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MDM2 RNA In Situ Hybridization for the Diagnosis of Atypical Lipomatous Tumor

A Study Evaluating DNA, RNA, and Protein Expression

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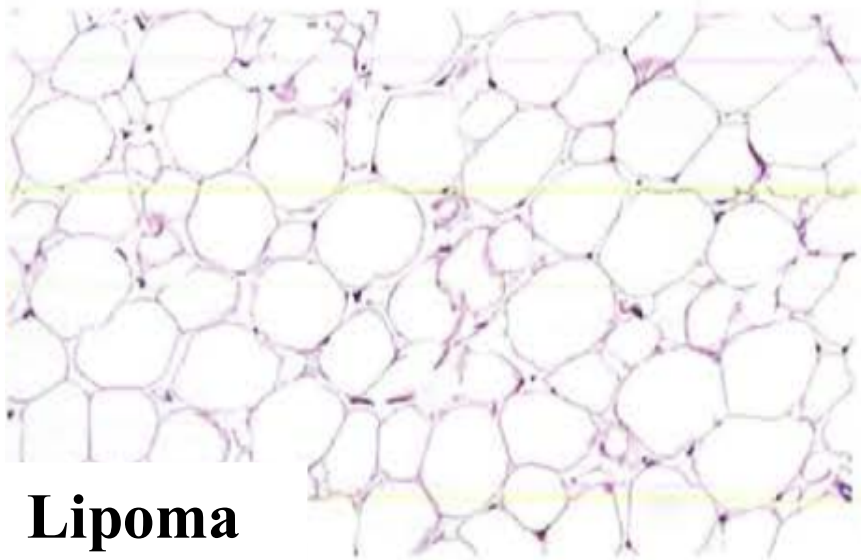
BACKGROUND

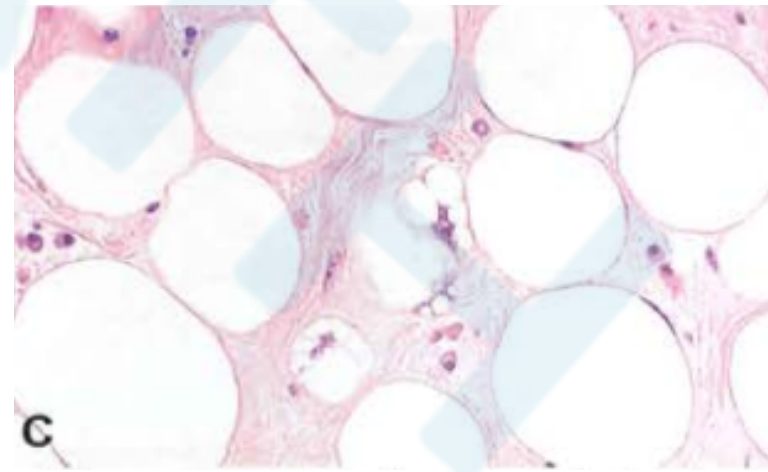
- Liposarcoma, a common soft tissue malignancy, accounts for 20% of all sarcomas
- The World Health Organization recognizes four subtypes of malignant lipomatous neoplasms:
  - (1) Atypical lipomatous tumor (ALT) and well-differentiated liposarcoma (WDL)
  - (2) Dedifferentiated liposarcoma (DDL)
  - (3) Myxoid liposarcoma
  - (4) Pleomorphic liposarcoma

BACKGROUND

- The distinction of ALT/WDL from its benign counterpart, lipoma, is generally accomplished on routine histopathology
- Benign lipomas rarely recur (1%), whereas the local recurrence rates for ALT/WDL are significantly higher (44%)



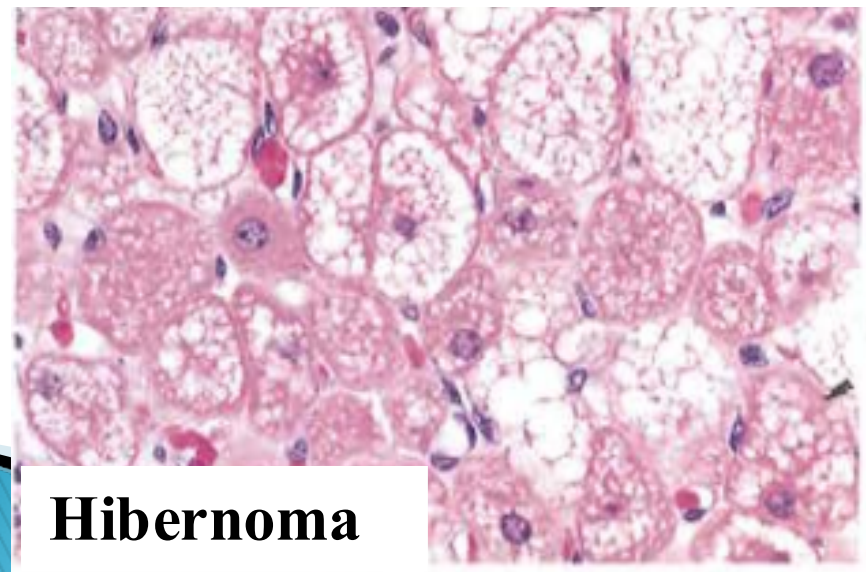
Lipoma



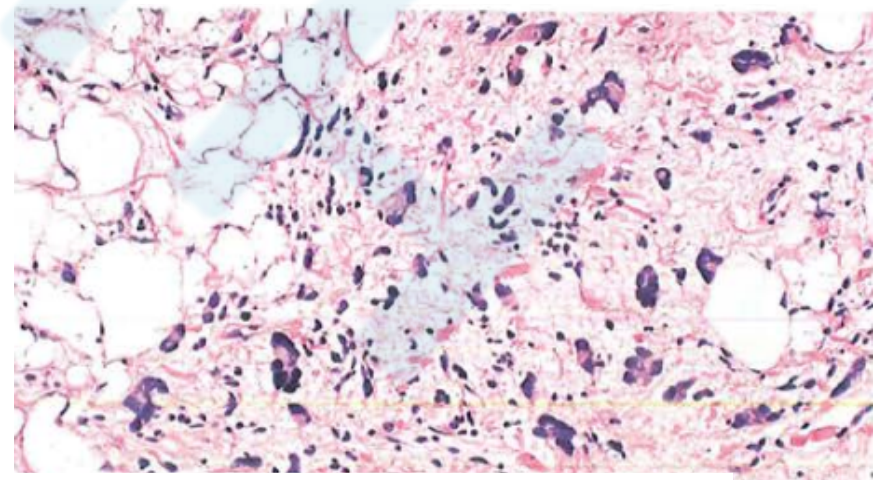
Atypical lipomatous tumor

BACKGROUND

- In some cases the morphologic distinction between lipoma and ALT/WDL is challenging
  - Poor representation of these atypical cells
  - Especially in the case of biopsies where only limited tissue is available
  - Hibernomas and spindle cell lipomas may also mimic ALT/WDL



