Histopathology

Histopathology 2021, **79**, 306–314. DOI: 10.1111/his.14250

Papillary-cystic neoplasms of the middle ear are distinct from endolymphatic sac tumours

Cecilia Taverna,[1,2] Federica Pollastri,[3] Rudi Pecci,[3] Beatrice Giannoni,[3] Caterina Fattorini,[2] Marco Santucci,[2] Sarina K Mueller,[4] Robert Stoehr,[1] Alessandro Franchi[5] & Abbas Agaimy[1]

[1]*Institute of Pathology, Friedrich-Alexander-University Erlangen-Nürnberg, University Hospital Erlangen, Erlangen, Germany,* [2]*Division of Pathological Anatomy, Department of Health Sciences, University of Florence, Florence,* [3]*Audiology Unit, Head and Neck Oncological and Robotic Surgery, Department of Oncological and Robotic Surgery, AOU Careggi, Florence, Italy,* [4]*Department of Otorhinolaryngology, Head and Neck Surgery, Friedrich-Alexander-University Erlangen-Nürnberg, University Hospital Erlangen, Erlangen, Germany, and* [5]*Department of Translational Research, University of Pisa, School of Medicine, Pisa, Italy*

Date of submission 3 March 2020
Accepted for publication 9 September 2020
Published online *Article Accepted* 9 November 2020

Taverna C, Pollastri F, Pecci R, Giannoni B, Fattorini C, Santucci M, Mueller S K, Stoehr R, Franchi A & Agaimy A (2021) *Histopathology* **79**, 306–314. https://doi.org/10.1111/his.14250

Papillary-cystic neoplasms of the middle ear are distinct from endolymphatic sac tumours

Aims: Papillary neoplasms of the middle and inner ear are rare and poorly characterised. The current World Health Organization classification divides them into two major subtypes: aggressive papillary tumours (APTs) and endolymphatic sac tumours (ELSTs). The aim of this article is to present two papillary neoplasms of the middle ear that do not fit into either the classic APT category or the classic ELST category, and compare them with three ELSTs.
Methods and results: The patients were a 48-year-old female and a 59-year-old male without a history of other neoplasms. Histology showed papillary-cystic growth of predominantly oncocytic (Case 1) or mucinous (Case 2) cells surrounded by a p63-positive basal layer. The overall histology was reminiscent of oncocytic sinonasal papilloma (Case 1) and pancreatobiliary or salivary intraductal papillary mucinous neoplasms (Case 2). Ovarian-type stroma, invasion and malignant features were absent. Immunohistochemistry revealed expression of cytokeratin (CK) 7, but not carbonic anhydrase IX (CAIX) or paired box gene 8 (PAX8) (except for very focal PAX8 expression in Case 1). The TST15 gene panel and *HRAS* sequencing revealed no pathogenic mutations in *BRAF*, *KRAS*, *EGFR*, *AKT1*, or *HRAS*. The TruSight RNA fusion panel revealed an *MKRN1–BRAF* fusion in Case 1. No fusion was detected in Case 2. The three ELSTs showed classic features of the entity, expressed CK7, epithelial membrane antigen, PAX8, and CAIX, and lacked a basal cell layer.
Conclusion: These novel cases suggest that papillary tumours of the ear represent a heterogeneous spectrum of distinct neoplasms unified by a prominent papillary-cystic pattern rather than a single entity. Future studies should clarify whether the *MKRN1–BRAF* fusion is a defining recurrent driver event, especially in those cases reported as sinonasal-type middle ear papillomas.

Keywords: aggressive papillary tumour, BRAF, ear, endolymphatic sac tumour, IPMN, oncocytic, papillary mucinous neoplasms, Schneiderian papilloma

Introduction

Papillary neoplasms of the middle and inner ear are rare. Because of their overlapping clinicopathological

Address for correspondence: A Agaimy, MD, Pathologisches Institut, Universitätsklinikum Erlangen, Krankenhausstrasse 8–10, 91054 Erlangen, Germany. e-mail: abbas.agaimy@uk-erlangen.de

and imaging characteristics, they are included in a single chapter in the 2017 4th edition of the World Health Organization (WHO) classification of head and neck tumours.[1,2] The WHO classification defines two categories of papillary tumour: aggressive papillary tumours (APTs)[1] and endolymphatic sac tumours (ELSTs).[2] Both types are locally aggressive low-grade lesions, composed of a single or double layer of bland columnar to cuboidal cells arranged in a papillary architecture. Immunohistochemistry (IHC) shows expression of pan-cytokeratin (CK), CK7, epithelial membrane antigen (EMA) (100% of cases), and paired box gene 8 (PAX8) (85% of cases), and negative staining for CDX2, CK20, and S100.[1–4] The diagnosis is based on a set of clinicopathological features and the exclusion of metastasis from more common papillary tumours of thyroid, pulmonary, renal and gastrointestinal origin.[1–4]

Some APTs and ELSTs are associated with von Hippel–Lindau (vHL) disease.[1,2,5–8] Germline *VHL* mutations are detectable in 39% of apparently sporadic cases, indicating initial manifestation of the syndrome.[8] In addition to molecular homology (both are predominantly driven by *VHL* defects), ELST and clear cell renal cell carcinoma express PAX8 and CAIX.[4]

We herein describe two rare variants of papillary middle ear tumour, one of them resembling oncocytic sinonasal papillomas and another one composed entirely of mucinous cells lacking ovarian-type stroma, mimicking salivary intraductal papillary mucinous neoplasms (IPMNs).[9] For comparison, three ELSTs are included. In addition to performing clinicopathological studies, we investigated the non-ELST tumours for mutations and gene fusions that are commonly encountered in comparable salivary or sinonasal tumours.

