Although *MDM2* aberrations were observed in three p-SCs (two with amplification and one with polysomy), angiosarcomas and CPTs lacked *MDM2* amplification. Furthermore, there was high concordance between FISH and DISH. In conclusion, we found that *MDM2* amplification strongly supports the diagnosis of intimal sarcoma, and *MDM2* DISH was a concordant method and an acceptable alternative to FISH. As *MDM2* amplification and p53 overexpression were mutually exclusive, disruption of the MDM2-p53 pathway may be an essential genetic event for this malignant tumor.

1. Introduction

Intimal sarcoma was first reported by Mandelstam in 1923 and is a rare malignant mesenchymal tumor originating from the intima of major large vessels and the heart. By definition, this tumor has predominantly intraluminal polypoid growth with obstruction of the vessel lumen [1,2]. Histologically, intimal sarcoma has features similar to undifferentiated to poorly differentiated sarcoma with or without focal heterologous components such as leiomyosarcoma, rhabdomyosarcoma, osteosarcoma, and chondrosarcoma [3,4]. The prognosis is poor because of the difficulty of complete excision, embolic dissemination, and resistance to chemotherapy and radiotherapy, and the mean survival is approximately 18 months [4,5].

The diagnosis of intimal sarcoma is challenging clinically and pathologically, and difficulty in diagnosis sometimes delays treatment. Intimal sarcoma of the pulmonary artery, the most common site of this tumor, is often misdiagnosed as pulmonary artery thromboembolism clinically. Radiologically, it is difficult to decide whether the tumor originated from the intima or invaded from outside. Furthermore, microscopic and immunohistochemical profiles vary greatly and lack specific diagnostic features or markers. Therefore, genetic characteristics are important for the correct diagnosis of intimal sarcomas.

Amplification or gain of 12q12–15, 4q12, and 7p12 are the most common chromosome abnormalities in intimal sarcomas [6,7]. At a genetic level, amplification or gain of *MDM2*, *CDK4*, *GLI* (located on 12q12–15), *PDGFRA* (4q12), and *EGFR* (7p12) frequently occur in intimal sarcomas based on fluorescence in situ hybridization (FISH) or microarray -based comparative genomic hybridization (CGH) [7,8]. Co-amplification or gain in *PGDFRA*, *EGFR*, and/or *MDM2* is frequently observed in intimal sarcomas by FISH analysis [9]. Amplification or gain of *MDM2* is found in 64–100% of intimal

Abbreviations: FISH, fluorescence in situ hybridization; DISH, dual-color in situ hybridization; IHC, immunohistochemistry; PCR, polymerase chain reaction; CGH, comparative genomic hybridization; CEP, chromosome enumeration probes; MDM2, mouse double minute 2; CDK4, cyclin-dependent kinase 4; GLI, glioma-associated oncogene homolog 1; PDGFA, platelet-derived growth factor receptor a; EGFR, epidermal growth factor receptor.

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sarcomas, whereas amplification or gain of *PDGFRA* and *EGFR* occurs in 37.5–100% and 6.7–76%, respectively [6–11]. Therefore, *MDM2* amplification is the most important genetic aberration of intimal sarcoma and may improve diagnostic accuracy. Moreover, confirming *MDM2* amplification may be important for novel targeted therapies such as small molecule *MDM2* inhibitors [12].

FISH, CGH, quantitative polymerase chain reaction (PCR), and immunohistochemistry (IHC) have been used to identify *MDM2* amplification or overexpression of intimal sarcoma. FISH-mediated detection of *MDM2* amplification is more sensitive and specific than quantitative-PCR and IHC for lipomatous tumors [13]. Therefore, FISH is the standard method to detect *MDM2* amplification. Automated brightfield dual-color in situ hybridization (DISH) has also been used to detect *MDM2* amplification [14–16]. In this study, we examined the usefulness of DISH for *MDM2* amplification for the diagnosis of intimal sarcoma as compared with FISH and IHC.

2. Materials and methods

2.1. Patient selection

We examined a total of 10 intimal sarcoma specimens (8 surgical specimens and 2 biopsies) from 10 patients between 2003 and 2018 at Kobe University Hospital. Angiosarcoma of the great vessels (n=5), pulmonary sarcomatoid carcinoma (p-SC) (n=14), and chronic pulmonary thrombosis (CPT) (n=3) were used for comparison. This study was approved by the ethics committee at Kobe University (no. 160095). All patients gave informed consent before collection of the samples.

