Novel Fusion \textit{KTN1-PRKD1} in Cribriform Adenocarcinoma of Salivary Glands Located in the Parotid Gland: Case Report Including Cytologic Findings

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Novel fusion KTN1-PRKD1 in cribriform adenocarcinoma of salivary glands located in the parotid gland: Case report including cytologic findings

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ABSTRACT

Background: Cribriform adenocarcinoma of salivary glands (CASG) is a rare, predominantly minor salivary gland tumor first described in 1999. Because the tumor shares morphologic and molecular features with polymorphous adenocarcinoma (PAC), in 2017, the World Health Organization (WHO) included CASG within the spectrum of PAC. Almost 75% of CASG harbor molecular alterations in the PRKD (protein kinase D) gene family, and some cases show ARID1A (AT-rich interaction domain 1A)-PRKD1 or DDX3X (DEAD-Box Helicase 3 X-Linked)-PRKD1 fusions.

Case presentation: A 39-year-old man presented with headache and painless right cheek mass of two years duration. Imaging showed a well-circumscribed, lobulated 1.7-centimeter mass located in the superficial lobe of the right parotid gland. Fine needle aspiration (FNA) of the mass revealed a “salivary gland neoplasm of uncertain malignant potential” (SUMP). Histopathology and immunohistochemical features of the resected tumor showed a primary salivary gland neoplasm with perineural invasion suggestive of cribriform adenocarcinoma of the salivary glands (CASG). Whole exome sequencing (WES) and transcriptome sequencing (RNAseq) of the tumor revealed a novel, intrachromosomal fusion: KTN1 (Kinectin1)-PRKD1. Sanger sequencing and fluorescent insitu hybridization (FISH) break apart probe results subsequently confirmed the presence of the fusion. The patient recovered from surgery without complications.

Conclusion: We report a novel fusion KTN1-PRKD1 in Cribriform Adenocarcinoma of the Salivary Glands located in the parotid gland. Importantly, this KTN1 fusion partner may account for other reports of intrachromosomal fusions in CASG in which the PRKD1 gene partner was not identified.

1. Introduction:

Cribriform adenocarcinoma of salivary glands (CASG) was first described in detail in 1999 as a distinctive salivary gland tumor limited to the tongue [1]. Subsequently, it was classified as a variant of polymorphous low-grade adenocarcinoma (PLGA) in 2005 by the WHO, as both entities share some histologic and molecular findings despite differing clinical profiles [2]. From 2005 until 2014, cribriform adenocarcinoma was reported at locations other than the tongue, and consequently the name was changed to cribriform adenocarcinoma of minor salivary glands (CAMSG) [3-5]. As familiarity with diagnostic criteria of CAMSG grew, rare cases were reported in the parotid gland. The name was then shortened to cribriform adenocarcinoma of the salivary glands (CASG). Although the clinical findings differ, the entity is still considered a type of polymorphous adenocarcinoma (low grade was dropped from its name) by the WHO. The 2017 edition of the WHO

Abbreviations: CASG, Cribriform adenocarcinoma of salivary glands; WHO, World Health Organization; PRKD, protein kinase D; ARID1A, (AT-rich interaction domain 1A); DDX3X, DEAD-Box Helicase 3 X-Linked; KTN1, Kinetin1; PLGA, Polymorphous low-grade adenocarcinoma; PAC, Polymorphous adenocarcinoma; CAMSG, Cribriform adenocarcinoma of minor salivary glands; FNA, Fine needle aspiration; STUMP, Salivary gland neoplasm of uncertain malignant potential; AJCC, American Joint Committee on Cancer; WES, Whole exome sequencing; SCFD 1, Sec1 Family Domain Containing 1; NBCI, National Center for Biotechnology Information; GRCh37, Genome Reference Consortium Human genome build 37; FFPE, Formalin-fixed paraffin embedded; PCR, Polymerase chain reaction; MAPK, Mitogen-activated protein kinase; JNK1, c-Jun N-terminal kinase1; AKT, Protein kinase B; ERK, Extracellular Signal-Regulated Kinase.

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groups CASG with polymorphous adenocarcinoma (PAC) and is considered part of the spectrum of architectural patterns that can be seen [2]. This classification may change in the future after additional evidence into this emerging entity is obtained.

CASG is histologically a low-grade malignant neoplasm exhibiting relatively indolent behavior. Incidence among primary salivary gland tumors is reported to be 0.5–1% [6]. Affected patients are adults with an approximately equal gender distribution. Patients typically present with cervical lymph node metastases. Despite this regional spread, CASG bears a favorable prognosis [7,8].