Materials and methods

The five cases were identified in our routine and consultation files. The clinicopathological features are summarised in Table 1. One ELST has been previously reported.[7] Samples were used in accordance with ethical guidelines for the use of retrospective tissue samples provided by the local ethics committee of the Friedrich-Alexander University Erlangen-Nuremberg (ethics committee statements 24 January 2005 and 18 January 2012). IHC was performed on 3-µm sections cut from paraffin blocks with a fully automated system (Benchmark XT System; Ventana Medical Systems, Tucson, AZ, USA). Details of antibodies are summarised in Table 2.

MOLECULAR STUDIES

Tumour DNA isolation from formalin-fixed paraffin-embedded (FFPE) tumour tissue, amplicon-based massive parallel sequencing with the TST15 panel (Illumina, San Diego, CA, USA) and *HRAS* hotspot (codons 12, 13, and 61) mutation analysis were performed as described previously.[10,11]

RNA isolated from FFPE tissue was analysed by use of the TruSight RNA Fusion panel (Illumina) as described previously.[12]

Fluorescence *in-situ* hybridisation (FISH) was performed with the ZytoLight SPEC BRAF Dual Color Break Apart Probe, which is designed to detect

Table 1. Clinicopathological data of endolymphatic sac tumours (ELSTs) and non-ELST papillary neoplasms

Case	Age (years)/sex	Site	Size (mm)	Histological pattern/diagnosis	Treatment	Follow-up (months)
Papillary middle ear tumour: Case 1	48/F	Tympanic cavity	10 × 8	Oncocytic-type papillary tumour	Complete surgical excision	NED (56)
Papillary middle ear tumour: Case 2	59/M	Mastoid and middle ear	16 × 105	Mucinous-type papillary tumour	Complete surgical excision and secondary petrosectomy for recurrence of disease	NED (25)
ELST: Case 1	39/M	Endolymphatic sac	18 × 8	ELST	Complete surgical excision	Two recurrences: NED (36)
ELST: Case 2	27/F	Petrous bone	28 × 14	ELST	Complete surgical excision	NED (15)
ELST: Case 3	69/M	Endolymphatic sac	17 × 10	ELST	–	NED (5)

F, Female; M, Male; NED, No evidence of disease.

Table 2. Antibodies employed in the immunohistochemical study

Antibody	Clone and provider	Species and dilution	Antigen retrieval
CK7	OV-TL; Biogenex, Fremont, CA, USA	Mouse, 1:1000	CC1
p63	SS16; DCS, Hamburg, Germany	Mouse, 1:100	CC1
S100	Polyclonal; Dako, Glostrup, Denmark	Mouse, 1:2500	CC1
AR	AR441; Dako, Glostrup, Denmark	Mouse, 1:50	CC1
MUC1/EMA	E29; Dako, Glostrup, Denmark	Mouse, 1:20	CC1
MUC2	CCP58; Dako, Glostrup, Denmark	Mouse, 1:50	CC1
MUC4	8G7; Santa Cruz, Heidelberg, Germany	Mouse, 1:500	CC1
MUC5AC	MRQ19; CellMarque, Rocklin CA, USA	Mouse, 1:200	CC1
MUC6	MRQ20; CellMarque, Rocklin, CA, USA	Mouse, 1:200	CC1
CK20	Ks20.8; Dako, Glostrup, Denmark	Mouse, 1:50	CC1
CDX2	CDX2-88; DCS, Hamburg, Germany	Mouse, 1:50	CC1
PAX8	Polyclonal; CellMarque, Rocklin, CA, USA	Mouse, 1:50	CC1
TTF1	8G7G3/1; Zytomed Systems, Berlin, Germany	Mouse, 1:500	CC1
CAIX	Polyclonal; Abcam, Cambridge, UK	Mouse, 1:1000	CC1
EGFR	3C6; Vantana Roche, Monza, Italy	Mouse, prediluted	CC1

AR, Androgen receptor; CAIX, Carbonic anhydrase IX; CK, Cytokeratin; EGFR, Epidermal growth factor receptor; EMA, Epithelial membrane antigen; MUC, Mucin; PAX8, Paired box gene 8; TTF1, Thyroid transcription factor 1.

rearrangements involving the 7q34 locus harbouring *BRAF* (ZytoVision, Bremerhaven, Germany). Amplification of the epidermal growth factor receptor (EGFR) gene (at 7p11.2) was assessed with a ZytoLight SPEC EGFR/CEN 7 Dual Color Probe (ZytoVision). All assays were performed according to the manufacturer's instructions.

Results

CLINICAL FEATURES OF PAPILLARY-CYSTIC NEOPLASMS OF THE MIDDLE EAR

Case 1

A 48-year-old woman presented with chronic catarrhal otitis and a persistent sensation of a plugged right ear. The audiogram showed conductive hearing loss of 20–30 dB and a type B tympanogram. On clinical examination, the tympanic membrane appeared to be everted. At surgery, mucinous material mixed with granulation tissue was found in the tympanic cavity. The patient had no previous or concurrent neoplasms. The patient was free of disease at last follow-up (56 months). Genetic workup revealed no evidence of vHL disease.