2.2. IHC

Formalin-fixed and paraffin-embedded specimens were used for histopathological and immunohistochemical examination. Four-micrometer-thick sections were cut for hematoxylin and eosin (H&E) and immunohistochemical staining. Immunostaining was performed on a Ventana Benchmark XT (Ventana Medical Systems, Tucson, AZ, USA) or Bond Max autostainer (Leica Microsystems, Wetzlar, Germany). The detailed information for the primary antibodies used in this study is listed in Table 1. The expression levels of these antibodies were estimated by a three-scale scoring system: negative (<10% positive cells), focal (10–50% positive cells), or diffuse (>50% positive cells). MDM2 and CDK4 IHC were also estimated semi-quantitatively based on immunostaining intensity and the percentage of immunopositive tumor cells to compare the scoring indices (IHC score) with the results of *MDM2* FISH and *MDM2* DISH. The immunostaining intensity was scored as 0 (negative), 1+ (weak to moderate), or 2+ (strong). The percentage of positive tumor cells in an entire section and the intensity of immunoreactivity were multiplied to give a scoring index (IHC score) ranging from 0 to 200 with 10 intervals.

2.3. FISH and DISH

Both FISH and DISH analyses were performed on all 32 specimens using formalin-fixed paraffin-embedded 4-μm-thick sections. FISH testing was performed with two DNA probes (Sure FISH; Agilent Technologies, Santa Clara, CA, USA). One probe was labeled with Cy3 (orange-red) hybridized with the 180-kb region on 12q15, which covers *MDM2*, and the other probe was labeled with FITC (green) hybridized with the 704-kb region on the centromere of chromosome 12 (CHR 12). After deparaffinization, slides were incubated in a pre-treatment solution at 98 °C for 10 min and then incubated using pepsin droplets to digest proteins at 37 °C for 10 min. After dehydration, the tissue sections were hybridized with probes using an IQFISH Fast Hybridization Protocol (incubation at 80 °C for 10 min followed by incubation at 45 °C for 90 min). Then, slides were washed with wash buffer at 63 °C for 10 min followed by counterstaining with DAPI. The reaction product was observed on a fluorescent microscope (AX80, Olympus, Tokyo, Japan).

DISH testing was performed with an automated staining system (Ventana BenchMark XT system, Ventana Medical Systems). The MDM2 Dual ISH probe cocktails (Ventana Medical Systems) consisted of an *MDM2* dinitrophenyl (DNP) probe, which was hybridized with an approximately 628-kb region of 12q15, and a CHR 12 digoxigenin (DIG) probe. *MDM2* was visualized as black signals with the ultraView SISH DNP Detection Kit (Ventana Medical Systems), whereas CHR12 signals were colored in red with the ultraView Red ISH DIG Detection Kit (Ventana Medical Systems).

The number of *MDM2* and CHR12 signals in the nuclei for both methods were counted for at least 20 nuclei for each specimen. When the signal ratio of *MDM2*/CHR 12 was over 2, *MDM2* was considered amplified. A parallel increase in the signal numbers of *MDM2* and CHR 12 of more than 3 was defined as polysomy.

2.4. Statistical analysis

We used Pearson correlation analysis and calculated p-values for two-variable correlation with the TDIST function in Microsoft Excel (Microsoft, Redmond, WA, USA) using the calculated t-values. A probability of P<.05 was considered significant.

3. Results

3.1. Clinical findings

The clinical information of patients with intimal sarcoma is listed in Table 2. Eight patients were female and two were male. The median age of the patients was 54 years (32–75 years). Clinically, five of nine tumors involving the pulmonary artery/trunk were correctly diagnosed as pulmonary artery tumors, but the rest were misdiagnosed as chronic pulmonary thrombosis (CPT) (2 cases) or

Table 1
Summary of the primary antibodies

Antibody	M/R	M/P	Clone number	Dilution	Resource
MDM2	M	M	1F2	1:100	Invitrogen, Carlsbad, USA
CDK4	M	M	DCS-31	1:100	Santa Cruz Biotechnology, Santa Cruz, USA
CK AE1/AE3	M	M	AE1/AE3	1:5	Dako Cytomation, Glostrup, Denmark
p53	M	M	DO-7	Prediluted	Dako Cytomation, Glostrup, Denmark
A-SMA	M	M	1A4	Prediluted	Dako Cytomation, Glostrup, Denmark
ERG	R	M	EPR 3864	Prediluted	Roche Diagnostics GmbH, Mannheim, Germany
CD31	M	M	JC70A	Prediluted	Dako Cytomation, Glostrup, Denmark
CD34	M	M	NU-4A1	Prediluted	Nichirei, Tokyo, Japan
S-100	R	P	-	1:2000	Dako Cytomation, Glostrup, Denmark
SOX-10	R	P	-	1:20	Cell Marque, Rocklin, USA
TTF-1	M	M	8G7G3/1	1:100	Dako Cytomation, Glostrup, Denmark
p40	M	M	BC28	Prediluted	Nichirei, Tokyo, Japan

