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CASE REPORTS

A Poorly Differentiated Non-keratinizing Sinonasal Squamous Cell Carcinoma with a Novel ETV6-TNFRSF8 Fusion Gene

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Abstract

Squamous cell carcinoma of the sinonasal tract is relatively rare and morphologically and genetically heterogeneous. We report the case of an adult male with a left sphenoid sinus mass. A biopsy revealed an undifferentiated carcinoma composed of sheets of epithelioid cells lacking keratinization and glandular formation. The tumor was associated with a prominent lymphoplasmacytic inflammatory infiltrate. Immunohistochemical staining demonstrated diffuse expression of pankeratin and p63; it was negative for p16. In addition, EBER was also negative. Morphologically the findings raised the possibility of non-keratinizing squamous cell carcinoma. RNA sequencing was undertaken to exclude the possibility of NUT carcinoma; interestingly, this revealed a novel *ETV6-TNFRSF8* fusion transcript, which was independently confirmed by fluorescence in situ hybridization. The current case is illustrative because it broadens our understanding of the molecular pathogenesis of non-keratinizing squamous cell carcinoma and adds to the diversity of *ETV6*-rearranged malignancies.

Keywords Sinonasal carcinoma · ETV6 · TNFRSF8

Introduction

Squamous cell carcinoma arising within the sinonasal tract is relatively uncommon and is currently divided by the World Health Organization into: keratinizing, non-keratinizing, spindle cell (sarcomatoid), and lymphoepithelial subtypes [1]. Risk factors vary from environmental exposures to infectious aetiologies (i.e., human papillomavirus and Epstein-Barr virus). The underlying molecular attributes tend to overlap with tumors at other anatomic sites, which mostly exhibit complex karyotypes and aneuploidy with frequent loss of *TP53* and *CDKN2A/B* tumor suppressor genes [2–6]. In contrast to salivary gland neoplasms, chromosomal translocations are relatively uncommon in squamous cell carcinoma. Herein we report the case of an adult male with a poorly differentiated carcinoma of the sphenoid sinus harboring a novel *ETV6-TNFRSF8* gene fusion.

Case Report

A 66-year-old male presented with diplopia and mild proptosis with no sinonasal symptoms. CT and MRI imaging showed an infiltrative mass in the base of skull centered

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along the left sphenoid sinus and extending into the left cavernous sinus and sella, which prompted an endoscopic sphenoidotomy and biopsy. Microscopic examination revealed a neoplasm composed of sheets of epithelioid cells with a vague fascicular-storiform pattern (Fig. 1a–c). The cytoplasm was abundant and eosinophilic and lacked overt keratinization. The nuclei were ovoid with mild-moderate pleomorphism and prominent small nucleoli; mitotic activity was conspicuous (Fig. 1d). There was a brisk lymphoplasmacytic inflammatory infiltrate and occasional neutrophils (Fig. 1c, d). Immunohistochemical staining demonstrated the tumor was diffusely positive for keratin (AE1/AE3), p40 and p63; it was negative for p16, CD30, CD45, CD56, and S100; INI-1 was intact (Fig. 2). In situ hybridization was negative for Epstein-Barr virus-encoded small RNAs (EBER) (not shown). Based on the findings, the differential diagnosis included non-keratinizing squamous cell carcinoma, NUT carcinoma, adamantinoma-like Ewing sarcoma, and adenoid cystic carcinoma. Targeted RNA sequencing was performed which excluded the presence of *NUTM1*, *EWSR1*, *FUS*, *MYB, MYBL1* and *NFIB* rearrangement, amongst others (Illumina TruSight RNA fusion panel; San Diego, CA). Surprisingly, it revealed a novel *ETV6-TNFRSF8* fusion product involving *ETV6* (exon 5 of 8; NCBI Reference Sequence: NM_001987.4) and *TNFRSF8* (exon 13 of 15; NM_001243.4). Independent validation of this event by fluorescence in situ hybridization (FISH)—using custom probes from bacterial artificial chromosomes (Supplementary Table 1)—confirmed the presence of *ETV6* rearrangement (Fig. 3). The molecular findings therefore suggest a novel form of non-keratinizing squamous cell carcinoma.