Molecular alterations of the PRKD gene family PRKD1, PRKD2, PRKD3 occur in both CASG and PAC. About 75% of PACs harbor an activating p.E710D point mutation in the PRKD1 gene, whereas 80% of CASG show gene fusions involving primarily PRKD1 but also PRKD2 and PRKD3 [8,9]. For CASG, ARID1A and DDX3X are common fusion partners of PRKD1. However, the fusion-partner genes are not always identified or reported [3,8,9].

Herein we describe a case of a novel gene fusion, KTN1-PRKD1, occurring in a cribriform adenocarcinoma located in the parotid gland of a 39-year-old man.

2. Case presentation

2.1. Clinical summary

A 39-year-old Caucasian man with a history of tobacco use and alcohol abuse presented to his family physician with a painless right cheek mass, diffuse headache, and ipsilateral hearing loss. He had noticed the mass two years previously and reported it was increasing in size. Imaging assessment by computed tomography (CT) of the head and neck revealed a well-circumscribed, lobulated 1.7-centimeter mass located in the superficial lobe of the right parotid gland (see Fig. 1). Adenopathy was absent, and additional imaging showed no evidence of metastatic disease.

Fine needle aspiration (FNA) of the mass was successfully performed. Using the Milan cytology diagnostic classification system for salivary glands, the specimen was diagnosed as a “salivary gland neoplasm of uncertain malignant potential” (SUMP).

2.2. Cytology findings

The FNA specimen from the right parotid mass was hypercellular and consisted of tightly cohesive, branching groups of monomorphic tumor cells showing nuclear overlapping and a moderate N/C ratio. Globules of matrix material adhered to fenestrated tumor structures or floated freely in the background. The cell block contained cribriform fragments as well as fragments consistent with ‘glomeruloid’ structures. At high magnification, tumor nuclei resembled the nuclei of papillary thyroid carcinoma with fine, dusty chromatin, eccentric micronucleoli and scattered nuclear grooves (see Fig. 2).

Immunohistochemical staining for c-kit (CD117) performed on the cell block showed nuclear and cytoplasmic reactivity in 80% of tumor cells. Cytologically, the differential diagnosis included adenoid cystic carcinoma, basal cell adenoma/ carcinoma and pleomorphic adenoma. Excision of the lesion was suggested for definitive characterization.

2.3. Histopathology and Immunohistochemistry

Intraoperatively, the tumor formed a single firm, ill-defined mass that was fixed within the tail of the right parotid gland. Upon resection, the unencapsulated tumor measured 2.2 cm maximally, and cut sections revealed a firm, white uniform solid interior. At low magnification, the tumor exhibited a focally infiltrative border and a mixed cribriform (20%), glomeruloid (50%) and solid (20%) architecture disposed as irregular nodules embedded in a fibrous to hyalinized stroma. The cribriform nodules featured globules of pale, blue gray myxoid material.Rare small papillae (~3% of tumor) and tubules (~5–7% of tumor) were scattered among solid nodules. Tumor cells were monomorphic with clear to pale frotthy eosinophilic cytoplasm and a medium N/C ratio. Tumor nuclei recapitulated resemblance to papillary thyroid carcinoma as noted previously on the cytology specimen with uniform features of overlapping nuclei with chromatin clearing, micronucleoli and nuclear grooving seen throughout the tumor. Mitotic figures were rare (<1/20 high magnification fields) and not atypical. Scattered psammoma bodies were also identified associated with tubules. Necrosis comprised less than 1% of tumor volume and was scattered as punctate foci. Perineural invasion was identified, but lymphovascular invasion was absent (see Fig. 3 for representative photomicrographs). Surgical margins were negative. The tumor was staged as pT2/pNX/pMX by the AJCC (American Joint Committee on Cancer) 8th Edition [10].

To further characterize the tumor, immunohistochemical (IHC) staining was performed on a Ventana Ultra automated platform (Roche Diagnostics, Indianapolis IN) with antigen retrieval using appropriate positive and negative controls and commercially available antibodies. Table 1 lists the resulting IHC profile (see Fig. 4 for representative IHC photomicrographs). The histologic features and IHC results suggested the tumor was primary to the salivary gland but not any of the entities suspected on the initial FNA biopsy exam. Therefore, additional molecular testing was performed.

