Secretory Myoepithelial Carcinoma: A Histologic and Molecular Survey and a Proposed Nomenclature for Mucin Producing Signet Ring Tumors

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Received: 5 July 2013 / Accepted: 10 January 2014 / Published online: 21 January 2014
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Abstract Signet ring cell (mucin producing) adenocarcinoma is a rare low grade salivary gland malignancy. While currently designated as an adenocarcinoma, myoepithelial differentiation has been implied in previously reported cases. We herein perform a survey of our cases of signet ring cell adenocarcinoma and review the literature in order to refine categorization of this rare tumor. Five cases were retrieved. One was reclassified as a mammary analogue secretory carcinoma, leaving four that fulfilled the criteria for signet ring cell adenocarcinoma: the presence of prominent signet ring or vacuolated cells arranged in islands, interconnecting strands, cords or sheets in a myxoid or hyaline stroma, or pools of mucin. An extensive panel of histochemical and immunohistochemical stains and fluorescence in situ hybridization (FISH) (modeled after common phenotypes and molecular alterations seen in signet ring and myoepithelial tumors at other sites) was

performed. The male-to-female ratio was 3:1. The mean age was 56 years (range 18–81). Sites involved included buccal mucosa (2), soft palate (1) and deep parotid (1). Perineural and angiolymphatic invasion were present in three and two cases respectively. One patient was lost to follow up and the remainder were alive and without disease at time of last follow up (mean 38 months). All cases showed mucicarmine positive vacuolated/signet ring cells embedded in a myxoid stroma. Three cases showed at least focal p63 staining and two cases showed positivity for calponin. Membranous E-cadherin was retained in all cases. FISH was negative for *ETV6*, *EWSR1*, and *ALK1* rearrangements in all four cases. Based on the current series and the previously reported cases, it is evident that signet ring adenocarcinomas have a dual secretory and myoepithelial phenotype and thus as a whole more appropriately designated as ‘secretory myoepithelial carcinoma.’ They behave in a fairly indolent fashion and do not share the major molecular alterations seen in other signet ring and myoepithelial tumor types.

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Keywords Secretory · Myoepithelial carcinoma ·
Myoepithelioma · Signet ring · Salivary · Head and neck

Introduction

Neoplasms with prominent mucin production and signet ring cells in the head and neck are extremely rare and generally invoke considerations for metastatic disease from distant sites such as the breast, lung or the gastrointestinal tract. However, in 1988, de Araujo et al. [1] reported a case of primary minor salivary gland adenocarcinoma with prominent signet ring cells and mucus production, and a microcystic and papillary growth pattern with prominent

intracytoplasmic lumina, involving the buccal mucosa of a 36 year old male. Later, in 2004, Ghannoum and Freedman [2] reported seven additional cases with prominent signet ring cells and similar morphologic features and labeled these as ‘signet ring cell (mucin producing) adenocarcinomas’ of minor salivary glands (SRCA). They believed their tumors differed from that of de Araujo’s in their growth pattern as they lacked the cystic and papillary growth patterns with interspersed microcystic spaces seen in de Araujo’s case. Their SRCAs have prominent signet ring cells arranged in islands and in interconnecting strands and cords with rare duct like structures lined by a single layer of cuboidal cells. The signet ring cells were also admixed with cells with somewhat eosinophilic and granular cytoplasm [2]. The stroma was myxoid and mucinous. Rarely, the stroma was sclerotic and hypocellular. One case showed these signet ring cells in pools of mucin [2].

Since then, only a few cases of SRCA have been reported and little is known about this extremely rare entity. Subsequent to the description of SRCA, other primary salivary tumor types such as salivary duct carcinoma [3] and adenoid cystic carcinoma [4] have been described with signet ring cell components. Additionally, the recently described mammary analog secretory carcinoma (MASC) may show a mucin rich vacuolated appearance resembling SRCA [5]. However, the apocrine features and nuclear pleomorphism of salivary duct carcinoma, the biphasic arrangement of abluminal myoepithelial cells and luminal ductal cells of adenoid cystic carcinoma are not seen in SRCA suggesting that this category is still distinct.

