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Alternate *PAX3-FOXO1* Oncogenic Fusion in Biphenotypic Sinonasal Sarcoma

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Abstract

Biphenotypic sinonasal sarcoma (SNS) is a low grade spindle cell sarcoma that affects middle-aged adults, in which the *PAX3-MAML3* chimeric transcription factor induces an aberrant dual myogenic and neuroectodermal phenotype. We report an alternate *PAX3-FOXO1* oncogenic fusion in SNS, confirming the crucial role of PAX3 in SNS oncogenesis. The presence of *PAX3-FOXO1* in SNS and alveolar rhabdomyosarcoma suggests that these two entities are genetically similar lesions arising from distinct progenitor cell pools. This finding has important implications for the molecular diagnosis of SNS and alveolar rhabdomyosarcoma, and underscores the critical contribution of the cell of origin to the phenotype induced by oncogenic transcription factor reprogramming.

Keywords

sarcoma; gene fusion; oncogene; sinonasal; spindle cell sarcoma

INTRODUCTION

Biphenotypic sinonasal sarcoma (SNS) is a recently recognized low grade sarcoma that affects middle-aged adults (Lewis et al., 2012). SNS is characterized by uniform spindle cell morphology and a t(2;4)(q35;q31) recurrent translocation, which results in a *PAX3-MAML3* oncogenic fusion (Wang et al., 2014). The *PAX3-MAML3* chimeric transcription factor encoded by this fusion gene induces an aberrant biphenotypic transcriptional program of dual neuroectodermal and myogenic differentiation, which recapitulates the developmental roles of PAX3 and which can be detected by immunohistochemistry for diagnosis. The biological contributions of MAML3 in this context remain unclear, and raise the important question of the relative contribution of a particular molecular alteration versus the cellular environment in which it occurs towards defining a specific tumor phenotype. We report an alternate *PAX3-FOXO1* oncogenic fusion in a case of SNS, which confirms the crucial role of PAX3 in SNS oncogenesis and underscores the critical contribution of the cell of origin to the tumor phenotype induced by aberrant transcription factor oncogenic reprogramming.

MATERIALS AND METHODS

Immunohistochemistry

Immunohistochemical analysis was performed on 4- μ m formalin-fixed paraffin-embedded (FFPE) tissue sections using the following antibodies and conditions: S-100 protein (Dako, Carpinteria, CA; polyclonal; 1:3000), smooth muscle actin (SMA; Sigma, St. Louis, MO; clone 1A4; 1:20,000), desmin (Sigma; clone D33; 1:500), myogenin (MYF4) (Novocastra, Newcastle, U.K.; Clone LO26; 1:600), EMA (Dako; clone E29; 1:200), TLE1 (Santa Cruz, Santa Cruz, CA; polyclonal; 1:400) and beta-catenin (Novocastra; clone 17C2; 1:50). The Envision Plus detection system (Dako) was used for all antibodies. Appropriate positive and negative controls were used throughout.

Fluorescence *in situ* Hybridization (FISH)

FISH evaluation for *FOXO1* rearrangement was performed on tumor nuclei using the LSI *FOXO1* Dual Color Break Apart Rearrangement Probe (Abbott, Des Plaines, IL) on 4- μ m-thick paraffin sections according to standard protocols in our laboratory.

RT-PCR and Sequencing

RNA was extracted from 20- μ m-thick paraffin sections of FFPE tissue using Qiagen RNeasy FFPE Kit according to the manufacturer's instructions. PCR was performed for 35 cycles (94°C for 30 seconds, 60°C for 30 seconds, and 72°C for 45 seconds) using the following primers: *PAX3* forward, CCTCCAACCCCATGAACCC; *FOXO1* reverse, CCTTCATTCTGCACACGAATGA. Primers for *PAX3-MAML3* are from reference 2.² Sanger sequencing was performed on PCR products using forward and reverse PCR primers.

Mutational Profiling

DNA extracted from FFPE tissue was subjected to targeted exon hybrid capture (Agilent, Santa Clara, CA) and next-generation sequencing using an Illumina HiSeq 2500 (Illumina, San Diego, CA). The exonic sequences of 300 cancer-associated genes were interrogated for mutations and copy number variations, and 113 introns across 35 genes were examined for gene rearrangements, using our institution's targeted sequencing platform (Oncopanel, details available upon request).

