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Clarifying the Distinction Between Malignant Peripheral Nerve Sheath Tumor and Dedifferentiated Liposarcoma

A Critical Reappraisal of the Diagnostic Utility of MDM2 and H3K27me3 Status

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Dedifferentiated Liposarcoma

- **DDLPS Definition**: An atypical lipomatous tumour(ALT)/well-differentiated liposarcoma showing progression, either in the primary or in a recurrence, to(usually non-lipogenic) sarcoma of variable histological grade. In most cases there is substantial amplification of MDM2. A well-differentiated component may not be identifiable. Rarely, the high-grade component may be lipogenic.

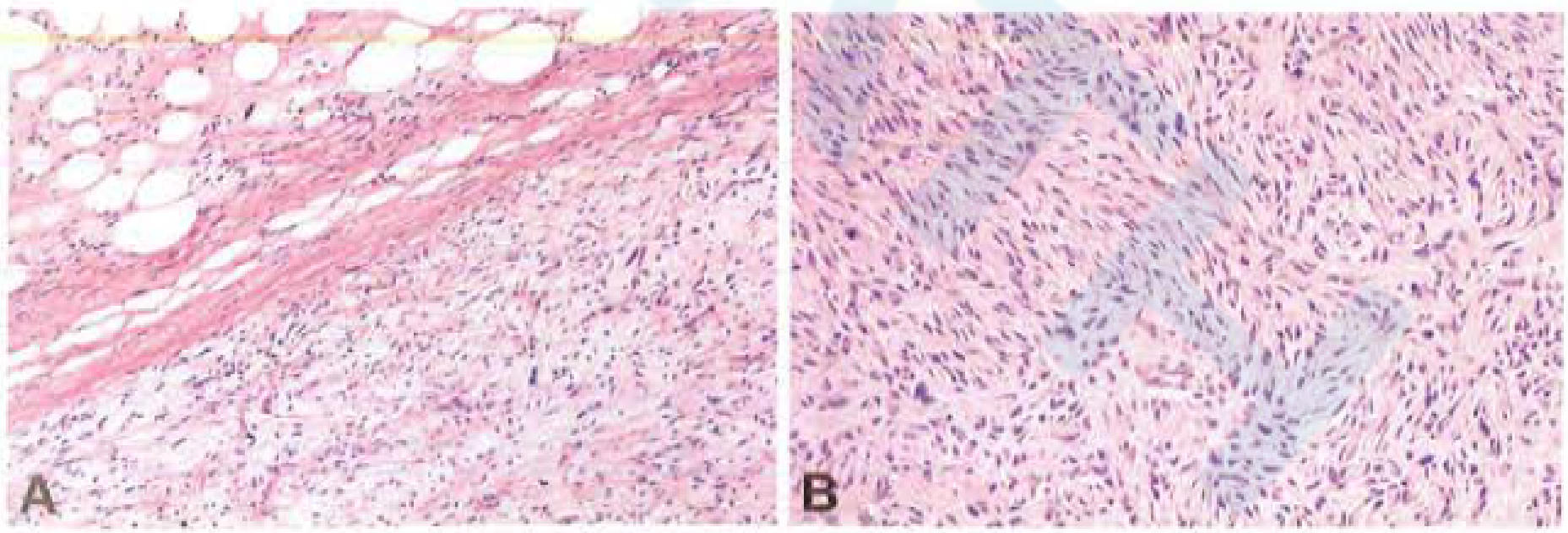
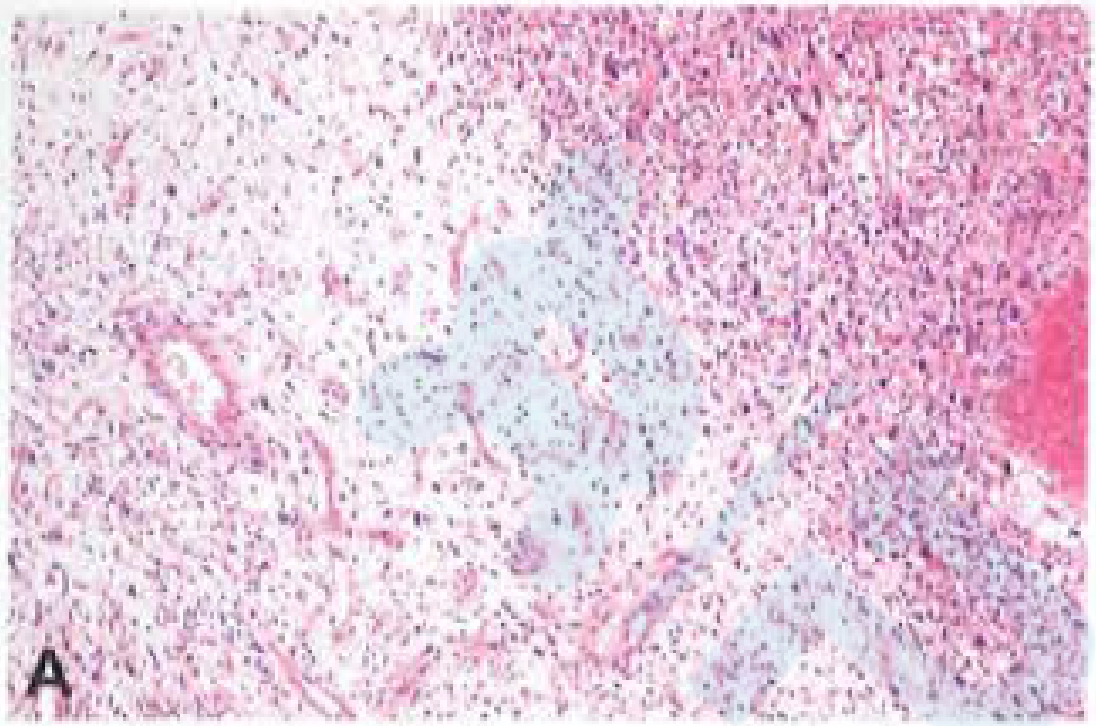