Interestingly, even in the series by Ghannoum and Freedman [2], a myoepithelial phenotype was implied, though the abundant mucin production and signet ring morphology stood at odds with the immunohistochemical profile. With the increased molecular characterization of signet ring cell rich and myoepithelial tumors, both salivary and extrasalivary, it is now possible to evaluate SRCA in the context of these newly described alterations. Specific entities of interest include: MASC mentioned above, and defined by an *ETV6-NTRK3* translocation akin to secretory carcinoma of the breast; *EML4-ALK* translocation positive lung adenocarcinomas, which prototypically demonstrate an acinar or signet ring appearance; and in the spectrum of tumors with a myoepithelial phenotype, particularly of soft tissue sites, *EWS* associated translocations have been increasingly described [6]. While any link between the aforementioned entities and SRCA beyond some morphologic mimicry is speculative, these translocations are readily testable in a clinical setting. We herein evaluate the clinicopathologic, ultrastructural, immunophenotypic and molecular features of four cases of primary SRCA and make a case for these tumors to be redesignated as ‘secretory myoepithelial carcinomas’. The histologic criteria for

inclusion are those described by Ghannoum and Freedman [2] and summarized above. Metastasis from other sites has also been ruled out clinically in all four cases.

Materials and Methods

Cases were retrieved from the archives of the Department of Pathology at the University of Pittsburgh (1983–2009) using the search terms ‘signet ring adenocarcinoma,’ ‘mucinous adenocarcinoma,’ and ‘mucin producing signet ring adenocarcinoma.’ Histologic criteria for retention in the SRCA category were as follows:

- Fulfilling criteria described by Ghannoum and Freedman [2] and Singh et al. [7] including: The presence of prominent signet ring or vacuolated cells arranged in either islands, interconnecting strands and cords, or solid sheets in a myxoid or hyaline stroma or pools of mucin [2, 7].
- Exclusion of other salivary neoplasms with prominent mucin production or those that may show vacuolated cells using morphologic, immunohistochemical, and molecular studies.
- Clinical, histologic and immunohistochemical exclusion of metastasis from a distant site.

Histochemical and immunohistochemical stains were performed on 4 µm sections cut from formalin-fixed paraffin-embedded (FFPE) tissue using a Ventana autostainer (Ventana systems, Tuscon, AZ). Labeling was performed using the i-VIEW 20-diaminobenzamide (DAB) detection kit (Ventana systems, Tucson, AZ) with a brown chromogen substrate for all antibodies except that to S100, which was labeled using ultraVIEW alkaline phosphatase (AP) red detection kit (Ventana systems, Tucson, AZ) with a red chromogen substrate. The antibody company, clone and dilutions are summarized in Table 1.

Ultrastructural analysis was performed on FFPE tissue from all four cases. A 3-mm³ section of the tumor was dissected from the paraffin block of each case, minced into smaller sections, deparaffinized and then fixed in 2 % osmium tetroxide, dehydrated and embedded in EMbed/Araldite resin mixture (Electron Microscopy Sciences, Hatfield, PA). 1 micron thick sections were cut and stained with toluidine blue. Suitable areas for ultrastructural analysis were then selected. Sections were cut at 80 nm and stained with uranyl acetate and lead citrate and examined under a FEI Philips EM208 electron microscope (FEI Company, Hillsboro, Oregon).

Fluorescence in situ hybridization for *ALK* gene rearrangement using a break-apart probe 2p23 and for *ETV6* gene rearrangement using break-apart probe 12p13 were performed (Abbott Molecular, Des Plaines, IL). Cases with more than 20 % of tumor cells showing rearrangement for *ALK* or *ETV6*

