Cryptic SYT/SSX1 fusion gene in high-grade biphasic synovial sarcoma with unique complex rearrangement and extensive BCL2 overexpression

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Abstract

Synovial sarcomas are high-grade malignant mesenchymal tumors that account for 10% of all soft-tissue sarcomas. Almost 95% of these tumors are characterized by a nonrandom chromosomal abnormality, t(X;18)(p11.2;q11.2), that is observed in both biphasic and monophasic variants. In this article, we present the case of a 57-year-old woman diagnosed with high-grade biphasic synovial sarcoma in which conventional cytogenetic analysis revealed the constant presence of a unique t(18;22)(q12;q13), in addition to trisomy 8. The rearrangement was confirmed by fluorescence in situ hybridization. The use of the whole chromosome painting probes WCPX did not detect any rearrangements involving chromosome X, although reverse-transcriptase polymerase chain reaction (PCR) analysis demonstrated the conspicuous presence of a SYT/SSX1 fusion gene. Spectral karyotyping (SKY) was also performed and revealed an insertion of material from chromosome 18 into one of the X chromosomes at position Xp11.2. Thus, the karyotype was subsequently interpreted as 47,X,der(X)ins(X;18)(p11.2;q11.2),der(18)del(18)(q11.2q11.2)t(18;22)(q12;q13),der(22)t(18;22). Real-time PCR analysis of BCL2 expression in the tumor sample showed a 433-fold increase. This rare finding exemplifies that thorough molecular—cytogenetic analyses are required to elucidate complex and/or cryptic tumor-specific translocations.

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1. Introduction

Synovial sarcoma (SS) is an aggressive mesenchymal malignancy that occurs most frequently in the lower limbs of young adults and accounts for 10% of all soft-tissue sarcomas [1]. According to histologic findings, this neoplasia can be classified into two main subtypes, biphasic and monophasic, defined by the presence or absence of areas of glandular epithelial differentiation, respectively, in a spindle cell background [2].

Cytogenetic studies on SS have shown consistently a nonrandom reciprocal t(X;18)(p11.2;q11.2) that fuses the SYT (synovial sarcoma translocation) gene on chromosome X with the distal portion of either SSX1 or SSX2 genes on chromosome 18. This aberration was first reported in 1986 [3], and since then, it has been demonstrated in approximately 95% of both biphasic and monophasic variants, either alone or in association with additional chromosome aberrations [4].

In this article, we report on a unique complex rearrangement resulting in the SYT/SSX1 fusion in a high-grade, biphasic synovial sarcoma occurring in a 57-year-old woman.

2. Materials and methods

2.1. Case report

A 57-year-old female patient was referred with complaints of intermittent pain in the left shoulder, lasting for several years. Ten months before admission, a soft, palpable mass was observed in the medial face of the left arm.

Upon physical examination, a painless, firm palpable mass was found in the left scapular region. The initial plain
film revealed a sclerotic lesion at the proximal right humerus. Computer tomography (CT) scan revealed a deep, soft-tissue expansive lesion at the dorsal left region, close to trapezium muscle. The magnetic resonance imaging confirmed a lobulated contour soft-tissue mass with better delimitation of the lesion and of the regional anatomy. The major diameters of the mass measured 9.0 × 6.2 × 4.4 cm. Bone scintigraphy revealed an intense vascularization at the left shoulder, and no additional areas of abnormal enhancement were present. Both abdominal and chest helical CT showed no metastasis or multifocal lesions.

The patient underwent a wide excision of the tumor by an extra-articular total scapulectomy and humeral head resection. The hematoxilin and eosin staining of paraffin-embedded specimen revealed a biphasic malignant tumor, presenting fusocellular, solid areas intermingled with epithelial, papillary areas, with glandular formation and cells presenting fusocellular, solid areas. Mitotic activity was conspicuous, with many atypical mitotic figures, and there were large necrotic areas. Immunohistochemical study revealed diffuse positivity to vimentin in the fusocellular areas and to epithelial membrane antigen (EMA) in the epithelial areas. Immunopositivity to B-cell CLL/lymphoma-2 (BCL2) was strong and diffuse in the stromal part of the epithelial areas. Immunopositivity to B-cell CLL/lymphoma revealed diffuse positivity to vimentin in the fusocellular areas with many atypical mitotic figures, and there was large necrotic areas. Immunohistochemical study revealed diffuse positivity to vimentin in the fusocellular areas and to epithelial membrane antigen (EMA) in the epithelial areas. Immunopositivity to B-cell CLL/lymphoma-2 (BCL2) was strong and diffuse in the stromal part of the tumor, and the epithelial part was totally negative (Fig. 1).

