CASE REPORTS

NUT Carcinoma of the Submandibular Gland: A Case at This Uncommon Site with Review of the Literature

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Abstract

Nuclear protein in testis (NUT) carcinoma is a rare, highly aggressive, undifferentiated carcinoma that harbors a characteristic rearrangement of the *NUTM1* gene. The majority arise in adolescents and young adults especially from the midline structures of the thorax, head, and neck. Until the present, there have only been three reported cases of NUT carcinoma of the submandibular gland, two of which were reported in children and another one in an adult from Korea. Here, we report the first case of NUT carcinoma arising in the submandibular gland of an adult female in the United States, representing the fourth case worldwide. A fine needle aspiration and biopsy was performed, and the diagnosis was confirmed by NUT immunohistochemical staining and fusion of the *BRD4* (19p13.12) and *NUTM1* (15q14) gene loci by fluorescence in-situ hybridization on the resection specimen. Salivary gland is an unusual site for NUT carcinoma and is rarely described in submandibular gland. We reviewed the clinicopathologic features of this entity at this site along with role of *NUTM1* gene rearrangements in NUT tumorigenesis.

Keywords Submandibular gland · Salivary gland · NUT carcinoma · NUT midline carcinoma · *NUTM1* · *BRD4-NUTM1*

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Introduction

Nuclear protein in testis (NUT) carcinoma, previously known as NUT midline carcinoma, is a rare, aggressive, translocation-associated carcinoma characterized by the presence of chromosomal rearrangement involving the NUT midline carcinoma family member 1 (*NUTM1*) gene, aka *NUT* gene, located on chromosome 15q14 [1, 2]. It was first described in 1991 in two case reports of mediastinal carcinomas harboring the t(15;19) chromosomal translocation [3, 4].

The *NUTM1* gene is commonly fused to the *BRD4* gene located on chromosome 19p13.12, a member of the BET family. This results in the most characteristic reciprocal translocation t(15;19) accounting for 70–88% of the cases [5–9]. About 15-30% have variant translocations involving the *BRD3* gene on chromosome 9q34.2 [2, 9]. Less frequently, other rarer (~ 6%) *NUTM1* fusion variants have been reported [9] including *NSD3* gene on chromosome 8p11.23 which is considered a BET-binding protein [10], zinc finger-containing proteins such as *ZNF532* [11] and *ZNF592* [12], and other unknown genes. This rare variant of poorly differentiated carcinoma was originally considered to affect

midline organs such as the mediastinum and the sinonasal tract; especially affecting children and young adults [7]. However, this entity has been identified in patients of all ages (0–81.7 years, median = 16, 22, 23, and 24 years in four meta-analysis studies) [5, 6, 8, 9], affecting females and males almost equally [5, 8, 9], and rarely arising outside of midline locations [5, 8, 9].

Outcomes are almost universally dreary, with a median overall survival (OS) of 6.5 months [9] and poor response to conventional chemotherapeutic agents or radiotherapy. Most patients (~ 50%) present with lymph node involvement or distant metastatic disease [1, 5], frequently seen in lung and bones, and rarely in adrenal glands, brain, bone marrow, and liver [8, 13–15].

NUT carcinoma (NC) arising from submandibular gland are extremely rare. As of today, only three cases of NC have been described to originate from the submandibular gland [15–17]. Here, we described the fourth case of NC arising from the submandibular gland and the first reported case in the United States in an adult female patient. The clinical and pathologic characteristics including molecular alterations of all four reported cases will be discussed as well.

Materials and Methods

Formalin-fixed, paraffin-embedded (FFPE) 4 μm-thick tissue sections were stained with hematoxylin and eosin (H&E). Immunohistochemistry (IHC) was performed on 3 to 5-μm-thick FFPE tissue sections using the DAKO Envision system (DAKO, Glostrup, Denmark) in an automated immunostainer (Dako, Autostainer plus). Antibodies used for IHC are summarized (Table 1). Heat induced antigen retrieval was used for all antibodies in appropriate buffers as per manufacturer's guidelines. Interpretation of NUT IHC was based on published data showing distinctive granular (punctate or speckled) nuclear immunoreactivity present in > 50% of neoplastic cell nuclei [18]. In-situ hybridization (ISH) for Epstein-Barr Virus-encoded small RNA (EBER-1) was performed using an EBER probe (Dako, Glostrup, Denmark) that was hybridized to proteinase-pretreated de-paraffinized 3 to 5-μm-thick tissue sections and prepared in RNase free conditions (performed as per manufacturer's guidelines). Visualization of a hybridized probe was achieved by chromogenic detection. Positive and negative controls were used throughout.

Fluorescence In-Situ Hybridization

Dual color bring-together fluorescence in-situ hybridization (FISH) to evaluate chromosome 19p13.1 *BRD4* and 15q14 *NUTM1* was performed on 5 μm-thick FFPE unstained sections of tumor as previously described [7]. FISH probes used for characterizing the *NUTM1*-fusion were as following: *NUTM1*: 3′ telomeric probes, RP11-1141P10 and RP11-477L8 (digoxigenin labeled, green); *BRD4*: 5′ centromeric probes, RP11-207i16 and RP11-3055m5 (biotin labeled, red). Two hundred nuclei were counted in four different