Table 1 Histochemical and immunohistochemical stains

Stain	Company	Clone	Dilution
Mucicarmine	Ventana, Tucson, AZ.		
PASD	ThermoFisher, Pittsburgh, PA.		
CK7	Dako, Carpinteria, CA.	OV-TL 12/30	1:200
CAM 5.2	Becton–Dickinson, San Jose CA.	CAM 5.2	1:10
CK5/6	Dako, Carpinteria, CA.	D5/16 B4	1:50
AE1/AE3	Dako, Carpinteria, CA.	AE1/AE3	1:100
P63	ThermoFisher, Pittsburgh, PA.	4A4	1:200
Calponin	Dako, Carpinteria, CA.	CALP	1:50
S-100	Dako, Carpinteria, CA.	N/A	1:500
GCDFP-15	Vector, Burlingame, CA.	23A3	1:25
E-Cad	Dako, Carpinteria, CA.	NCH-38	1:25
Mammaglobin	Zeta Co.	304-1A5 & 51A5	Prediluted
Ki-67	Dako, Carpinteria, CA.	MIB-1	1:100
SMA	Cell Marque, Rocklin, CA.	IA4	Prediluted
Androgen receptor	Dako, Carpinteria, CA.	AR441	1:100
HER2/neu	Dako, Carpinteria, CA.	N/A	1:1500
CK20	Dako, Carpinteria, CA.	Ks20.8	1:50
TTF	Dako, Carpinteria, CA.	8G7G3/1	1:50
Thyroglobulin	Dako, Carpinteria, CA.	N/A	1:8000
CDX2	Biogenex, Fremont, CA.	CDX2-88	1:200
AMA	Dako, Carpinteria, CA.	HHF35	1:100
Synaptophysin	Cell Marque, Rocklin, CA.	N/A	Prediluted
Chromogranin	Ventana, Tucson, AZ.	LK2H10	Prediluted
ER	Ventana, Tucson, AZ.	SP1	Prediluted
PR	Ventana, Tucson, AZ.	1E2	Prediluted
PSA	Ventana, Tucson, AZ.	N/A	Prediluted
PsAP	Ventana, Tucson, AZ.	PASE/4LJ	Prediluted

N/A not available

were considered positive for the particular gene rearrangement. Also, fluorescence in situ hybridization for *EWSR1* gene rearrangement using a break-apart probe 22q12 was performed (Abbott Molecular, Des Plaines, IL). Cases with more than 13 % of tumor cells showing rearrangement were considered positive for the *EWSR1* gene rearrangement. Cutoffs for positivity were derived from extensive in-house testing of cases, positive and negative controls.

Results

One of the five cases had cells with more abundant, more heavily multivacuolated cytoplasm and microcystic growth, as well as an immunophenotype and *ETV6-NTRK3* rearrangement leading to the reclassification as MASC (see also below) [8], leaving four cases of primary SRCA. This case of MASC is still retained for the study for comparative purposes. The remaining four cases included two in-house cases and two cases which were received for consultation. One case (Case 1) was previously reported [9], and the

MASC case was previously included in the recent series [5] of this entity from our institution.

Clinical Findings

The clinical features of the four cases are summarized in Table 2. Interestingly, one patient reported a history of repeated trauma to the area (case 1) and one patient reportedly had received radiation therapy to the head and neck as a child for ring-worms (case 3). The mean patient age was 56.3 years (range 18–81 years). The male to female ratio was 3:1. All but one case were of minor salivary glands origin, while case 2 was in the left deep parotid lobe. The average size was 2.7 cm (range 1.5–4.5 cm). Case 4 demonstrated a positive lymph node on presentation, while the remaining cases were clinically node negative. All patients underwent resection, and case 4 also had a selective neck dissection. Case 4 was lost to follow up. The remaining three patients were alive with no evidence of disease with an average follow up of 38 months (Table 2). The case of MASC was a right parotid tumor from a 65 year old female.

Pathologic Features

The morphologic features of SRCA are illustrated in Fig. 1. While all cases showed some infiltration at low magnification, three cases were still relatively well-circumscribed with rounded pushing borders (Fig. 1a) while

one case was frankly infiltrative, with thin strands and trabeculae of tumor cells infiltrating deep into densely collagenous stroma (Fig. 1b).

All four cases consisted mainly of vacuolated and signet ring cells arranged in cords and trabeculae and embedded in a myxoid stroma (Fig. 1c). Of note, one case (case 2)

Table 2 Clinical features

Case	1	2	3	4
Age (years)	18	81	61	65
Sex	Male	Female	Male	Male
Location	Left buccal vestibule	Left deep parotid	Left buccal mucosa	Left soft palate
Size	4.5 cm	2.0 cm	N/A	1.5 cm
Lymph node status	Negative	Negative	Negative	Positive
Treatment	Resection	Resection	Resection	Resection and bilateral neck dissection
Margin status	Negative	Negative	Negative	Negative
Follow up	24 months, Alive, NED	39 months, Alive, NED	52 months, Alive, NED	Lost to follow up