Case 2

A 59-year-old man presented with a 4-month history of left facial nerve palsy (House Brackmann grade IV–V[13]) and a 2-month history of otorrhea associated with microperforation of the tympanic membrane. He had no history of other malignancies or evidence of another primary tumour. The audiogram showed conductive hearing loss of 40 dB, and a type B tympanogram. Preoperative computed tomography (CT) revealed diffuse opacity of the mastoid bone. The patient underwent canal wall down mastoidectomy. Mucinous material was found in mastoid cells and in the middle ear without temporal bone erosion or invasion of the middle ear structures. Positron emission tomography examination 4 months later showed limited persistent disease in the temporal bone, justifying subtotal petrosectomy. There was no evidence of recurrence at last follow-up (25 months). Genetic workup revealed no evidence of vHL disease.

PATHOLOGICAL FINDINGS OF PAPILLARY-CYSTIC NEOPLASMS OF THE MIDDLE EAR

Case 1 showed exophytic and endophytic growth of linear and complex branching papillae covered by multiple layers of columnar cells with abundant eosinophilic granular cytoplasm and vesicular to hyperchromatic nuclei. Scattered mucinous cells with basally oriented nuclei were seen. There were intraepithelial microcysts filled with mucinous material and neutrophils reminiscent of sinonasal papilloma. Periodic acid–Schiff with diastase predigestion (PAS-D) stain confirmed intracytoplasmic mucin in the scattered mucinous cells. Immunohistochemically, both cell types expressed CK7 and were negative for CDX2, CK20, S100, thyroid transcription factor 1 (TTF1), mucin (MUC) 2, MUC6, carbonic anhydrase IX (CAIX), EGFR, and androgen receptor (AR). The oncocytic component stained focally for PAX8, MUC1, and MUC4, whereas only a few scattered mucinous cells were positive for these markers. MUC5AC stained the mucinous but not the oncocytic cells. Representative images are shown in Figure 1.

Case 2 showed prominent papillae lined by a single, partially pseudostratified layer of tall columnar epithelial cells with hyperchromatic, basally oriented nuclei and pale eosinophilic to clear mucin-containing apical cytoplasm (PAS-D). A small cholesteatoma was seen at the periphery. The neoplastic cells diffusely expressed CK7 and EGFR, and were negative for S100, AR, PAX8, CAIX, CDX2, CK20, and TTF1. p63 staining demonstrated an incomplete basal layer. MUC1, MUC2, MUC4, MUC5AC and MUC6 were immunohistochemically negative. The immunohistochemical results are summarised in Table 3, and representative histological and immunohistochemical images are shown in Figure 2.

MOLECULAR FINDINGS OF PAPILLARY-CYSTIC NEOPLASMS OF THE MIDDLE EAR

No mutations were detected in *KRAS*, *AKT1*, or *EGFR*, which are often mutated, respectively, in pancreatic IPMN[14,15] and oncocytic and inverted sinonasal papillomas,[16] salivary gland IPMN,[17] and papillary lesions of the ear in a murine model.[18] Also, no *HRAS* mutations were found.[9]

The next-generation sequencing (NGS) RNA fusion analysis revealed an *MKRN1–BRAF* fusion in Case 1, in which exons 1–3 (NM_013446) of *MKRN1* were fused to exon 10 (NM_001354609) of *BRAF* (Figure 3). Case 2 showed no gene fusion in any of the

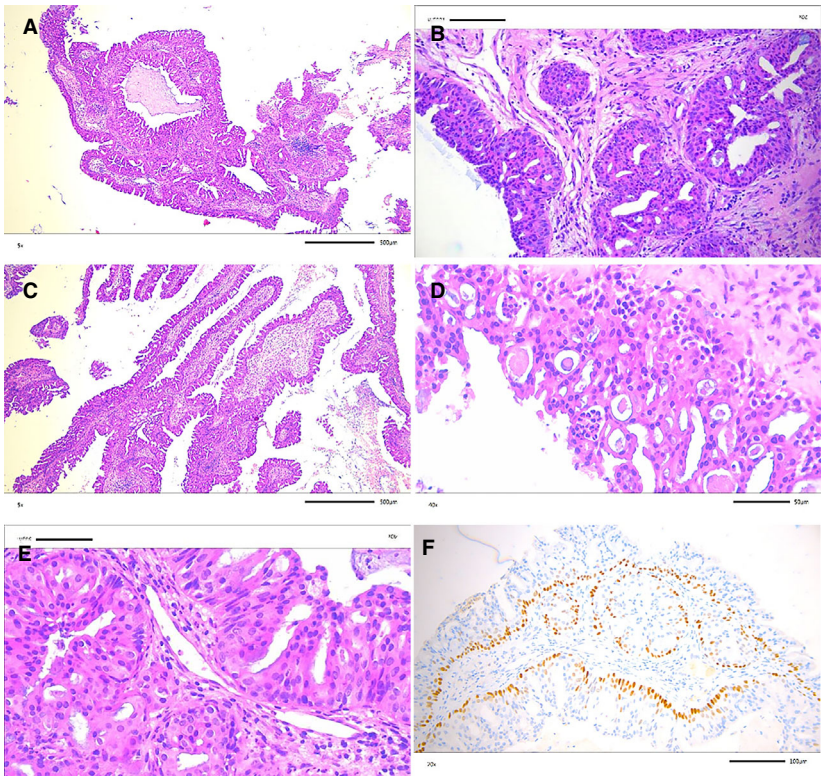

Figure 1. Case 1 of the non-endolymphatic sac tumour papillary tumours (predominantly oncocytic variant) was composed of an admixture of simple and complex branching papillae, and pseudocribriform aggregates of predominantly oncocytic cells with scattered mucinous elements (**A**,**B**). A prominent villous pattern was seen focally (**C**). Microlumina reminiscent of sinonasal papilloma are seen (**D**). At higher magnification, the oncocytic pattern is seen; note the lack of significant atypia or mitotic activity (**E**). A continuous basal layer is highlighted by p63 immunostaining (**F**).