Table 2
Summary of the clinical information of the intimal sarcoma patients

Case no.	Age	Sex	Initial clinical diagnosis	Tumor site	Additional involvement	Diagnostic procedure	Additional treatment	Outcome, survival period
1	56	M	Pulmonary artery tumor	PT, PA	Lung	PEA	CT + RT	Died, 75 m
2	55	F	Pulmonary artery tumor	PV, PT	None	PEA	None	Died, 60 m
3	54	F	CPT	PT, PA	None	PEA	CT	Died, 6 m
4	69	F	Pulmonary artery tumor	PT, PA	Lung, right ventricle	PEA	None	Died, 1 m
5	73	F	Pulmonary parenchymal tumor	PA	Lung	Open biopsy	RT	Died, 18 m
6	53	F	CPT	PA	Lung	PEA	PNX + CT + RT	Died, 20 m
7	32	M	Pulmonary parenchymal tumor	PA	Lung	VATS	PNX + RT + CT + TKI	Died, 18 m
8	44	F	Pulmonary artery tumor	PA	Lung	PEA	Lobectomy+CT + TKI	Alive, 13 m
9**	75	F	Gastric tumor	Left atrium	Stomach	Gastric biopsy	CT	Died, 17 m
10	46	F	Pulmonary artery tumor	PA	None	PEA	PNX	Alive, 5 m

M, male; F, female; CPT, chronic pulmonary thrombosis; PT, pulmonary trunk; PV, pulmonary valve; PA, pulmonary artery; PEA, pulmonary endarterectomy; VATS, video-assisted thoracic surgery; PNX, pneumonectomy; CT, chemotherapy; RT, radiotherapy; TKI, thyrosin kinase inhibitor; m, months from the time of the diagnostic procedure. **Autopsy case.

pulmonary parenchymal tumor. Of note, one (Case 6) of two suspected CPT patients had received treatment for CPT for more than 1 year. In addition, it took 10 months to diagnose intimal sarcoma in case 9. The pulmonary artery system was involved in nine cases, and only one tumor was predominantly located in the left atrium (case 9). Seven tumors also involved other organs, including the lung, right ventricle, and stomach, at the initial diagnosis. The most common diagnostic procedure was pulmonary endarterectomy (PEA). One patient (case 7) received video-assisted thoracoscopic surgery (VATS) followed by transbronchial biopsy (TBB). Two tumors (cases 5 and 9) were diagnosed as intimal sarcoma from small biopsy samples. Four of 10 patients underwent pneumonectomy (n=3) and lobectomy (n=1). Seven of 10 patients received adjuvant therapy, including chemotherapy (n=6), radiotherapy (n=4), and molecular target therapy (n=2), in varying combinations. All cases had follow-up data. The median survival period was 18 months (1–75 months). Seven of 10 patients died from persistent, recurrent, or metastatic tumors, and one patient (case 2) died from septic shock and brain damage associated with anaphylactic shock from antibiotics.