Fig. 2.28 Dedifferentiated liposarcoma. **A** Abrupt transition between well-differentiated liposarcoma and a high-grade non-lipogenic area is seen. **B** The morphology of the dedifferentiated component usually overlaps with undifferentiated sarcoma.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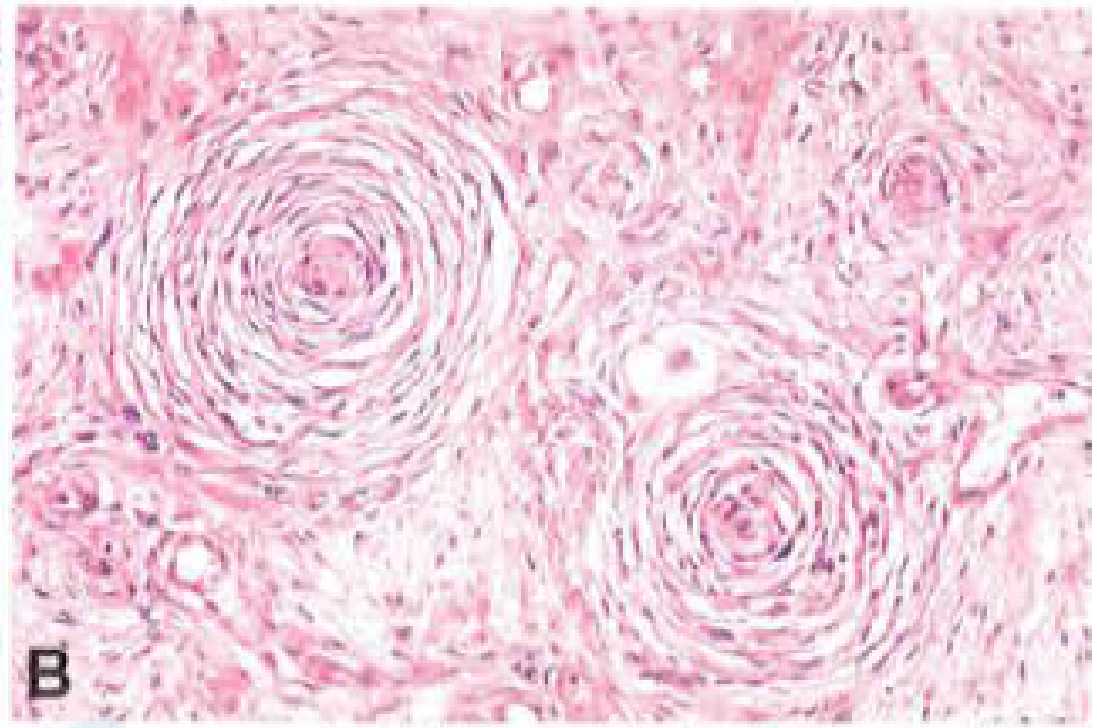
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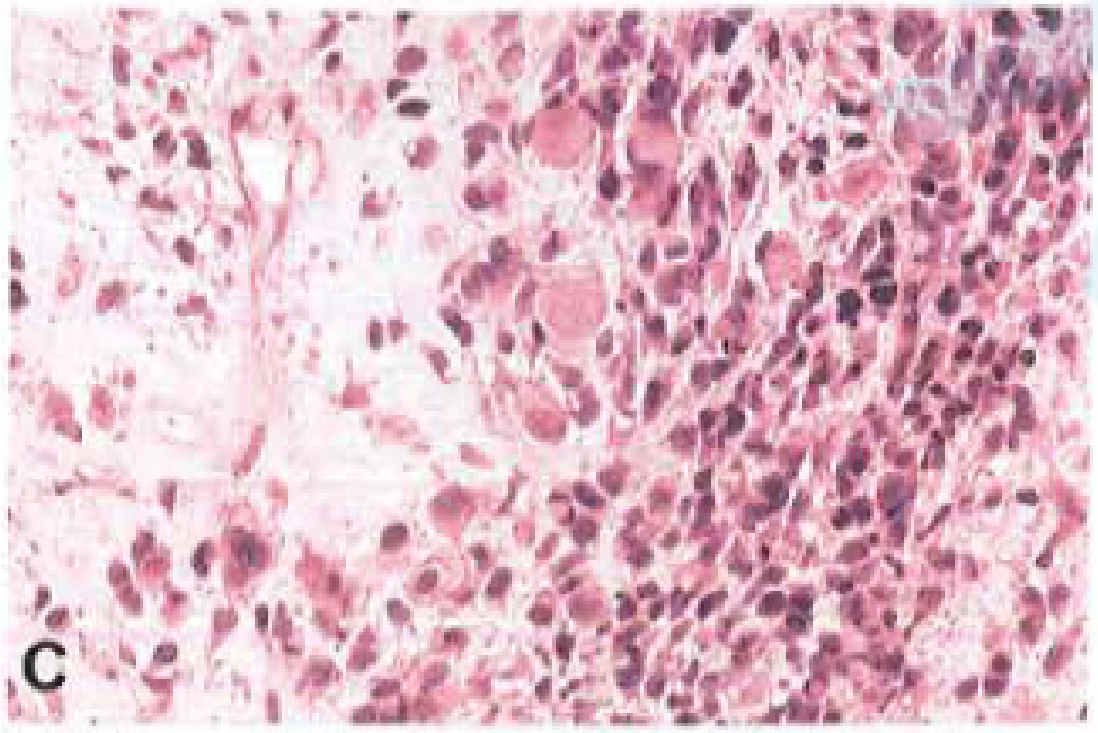
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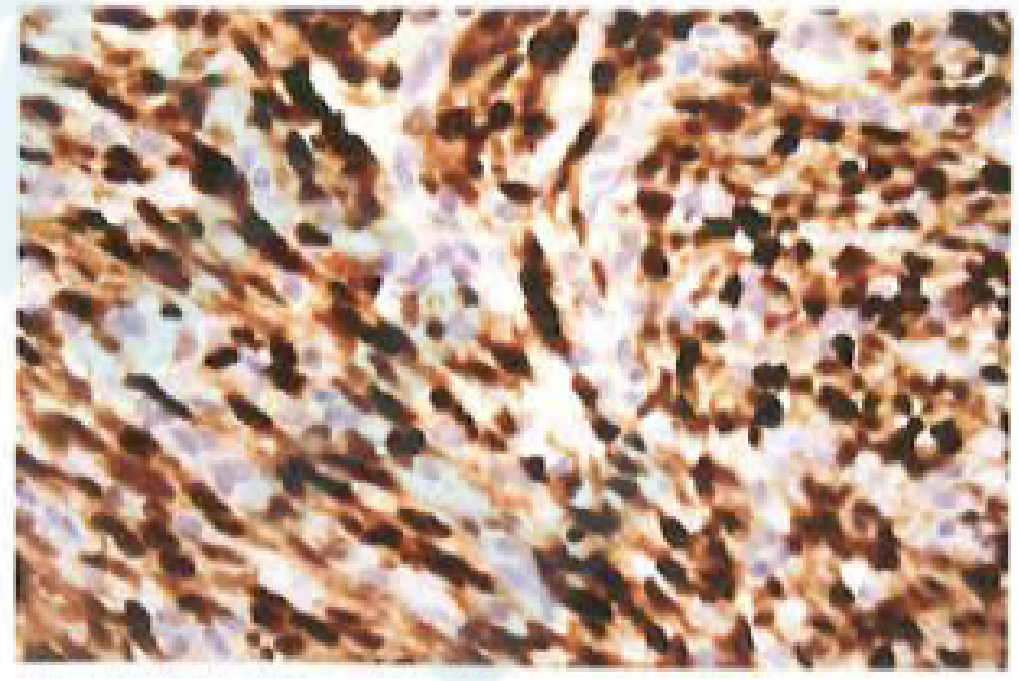


Malignant Peripheral Nerve Sheath Tumor

- **MPNSTs Definition**: A malignant nerve sheath tumour arising from a peripheral nerve, from a pre-exist-ing benign nerve sheath tumour (usually neurofibroma) or in a patient with neurofibromatosis type 1(NF1). In the absence of these settings, the diagnosis is based on the constellation of histological, immunohistochemical and ultrastructral features suggesting Schwann-cell differentiation.