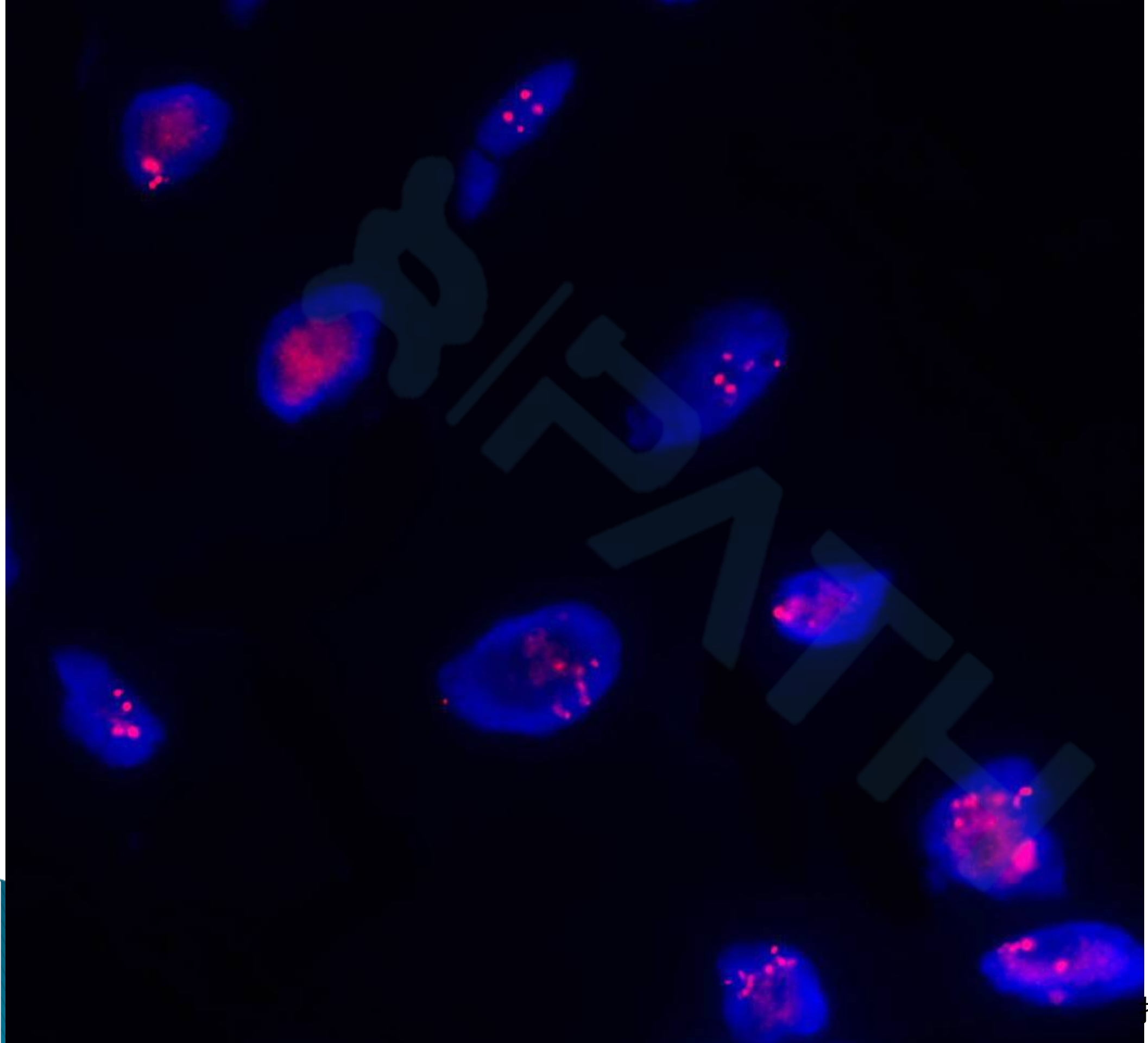
Hibernoma



Pleomorphic lipoma

BACKGROUND

- *MDM2* amplification, a characteristic feature of ALT/WDL and DDL, is absent in benign adipocytic tumors
- Fluorescence in situ hybridization (FISH) for MDM2 is considered the gold standard
  - High sensitivity and specificity



MDM2 FISH

摘自王哲主任课件

BACKGROUND

- The disadvantages of *MDM2* FISH are widely acknowledged:
  - Expense and slow turnaround time
  - Requires a specialized fluorescence microscope and well-trained technologists
  - The manual protocol can introduce human error during the lengthy assay and exhibits a high failure rate
  - Autofluorescence may create technical difficulties in interpretation
  - FISH signal is difficult to preserve during long-term storage
  - The relative lack of community- based pathology experience with this technology

BACKGROUND

- Clay and colleagues recommend the selective use of FISH
  - Restrict the use to recurrent tumors
  - Deep extremity tumors >10 cm
  - Patients in over 50 years of age
  - Retroperitoneal tumors
  - Cases with equivocal cytologic atypia

BACKGROUND

- MDM2 immunohistochemistry lacks specificity and sensitivity
  - Sensitivity has ranged from 45% to 100%
  - Specificity from 59% to 100%
  - 11% of lipomas showed some degree of MDM2 protein expression
- Other antibodies include CDK4 and p16

Diagnostic Utility of p16, CDK4, and MDM2 as an Immunohistochemical Panel in Distinguishing Well-differentiated and Dedifferentiated Liposarcomas From Other Adipocytic Tumors

Khin Thway, FRCPath, Rashpal Flora, FRCPath,* Chirag Shah, FRCPath,* David Olmos, MBBS, PhD,† and Cyril Fisher, MD, DSc, FRCPath**

The sensitivity and specificity of the trio for detecting WDLs/DDLs were 71% and 98%, respectively.

