Histopathology

Histopathology 2021, **79**, 315–324. DOI: 10.1111/his.14251

MUC4 is a valuable marker for distinguishing secretory carcinoma of the salivary glands from its mimics

Cecilia Taverna,[1,2] Martina Baněčková,[3] Monica Lorenzon,[4] Annarita Palomba,[5] Alessandro Franchi,[6] Alena Skalova[3] & Abbas Agaimy[1]

[1]*Institute of Pathology, Friedrich-Alexander-University Erlangen-Nürnberg, University Hospital, Erlangen, Germany,* [2]*Department of Laboratory Medicine, Unit of Anatomical Pathology, Pisa University Hospital, Pisa, Italy,* [3]*Department of Pathology, Faculty of Medicine in Plzen, Charles University, Plzen, Czech Republic,* [4]*The Molecular Foundry, Lawrence Berkeley National Laboratory, Berkeley, CA, USA,* [5]*Unit of Histopathology and Molecular Diagnostics, Careggi University Hospital, Florence, and* [6]*Department of Translational Research, School of Medicine, University of Pisa, Pisa, Italy*

Date of submission 3 June 2020
Accepted for publication 9 September 2020
Published online *Article Accepted* 15 September 2020

Taverna C, Baněčková M, Lorenzon M, Palomba A, Franchi A, Skalova A & Agaimy A. (2021) *Histopathology* **79**, 315–324. https://doi.org/10.1111/his.14251

MUC4 is a valuable marker for distinguishing secretory carcinoma of the salivary glands from its mimics

Aims: Secretory carcinoma (SC) (synonym: mammary analogue secretory carcinoma) is a low-grade salivary gland tumour that occurs in both major and minor salivary glands. SC is known for its wide morphological, architectural and immunohistochemical spectrum, which overlaps with those of several salivary gland neoplasms, including acinic cell carcinoma (AciCC) and intercalated duct-type intraductal carcinoma (IDC) in major salivary glands, and polymorphous adenocarcinoma (PAC) in minor salivary glands. These tumours share with SC some morphological features and SOX10 immunoreactivity; also, with the exception of AciCC, they all coexpress S100 and mammaglobin.

Methods and results: We compared MUC4 and mammaglobin expression in 125 salivary gland carcinomas (54 genetically confirmed SCs, 20 AciCCs, 21 PACs, and 30 IDCs) to evaluate the potential of these two markers to differentiate these entities. Moderate to strong diffuse MUC4 positivity was detected in 49 SCs (90.7%), as compared with none of the IDCs and PACs. In contrast, mammaglobin was frequently expressed in SCs (30 of 36 cases; 83.3%), IDCs (24/28; 85.7%), and PACs (7/19; 36.8%). Two of three high-grade SCs lost MUC4 expression in the high-grade tumour component. No significant correlation was found between MUC4 expression and the fusion variant in SC (*ETV6–NTRK* versus non-*ETV6–NTRK*).

Conclusion: The results of our study identify MUC4 as a sensitive (90.7%) and specific (100%) marker for SC, with high positive (100%) and negative (93.4%) predictive values. Thus, MUC4 may be used as a surrogate for SC in limited biopsy material and in cases with equivocal morphology.

Keywords: acinic cell carcinoma, immunohistochemistry, intraductal carcinoma, mammaglobin, MUC4, polymorphous adenocarcinoma, secretory carcinoma

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

Introduction

Salivary gland secretory carcinoma (SC) is a recently described neoplasm; it was first reported by Skalova *et al.* in 2010, and characterised by *ETV6* rearrangements.[1] Many salivary gland carcinomas that had been initially diagnosed as other histotypes or 'adenocarcinoma not otherwise specified' were recategorised as SC upon histological review, with the support of molecular testing. SC shows a variety of morphological patterns,[2] frequently with a lobular arrangement and limited invasion. Microcystic, tubular and solid areas can be found within the same tumour. A minority of cases present as multiple multilocular macrocysts with or without other patterns. The tumour cells are uniform, with bland round or oval vesicular nuclei, finely granular chromatin, and inconspicuous central nucleoli. Mitotic figures are infrequent.[1,3] Rare cases show high-grade transformation.[4–6]

Immunohistochemically, the tumour cells are usually positive for cytokeratin, epithelial membrane antigen, vimentin, and S100. Moreover, mammaglobin expression is considered to be specific for this tumour.[1,2,7] However, SC is known to show significant overlap, both histologically and immunohistochemically, with acinic cell carcinoma (AciCC), polymorphous adenocarcinoma (PAC) and intercalated duct-type intraductal carcinoma (IDC) [synonym in the current World Health Organization (WHO) classification: 'intraductal carcinoma'].

Accordingly, molecular testing is considered to be the gold standard for confirming the diagnosis, especially if the morphology and immunohistochemistry (IHC) findings are equivocal. Like its mammary counterpart, SC harbours a recurrent balanced chromosomal translocation, t(12;15) (p13;q25), leading to fusion of *ETV6* on chromosome 12 with *NTRK3* on chromosome 15.[1] Alternative fusion variants have been reported, including *ETV6–RET*,[8] *ETV6–MET*,[9] and, more recently, concurrent *ETV6–RET* and *EGFR–SEPT14*[10] and *VIM–RET*[11] fusions.

However, molecular testing is not routinely performed in all laboratories, so some cases are still misdiagnosed. Thus, the identification of new, reliable, widely available and low-cost markers for diagnosing SC is of utmost importance, especially in small biopsies and in the presence of uncommon histological features.