N/A not available, NED no evidence of disease

Fig. 1 **a** Low magnification showing a well-circumscribed tumor with pushing borders (hematoxylin and eosin, $\times 4$). **b** An ill-defined tumor (*right*) with extensively infiltrative strands and trabeculae of tumor cells (*left*) (hematoxylin and eosin, $\times 4$). **c** High magnification showing trabeculae of vacuolated tumor cells in a myxoid stroma (hematoxylin and eosin, $\times 40$). **d** Infiltrative trabeculae and strands of predominantly plasmacytoid tumor cells in a hyalinized stroma (hematoxylin and eosin, $\times 20$). **e** This particular case shows trabeculae of vacuolated tumor cells in a pool of mucin. Focal tubule formation is also illustrated (hematoxylin and eosin, $\times 40$). **f**, **g** Perineural and angiolymphatic invasion respectively (hematoxylin and eosin, $\times 20$)

Table 3 Histochemical and immunohistochemical profile

Case	1	2	3	4
Mucicarmine	Extracellular and cytoplasmic staining	Extracellular and cytoplasmic staining	Extracellular and cytoplasmic staining	Extracellular and cytoplasmic staining
PASD	Extracellular and cytoplasmic staining	Extracellular and cytoplasmic staining	Extracellular and cytoplasmic staining	Extracellular and cytoplasmic staining
CK7	Positive	Positive	Positive	Positive
CK20	Negative	Negative	Negative	Negative
CAM 5.2	Positive	Positive	Positive	Positive
CK5/6	Focally positive	Focally positive	Negative	Negative
AE1/AE3	Positive	Focally positive	Positive	Positive
P63	Positive	positive	Rare positive cells	Negative
Calponin	Focally positive	Focally positive	Negative	Negative
Actin	Negative	Negative	Negative	Negative
TTF	Negative	Negative	Negative	Negative
Thyroglobulin	Negative	Negative	Negative	Negative
CDX2	Negative	Negative	Negative	Negative
S-100	Negative	Negative	Negative	Negative
AMA	Negative	Negative	Negative	Negative
Synaptophysin	Negative	Negative	Negative	Negative
Chromogranin	Negative	Negative	Negative	Negative
AR	Negative	Negative	Negative	Negative
ER	Negative	Negative	Negative	Negative
PR	Negative	Negative	Negative	Negative
HER-2/neu	Negative	Negative	Negative	Negative
GCDFP	Negative	positive	Negative	Focally positive
PSA	Negative	Negative	Negative	Negative
E-Cadherin	Positive	Focal weak staining	Positive	Positive
Mammaglobin	Weakly positive	Focally positive	Focally positive	Positive
PsAP	Negative	Negative	Negative	Negative
Ki-67	5 %	5 %	60 %	60 %

also showed plasmacytoid cell component and focal hyalinized stroma (Fig. 1d). One case (case 4) had a particularly prominent myxoid stroma with tumor cells often embedded in acellular, coalescing pools of mucin with thin fibrous septae, as previously described [2] (Fig. 1e). This case also had focal tubule formation. Perineural and angiolymphatic invasions were present in two of four and three of four cases respectively (Fig. 1f, g).

Histochemical, Immunohistochemical and Molecular Features

The histochemical and immunohistochemical profile of the four cases is summarized in Table 3. All four cases demonstrated abundant cytoplasmic and stromal mucin on mucicarmine and periodic acid schiff (PAS) after diastase staining (Fig. 2a, b). By immunohistochemical staining, all cases were positive for cytokeratins CAM 5.2 and AE1/AE3. Three of four cases were positive for p63 (Fig. 2c), and two

of four cases were positive for calponin and cytokeratin (CK) 5/6 (Fig. 2d). Other ‘myoepithelial markers’ all muscle actin (AMA), smooth muscle actin (SMA), and S100 were however negative. Additionally, all cases were negative for: CK20, TTF-1, thyroglobulin, CDX2, synaptophysin, chromogranin, androgen receptor (AR), estrogen receptor (ER), progesterone receptor (PR), Her-2/neu, prostate-specific antigen (PSA) and prostatic acid phosphatase (PSAP). Interestingly, all cases showed at least focal staining for mammaglobin, two of four cases were focally GCDFP-15 positive as well (Fig. 2e). E-cadherin was retained and membranous in pattern in all cases (Fig. 2f). *ALK*, *ETV6* and *EWSR1* rearrangements were absent in all four cases.

