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Case report

A case of uterine atypical leiomyoma with excess p53 staining diagnosed as benign by p53-Polymerase chain reaction (PCR) followed by Single-stranded conformational polymorphism (p53-SSCP) in the formalin-fixed paraffin-embedded specimen (FFPE)

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Background : Generally the macroscopic necrosis, the pathologic atypism, mitotic index (MI), and p53-stainability are considered to be the reliable histologic diagnostic criteria of cancer. But several uterine atypical leiomyoma of benignancy could show marked cellular atypism and p53 reaction so that a further additional study was required to diagnose its malignancy. We had an opportunity to study a case of uterine atypical leiomyoma by the genetic method with p53-PCR-SSCP and confirm its benignancy.

Case : 54-year-old female patient revealed atypical endometrial hyperplasia and myoma uteri of multiple leiomyomatous nodules, one intramural nodule of which was finally diagnosed as atypical leiomyoma. It was 4.2cm in diameter without any macroscopic necrosis or hemorrhage, but there were microscopic necrosis surrounded by aggregates of atypically bizarre and multinucleated cells in leiomyomatous elements. Total count of mitotic figure (MF) in 6 high power fields of x400 (HPF) with microscopic ocular lens of the field number (FN) 26.5, Mitotic index (MI), was less than 5/2mm^2. Immunohistochemically there were a slight increment of Ki-67-positive cells and abundant p53-positive cells. The genomic analysis of p 53 suppressor gene was done by p53-PCR-SSCP to differentiate atypical leiomyoma from leiomyosarcoma, which failed to show any evidence of genetic anomaly.

Conclusion : No genomic mutation of p53 suppressor gene could disclose this leiomyomatous lesion as benign.

Key words : uterine atypical leiomyoma, histologic criteria of leiomyosarcoma, excess p53 stainability, necrosis, marked cell atypism, mitotic index, ocular lens of the field number (FN) 26.5, accurate identification of mitotic figure, p53-polymerase chain reaction and single-stranded conformational polymorphism (p53-PCR-SSCP), p53-SSCP, formalin-fixed paraffin-embedded specimen, immunohistochemistry (FFPE), Ki-67

Case report

54-year-old female patient revealed atypical endometrial hyperplasia and myoma uteri of multiple leiomyomatous nodules, and total abdominal hysterectomy with bilateral adnexectomy was performed. Postoperative pathologic study showed atypical endometrial hyperplasia and myoma uteri with cytologic atypism. This intramural atypical nodule was 4.2cm in diameter without any macroscopic necrosis or hemorrhage, but there were microscopic necrosis surrounded by aggregates of atypically bizarre and multinucleated cells in leiomyomatous elements (Fig.1). Our recent microscope with FN 26.5 required 6 fields to count MI, and the MI of this case was less than 5/2mm^2. Immunohistochemically there were a slight increment of Ki-67-positive cells and abundant p53-positive cells (Fig. 2, 3). The genomic analysis of p53 suppressor gene, exon 5－8, was done by p53-PCR-SSCP to differentiate atypical leiomyoma from leiomyosarcoma, which failed to show any evidence of genetic anomaly (3, Fig. 4－8). In p53-PCR-SSCP analysis, no genomic mutation of p53 suppressor gene could disclose this leiomyomatous lesion as atypical leiomyoma ; a benign counterpart of leiomyogenic tumor group (Table 2, 3).

Discussion

The histologic findings of malignancy consisted of necrosis, atypia, and MI (Table 3). Macroscopic necrosis has been regarded as important (Table 3). Concerning microscopic necrosis in our case, it was difficult how we should handle it, and we did not emphasize the signifi-

cance of microscopic necrosis.

The MI has been one of the most reliable methods in diagnosing malignancy histologically, and we should no longer count the illegible MF-like apoptotic one to improve the precision of MI value (Table 1). On the previous articles before 30 to 40 years the total MF in 10HPF was counted with the ocular lens FN15 to 20, and its total area was 1－2mm^2(1). The MI of our case was counted with the ocular lens FN26.5 and we counted 6 HPF (x 400) as the total area of 2mm^2 according to some advice by Miettinen et al (1, Acknowledgement, Table 1, 2). The MI was less than 5/2mm^2, which was short of malignancy (Table 3).