Mucins constitute a family of high molecular weight glycoproteins secreted by epithelia lining various organs (respiratory, gastrointestinal, and others).[12,13] They are classified into secreted (MUC2, MUC5AC, MUC5B, MUC6, and MUC7) and membrane-associated (MUC1, MUC3, MUC4, and MUC12)[14] mucins. In human tissues, their expression varies among organs and among different cell types within the same organ.[15–18]

MUC4 is one of the best-characterised salivary gland mucins, both in major salivary glands[19] and in minor salivary glands.[14,20] It is diffusely expressed in cells lining striated and major excretory ducts, and it is variably expressed in serous acinar cells.[14,19]

However, only a few studies have investigated MUC4 expression in salivary gland neoplasms. Mucoepidermoid carcinoma frequently shows membranous and cytoplasmic MUC4 expression in all cell types (epidermoid, intermediate, mucinous, clear, and columnar), with a positive correlation with low-grade histology and a good prognosis.[21–23] In pleomorphic adenoma, MUC4 expression is uncommon and usually weak (14.2%).[24] Other salivary gland neoplasms with anecdotally reported MUC4 expression include adenoid cystic carcinoma, PAC, papillary adenocarcinoma,[22] papillary cystadenoma,[22,25] and low-grade salivary duct carcinoma (synonym in the current WHO classification: 'intraductal carcinoma').[26] MUC4 expression in SC has been evaluated in a few studies[1,27–29] (Table 1).

We analysed MUC4 expression in a cohort of SCs and SC mimics to evaluate its possible role as a surrogate marker for SC. In addition, we evaluated mammaglobin expression in the same cohort to establish whether combined expression of these two markers can differentiate SC from its mimics, in particular IDC and PAC.

Patients and methods

PATIENTS

The cohort ($n = 125$) included 54 genetically confirmed SCs (three with a dedifferentiated component),

Table 1. Published data on MUC4 expression in secretory carcinoma of the salivary glands

Author	SC cases (*n*)	MUC4-positive cases, *n* (%)
Skalova *et al.* (2010)[1]	16	9/11 (81.8)
Bissinger *et al.* (2017)[27]	3	2/3 (66.6)
Khurram *et al.* (2016)[28]	42	37/37 (100)*
Luk *et al.* (2015)[29]	9	9/9 (100)
Total	70	57/60 (95)

SC, secretory carcinoma.
*Khurram *et al.* reported positive staining for MUC4 in ducts and secretory material, with variable positivity in luminal cells.

Table 2. Clinicopathological features of the study cohort

	SC (54)	IC (30)	AciCC (20)	PCA (21)
Age (years), range (mean)	(*N* = 54) 28–86 (54.3)	(*N* = 29) 24–71 (50)	(*N* = 10) 14–81 (44.9)	(*N* = 20) 25–82 (57.3)
Sex (*n*)	(*N* = 54)	(*N* = 29)	(*N* = 10)	(*N* = 20)
Male	29	19	2	8
Female	25	10	8	12
Site (*n*)	(*N* = 54)	(*N* = 29)	(*N* = 20)	(*N* = 20)
Major salivary glands				
Parotid	28	25	18	0
Submandibular	4	2	0	0
Sublingual	1	0	0	0
Minor salivary glands	19	2	2	20
Other	2	0	0	0

AciCC, acinic cell carcinoma; IC, intraductal carcinoma; PCA, polymorphous adenocarcinoma; SC, secretory carcinoma.

30 intercalated duct-type IDCs, 21 PACs, and 20 AciCCs. Clinicopathological data are summarised in Table 2. 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).

IHC

IHC was performed on 3-μm sections prepared from formalin-fixed paraffin-embedded tumour blocks with a BenchMark Ultra automated instrument (Ventana Medical Systems, Tucson AZ, USA) and the antibodies anti-S100 (1:6000; Dako, Glostrup Denmark), anti-mammaglobin (1:100; Menarini Diagnostics, Florence, Italy), and anti-MUC4 (1:300; Santa Cruz, Heidelberg, Germany), according to routine laboratory standards and the manufacturer's instructions. Heat-induced epitope retrieval was performed with CC1 antigen retrieval buffer at 95°C (36 min for S100 and 64 min for mammaglobin and MUC4). Antibody incubation was then performed at 37°C for 32 min. Binding of the antibodies to the antigen was visualised with the UltraView Universal DAB Detection Kit, and sections were then counterstained with haematoxylin and Bluing Reagent (Ventana) Medical Systems. Positive and negative controls were used throughout.

The proportion of positive cells was scored as 0 (negative stain), 1 (1–24%), 2 (25–49%), and 3 (>50%). For statistical analysis, cases with a score of ≥ 2 were considered to be positive.

Molecular analyses were performed in two laboratories using established methods. All 17 SC cases from Plzen were tested with a combination of fluorescence *in-situ* hybridisation (FISH), next-generation sequencing, and polymerase chain reaction, as described previously.[11] Eleven SCs from Erlangen were tested for *ETV6* fusions by use of the TruSight RNA Fusion panel (Illumina, San Diego, CA, USA), as described previously.[30] Twenty-eight SCs were tested with FISH by use of an ETV6 Dual Color Breakapart Probe (ZytoVision, Bremerhaven, Germany), according to the manufacturer's protocol. Hybridised slides were examined with a Zeiss AxioScope fluorescence microscope with × 10 and × 63 oil objectives (Zeiss Company, Oberkochen, Germany). Fifty randomly selected non-overlapping tumour cell nuclei were examined for the presence of translocation signals.

STATISTICAL ANALYSIS

Statistical tests were performed with SPSS software (release 21.0). Associations between categorical variables were assessed with the chi-square test and Fisher's exact test. The differences between categories were assessed with the log-rank (Mantel–Cox) chi-square test. Sensitivity, specificity, positive predictive value and negative predictive value were calculated. Two-tailed *P*-values of < 0.05 were considered to be significant.

Table 3. Immunohistochemical results

Tumour type/results	S100	MUC4	Mammaglobin	MUC4 and mammaglobin coexpression	*P*-value
Secretory carcinoma, *n* (%)	52/54 (96.3)	49/54 (90.7)	30/36 (83.3)	26/36 (72.2)	<0.001
Intraductal carcinoma	27/30 (90)	0/30 (0)	24/28 (85.7)	0/28 (0)	<0.001
Acinic cell carcinoma	0/4 (0)	0/20 (0)	0/6 (0)	0/6 (0)	<0.001
Polymorphous adenocarcinoma	21/21 (100)	0/21 (0)	7/19 (36.8)	0/19 (0)	<0.001

Results

Clinicopathological features and immunohistochemical findings are summarised in Tables 2 and 3.