Distinction Between MASC and SRCA

Figure 3 shows a side by side comparison between SRCA and the one case of MASC that was initially misclassified as SRCA. While morphologically similar, SRCA consists mainly

Fig. 2 **a** Mucicarmine histochemical stain highlighting intracytoplasmic and stromal mucin (Mucicarmine, $\times 20$). **b** PASD histochemical stain highlighting intracytoplasmic and stromal mucin (PASD, $\times 20$). **c** Strong and diffuse nuclear staining with p63 (p63, $\times 20$). **d** Calponin stain is focally positive in two of four tumors (Calponin, $\times 20$). **e** Focal staining for GCDFP which was positive in two or four cases (GCDFP, $\times 20$). **f** Membranous staining for E-Cadherin was retained in all four cases (E-Cadherin, $\times 20$)

of tight cords and trabeculae composed of predominantly univacuolated signet ring cells (Fig. 3a), while the case of MASC showed solid and microcystic growth with more abundant cytoplasm and a mixture of univacuolated and multivacuolated cells (Fig. 3b). Additionally, the nuclei of SRCA are smaller and less vesicular than those of MASC. Immunophenotypically, S100 is negative in our SRCA while it is intensely positive in MASC (Fig. 3c, d). While mammaglobin can be positive in both tumors, only one of four SRCA showed diffuse staining (Fig. 3e, f). Finally, none of the SRCA showed an *ETV6* translocation while the MASC did, as expected (Fig. 3g, h). Admittedly, while comparison of this one MASC with the cases retained as SRCA has limitations, it appears that the distinguishing features noted above are commonly seen even in our and other prior series [8, 10–12].

Ultrastructural Analysis

Since the material was processed for light microscopy, ultrastructural analysis was limited. However, by electron

microscopy, tumor cells did show evidence of secretory differentiation as evidenced by numerous secretory vesicles (Fig. 4a) displacing the nucleus in an eccentric manner. Additionally, primitive lumina formation with apical microvilli was noted as well (Fig. 4b). Where cytoplasm is preserved, there were haphazardly arranged intermediate filaments, microfilaments, and perinuclear structures compatible with tonofilaments, which are in keeping with myoepithelial differentiation (Fig. 4c). Some areas also showed thickened basal lamina (Fig. 4d).

Discussion

Our cases of SRCA show clinical, morphologic and immunophenotypic similarities to those reported previously (Table 4). We excluded the case from de Araujo et al. [1] from our review since the morphologic features, namely the papillary and cystic growth, were different from the other tumors and in fact raise concern that this is a

Fig. 3 **a** Trabeculae of vacuolated and epithelioid secretory myoepithelial tumor cells in a myxoid stroma (hematoxylin and eosin, $\times 20$). *Inset*: On higher magnification the nuclei are fairly round and monomorphic (hematoxylin and eosin, $\times 40$). **b** MASC, exhibiting sheets of epithelioid tumor cells with scattered vacuolated cells (hematoxylin and eosin, $\times 20$). *Inset*: In contrast to SRCA, the nuclei are more vesicular, and though there are signet ring cells here, the cytoplasm is also somewhat more abundant and granular. (hematoxylin and eosin, $\times 40$). **c, d** Negative S100 staining in the tumor cells compared to the strong and diffuse S100 staining in the MASC respectively (S100, $\times 20$). **e** Diffuse and strong staining with mammaglobin was seen in 1 of 4 cases; the other three cases had focal staining (Mammaglobin, $\times 20$). **f** Mammaglobin staining is typically strong and diffuse in MASC (Mammaglobin, $\times 20$). **g** *ETV6* FISH in the SRCA showing no translocation. **h** *ETV6* FISH in the MASC showing evidence of a translocation

MASC instead. Overall this class of tumors appears to have both a secretory and myoepithelial phenotype, hence our proposal to use the term ‘secretory myoepithelial carcinoma.’ This has also been recently validated by Esteva et al. [13] who have used the term ‘mucinous myoepithelioma’ to describe their series of tumors. Of the handful of reported cases, including 4 cases in this study, 11 arose in minor salivary glands and 3 in the parotid. One of the three parotid tumors was in fact considered benign [13]. The

average age at presentation is 57 years (range 18–81 years) with a slight female predominance and a 1:1.33 male to female ratio. The average size of the tumors was approximately 2.0 cm (range 1–4.5 cm). Most tumors were treated with surgical excision alone with negative margins. One patient presented with neck metastasis at the time of initial presentation. Six patients available for follow-up had no evidence of disease with an average follow-up of 33 months. Based on these cumulative findings, it appears

Fig. 4 **a** Tumor cell with abundant secretory vesicles and apical microvilli ($\times 7,100$). **b** Higher magnification of the microvilli ($\times 22,000$). **c** Electron photomicrograph of an epithelioid tumor cell. Note the tonofilaments (*arrow heads*) and intermediate filaments ($\times 7,100$). **d** Epithelioid tumor cells showing thickened basal lamina (*arrow heads*) ($\times 4,400$)

that SRCA behaves in an indolent fashion with a low risk of recurrence and metastasis.