RESULTS

Clinical Course

A 33 year-old man with no significant medical history presented with recurrent brisk epistaxis. A left intranasal mass was appreciated on nasal endoscopy. Computed tomography demonstrated an enhancing 3.7 cm mass in the left nasal cavity, with extension into the left sphenoid sinus. The patient underwent endoscopic resection of the tumor with adjuvant chemoradiation therapy, and is free of disease 5 months after resection.

Morphology and Immunohistochemistry

The resection specimen consisted of a firm, white-tan, polypoid mass with scant attached bone fragments. Histologically, the tumor was a homogeneous and highly cellular proliferation of uniform spindle cells, arranged in short fascicles, with bland, elongated nuclei and scant cytoplasm (Fig. 1A). The overlying epithelium was unremarkable. There were no mitotic figures or necrotic areas. Bone infiltration was present. The tumor cells demonstrated a characteristic dual neural and myogenic phenotype, expressing S100 protein, smooth muscle actin, and desmin. Scattered cells expressed myogenin, corresponding to focal rhabdomyoblastic differentiation (Fig. 1B). There was diffuse weak expression of TLE1 and no expression of EMA.

Genetics

Giemsa-banding analysis of metaphase cells revealed a balanced translocation involving chromosome bands 2q35 and 13q14. Rearrangement of the *FOXO1* locus in chromosome 13q14 was demonstrated by fluorescence *in situ* hybridization (FISH) using a break-apart probe (Fig. 2A). RT-PCR, followed by Sanger sequencing, demonstrated an in-frame fusion between exon 7 of *PAX3* and exon 2 of *FOXO1* (Fig. 2B and 2C). In contrast, *PAX3-MAML3* gene fusion was absent (Fig. 2B). The RNA sequence predicts a PAX3-FOXO1 chimeric protein containing the paired box and homeobox DNA-binding domains of PAX3 fused to the transactivation domain of FOXO1, which is the chimeric fusion protein characteristic of alveolar rhabdomyosarcoma (Galili et al., 1993).

Targeted exome sequencing of 300 commonly mutated cancer-associated genes revealed a nonsense mutation in *CDC73*, predicted to produce a truncated protein with a premature stop codon at residue 448. *CDC73* encodes parafibromin, a tumor suppressor and transcriptional regulator of Wnt/beta-catenin signaling (Mosimann et al., 2006). Accordingly, immunohistochemistry demonstrated nuclear accumulation of beta-catenin, indicating activation of Wnt signaling (Fig. 1D). No other significant single nucleotide variants or copy number alterations were identified.

DISCUSSION

SNS is a distinctive low-grade sarcoma that most often arises in the upper sinonasal tract (Lewis et al., 2012). Based on its high cellularity and uniform spindle cell morphology, the differential diagnosis of SNS includes monophasic spindle cell synovial sarcoma and low-grade malignant peripheral nerve sheath tumor (MPNST), both of which can occur in the sinonasal cavity.

Monophasic spindle cell synovial sarcoma is composed of solid sheets or fascicles of spindle cells with scant cytoplasm, indistinct cell borders, and high nucleus-to-cytoplasm ratio that results in a characteristic appearance of overlapping nuclei at low magnification. Characteristic stromal features of synovial sarcoma such as hyalinized 'wiry' collagen bundles or stromal calcification are typically absent in SNS, but distinguishing synovial sarcoma from SNS based on morphology alone remains challenging. There is also substantial immunophenotypic overlap between these two entities, since up to 30% of

synovial sarcoma cases express S100 (Fisher et al., 1998), and SNS can express TLE1 diffusely, such as in this case. Epithelial differentiation in synovial sarcoma, detected by expression of keratins or EMA, and the expression of desmin and SMA in SNS are the most useful immunohistochemical features to resolve this differential diagnosis. A definitive diagnosis of synovial sarcoma can be obtained by molecular identification of *SS18* rearrangement.

MPNST is most commonly a high grade lesion, which can show heterologous rhabdomyoblastic differentiation (so-called *malignant 'triton' tumor*), often affecting patients with neurofibromatosis type 1 (Ducatman et al., 1984). Low-grade examples of MPNST with rhabdomyoblastic differentiation have been described, although some cases occurring in the sinonasal region may actually represent unrecognized SNS (Lewis et al., 2012). The distinction between SNS and low-grade MPNST in the sinonasal region may be extremely challenging, and in the absence of ultrastructural evidence of Schwannian differentiation, clinical evidence of neurofibromatosis, or demonstrable origin from a nerve, molecular studies may be required to reach a definitive diagnosis.