MUC4, MAMMAGLOBIN AND S100 EXPRESSION IN SC (*N* = 54)

The patients were 29 men and 25 women, aged 28–86 years (mean, 54.3 years). Thirty-three tumours originated in major salivary glands (28 parotid glands, four submandibular glands, and one sublingual gland), and 19 originated in minor salivary glands. One case originated in a neck lymph node, and one case originated in the nasal cavity.[31]

Molecular findings were available for all cases. Fifty-one cases (94.4%) harboured the canonical *ETV6–NTRK3* fusion, two cases (3.7%) harboured the *ETV6–RET* fusion, and one case (1.8%) harboured a novel *ETV6–NTRK2* fusion.

MUC4 was positive in 49 (90.7%) cases and negative in five (9.3%) cases, with a sensitivity of 90.7%, a specificity of 100%, a positive predictive value of 100%, and a negative predictive value of 93.4%. Among the five negative cases, two were completely negative and three showed only scattered positive cells. One of the two cases with complete absence of MUC4 staining was an intranodal classic SC, and the other was a high-grade SC of the parotid. Both harboured the *ETV6–NTRK3* fusion. The three cases with low MUC4 expression in a few cells were classic SCs (two harboured the *ETV6–NTRK3* fusion, and one harboured the *ETV6–RET* fusion).

Mammaglobin was positive in 30 of 36 (83.3%) cases and negative in six (16.7%) cases. It was negative in two of three cases with a high-grade component, and in the one case arising in the cervical lymph node.

Among the 36 SCs tested for both mammaglobin and MUC4, 26 (72.2%) expressed both markers, four (11.1%) expressed only MUC4, four (11.1%) expressed only mammaglobin, and two (5.5%) were negative for both markers. The two double-negative cases harboured the classic *ETV6–NTRK3* fusion. Combined MUC4 and mammaglobin expression in SC had a sensitivity of 75%, a specificity of 100%, a positive predictive value of 100%, and a negative predictive value of 0%.

S100 was positive in 52 of 54 (96.3%) cases and negative in two cases (3.7%) ($P < 0.001$). Both S100-negative cases showed classic histology, without a dedifferentiated component, and they harboured the *ETV6–NTRK3* fusion.

Representative examples of immunohistochemical findings in SC are shown in Figures 1 and 2.

MUC4, MAMMAGLOBIN AND S100 EXPRESSION IN INTERCALATED DUCT-TYPE IDC (*N* = 30)

Clinicopathological data were available for 29 of 30 IDCs. There were 19 males and 10 females, aged 24–71 years (mean, 50 years). Most tumours were located in major salivary glands (27 cases; 25 cases in the parotid and two in the submandibular gland). Only two cases involved the minor salivary glands.

Molecular data were available for 10 cases: nine harboured the *NCOA4–RET* fusion (eight of them previously published),[32] and one harboured a *TRIM27–RET* fusion.[32]

MUC4 was negative in all cases, whereas mammaglobin was positive in 24 of 28 cases (85.7%) ($P < 0.001$).

S100 was positive in 27 of 30 (90%) cases and negative in three (10%) cases ($P < 0.001$). Molecular tests were not performed in the S100-negative cases. Representative examples of immunohistochemical findings in IDC are shown in Figure 3.

MUC4, MAMMAGLOBIN AND S100 EXPRESSION IN ACICC (*N* = 20)

Clinicopathological data were collected for 10 cases. There were two males and eight females, aged 14–81 years (mean, 44.9 years). Tumours occurred mainly in the parotid (18 cases), and two cases originated in minor salivary glands. MUC4 and

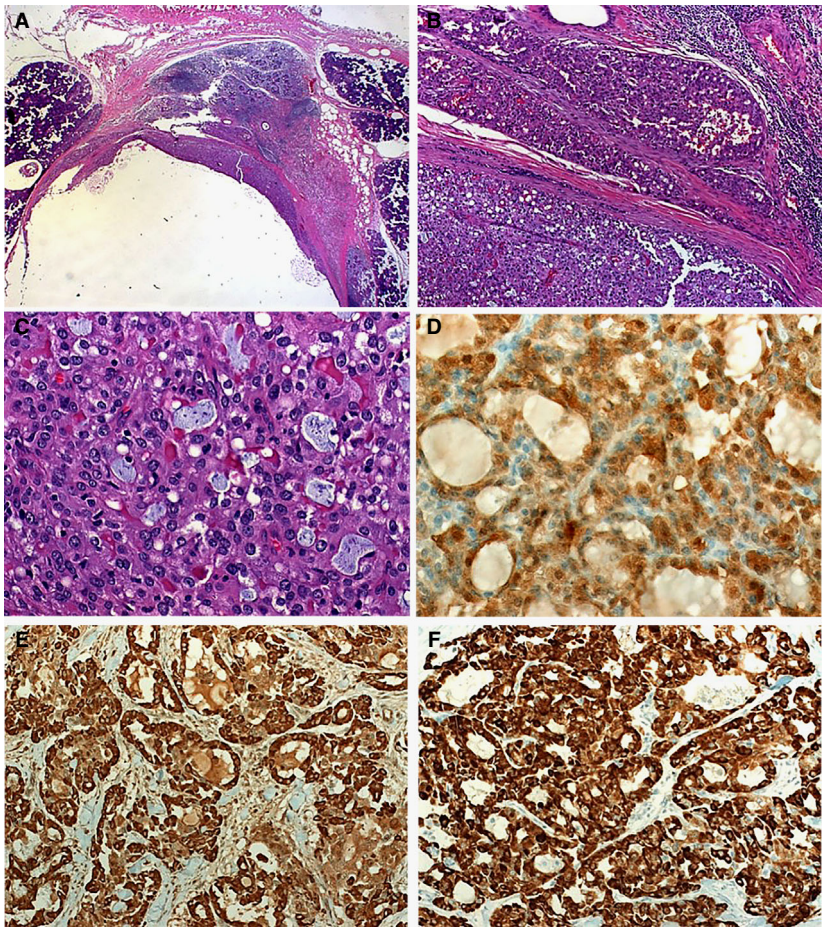

Figure 1. A,B, Secretory carcinoma may present as a cystic mass (A) surrounded by solid invasive nests (B), closely mimicking the pattern seen in intraductal carcinoma, but lacking p63-positive basal cells (not shown). C, Monomorphic rounded cells interrupted by small mucin-filled lumina are seen at higher magnification. D–F, Diffuse and strong expression of S100 (D), mammaglobin (E) and MUC4 (F) is characteristic of secretory carcinomas. The illustrated tumour harboured the classic *ETV6–NTRK3* fusion

mammaglobin were negative in all cases tested. S100 was tested in only four cases; all were negative ($P < 0.001$).

