厚生連医誌　第25巻　1号　69～73　2016

Case report

Two cases of solitary fibrous tumor and gastrointestinal stromal tumor with an unexpected expression of SYT-SSX fused gene as a specific marker for synovial sarcoma

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Abstract

Background : There were three difficult-to-diagnose fibrous tumors ; (1)synovial sarcoma (SS), (2)solitary fibrous tumor (SFT), and (3)gastrointestinal stromal tumor (GIST). These tumors could be differentiated pathologically by the following three viewpoints ; the clinical information especially with respect to the favorable site of occurrence, the characteristic immunostain, and the genetic analysis. However, SS could arise in unusual sites and the immunostain was sometimes useless because of its low discriminating specificity. Consequently SS could be differentiated from the other tumors only by the genetic demonstration of synaptotagmin-synovial sarcoma X breakpoint fused gene of t (X ; 18) (p11.2 ; q11.2) mutual translocation (SYT-SSX fused gene), but then a problem was left in the certainty of this genetic discrimination. We experienced two non-SS cases positive for SYT-SSX fused gene confirmed by the reverse transcriptase-polymerase chain reaction (RT-PCR) with formalin-fixed paraffin-embedded tissue sections (FFPE), and reported in this paper.

Case report : Case 1 ; The pathology was SS of monophasic fibrous type, which originated from lung of an unusual site and diagnosed by the positive imunostain for epithelial membrane antigen (EMA) and the positive SYT-SSX1fused gene. Case 2, 3 : They were identified respectively as serosa-originating SFT and colon-originating GIST by the positive immunostain for CD34 and CD117 (c-kit). Interestingly SYT-SSX1fused gene was identified in both tumors as well as SS.

Conclusion : In the present study, we have demonstrated that both SFT and GIST can also demonstrate SYT -SSX fused gene regarded as the essential tumor marker of SS, so that some of these tumors and SS are reclassified into "the fibrous tumor group expressing SYT-SSX fused gene", or "the SYT-SSX fused gene related tumor."

Key words : synovial sarcoma (SS), solitary fibrous tumor (SFT), gastrointestinal stromal tumor (GIST), immunostain, epithelial membrane antigen (EMA), CD34, CD117 (c-kit), fluorescence in situ hybridization (FISH), reverse transcriptase-polymerase chain reaction (RT-PCR), formalin-fixed paraffin-embedded tissue sections (FFPE), synaptotagmin-synovial sarcoma X breakpoint fused gene of t (X ; 18) (p11.2 ; q11.2) mutual translocation (SYT-SSX fused gene), differential diagnosis, specificity, the fibrous tumor group expressing SYT-SSX fused gene, the SYT-SSX fused gene related tumor

Background

The rare undiagnosable fibrous tumors of borderline malignancy consisted of SS, SFT (hemangiopericytoma, localized fibrous tumor, or fibrous mesothelioma as a synonym), and GIST. These three tumors could be pathologically differentiated by the favored site of localization, the immunostain for CD34 and CD117, and SYT-SSX fused gene (Table 2).

Although SS was often found in the orthopedic regions, recent references revealed that SS could also develop in several internal organs as an unusual origin. Immunohistochemically EMA, CD34, and CD117 were stainable in all tumors and their stainability was various and weak for the definitive diagnosis (Table 2). In these diagnostically troublesome tumors the genetic analysis became the most reliable method, and we reexamined about its differentiating efficacy in this report (1).

As to our materials and methods, neutral 10% FFPE was used for immunostain and RT-PCR study. Total ribonucleic acid (RNA) was collected by NucleoSpin® RNA FFPE (Macherey-Nagel, Germany ; provided by Takara Bio Inc, Japan) and DNA was amplified by PrimeScript® One Step RT-PCR Kit Ver. 2 (Takara Bio Inc, Japan). The electrophoretic blotting size of final PCR products was as follows : SYT-SSX of common type, 98 bp ; SYT-SSX1of one subtype, 118 bp ; and SYT-SSX2 of another subtype, 118 bp (1).

Case report（Table 3）

Case 1 was 53 y/o female with left intrapulmonary carcinoma. She was histologically diagnosed as synovial sarcoma, monophasic type, on the basis of the presence of a small focus of epithelial cells of biphasic type and focally positive EMA cells. SS was confirmed by positive SYT-SSX immunofluorescence in situ hybridization（FISH）study by the pathologic consultation of The Japanese Society of Pathology（＃14-262, 2014/08/27）. SYT-SSX1fused gene was also positive in our RT-PCR study（Figure 1）.

Case 2 was 63 y/o female with huge spherical mass on serosa involving omentum and mesocolica. She was immunologically diagnosed as solitary fibrous tumor by the positive CD34. Our RT-PCR study revealed SYT-SSX1fused gene（Figure 2）.