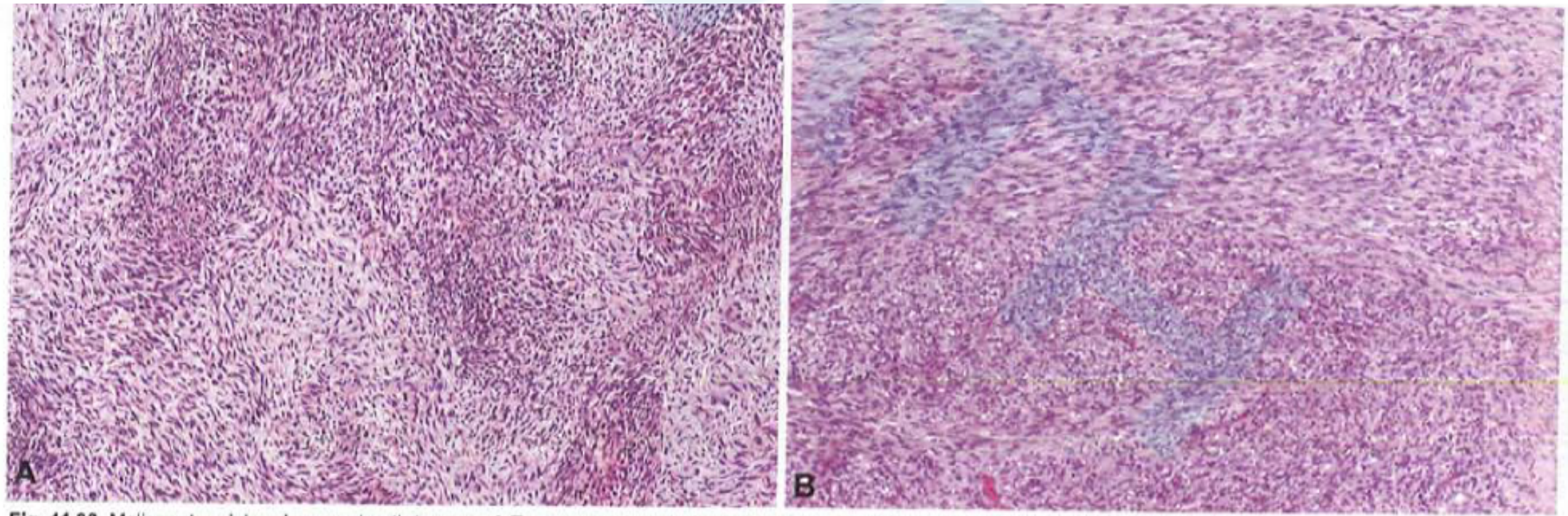
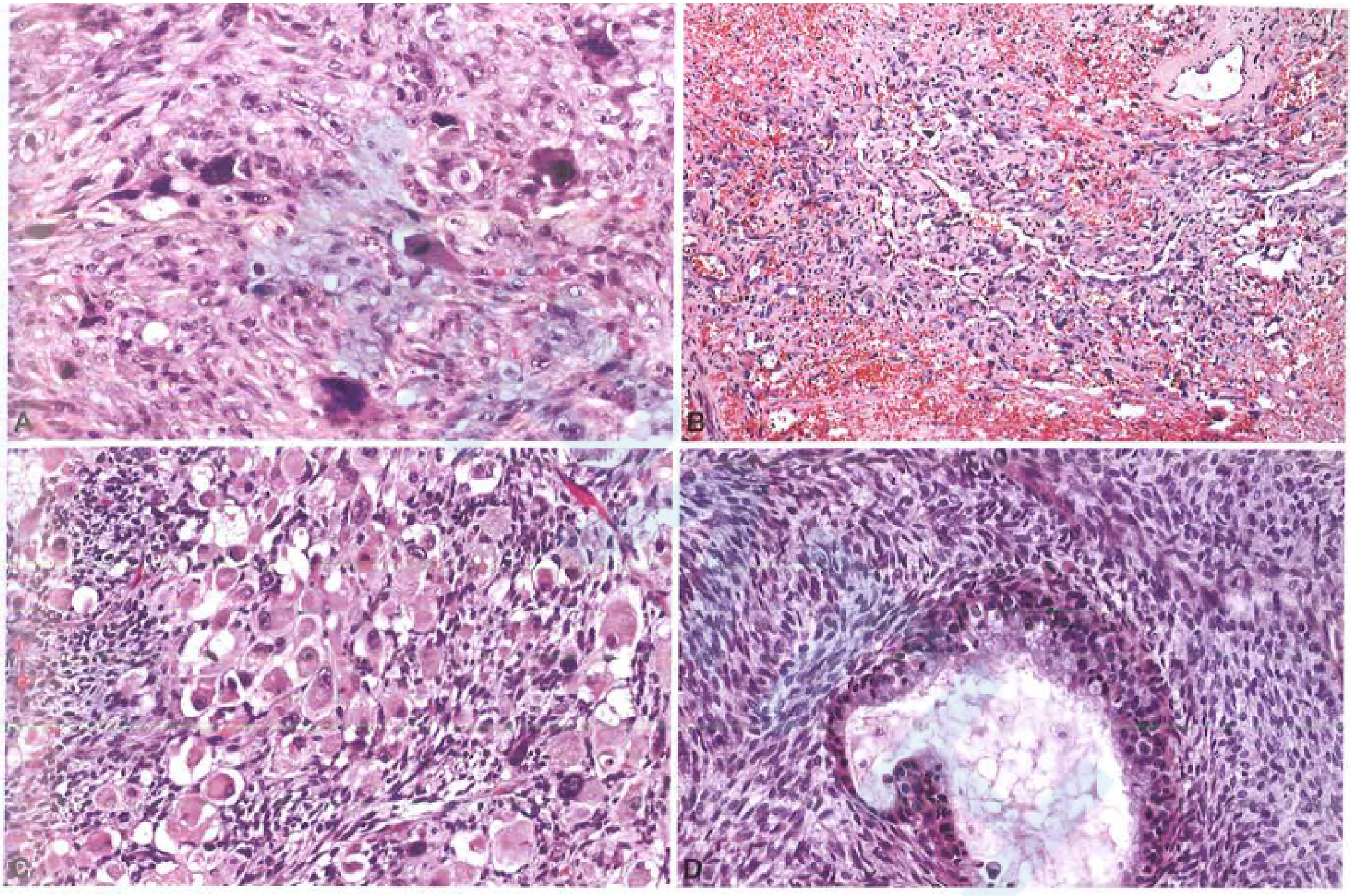


Fig. 11.33 Malignant peripheral nerve sheath tumour. **A** Tumour composed of cellular areas alternating with less cellular areas ("tapestry" appearance or "marble-like" pattern). **B** The tumour is cellular and has a fascicular growth pattern mimicking a fibrosarcoma or a synovial sarcoma.