Table 3. Immunohistochemical results

Antibody	Papillary cystic tumour: Case 1	Papillary cystic tumour: Case 2	ELST: Case 1	ELST: Case 2	ELST: Case 3
CK7	+	+	+	+	+
p63	+ Basal	+ Basal	–	–	–
S100	–	–	–	–	–
AR	–	–	–	–	–
MUC1/EMA	Focal in oncocytic cells	–	+	+	+
MUC2	–	–	–	–	–
MUC4	Focal in oncocytic cells	–	–	–	–
MUC5AC	Focal in mucinous cells	–	–	Focal	Focal
MUC6	–	–	–	–	Focal
CK20	–	–	–	–	–
CDX2	–	–	–	–	–
PAX8	Focal	–	+	+	+
TTF1	–	–	–	–	–
CAIX	–	–	+	+	+
EGFR	–	+	+	+	+

AR, Androgen receptor; CAIX, Carbonic anhydrase IX; CK, Cytokeratin; ELST, Endolymphatic sac tumour; EGFR, Epidermal growth factor receptor; EMA, Epithelial membrane antigen; MUC, Mucin; PAX8, Paired box gene 8; TTF1, Thyroid transcription factor 1.

507 genes included in the panel. FISH testing revealed no *BRAF* translocation signals. FISH showed no *EGFR* amplification in either of the two cases.

CLINICAL FEATURES OF ELSTS

Case 1

A 39-year-old male presented with vertigo in 2009 and hearing loss in 2012. Magnetic resonance tomography (MRT) showed a lesion within the posterior semicircular canal measuring 18 × 8 mm. Transmastoidal excision was performed, and a diagnosis of ELST was made. The patient underwent surgical excision of two recurrences (2014 to 2017). Since then, he has remained free of disease until now. Genetic counselling ruled out vHL syndrome.

Case 2

A 27-year-old woman presented in 2012 with right-sided tinnitus and hearing loss, followed by occasional vertigo 3 years later. In 2018, CT showed a petrous bone lesion, measuring 28 × 14 mm. Surgical excision and histopathology confirmed ELST. The patient remained disease-free 15 months after surgery. There was no clinical evidence of vHL disease.

Case 3

A 69-year-old male with a 30-year history of hearing loss in the right ear presented with right post-auricular swelling in 2018. MRT showed an inner ear lesion invading the internal acoustic meatus and cerebellum, measuring 17 × 10 mm. Surgical excision was performed, and ELST was confirmed. The patient had no recurrence at 5 months. There was no clinical evidence of vHL disease.

PATHOLOGICAL FINDINGS OF ELSTS

The three ELSTs showed similar histological and immunohistochemical findings. They were composed of simple papillae with oedematous fibrovascular cores covered by a single or double layer of bland columnar to cuboidal cells with eosinophilic to clear cytoplasm and central small, round nuclei. Atypia and mitoses were absent. The cells expressed CK7, PAX8, CAIX, EMA (MUC1), and EGFR, and were negative for CDX2, CK20, S100, TTF1, AR, and p63. Mucin IHC revealed focal apical positivity for MUC5AC in Cases 2 and 3, and for MUC6 in Case 3. No p63-positive basal cell layer was noted. The immunohistochemical results are summarised in

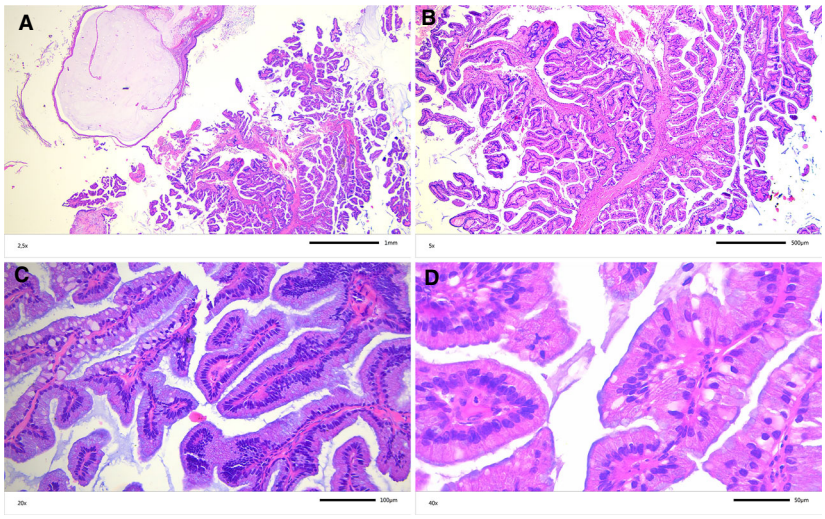

Figure 2. Case 2 of the non-endolymphatic sac tumour papillary tumours (purely mucinous variant) was composed of an admixture of complex branching papillary proliferations (**A**,**B**); note cholesteatoma at the upper left in (**A**). At higher magnification, the neoplastic cells are large and columnar, and are arranged in a single layer or pseudostratified layers with basally oriented nuclei and mucinous apical cytoplasm closely mimicking gastric foveolar (**C**, right) or intestinal (**C**, left) epithelia. Atypia and mitoses are lacking (**D**).

