Whole-Exome Sequencing of Sinonasal Small Cell Carcinoma Arising within a Papillary Schneiderian Carcinoma In Situ

Joshua Smith¹, Aditi Kulkarni, MS¹, Andrew C. Birkeland, MD¹, Jonathan B. McHugh, MD¹,²,³, and J. Chad Brenner, PhD¹,³,⁴,⁵

Sponsorships or competing interests that may be relevant to content are disclosed at the end of this article.

Abstract

Objective. The pathogenetic underpinnings of extrapulmonary small cell carcinomas (EPSCCs) of the head and neck are poorly understood. We sought to describe the clinical case and whole-exome DNA sequencing data of a patient with sinonasal Schneiderian carcinoma in situ whose tumor progressed to small cell carcinoma (SCC).

Study Design. Case report and whole-exome sequencing of tumor DNA.

Setting. Academic medical center.

Subjects and Methods. A 52-year-old man with sinonasal Schneiderian carcinoma in situ whose tumor progressed to small cell carcinoma. We performed whole-exome genetic sequencing and copy-number variation (CNV) analysis of tumor and normal DNA extracted from flash-frozen, paraffin-embedded (FFPE) samples.

Results. A total of 93 high-confidence, nonsynonymous somatic mutation events were identified in sinonasal SCC, including loss-of-function mutations in TP53, MAML3, a transcriptional coactivator of the Notch pathway, and GAS6, an activating ligand of the TAM family of tyrosine kinase receptors. Focal amplifications of chromosomal regions 6p25-11.1, containing SOX4 and VEGFA, and 14q32.1-32.3, containing AKT1 and the Notch inhibitory ligand DLK1, were also seen. Further CNV analysis revealed deletions in the critical cell cycle regulators CDKN2A, RB1, RBL1, and RBL2 and the chromatin modifier EP300.

Conclusions. Small cell carcinoma may rarely arise from sinonasal Schneiderian carcinoma in situ and exhibits similar genomic aberrations (eg, SOX amplification, Notch pathway inactivation) to pulmonary small cell carcinoma.

Keywords

sinonasal SCC, NOTCH, SOX4

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Extrapulmonary small cell carcinomas (EPSCCs) are exceptionally rare neoplasms that arise from primary sites of the genitourinary tract, gastrointestinal tract, or head and neck (eg, oral cavity, salivary glands, larynx, nasal cavity, and paranasal sinuses).¹⁻³ EPSCCs of the head and neck are clinically aggressive; patients typically present with distant metastatic disease and exhibit dismal 5-year overall and disease-specific survival rates of less than 25%.⁴⁻⁵ In contrast to the genetic understanding of many other cancers of the head and neck region that have driven advances in precision medicine,⁶⁻⁸ the pathogenetic basis of head and neck EPSCCs is currently unknown, limiting the utility of advancing targeted therapy for this disease.

EPSCCs appear to share some common genomic aberrations with small cell lung carcinoma (SCLC), a disease characterized by an extraordinary mutational burden and frequent inactivation of RB1 and TP53.⁹,¹⁰ These cancers are thought to either clonally proliferate from a multipotent stem cell progenitor, as in SCLC, or arise as a late-stage evolution in the genetic progression of more organ-typical cancers.¹¹ The latter hypothesis is supported by genetic and histologic evidence of tumor evolution into EPSCC. EPSCCs of the breast demonstrate loss of heterozygosity at BRCA-1, BRCA-2, 8p21-24, 11q23.3, and 12q25 typical of invasive ductal carcinoma.¹² Histologically, EPSCCs of the appendix and urinary bladder often arise focally in a
background of adenocarcinoma and urothelial carcinoma, respectively. However, these posited molecular associations are derived from limited genomic sequencing data of genitourinary and gastrointestinal EPSCCs. Little is known about the pathogenetic underpinnings of EPSCCs of the head and neck.

Inverted papillomas (IPs) of the paranasal sinuses are pathologically benign tumors that tend to recur after surgical resection. Synchronous or metachronous development of in situ or invasive carcinoma (eg, Schneiderian carcinoma) develops in 10% to 25% of IP cases. Most often, these cancers are of squamous differentiation (ie, sinonasal squamous cell carcinoma), although isolated cases of other malignant pathologies associated with IPs have been reported. Herein, we report a case of a patient with a multiply recurrent papillary Schneiderian carcinoma in situ of the sphenoethmoidal sinus who developed a subsequent small cell carcinoma in the surgical resection bed that was subjected to whole-exome sequencing. We posit clinical evidence that this patient’s original tumor devolved into an EPSCC and present the first comprehensive sequencing data on EPSCCs of the head and neck.