Immunophenotype: S100 (<50%)、GFAP(20-30%)

Histopathology

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Immunohistochemistry for trimethylated H3K27 in the diagnosis of malignant peripheral nerve sheath tumours

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- **With the monoclonal antibody, 56% of 54 conventional MPNSTs showed complete loss of staining.**
- **Among 232 non-MPNSTs, only two (0.9%) showed complete loss of staining.**

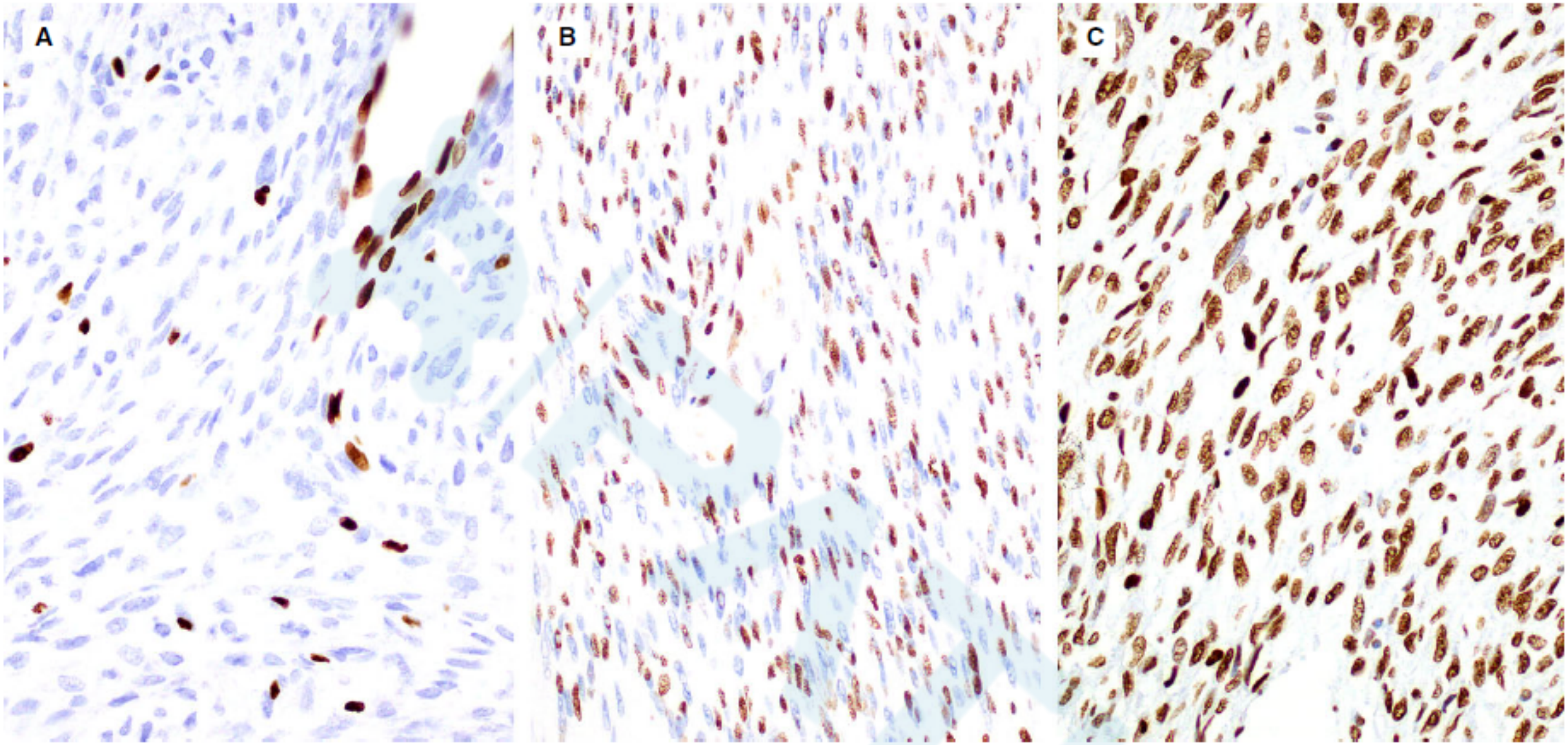

Figure 2. Immunohistochemistry for H3K27me3 in malignant peripheral nerve sheath tumours (MPNSTs), with a monoclonal antibody. Over half of conventional MPNSTs completely lacked staining (A) (note strongly labelled vascular endothelial cells as an internal positive control), whereas the remainder showed either mosaic loss (B) or intact (C) staining.

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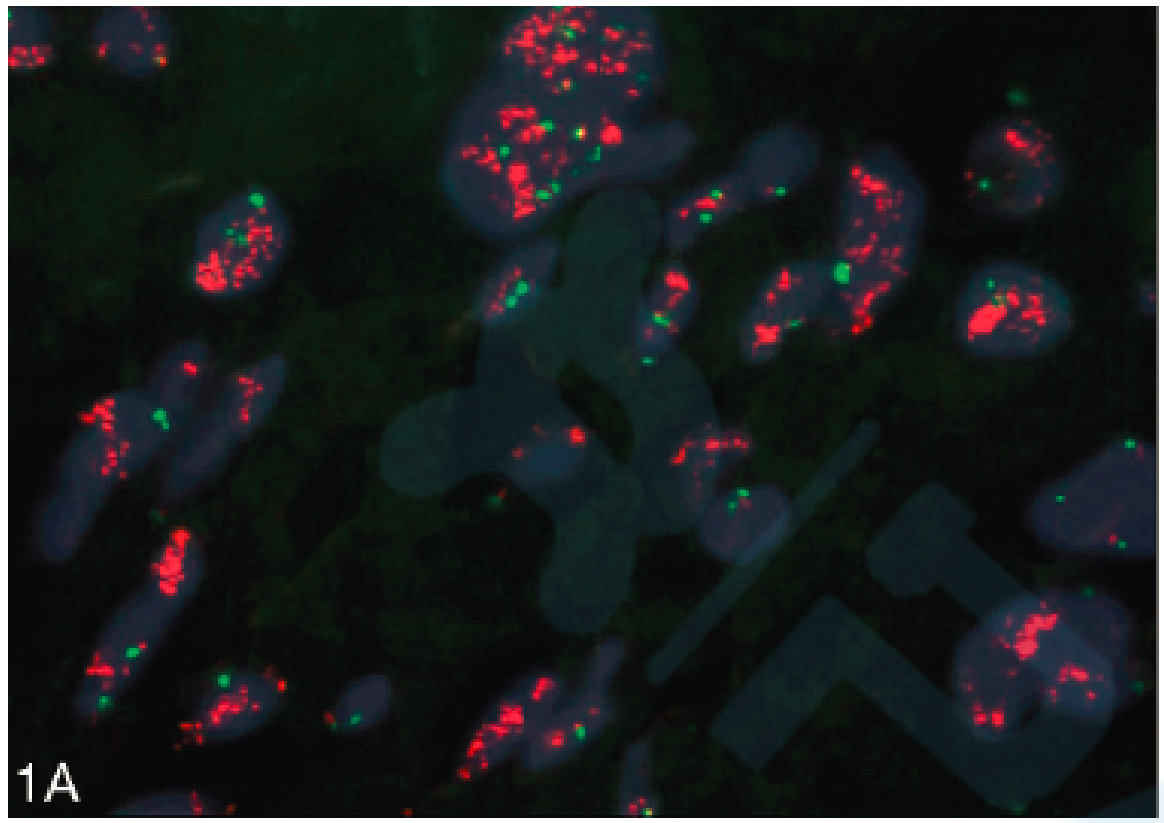