Table 1 Antibodies used for immunohistochemical stains

Antibody	Clone	Manufacturer	Species	Dilution	Type
Pan-CK AE1/AE3	AE1/AE3	Agilent	Mouse	RTU	Monoclonal
CK7	OV-Tl 12/30	Agilent	Mouse	RTU	Monoclonal
CK5/6	D5/16 B4	Agilent	Mouse	RTU	Monoclonal
p63	4A4	Biocare	Mouse	RTU	Monoclonal
p40	Anti-p40	Biocare	Rabbit	1:200	Polyclonal
Synaptophysin	DAK-SYNAP	Agilent	Mouse	RTU	Monoclonal
Chromogranin A	DAK-A3	Agilent	Mouse	1:100	Monoclonal
Desmin	D33	Agilent	Mouse	RTU	Monoclonal
S100	Anti-S100	Agilent	Rabbit	RTU	Polyclonal
SOX-10	BC34	Biocare	Mouse	1:100	Monoclonal
CD45 (LCA)	2B11+PD7/26	Agilent	Mouse	RTU	Monoclonal
CD99	12E7	Agilent	Mouse	RTU	Monoclonal
GATA3	L50-823	Biocare	Mouse	1:400	Monoclonal
AR	Anti-AR	Cell Signaling	Rabbit	1:200	Polyclonal
BRG1	EPNCIR111A	Abcam	Rabbit	1:100	Monoclonal
INI-1/BAF-47	25/BAF47	BD Biosciences	Mouse	1:200	Monoclonal
NUT	C52B1	Cell Signaling	Rabbit	1:400	Monoclonal
Ki-67	MIB-1	Agilent	Mouse	RTU	Monoclonal

Pan-CK pancytokeratin, *CK* cytokeratin, *LCA* leukocyte common antigen, *AR* androgen receptor, *NUT* nuclear protein of testis, *RTU* ready to use

areas of the tumor. 80% positive interpretable nuclei were defined as positive for a rearrangement.

Molecular Study

Next Generation Sequencing (NGS) study was performed using the Oncomine Precision Assay in a Genexus instrument (ThermoFischer Scientific) on FFPE sections by a validated polymerase chain reaction (PCR)-based sequencing assay for detection of DNA sequence variants in a panel of genes (*AKT1, AKT2, AKT3, ALK, AR, ARAF, BRAF, CDK4, CDKN2A, CHEK2, CTNNB1, EGFR, ERBB2, ERBB3, ERBB4, ESR1, FGFR1, FGFR2, FGFR3, FGFR4, FLT3, GNA11, GNAQ, GNAS, HRAS, IDH1, IDH2, KIT, KRAS, MAP2K1, MAP2K2, MET, MTOR, NRAS, NTRK1, NTRK2, NTRK3, PDGFRA, PIK3CA, PTEN, RAF1, RET, ROS1, SMO, TP53*); detection of copy number variations (*ALK, AR, CD274, CDKN2A, EGFR, ERBB2, ERBB3, FGFR1, FGFR2, FGFR3, KRAS, MET, PIK3CA, PTEN*); detection of inter-genetic fusions in *ALK, AR, BRAF, EGFR, ESR1, FGFR1, FGFR2, FGFR3, MET, NRG1, NTRK1, NTRK2, NTRK3, NUTM1, RET, ROS1, RSPO2,* and *RSPO3*; as well as detection of intra-genetic fusions in *AR, EGFR* and *MET* in selected hot-spots.

Results

Clinical and imaging findings

A 49-year-old woman presented with a rapidly enlarging, firm, non-mobile, and painful 4.2 cm mass in the right lower jaw for about 2–4 months. She denied history of weight loss, night sweats, fever, ear pain, hoarseness, odynophagia, dysphagia, or dyspnea. Her past medical history was significant for hyperlipidemia, hypertension, asthma, gastroesophageal reflux with hiatal hernia, and morbid obesity. Her prior surgical history comprises two cesarean sections in 2001 and 2004, hiatal hernia repair and laparoscopic sleeve gastrectomy in 2012. She had used phentermine (ionamine/adipex) for about 24 months since 2011. She denied history of tobacco/cigarette smoking, regular drinking, or recreational drug use. Her family history was significant for lung cancer in her grandparent. Her clinical examination was normal except for tenderness to palpation of the neck mass. She also had right neck level II lymphadenopathy. The overlying skin was non-erythematous.

A computed tomography (CT) scan with contrast of the neck showed a mass lesion ($42 \times 34 \times 35$ mm; AP $\times$ TV $\times$ CC) inseparable from the right submandibular gland (Fig. 1A). The mass was abutting the mylohyoid sling posteriorly. A prominent lymph node ($21 \times 19 \times 28$ mm; AP $\times$ TV $\times$ CC) within the right level IIA was observed, concerning

for metastatic disease. A few subcentimeter right level III lymph nodes were also noted. A full mouth panorex showed no acute abnormality. Imaging studies including chest, abdomen/pelvis CT scan with contrast demonstrated no evidence of malignancy or metastatic disease.

Cytology Findings

The patient underwent ultrasound-guided fine needle aspiration (FNA) which showed moderate to hypercellular smears comprised of single cells admixed with loose aggregates of monotonous, primitive-appearing tumor cells with stripped cytoplasm. Overall, the tumor cells showed scant, pale cytoplasm, naked round nuclei with finely granular chromatin and distinct nucleoli. Focal areas showed nuclear molding. Neutrophilic infiltrate was noted in the background along with scant necrotic debris (Fig. 2A–C). Based on cytologic features the differential diagnoses included small cell carcinoma, poorly differentiated carcinoma, NC, sarcoma, and lymphoma. Concurrent thin-needle core biopsy showed sheets of monotonous, small to medium sized round blue tumor cells infiltrating salivary tissue with frequent mitoses and apoptosis. Immunostains showed the tumor cells were positive for pancytokeratin AE1/AE3 (focal), CK5/6 (focal), p63 (diffuse), and NUT (diffuse), while negative for CK7, GATA3, SOX-10, S100, p40, CD45, and synaptophysin. Based on morphologic and IHC results the findings were consistent with NC, and within 48 h the patient underwent urgent right submandibular gland resection with right neck lymph node (midline level IA, and levels IB, II, III, IV and V) dissection.