We disclosed the importance of abnormal expression of the suppressor gene p53 in diagnosing malignancy histologically (2). But this benign uterine abnormal leiomyoma also showed excessive p53 reaction immunohistochemically, which required further study on genetic mutation of p53 to differentiate the malignant change (3). The p53-SSCP study was very useful to confirm this case as benign atypical leiomyoma with the evidence of negative p53-PCR-SSCP result (Table 3).

Acknowledgement

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References

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症例報告

免疫染色上 p53が強陽性であった子宮異型筋腫の１症例－ホルマリン固定－パラフィン包埋材料を使った p53-polymerase chain reaction and single-stranded conformational polymorphism (p53-PCR-SSCP) 検査で p53変異が認められず平滑筋肉腫と鑑別できた１症例－

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背景：子宮の平滑筋腫瘍に関して、組織学的悪性基準には、肉眼的壊死、組織学的異型性、細胞分裂像数、免疫染色における p53強陽性等の所見がある。今回、高度の異型性と p53強陽性を示す筋腫症例を経験したので、組織学的鑑別と遺伝子補助検査による検討を加えたので報告する。

症例：症例は54才の女性で、異型子宮内膜増殖症と多発性子宮筋腫で、腹式単純子宮全摘出術と両側付属器摘出術が施行された。多発子宮筋腫の内の4.2cm 大の筋腫結節関して、顕微鏡的壊死巣とそれを囲繞する高度異型細胞集簇、細胞分裂像数５未満／2mm^{2}相当、免疫染色上 p53強陽性像が認められた。平滑筋肉腫との鑑別上、ホルマリン固定パラフィン包埋標本 (FFPE) を使った p53－ポリメラーゼ連鎖反応法－１本鎖 DNA 高次構造多型分析法 (p53-PCR-SSCP, p53-SSCP) を実施し、p53の遺伝子変異が無いことを確認し、異型子宮筋腫と診断した。

結論：子宮異型筋腫と子宮平滑筋肉腫の鑑別において、p53-PCR-SSCP による p53抑制遺伝子の変異の同定が有効であった。

キーワード：子宮異型筋腫、平滑筋肉腫との組織鑑別基準、p53強陽性、壊死、高度異型、2mm^{2}当りの細胞分裂数、視野番号 (FN) 26.5の接眼レンズ、細胞分裂像の明確な同定、p53－ポリメラーゼ連鎖反応法－１本鎖 DNA 高次構造多型分析法 (p53-PCR-SSCP、p53-SSCP)、ホルマリン固定パラフィン包埋標本 (FFPE)、免疫染色、Ki-67

Table 1. The relationship between Field number of ocular lens（FN）and the area（mm2/field）at using x 40-objective lens

occular field number (FN)	field area (mm3/x400)
14	0.10
15	0.11
16	0.13
17	0.14
18	0.16
19	0.18
20	0.20
21	0.22
22	0.24
23	0.26
24	0.28
25	0.31
26	0.33
26.5	0.34
27	0.36

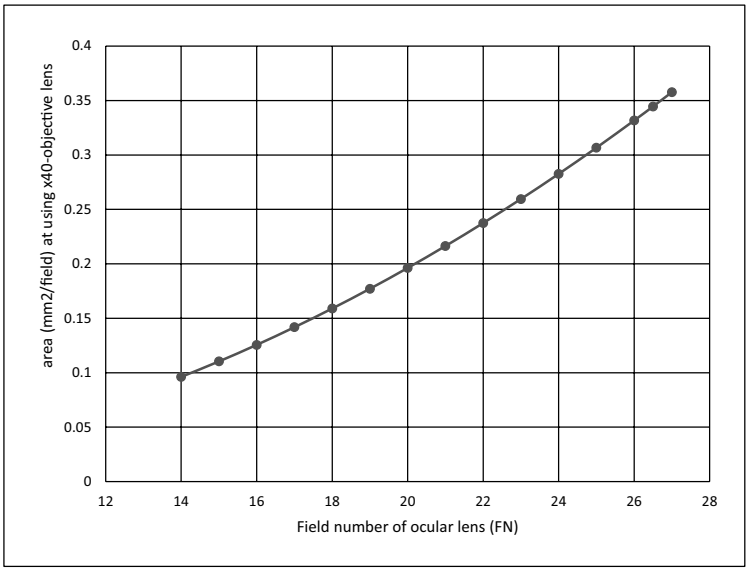

Table 2. The number of the necessary microscopic fields necessary for pathologic diagnosis