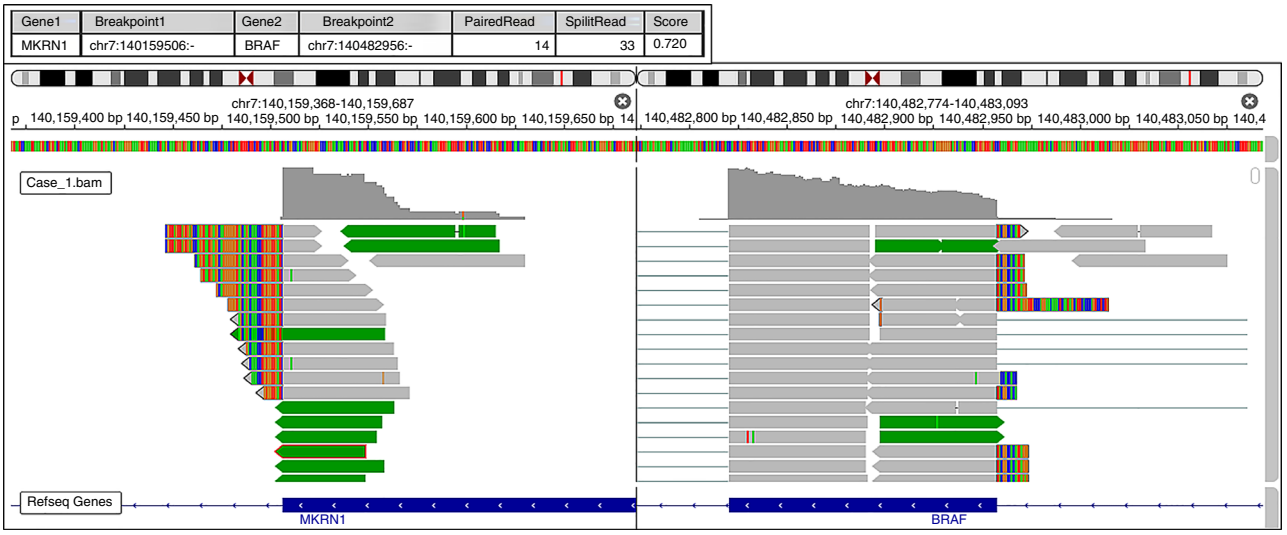

Figure 3. Integrated Genome Viewer split-screen view of read alignments of the identified *MKRN1–BRAF* fusion event in case 1. Shown are the breakpoints in the *MKRN1* locus (left) and the *BRAF* locus (right), respectively. Alignments whose mate pairs are mapped to the fusion sequence on the other chromosome are colour-coded. The green-coloured alignments on the left side and on the right side are mate pairs, illustrating the fusion event. The multicoloured alignments are split reads. All other alignments are coloured grey. [Colour figure can be viewed at wileyonlinelibrary.com]

Table 3, and representative images are shown in Figure 4.

Discussion

The histogenesis and nomenclature of papillary tumours of the middle and inner ear have been controversial. In 1988, Gaffey *et al.*[19] reported 10 locally aggressive papillary middle ear tumours, and this was followed by single cases and small series.[20–28] An endolymphatic sac origin was suggested by Michaels *et al.*[25] in 1987, and verified in a large series ($n = 20$) by Heffner[29] in 1990. The endolymphatic sac origin was supported by others.[30]

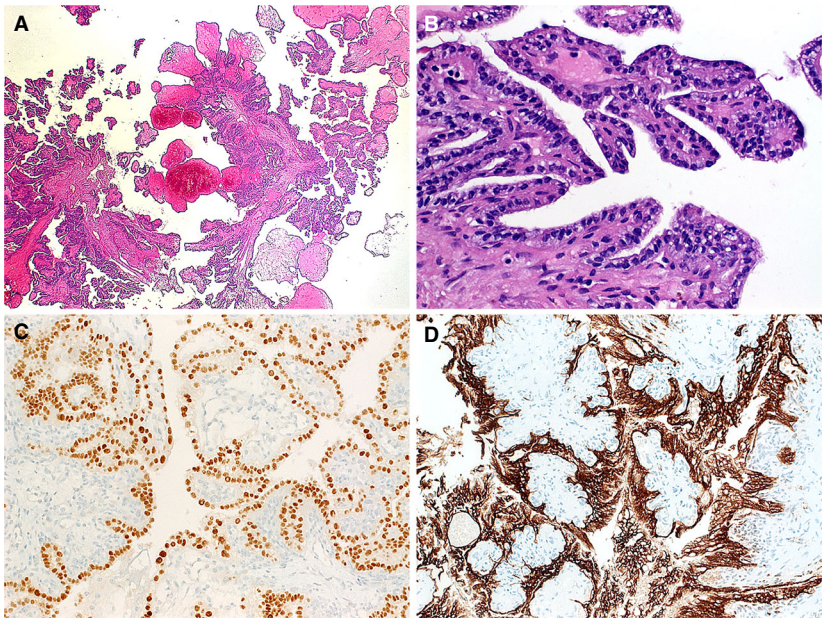

Figure 4. An example of classic endolymphatic sac tumour showing prominent branching and variably oedematous papillae (**A**) covered by low-columnar to cuboidal monomorphic cells lacking mucinous features or a basal layer (**B**). The neoplastic cells are homogeneously positive for paired box gene 8 (PAX8) (**C**) and carbonic anhydrase IX (CAIX) (**D**). [Colour figure can be viewed at wileyonlinelibrary.com]