Gross, Histopathologic, IHC, FISH and Molecular Findings

Grossly, the resection specimen consisted of a submandibular gland diffusely involved by a tan-gray, firm, irregular nodular mass ($5.3 \times 4.0 \times 3.4$ cm) with pushing borders and areas of necrosis. The mass was focally abutting the inked surgical margins with small remnant of submandibular gland and skeletal tissue at the periphery (Fig. 2D).

Histologically, the sections showed morphologic features similar to those seen on FNA/core biopsy with an infiltrating, undifferentiated, high-grade tumor diffusely involving the submandibular gland in a background of extensive necrosis (Fig. 2E, F). The tumor was characterized by cohesive sheets of medium to large-sized, monomorphic tumor cells with scant to moderate cytoplasm; scattered cells showed cytoplasmic clearing (Fig. 3A). The nuclei were round to oval with vesicular chromatin and distinct nucleoli (Fig. 3B). Also, the tumor showed intra-tumoral and peri-tumoral inflammatory infiltrate mainly composed of neutrophils admixed with few lymphocytes and plasma cells (Fig. 3B).

Fig. 1 Imaging findings, pre- and post-surgical: **A** Pre-operative, axial neck CT scan with contrast showing a 4.2×3.5×3.4 cm mass (yellow arrow) involving the right submandibular gland. **B** Two-weeks post-operative PET-CT scan of the neck showing focal radiotracer uptake (bright area) at the right neck surgical resection site (yellow arrow), which could represent either residual tumor or post-surgical changes. **C** Post-operative (2 weeks after surgery) PET-CT scan of the whole body show no metastatic disease with lack of FDG-uptake (bright areas) elsewhere in the body
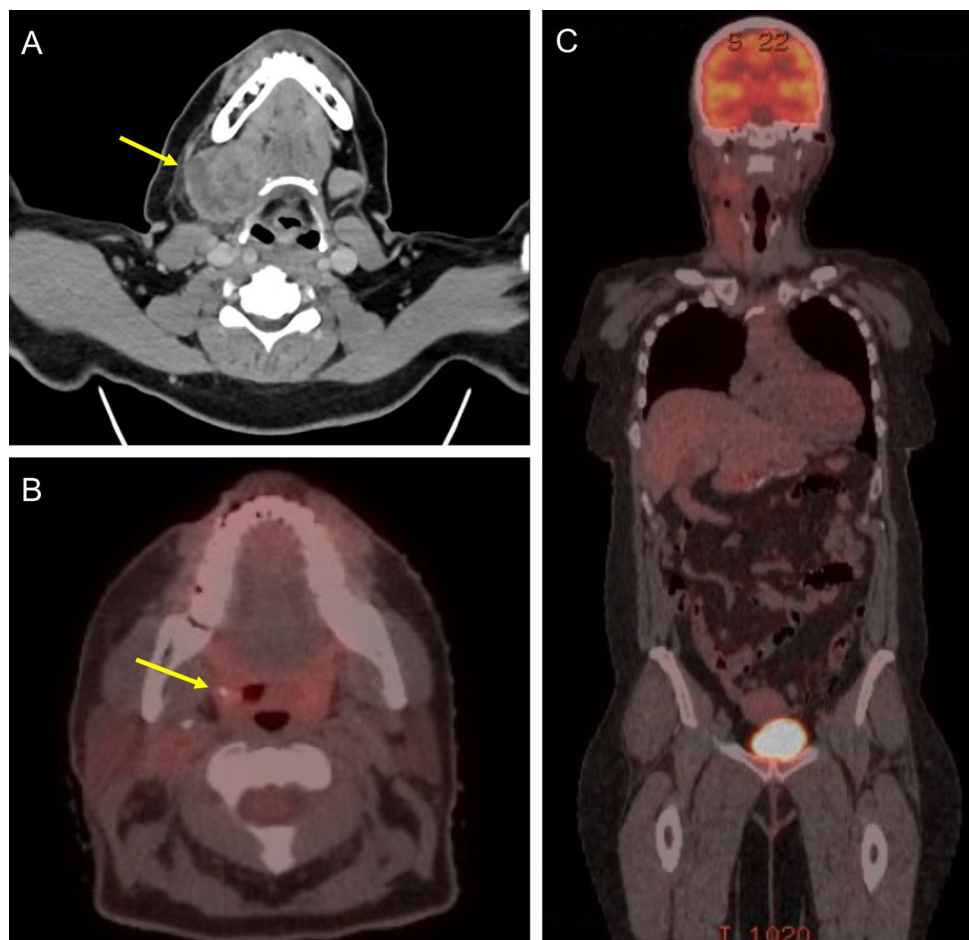
Brisk mitotic activity including atypical forms were noted. No definitive areas of abrupt keratinization were appreciated, though focal small ducts showed squamous metaplasia. Lymphovascular and perineural invasion (Fig. 3C) was identified. Of the total 42 regional lymph nodes, 11 were involved by metastatic carcinoma (Fig. 3D) with a 3.1 cm largest metastatic deposit along with focal extranodal extension. Although a diagnosis of NC was suggested on FNA/core biopsy, due to the rarity of this type of tumor at this specific location along with the clinical implications for the patient, other differential diagnosis were considered including poorly differentiated carcinoma of salivary gland origin, Ewing sarcoma (ES), rhabdomyosarcoma, members of the SWItch/sucrose non-fermentable (SWI-SNF) family deficient carcinoma (i.e., SMARCB1/INI-1- or SMARCA4/BRG1-deficient carcinoma), and metastatic carcinoma from oropharyngeal or nasopharyngeal site such as human papillomavirus (HPV)-related squamous cell carcinoma or Epstein–Barr virus (EBV)-positive nasopharyngeal carcinoma. Therefore, a repeat IHC panel and further workup was performed to exclude the other differential diagnoses.