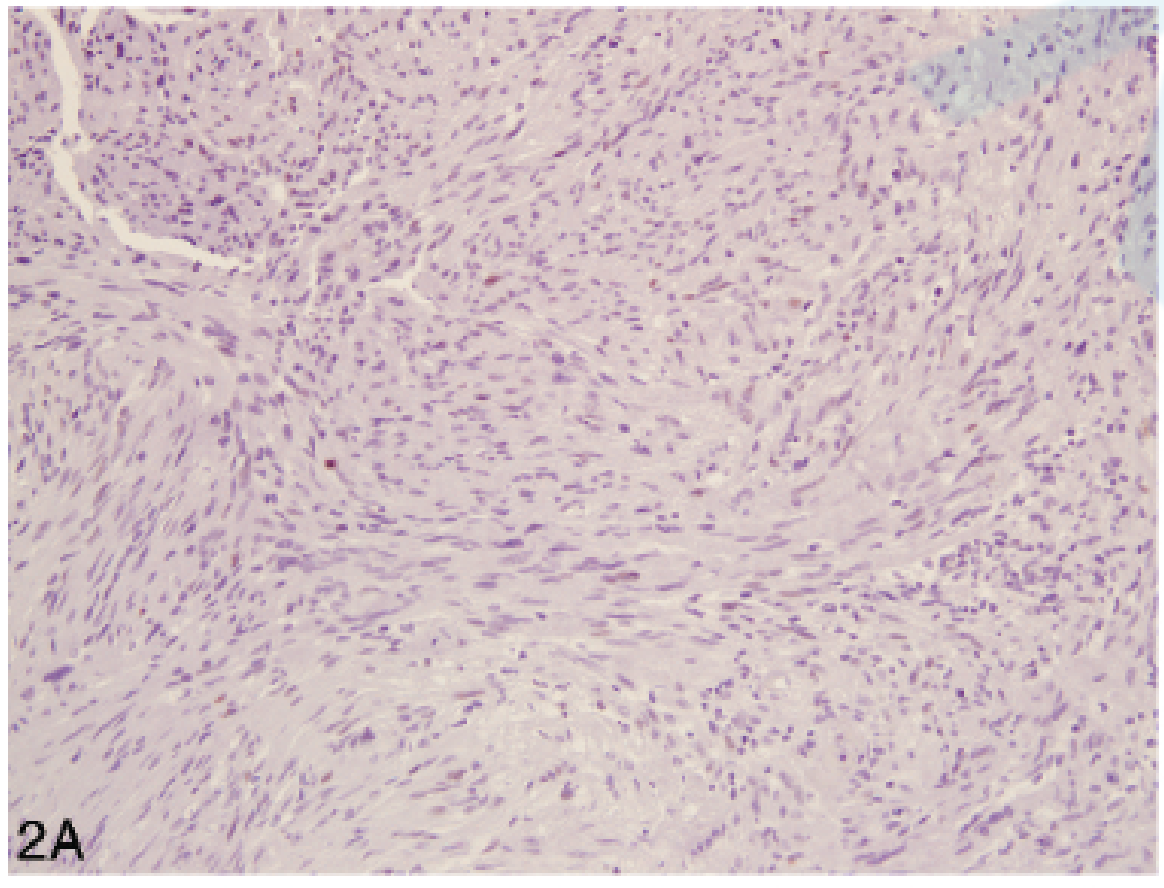

Results:

15 MPNSTs, 3 (20%) demonstrated amplification of the MDM2 gene.

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MATERIALS AND METHODS

- Case Selection
  - 68 cases of nonepithelioid MPNSTs
  - 47 cases of DDLPS

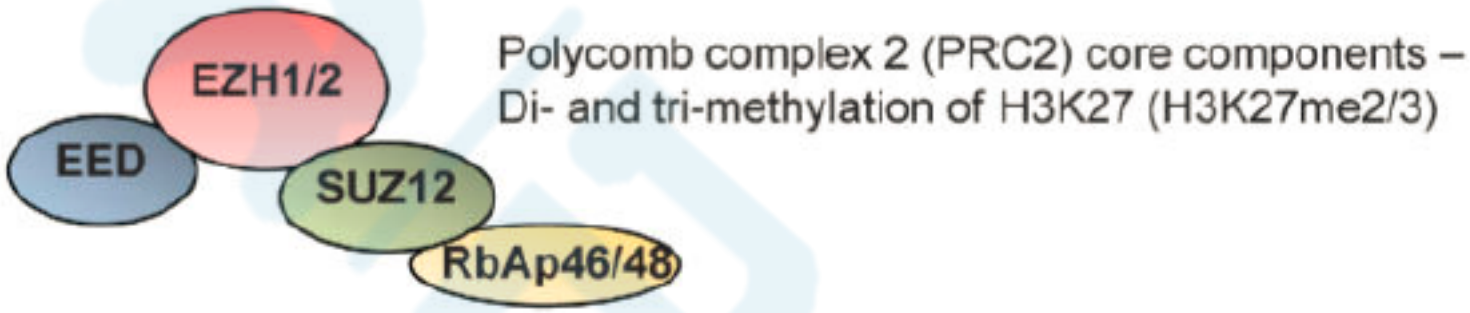
（All cases of DDLPS were associated with an unequivocal WDLPS component）

- Immunohistochemistry

MDM2、CDK4： the extent of staining was classified as negative (0% or <1%), focal (1% to 10%), or diffuse (11% to 100%).

H3K27me3： Only complete loss (global or geographic) of staining was considered significant.

SUZ12: <5% (complete loss), 5% to 95% (focally positive), and >95% (diffusely positive).



(Nuclear staining of endothelial cells served as a positive internal control for H3K27me3 and SUZ12.)

- Fluorescence In Situ hybridization
- Targeted Next-generation Sequencing

NGS was performed in a single case of H3K27me3-deficient DDLPS.