Staging revealed T4N2M0 disease. The patient received induction chemotherapy followed by concurrent chemoradiotherapy with high-dose cisplatin. There was no evidence of recurrence at the 1-year follow-up.

Discussion

In contrast to salivary gland neoplasms, oncogenic fusion genes are relatively uncommon in squamous carcinoma of the head and neck. A notable exception includes NUT carcinoma—characterized by *NUTM1*-rearrangement—which may represent an aggressive subtype of squamous cell carcinoma [6]. More recently, squamous cell carcinomas with other gene fusions have been reported in the literature. For example, the *DEK-AFF2* fusion gene in a subset of non-keratinizing squamous cell carcinoma of the middle ear and temporal bone [7, 8]. These neoplasms can be morphologically undifferentiated, necessitating immunohistochemistry to highlight evidence of squamous differentiation. Herein

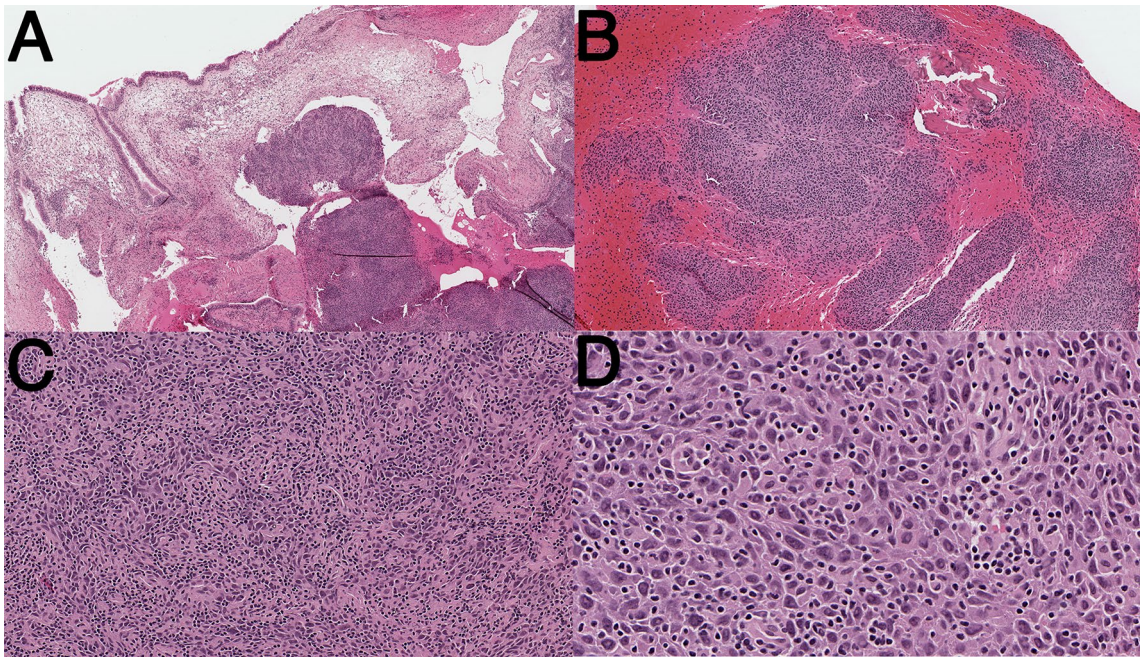

Fig. 1 Representative photomicrographs of *ETV6-TNFRSF8* associated carcinoma (Hematoxylin and Eosin). **a** Low-power magnification showing respiratory-type mucosa overlying neoplasm. **b** Intermediate power magnification demonstrating infiltration of bone. **c** Intermediate magnification showing sheets of epithelioid to spindled cells with a prominent lymphoplasmacytic inflammatory infiltrate. **d** High power magnification highlighting moderate nuclear atypia and numerous mitotic figures. There is no evidence of overt keratinization, or glandular formation