IHC stains, with appropriate controls, showed the tumor cells were positive for pancytokeratin AE1/AE3 (focal)

(Fig. 3E), CK7 (focal), CK5/6 (focal), p63 (diffuse), NUT (diffuse, speckled pattern) (Fig. 3F), p40 (rare cells), synaptophysin (patchy) and chromogranin A (focal). The tumor cells were negative for desmin, S100, SOX-10, CD99, GATA3, androgen receptor, CD45, and INI-1/BAF-47 and BRG1 (both with retained nuclear staining). Ki-67 labeling index was 70% in the tumor cells. ISH for EBER-1 was negative in tumor cells. Based on the above morphologic and IHC evaluation, the findings were compatible with NC of the submandibular gland. Subsequent FISH study with probes to detect the telomeric side of the *NUTM1* locus breakpoint and the centromeric side of the *BRD4* locus breakpoint was positive for *BRD4-NUTM1* fusion, confirming the presence of the t(15;19) chromosomal translocation (Fig. 4). NGS did not reveal genetic alterations (single nucleotide changes, small insertions and deletions, copy number alterations, and gene fusions) in selected hotspot regions from the above-mentioned genes.

Clinical Management and Follow-Up

Two weeks after the surgery patient underwent fluorodeoxyglucose (FDG) positron-emission tomography/computed
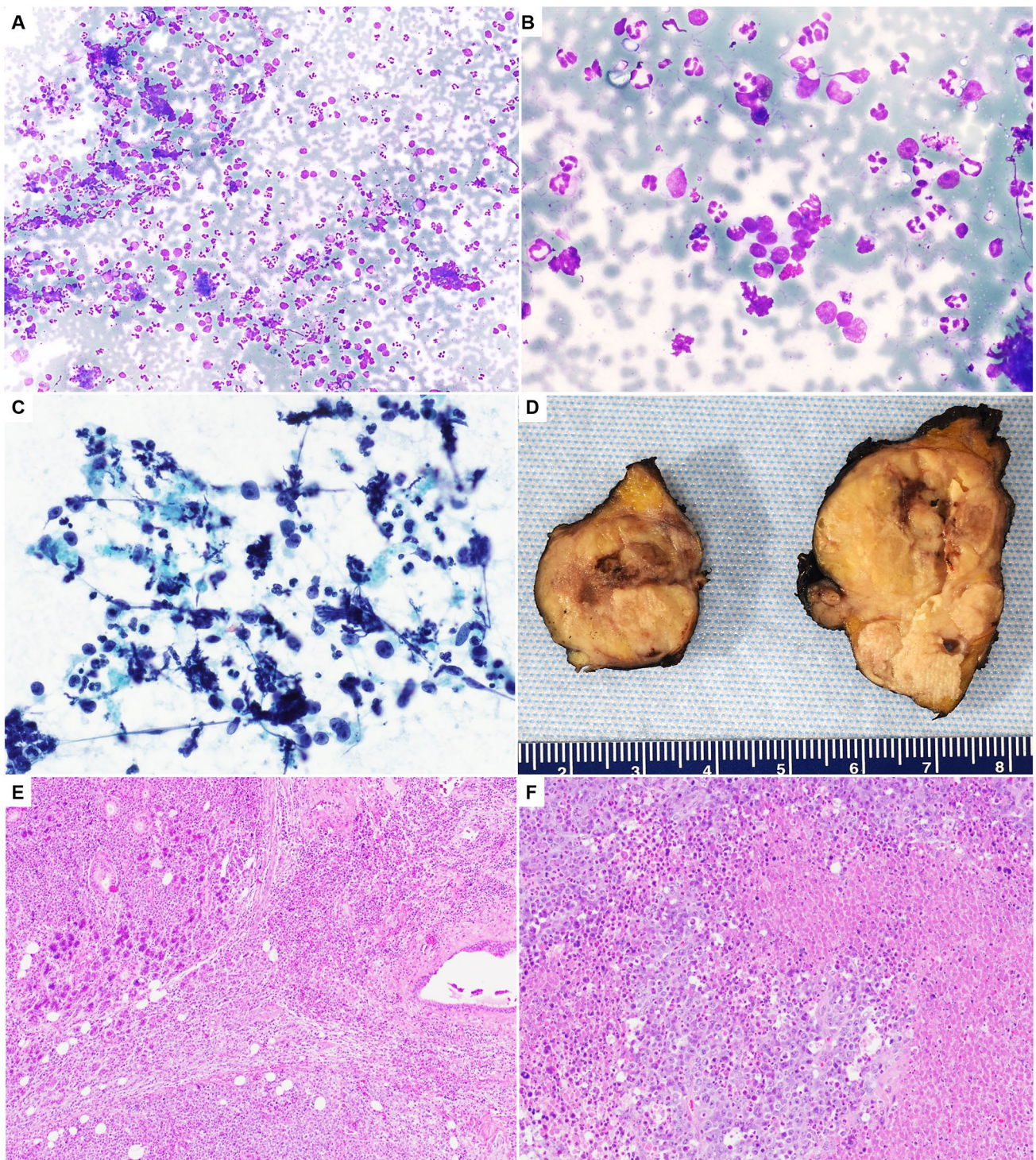
Fig. 2 Gross, cytology, and microscopic findings: **A** Cytology smear from FNA showing moderate to hypercellular lesion with tumor cells either forming loose aggregates or dispersed as single cells (Diff-Quik stain, ×100). **B** Tumor cells are monotonous, primitive appearing with round to oval nuclei, prominent nucleoli, and scant cytoplasm. Background neutrophilic infiltrate is prominent. The loosely aggregated tumor cells focally