TABLE 1. The Summary of the Primary Antibody Used

Antibody	Clone	Dilution	Pretreatment	Solution	LINKER	Source
MDM2	IF2	1:100	Autoclaving	Targeted Retrieval Solution (Dako, Glostrup, Denmark)	No	Zymed Laboratories, San Francisco, CA
CDK4	DCS-31	1:200	Autoclaving	Citrate buffer	No	Biosource International, Camarillo, CA
p16	G175-405	1:10	Water bath	Targeted Retrieval Solution, pH9 (Dako)	No	BD Biosciences, San Jose, CA
H3K27me3	C36B11	1:200	Autoclaving	Citrate buffer	No	Cell Signaling Technology, Danvers, MA
SUZ12	ab126577	1:200	Water bath	Targeted Retrieval Solution, pH9 (Dako)	Yes	Abcam, Cambridge, UK
H3K27M	Polyclonal	1:8000	Autoclaving	Citrate buffer	Yes	Millipore, Billerica, MA

RESULTS

- Clinical Information

	male	female	age	NF1	sporadic	Radiation-induced
MPNST (68)	34 (50%)	34 (50%)	12-75 years（median，40y）	38（56%）	29（43%）	2（3%）
DDLPS (47)	32（68%）	15（32%）	38-79 years（median，64y）	none	-	none

MDM2 Status of MPNST

- 68 cases of nonepithelioid MPNSTs

	Immunohistoch-emistry cases	Positive cases	Positive for both markers	FISH
MDM2	62	22	4	21/22
CDK4	68	12		

- Most of which were focal and/or weak to moderate in quality.

TABLE 2. The Summary of MDM2-overexpressing MPNST

	Clinicopathologic Data			Immunohistochemistry				*MDM2* FISH				
Diagnosis	NF1	Coexisting NF	Heterol. diff.	H3K27me3	SUZ12	MDM2	CDK4	Classification	Ratio of Cells With *MDM2*/*CEP12* ≥5.0 (%)	Ratio of Cells With *MDM2*/*CEP12* ≥2.0 (%)	Ratio of Cells With *MDM2*/*CEP12* ≥1.5 (%)	Median Copy Number
MPNST	No	No	No	Global loss	Lost	3D	0	High-amp	17	95	99	11
MPNST	No	No	No	Retained	ND	3F	0	Low-amp	2.6	31	68	4
MPNST	Yes	No	RMS	Global loss	Focal	2F	0	Low-amp	0	29	64	4
MPNST	Yes	Yes	No	Geographic loss	Focal	3D	1F	Low-amp	0	29	48	4
MPNST	Yes	No	OS	Global loss	Lost	1F	0	Low-amp	0	27	39	4
MPNST	Yes	Yes	No	Retained	ND	3F	1D	Low-gain	0	8.9	30	3
MPNST	No	No	No	Global loss	ND	3D	0	Low-gain	0	4.7	27	3
MPNST	Yes	Yes	No	Global loss	Lost	1D	0	Low-gain	0	2.6	36	2
MPNST	No	No	RMS	Global loss	Focal	3F	0	Polysomy	0	3.3	9.9	4
MPNST	Yes	Yes	No	Retained	ND	1F	1D	Polysomy	0	2.5	11	4
MPNST	Yes	No	No	Retained	ND	3F	0	Polysomy	0	0	2.6	4
MPNST	Yes	No	No	Global loss	Focal	3F	0	Polysomy	0	4.8	12	3
MPNST	Yes	No	No	Retained	ND	3F	0	Polysomy	0	2.6	9.2	3
MPNST	Yes	No	No	Retained	ND	2D	0	Polysomy	0	0	7.1	3
MPNST	Yes	Yes	No	Retained	ND	2F	0	Polysomy	0	0	2.5	3
MPNST	No	No	No	Global loss	Focal	2D	0	Disomy	0	3.9	10	2
MPNST	Yes	Yes	No	Global loss	Focal	3F	0	Disomy	0	1.3	13	2
MPNST	No	No	No	Geographic loss	ND	3F	0	Disomy	0	1.3	1.3	2
MPNST	Yes	Yes	No	Retained	ND	3F	0	Disomy	0	1.2	4.7	2
MPNST	Yes	Yes	No	Retained	ND	3F	0	Disomy	0	0	3.7	2
MPNST	No	Yes	No	Global loss	Lost	1F	1F	Disomy	0	0	0	2
MPNST	No	No	No	Global loss	Lost	1F	0	Failure	—	—	—	—

0 indicates negative; 1, weak; 2, intermediate; 3, strong; D, diffuse; F, focal; Heterol. diff; heterologous differentiation; High-amp, high-level amplification; Low-amp, low-level amplification; Low-gain, low-level selective gain; ND, not done; NF, neurofibroma; OS, osteosarcoma; RMS, rhabdomyosarcoma.

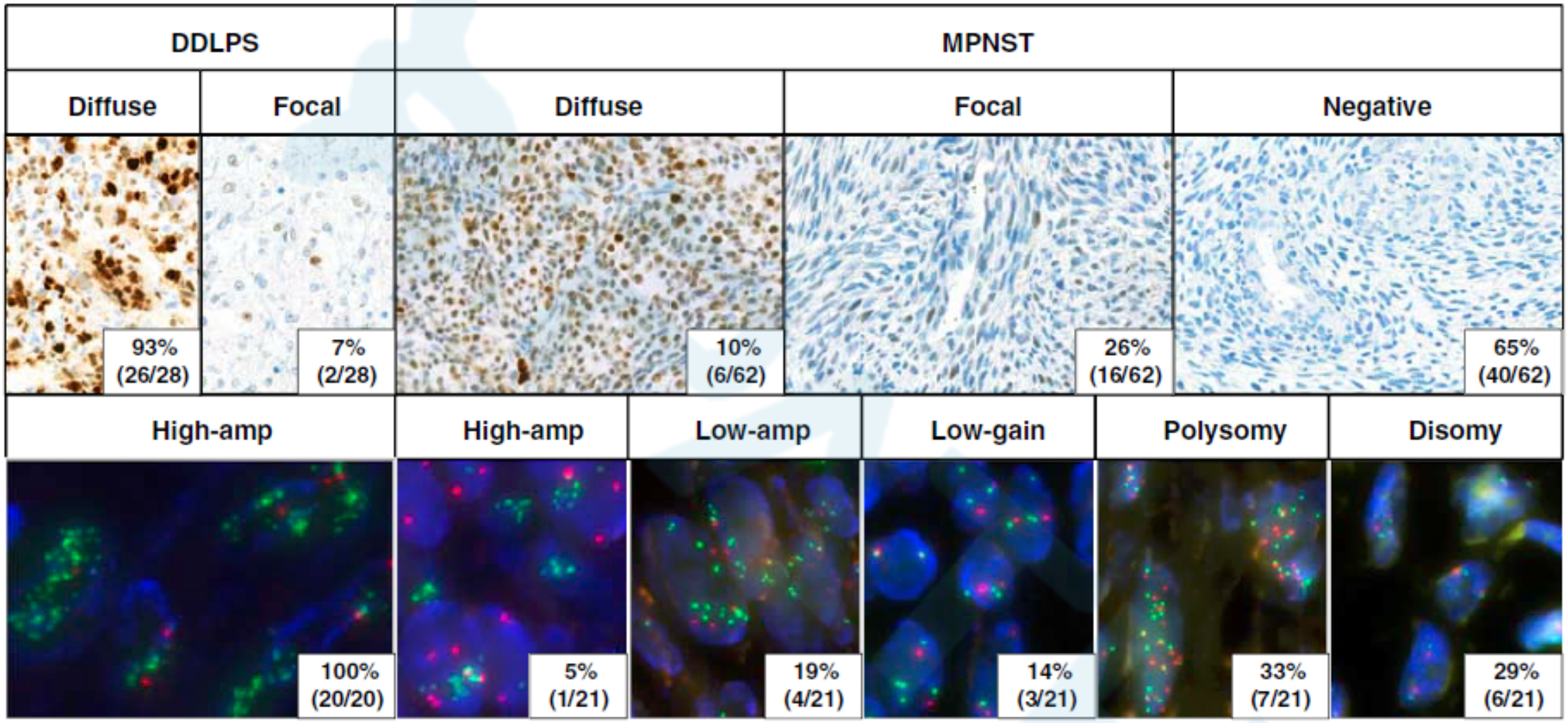


FIGURE 1. MDM2 immunohistochemistry (top) and *MDM2* FISH (bottom; green signals indicate *MDM2*) in MPNST and DDLPS.