Case 3 was 81 y/o male with carcinoma of transverse colon, protuberant-ulcerative tumor with well demarcated expansive growth. He was immunohistologically diagnosed as malignant GIST because of positive CD34 and CD117. Our RT-PCR study, furthermore, showed SYT-SSX1fused gene（Figure 2）.

Discussion

In the pathological differentiation among SS, SFT, and GIST, the following clinical findings were important : the site of origin, the immunostain for EMA, CD34 and CD117, and the SYT-SSX fused gene（Table 2）(1).

"Synovial" of SS is a misnomer, because a developmental origin is unknown and a site of origin is unrelated to synovium. As to case 1 SS developed in lung, which was an unusual site for SS. In these heterotopically occuring SS the theoretically coexisting epithelial cells were required to diagnose SS histologically even if there were few cells among massive fibrous spindle cells. Although positive EMA was the immunohistochemical evidence of SS, the degree of positivity and its reliability of positive judgment in non-epithelial spindle cells were lower than that in epithelial cells. The pure fibrous monophasic subtype of ectopic SS or the dedifferentiated subtype of SS was very difficult to diagnose immunologically, and both SFT and GIST could, furthermore, reveal positive EMA like SS (2, 4). In those situations, the confirmation of SYT-SSX fused gene is necessary to diagnose SS pathologically.

Case 2 was diagnosed as SFT because of the subserosal location as a favorable location and the strong immunostaining for CD34. Case 3 was diagnosed as GIST because of the wall of digestive tract as a favorable location and the strong immunostaining for CD34- and CD 117. The tumor-originating location was, however, often ambiguous in extramural overgrowing tumors : the tumor arising from external proper muscle close to subserosa could not be correctly discriminated from the subserosal tumor infiltrating into mural wall. CD34 could be shown in SS as well as GIST and SFT(3). In an immunostaining analysis the decreased staining for CD34 and CD117, furthermore, disturbed confirming GIST and SFT tumors. In those situations, the confirmation of SYT-SSX fused gene for SS is useful to rule out SS(1). Because a reliability of this genetic screening has been emphasized, the positive tumors are automatically classified into SS. However, our two cases of SFT and GIST also showed positive SYT-SSX1 fused gene, and the positive cases suggested not only SS but also other tumors. That being the case, the confirmation of SYT-SSX1 fused gene was not the sufficient evidence to diagnose SS pathologically.

SYT-SSX fused gene were not diagnostic in our cases of SFT and GIST, so that the tumors with positive SYT-SSX fused gene could be reclassified into "the broader category including SS" or "the fibrous tumor group expressing SYT-SSX fused gene" named after this genetic feature.

Conclusion

SS is not a disease specialized in synovial tissue, and the demonstration of SYT-SSX fused gene is not the most reliable discriminating marker for SS. Both SS and a part of SFT and GIST could be tentatively grouped together into "the fibrous tumor group expressing SYT-SSX fused gene", or "the SYT-SSX fused gene relating tumor," to remove a further meaningless discriminating effort.

Reference

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和　文　抄　録

症例報告

滑膜肉腫に特異的な SYT-SSX 融合遺伝子の異常発現

を認めた弧在性線維性腫瘍と消化管間質腫瘍の２症例

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背景：組織鑑別が困難な境界悪性型線維性腫瘍として以下の３腫瘍がある；すなわち、滑膜肉腫（synovial sarcoma, SS）、弧在性線維性腫瘍（solitary fibrous tumor, SFT）、消化管間質腫瘍（gastrointestinal stromal tumor, GIST）である。これらの腫瘍の組織学的鑑別診断の手掛りには３項目がある；すなわち、好発発生部位などの臨床所見と、免疫染色検査における上皮性膜抗原（epithelial membrane antigen, EMA）、CD34、CD117（c-kit）発現と、そして、遺伝子検査における t（X；18）（p11.2；q11.2）相互転座 synaptotagmin-synovial sarcoma X breakpoint fused gene（SYT-SSX 融合遺伝子）の発現が鑑別点となる（表２）。SS は四肢関節部が好発部位とされ、EMA 陽性により診断される。が、好発以外の異所性に発生する SS が報告されるようになり、EMA の陽性所見が不明瞭な症例においては、SS に特異的とされる SYT-SSX 融合遺伝子の発現が唯一の鑑別診断根拠となった。本論文において、その SYT-SSX 融合遺伝子発現の特異性に関して検討した。SS、SFT、GIST と診断された３症例について、そのホルマリン固定―パラフィン包埋組織切片（formalin-fixed paraffin-embedded tissue sections, FFPE）を材料として、全 ribonucleic acid（RNA）を抽出・精製し、逆転写ポリメラーゼ連鎖反応（reverse transcriptase-polymerase chain reaction, RT-PCR）により検討を加えた。その結果、SFT と GIST の２症例にも SS 同様の SYT-SSX 融合遺伝子を確認できたので報告した。