show nuclear "molding", mimicking neuroendocrine neoplasm (Diff-Quik stain, ×200). **C** Tumor cells are discohesive and show similar features as described above (Papanicolaou stain, ×200). **D** Grossly, sections show an infiltrating tumor with pushing borders, tan-gray to tan-yellow cut surfaces with areas of necrosis. **E** Histologic section shows sheet of undifferentiated tumor diffusely infiltrating the submandibular gland (H&E, ×40). **F** Extensive areas of necrosis are present (H&E, ×100)
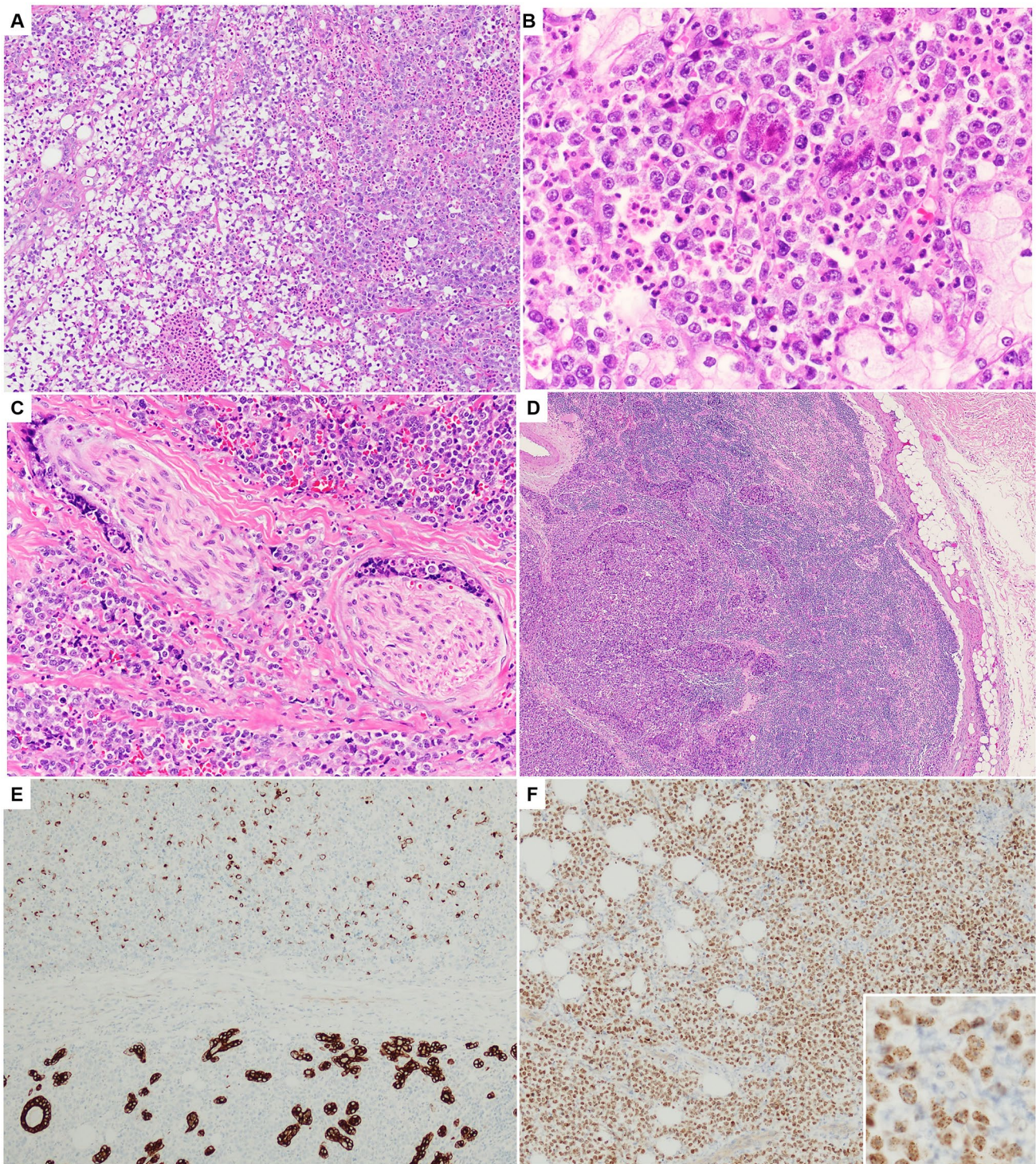
Fig. 3 Microscopic and immunohistochemical findings: **A** tumor exhibiting areas with clear cell features (H&E, ×100). **B** Discohesive, medium to large-sized, monomorphic tumor cells associated with tumoral neutrophilic inflammatory infiltrate. Entrapped salivary serous and mucinous acini are noted (H&E, ×200). **C** Perineural invasion (H&E, ×100). **D** Tumor metastasis involving level II lymph nodes (H&E, ×20). **E** Tumor cells demonstrating patchy keratin staining. Normal ducts showing strong complete staining as internal control (pancytokeratin AE1/AE3, ×100). **F** Tumor cells diffusely positive for anti-NUT antibody (NUT stain, ×100); inset (lower right) shows characteristic "speckled" nuclear staining (NUT stain, ×200)
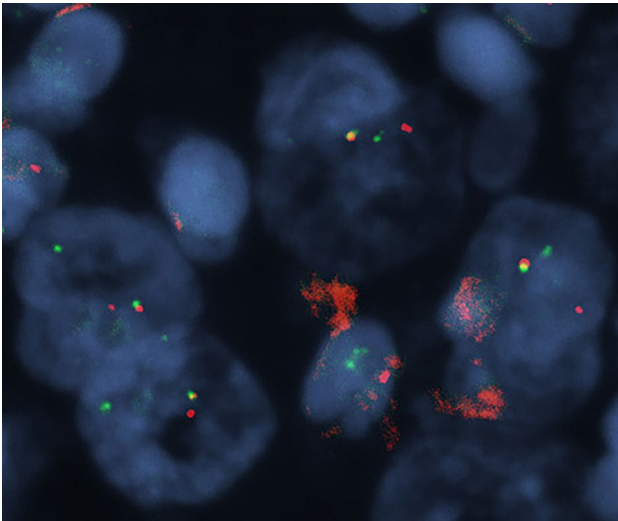
Fig. 4 Detection of *BRD4-NUTM1* fusion in the tumor by dual color, bring-together FISH. Green probes detect the telomeric side of the *NUTM1* locus breakpoint, whereas red probes detect the centromeric side of the *BRD4* breakpoint. The tumor cells are diploid: overlapping red-green signals indicate *BRD4-NUTM1* fusion loci, whereas non-overlapping signals indicate wild type alleles of the respective genes