Methods
Whole-Exome Sequencing
All patient material was collected under an institutional review board (IRB)–approved protocol from the University of Michigan pathology archive. Regions of tumor or adjacent normal were identified using a hematoxylin and eosin (H&E) slide, and punch cores were taken from the block as described. In situ hybridization for Epstein-Barr virus was performed and negative. Genomic DNA was isolated from the sinonasal small cell carcinoma and adjacent normal mucosa using the Qia-gen (Hilden, Germany) Allprep DNA/RNA FFPE kit as previously described and quantified using a qubit and bioanalyzer to determine the quality DNA yields using previously defined thresholds for molecular analysis. An Illumina (San Diego, CA) gDNA library was prepared from 300 ng DNA and enriched for exomes using the Roche Nimblegen SeqCap EZ v3.0 kit (Roche Nimblegen, Indianapolis, Indiana) as described. Exome-enriched libraries were then sequenced on an Illumina HiSeq 2500 System at the University of Michigan sequencing core, resulting in 86 million (mean of 50.3 reads/base) and 76 million (mean of 34.7 reads/base) mapped reads for tumor DNA and normal DNA, respectively.

Variant Calling and Copy Number Analysis
FastQC (v.0.11.5) was used to assess quality of the exome sequencing data. The sequencing reads were mapped to the hg19 version of the human genome using BWA (v.0.7.15). GATK best practices were followed to prepare BAM files for variant calling. VarScan2 (v.2.4.1) was employed for variant calling. VarSeq was used to annotate and filter variants of interest. All variants in introns and intergenic regions were eliminated from the analysis. Only variants with 5 or more alternate reads were considered as true positives. ADTEx was used to estimate the copy number variations (CNVs) seen in the sinonasal small cell carcinoma.

Results
Case Description
A 52-year-old man presented with a 6-month history of nasal congestion, rhinorrhea, and dysosmia; magnetic resonance imaging (MRI) revealed an inhomogeneous, enhancing mass in the sphenoethmoidal sinus (Figure 1). Tissue biopsy was consistent with a poorly differentiated, papillary Schneiderian carcinoma in situ, also known as transitional cell carcinoma in situ or nonkeratinizing (cylindrical cell) carcinoma in situ. This pathologic diagnosis was made on the basis of a distinct papillary tumor architecture with a distinctive ribbon-like growth pattern with absent to limited cytoplasmic maturation and containing occasional cytoplasmic mucin with strong positive nuclear staining for p63 and lack of infiltrative stromal invasion. The tumor was negative for human papillomavirus (HPV) by in situ hybridization. Roughly 20% to 30% of these tumors contain HPV DNA, although it is unclear whether this represents an incidental mucosal colonization or a direct etiologic contributor to development of IP or malignant transformation. Interestingly, the tumor had a dimorphic population of typical Schneiderian papilloma epithelial cells and a subpopulation with more cohesive morphology reminiscent of small cell carcinoma (Figure 2). A diagnosis of small cell carcinoma was entertained but was ultimately excluded on the basis of large cell size, minimal mitotic activity, lack of invasive growth, and absence of staining for neuroendocrine markers and thyroid transcription factor 1 (TTF-1). The tumor was resected via an anterior subcranial approach with negative margins. Over the ensuing 5 years of clinical surveillance, the patient developed 2 consecutive recurrences of sphenoidotymal masses, both resected endoscopically with pathologic confirmation of recurrent papillary Schneiderian carcinoma in situ.

Six years after original diagnosis, surveillance MRI noted a new polyoid, enhancing mass within the sphenoidotymal surgical defect with infiltration into the cribriform plate and right medial orbital wall (Figure 1). Small
cell carcinoma with extensive in situ component was diagnosed on the basis of distinct pathologic features, including sheets of small, ovoid cells with minimal cytoplasm and nuclei with neuroendocrine-type chromatin with scant stroma and aggressive bone invasion (Figure 3). Importantly, contrast-enhanced computed tomography (CT) of the chest did not demonstrate any evidence of metastatic disease prior to surgical resection. The patient underwent repeat subcranial resection with calvarial bone graft inset of medial orbital wall and radial forearm free flap reconstruction. Postoperatively, 3 cycles of adjuvant cisplatin and etoposide and skull base radiation (54 Gy) were administered. MRI of the head and neck and whole-body positron emission tomography (PET)–CT scans were performed at routine 6-month intervals after adjuvant treatment completion for surveillance of recurrence. At the time of manuscript preparation, the patient was 5 years out from treatment completion with no evidence of small cell carcinoma recurrence or metastases.