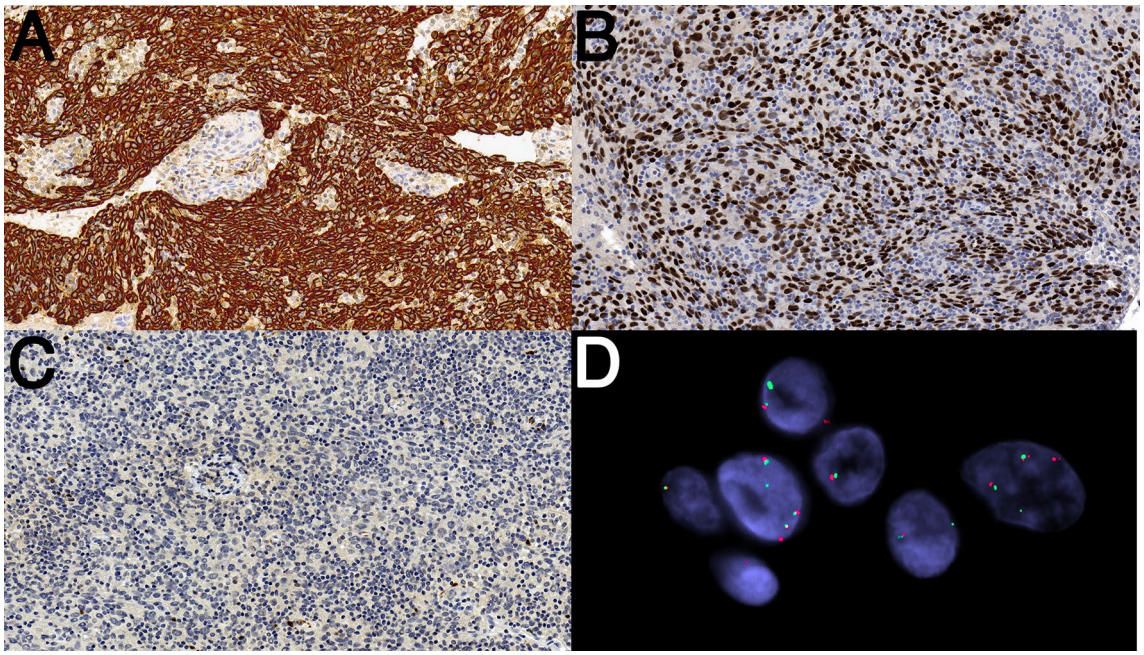

Fig. 2 Immunohistochemical staining and fluorescence in situ hybridization of *ETV6-TNFRSF8* associated carcinoma. **a** Diffuse cytoplasmic immunostaining for pan-cytokeratin. **b** Diffuse nuclear immunostaining for p63. **c** The tumor is negative for p16. **d** Florescence in situ hybridization showing unbalanced *ETV6* gene rearrangement with deletion of the centromeric signal (red), in cells with 2n, 3n, and 4n copies (polysomic) of *ETV6* (red, centromeric; green, telomeric)