2.4. Molecular testing and Results:

Whole exome sequencing (WES) and transcriptome sequencing (RNAseq) of a representative formalin-fixed paraffin embedded (FFPE) block of tumor (70% tumor cellularity with <1% necrosis) was carried out in a CLIA-certified laboratory (ASHION, Phoenix AZ). The custom xGEN target capture (Integrated DNA Technologies, Coralville IA) was used for WES. RNA libraries were prepared using KAPA HyperPrep with Riboerase (Kapa Biosystems, Wilmington, MA). Libraries were then sequenced using the Illumina HiSeq 2500 platform (Illumina, San Diego, CA). Sequence data were analyzed using GEM ExTra pipeline 4.0 (ASHION), and the NCBI (National Center for Biotechnology Information) GRC37 (Genome Reference Consortium Human genome build 37) was the reference genome assembly used for alignment. Mean coverage for WES was approximately 440x. Approximately 120 million reads were obtained for RNAseq. Aggregated results showed that the tumor harbored a novel KTN1-PRKD1 fusion. In addition, eleven other variants of unknown significance were detected including nine missense variants, a splice donor variant and an SCF-D1 (Sec1 Family Domain Containing 1)-KTN1 fusion.

Confirmatory Sanger Sequencing: Because of the diagnostic significance of the KTN1-PRKD1 fusion, we performed Sanger sequencing to

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Fig. 1. CT scan shows a 1.7 cm mass in the superficial lobe of the right parotid gland (yellow circled region, coronal view).
Fig. 2. Cytologic features on fine needle aspiration biopsy: A) The tumor consisted of complex cohesive cellular fragments (DQ, 100X); B) Twisted fragment suggesting a cribriform architecture with attached and free-floating pale metachromatic matrix globules (DQ, 400X); C) Nuclear features suggestive of PTC included chromatin clearing, eccentric micronucleoli and nuclear grooves (DQ, 600X); D) Tumor fragment with globular matrix and dense stromal fragment (Papanicolaou, 400x); E) Glomeruloid structure on cell block (H&E, 600X); F) PTC-like nuclear features on the cell block (H&E, 600X).

Fig. 3. Histologic features of excised tumor: A) The tumor comprised nodules of varying size embedded in a fibrotic to hyalinized stroma (H&E, 100X); B) Papillae were rare comprising ~3% of total tumor epithelial component (H&E, 200X); C) Glomeruloid nodule with rim of hyperchromatic cells and varying hyalinization (H&E, 400X); D) Cribriform nodule with myxoid pools (H&E, 400X); E) Psammoma bodies (H&E; 600X); F) Tumor cell nuclei with open chromatin mimicking papillary thyroid carcinoma (H&E, 600X).
alternately confirm the presence of the fusion in the tumor. RNA extracted from FFPE tumor sample was used as template for cDNA synthesis (Ampliseq™ cDNA Synthesis, Illumina, San Diego CA). The sequence of KTN1-PRKD1 fusion primers were forward primer: 5′-CAAGTTTCGTGAGCAGATGGA-3′, reverse primer: 5′-CGAAGCTGGCTTTCTTGTTT-3′. Fusion PCR (Polymerase chain reaction) was carried out using the following program: initial denature at 94°C for 5 min followed by 30 cycles of denature-annealing-extension at 94°C for 60 sec, 60°C for 40 sec, 72°C for 30 sec respectively and final extension at 72°C for 5′. The PCR fragments were purified using Exo-SapIT™ (ThermoFisher Scientific, Waltham MA) and Sanger sequenced using BigDye™ terminator V3.1 (ThermoFisher Scientific). The PCR yielded a 392 bp product, and the fusion was confirmed by Sanger sequencing (see Fig. 5).

For further confirmation of the genetic results, we performed Fluorescent in situ hybridization (FISH) on formalin fixed, paraffin-embedded tissue using dual-color, break-apart probes. The FISH pretreatment protocol from Abbott Molecular (Abbott Molecular Inc) and hybridization protocol from Empire Genomics were followed. Probe location studies were validated by metaphase chromosomes. Standard FISH quality assurance and control procedures were used in the CLIA and CAP accredited Clinical Cytogenetics Laboratory at University of Kentucky. The FISH results showed separation of the KTN1 (5′, 3′) in 45% of the tumor cells (see Fig. 6) and showed separation of the PRKD1 (3′, 5′) in 47.5% of tumor cells (see Fig. 7), thus successfully confirmed the previous results.

2.5. Clinical course

The patient recovered from surgery without complications. Subsequently, he was lost to follow-up.

