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Original article

Malignant solitary fibrous tumor with high-grade nuclear atypia: An alternate entity for the undetermined tumor group

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ABSTRACT

Recently, a novel fusion transcript, NAB2–STAT6, and its variants have also been reported to be specific diagnostic markers for solitary fibrous tumors (SFTs). In this study, we validated the existence of the NAB2–STAT6 fusion gene in SFTs and examined its relation with the pathological features. Frozen samples from 9 tumors were assessed for fusion gene. The detected fusion genes exhibited large intron sequences and the insertion of unknown and previously unreported sequences. The fusion genes were not detected in the 2 malignant cases with high-grade nuclear atypia, nuclear pleomorphism and necrosis, that was confirmed by multiplex PCR method. In addition, 1 of the 2 NAB2–STAT6 fusion gene-negative tumors showed amplification of the MDM2 and CDK4 genes. It was suggested that a certain proportion of tumors previously diagnosed as malignant SFTs with high-grade nuclear atypia lacking NAB2–STAT6 should be categorized into a special subtype of SFT, which is genetically different from conventional SFTs, and which cannot be apparently distinguished from dedifferentiated liposarcoma or undifferentiated pleomorphic sarcoma.

Introduction

Solitary fibrous tumor (SFT) is a rare fibrous tumor that possesses distinct histopathological features such as hemangiopericytomatous branching vessels, patternless architecture, fibro-collagenous stroma and immunoreactivity for CD34, but does not show consistent immunoreactivity for other markers such as smooth muscle actin, desmin, S-100 and cytokeratins (CKs) [1]. It is difficult to make a definitive diagnosis in cases without typical histopathological findings by microscopic examination or immunohistochemical analysis alone. In addition, other soft tissue tumors with CD34 immunoreactivity, such as gastrointestinal stromal tumors (GIST) or dermatofibrosarcoma protuberans (DFSP), or morphological mimics such as synovial sarcoma, glomangiopericytoma, angiofibroma of soft tissue or mesenchymal chondrosarcoma, make it difficult to differentiate these tumor types definitively.

In 2013, Chmielecki et al. and Robinson et al. introduced a new fusion gene, NAB2–STAT6, and its variants as specific diagnostic markers for SFTs [2,3]. Subsequently, STAT6-nuclear expression was picked up as a diagnostic tool to detect the fusion gene by immunostaining [4,5]. Together, these investigations provided novel strategies for the diagnosis of soft tissue tumors. However, it remains to be examined whether there is any difference between the fusion gene-positive SFTs and so-called solitary fibrous tumors which are CD34 immunostaining and morphologically defined regardless of fusion gene. In this study, we confirmed the existence of the NAB2–STAT6 fusion gene in SFTs by using frozen samples and examined its relation to the clinicopathological and histopathological features.

Materials and methods

Materials

Nine cases of previously diagnosed SFTs, for which frozen samples were available, were retrieved from the soft tissue tumors registered in the files of the Department of Anatomic Pathology, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan. Each tumor was classified according to its histology and

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immunohistochemical results by reference to the recent World Health Organization classification [1]. Only one sample was from a recurrent tumor. A frozen sample from each of the 9 patients was prepared for gene analysis. Informed consent was obtained from the patients or guardians. This study was conducted in accordance with the principles embodied in the Declaration of Helsinki. The study was also approved by the Ethics Committee of Kyushu University (No. 25-111, 25-143).

Histological malignancy of SFT was evaluated as follows. Samples were judged as England's malignant SFT if they fulfilled at least one of the diagnostic criteria of malignant SFT proposed by England et al.: (a) high cellularity, (b) >4 mitotic figures per 10 high-power fields (HPFs), (c) nuclear pleomorphism, (d) hemorrhage and (e) tumor necrosis [6]. They were judged as Fletcher's malignant SFT if they fulfilled at least one of the malignant features indicated by Fletcher et al.: (a) high cellularity, (b) more than 4 mitotic figures per 10 HPFs, (c) more than intermediate nuclear atypia and (d) tumor necrosis [7]. In this study, "intermediate nuclear atypia" were defined as definite nuclear atypia that were not high-grade atypia. In brief, the tumor nuclei had irregular shapes, slight differences in size and shape, and hyperchromatism, but lacked a rough chromatin pattern or prominent deviation in size and shape. On the other hand, "high-grade nuclear atypia" were those that met the criteria for intermediate nuclear atypia and also had a rough chromatin pattern, but did not present with bizarre nuclei. Finally, "nuclear pleomorphism" was defined by the presence of bizarre nuclei, i.e., nuclei of bizarre size and shape.

Immunohistochemistry

Immunohistochemical staining was carried out for all 9 specimens. Formalin-fixed, paraffin-embedded tissue was sectioned at 3 μm thickness. The primary antibodies and their dilutions and antigen retrievals are summarized in Supp. 1. The immune complex was detected with the DAKO EnVision Detection System.