MUC4, MAMMAGLOBIN AND S100 EXPRESSION IN PAC ($N = 21$)

Clinicopathological data were available for 20 cases. There were eight men and 12 women, aged 25–82 years (mean, 57.3 years). All tumours originated in minor salivary glands. Only a subset of PACs were tested for molecular alterations. Six of them were studied for *ETV6–NTRK* rearrangement, two for *MYB* rearrangement, and one for both. All cases tested negative for these genetic alterations.

All cases were negative for MUC4, and seven of 19 cases (36.8%) were positive for mammaglobin. All cases were positive for S100 (100%) ($P < 0.001$). Representative examples of immunohistochemical findings in PAC are shown in Figure 4.

Discussion

This is the largest study that has analysed and compared MUC4 and mammaglobin expression in genetically confirmed SCs and cohorts of salivary gland carcinomas that are frequently confused with SC, including, in particular, AciCC, intercalated duct-type IDC, and PAC. MUC4 expression in SC has been evaluated in only a few studies,[1,27–29] and, besides S100 and mammaglobin, is considered to be a marker to support an SC diagnosis. In our study, MUC4 was expressed in the majority of SCs (90.7%), but in none of its mimics, confirming it as a reliable context-specific marker for SC. Mammaglobin was typically

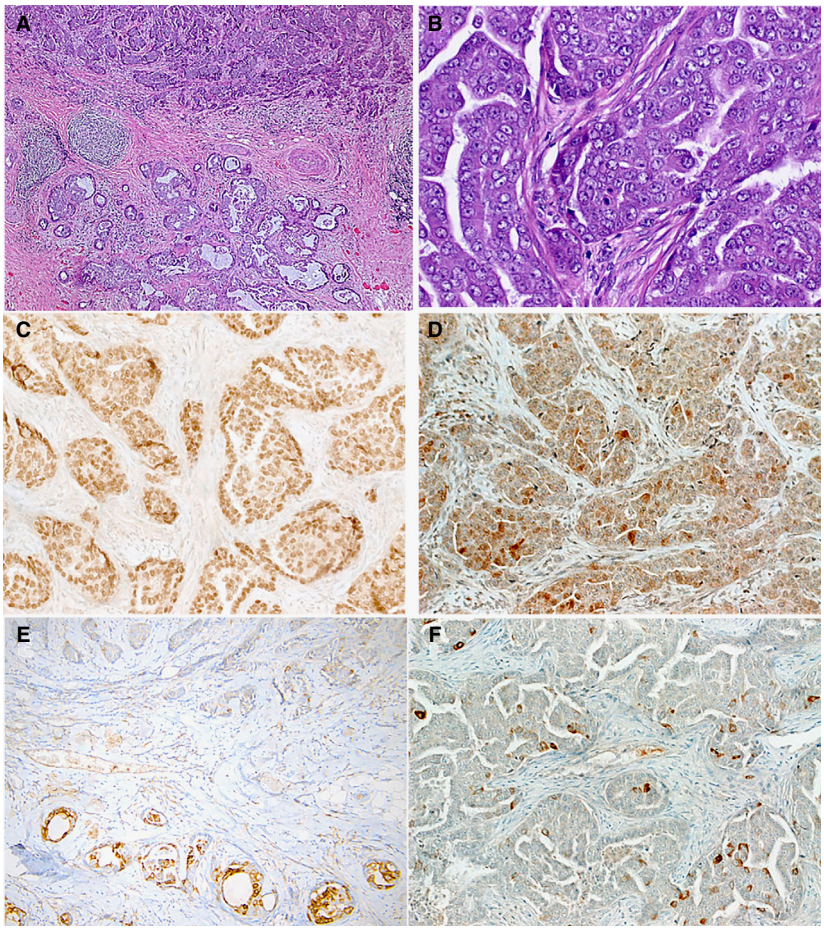

Figure 2. A, A secretory carcinoma with high-grade transformation, showing transition from a conventional (lower field) to an undifferentiated solid high-grade (upper field) clone. B, Higher magnification of the high-grade component shows epithelioid cells with rounded vesicular nuclei and prominent nucleoli. C,D, Whereas diffuse and strong SOX10 expression is still retained in the high-grade clone (C), only limited and weak S100 reactivity is seen (D). E,F, Near-total loss of MUC4 expression is seen in the high-grade component (E, upper field; F, higher magnification), in contrast to strong expression in the conventional low-grade tumour area (E, lower field). The illustrated tumour harboured the classic *ETV6–NTRK3* fusion

diffusely positive in SC (83.3% of cases), similarly to what has been found in previous studies.[1–4,33] Focal or weak positivity in a few cases can be ascribed to fixation or tissue preservation issues, as we included cases from different hospitals, and only unstained slides were available for some tumours.

Few studies have assessed whether histological features and immunohistochemistry are sufficient for the diagnosis of SC, without molecular testing. Shah *et al.*[34] found coexpression of S100 and mammaglobin in 94.7% of SC cases. Their finding was confirmed by Montalvo *et al.*[35] However, S100 expression and mammaglobin expression were absent in some SCs.[3]

Distinguishing between SC and intercalated duct-type IDC can be challenging, as these entities share histological, cytological and immunohistochemical (expression of S100, SOX10, and mammaglobin) features.[7,36–40] In our study, mammaglobin expression was present in both SC and intercalated duct-type IDC (83.3% and 85.7%, respectively), whereas MUC4 was restricted to SC (all 30 IDCs were negative).