**Exome Sequencing Analysis of Sinonasal Small Cell Carcinoma**

Deep whole-exome sequencing identified 93 high-confidence, nonsynonymous somatic mutation events in the sinonasal squamous cell carcinoma (SCC) (Suppl. Figure S1 and Suppl. Table S1 at www.otojournal.org/supplemental). Sixty-three (67.8%) were single-nucleotide substitutions and 30 (32.2%) were disruptive insertion-deletion (indel) events. Only 18 (19.4%) of these mutational events have been previously documented in the Catalogue of Somatic Mutations in Cancer (COSMIC).30

A heterozygous missense mutation in TP53 (c.528C>G; p.C176W) was the most significant mutational event confirmed in the small cell carcinoma sample. This TP53 variant is pathogenic and frequently present across multiple human cancers.31,32 Importantly, no single-nucleotide substitution or indel events were identified in genes involved in cell cycle regulation (RB1, RBL1, RBL2, TP73), chromatin modification (EP300, CREBBP), or kinase signaling (KIT, PIK3CA, BRAF) pathways recurrently implicated in SCLC.9,10,33 However, we identified a disruptive in-frame indel mutation in MAML3 (c.2302_2304delCAG), which may play a role in regulating downstream NOTCH signaling.34 We also noted a missense mutation in GAS6 (c.629C>T,p.A210V), a secreted peptide that binds to and activates the TAM family of tyrosine kinase receptors.35 Whole-exome CNV analysis identified a focal amplification of the chromosomal region 6p25-11.1 containing SOX4, a transcription factor essential to embryologic differentiation, and VEGFA (vascular endothelial growth factor–α). Focal amplification of 14q32.1-32.3 containing AKT1, a serine/threonine protein-kinase and known oncogene, was

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**Figure 2.** Papillary Schneiderian carcinoma in situ with intraepithelial small cell component at ×10 (left) and ×20 (right) magnification. Hyperplastic respiratory epithelium with papillary architecture (left) and mucin-containing cells (right) encircled.

**Figure 3.** Sinonasal squamous cell carcinoma (SCC) at ×10 (left) and ×20 (right) magnification. On the left, SCC (central) shown replacing marrow space with erosion of surrounding bone (encircled).
also present. Genomic losses at 3p14.3-14.2 (harboring *FHIT*) and 3p12.3-12.2 (harboring *ROBO1*), typical of SCLC, were not identified in the sinonasal SCC, but we did identify an amplification of the Notch pathway inhibitor *DLK1*, further suggesting that inhibition of NOTCH signaling is an important step in the pathogenesis of small cell carcinoma (*Figure 4*).37,38

Significant CNVs were identified in several genes central to SCLC pathogenesis (*Figure 5*). Recurrent amplification of the progrowth genes *CCND1*, *E2F1*, and *MDM2* and hemizygous deletion of tumor suppressors *CDKN2A*, *RB1*, *RBL1*, and *RBL2* contributed to aberrant cell cycle regulation and cellular proliferation. Amplification of Notch pathway members, *MYC* family genes, and CNVs of chromatin modifiers *CREBBP* and *EP300* were similarly important genomic events in sinonasal SCC pathogenesis.

**Discussion**

Benign IP, Schneiderian carcinoma, and sinonasal SCC exist on a spectrum of tumor evolution. Specific molecular drivers, including oncogenic *EGFR* mutations and high-risk HPV infection, provide a clear pathogenetic framework for malignant progression in these neoplasms. However, the molecular mechanisms and clinical prediction of malignant transformation from IP and Schneiderian carcinoma into nonsquamous tumors such as SCC remain nebulous. Our study does suggest that IP and Schneiderian carcinoma have the potential to devolve into sinonasal SCC, although certainly robust investigations of genetic associations between these tumor types are essential to confirm direct tumor progression.

The patient described herein had a multiply recurrent Schneiderian carcinoma in situ of the paranasal sinuses with subsequent tumor devolution into sinonasal SCC. The development of SCC within the previously resected sinonasal surgical defect and absence of distant metastatic disease and potential primary SCC foci support a direct progression from Schneiderian carcinoma in situ to sinonasal SCC. In addition, the patient’s continued survival with no evidence of disease recurrence concurs with superior prognosis of

![Figure 4](Circos plot showing amplification (green dots) of SOX4 on chr 6 (yellow arrow), DLK1 on chr 14 (orange) and deletion (red dots) of CDKN2A on chr 9 (blue), RB1 on chr 13 (black).)
sinonasal SCC compared to SCLC and EPSCCs as reported in the literature.4

EPSCCs of the head and neck and other anatomic sites share identical histopathological features with SCLC and are treated with similar chemoradiation protocols. However, recent evidence suggests that EPSCCs are a heterogeneous group of neuroendocrine neoplasms harboring unique genetic drivers of oncogenesis, making comparative genomic analyses with SCLC imperative.40 Whole-exome sequencing and CNV analysis of this rare case of sinonasal SCC revealed several unique genetic aberrations but also important shared mutational signatures with SCLC.