3. Discussion and conclusion

To our knowledge, this is the first reported case of cribriform adenocarcinoma of the salivary gland showing a KTN1-PRKD1 fusion (about 75 cases of CASG have been reported in the literature) [8,11]. Given the relatively small number of published cases, it may be anticipated that additional novel findings, including molecular alterations, will be reported.

In fact, investigators recently have further characterized molecular alterations in the CASG-PLGA spectrum of tumors substantiating an overlap in molecular pathogenesis comparable to the histologic overlap between the two carcinomas. The molecular alterations affect the same gene family, PRKD, but predominantly show point mutations in PAC and fusions in CASG [3,4,8,9,11,12]. In CASG, tumors display fusions involving PRKD1, PRKD2, PRKD3 (PRKD1/2/3). PRKD1 hotspot mutations mainly occur in PAC, whereas fusions involving PRKD1, PRKD2, or

<table>
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<tr>
<th>Antibody</th>
<th>Reactivity in Our Patient Clone Manufacturer</th>
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<tr>
<td>Cytokeratin 7</td>
<td>Positive (90%) OV-TL 12/30 DAKO</td>
</tr>
<tr>
<td>Cytokeratin 5/6</td>
<td>Positive (60%) D5/16 B4 DAKO</td>
</tr>
<tr>
<td>p63</td>
<td>Positive (60%) DO-7 DAKO</td>
</tr>
<tr>
<td>p40</td>
<td>Negative B628 BIOCARE</td>
</tr>
<tr>
<td>Smooth Muscle Actin</td>
<td>Positive (40%) 1 A4 DAKO</td>
</tr>
<tr>
<td>S-100 protein</td>
<td>Positive (100%; nuclear and cytoplasmic) Polyclonal DAKO</td>
</tr>
<tr>
<td>CD117 (c-kit)</td>
<td>Positive (80%) Polyclonal DAKO</td>
</tr>
<tr>
<td>SOX-10</td>
<td>Positive (100%) EP 268 MARQUE</td>
</tr>
<tr>
<td>Mammaglobin</td>
<td>Negative 304-1 A5 DAKO</td>
</tr>
<tr>
<td>Beta-Catenin</td>
<td>Normal (membranous) Beta-catenin-1</td>
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Table 1 Antibody reactivity in parotid tumor.

DAKO (Agilent DAKO), Santa Clara CA; Biocare Medical, Pacheco CA; Cell Marque Corporation, Rocklin CA.

Fig. 4. Immunohistochemical stains performed on excised tumor: A) Diffuse SOX10 nuclear expression (100X) B) Diffuse Keratin 7 expression of varying intensity (200X); C) Diffuse S-100 protein with variable nuclear and cytoplasmic expression; D) Patchy smooth muscle actin expression (600X); E) Patchy p63 expression (200X); F) No tumor expression of p40 with normal salivary gland expression at right (100X).
PRKD3 occur in CASG; however, these molecular events are not exclusive, as 13% of histologically compatible CASG have point mutations, and 7% of histologically compatible PAC feature fusions [11]. Histologically indeterminate cases (sharing mixed features of PAC and CASG) also variably show PRKD1 point mutations or PRKD1/3 fusions further supporting a shared pathogenesis between PAC and CASG [8,9,11].

The novel fusion discovered in our case is a KTN1-PRKD1. KTN1 is located on chromosome 14q22 and encodes an integral membrane protein of the Kinectin protein family. Kinectin also accumulates in integrin-based adhesion complexes (IAC) [13,14]. In our patient’s tumor, the fusion occurs between exon 12 of PRKD1 and exon 9 of KTN1. As the previously reported ARID1A-PRKD1 and DDX3X-PRKD1 fusions lead to an over-expression of PRKD1 [9], the KTN1-PRKD1 fusion may also lead to PRKD1 over-expression. This is also supported by the gain-of-function point mutation (p.E710D) in PRKD1 found in 75% of PACs. Intriguingly, two other PRKD1-rearranged tumors have been described that showed “evidence of intrachromosomal rearrangement” [9]. Possibly, those two cases may also contain KTN1-PRKD1 fusions.