The sensitivity and specificity of CDK4 for detecting WDLs/DDLs were 86% and 89%, those of MDM2 were 86% and 74%, and those of p16 were 93% and 92%, respectively.

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BACKGROUND

- *MDM2* DNA amplification strongly correlated with *MDM2* mRNA expression (P = 0.0001)
  - Suggesting that the latter could be used as a marker of ALT/WDL
  - Supports the use of the latter test in the clinical laboratory
- Performed on instruments used in most clinical laboratories and is read at a brightfield microscope

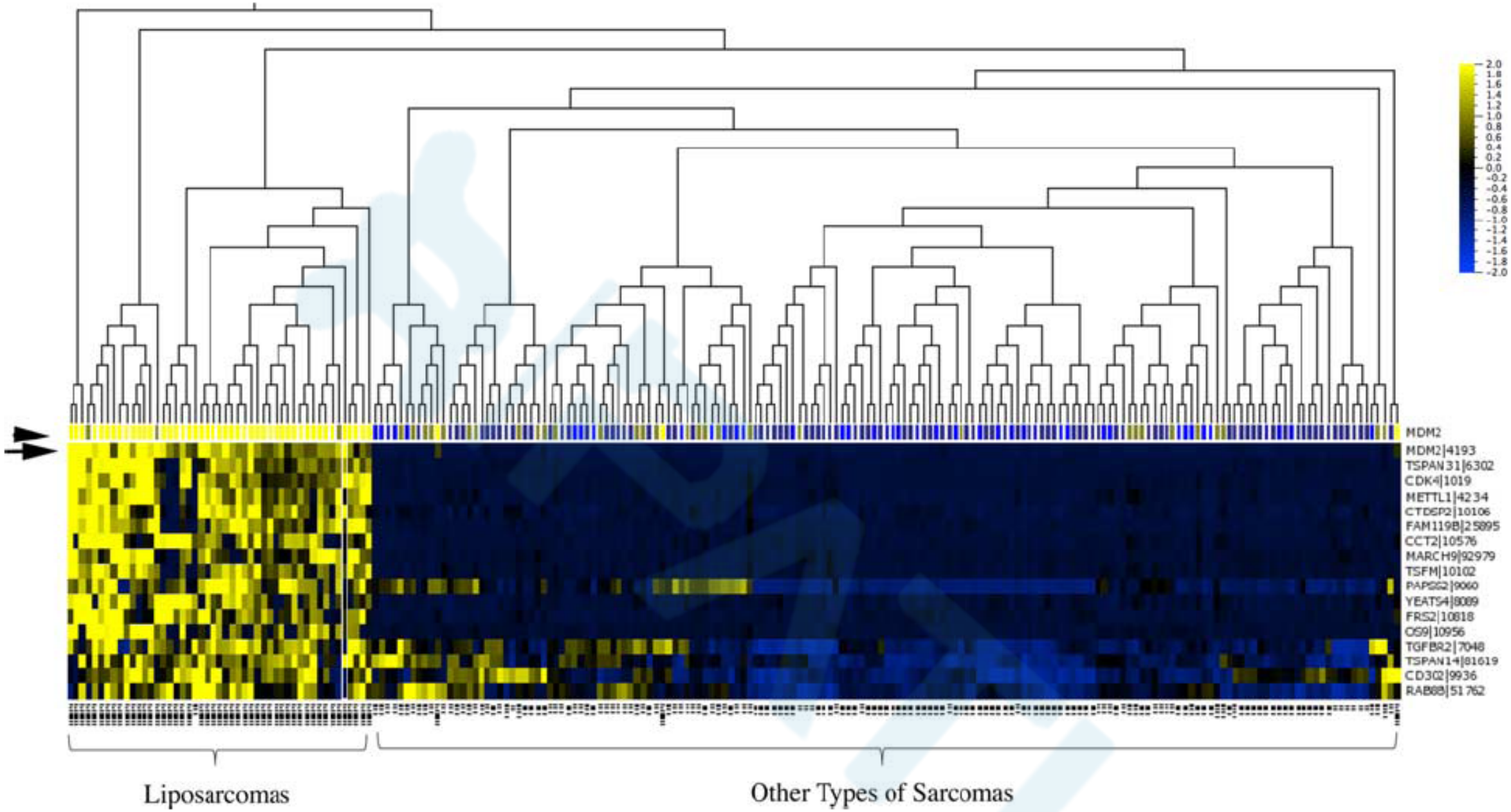


FIGURE 1. Unsupervised hierarchical clustering of sarcomas from TCGA showing overexpression of *MDM2* mRNA (arrow) correlates strongly with *MDM2* amplification (arrowhead) at the DNA level in dedifferentiated liposarcomas. The overwhelming majority of dedifferentiated liposarcomas show overexpression of *MDM2* mRNA as well as *MDM2* DNA amplification. The yellow bars on the top row (arrowhead) indicates tumors with high-level *MDM2* DNA amplification; nonyellow bars indicate low-level or no-amplification of *MDM2* DNA. The 17 genes with the highest correlation (in descending order of significance) with *MDM2* amplification are shown.

BACKGROUND

- Investigate the diagnostic utility of *MDM2* RNA in situ hybridization (RNA-ISH)
- Compare the test with MDM2 immunohistochemistry and *MDM2* DNA fluorescence in situ hybridization (FISH)
- Validate our finding on a series of diagnostically challenging lipomatous neoplasms

MATERIALS AND METHODS

- Selection of Cases(109 neoplasms)
  - 27 lipomas
  - 25 spindle cell lipomas
  - 32 ALTs/WDLs
  - 25 dedifferentiated liposarcomas (DDL)
  - 14 lipoma-like neoplasms
    - Lacked unequivocal features of ALT/ WDL
    - MDM2 immunohistochemistry was either equivocal, negative or falsely positive
- Immunohistochemistry, automated RNA-ISH and DNA-FISH for MDM2

MATERIALS AND METHODS

- *MDM2* RNA In Situ Hybridization
  - Nuclear MDM2 staining in >2% of cells was interpreted as positive
  - Diffuse nuclear staining or >50 dots per cell were considered positive
  - Scored semiquantitatively based on the lowest objective magnification at which positivity was ascertained:

negative at×40=0　　positive at ×40=1+　　positive at ×20=2+

positive at×10=3+　　positive at ×4=4+　　positive at ×2=5+

RESULTS

TABLE 1. Demographic and Clinical Information of the Cohort

Diagnosis	Total Number of Cases	Mean Age (Range) (y)	Sex M:F	Location Extremity: Intra-Abdominal	Mean Tumor Size (Range) (cm)
Lipoma	27	53 (25-76)	2:1	3.5:1	10.6 (1.9-26)
Spindle cell lipoma	25	53 (26-73)	3.2:1	25:0	5.7 (1.3-12.5)
Atypical lipomatous tumor/well-differentiated liposarcoma	32	64 (41-80)	1:2.3	1.3:1	20.2 (3.2-40)
Dedifferentiated liposarcoma	25	67 (56-87)	1:1	1:2.6	17.6 (5-45)

F indicates female; M, male.