Another entity in the differential diagnosis of SC is AciCC, especially the zymogen-poor variant. Indeed, SC has been historically confused with AciCC, and both were considered to be variants on the same tumour spectrum. Most studies have reported the absence of S100 and mammaglobin expression in AciCC. Hsieh *et al.*[41] found S100 and mammaglobin coexpression in SC, but not in AciCC (20 of 21 AciCCs were S100-negative, and 18 of 21 were mammaglobin-negative). Negativity of AciCC for S100 and mammaglobin has been confirmed by others.[42,43]

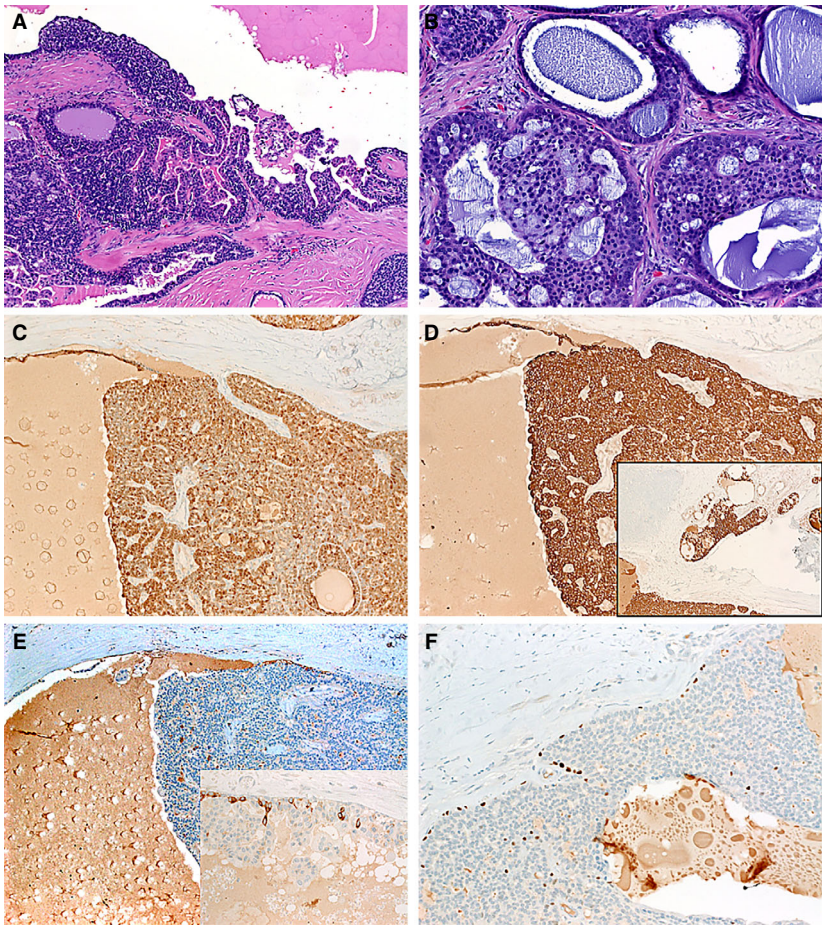

Figure 3. A,B, Intercalated duct-type intraductal carcinoma frequently presents with a solid and cystic component (A) surrounded by solid nests mimicking invasion (A, lower right corner). B, Higher magnification of the lobular cancerisation surrounding the main mass of the intraductal carcinoma shows monomorphic cells with multiple mucin-filled lumina mimicking secretory carcinoma. C,D, Strong expression of SOX10 (not shown), S100 (C) and mammaglobin (D) characterises the majority of intraductal carcinomas (note the lobular cancerisation highlighted by the mammaglobin stain in the D inset). E, All intraductal carcinomas are MUC4-negative. A few positively staining cells seen adjacent to the cyst wall in some cases probably represent native ductal cells detached from the cyst lining (inset). F, Non-invasive intraductal carcinoma shows a retained basal cell layer surrounding the main mass and the adjacent intralobular non-invasive solid nests, highlighted by p63 immunostaining. The illustrated tumour harboured the *NCOA4–RET* fusion [Colour figure can be viewed at wileyonlinelibrary.com]

Conversely, Baghai *et al.*[33] found S100 and mammaglobin expression in a small subset of zymogen-poor AciCCs. Urano *et al.*[7] reported S100 and mammaglobin coexpression in 90% of SCs, but also in two of six AciCCs. Notably, comparison of studies pre-dating the redefinition of SC as a specific molecular entity is not reliable, and some recent studies might have accepted archival diagnoses of AciCC.

MUC4 status and coexpression of MUC4 and mammaglobin have been rarely studied in AciCC and IDC as compared with SC.[1,29] All AciCCs and IDCs lacked MUC4 expression in our study. Mammaglobin was also negative in AciCC. Thus, none of the AciCCs coexpressed these two markers, whereas 72% of SCs did.

Another SC mimic is PAC, especially in minor salivary glands. PACs show histological similarities and overlaps in their immunoprofile with SC, including S100 expression[44,45] and mammaglobin expression.[46,47] Patel *et al.*[48] found S100 and mammaglobin coexpression in 60% of PACs. MUC4 expression has been evaluated in only two PACs; both were negative.[29] We have confirmed this finding in a larger series. MUC4 was negative in all PACs, whereas mammaglobin was positive in 36.8%. Coexpression of both markers was absent in PAC, confirming MUC4 as a reliable marker for differentiating these two entities.