RT-PCR, multiplex PCR and direct sequencing

Total RNA was extracted from the frozen samples using a TRIzol reagent (Invitrogen, Carlsbad, CA) and was reverse transcribed using Superscript III reverse transcriptase (Invitrogen) to prepare the first-strand complementary DNA. An NAB2–STAT6 fusion assay was performed using a newly designed set of primers (Supp. 2) that specifically amplify the fusion gene transcripts of NAB2–STAT6. Each PCR product (5 μL) was loaded onto 2% agarose gel with ethidium bromide and visualized under UV illumination. The PCR products were also evaluated by direct sequence analysis using the Big-Dye terminator method (version 1.1; Applied Biosystems, Foster City, CA) to confirm the breakpoints of fusion transcripts.

To confirm the absence of the NAB2–STAT6 fusion gene, the multiplex PCR method was adopted using the primer set that covered almost entire sequence of each of the two genes. Combinations of one forward primer and three reverse primers employed in each of the PCR trials were shown in Supp. 3. In addition, we validated the detectability of RT-PCR for large-sized targets, utilizing the more distant primers for NAB2 and STAT6 and the primers for the PGK gene designed for large-sized products.

Fluorescence in situ hybridization (FISH)

To assay amplifications of the MDM2 and CDK4 genes, dual-color FISH was performed for all 9 cases using FISH probes (GSP Lab., Kawasaki, Japan). Paraffin-embedded tissue sections were used for FISH. MDM2 and CDK4 FISH were evaluated according to the past reports, which defined amplification as greater than 5 or 12 signals per cell [8,9]. For each of the 9 cases, the MDM2 or CDK4 and centromere signals were counted and the MDM2 or CDK4/centromere ratio was calculated. MDM2 or CDK4/centromere ratio of >12 was defined as amplification-positive.

Results

Clinicopathological and histopathological findings

The clinicopathological data of the presented cases are shown in Table 1. Two of the nine cases were pleural (i.e., included visceral pleura), and seven were extra-pleural cases. Cases 4, 6 and 9 were recent cases and had inadequate follow-up information.

Representative histologic findings are shown in Fig. 2 and summarized in Table 2. Histopathologically, each of the 9 tumors was composed of oval, polygonal or spindle-shaped tumor cells with various patterns of degenerative or atypical features, arranged in a patternless architecture, accompanied by hemangiopericytomatous staghorn-like blood vessels and fibro-collagenous stroma. The degenerative or atypical findings were as follows: 3 myxoid degeneration, 2 cystic degeneration, 5 hyalinization, 2 necrosis, 2 hemorrhage, 5 intermediate nuclear atypia, 2 nuclear pleomorphism, 3 high cellularity, and 3 high mitotic rate (>4/10 HPF). Four and five SFTs fulfilled the criteria of malignant features proposed by England et al. and Fletcher et al., respectively.

Immunohistochemistry

The representative status of immunohistochemical profile was shown in Fig. 1(i)–(r). All 9 tumors were diffusely positive for CD34 (9/9 cases), bcl-2 (6/9 cases), and/or CD99 (6/9 cases), and some tumors were focally positive for alpha-SMA (2/9 cases) and muscle-specific actin (3/9 cases), while no tumor revealed positivity for EMA, desmin, c-kit, DOG1 or HMB45. Two tumors had immunoreactivity for MDM2. The tumor had scattered and strong positivity for MDM2 in Case 9, but the others showed focal and weak positivity, and 5 tumors appeared positive for CDK4. Seven tumors strongly expressed STAT6 in the tumor cell nuclei, while the tumor presented weak and none nuclear staining in Case 9 and Case 8. In all the 9 tumors, three tumors strongly and diffusely expressed GRIA2 in the tumor cell membrane or cytoplasm, 2 tumors expressed weakly and diffusely, 3 tumors expressed strongly and focally, and 1 tumor was completely negative. GRIA2 was negative in Case 8, and focally positive in Case 9.

RT-PCR and sequence analysis

The results of the RT-PCR and direct sequencing are shown in Fig. 2A–C. Fusion gene products and used primers were as follows: Case 1: NAB2(exon4)-Unknown sequence(GCCCAAAAG)-STAT6(exon3), Fex3 + Rex3; Case 2:NAB2(exon6)-STAT6 intron (42 bp)-STAT6(exon1), Fex6A + Rex3; Case 3: NAB2(exon4)-STAT6(exon3), Fex3 + Rex3; Case 4: NAB2(exon3)-NAB2 intron (210 bp)-STAT6(exon18), Fex3 + Rex18; Case 5: NAB2(exon5)-STAT6(exon 1b, 17 bp)-STAT6(exon3), Fex5 + Rex3; Case 6: NAB2(exon5)-STAT6(exon17), Fex5 + Rex17; Case 7: NAB2(exon5)-STAT6(exon17), Fex5 + Rex17, Case 8: NAB2–STAT6-negative; Case 9: NAB2–STAT6-negative. In 7 of the 9 cases, UV illumination revealed conspicuous bands of PCR product, and fusion genes were demonstrated by direct sequencing. Three of the seven fusion gene-positive cases had the sequence previously reported [10], while the four remaining cases had other patterns of gene fusion, intron sequence or insertion of unknown sequence as follows: Case 5 had deletion of 20 base pairs in STAT6 mRNA-cDNA, Case 1