tomography (PET-CT) scan which revealed focal radiotracer uptake at the right neck surgical resection site (Fig. 1B), the differential being possible residual malignancy versus post-surgical inflammation. The PET-CT scan was negative for any other nodal or metastatic disease (Fig. 1C).

Post-surgery, she had received 7 of 7 cycles of adjuvant chemotherapy (carboplatin and paclitaxel) along with radiotherapy of 66 Gy in 33 fractions to the surgical site and bilateral regional nodal areas. After completion of local therapy, she is being planned for carboplatin-fluorouracil-pembrolizumab therapy. At the time of writing this report, 17 weeks after surgery, patient is alive with no evidence of tumor recurrence.

Discussion

NUT carcinoma is a rare tumor characterized by an aggressive clinical behavior. Although the cell of origin is unknown, it has been speculated that NC may arise from primitive neural crest-derived cells [19]. The thorax has been found to be the most common location of primary NC (~ 50%) followed by head and neck (~ 40%) [9]. However, it can rarely arise outside of midline locations such as bladder [7], ocular globe [7], salivary glands [13, 15–17, 20–29], brain [30], kidney [30–32], stomach [30], adrenal gland [24], pancreas [33], soft tissue [30] and bone locations like iliac bone [34]. NC arising from salivary glands, especially from the submandibular gland, is extremely rare.

To best of our knowledge, a total of 17 salivary gland NCs have been reported in the literature. This tumor, originating from salivary glands, have shown exclusive occurrence in males in the pediatric age group (< 18 years; n = 6) compared to adult patients with a slight female predominance (n = 11; F:M = 1.2:1). The most common location has been described in the parotid gland (n =11; 8 adults/3 pediatric patients), followed by the sublingual (n = 3; 2 adults/1 child) and the submandibular gland (n = 3; 1 adult/2 pediatric patients) [13, 15–17, 20–29].

The clinical, pathological, and molecular findings of the previous 3 reported cases in the submandibular gland [15–17] along with the current case are summarized (Tables 2 and 3). Of the NC arising from submandibular gland females and males are equally affected (F:M = 1:1); both male patients being in the pediatric age group. The median age was 26. All tumors presented as rapidly growing painful masses with a mean tumor size of 3.7 cm. Regional lymph nodes metastases were noted in all 4 patients, and 2 of them had distant metastases. One of the pediatric patients had metastasis to bone, liver, and brain. He received neoadjuvant chemotherapy followed by surgery and adjuvant radiotherapy. Despite treatment, he developed tumor recurrence and disseminated disease; subsequently died of disease at 8 months after initial presentation [15]. On the other hand, one of the long-term survivors is a Korean 29-year-old female with distant metastasis to para-aortic lymph node who underwent submandibular gland resection with modified radical neck dissection and para-aortic lymphadenectomy [16]. She received adjuvant chemotherapy (6 cycles of docetaxel and cisplatin) followed by consolidation radiotherapy and has been alive without disease for 27 months [16, 35]. Our patient underwent surgery and has received adjuvant chemotherapy (carboplatin and paclitaxel) and radiotherapy; she has been alive free of disease at seventeen weeks after initial diagnosis.