症例報告：第１症例：EMA 陽性の異所性肺原発の単相線維型一部二相型の SS で、SYT-SSX 融合遺伝子が陽性であった。第２、３症例：CD34強陽性の漿膜原発の SFT と、CD34と CD117が強陽性の消化管固有筋層原発の GIST であった。２例ともに SYT-SSX 融合遺伝子発現が陽性であった。

結論：３腫瘍（SS と SFT と GIST）の鑑別点としての発生部位、免疫染色（EMA、CD34、CD117）、及び SYT-SSX 融合遺伝子に関して再評価した。SS に特異的とされる SYT-SSX 融合遺伝子が SFT と GIST の２症例に共通に発現し、この遺伝子発現による病理学的鑑別が困難であることがわかった。SS 及び鑑別が困難な腫瘍を含めたより広義の疾患単位として、暫定的に、『SYT-SSX 融合遺伝子発現線維性腫瘍群』または『SYT-SSX 融合遺伝子関連腫瘍』として捉えることを提唱した。

キーワード：滑膜肉腫（synovial sarcoma, SS）、孤在性線維性腫瘍（solitary fibrous tumor, SFT）、消化管間質腫瘍（gastrointestinal stromal tumor, GIST）、免疫染色、上皮性膜抗原（epithelial membrane antigen, EMA）、CD34、CD117（c-kit）、蛍光 in situ ハイブリダイゼーション（immunofluorescence in situ hybridization, FISH）、逆転写ポリメラーゼ連鎖反応（reverse transcriptase-polymerase chain reaction, RT-PCR）、ホルマリン固定―パラフィン包埋組織切片（formalin-fixed paraffin-embedded tissue sections, FFPE）、t（X；18）（p11.2；q11.2）相互転座 synaptotagmin-synovial sarcoma X breakpoint fused gene 融合遺伝子（SYT-SSX fused gene）、特異性、SYT-SSX 融合遺伝子発現線維性腫瘍群、SYT-SSX 融合遺伝子関連腫瘍、病理学的鑑別診断

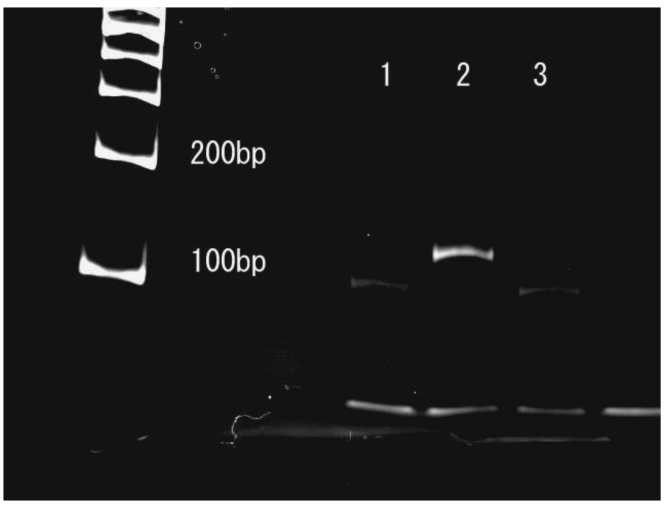

Figure 1. Electrophoresis of Case 1,
1 : SYT-SSX, 2 : SYT-SSX1, 3 : SYT-SSX2.
Lane 2 : SYT-SSX1 fused gene is confirmed in 118bp.

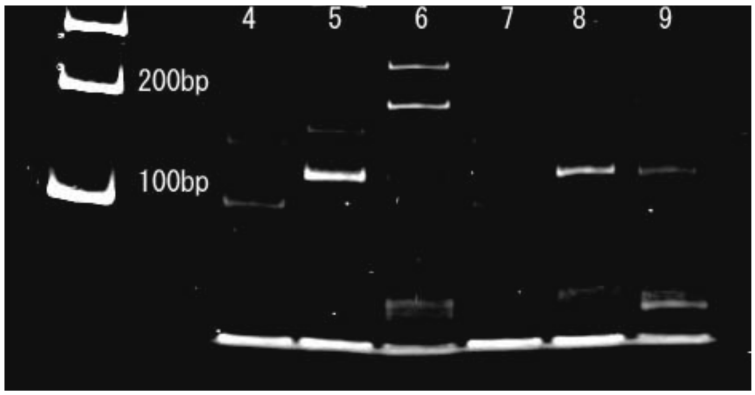

Figure 2. Electrophoresis of Case 2 and 3,
4-6 : Case 2, 7-9 : Case 3, 4, 7 : SYT-SSX, 5, 7 : SYT-SSX1, 6, 9 : SYT-SSX2.
Lane 5 and 8 : SYT-SSX1 fused gene is confirmed in 118bp.