RESULTS

TABLE 2. Results of MDM2 Immunohistochemistry, RNA ISH and DNA FISH

	MDM2 RNA-ISH (n [%])			n/N (%)	
	Negative	2-6 dots/ cells	> 50 dot/cell or Diffuse Nuclear Staining	MDM2 IHC	*MDM2* DNA-FISH
Lipoma (n = 27)	11 (41)	16 (59)	0	0/13 (0)	0/9 (0)
Spindle cell lipoma (n = 25)	21 (16)	4 (84)	0	0/25 (0)	0/25 (0)
Atypical lipomatous tumor/well-differentiated liposarcoma (n = 32)	0	0	32 (100)	24/30 (80)*	24/26 (92.3)
Dedifferentiated liposarcoma (n = 25)	0	0	25 (100)	22/24 (92)†	22/22 (100)

*Among the positive cases 10 (42%) cases showed focal or weak positive reactivity and 14 (58%) were diffuse strong.
†Among the positive cases 2 cases showed (8%) weak/focal reactivity and 20 (92%) cases showed diffuse strong reactivity.

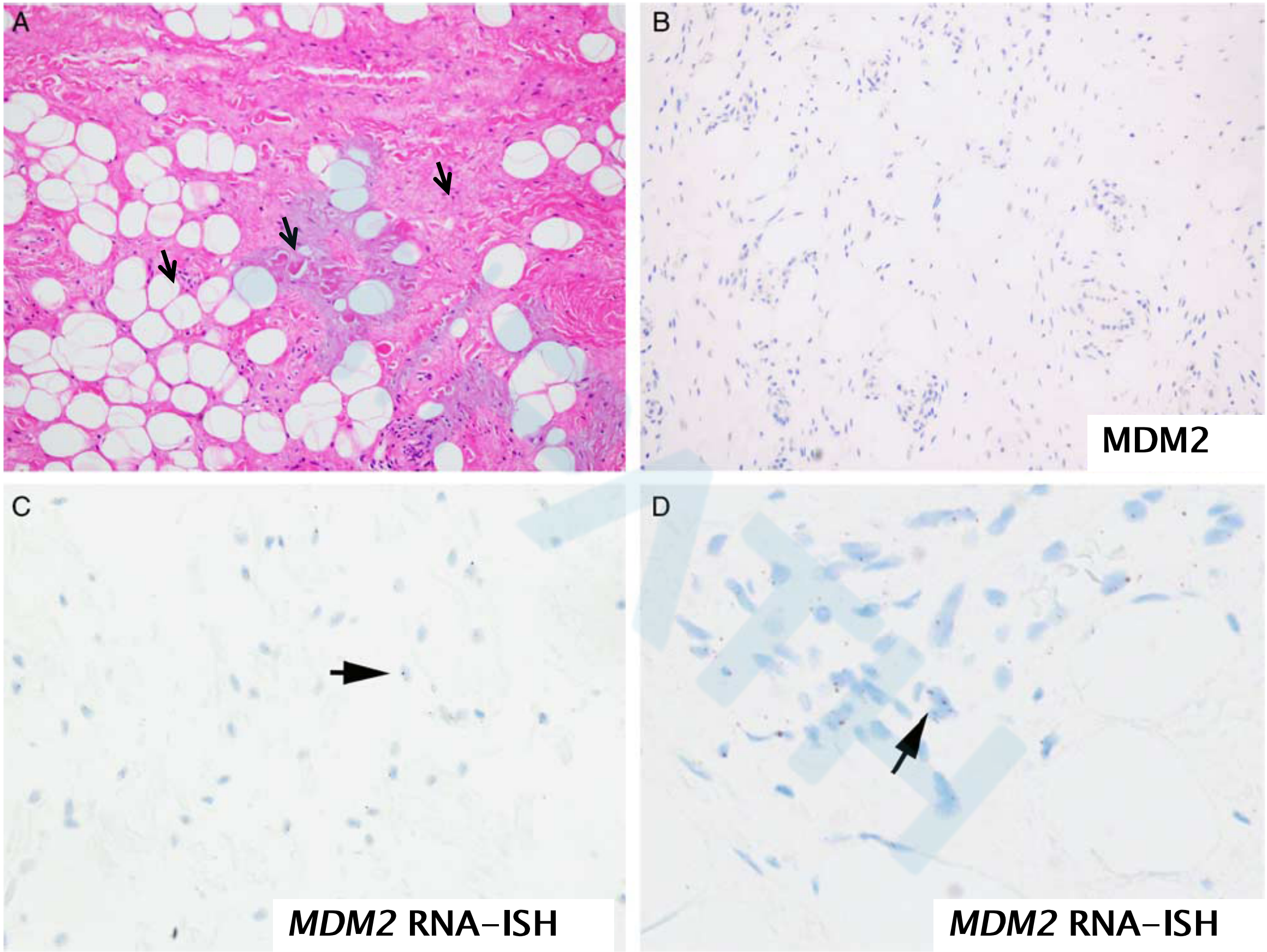


FIGURE 2. Spindle cell lipoma (A), negative for MDM2 immunohistochemistry (B), *MDM2* RNA-ISH shows 1 to 2 dots (arrows) per adipocyte, negative staining pattern (C, D).