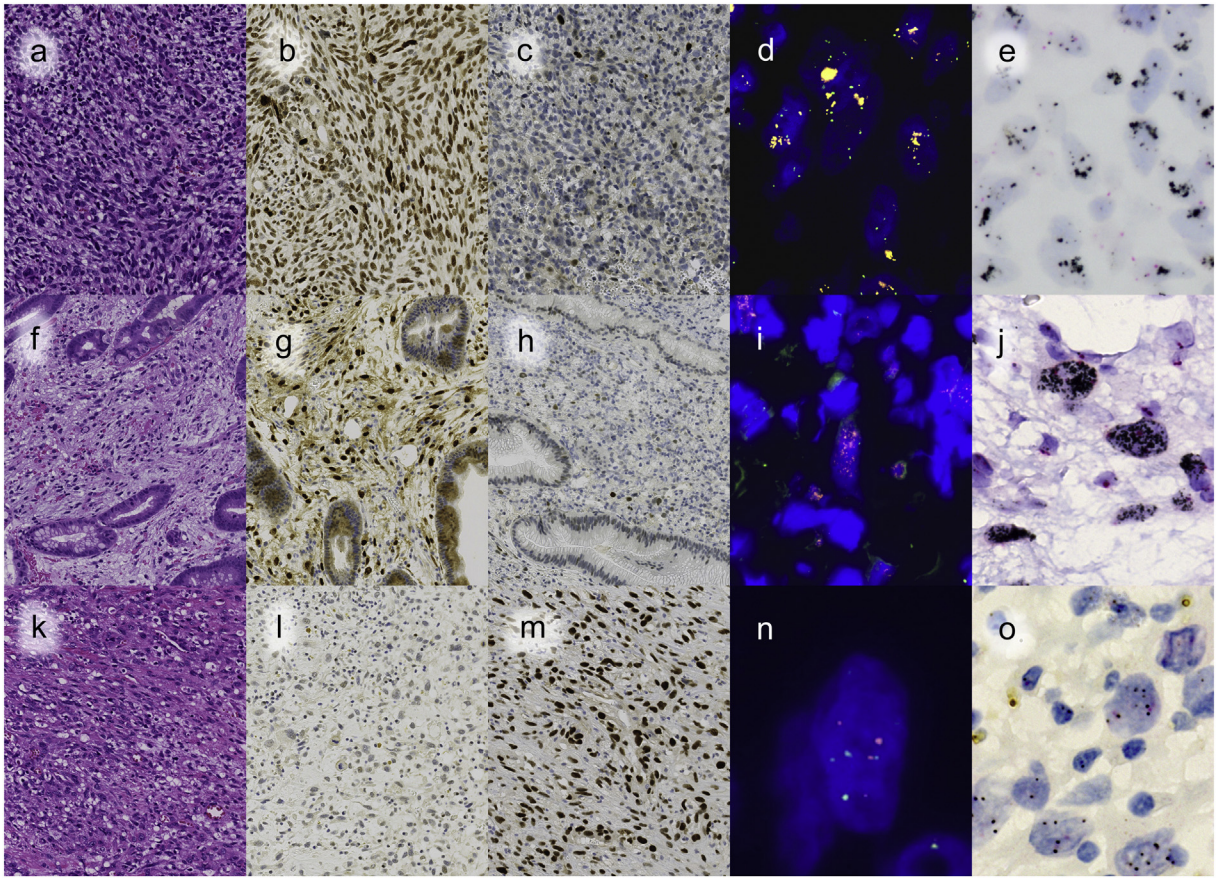

Fig. 1. Representative findings of intimal sarcoma. Intimal sarcoma case 6 (a-e): Resected sample of pulmonary intimal sarcoma with *MDM2* amplification. Note the diffuse proliferation of atypical spindle cells (a). Tumor cells were strongly positive for MDM2 (IHC score=160) (b) and almost negative for p53 (c). FISH and DISH showed high-level *MDM2* amplification (orange-red or black signal) as compared with chromosome 12 (green or red signal) (d, e). Intimal sarcoma case 9 (f-j): Gastric biopsy sample of metastatic intimal sarcoma with *MDM2* amplification. Atypical spindle cells had focally infiltrated between non-neoplastic gastric epithelium (f). Tumor cells were strongly positive for MDM2 (IHC score=140) (g) and almost negative for p53 (h). FISH and DISH showed high-level *MDM2* amplification (orange-red or black signal) as compared with chromosome 12 (green or red signal) (i, j). Intimal sarcoma case 2 (k-o): Resected sample of pulmonary intimal sarcoma with *MDM2* polysomy. Atypical spindle cells had diffusely infiltrated (k). Tumor cells were weakly and focally positive for MDM2 (IHC score=30) (l), and diffusely positive for p53 (m). FISH and DISH showed polysomy of chromosome 12 (green or red signal) without *MDM2* amplification (red or block signal) (n, o).

3.2. Pathological findings

Nine of 10 intimal sarcomas were macroscopically confirmed to exhibit intraluminal polypoid growth at the operation or autopsy (case 9), whereas the remaining tumor (case 5) was found to exhibit intraluminal growth in the pulmonary artery only by computed tomography (CT).

Histologically, all intimal sarcomas consisted of moderate-to-severe atypical spindle cells arranged focally in a storiform pattern with or without nuclear pleomorphism (Fig. 1a, f, k). Tumor cells were aggregated around and inside the vessel wall. All of the nine pulmonary intimal sarcomas invaded the media of the pulmonary artery, and three tumors also involved the adventitia. Three tumors had epithelioid cell morphology. The matrix was mainly collagenous and myxoid. Foci of osteosarcoma and chondrosarcoma areas were observed in one tumor (case 7).

All angiosarcomas consisted of atypical spindle cells, which formed irregular vascular lumens (Fig. 2a). Ten of 14 p-SCs contained differentiated non-small cell carcinoma components such as adenocarcinoma and squamous cell carcinoma, as well as sarcomatoid components, whereas the remaining consisted of pure spindle cell carcinoma (Fig. 2f, k).

3.3. Immunohistochemistry

Immunohistochemical results are shown in Tables 3 and 5. All cases of intimal sarcomas were positive for MDM2 (Fig. 1b, g, l) and CDK4, whereas half or less than half the cases of pulmonary sarcomatoid carcinomas (p-SCs) were positive. The expression of MDM2 and CDK4 was stronger and more diffuse in intimal sarcomas than in p-SCs (Table 3). The median IHC scores of MDM2 and CDK4 for intimal sarcomas were 100 (30–160) and 40 (30–200), respectively (Table 5). On the other hand, those of MDM2 and CDK4 for p-SCs were 25 (20–120) and 10 (0–40), respectively (data not shown). The expression of cytokeratin (AE1/AE3) was less frequent and less strong in intimal sarcomas than in p-SCs. P53 was diffusely positive in 2 of 10 intimal sarcomas and 6 of 14 p-SCs. A-SMA, ERG, CD31, CD34, S-100, and SOX10 were positive in 9, 5, 1, 2, 0, and 0 of 10 intimal sarcomas, respectively, and 11, 0, 1, 0, 3, and 0 of 14 p-SCs, respectively (Table 3). Among the cases of p-SCs, TTF-1 and p40 were positive in 3 and 3 of 14 tumors, respectively. The cases of angiosarcomas and CPTs demonstrated limited or no immunoreactivity for MDM2, CDK4, CK AE1/AE3, and p53. The immunophenotype of angiosarcomas was relatively uniform in contrast to intimal sarcomas as A-SMA, ERG, CD31, and CD34 were diffusely positive in most cases, and S-100 and SOX10 were negative in all cases. (See Table 3).