MDM2 Status of DDLPS

- MDM2 and CDK4 were immunohistochemically positive for 28 (100%) of 28 and 25 (89%) of 28 DDLPS cases tested, respectively, and most of the staining was strong and/or diffuse in quality.

- Coexpression of MDM2 and CDK4 in DDLPS was significantly more frequent than in MPNST ($P < 0.0001$, Fisher exact test).

TABLE 3. The Summary of H3K27me3-deficient DDLPS

	Pathologic Information		Immunohistochemistry				*MDM2* FISH				
Diagnosis	WDLPS	Heterol. diff.	H3K27me3	SUZ12	MDM2	CDK4	Classification	Ratio of Cells With *MDM2*/*CEP12* ≥5.0 (%)	Ratio of Cells With *MDM2*/*CEP12* ≥2.0 (%)	Ratio of Cells With *MDM2*/*CEP12* ≥1.5 (%)	Median Copy Number
DDLPS*	Yes	Epithelium	Geographic loss	Focal	3D	3D	High-amp	91	100	100	14
DDLPS†	Yes	RMS	Global loss	Focal	3D	3D	High-amp	56	100	100	19
DDLPS	Yes	CS	Geographic loss	Diffuse	2D	3D	High-amp	85	100	100	20

*This case was previously reported.[31]

†Homozygous *EED* deletion was detected by target NGS in the dedifferentiated component, while it was absent in the WDLPS component.

2 indicates intermediate; 3, strong; CS, chondrosarcoma; D, diffuse; Heterol. diff; heterologous differentiation; High-amp, high-level amplification; RMS, rhabdomyosarcoma.

- Twenty selected DDLPSs were tested by FISH, including 3 cases with H3K27me3 deficiency.

H3K27me3 Status of MPNST

- Of 68 cases of MPNST, 42 cases (62%) exhibited complete loss of H3K27me3 (38 global and 4 geographic,Fig. 2).

- Heterologous differentiation was present in 13 cases, and all these cases were deficient in H3K27me3.

- Of 37 H3K27me3-deficient MPNSTs successfully tested, SUZ12 staining was also lost in 19 (51%) cases, whereas it was focally positive in 18 (49%) cases.

	Global loss	Geographic loss	Retained
DDLPS	2% (1/47)	4% (2/47)	94% (44/47)
MPNST	56% (38/68)	6% (4/68)	38% (26/68)

FIGURE 2. H3K27me3 immunohistochemistry in MPNST and DDLPS. For geographic loss, the staining is lost in the right half.

H3K27me3 Status of DDLPS

- Of the 47 cases of DDLPS, 3 cases (6%) exhibited complete loss of H3K27me3.

- All 3 H3K27me3-deficient cases exhibited heterologous differentiation.

- Of those 3 cases, SUZ12 was focally positive in 2 cases and diffusely positive in 1 case.

- H3K27me3 was retained in the WDLPS components in all 3 cases.

- p16 Status in Selected Cases of MPNST and DDLPS

(1)Immunohistochemistry for p16 was performed in a single case of MPNST with high-level MDM2 amplification, and in 3 cases of H3K27me3-deficient DDLPS.

(2)The MPNST was negative for p16, whereas all 3 cases of DDLPS were diffusely positive.