A few SCs undergo high-grade transformation.[4–6] Mammaglobin expression has been analysed in only a few of them. Skalova *et al.*[4] and Majewska *et al.*[6]

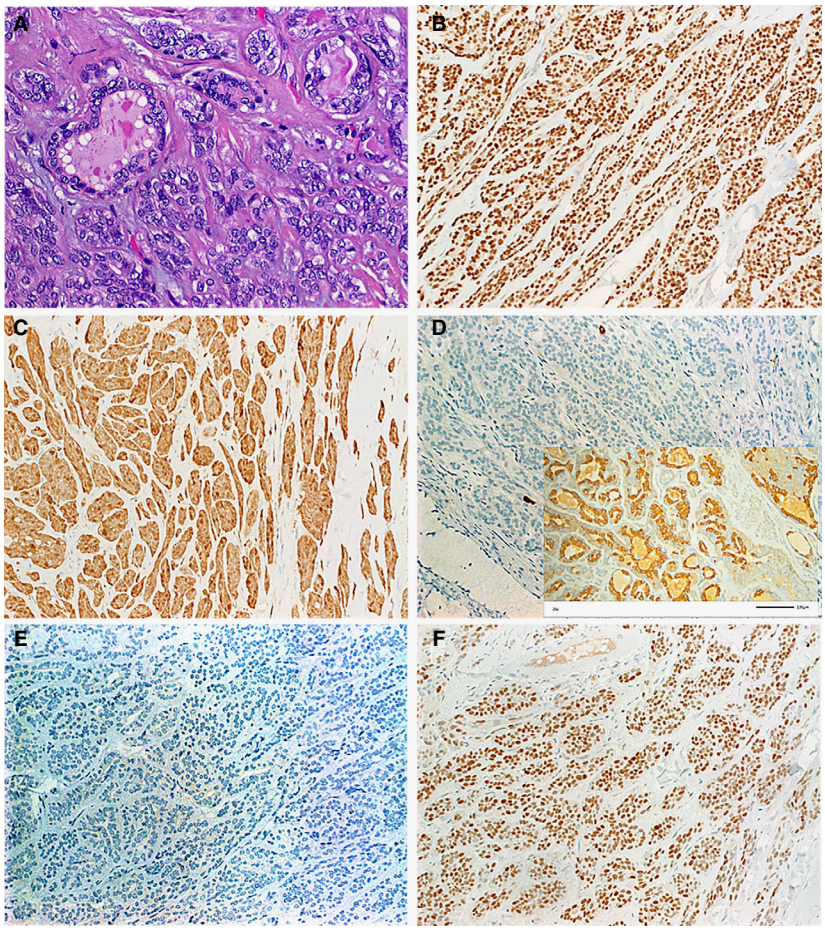

Figure 4. Polymorphous adenocarcinoma (PAC) shows sweeping fascicles of monomorphic elongated or oval cells with a variable glandular component (A). Consistent expression of SOX10 (B) and S100 (C) is characteristic. Mammaglobin is variably expressed in PAC, in this example being negative in the solid areas (D, main image) and positive in the glandular areas (D, inset). MUC4 is consistently negative in PAC, except for a few scattered native ductal cells (E). p63 is variably expressed in the neoplastic cells of PAC (F), which is in contrast to its absence in secretory carcinoma and its strictly basal expression in intraductal carcinoma [Colour figure can be viewed at wileyonlinelibrary.com]

observed mammaglobin expression in both components of high-grade SC. Luo *et al.* reported focal mammaglobin expression in the low-grade component, but did not report the high-grade component. Two of three high-grade SCs in our study lost MUC4 and mammaglobin expression in the high-grade component.

When we compared MUC4 expression with SC genotypes, we observed limited MUC4 expression in one of two *ETV6–RET*-rearranged cases. The *ETV6–NTRK2*-rearranged case was strongly and diffusely positive for MUC4. Whether lack of MUC4 expression is overrepresented among SCs harbouring the rare *ETV6–RET* fusion variant remains to be addressed in larger future series.

Pan-TRK IHC is emerging as a surrogate marker for *NTRK* fusion-positive SC, but data are still limited.[49–51] Notably, pan-TRK expression is probably restricted to those tumours harbouring the canonical *ETV6–NTRK3* or *ETV6–NTRK2* fusions, underlying its limited value in negative cases (e.g. those carrying the *RET* or other rare non-*NTRK* fusion variants). In addition, NR4A3 (NOR-1) has recently been described as a valuable novel marker for AciCC, and is absent in SC.[52] All of these recent developments underline the emerging roles of proteins as surrogate molecular markers for the subclassification of salivary gland carcinoma, and point to their value in complementing molecular tools and in identifying cases for targeted and rational molecular testing.

In conclusion, we have shown that both MUC4 expression and combined expression of MUC4 and mammaglobin are reliable adjuncts in screening for SC as well as in discriminating this tumour type from

its most common mimics with high sensitivity, high specificity and high positive predictive values. In typical cases, morphology in combination with these markers can be sufficient to diagnose secretory carcinoma.

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 and A. Agaimy. Data collection and interpretation: C. Taverna, M. Baněčková, M. Lorenzon, A. Palomba, A. Franchi, A. Skalova 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, M. Baněčková, M. Lorenzon, A. Palomba, A. Franchi, A. Skalova and A. Agaimy.

Acknowledgment

Open access funding enabled and organized by ProjektDEAL.

