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Case report

A case of low-grade fibromyxoid sarcoma diagnosed by FUS-CRREB3L2 fusion transcript using reverse transcription-polymerase chain reaction in formalin-fixed paraffin-embedded tissue specimen

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Background : Genetic analysis is useful for the differentiation of soft part tumors. We could diagnose a case of low-grade fibromyxoid sarcoma (LGFMS) by detecting FUS-CREB3L2 fusion transcript (FUS-CREB3L2) and reported in this paper.

Case report : The 76 years old female case had noticed an egg-shaped tumor in left groin subcutis for three years. At enucleation of tumor, the tumor was 5.5 cm in size and accompanied a central mucinous lake. Microscopically the tumor consisted of fibrous tumor cells of weak atypism and intercellular myxoid stroma. The mitotic count was less than 1 %/2 mm2 and there were no characteristic findings immunohistochemically. The FP3-RP3 type of FUS-CREB3L2 was detected by reverse transcription-polymerase chain reaction (RT-PCR) using formalin-fixed paraffin-embedded specimens (FFPE) and LGFMS was able to be diagnosed.

Conclusion : In the case that the pathological differentiation among fibrous soft part tumors was difficult, the genetic screening using FFPE was very effective, especially of LGFMS.

Key words : low-grade fibromyxoid sarcoma (LGFMS), FUS gene on chromosome 16p11.1 (FUS), forward primer FP2-fc2 on in exon 6 of FUS (FP2), forward primer FP3-fc2 in exon 6 of FUS (FP3), CREB3L2 gene on chromosome 7q34 (CREB3L2), reverse primer RP1-fc2 in exon 5 of CREB3L2 (RP1), reverse primer RP2-fc2 between exon 5 and exon 6 of CREB3L2 (RP2), reverse primer RP3-fc2 in exon 6 of CREB3L2 (RP3), FUS-CREB3L2 fusion transcript (FUS-CREB3L2), reverse transcription-polymerase chain reaction (RT-PCR), formalin-fixed paraffin-embedded tissue specimen (FFPE), deoxyribonucleic acid (DNA), ribonucleic acid (RNA)

Background

Genetic analysis is useful for the differentiation of soft part tumors because each histological type shows the specific genetic fusion. LGFMS reveals the FUS-CREB3L2 fusion transcript.(1) A formalin fixation is an essential process in an examination of histopathology, and the genetic resolution with the formalin disturbs the subsequent genetic examination using FFPE specimens. We examined the possibility of the clinical genetic screening in LGFMS using the FFPE specimen and reported in this paper.

Case report

The patient first noticed tumor three years ago, diagnosed as fibroma, and tumor was extirpated in her 76 years old at 3 years later after noticing her tumor mass, The left inguinal subcutaneous lambda-shaped oval tumor was expansive growth and 5.5x4.3x3cm in size. Microscopically tumor consisted of compact fibrous cells of weak atypism, low mitotic index of 1 %, and prominent intercellural myxoid change with mucinous retention in the center of tumor mass. There was no immunohistochemical characteristics, no imaging evidence of infiltration and metastasis, and no involvement of femoral nerve. Her histopathological findings suggested the mixed tumor of fibrous and myxoid elements without any aggressive malignancy. The genetic examination of the fusion pattern was accomplished as a definitive diagnosis as following 3 steps.(1,2) (i) Total ribonucleic acid (RNA) was extracted from 10% neutral formalin-FFPE with NucleoSpin ®total RNA FFPE (Takara, Tokyo), (ii) RT-PCR was done with PrimeScript TM One Step RT-PCR Kit Ver.2 (Takara, Tokyo). The reaction mixture was produced based on Takara's commercial manual, The amplification profile of PCR consisted of 35 cycls of denaturation at 94℃ for 30", annealing and elongation at 60℃ for 30" and 72℃ for 60" rspectively, and followed by a final ex-

tension at 72℃ for 10'. The forward and reverse primers were same as those in the previous report and our selection of primers limited to the most frequent five ones in LGFMS(1) ; namely, primers consisted of forward primer FP2-fc2 on in exon 6 of FUS (FP2), forward primer FP3-fc2 in exon 6 of FUS (FP3), reverse primer RP1-fc2 in exon 5 of CREB3L2 (RP1), reverse primer RP2-fc2 between exon 5 and exon 6 of CREB3L2(RP2), and reverse primer RP3-fc2 in exon 6 of CREB3L2 (RP3). (iii) FUS-CREB3L2 fusion products by PCR, 72-203bp, were identified by polyacrylamide gel electrophoresis. This case showed FUS-CREB3L2 fusion transcript as FP3-RP3 pattern between forward primer FP3-fc2 in exon 6 of FUS (FP3) and reverse primer RP3-fc2 in exon 6 of CREB3L2 (RP3) and could finally be diagnosed as LGFMS. Poly-acrylamide gel electrophoresis (PAGE) was done under 200V, 30'with Mini-gel Slab-type electrophoresis of 106 x100x1mm in plate size (Nihn-eido, Tokyo).

Discussion

An examination for RT-PCR using FFPE materials included two serious problems derived from a gene destruction by formalin fixation ; i.e., the inability of gene amplification and the nonspecific amplification bands. In this case the amplification of objective gene was possible by short fixation period with neutral formalin in spite of fewer reliable primers, but we were troubled with the nonspecific amplification bands. Eliminating these nonspecific extra-band, we tried to reduce the number of cycles of the PCR and increase the annealing temperature. We ultimately coped with this problem by the alteration of PAGE condition from 150V to 200V.

Although it was very difficult to differentiate LGFMS from other fibromatous tumors, the genetic analysis of FUS-CREB3L2 fusion was the most reliable technique to diagnose LGFMS.