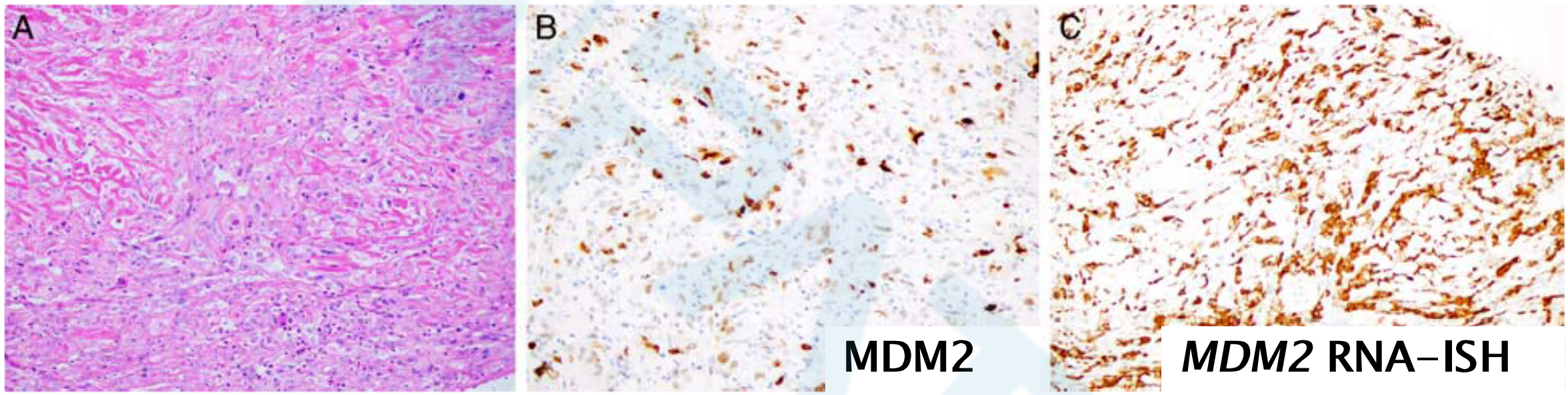


FIGURE 3. Dedifferentiated liposarcoma (A). Although both immunohistochemical stain for MDM2 (B) and *MDM2* RNA-ISH (C) are positive, a higher percentage of neoplastic cells are positive on the latter stain.

RESULTS

TABLE 3. Comparing Signal Performance of *MDM2* RNA-ISH and MDM2 IHC on TMAs

	n/N (%)				
	RNA-ISH+	IHC+	ISH (Visible at ≤4 Objective)	ISH (Mean % of Cells+)	IHC (Mean % of Cells)
Well-differentiated liposarcoma (n=10)	10/10 (100)	9/10 (90)	9/10 (90)	72.5	23.7
Dedifferentiated liposarcoma (n=22)	22/22 (100)	21/22 (95.7)	20/22 (90.9)	69.8	32.3

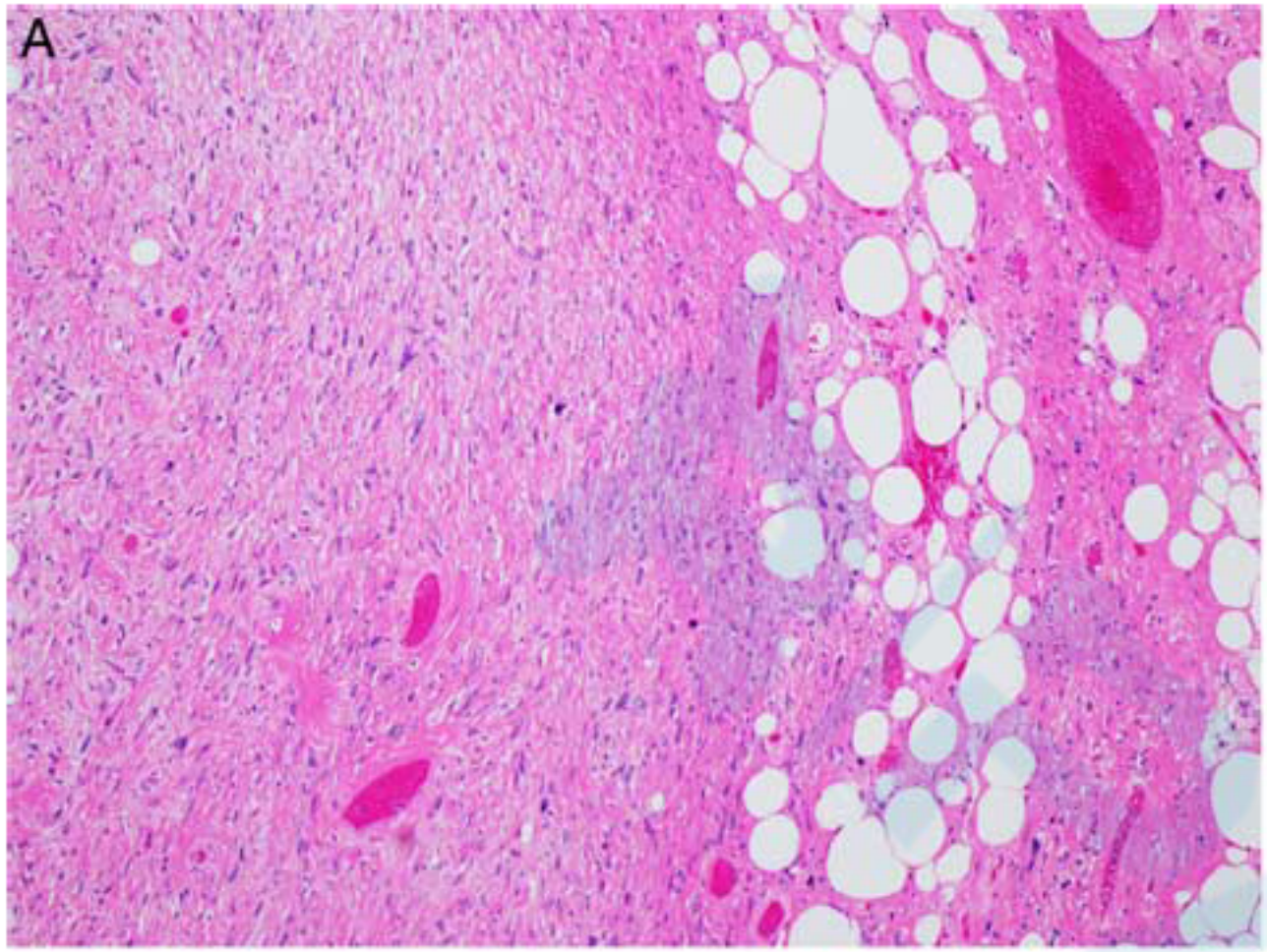
IHC indicates immunohistochemistry.