3.4. DISH and FISH

Hybridization for both *MDM2* FISH and *MDM2* DISH was successful in 29 of 32 (90.6%) specimens, including small biopsy specimens (cases 5 and 9). No signals were found in two angiosarcomas and one p-SC. (Table 4). *MDM2* status was identical by

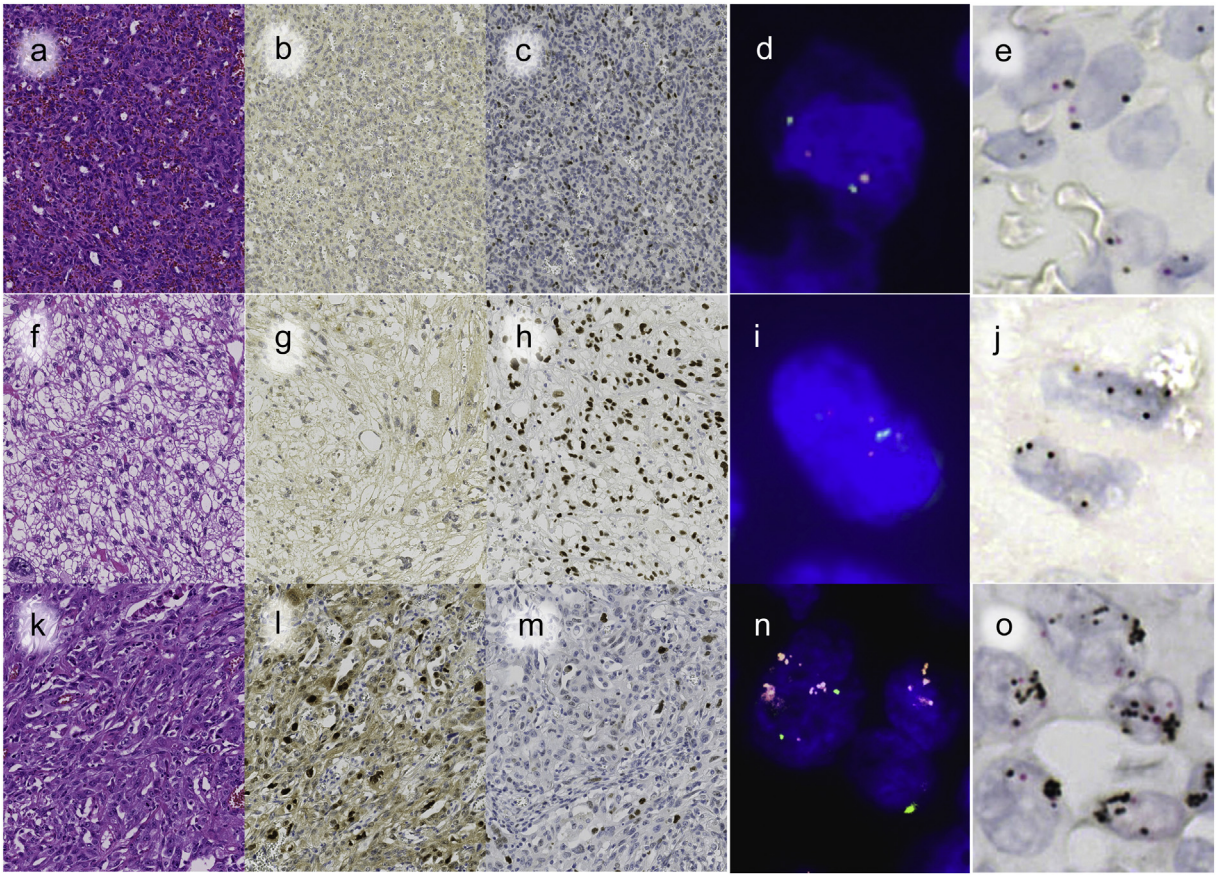

Fig. 2. Representative findings of angiosarcoma and p-SCs. Angiosarcoma (a-e): Resected sample of the right atrium with no *MDM2* amplification. Irregular vascular lumens were lined by spindle cells with moderate cytological atypia (a). Tumor cells were negative for MDM2 (IHC score=0) (b) and almost negative for p53 (c). FISH and DISH showed no *MDM2* amplification (orange-red or black signal) as compared with chromosome 12 (green or red signal) (d, e). p-SC (f-j): Resected sample of the right lower lung with *MDM2* polysomy. Atypical polygonal or spindle cells were arranged in sheets or nests (f). Tumor cells were almost negative for MDM2 (IHC score=20) (g), but diffusely positive for p53 (h). FISH and DISH showed polysomy of chromosome 12 (orange-red or black signal) without MDM2 amplification (green or red signal) (i, j). p-SC (k-o): Resected sample of the right lower lung with *MDM2* amplification. Atypical spindle cells had proliferated diffusely (k). Tumor cells were diffusely positive for MDM2 (IHC score=120) (l), but almost negative for p53 (m). FISH and DISH showed *MDM2* amplification (orange-red or black signal) as compared with chromosome 12 (green or red signal) (n, o).