Subsequent reports, however, generated a continuous debate regarding the origin and histogenesis of APTs.[31–33] A subset of cases were confined to the middle ear, suggesting a dual histogenetic theory: endolymphatic sac and middle ear mucosa.[34]

Our two cases showed morphological and immunohistochemical differences from classic APT and ELST, but they also differed from each other. Case 1 showed histological and immunohistochemical features (the presence of a p63-positive basal layer and the lack of homogeneous CAIX and PAX8 reactivity) that are not seen in classic ELST or APT. Instead, its morphology was reminiscent of oncocytic IPMN of the pancreas and oncocytic sinonasal papilloma.[14–16,35–37]

The majority of previously reported sinonasal-type papillomas of the middle ear/temporal bone represented either genuine squamous papillomas/papillary squamous carcinomas[38] or extension from sinonasal papillomas,[39] or lacked histological illustration. Very few cases seem to be identical to our Case 1, including most of the cases reported by Wenig[40] (illustrated in Figures 1 and 4 of that article) and three case reports.[41–43]

The lack of *EGFR* and *KRAS* mutations in our Case 1, despite its histological similarity to oncocytic sinonasal papillomas, suggests a distinct molecular pathogenesis.[16] We detected an *MKRN1–BRAF* fusion in this case. The makorin ring finger protein 1 (MKRN1) encoded by *MKRN1* is a co-regulator of transcription that controls cell cycle arrest and apoptosis.[44] Both *MKRN1* and *BRAF* have been mapped to chromosome 7q34. The *MKRN1–BRAF* fusion is rare, and has been reported only recently in two papillary thyroid carcinomas,[45,46] an anaplastic thyroid carcinoma cell line,[47] and *EGFR*-mutant metastatic non-small-cell lung carcinoma resistant to osimertinib.[48] Its fusion product probably leads to constitutive activation of the kinase.[47] Although sufficient tumour tissue was not available for validation studies, the negative FISH result can be explained by the close proximity of the two genes on chromosome 7q34, which makes the FISH method suboptimal for detection of the translocation. The absence of additional genetic alterations in this tumour suggests *MKRN1–BRAF* fusion as its driver.

Case 2 has similarities to gastric-type pancreatic IPMN and the recently described minor salivary gland IPMN.[17] Ovarian-type stroma was absent, ruling out mixed epithelial and stromal tumour, of which only a single case has been reported in the middle ear.[49] Furthermore, the uniformly mucinous-type epithelium and lack of AR and S100 immunoreactivity exclude salivary intraductal carcinoma of the apocrine and the intercalated duct type, respectively.[50] Also, the lack of *NCOA4–RET* and *TRIM27–RET* fusions as shown by NGS represents another argument against this possibility.[50]

Pancreatic and salivary IPMNs harbour *KRAS* and *AKT1* mutations, respectively.[14–16] The *AKT1* p.Glu17Lys activating mutation was reported in three of three[17] and eight of nine[9] minor salivary gland IPMNs. A subset of salivary papillary lesions harbour

HRAS mutations.[9] Our cases tested negative for mutations in all of these genes, suggesting a different molecular pathogenesis.

The development of APTs of the middle ear (similar to Case 2) in mouse models harbouring mutant *EGFR* suggested a role for EGFR.[18] Studies on human APTs and ELST, however, found no *EGFR* mutations, suggesting different mechanisms.[18]

In conclusion, this study highlights two putative novel variants in the spectrum of papillary middle ear neoplasms: one variant overlapping with oncocytic sinonasal papilloma, but lacking *KRAS* mutations and, instead, harbouring a *MKRN1–BRAF* fusion; and another closely mimicking salivary IPMN but lacking *AKT1/HRAS* mutations. These tumours should be separated from ELSTs. Their relationship to APTs of the middle ear remains to be addressed in future studies.

Acknowledgement

Open access funding enabled and organized by Projekt DEAL.

Conflicts of interest

The authors state that they have no conflicts of interest. No funding was received.

Author contributions

Study conception and design: C. Taverna, A. Franchi, and A. Agaimy. Data collection and interpretation: C. Taverna, A. Franchi, F. Pollastri, R. Pecci, B. Giannoni, C. Fattorini, M. Santucci, S. K. Mueller, R. Stoehr, and A. Agaimy. Drafting of the manuscript: C. Taverna and A. Agaimy. Discussion of results, critical reading of the manuscript, intellectual editing and comments, and approval of the manuscript: C. Taverna, F. Pollastri, R. Pecci, B. Giannoni, C. Fattorini, M. Santucci, S. K. Mueller, R. Stoehr, A. Franchi, and A. Agaimy.