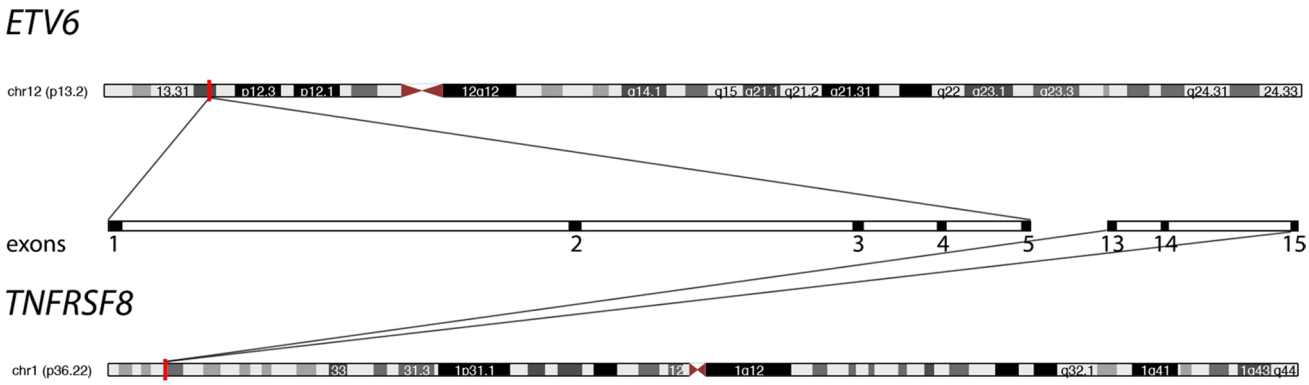

Fig. 3 Diagrammatic representation of *ETV6-TNFRSF8* fusion transcript. The gene location is highlighted by red band on each chromosome (http://genome.ucsc.edu) [31]. The expanded view in the center of the image depicts the spatial relationship of the exons for each gene

we describe another carcinoma, arising at the base of skull, which harbored a novel *ETV6-TNFRSF8* fusion product.

ETV6 encodes ETS variant transcription factor 6, which is normally involved in hematopoiesis through the regulation of stem cells and maintenance of vascular networks [9]. *ETV6* fusions with various partner genes are well-documented in hematologic malignancies; however, *ETV6* rearrangements in solid tumors are increasingly recognized [9]. The *ETV6-NTRK3* fusion gene has been demonstrated in secretory carcinoma of the breast [10], salivary gland [11], thyroid gland [12, 13] and skin [14] as well as in low-grade sinonasal adenocarcinoma [15, 16], infantile fibrosarcoma [17], congenital mesoblastic nephroma [18], ALK-negative inflammatory myofibroblastic tumor [19], gastrointestinal stromal tumor [20], radiation-associated papillary thyroid carcinoma [21], pediatric high grade glioma [22] and melanocytic neoplasms such as Spitz tumors [23]. Alternate *ETV6* fusion partners have also been described. These include *ALK* in epithelioid fibrous histiocytoma [24] as well as *RET*, *MET* and *MAML3* in secretory carcinoma of the salivary glands [25, 26]. To the

best of our knowledge, *TNFRSF8* has not previously been reported as an *ETV6* fusion partner.

The *TNFRSF8* gene is a member of the tumor necrosis factor receptor superfamily. It encodes for CD30 protein, a well-known marker of Hodgkin and anaplastic large cell lymphoma [27]. CD30 interacts with its ligand, CD30L, to activate NF-κB, MAP kinase, and Akt signaling pathways [27, 28]. Depending on the specific cell type and costimulatory signals involved, signal transduction can either promote cell proliferation and survival or promote apoptosis and cell cycle arrest [28, 29]. Rare cases of lung adenocarcinoma and breast carcinoma harboring *TNFRSF8- PLEKHG5* and *TNFRSF8-STARD13* fusions, respectively, have been reported in the literature [30].

The tumor in our patient was morphologically undifferentiated and characterized by sheets of epithelioid cells lacking keratinization. Immunohistochemical stains revealed squamous differentiation. Interestingly, the tumor contained a brisk lymphoplasmacytic infiltrate, raising the possibility of lymphoepithelial carcinoma; however, EBER was negative. Targeted RNA sequencing was performed to exclude the possibility of NUT carcinoma, amongst other considerations; surprisingly, it revealed a novel *ETV6-TNFRSF8* fusion gene. While the presence of *ETV6*-rearrangement in a tumor at this location raises the possibility of secretory carcinoma or a low-grade sinonasal adenocarcinoma with *ETV6* rearrangement, these tumors can be readily differentiated based on their distinctive morphologies and immunophenotypes. Based on its undifferentiated morphology and immunohistochemical evidence supporting squamous differentiation, this tumor is believed to be best classified as an undifferentiated carcinoma given the information available to date. This fusion product is potentially diagnostically relevant; however, at present, it does not appear to have direct therapeutic relevance.

In summary, we report an undifferentiated carcinoma arising in the sphenoid sinus that harbored a novel *ETV6-TNFRSF8* fusion gene. This finding expands the spectrum of neoplasms characterized by *ETV6*-rearrangement. Further studies are necessary to determine the prevalence of this finding, and ascertain whether this is restricted to carcinoma of the sinonasal tract.

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Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

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