TABLE 4. Validation Cohort

No.	Age	Sex	Location	Biopsy/Resection	Size (cm)	MDM2 RNA-ISH	MDM2 IHC	CG/FISH	Final Diagnosis
1	69	M	Thigh	Excision	9	Positive	Equivocal	CG positive	ALT
2	63	M	Chest wall	Excision	13	Positive	Equivocal	CG positive	ALT
3	62	F	Retroperitoneum	Excision	8.5	Positive	Equivocal	CG positive	ALT
4	46	M	Thigh	Excision	26.5	Positive	Equivocal	CG positive	ALT
5	71	F	Gluteal	Needle biopsy	21	Positive	Negative	FISH positive	ALT
6	58	F	Shoulder	Excision	14	Positive	Equivocal	FISH positive	ALT
7	77	F	Shin	Needle biopsy	4	Positive	Equivocal	CG positive	ALT
8	42	F	Groin	Biopsy	11	Positive	Equivocal	NA*	ALT
9	65	F	Retroperitoneum	Excision	16.2	Positive	Negative	FISH positive	WDL
10	82	F	Thigh	Biopsy	12.9	Positive	Equivocal	FISH positive	WDL and DDL
11	74	F	Thigh	Excision	26	Negative	Equivocal	CG negative	Lipoma
12	43	M	Periscapular	Excision	8.5	Negative	Positive	FISH negative	Lipoma†
13	51	F	Retroperitoneum	Excision	7	Negative	Negative	FISH negative	Lipoma
14	68	M	Thigh	Resection	10	Negative	Equivocal	CG negative	Lipoma

*Resection was morphologically typical for atypical lipomatous tumor.
†Initially diagnosed as atypical lipomatous tumor.
CG indicates cytogenetics; F, female; IHC, immunohistochemistry; M, male; MDM2, murine double minute 2; NA, not available.