Table 3
Results of immunohistochemistry

	Intimal sarcoma(n=10)		Angiosarcoma(n=5)		p-SC(n=14)		CPT(n=3)	
	Positive case	Focal/diffuse	Positive case	Focal/diffuse	Positive case	Focal/diffuse	Positive case	Focal/diffuse
MDM2	10	f1/d9	0	-	7	f5/d2	0	-
CDK4	10	f3/d7	0	-	5	f5/d0	0	-
CK AE1/AE3	5	f4/d1	0	-	14	f1/d13	0	-
p53	5	f3/d2	3	f3/d0	8	f2/d6	0	-
A-SMA	9	f4/d5	5	f1/d4	11	f11/d0	N/A	-
ERG	5	f4/d1	5	f0/d5	0	-	N/A	-
CD31	1	f1/d0	5	f0/d5	1	f0/d1	N/A	-
CD34	2	f1/d1	5	f0/d5	0	-	N/A	-
S-100	0	0	0	-	3	f3/d0	N/A	-
SOX10	0	0	0	-	0	-	N/A	-
TTF-1	N/A	-	N/A	-	3	f3/d0	N/A	-
p40	N/A	-	N/A	-	3	f2/d1	N/A	-

p-SC, pulmonary sarcomatoid carcinoma; CPT, chronic pulmonary thrombosis; CK, cytokeratin; A-SMA, alpha-smooth muscle actin; N/A, not applicable; negative, <10% positive cells; focal, 10–50% positive cells; and diffuse, >50% positive cells.

both FISH and DISH in all specimens (Table 4). All intimal sarcomas had *MDM2* abnormality. Eight of 10 tumors (80%) had high-level amplification of the *MDM2* gene (Fig. 1d, e, i, j; cases 6 and 9). The median ratios of *MDM2*/CEP12 in FISH and *MDM2*/CHR12 in DISH in these cases were 6.33 (3.6–13.4) and 7.93 (5.1–17.23), respectively (Table 4). More than 10 dot-like signals, each of which corresponded to one copy of the MDM2 gene, were often observed in single nuclei of tumor cells. The other two tumors (cases 2 and 10) had *MDM2*/CHR 12 polysomy (Fig. 1n, o). Of note, *MDM2*-amplified intimal sarcomas (8 tumors) exhibited no significant p53 immunoreactivity, whereas intimal sarcomas with *MDM2*/CHR12 polysomy demonstrated diffuse and strong p53 immunoreactivity, which suggested *TP53* gene aberration, and no MDM2 immunoreactivity (Fig. 1c, h, m; Table 5). These findings suggested that *MDM2* amplification and mutation of *TP53* were genetically exclusive. For the 22 cases that were not intimal sarcomas, two p-SC tumors had *MDM2* amplification and one p-SC had *MDM2*/CHR 12 polysomy. Two cases of *MDM2*-amplified p-SC tumors exhibited no significant p53 immunoreactivity (Fig. 2m), whereas one p-SC tumor with *MDM2*/CHR 12 polysomy exhibited strong p53 immunoreactivity (Fig. 2h). Ten cases of p-SCs with no *MDM2* abnormalities exhibited negative (n=6) or diffuse (n=4) p53 immunoreactivity.

Three p-SC cases with *MDM2* aberrations (two with amplification and one with polysomy) contained both sarcomatoid and adenocarcinoma components with diffuse immunoreactivity for CK AE1/AE3. Sarcomatoid components of three cases demonstrated no significant immunoreactivity for ERG, CD31, CD34, S-100, or SOX10, but two were focally positive for A-SMA (data not shown). No *MDM2* amplification was noted in any angiosarcomas or CPTs.

Table 4
Summary of *MDM2* FISH and DISH

		Intimal sarcoma (n=10)	Angiosarcoma (n=5)	p-SC (n=14)	CPT (n=3)
MDM2-FISH	Amplification	8	0	2	0
	Polysomy	2	0	1	0
	Negative*	0	3	10	3
	No signal	0	2	1	0
MDM2-DISH	Amplification	8	0	2	0
	Polysomy	2	0	1	0
	Negative*	0	3	10	3
	No signal	0	2	1	0

p-SC, pulmonary sarcomatoid carcinoma; CPT, chronic pulmonary thrombosis. *No copy number changes.