primary organ	evidence	total area (mm2)	required counting filelds by using FN26.5 ocular lens (0.345mm2/x400HPF)	atypia	
				histological grading (moderate grade)	cytological grading (moderate grade)
breast	JSC	2	6	8～14	5～10
NET	WHO	2	6	2～20 (Ki67: 3～20)	
soft tissue	WHO	0.1734/x400HPF x10HPF=1.743	5	10～19	
uterus, smooth		FN=20(1990年代)x10HPF=2	6	(malignant: 10≦)	
stomach, smooth		FN=20(1995) 4	12	(malignant: 1≦)	
stomah, GIST	JSC Mittinen	4.8～5	14	*	
intestine, GIST	JSC Mittinen	4.8～5	14	*	

*

tumor diameter (cm)	MF			
	gastric GIST		entero-colic GIST	
	≦5	5<	≦5	5<
≦2	very low risk	low r.	very low risk	high r.
≦5	low r.	intermediate r.	low r.	high r.
≦10	low r.	high r.	intemediate r.	high r.
10<	intemediate r.	high r.	high r.	high r.

FN	field number		
JSC	Japanese Society for Cancer		
MF	exception	apoptosis	perinuclear vacuolar degeneration (perinuclear halo)
		difficult	ref: immunostain of Ki67
	report	mean (minimal～maximal in hot spot)	
WHO	World Health Organization		

Table 3. Uterine smooth muscle tumors, histological differentiation of malignancy

Histologic criteira for the diagnosis of uterine smooth muscle tumors

necrosis	atypia		MF/10HPF				
			<5	atypical	5–9	<10	10≦
+	diffuse	mod–severe	leiomyosarcoma				
		no–mild				STUMP (R/O infarcted leiomyoma)	leiomyosarcoma
–	diffuse	mod–severe	atypical leiomyoma with low risk of recurrence	STUMP			leiomyosarcoma
		no–mild	leiomyoma		mitotically active leiomyoma		
	focal	mod–severe	atypical leiomyoma		STUMP		

cellularity	atypism					
			5	10	15	20
	severe	atypical leiomyoma (syncytial leiomyoma)	STUMP	leiomyosarcoma		
	moderate	epithelioid leiomyoma				
increased	mild	cellular leiomyoma	STUMP			
normal	no	usual leiomyoma	mitotically active leiomyoma		STUMP	

upper table: current criteria based on necrosis, atypia, and MF.
lower table: previous classical criteira based on cellularity, atypia, and MF.
abbr. STUMP smooth muscle tumor of uncertained malignant potential
MF mitotic figures

cf. Uterine smooth muscle tumors, histological differentiation of malignancy, eds. Kurman, RJ and Ronnett BM, Blaustain's pathology of the female genital tract, 6th ed. pp 471–2, 2010, Springer New York.

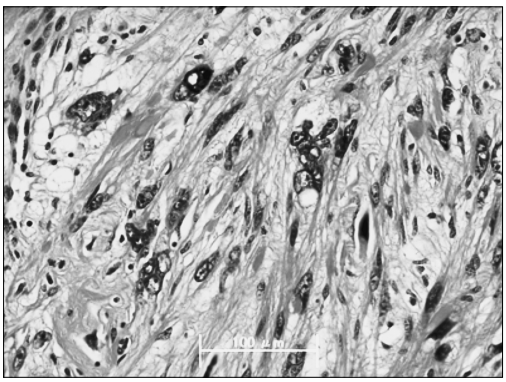

Figure 1. Marked microscopic cellular atypism (HE)
HE : hematoxylin-eosin stain
bar : 100μm

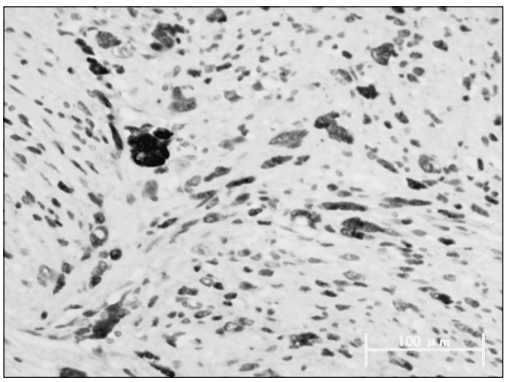

Figure 2. Immunostain of p53
Marked positive stainability.