We identified a total of 93 high-confidence, nonsynonymous somatic alterations in exomes of sinonasal SCC, a relatively modest mutational burden compared to that typically seen in SCLC.9,10,33 The 75 (80.6%) somatic alterations not presently listed in COSMIC are potential targets for future elucidation of their role in sinonasal SCC initiation and progression. We did not identify any single-nucleotide substitution or indel events in the majority of genes recurrently altered in SCLC, although significant CNVs in these genes were almost uniformly present (Figure 5; Suppl. Table S1 at www.otojournal.org/supplemental). While a heterozygous, loss-of-function mutation in TP53 (c.528C>G; p.C176W) was confirmed in our sample, 1 allele remained intact with no loss of heterozygosity seen for this critical tumor suppressor.

Focal amplification of chromosomal regions 6p25-11.1 and 14q32.1-32.3 was seen in our sinonasal SCC sample. The former is a known genomic fragile site and amplification hotspot containing the SOX4 gene.41 Notably, amplification of SOX family genes is a recurrent driver of SCLC initiation and proliferation.33 Amplification of 14q31.2-32.3 resulted in activation of DLK1, an inhibitory ligand of the Notch signaling pathway. Overexpression of DLK1 and recurrent inactivating mutations in NOTCH1 are typical of SCLC, supporting an important tumor suppressor function of the Notch pathway in these neuroendocrine neoplasms.

Homozygous deletions of FHIT at 3p14.3-14.2 and ROBO1 at 3p12.3-12.2, genes posited to be critical tumor suppressors in SCLC and other human malignancies, were not present in sinonasal SCC.42,43

Figure 5. Whole-exome copy number variation analysis of sinonasal squamous cell carcinoma (SCC) reveals significant amplification and deletion events in genes recurrently implicated in small cell lung carcinoma (SCLC) pathogenesis9,10,30 (frequency of individual gene alterations in SCLC calculated using cBioPortal).34,35

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Homozygous deletions of FHIT at 3p14.3-14.2 and ROBO1 at 3p12.3-12.2, genes posited to be critical tumor suppressors in SCLC and other human malignancies, were not present in sinonasal SCC.42,43
Whole-exome CNV analysis of sinonasal SCC revealed numerous amplification and deletion events in genes critical to SCLC pathogenesis (Figure 5). Hemizygous deletion of CDKN2A, RB1, RBL1, and RBL2 was present in sinonasal SCC. Therefore, inactivating alterations of these critical cell cycle regulators are likely essential for both sinonasal SCC and SCLC pathogenesis. In the Notch signaling pathway, DLK1 amplification and inactivation of the downstream transcriptional coactivator MAML3 likely resulted in net inactivation of Notch signaling in sinonasal SCC. Notch signaling has been posited to have a critical tumor suppressor function in SCLC and other human malignancies, and thus this pathway is an attractive target for future mechanistic studies in sinonasal SCC, EPSCCs of other anatomic sites, and SCLC. Finally, copy gain of the chromatin modifier CREBBP in sinonasal SCC diverged from the predominant pattern of inactivation of chromatin modifiers in SCLC.

Conclusions
Sinonasal SCC may rarely arise as a late-stage evolution in the genetic progression of sinonasal IP and Schneiderian carcinoma. Whole-exome sequencing of sinonasal SCC revealed inactivating alterations in TP53, CDKN2A, and RB1 similar to SCLC. Likewise, the probable inactivation of NOTCH signaling through an indel in MAML3 and amplification of the pathway inhibitor DLK1 are consistent with the inactivation of NOTCH signaling commonly seen in SCLC. Future comprehensive genomic studies on sinonasal SCC and EPSCCs of the head and neck and other anatomic sites are needed to guide therapeutic strategies in these rare neoplasms.

Author Contributions
Joshua Smith, data analysis, drafting, final approval, accountability for all aspects of the work; Aditi Kulkarni, data analysis, drafting, final approval, accountability for all aspects of the work; Andrew C. Birkenland, data analysis, drafting, final approval, accountability for all aspects of the work; Jonathon B. McHugh, data analysis, drafting, final approval, accountability for all aspects of the work; J. Chad Brenner, data analysis, drafting, final approval, accountability for all aspects of the work.

Disclosures
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