MDM2 RNA-ISH can be of diagnostic value in histologically challenging lipomatous neoplasms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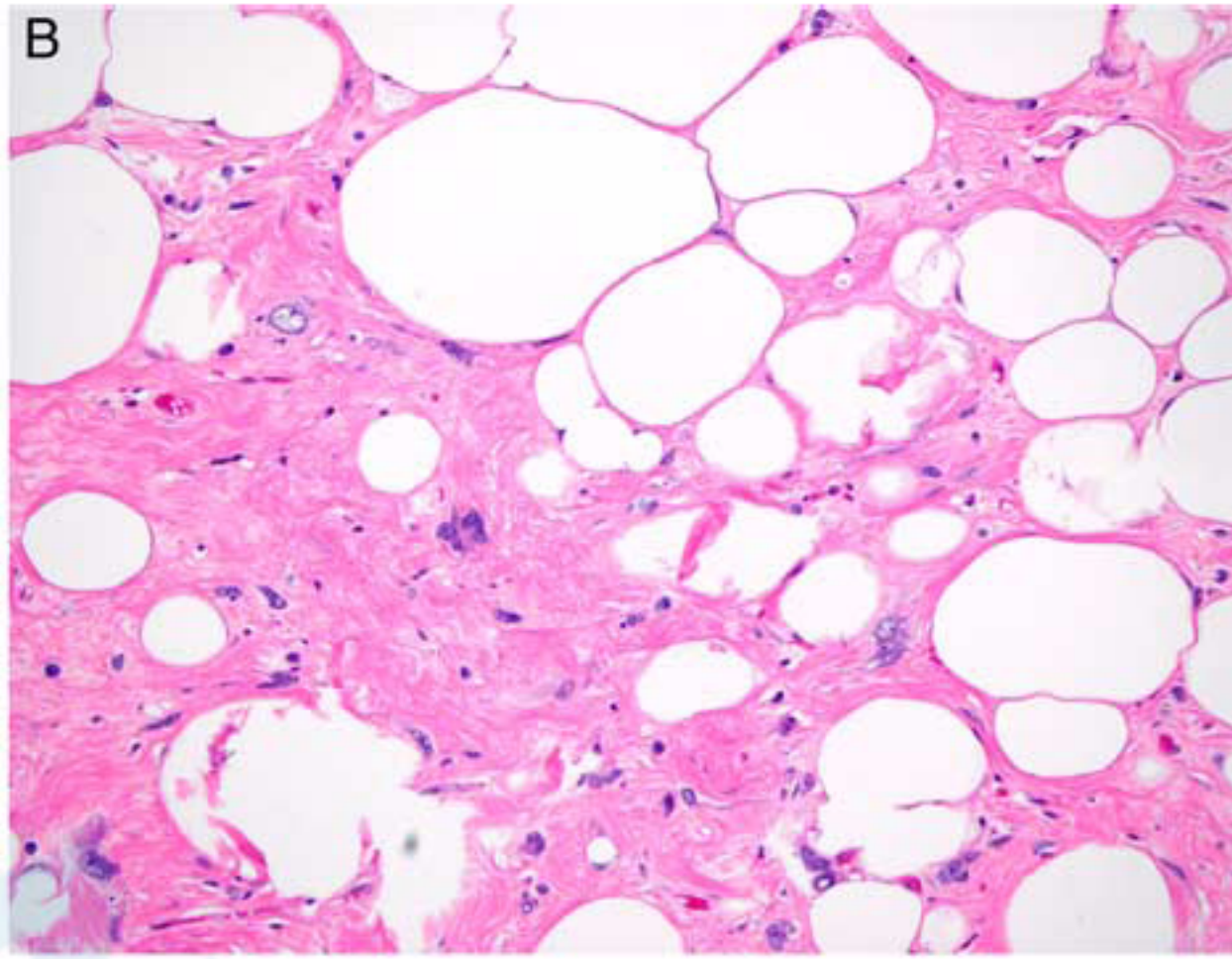
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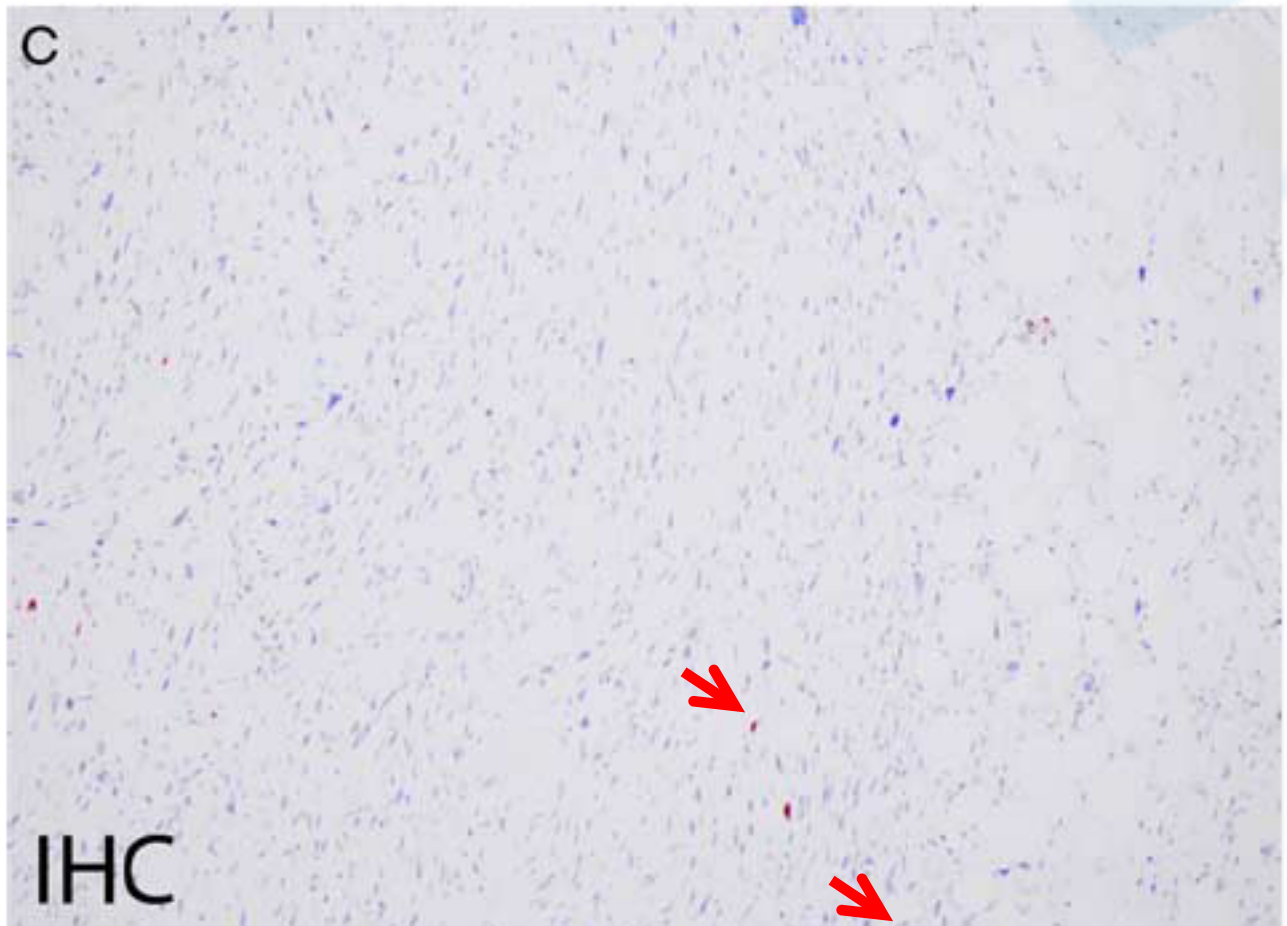
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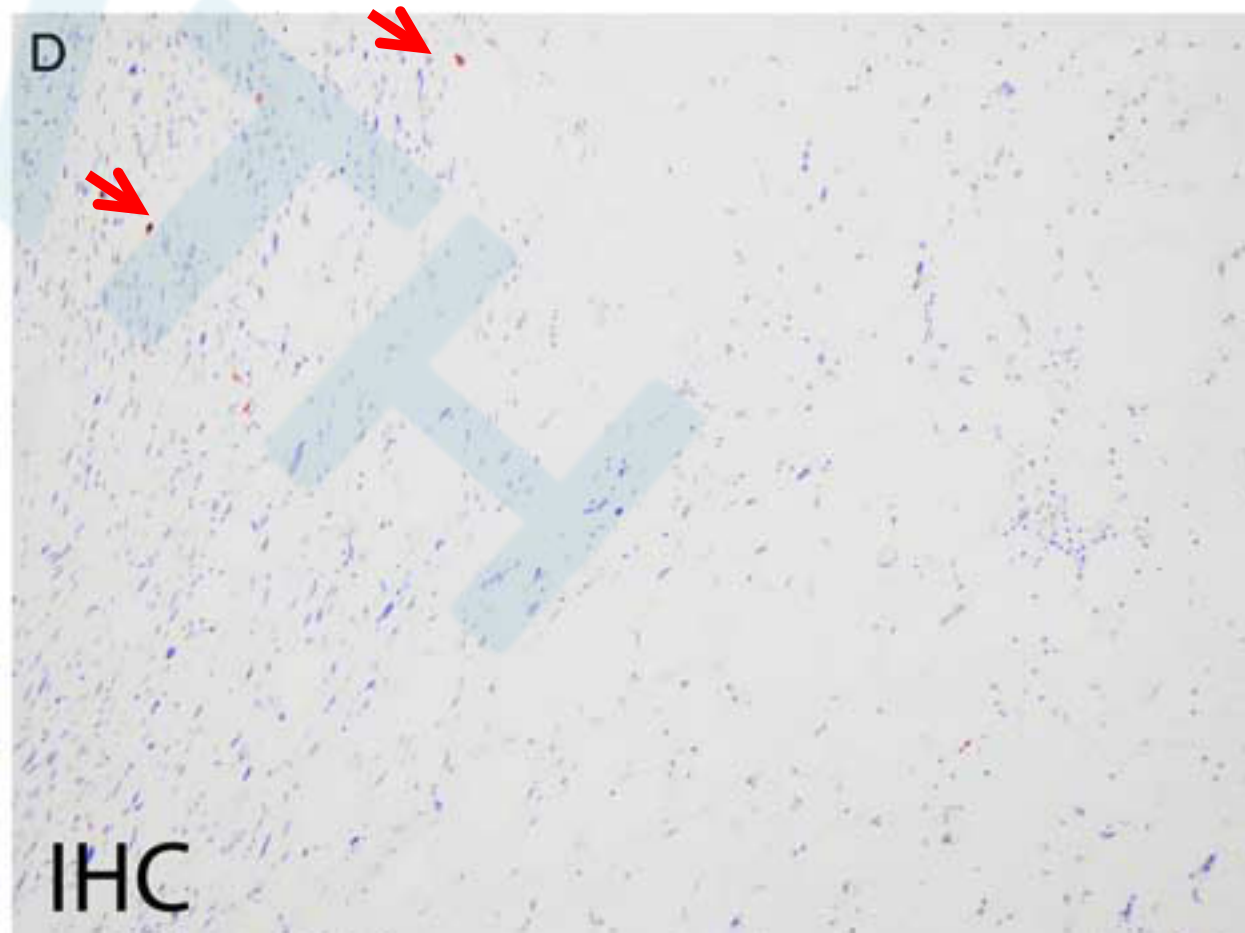
IHC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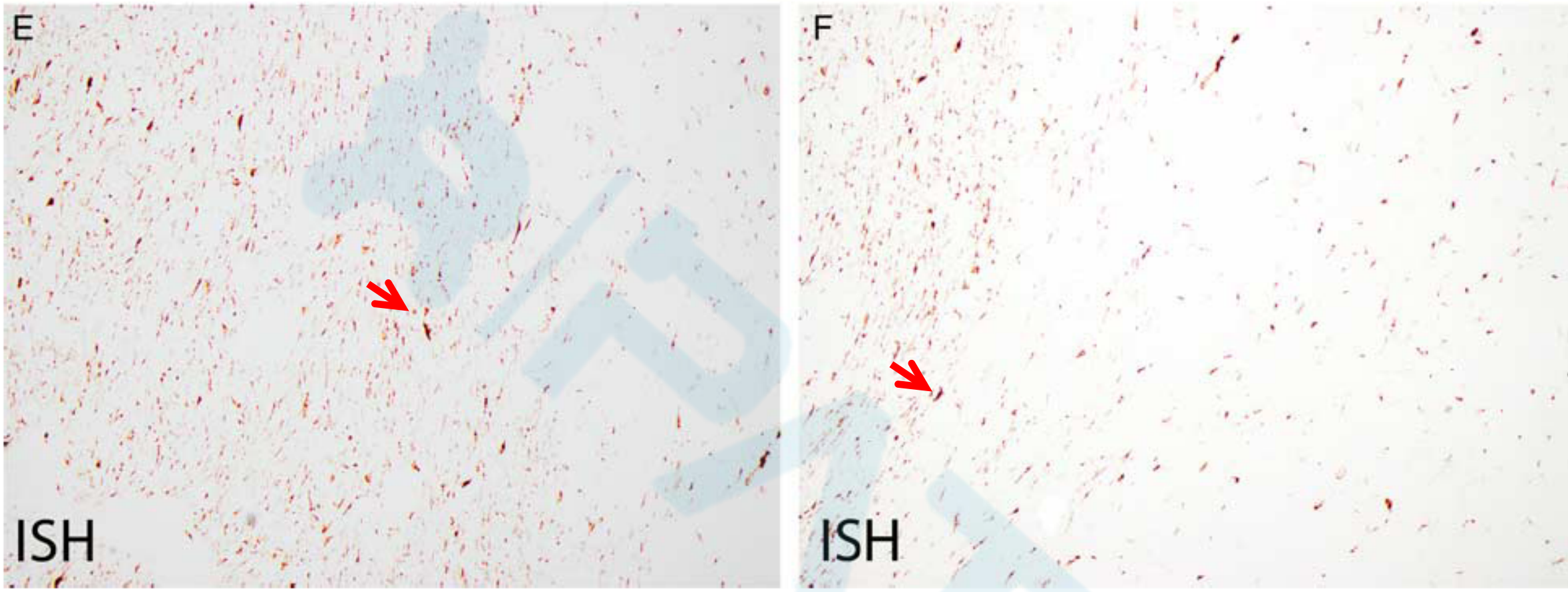