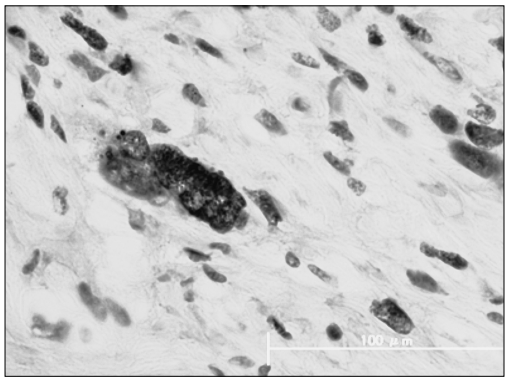

Figure 3. Immunostain of p53
Magnified view of Figure 2.

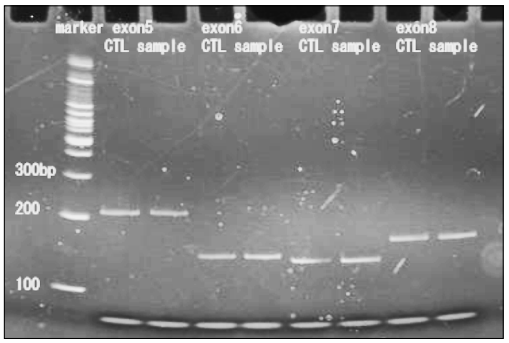

Figure 4. Electrophoresis of p53 products after PCR
PCR : polymerase chain reaction,
CTL : normal control
sample : this case

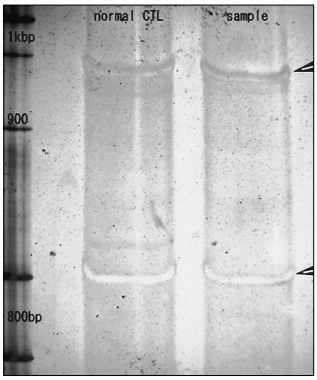

Figure 5. Exon 5 after p53-SSCP
Two same bands were confirmed in both normal control (CTL) and this case (sample)
SSCP : single-stranded conformational polymorphism

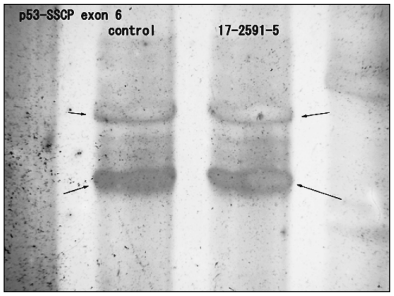

Figure 6. Exon 6 after p53-SSCP
Two same bands were confirmed in both normal control (CTL) and this case (sample)
SSCP : single-stranded conformational polymorphism

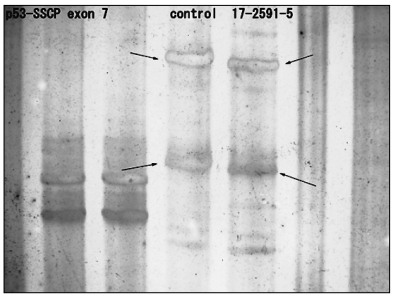

Figure 7. Exon 7 after p53-SSCP
Two same bands were confirmed in both normal control (CTL) and this case (sample)
SSCP : single-stranded conformational polymorphism

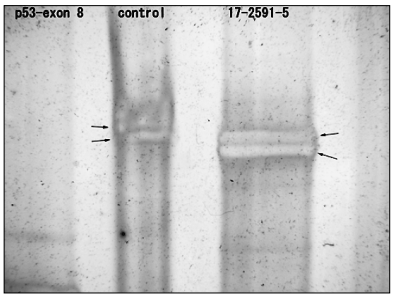

Figure 8. Exon 8 after p53-SSCP
Two same bands were confirmed in both normal control (CTL) and this case (sample)
SSCP : single-stranded conformational polymorphism

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