3.5. Statistical analysis

The relationships between *MDM2* DISH and *MDM2* FISH, between *MDM2* FISH and MDM2 IHC, between *MDM2* DISH and MDM2 IHC, and between *MDM2* DISH and CDK4 IHC are shown in Fig. 3. There was a strong correlation between *MDM2* DISH and *MDM2* FISH (R^2=0.885, P=3.29E-14), *MDM2* FISH and MDM2 IHC (R^2=0.707, P=1.13E-08), and *MDM2* DISH and MDM2 IHC (R^2=0.678, P=4.21E-08), and there was a weak correlation between *MDM2* DISH and CDK4 IHC (R^2=0.338, P=.00093).

4. Discussion

Intimal sarcoma is a rare malignant sarcoma that arises from the intima of great vessels and usually has microscopic features of undifferentiated sarcoma, sometimes associated with focal heterologous elements. The diagnosis of intimal sarcoma is often difficult because of inconsistent clinical findings, and a lack of specific microscopic features and immunohistochemical markers. In our study, five of 10 cases were clinically suspected for other diseases, such as CPTs and pulmonary parenchymal tumors, instead of intimal sarcoma because radiological analyses failed to demonstrate the characteristic intraluminal polypoid growth pattern. Especially, it was difficult to reach final diagnosis in case 9. The patient had multiple gastric tumors, and the biopsy specimens demonstrated undifferentiated malignant tumor cells with high-level *MDM2* amplification. Tumor cells were negative for c-kit, DOG-1, CD31, CD34, S-100, HMB-45, SOX-10, and desmin, whereas they were diffusely positive for MDM2 and CDK4, and focally

Table 5
Immunohistochemistry (MDM2, CDK4, and p53), and *MDM2* FISH and DISH results of intimal sarcomas

Case no.	MDM2 IHC		CDK4 IHC		p53 IHC	*MDM2* FISH	*MDM2* DISH
	3-scale	IHC score	3-scale	IHC score	3-scale	*MDM2*/CEP12	*MDM2*/CHR12
1	Diffuse	100	Focal	40	Focal	3.6	5.1
2	Focal	30	Diffuse	140	Diffuse	1.56*	1.9*
3	Diffuse	80	Diffuse	90	Negative	3.85	6.35
4	Diffuse	70	Focal	30	Focal	4.13	6.12
5	Diffuse	120	Diffuse	200	Focal	5.57	7.84
6	Diffuse	160	Focal	40	Negative	7.09	8.19
7	Diffuse	80	Diffuse	80	Negative	7.9	8.02
8	Diffuse	160	Diffuse	90	Negative	13.4	9.5
9	Diffuse	140	Diffuse	160	Negative	12.25	17.23
10	Diffuse	100	Diffuse	120	Diffuse	1.1*	0.82*

IHC, immunohistochemistry. *Polysomy of chromosome 12.