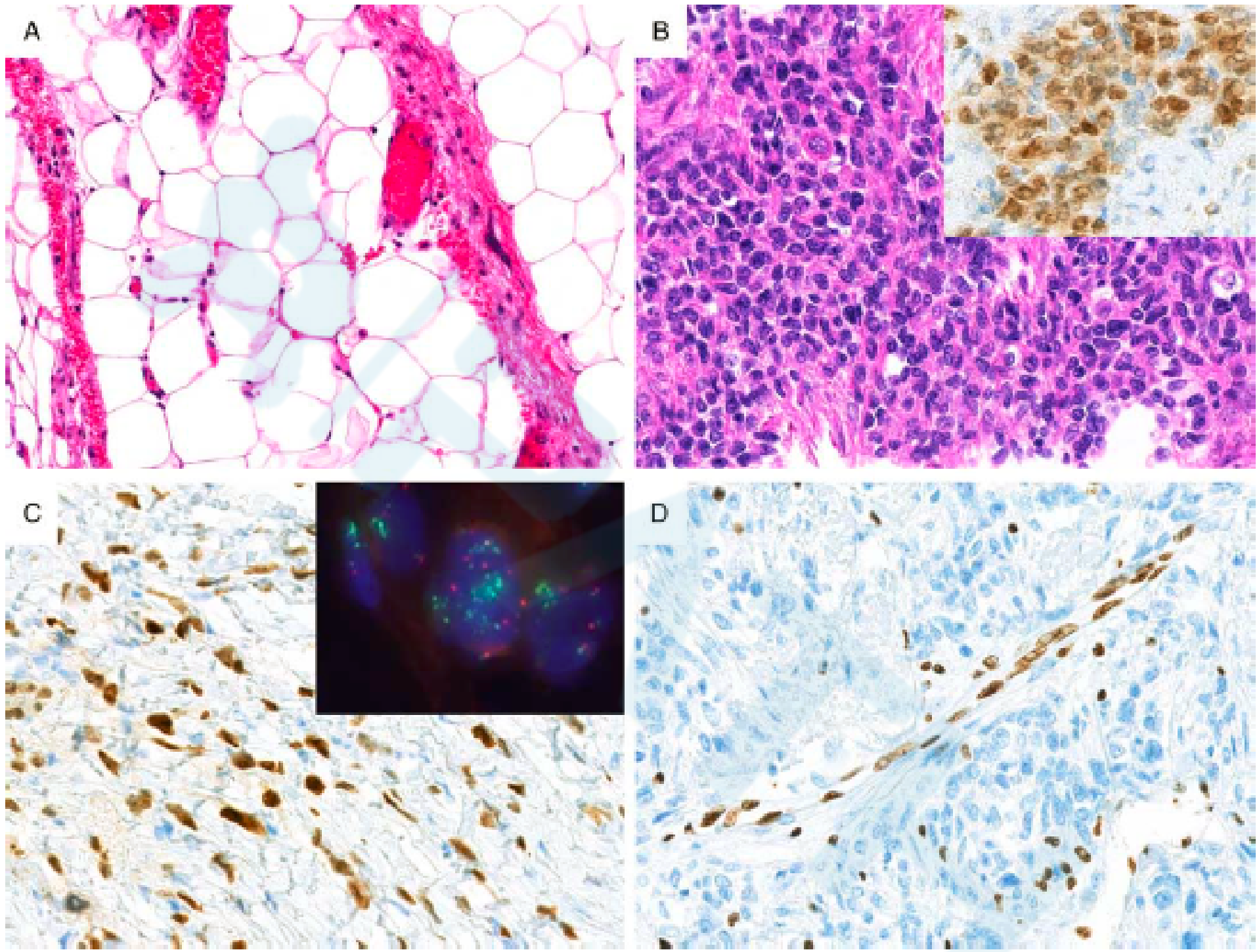


FIGURE 3. A case of DDLPS with global complete loss of H3K27me3 and homozygous *EED* deletion. A, This tumor contained an unequivocal WDLPS component. B, The DDLPS component of this tumor consisted of pleomorphic spindle cells with some focus of island-like proliferation of round to short-spindled cells. Immunohistochemically, the tumor cells in the islands were positive for myogenin (B, inset), supporting rhabdomyoblastic differentiation. Both MDM2 (C) and CDK4 were diffusely positive, and FISH revealed high-level *MDM2* amplification (C, inset; green signals indicate *MDM2*). D, H3K27me3 staining showed global complete loss.

Targeted NGS

DISCUSSION

- MDM2 immunohistochemistry alone was not very useful for this distinction, because as many as 35% of MPNSTs overexpressed this marker.

- MPNSTs (16/62, 26%) did demonstrate strong or diffuse MDM2 expression.

- By using FISH, high-level MDM2 amplification was observed in only 1 of the 21 MDM2-overexpressing MPNSTs.

- As expected, the majority (62%) of the 68 MPNSTs exhibited complete loss of staining (38 global and 4 geographic). However, we also found that 3 (6%) of the 47 DDLPSs were deficient in H3K27me3.

- Interestingly, all 3 H3K27me3-deficient DDLPSs harbored heterologous differentiation.

- The WDLPS component of that tumor retained H3K27me3 and lacked the EED deletion, suggesting that PRC2 abnormality may play a role in the dedifferentiation or progression thereafter.

- Therefore, distinguishing MDM2-amplified MPNST from H3K27me3-deficient DDLPS could still be problematic.

- For example, p16 expression is reportedly negative in 73% of MPNSTs, whereas it is expressed in most (93% to 98%) DDLPS.

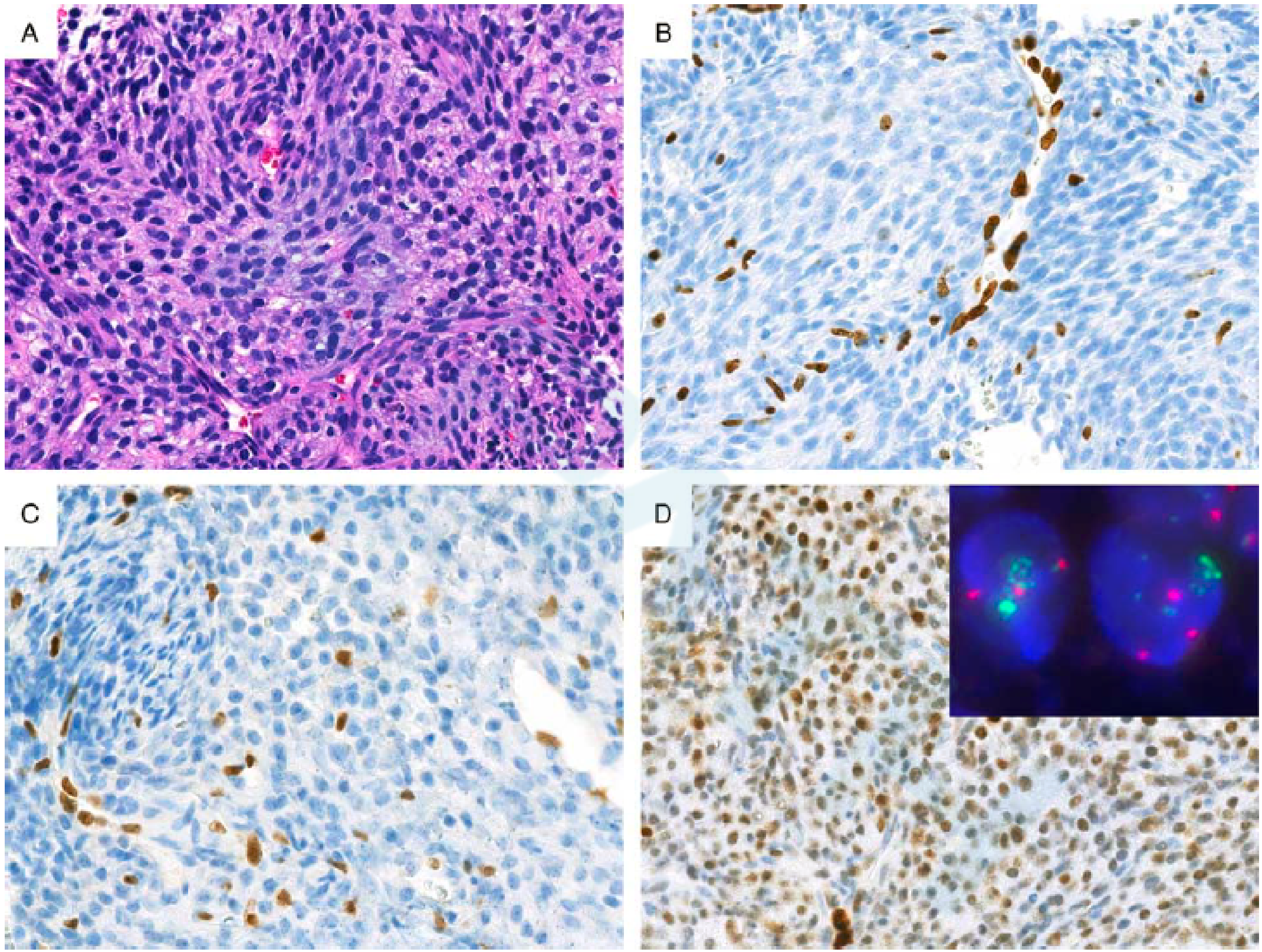


FIGURE 4. A case of MPNST with high-level *MDM2* amplification. The tumor exhibited characteristic histology comprising swirling fascicles of mildly pleomorphic cells (A), and showed global complete loss of H3K27me3 (B) and SUZ12 (C). MDM2 staining was diffuse and strong (D) and FISH demonstrated high-level *MDM2* amplification (D, inset; green signals indicate *MDM2*).

谢谢！