References

1. Sandison A. Tumors of the middle and inner ear: aggressive papillary tumor. In El-Naggar AK, Chan JKC, Grandis JR, Takata T, Slootweg PJ eds. *World Health Organization classification of head and neck tumours*. 4th ed. Lyon: IARC Press, 2017; 266–267.
2. Sandison A. Tumors of the middle and inner ear: endolymphatic sac tumor. In El-Naggar AK, Chan JKC, Grandis JR, Takata T, Slootweg PJ eds. *World Health Organization classification of head and neck tumours*. 4th ed. Lyon: IARC Press, 2017; 266–267.
3. Duderstadt M, Förster C, Welkoborsky HJ, Ostertag H. Adenomatous tumors of the middle ear and temporal bone: clinical, morphological and tumor biological characteristics of challenging neoplastic lesions. *Eur. Arch. Otorhinolaryngol.* 2012; **269**; 823–831.
4. Thompson LDR, Magliocca KR, Andreasen S *et al.* CAIX and pax-8 commonly immunoreactive in endolymphatic sac tumors: a clinicopathologic study of 26 cases with differential considerations for metastatic renal cell carcinoma in von Hippel-Lindau patients. *Head Neck Pathol.* 2019; **13**; 355–363.
5. Choo D, Shotland L, Mastroianni M *et al.* Endolymphatic sac tumors in von Hippel-Lindau disease. *J. Neurosurg.* 2004; **100**; 480–487.
6. Gaffey MJ, Mills SE, Boyd JC. Aggressive papillary tumor of middle ear/temporal bone and adnexal papillary cystadenoma. Manifestations of von Hippel-Lindau disease. *Am. J. Surg. Pathol.* 1994; **18**; 1254–1260.
7. Künzel J, Agaimy A, Hornung J *et al.* Sporadic endolymphatic sac tumor—a diagnostic and therapeutic challenge. *Int. J. Clin. Exp. Pathol.* 2014; **7**; 2641–2646.
8. Bausch B, Wellner U, Peyre M *et al.* International Endolymphatic Sac Tumor (ELST) Consortium. Characterization of endolymphatic sac tumors and von Hippel-Lindau disease in the International Endolymphatic Sac Tumor Registry. *Head Neck* 2016; **38**(Suppl. 1); E673–E679.
9. Nakaguro M, Urano M, Ogawa I *et al.* Histopathological evaluation of minor salivary gland papillary–cystic tumours: focus on genetic alterations in sialadenoma papilliferum and intraductal papillary mucinous neoplasm. *Histopathology* 2020; **76**; 411–422.
10. Maisch S, Mueller SK, Traxdorf M *et al.* Sinonasal papillomas: a single centre experience on 137 cases with emphasis on malignant transformation and EGFR/KRAS status in 'carcinoma ex papilloma'. *Ann. Diagn. Pathol.* 2020; **46**; 151504.
11. Kompier LC, Lurkin I, van der Aa MN, van Rhijn BW, van der Kwast TH, Zwarthoff EC. FGFR3, HRAS, KRAS, NRAS and PIK3CA mutations in bladder cancer and their potential as biomarkers for surveillance and therapy. *PLoS One* 2010; **5**; e13821.
12. Agaimy A, Tögel L, Haller F, Zenk J, Hornung J, Märkl B. YAP1-NUTM1 gene fusion in porocarcinoma of the external auditory canal. *Head Neck Pathol.* 2020. E-pub ahead of print, 20 May. https://doi.org/10.1007/s12105-020-01173-9
13. House JW, Brackmann DE. Facial nerve grading system. *Otolaryngol. Head Neck Surg.* 1985; **93**; 146–147.
14. Chang XY, Wu Y, Li Y, Wang J, Chen J. Intraductal papillary mucinous neoplasms of the pancreas: clinical association with KRAS. *Mol. Med. Rep.* 2018; **17**; 8061–8068.
15. Wu JX, Arcega R, Donahue TR, Tomlinson JS, Reber HA, Hines J. Pancreatic oncocytic intraductal papillary mucinous neoplasms: three case reports and review of cases in literature. *J. Pancreas* 2015; **16**; 626–632.
16. Udager AM, McHugh JB, Betz BL *et al.* Activating KRAS mutations are characteristic of oncocytic sinonasal papilloma and associated sinonasal squamous cell carcinoma. *J. Pathol.* 2016; **239**; 394–398.
17. Agaimy A, Mueller SK, Bumm K *et al.* Intraductal papillary mucinous neoplasms of minor salivary glands with AKT1 p.Glu17Lys mutation. *Am. J. Surg. Pathol.* 2018; **42**; 1076–1082.