The patient is currently under treatment with conformal local radiotherapy, and adjuvant cycles of chemotherapy with ifosfamide plus doxorubicin are planned as part of the treatment strategy.

2.2. Tumor culture and cytogenetic analysis

For cytogenetic studies, a fresh SS sample (adjacent to areas of tumor verified by frozen section) was collected aseptically in the operating room and processed, as described previously [5]. The subsequent analysis was performed by GTG banding, and the results were interpreted according to the International System for Human Cytogenetic Nomenclature (2005) guidelines [6].

Fluorescent in situ hybridization (FISH) analyses were also performed on the same cell preparations using commercially available probes. For the detection of the chromosome 18 centromere, a mixture of CEP 18 (SpectrumOrange; Abbott Molecular, Des Plains, IL) and SE18 (D18S1 marker) PlatinumBright495-labeled probes (Kreatech Diagnostics, Amsterdam, The Netherlands) was used. Centromeric PlatinumBright495-labeled probes were also used for the detection of chromosome 22 (SE14/22; Kreatech Diagnostics), whereas the BCL2 gene was detected by the use of LSI IGH/BCL2 dual-color (Abbott Molecular). Whole chromosome painting probes WCP8 and WCPX (Abbott Molecular) were also used. The tests were performed according to the protocol of the manufacturer.

The spectral karyotyping (SKY) assay was performed using the SKY probe cocktail [Applied Spectral Imaging (ASI), Migdal Ha’Emek, Israel] according to the manufacturer’s instructions. Image acquisition was performed using a SD200 SpectraCube system (ASI) mounted on an Olympus BX60 microscope with a custom-designed optical filter (SKY-1; Chroma Technology, Brattleboro, VT). SKY analysis was performed with SkyView 1.6.2 software (ASI).

2.3. Reverse-transcriptase polymerase chain reaction (RT-PCR)

Qualitative RT-PCR for the detection of the SYT-SSX1 and SYT-SSX2 chimeric transcripts was performed by using the primers SYT, 5′-ATCCACCCCCAGCAG CAGTA-3′, SSX1 5′-GGTGCACTTGTTCCATCG-3′ and SSX2 5′-GGCACAGCTTCCCATCA 3′ [7]. The case was studied with simultaneous analysis of positive controls for both fusion genes.

2.4. Quantitative polymerase chain reaction (Q-PCR)

Real-time quantitative analysis for the BCL2 gene expression was performed in a 7500 Real Time PCR System (Applied Biosystems, Foster City, CA) by using the SYBR Green PCR Master Mix (Applied Biosystems) and the following set of primers: forward 5′-ATGTTGTGTTGGA-GAGCGTCAA-3′ and reverse 5′-GCCGTACAGTCCCAA-
CAAAAG3′. The endogenous housekeeping gene coding for glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as internal control. Relative expression of BCL2 was calculated using the comparative standard curve method ΔΔCT [8]. Blank and standard controls were run in parallel to verify amplification efficiency within each experiment. In this study, cDNA from normal fibroblast culture was used as calibrator. The differences in gene expression levels were analyzed by the Student’s t-test, using GraphPad Prism software (version 4.0; GraphPad Software, San Diego, CA). Differences were considered to be significant at P < 0.05.

3. Results

Twenty-nine metaphase cells were examined and no cytogenetically normal cells were found. Chromosome banding analysis revealed the following karyotype: 47,XX,+8,t(18;22)(q12;q13) (Fig. 2a). Trisomy of chromosome 8 and the translocation between chromosomes 18 and 22 were confirmed by FISH (Fig. 2, b and c). The use of whole chromosome painting probes WCPX did not detect any involvement of chromosome X (Fig. 2d). However, since the analysis carried out by RT-PCR demonstrated the conspicuous presence of the SYT/SSX1 fusion gene (Fig. 2e). Additional studies using SKY were performed and revealed a more complex rearrangement consisting of an insertion of material from chromosome 18 into one of the X chromosomes (Fig. 2f). Thus, the karyotype was subsequently interpreted as: 47,X,der(X)ins(X;18)(p11.2;q11.2q11.2),der(18)del(18)(q11.2q11.2)t(18;22)(q12;q13),der(22)t(18;22).
Real-time PCR analysis of BCL2 expression in the tumor sample showed a highly significant statistical difference with a 433-fold increase in relation to control fibroblasts (data not shown).

4. Discussion

Synovial sarcoma is a malignant mesenchymal neoplasm of young adults, typically occurring in the para-articular tissues of the extremities, most commonly around the knee [1].