The protein kinase D gene (PRKD; 14q12) encodes the protein kinase D (PRKD) family of serine/threonine protein kinases. Three isoforms occur in humans: PRKD1, PRKD2 and PRKD3. PRKD1 alterations occur in colorectal, breast, esophageal, laryngeal, and numerous other cancers [9,15]. The isoforms variably interact with several signaling pathways including the MAPK8 (mitogen-activated protein kinase)/JNK1 (c-jun N-terminal kinase1), AKT (protein kinase B)/ERK (Extracellular Signal-
Regulated Kinase), NF-kappaB and RAS regulated pathways [15]. Via these pathway interactions, PRKD isoforms play significant roles in fundamental cell activities including proliferation, apoptosis, epithelial to mesenchymal transition, angiogenesis, vesicle transport through the Golgi network, invasion, metastasis, and the innate immune response [15–17]. The effects of PRKD enzyme isoforms in tumors are complex, as they can promote or suppress tumors according to cancer type and isoform type. Within a single tumor type, such as breast ductal adenocarcinoma, one isoform exerts pro-tumoral effects, while another exerts anti-tumoral effects [15]. Even the same isoform may promote or suppress tumor growth in a single cancer type, based on results of various investigations [15]. Remarkably, despite the appositional complexity of PRKD isoform effects, in-vitro and in-vivo cancer models have demonstrated that PRKD inhibitors show promise as an effective targeted therapy [15].

Importantly, given the novel KTN1 fusion partner, our case conformed to published histologic descriptions of CASG, and immunohistochemical staining results also supported the diagnosis. Key diagnostic histologic findings present in our patient’s tumor that align with original diagnostic criteria by Michal et al (and confirmed in subsequent studies by Michal and others) include: 1) glomeruloid structures (nodules with irregular ‘flagpole’ clefts and often a peripheral, palisading rim of smaller, hyperchromatic cells) and 2) nuclear features strongly reminiscent of the nuclear features of papillary thyroid carcinoma: ‘Orphan Annie’ change, chromatin clearing, eccentric micronucleoli and nuclear grooves [1,2,18]. Features consistent with classic polymorphous adenocarcinoma, including marked diversity of architectural patterns, targetoid whirling of single file tumor cells and slate blue stroma, were absent [7,18]. Psammoma bodies, found focally in our patient’s tumor, also uncommonly occur in CASG [18]. Tumors with at least 10% papillae and/or 30% cribriform pattern are associated with a worse disease-free survival [8,12]. At 3% papillae and 20% cribriform nodules, our patient’s tumor may have a lower risk for recurrence.

As with the histologic features of our patient’s tumor, the immunohistochemical profile also conformed with published descriptions [3,18]. Our patient’s tumor expressed markers of basal/myoepithelial cells, CK5/6, S-100 protein, SMA, CK7 and SOX10, and showed the distinctive p63+/p40- pattern reported as a characteristic feature of PAC including CASG [19].

Publications detailing the cytologic features of CASG are few, consisting of four, well-described cases [20,21,22]. Our patient’s cytology specimen contained cribriform structures, matrix globules and tumor cells with nuclear features suggestive of papillary thyroid carcinoma; these features were also uniformly reported in the three other cases. Additionally, our case and one of the three other cases contained glomeruloid structures [22]. Their presence in smears and minimally processed cell blocks suggests glomeruloid structures are not artifacts of processing [1,18] but instead comprise a peculiar, innate growth pattern of CASG.

Given the fact that there is morphologic overlap between PAC and CASG, this tumor would be best classified according to the WHO as polymorphous adenocarcinoma, cribriform variant, but we favor the terminology that is frequently seen throughout the literature of CASG due to the molecular findings and morphology.

In summary, we describe a case of cribriform adenocarcinoma of the salivary glands that was located unusually in the parotid gland and showed a novel fusion gene, KTN1-PRKD1. The finding of a PRKD1 gene fusion in a parotid tumor with histologic and cytologic features of cribriform adenocarcinoma further supports the rare occurrence of this tumor in major salivary glands and continues to confirm the significant role of PRKD1 fusions in CASG. Possibly, given the report of other intrachromosomal PRKD1 gene rearrangements in CASG or CASG-like tumors, KTN1 may prove to be a recurrent fusion partner with PRKD1 in cribriform adenocarcinoma of the salivary glands.

4. Patient consent statement
Only non-identifiable images and pathology slides pictures are used in this manuscript. All authors have approved the final manuscript.

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There was no external funding for this case report.

6. Authors’ contributions
All authors have made substantial contributions to the paper and they approved the final manuscript.

Declaration of Competing Interest
The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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ASHION, Phoenix, AZ, for return of residual extracted RNA.

References

[19] L. Rooper, R. Sharma, J.A. Bishop, Polymorphous low grade adenocarcinoma has a consistent p63+/p40− immunophenotype that helps distinguish it from adenoid cystic carcinoma and cellular pleomorphic adenoma, Head Neck Pathol. 9 (1) (2015) 79–84.