Table 1. Abbreviation list

Japanese	English	Abbreviation
ホルマリン固定―パラフィン包埋組織切片	formalin-fixed paraffin-embedded tissue sections	FFPE
蛍光in situハイブリダイゼーション	immunofluorescence in situ hybridization	FISH
消化管間質腫瘍	gastrointestinal stromal tumor	GIST
ヘマトキシリン―エオジン染色	hematoxylin-eosin stain	HE
逆転写ポリメラーゼ連鎖反応	reverse transcriptase-polymerase chain reaction	RT-PCR
孤立性線維性腫瘍	solitary fibrous tumor	SFT
滑膜肉腫	synovial sarcoma	SS
SYT-SSX融合遺伝子	t(X;18)(p11.2;q11.2) mutual translocation synaptotagmin-synovial sarcoma X breakpoint fused gene	SYT-SSX fused gene

Table 2. Routine differential diagnosis based on references

pathologic diagnosis synonym		SS	SFT hemangiopericytoma, localized fibrous tumor, fibrous mesothelioma	GIST
suspicious origin		unknown	Interstitial cells in subserosal area	Interstitial cells of Cajal in proper muscle layer of gastrointestinal wall
involved site	favorable site of occurren	extremities	visceral pleura, soft tissue	digestive tract
	unusual site of location	gastrointestinal tract, head, heart, kidney, lung, mediastinum, pleura, prostate, retroperitoneum, retropharynx,vulva, stomach, vessel	adrenal gland, bone, breast, central nervous system, eylid, kidney, lacrimal gland, larynx, liver, lung, mediastinum, meninges, nasopharynx, oral cavity, orbit, pancreas, peritoneum, pleura, prostate, renal pelvis, ureter, retroperitoneum, salivary gland, skin, spermatic cord, thorax, thyroid, urinary bladder, uterus,	gallbladder, mesentery, omentum, pancreas, pleura, prostate, retroperitoneum, uterus, vulva
immunostain	EMA	++ 50-100%	― (+)*	― (+)**
	CD34	± <6%	++ 90-100%	++ 60-89%
	CD117 (c-kit)	＋ <10%	―	++ 90-100%
	CD99	++ 20-60%	++ 90-100%	＋ 89%
genetic study	SYT-SSX fused gene	++ 60-100%	―	―

listed on the basis of Rosai J ed. Rosai and Ackerman's Surgical Pathology. 10th ed. Edinburgh: Mosby Elsevier Health Science: 2011. and Pathology Outlines. Com (available from; URL: http://www.pathologyoutlines.com/)

% positivity rate regardless of its severity and reliablity
+ effective degree
* reference 2
** reference 4

Table 3. Case record

case numbrer			case 1	case 2	case 3
histogogical number			14-8975	15-4629	12-4179
age (years/old), sex			53 y/o, female	63 y/o, female	81 y/o, male
origin			intrapulmonary	serosa (omentum/mesocolica)	transverse colon (proper muscle layer and subserosa)
tumor size (cm)			5	22	8
macroscopy			expansive, nodular, solid, hemorrhage, necrosis	expansive, globular, solid, degeneration	epansive, ulcerative, endophytic, solid
HE stain			fibrous, spindle cell, cell atypism: mild, few small epithelial	fibrous, spindle cell, cell atypism; moderate	fibrous, spindle cell, cell atypism: moderate
mitotic counts/mm2			18	2	11
lymph node metastasis			−	−	+
distant metastasis			−	−	+: liver, peritoneum
immunostain		myoglobin	−	−	−
		desmin	−	−	−
		HHF35	−	−	−
		aSMA	−	−	−
		S100	−	−	−
		CD34	−	++	++
		CD117 (c-kit)	−	−	+
		ALK	−	−	−
		CD68	−	−	−
		HMB45	−	−	−
		chromogranin A		−	
		EMA	± few epithelial cells	−	−
		AE1/3	± few epithelial cells	−	−
		CAM5.2		−	
		BerEP4		−	
		D2-40		−	
		WT1		−	
		calretinin		−	
		p63		−	
		CD99	−	−	−
		p53	−	+	
		Ki67		± <5%	+ 40%
		bcl-2		+	
genetic study	FISH	SYT-SSX fused gene	+		
	RT-PCR	SYT-SSX fused gene	SSX1	SSX1	SSX1
final diagnosis			synoial sarcoma; monophasic fibrous>>biphasic	solitary fibrous tumor, including hemangiopericytoma	GIST, malignant

（2015／05／31受付）