In this study, we report the distinctive cytogenetic findings of a high-grade biphasic SS occurring in the left shoulder of a 57-year-old woman. GTG-banding analysis of the tumor sample showed trisomy 8 and an apparently balanced t(18;22)(q12;q13) without evidence of the typical t(X;18) (p11.2;q11.2) associated with this tumor. FISH analysis performed on the same chromosome preparations confirmed the rearrangement, as well as trisomy 8 and also failed to detect any involvement of chromosome X.

Cytogenetic studies of SS have shown that about one third of the cases display the t(X;18) as the only cytogenetic change [9]. Secondary genetic anomalies including additional chromosomes are sometimes seen, but these changes are often variable and inconsistent. Comparative genomic hybridization studies have shown gains of 8q as the most frequent aberrations in SS [10], followed by gains at 12q, loss of 13q21–q31, and loss of 3p. Interestingly, gains of the whole or parts of chromosome 8 were found to be significantly overrepresented in large tumors (> 5 cm), suggesting that this genetic abnormality might render an increased growth rate [11].

On the other hand, it has been shown that about 10% of previously reported SS cases show complex translocations that may involve three or more chromosomes [4], and cryptic rearrangements resulting in the same gene fusions are occasionally found in cases with no apparent translocations between Xp11.2 and 18q11.2 [12,13], turning the molecular analysis of these chimeric transcripts into an obligatory and complementary diagnostic tool.

The subsequent reverse-transcriptase polymerase chain reaction analysis of our tumor sample showed the presence of an SYT/SSX1 fusion transcript, confirming the presence of a cryptic t(X;18). Further analysis by spectral karyotyping corroborated this result, showing an insertion of material from chromosome 18 into one of the X chromosomes.

The underlying molecular mechanisms of chimeric SYT/SSX products in SS are still poorly understood. The inherent transforming activity of SYT/SSX1 has been demonstrated [14], while SYT/SSX2 seems to exert part of its oncogenic effect by altering cytoskeletal architecture [15]. Nonetheless, both fusion proteins contain the putative transcriptional activation regions of the SYT along with the repression domain of SSX, thus SYT–SSX fusions are thought to function as transcriptional proteins that deregulate gene expression [2,16]. In synovial sarcoma, CCND1 activity is increased.
Altering in other genes include ERBB2 expression in epithelial areas, IGF2 upregulation, and PTEN inactivation, among others [17]. However, there is a great variability in expression of genes in SS, which might be related to the variable secondary (additional) chromosomal changes besides the t(X;18). It is generally thought that these secondary changes are frequently associated with tumor progression and affect the prognosis negatively [11].

An almost general constitutive alteration in SS is the expression of BCL2. The initial analysis of the tumor-cultured cells in our sample pointed the band 18q21 as a translocation breakpoint. FISH analysis showed that, even translocated to the derivative chromosome 22, the BCL2 gene presented normal hybridization patterns.

The BCL2 proto-oncogene expression in SS was first described by Hirakawa et al. [18] in 79% of the tumors analyzed by immunohistochemistry. Kawauchi et al. [19] later showed that almost all cases of SS are positive for this marker, but it shows different staining patterns that range from moderate to extensive perinuclear and/or cytoplasmic reactivity. Also, in biphasic SS, its expression is restricted to the spindle cell component [20,21].

Mancuso et al. [24] demonstrated that this gene is not

Fig. 2. (a) Giemsa-trypsin-banded karyogram of a high-grade synovial sarcoma showing trisomy 8 and an apparently balanced t(18;22)(q12;q13). (b) Metaphase showing chromosome 8 trisomy. (c) Partial FISH results showing normal chromosomes 18 and 22 and the translocated derivatives (note the BCL2 gene in SpectrumOrange). (d) Metaphase showing normal hybridization patterns for chromosome X. (e) RT-PCR analysis showing molecular weight marker (lane 1), positivity for the SYT/SSX1 rearrangement, and negativity for SYT/SSX2 in the tumor sample (lanes 2 and 3, respectively), together with blanks (lanes 4 and 6) and positive controls (lanes 5 and 7) for both rearrangements. (f) Partial hybridized SKY paints showing the chromosomes involved in the complex rearrangement as well as the insertion (X;18)(p11.2;q11.2).
rearranged or amplified at the genomic level, but rather, the high amplification may result from transcriptional activation, as shown by the 433-fold change in BCL2 expression increase observed in the case we report here.

The complex three-way cryptic ins(X;18) resulting in a SYT/SSXI fusion and extensive BCL2 overexpression in the high-grade, biphasic SS reported here demonstrate that thorough molecular—cytogenetic analyses are required to uncover complex and/or cryptic tumor-specific translocations.

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