References

1. Skalova A, Vanecek T, Sima R *et al.* Mammary analogue secretory carcinoma of salivary glands, containing the ETV6-NTRK3 fusion gene: a hitherto undescribed salivary gland tumor entity. *Am. J. Surg. Pathol.* 2010; **34**; 599–608.
2. Skalova A. Mammary analogue secretory carcinoma of salivary gland origin: an update and expanded morphologic and immunohistochemical spectrum of recently described entity. *Head Neck Pathol.* 2013; **7**(Suppl. 1); S30–S36.
3. Connor A, Perez-Ordonez B, Shago M, Skalova A, Weinreb I. Mammary analog secretory carcinoma of salivary gland origin with the ETV6 gene rearrangement by FISH: expanded morphologic and immunohistochemical spectrum of a recently described entity. *Am. J. Surg. Pathol.* 2012; **36**; 27–34.
4. Skalova A, Vanecek T, Majewska H *et al.* Mammary analogue secretory carcinoma of salivary glands with high-grade transformation: report of 3 cases with the ETV6-NTRK3 gene fusion and analysis of TP53, β-catenin, EGFR, and CCND1 genes. *Am. J. Surg. Pathol.* 2014; **38**; 23–33.
5. Luo W, Lindley SW, Lindley PH, Krempl GA, Seethala RR, Fung KM. Mammary analog secretory carcinoma of salivary gland with high-grade histology arising in hard palate, report of a case and review of literature. *Int. J. Clin. Exp. Pathol.* 2014; **7**; 9008–9022.
6. Majewska H, Skalova A, Stodulski D *et al.* Mammary analogue secretory carcinoma of salivary glands: a new entity associated with ETV6 gene rearrangement. *Virchows Arch.* 2015; **466**; 245–254.
7. Urano M, Nagao T, Miyabe S, Ishibashi K, Higuchi K, Kuroda M. Characterization of mammary analogue secretory carcinoma of the salivary gland: discrimination from its mimics by the presence of the ETV6-NTRK3 translocation and novel surrogate markers. *Hum. Pathol.* 2015; **46**; 94–103.
8. Skalova A, Vanecek T, Martinek P *et al.* Molecular profiling of mammary analog secretory carcinoma revealed a subset of tumors harboring a novel ETV6-RET translocation: report of 10 cases. *Am. J. Surg. Pathol.* 2018; **42**; 234–246.
9. Rooper LM, Karantanos T, Ning Y, Bishop JA, Gordon SW, Kang H. Salivary secretory carcinoma with a novel ETV6-MET fusion: expanding the molecular spectrum of a recently described entity. *Am. J. Surg. Pathol.* 2018; **42**; 1121–1126.
10. Black M, Liu CZ, Onozato M *et al.* Concurrent identification of novel *EGFR–SEPT14* fusion and *ETV6–RET* fusion in secretory carcinoma of the salivary gland. *Head Neck Pathol.* 2020; **14**; 817–821.
11. Skálová A, Banečkova M, Thompson LDR *et al.* Expanding the molecular spectrum of secretory carcinoma of salivary glands with a novel VIM-RET fusion. *Am. J. Surg. Pathol.* 2020; **44**; 1295–1307.
12. Davies JR, Herrmann A, Russell W, Svitacheva N, Wickstrom C, Carlstedt I. Respiratory tract mucins: structure and expression patterns. *Novartis Found. Symp.* 2002; **248**; 76–88; discussion 88–93, 277–282.
13. Seregni E, Botti C, Massaron S *et al.* Structure, function and gene expression of epithelial mucins. *Tumori* 1997; **83**; 625–632.
14. Offner GD, Troxler RF. Heterogeneity of high-molecular-weight human salivary mucins. *Adv. Dent. Res.* 2000; **14**; 69–75.
15. Lau SK, Weiss LM, Chu PG. Differential expression of MUC1, MUC2, and MUC5AC in carcinomas of various sites: an immunohistochemical study. *Am. J. Clin. Pathol.* 2004; **122**; 61–69.
16. Ho SB, Niehans GA, Lyftogt C *et al.* Heterogeneity of mucin gene expression in normal and neoplastic tissues. *Cancer Res.* 1993; **53**; 641–651.
17. Audie JP, Janin A, Porchet N, Copin MC, Gosselin B, Aubert JP. Expression of human mucin genes in respiratory, digestive, and reproductive tracts ascertained by in situ hybridization. *J. Histochem. Cytochem.* 1993; **41**; 1479–1485.
18. Hollingsworth MA, Swanson BJ. Mucins in cancer: protection and control of the cell surface. *Nat. Rev. Cancer* 2004; **4**; 45–60.
19. Liu B, Offner GD, Nunes DP, Oppenheim FG, Troxler RF. MUC4 is a major component of salivary mucin MG1 secreted by the human submandibular gland. *Biochem. Biophys. Res. Commun.* 1998; **250**; 757–761.
20. Alos L, Lujan B, Castillo M *et al.* Expression of membrane-bound mucins (MUC1 and MUC4) and secreted mucins (MUC2, MUC5AC, MUC5B, MUC6 and MUC7) in mucoepidermoid carcinomas of salivary glands. *Am. J. Surg. Pathol.* 2005; **29**; 806–813.
21. Li P, Arango ME, Perez RE *et al.* Expression and localization of immunoreactive-sialomucin complex (Muc4) in salivary glands. *Tissue Cell* 2001; **33**; 111–118.
22. Weed DT, Gomez-Fernandez C, Bonfante E *et al.* MUC4 (sialomucin complex) expression in salivary gland tumors and squamous cell carcinoma of the upper aerodigestive tract. *Otolaryngol. Head Neck Surg.* 2001; **124**; 127–141.

