Introduction

The sinonasal tract is an uncommon location for head and neck malignancies, accounting for less than 5% of head and neck cancers (14). Moreover, the overall incidence has been declining for the past 30 years (14). Sinonasal cancer most commonly occurs in patients between 50 and 60 years of age and is twice as likely to occur in males than females (7). As with malignancies in other subsites of the head and neck, squamous cell carcinoma (SCC) is the prevailing histopathology, comprising greater than 50% of all sinonasal tumors in the United States (15).

SINONASAL SCC (SNSSC) most frequently arises in the maxillary sinus, with the second most common location being the nasal cavity. It rarely originates in the frontal or sphenoid sinus (2, 4, 7, 12, 14). These tumors most often present at an advanced stage—one study demonstrated 48.9% of tumors examined were T4 at diagnosis—most likely because the earliest symptoms are secondary to mass effect (12). 5-year survival is 30 – 50% regardless of treatment, with T1 SNSSC having 5-year survival of 80% and T4 SNSSC having a 5-year survival of 30% (7). Local recurrence is the most common cause of mortality (7).

Risk factors for the development of SNSSC include tobacco use, occupational exposures such as nickel, soft wood dust, radium, and mustard gas (4.7). In fact, occupational exposures are associated with approximately 30% of SNSSC, (4, 7).

Though SNSSC is histologically similar to tumors in other areas of the aerodigestive tract, they are genetically distinct (7). This has limited not only our understanding of the mutations that drive the development of SNSSC but also our ability to identify prognostic biomarkers for the disease. At present, there is a glaring lack of understanding regarding common driver mutations that arise during the genesis of SNSSC. The goal of this study was to comprehensively catalog common somatic mutations in SNSSC tumors using Next Generation (high-throughput) whole-exome sequencing.

Methods

Methods and Materials

The study is retrospective, utilizing whole-exome tumor tissue from 10 SNSSC patients with paired adjacent normal tissue available through the University of Cincinnati Cancer Institute Tumor Bank. Tumor samples were all collected prior to initiation of chemotherapy or radiation from patients diagnosed between 2012 and 2014. Histopathologic classification and grading was confirmed by a board-certified Anatomic Pathologist who specializes in head and neck cancer. The sequencing library was prepared using the SureSelectXT Target Enrichment System (Agilent, Santa Clara, CA). Clustered libraries were paired-end sequenced for 2X100 cycles using the TruSeq SBS HS 200-cycles kit v3 on an Illumina HiSeq 1000 system. Bioinformatic analysis of point mutations was performed via Mutect HC the Genome Analysis Toolkit (GATK; Broad Institute, Boston, MA).

Results

The Q30 distribution score for the sequencing of SNSSC and adjacent tissue is 92.2%, indicating high quality sequencing data. The most frequently mutated genes were KMT2C (5/10), PIK3CA (3/10), and GNAS-A1 (3/10). TPS3 was mutated in 2/10 of the samples.

Conclusions

Prior studies have evaluated SNSSC for several common somatic mutations that arise in head and neck squamous cell carcinoma (HNSCC) but with little concordance beyond EGFR and PS3. To date, there have been no reports involving comprehensive somatic profiling of SNSSC in the literature. This study performed whole-exome sequencing of head and neck SCC at all anatomic sites, including only 2 patients with SNSSC, but without stratifying this information by sub-site. This study demonstrated mutations in genes previously implicated in HNSCC, including TP53, CDKN2A, PTEN, HRAS, and PIK3CA, but also identified mutations in NOTCH1 (26). The NOTCH1 mutation was substantiated in a second study in which whole-exome sequencing in HNSCC patients were also performed (4). This study was performed on patients with head and neck SCC (HNSCC). A third study examined genome-wide copy number abnormalities in patients with SNSSC, demonstrating alterations most commonly in BRCA1, CDH1, EMS1, BCL2L2, EGFR, and CD44 (8). This study was the first and, despite the modest sample size, the largest to perform whole-exome sequencing of only patients with SNSSC examining all mutations.

The most commonly mutated gene in our study was KMT2C (MLL3), which was mutated in 50% of SNSSC tumors compared with only 7.3% of patients with HNSCC. KMT2C is a lysine methyltransferase involved in methylation of H3K4. This gene is commonly deleted in malignant myeloid disorders and mutations are associated with 24.5% of bladder urothelial carcinoma, 18.4% of lung adenocarcinoma, 15.5% of lung SCC, and 7.3% of HNSCC (6).

Our study demonstrated that the lone sample harboring an EGFR mutation (data not shown; Table 2) was restricted to mutations in > 2 samples). This is in-line with the literature suggesting that the majority of SNSSC arising from inverted papilloma contain such mutations but not in patients with SNSSC without a preceding inverting papilloma (17). However, PIK3CA, a downstream effector of EGFR, was one of the most common mutations encountered at 3/10 compared with 17.8% in head and neck SCC overall. PIK3CA (Phosphatidylinositol 3-kinase) regulates the activation of AKT downstream from EGFR. It is involved in cell growth, survival, proliferation, motility, and morphology. This elevated rate of mutations is consistent with a previous study that demonstrated mutations of PIK3CA in 2/5 samples of nasal cavity/sinus SCC (10). This is in contra-distinction to other downstream effectors of EGFR, including KRAS and BRAF, which are not mutated in SNSSC (9).

Our study demonstrated a low mutation rate of TP53 (p53) at 2/10 compared with a mutation frequency of 7% in HNSCC. A study investigating TP53 mutations in patients with both sinonasal adenocarcinoma demonstrated a mutation rate of 77% of TP53 in combined SNSSC and sinonasal adenocarcinoma but was less in SCC compared with adenocarcinoma (5). This finding supports the idea that SNSSC is genetically distinct from SCC in other areas of the upper aerodigestive tract.

Finally, this study demonstrated a mutation in GNAS-A3 in 3/10 patients. GNAS-A3 is a non-coding RNA with as of yet unknown function. A recent Chinese study, however, identified mutations in GNAS-A3 in patients with papillary thyroid carcinoma (13). It has not previously been associated with HNSCC.

Conclusion

This study sheds light on the mutational landscape of SNSSC, including many genes that are common with HNSCC, as well as others that have not been described in upper aerodigestive tract cancers. Moreover, the observation of frequent mutations in a H3K4 methyltransferase gene warrants further investigation, as H3K4 is a potentially targetable epigenetic modification (11). This information advances our understanding of the genesis of an uncommon, though important, malignancy.

References