Molecularly, recurrent chromosomal translocations that create neomorphic chimeric fusion genes are common events in cancer, and are thought to be tumor-specific initiating oncogenic events in a growing number of sarcoma and carcinoma types (Mertens et al., 2015). Gene fusions involving transcription factors, such as *PAX3-FOXO1*, can induce transcriptional deregulation both as a result of juxtaposition of modular functional elements and haploinsufficiency of the genes involved (Keller et al., 2004a). The distinctive tumor phenotypes associated with various transcription factor fusions may reflect fundamental differences in the biological properties of the component and chimeric proteins, but the cell of origin and the genomic context in which fusion events occur are also critical. Thus far, *PAX3-FOXO1* has been exclusively associated with alveolar rhabdomyosarcoma, hence serving as a diagnostic and prognostic biomarker (Sorensen et al., 2002). High *PAX3-FOXO1* expression in murine myogenic precursor cells causes alveolar rhabdomyosarcoma (Xia et al., 2009), when activated as a sole aberration (in differentiating myoblasts) or in collaboration with additional genetic hits, such as cell cycle checkpoint loss (Keller et al., 2004a).

The discovery of a *PAX3-FOXO1* gene fusion in SNS indicates a significant tumorigenic overlap with *PAX3-MAML3*, at least in some cellular contexts. In the original series describing the molecular genetics of SNS, up to 79% of SNS were associated with *PAX3-MAML3*, while *PAX3-FOXO1* was not detected in the subset of *PAX3-MAML3*-negative cases with *PAX3* rearrangement (Wang et al., 2014). Interestingly, *PAX3-FOXO1* has mitogenic effects on neuroectodermal progenitor cells when it is expressed during early embryogenesis, in *PAX3-FOXO1* knock-in mouse models, but remarkably these animals do not develop tumors of any type (Keller et al., 2004b). Furthermore, the FOXO1 transcription factor and the MAML3 transcriptional co-activator do not share known structural properties or target genes. Transcriptional reprogramming induced by *PAX3-FOXO1* in mouse myogenic precursor cells, and in human alveolar rhabdomyosarcoma, induces expression of early myogenic markers including myogenin and myoD1, which are typically absent or expressed at only low levels in SNS. Conversely, *PAX3-MAML3* induces the expression of

genes involved in neurogenic differentiation, characteristic of SNS and largely absent in alveolar rhabdomyosarcoma (Wang et al., 2014). The current case demonstrates that the expected functional consequences of different PAX3 fusion partners (MAML3 and FOXO1) may be minimized in certain cellular contexts, resulting in phenotypic convergence, as evidenced by SNS. The dramatic clinical differences between SNS – a locally aggressive, rarely metastasizing tumor – and alveolar rhabdomyosarcoma – a lethal malignancy, with a high metastatic rate and >50% mortality – further illustrate the relevance of cellular context in PAX3-driven transformation.

The occurrence of *PAX3-FOXO1* in SNS confirms that multiple translocation partners can create oncogenic PAX3 forms, contributing to SNS tumorigenesis, and suggests that SNS and alveolar rhabdomyosarcoma are genetically similar lesions arising in distinct progenitor cell pools. These findings have important diagnostic implications, and highlight the paramount contribution of the cellular environment to translocation-mediated oncogenesis.

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Figure 1.

Histological features of biphenotypic sinonasal sarcoma. The tumor consists of a highly cellular submucosal proliferation (A) of uniform spindle cells with bland nuclear features (B), arranged in short fascicles, with bone invasion (C) and no mitotic figures or necrotic areas. D, Multifocal expression of S100 protein, smooth muscle actin (SMA), and scattered myogenin (MYOG) demonstrating focal rhabdomyoblastic differentiation. Diffuse expression and nuclear accumulation of beta-catenin (β -CAT) indicates activation of Wnt signaling.

Figure 2.

A, Break-apart FISH demonstrating split of 5' (red) and 3' (green) ends of one copy of *FOXO1*. **B**, RT-PCR demonstrates presence of *PAX3-FOXO1* fusion transcript in biphenotypic sinonasal sarcoma (SNS) and alveolar rhabdomyosarcoma (RMS, control). No *PAX3-MAML3* fusion transcript was detected using two independent previously published sets of primers (Wang et al., 2014). GAPDH served as control. **C**, Predicted exon structure and transcript Sanger sequencing demonstrating an in-frame fusion between exon 7 of *PAX3* and exon 2 of *FOXO1*.