NUT carcinoma of the submandibular gland showed consistent expression for pancytokeratin and p63 in all four cases. Variable expression of neuroendocrine markers either focal or patchy for synaptophysin (2/4 cases) and chromogranin A (1/4 cases) was noted, while CD56 was negative in all cases performed (3/3 cases). Other markers tumor was negative for included SMA, desmin, CD34, AR, CD45, HMB-45, SOX-10, GFAP, GATA3, and NB84. All cases were consistently negative for EBER by ISH; and showed nuclear retention for INI-1 and BRG1 stains, when performed. All tumor cases stained for anti-NUT antibody showed positive nuclear staining, when performed (3 cases). FISH analysis was performed in the case without anti-NUT IHC which showed *NUTM1* rearrangement, confirming the diagnosis. Molecular data was available in 3 cases; one of the pediatric cases harbored *NUTM1*-variant fusion (negative for *BRD3/BRD4* rearrangement) and the other two cases

Table 2 Summary of clinicopathologic features of all reported submandibular gland NUT carcinomas

Case	Age (years)	Sex	Site	Size (cm)	Symptoms	Onset (months)	TNM stage	Treatment	Metastases/organs	Outcome	OS (months)	References
1	15	M	Left	2.5	Mass and pain	3	pT2 pN1 pM0	Surgery ◇ aRT	No	ANED	14	Ziai et al. [17]
2	29	F	Left	1.5	Mass and pain; pregnant 19 weeks	1	pT1 pN2a pM1	Surgery ◇ aRT, aCT	Yes (Portal lymph node)	ANED	27	Cho et al. [16] and Jung et al. [35]
3	11	M	Left	5.3	Rapidly growing and painful mass	2	ypT3 ypN2 ypM1	neo-aCT ◇ Surgery ◇ aRT	Yes (Liver, brain, bone)	DOD	8	Wang et al. [15]
4	49	F	Right	5.5	Rapidly growing and painful mass	2	pT3 pN3b pM0	Surgery ◇ aRT, aCT	No	ANED	4	Current report

M male, *F* female, *ANED* alive with no evidence of disease, *DOD* died of disease *a* adjuvant, *neo-a* neoadjuvant, *CT* chemotherapy, *RT* radiotherapy, *OS* overall survival

demonstrated *BRD4-NUTM1* translocation, including our current case (Table 3).

Overall, the gross features of NC are non-specific; however, invasive margins and areas of necrosis are frequently seen [14]. Cytology smears of NC are usually highly cellular with tumor arranged either in clusters or dispersed as single cells. The tumor cells are round, oval, or spindled, small to intermediate size with moderate to scant cytoplasm and round to oval nuclei [31]. Morphologically, on histologic sections, this tumor has a poorly differentiated or undifferentiated appearance and grows as nests and sheets of primitive cells that are widely infiltrative without an overlying in-situ component [36]. It shows high mitotic rate and frequent confluent necrosis [36, 37]. One clue to its diagnosis is that despite demonstrating such aggressive histologic features, the nuclei are quite monotonous, in contrast to what it is typically encountered in high-grade carcinomas [1, 37]. Abrupt keratinization (i.e., undifferentiated cells immediately adjacent to highly differentiated squamous cells), though characteristic, is only observed in 1/3rd of cases [9]. This peculiar feature is another clue to the diagnosis, but it can also be seen in HPV-associated basaloid squamous cell carcinomas [1, 37]. Our case did not show any abrupt squamous differentiation; however, focal squamous metaplasia of the intercalated and striated ducts was noted on the resection specimen. The background stroma varies from edematous, slightly myxoid to fibrous but it can also present with variable amount of desmoplastic stroma [13, 14]. Some tumors may exhibit features mimicking myoepithelial carcinoma with myxoid matrix, and rarely mesenchymal differentiation as described in one pediatric salivary gland NC [21]. Although infiltrating intraepithelial and stromal lymphocytes can occasionally be found, the presence of neutrophilic infiltrate is more common and can be prominent [13].

NUT carcinoma is presumed to have a different pathogenesis than squamous cell carcinoma (SCC), characterized by translocation-associated fusion oncoproteins that block cell differentiation. This characteristic of single chromosomal translocation resembles those found in hematopoietic and mesenchymal malignancies rather than those typically found in SCC or other epithelial cancers where multiple sequential mutations are required to progress to carcinogenesis [10]. The BRD4 protein, encoded by the *BRD4* gene, is the most extensively studied member of the BET protein family. This latter group contain bromodomains that bind to transcriptionally active chromatin and appear to affect cell cycle progression and cellular proliferation [2]. Furthermore, several in vitro studies with patient-derived tumor cells using knockdown of *BRD3/4-NUTM1* and *NSD3/NUTM1* genes have provided evidence of terminal and irreversible squamous differentiation and growth arrest [2, 10, 38]. This observation indicates that *NUTM1* fusion proteins act to maintain growth and block squamous-cell differentiation, in a mechanism

Table 3 Pathologic and molecular findings of all reported submandibular gland NUT carcinomas