FIGURE 4. Dedifferentiated liposarcoma (A) arising in a well-differentiated liposarcoma (B) (case 10). The well-differentiated component is also seen on (A) (right). Immunohistochemical stains for MDM2 and RNA-ISH for *MDM2* on the dedifferentiated component are illustrated in (C) and (E), respectively. The well-differentiated component is also visible on the right half of the images. Immunohistochemical stains for MDM2 and RNA-ISH for *MDM2* on the well-differentiated component are illustrated in (D) and (F), respectively. The dedifferentiated component is also visualized on the left half of the image. The immunohistochemical stain for MDM2 was equivocal in both components while *MDM2* RNA-ISH was diffusely positive in both the well-differentiated as well as the dedifferentiated portion of the neoplasm. IHC indicates immunohistochemistry.

FIGURE 5. Lipomatous tumor (A), negative for MDM2 immunohistochemistry (B) and positive for *MDM2* RNA-ISH (C) (case #9). The tumor is morphologically indistinguishable from a lipoma. FISH for *MDM2* amplification was positive.

DISCUSSION

- Lipomas may show positive staining for *MDM2* RNA-ISH, this is confined to 4 to 6 dots per nucleus
- ALTs/WDLs show >50 dots and often coalesce to occupy the entire nucleus
- *MDM2* RNA-ISH reactivity is present in both atypical cells as well as lesional cells that resemble mature adipocytes
- *MDM2* RNA-ISH reactivity in DDLs tends to be stronger than ALTs/WDLs

DISCUSSION

- *MDM2* RNA-ISH successfully resolved the diagnostic dilemma associated with a series of challenging lipomatous tumors and could have circumvented issues related to delays of *MDM2* DNA FISH

- While *MDM2* DNA amplification correlates with *MDM2* RNA expression, protein expression is often undetectable; the underlying reasons for this lack of correlation remain uncertain

DISCUSSION

- This automated RNA-ISH *MDM2* assay suitable for routine use in the clinical laboratory
  - The short turnaround time (30 slides can be completed overnight)
  - Brightfield microscopy will allow for the detailed analysis of tissue morphology and tumor heterogeneity
- We did not address the specificity of *MDM2* RNAISH in the context of dedifferentiated liposarcomas

CONCLUSION

- *MDM2* RNA-ISH can be of diagnostic value in histologically borderline and diagnostically challenging lipomatous neoplasms
- The accuracy of *MDM2* RNA-ISH and *MDM2* DNA-FISH are equivalent
- The easy availability and quick turnaround time should facilitate the more widespread use of *MDM2* RNA-ISH in the evaluation of lipomatous tumors

THANK YOU