18. Kawabata S, Hollander MC, Munasinghe JP *et al*. Epidermal growth factor receptor as a novel molecular target for aggressive papillary tumors in the middle ear and temporal bone. *Oncotarget* 2015; **6**; 11357–11368.
19. Gaffey MJ, Mills SE, Fechner RE, Intemann SR, Wick MR. Aggressive papillary middle-ear tumor. A clinicopathologic entity distinct from middle-ear adenoma. *Am. J. Surg. Pathol.* 1988; **12**; 790–797.
20. Benecke JE, Noel FL, Carberry JN, House JW, Patterson M. Adenomatous tumors of the middle ear and mastoid. *Am. J. Otol.* 1990; **11**; 20–26.
21. Cilluffo JM, Harner SG, Miller RH. Intracranial ceruminous gland adenocarcinoma. *J. Neurosurg.* 1981; **55**; 952–956.
22. Pallanch JF, Weiland LH, McDonald TJ, Facer GW, Harner SG. Adenocarcinoma and adenoma of the middle ear. *Laryngoscope* 1982; **92**; 47–54.
23. Schuller DE, Conley JJ, Goodman JH *et al*. Primary adenocarcinoma of the middle ear. *Otolaryngol. Head Neck Surg.* 1983; **91**; 280–283.
24. Robson AK, Eveson JW, Smith IM, Maw AR. Papillary adenocarcinoma of the middle ear. *J. Laryngol. Otol.* 1990; **104**; 91–96.
25. Michaels L. *Ear, nose and throat pathology*. London: Springer-Verlag, 1987; 124.
26. Gussen R. Meniere's disease: new temporal bone findings in two cases. *Laryngoscope* 1971; **81**; 1695–1707.
27. Schuknecht HF. *Pathology of the ear*. Cambridge, MA: Harvard University Press, 1974; 444.
28. Hassard AD, Boudreau SF, Cron CC. Adenoma of the endolymphatic sac. *J. Otolaryngol.* 1984; **13**; 213–216.
29. Heffner DK. Low-grade adenocarcinoma of probable endolymphatic sac origin: a clinicopathologic study of 20 cases. *Cancer* 1990; **64**; 292–302.
30. Megerian CA, McKenna MJ, Nuss RC *et al*. Endolymphatic sac tumors: histologic confirmation, clinical characterization, and implication in von Hippel-Lindau disease. *Laryngoscope* 1995; **105**; 801–808.
31. Heffner DK. Are papillary adenomas endolymphatic sac tumors? *Ann. Otol. Rhinol. Laryngol.* 1996; **105**; 251–252.
32. Lavoie M, Morency RM. Low-grade papillary adenomatous tumors of the temporal bone: report of two cases and review of the literature. *Mod. Pathol.* 1995; **8**; 603–608.
33. Wenig BM, Heffner DK. Endolymphatic sac tumors: fact or fiction? *Adv. Anat. Pathol.* 1996; **3**; 378–387.
34. Tysome JR, Harcourt J, Patel MC, Sandison A, Michaels L. Aggressive papillary tumor of the middle ear: a true entity or an endolymphatic sac neoplasm? *Ear Nose Throat J.* 2008; **87**; 378–393.
35. Hunt JL, Chiosea S, Sarioglu S. Sinonasal papilloma, oncocytic type. In El-Naggar AK, Chan JKC, Grandis JR, Takata T, Slootweg PJ eds. *World Health Organization classification of head and neck tumours*. 4th ed. Lyon: IARC Press, 2017.
36. Karligkiotis A, Bignami M, Terranova P *et al*. Oncocytic Schneiderian papillomas: clinical behavior and outcomes of the endoscopic endonasal approach in 33 cases. *Head Neck* 2014; **36**; 624–630.
37. Barnes L, Benedetti C. Oncocytic Schneiderian papilloma: a reappraisal of cylindrical cell papilloma of the sinonasal tract. *Hum. Pathol.* 1984; **15**; 344–351.
38. Stone DM, Berktold RE, Ranganathan C, Wiet RJ. Inverted papilloma of the middle ear and mastoid. *Otolaryngol. Head Neck Surg.* 1987; **97**; 416–418.
39. Haywood EB, Fuller C, Hill GW 3rd, Olobatuyi F, Clark DW. Multifocal sinonasal inverted papilloma with middle ear involvement. *Proc. (Bayl. Univ. Med. Cent.)* 2017; **30**; 457–458.
40. Wenig BM. Schneiderian-type mucosal papillomas of the middle ear and mastoid. *Ann. Otol. Rhinol. Laryngol.* 1996; **105**; 226–233.
41. De Filippis C, Marioni G, Tregnaghi A, Marino F, Gaio E, Staffieri A. Primary inverted papilloma of the middle ear and mastoid. *Otol. Neurotol.* 2002; **23**; 555–559.
42. Inoue R, Kanazawa T, Morita M, Iino Y, Yamada S, Ishida T. Inverted papilloma of the middle ear. *Otol. Neurotol.* 2010; **32**; e7–e8.
43. Van der Putten L, Bloemena E, Merkus P, Hensen EF. Schneiderian papilloma of the temporal bone. *BMJ Case Rep.* 2013; **2013**; bcr2013201219.
44. Lee EW, Lee MS, Camus S *et al*. Differential regulation of p53 and p21 by MKRN1 E3 ligase controls cell cycle arrest and apoptosis. *EMBO J.* 2009; **28**; 2100–2113.
45. Cancer Genome Atlas Research Network. Integrated genomic characterization of papillary thyroid carcinoma. *Cell* 2014; **159**; 676–690.
46. Pfeifer A, Rusinek D, Żebracka-Gala J *et al*. Novel *TG-FGFR1* and *TRIM33-NTRK1* transcript fusions in papillary thyroid carcinoma. *Genes Chromosomes Cancer* 2019; **58**; 558–566.
47. Kasaian K, Wiseman SM, Walker BA *et al*. The genomic and transcriptomic landscape of anaplastic thyroid cancer: implications for therapy. *BMC Cancer* 2015; **15**; 984.
48. Roper N, Brown AL, Wei JS *et al*. Clonal evolution and heterogeneity of osimertinib acquired resistance mechanisms in EGFR mutant lung cancer. *Cell Rep. Med.* 2020; **1**; 100007.
49. Michal M, Skálová A, Kazakov DV *et al*. Mixed epithelial and stromal tumor of the middle ear: the first case report. *Hum. Pathol.* 2017; **61**; 199–204.
50. Skálová A, Ptáková N, Santana T *et al*. *NCOA4-RET* and *TRIM27-RET* are characteristic gene fusions in salivary intraductal carcinoma, including invasive and metastatic tumors: is 'intraductal' correct? *Am. J. Surg. Pathol.* 2019; **43**; 1303–1313.