Case	Histologic Pattern	Immunohistochemistry											EBER ISH	NUT	
		CK	p63	SMA	Desmin	S100	CD34	NE Markers	INI-1	BRG1	Ki-67	Other Markers		IHC	FISH
1	Poorly differentiated cohesive cells with necrosis, very focal squamous differentiation	+	+	−	NA	+ (f)	−	(−): Syn, ChA, CD56	NA	NA	> 50%	(+): CAM5.2, p16, CD117 (f) (−): Calponin	−	NA	NUTM1-variant (other than BRD4/BRD3)
2	Poorly differentiated small cells	+	+	−	NA	−	NA	(−): Syn, ChA, CD56	NA	NA	Partly +	(+): EMA, p16, vimentin, calponin (p) (−): AR, CD5, CD117	−	+	NA
3	Cohesive sheets of small blue round cells, no squamoid foci, reactive lymphocytic infiltrate	+ (p)	+ (p)	−	−	+ (p)	−	(+): Syn (p) (−): ChA, CD56	NR	NA	NA	(+): CK5/6 (p), CK19 (p), CD99, BCL-2 (−): EMA, myogenin, Melan-A, HMB-45, GFAP, NB84, CD117	−	+	NUTM1-BRD4
4	Cohesive sheets of undifferentiated cells; no foci of abrupt squamous differentiation; neutrophilic infiltrate	+ (p)	+	NA	−	−	NA	(+): Syn (p), ChA (f)	NR	NR	70%	(+): p40 (f), CK5/6 (f), CK7 (f) (−): CD45, SOX-10, CD99, AR, GATA3	−	+	NUTM1-BRD4

CK pancytokeratin, *SMA* smooth muscle actin, *NE* neuroendocrine, *INI-1* also knowns as BAF47 is a marker for SMARCB1-deficient tumors, *BRG1* marker for SMARCA4-deficient tumors, *EBER ISH* Epstein–Barr virus-encoded small RNAs in situ hybridization, *NUT* nuclear protein of testis, *IHC* immunohistochemistry, *FISH* fluorescent in situ hybridization, (+) positive, (−) negative, *f* focally, *p* patchy, *NA* not available, *NR* nuclear retention, *Syn* synaptophysin, *ChA* chromogranin A, *CPS* combined positive score, *EMA* epithelial membrane antigen, *AR* androgen receptor, *GFAP* glial fibrillary acidic protein, *NB84* neuroblastoma marker, *NUTM1* NUT gene. *BRD* bromodomain gene

dependent on the targeting of *MYC, SOX2,* and *TP63* genes by *BRD-NUT* [8, 11, 38, 39].

NUT carcinoma may be an under recognized diagnostic consideration of any poorly differentiated tumor or "small round blue cell tumor" involving the salivary gland due to its rarity and non-midline location. The entities usually considered as differential diagnoses are poorly differentiated SCC, small cell carcinoma, lymphoepithelial carcinomas, mucoepidermoid carcinoma, myoepithelial carcinoma, adamantinoma-like variant of ES, alveolar rhabdomyosarcoma, lymphoma, melanoma, thymic carcinoma, SMARCB1/INI-1- or SMARCA4/BRG1-deficient carcinoma, and undifferentiated epithelioid/round cell malignant neoplasms [4, 17, 18, 34, 36, 37, 40]. Because of its occurrence in younger patients with minimal smoking history, midline NC can masquerade germ cell tumor of the mediastinum, primitive neuroectodermal tumor/Ewing sarcoma (PNET/ES), or sinonasal undifferentiated carcinoma. IHC can be helpful in these differential diagnoses. NC is variably positive for cytokeratins, p63, p40 [41], and about half of the cases are positive for CD34[37]. Meanwhile, this tumor is usually negative for neuroendocrine markers like synaptophysin and chromogranin. Similarly, testing for HPV and EBV are consistently negative [37]. However, one should be careful with the expression of cytokeratins, EMA, and myoepithelial markers, or the lack thereof in NC with some of them showing immunoreaction for CD99 since they may overlap with myoepithelial carcinoma and PNET/ES, respectively [7, 18].

A definitive diagnosis of NC requires presence of *NUTM1* gene rearrangement, which can be confirmed by IHC with NUT specific monoclonal antibody (clone C52, Cell Signaling). The IHC stain detects the presence of NUTM1 protein and is relatively sensitive (87%) and highly specific (nearly 100%) for the diagnosis of NC [18]. In fact, diffuse (> 50%) and strong nuclear positivity for NUTM1 is considered sufficient evidence for *NUTM1* rearrangement, obviating the need for highly specialized genetic testing [18]. An alternative to NUT IHC is molecular analysis to detect rearrangement of *NUTM1* gene using FISH, reverse-transcriptase PCR, cytogenetics, or NGS-based approaches. These methods should be considered if the monoclonal antibody for NUT IHC is not available or if the result is negative or equivocal, but a high suspicion of NC persists [30]. Although not required for diagnosis, molecular techniques can be used to determine the specific NUT fusion partner which could be of potential prognostic and therapeutic significance. Recently, it has been found that the OS significantly correlates with anatomic site and *NUTM1*-fusion partner. For instance, *NSD3-* or *BRD3-NUTM1*-positive tumors of nonthoracic origin has been associated with significantly better OS than those with *BRD4-NUTM1* [9]. Up today, there is not a standard treatment for this rare and aggressive form of cancer. However, a multimodal approach with surgery,

systemic chemotherapy, and radiation therapy is currently adopted in clinical practice. Additionally, targeted therapy using small-molecule bromodomain and extra-terminal motif (BET) inhibitors (BETis) have shown activity, but no obvious survival benefit [42–45].

In summary, salivary gland NC is rare, diagnostically challenging, and probably under recognized due to overlapping histopathologic features with other poorly differentiated neoplasms. Hence, the awareness of this entity is paramount as it has been initially misdiagnosed in more than half of the reported cases. Moreover, children with salivary gland NC may represent a distinct subset with male predilection. Herewith, we reported the first case of submandibular gland NC in an adult described in the United States, being the fourth case worldwide, and emphasizing the importance to include this entity in the differential diagnosis of poorly differentiated salivary gland carcinomas, particularly in cases of poorly differentiated SCC of presumably salivary gland origin or of unknown origin.

Declarations

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