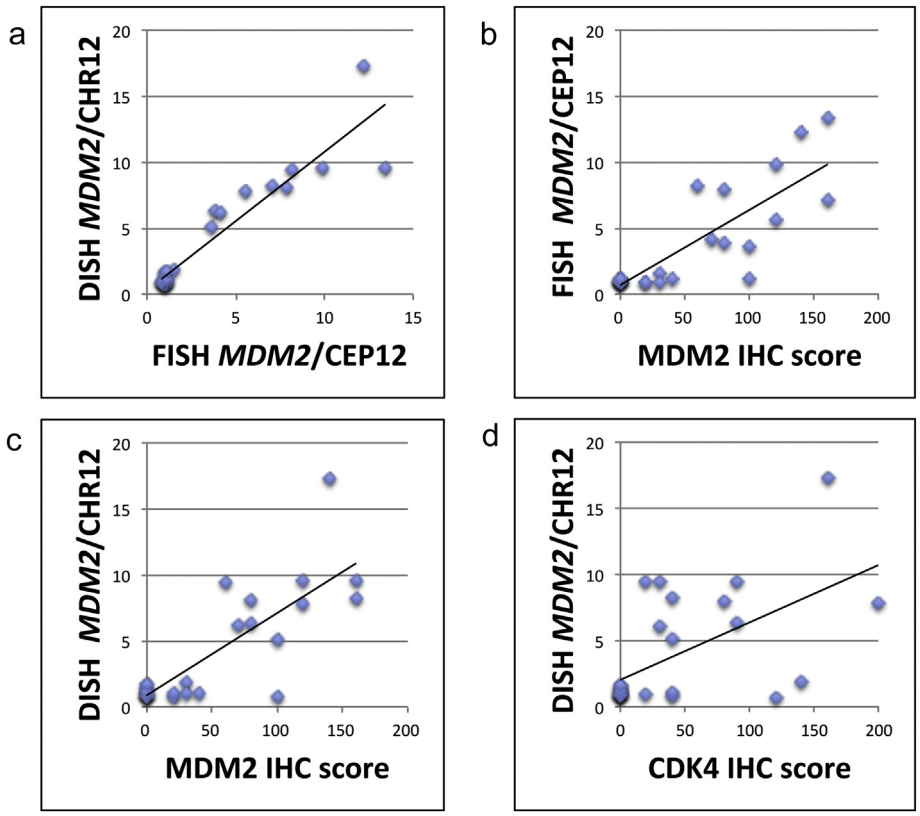

Fig. 3. The correlation among *MDM2* DISH, *MDM2* FISH, MDM2 IHC, and CDK4 IHC in all 29 specimens with in situ hybridization of *MDM2*.
Each dot represents a comparison of two variables in each patient, including the mean *MDM2*/CHR12 in DISH and *MDM2*/CEP12 in FISH (a), *MDM2*/CEP12 in FISH and MDM2 IHC score (b), *MDM2*/CHR12 in DISH and MDM2 IHC score (c), and *MDM2*/CHR12 in DISH and CDK4 IHC score (d). Each solid line shows the approximate linear curve of the relationship between two variables. There was a strong correlation between *MDM2* DISH and *MDM2* FISH (R^2=0.885), *MDM2* FISH and MDM2 IHC (R^2=0.707), and *MDM2* DISH and MDM2 IHC (R^2=0.678), but there was a weak correlation between *MDM2* DISH and CDK4 IHC (R^2=0.338).

positive for A-SMA. Systemic screening including PET-CT, CT, MRI, and transesophageal echocardiography revealed masses only in the stomach and left atrium. There was no clear evidence of lipomatous components in stomach, heart lesions or retroperitoneum by imaging studies. We concluded that the gastric lesions were metastases of the cardiac intimal sarcoma, which were eventually confirmed on autopsy.

To improve the diagnostic accuracy for this rare tumor, we performed FISH and DISH to detect *MDM2* amplification, which is an important molecular aberration in intimal sarcoma. *MDM2* abnormalities were observed in all intimal sarcomas. *MDM2* amplification was detected in 80% (8 of 10 cases), similar to that reported by other investigators [6–8]. In contrast, *MDM2* amplification was detected in only 14.2% of p-SCs and was not present in any angiosarcomas or CPTs. Although amplification is not specific to intimal sarcoma [17], the presence of *MDM2* amplification may support the diagnosis of intimal sarcoma. In this study, ERG expression in the one case of intimal sarcoma was diffuse (>50% positive cells); however, the immunohistochemical intensity was weaker than angiosarcoma. *MDM2* amplification and the lack of CD31 and CD34 immunostaining help the diagnosis of intimal sarcoma in this case.

Intimal sarcoma is one of the tumors associated with *MDM2* amplification (so-called MDM2-oma), which include dedifferentiated liposarcoma, atypical lipomatous tumor/well differentiated liposarcoma, low-grade central osteosarcoma, and parosteal osteosarcoma.

MDM2 is an important oncogene involved in tumor development and progression by inhibiting tumor suppressor *TP53* function. The frequency of *MDM2* amplification in our study was 80%. Of note, the remaining two tumors (20%) had *MDM2* polysomy and p53 overexpression. Our study suggested that *MDM2* amplification and p53 overexpression (*TP53* gene aberrations) were mutually exclusive. Therefore, abnormality in the MDM2-p53 pathway is the major pathogenesis of intimal sarcoma, and genetic analysis may be useful to differentiate intimal sarcomas from other pathological mimics. Furthermore, some MDM2 inhibitors that block MDM2-p53 interaction, such as nutlin-3 and MI-219, are being evaluated in early clinical trials [12]. Therefore, it is important to distinguish *MDM2*-amplified intimal sarcoma from undifferentiated sarcoma without *MDM2* amplification.

We also demonstrated a high concordance between DISH and FISH in detecting *MDM2* amplification. Therefore, FISH can be replaced by DISH to detect *MDM2* amplification. There are some advantages of *MDM2* DISH over FISH. *MDM2* DISH can be conducted with the same autostainer (Ventana Benchmark XT) as IHC and does not require a florescence microscope. Hybridized slides can be kept for a long time without fading of the signal intensity. Moreover, DISH is superior to evaluate the morphology and atypia of nuclei containing hybridization signals and to observe wide areas of specimens. Taken together, *MDM2* DISH is a concordant method and an acceptable alternative to FISH for the diagnosis of intimal sarcomas.

In summary, DISH was used to demonstrate *MDM2* amplification in intimal sarcoma, and the results were compared with those of FISH and IHC. *MDM2* amplification was detected in 80% of intimal sarcomas by both DISH and FISH, and polysomy of chromosome 12 was found in 20%. The former was mostly immunohistochemically negative for p53, whereas the latter was diffusely positive, which suggests that aberrations in the MDM2-p53 pathway are the main pathogenetic event in intimal sarcoma. *MDM2* DISH was a

concordant method and an acceptable alternative to FISH for the diagnosis of intimal sarcoma and detection of *MDM2* amplification.

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