23. Weed DT, Gomez-Fernandez C, Pacheco J *et al.* MUC4 and ERBB2 expression in major and minor salivary gland mucoepidermoid carcinoma. *Head Neck* 2004; **26**; 353–364.
24. Hamada T, Matsukita S, Goto M *et al.* Mucin expression in pleomorphic adenoma of salivary gland: a potential role for MUC1 as a marker to predict recurrence. *J. Clin. Pathol.* 2004; **57**; 813–821.
25. Lanzel EA, Pourian A, Sousa Melo SL, Brogden KA, Hellstein JW. Expression of membrane-bound mucins and p63 in distinguishing mucoepidermoid carcinoma from papillary cystadenoma. *Head Neck Pathol.* 2016; **10**; 521–526.
26. Kusafuka K, Itoh H, Sugiyama C, Nakajima T. Low-grade salivary duct carcinoma of the parotid gland: report of a case with immunohistochemical analysis. *Med. Mol. Morphol.* 2010; **43**; 178–184.
27. Bissinger O, Gotz C, Kolk A *et al.* Mammary analogue secretory carcinoma of salivary glands: diagnostic pitfall with distinct immunohistochemical profile and molecular features. *Rare Tumors* 2017; **9**; 7162.
28. Khurram SA, Sultan-Khan J, Atkey N, Speight PM. Cytogenetic and immunohistochemical characterization of mammary analogue secretory carcinoma of salivary glands. *Oral Surg. Oral Med. Oral Pathol. Oral Radiol.* 2016; **122**; 731–742.
29. Luk PP, Selinger CI, Eviston TJ *et al.* Mammary analogue secretory carcinoma: an evaluation of its clinicopathological and genetic characteristics. *Pathology* 2015; **47**; 659–666.
30. 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.
31. Baneckova M, Agaimy A, Andreasen S *et al.* Mammary analog secretory carcinoma of the nasal cavity: characterization of 2 cases and their distinction from other low-grade sinonasal adenocarcinomas. *Am. J. Surg. Pathol.* 2018; **42**; 735–743.
32. Skalova A, Ptakova 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.
33. Baghai F, Yazdani F, Etebarian A, Garajei A, Skalova A. Clinicopathologic and molecular characterization of mammary analogue secretory carcinoma of salivary gland origin. *Pathol. Res. Pract.* 2017; **213**; 1112–1118.
34. Shah AA, Wenig BM, LeGallo RD, Mills SE, Stelow EB. Morphology in conjunction with immunohistochemistry is sufficient for the diagnosis of mammary analogue secretory carcinoma. *Head Neck Pathol.* 2015; **9**; 85–95.
35. Montalvo N, Galarza D, Redroban L. Secretory carcinoma of the parotid: making the correct diagnosis of a rare salivary gland carcinoma when molecular biology testing is not available. *Case Rep. Pathol.* 2019; **2019**; 5103496.
36. Delgado R, Klimstra D, Albores-Saavedra J. Low grade salivary duct carcinoma. A distinctive variant with a low grade histology and a predominant intraductal growth pattern. *Cancer* 1996; **78**; 958–967.
37. Giovacchini F, Bensi C, Belli S *et al.* Low-grade intraductal carcinoma of salivary glands: a systematic review of this rare entity. *J. Oral Biol. Craniofac. Res.* 2019; **9**; 96–110.
38. Brandwein-Gensler M, Hille J, Wang BY *et al.* Low-grade salivary duct carcinoma: description of 16 cases. *Am. J. Surg. Pathol.* 2004; **28**; 1040–1044.
39. Weinreb I, Seethala RR, Hunt JL, Chetty R, Dardick I, Perez-Ordonez B. Intercalated duct lesions of salivary gland: a morphologic spectrum from hyperplasia to adenoma. *Am. J. Surg. Pathol.* 2009; **33**; 1322–1329.
40. Nishijima T, Yamamoto H, Nakano T *et al.* Low-grade intraductal carcinoma (low-grade cribriform cystadenocarcinoma) with tumor-associated lymphoid proliferation of parotid gland. *Pathol. Res. Pract.* 2017; **213**; 706–709.
41. Hsieh MS, Chou YH, Yeh SJ, Chang YL. Papillary-cystic pattern is characteristic in mammary analogue secretory carcinomas but is rarely observed in acinic cell carcinomas of the salivary gland. *Virchows Arch.* 2015; **467**; 145–153.
42. Bishop JA, Yonescu R, Batista D, Eisele DW, Westra WH. Most nonparotid 'acinic cell carcinomas' represent mammary analog secretory carcinomas. *Am. J. Surg. Pathol.* 2013; **37**; 1053–1057.
43. Naous R, Zhang S, Valente A, Stemmer M, Khurana KK. Utility of immunohistochemistry and *ETV6* (12p13) gene rearrangement in identifying secretory carcinoma of salivary gland among previously diagnosed cases of acinic cell carcinoma. *Pathol. Res. Int.* 2017; **2017**; 1497023.
44. Regezi JA, Zarbo RJ, Stewart JC, Courtney RM. Polymorphous low-grade adenocarcinoma of minor salivary gland. A comparative histologic and immunohistochemical study. *Oral Surg. Oral Med. Oral Pathol.* 1991; **71**; 469–475.
45. Gonzalez-Garcia R, Rodriguez-Campo FJ, Munoz-Guerra MF, Nam-Cha SH, Sastre-Perez J, Naval-Gias L. Polymorphous low-grade adenocarcinoma of the palate: report of cases. *Auris Nasus Larynx* 2005; **32**; 275–280.
46. Batsakis JG, Pinkston GR, Luna MA, Byers RM, Sciubba JJ, Tillery GW. Adenocarcinomas of the oral cavity: a clinicopathologic study of terminal duct carcinomas. *J. Laryngol. Otol.* 1983; **97**; 825–835.
47. Montalli VA, Passador-Santos F, Martinez EF *et al.* Mammaglobin and DOG-1 expression in polymorphous low-grade adenocarcinoma: an appraisal of its origin and morphology. *J. Oral Pathol. Med.* 2017; **46**; 182–187.
48. Patel KR, Solomon IH, El-Mofty SK, Lewis JS Jr, Chernock RD. Mammaglobin and S-100 immunoreactivity in salivary gland carcinomas other than mammary analogue secretory carcinoma. *Hum. Pathol.* 2013; **44**; 2501–2508.
49. Hung YP, Jo VY, Hornick JL. Immunohistochemistry with a pan-TRK antibody distinguishes secretory carcinoma of the salivary gland from acinic cell carcinoma. *Histopathology* 2019; **75**; 54–62.
50. Bell D, Ferrarotto R, Liang L *et al.* Pan-Trk immunohistochemistry reliably identifies ETV6-NTRK3 fusion in secretory carcinoma of the salivary gland. *Virchows Arch.* 2020; **476**; 295–305.
51. Xu B, Haroon Al Rasheed MR, Antonescu CR *et al.* Pan-Trk immunohistochemistry is a sensitive and specific ancillary tool for diagnosing secretory carcinoma of the salivary gland and detecting ETV6-NTRK3 fusion. *Histopathology* 2020; **76**; 375–382.
52. Haller F, Skalova A, Ihrler S *et al.* Nuclear NR4A3 immunostaining is a specific and sensitive novel marker for acinic cell carcinoma of the salivary glands. *Am. J. Surg. Pathol.* 2019; **43**; 1264–1272.