References

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和 文 抄 録

症例報告

ホルマリン固定・パラフィン包埋病理標本（FFPE）を使った逆転写ポリメラーゼ連鎖反応（RT-PCR）によるFUS-CRREB3L2融合転写物（FUS-CREB3L2）を検出することによって診断された低悪性線維粘液肉腫（LGFMS）の1症例

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背景：線維性軟部腫瘍の診断鑑別には遺伝子診断が有用である。今回、FUS-CREB3L2を検出することによりLGFMSと診断された症例を経験したので報告した。
症例内容：症例は76才女性で、3年来、左の鼠径部皮下に卵型腫瘍を自覚した。腫瘍摘出時、腫瘍は5.5x4.3x3cmで中心性粘液性停留を示し、顕微鏡所見上粘液背景の強い線維性腫瘍細胞の細胞異型は弱く、1％の低い分裂指数で、免疫組織化学的特徴でなかった。FFPEから抽出したRNAを使ったRT-PCRによりFUS-CREB3L2のFP3-RP3型が検出され、LGFMSと診断できた。
結論：線維性軟部腫瘍の病理学的鑑別が困難な症例において、FFPEを使った遺伝子検査は有効な手段であった。

キーワード：低悪性線維粘液肉腫（LGFMS）、染色体16p11.1のFUS遺伝子（FUS）、FUS遺伝子エクソン6の順行プライマーFP2-fc2(FP2)、FUS遺伝子エクソン6の順行プライマーFP3-fc2（FP3）、染色体7q34のCREB3L2遺伝子（CREB3L2）、CREB3L2遺伝子エクソン5の逆行プライマーRP1-fc2（RP1）、CREB3L2遺伝子エクソン5・6間の逆行プライマーRP2-fc2（RP2）、CREB3L2遺伝子エクソン6の逆行プライマーRP3-fc2（RP3）、FUS-CREB3L2融合転写（FUS-CREB3L2）、逆転写ポリメラーゼ連鎖反応（RT-PCR）、ホルマリン固定・パラフィン包埋病理標本（FFPE）、デオキシリボ核酸（DNA）、リボ核酸（RNA）

List of abbreviation

abbreviation	formal name, sequence
LGFMS	low-grade fibromyxoid sarcoma
FUS	FUS gene on chromosome 16p11.1
FP2-fc2	forward primer FP2-fc2 on in exon 6 of FUS, 5'-cAg tgg tgg cgg ttA tgg cAA-3'
FP3-fc2	forward primer FP3-fc2 in exon 6 of FUS, 5'-tgg tgg ttA cAA ccg cAg cA-3'
CREB3L2	CREB3L2 gene on chromosome 7q34
RP1	reverse primer RP1-fc2 in exon 5 of CREB3L2, 5'-ctg gAg ggg ctg tgg gtc tgA-3'
RP2	reverse primer RP2-fc2 between exon 5 and exon 6 of CREB3L2, 5'-Agt ttA tgA ggA gcc gtg Agg-3'
RP3	reverse primer RP3-fc2 in exon 6 of CREB3L2, 5'-tct tct cct cct ctg tcA ggA c-3'
FUS-CREB3L2	FUS-CREB3L2 fusion transcript
RT-PCR	reverse transcription-polymerase chain reaction
FFPE	formalin-fixed paraffin-embedded tissue specimen
DNA	deoxyribonucleic acid
RNA	ribonucleic acid
PAGE	poly-acrylamide gel electrophoresis

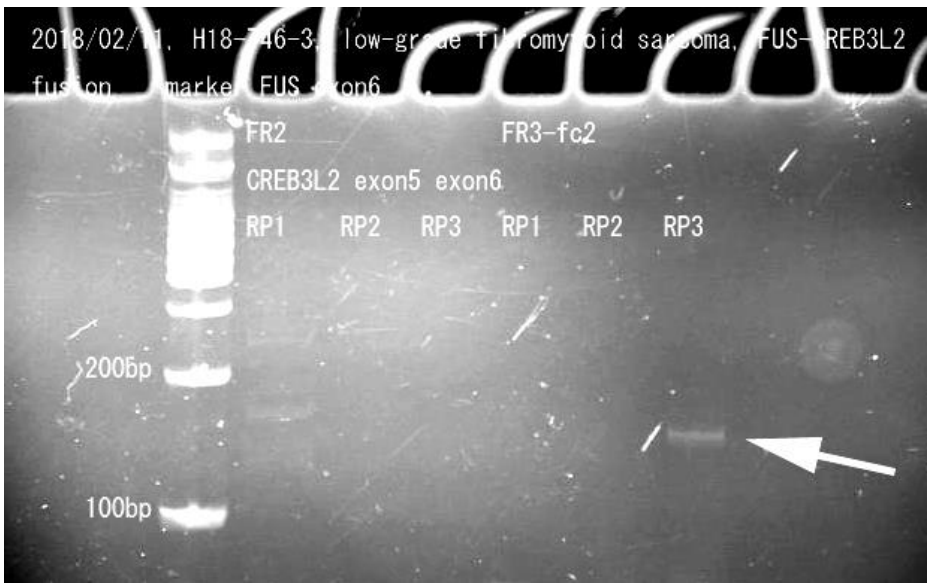

Fig 1. Electrophoresis of FUS-CREB3L2 fusion transcript（FUS-CREB3L2）.
FP3-RP3 fusion pattern was found in the 6th lane.
0th lane : 100bp markers, 1st lane : FP2-RP1 fusion, 2nd lane : FR2-RP2 fusion, 3rd lane : FR2-RP3 fusion, 4th lane : FR3-RP1 fusion, 5th lane : FR3-RP2 fusion, 6th lane : FR3-RP3 fusion.

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