On review of the literature, all but one case had intracytoplasmic positive staining with mucicarmine. Of the 13 cases tested with PAS after diastase, 100 % had intracytoplasmic staining. All 14 cases stained with cytokeratin. Smooth muscle actin and all muscle actin were positive in 9 and in 7 cases respectively. Calponin was positive in 3 cases, p63 was positive in 10 cases and S100 was positive in 9 cases. With the ‘histogenesis’ and ‘reserve cell’ models of tumorigenesis out of vogue for the past few decades [14], SRCA does not necessarily need to be explained in terms of normal cell constituents of salivary gland. Unlike prototypical biphasic tumors, which also show dual differentiation, but instead with two distinctive cell types arranged in ductal and abluminal tubular arrangements, SRCA is unique in that it is morphologically monotypic, but each cell shows both lines of differentiation. Referring back to early ultrastructural studies, however, and accepting the notion that neoplastic myoepithelial cells are not necessarily subject to the same rules as normal myoepithelial cells in terms of ultrastructural features and marker expression, it is reasonable to predict the occurrence of rare ‘biphenotypic’ tumors such as SRCA.

Neoplastic or ‘modified’ myoepithelial cells may not express all myoepithelial markers, and may lose some ultrastructural hallmarks that are expected in normal myoepithelial cells though immune-electron microscopy may still show muscle marker expression [15, 16]. In fact, Dardick et al. [17] even describe a metastasis from a myoepithelial carcinoma showing early acinar differentiation by electron microscopy, suggesting that neoplastic myoepithelial cells may not only have incomplete myoepithelial characteristics, but in some cases may show transdifferentiation towards a luminal phenotype. We believe that SRCA represents an extreme form of this. And of note, this is not the first tumor type described with this form of dual differentiation. The concept of secretory myoepithelial cells has already been proposed for the entity ‘cribriform adenocarcinoma of minor salivary gland origin,’ and has been substantiated by immunohistochemical p63 expression and the detection of myofilaments ultrastructurally.

Of note however, one of our cases (#4) tested negative for all muscle markers by immunohistochemistry though it showed evidence of myoepithelial differentiation on ultrastructural analysis. Of further interest, this particular tumor behaved more aggressively than the others, with

Table 4 Clinical, histologic and immunohistochemical features of the reported cases

Report	case	Age/ Sex	Location	Size	Follow-up	Histologic features	IHC
Freedman [2]	1	72 F	Buccal mucosa	NS	Lost to follow-up	All seven cases had prominent signet ring cells arranged in varying degrees of islands, strands and tightly packed cords in somewhat myxoid to hyalinized stroma. The signet ring cells were relatively sparse in case 5 on account of the relative small size of the tumor. Tumors were intimately associated with the surface mucosa where it showed pseudoepitheliomatous hyperplasia. No pagetoid spread of tumor cells into the surface epithelium was observed. Perineural invasion was present in three cases but no angiolymphatic invasion was present. Cases 1–4 showed tumor cells infiltrating adjacent normal salivary gland tissue and muscle. None of the seven cases were encapsulated, though case 3 was focally delineated from the normal stroma. The seven tumors showed cytoplasmic and stromal mucin proven by positive staining with mucicarmine and PASD.	Positive for CAM 5.2, SMA, s100, HHF-35, p63. Negative for GFAP, calponin, chromogranin.
	2	32 F	Palate	1.0 cm	8 months: NED		Positive for CAM 5.2, SMA, GFAP, s100, HHF-35, p63. Negative for calponin, chromogranin.
	3	53 F	Warthin's duct	1.0 cm	5 months: NED		Positive for CAM 5.2, SMA, s100, HHF-35, p63. Negative for GFAP, chromogranin.
	4	63 M	Palate	2.0 cm	NS		Positive for CAM 5.2, SMA, s100, HHF-35, p63. Negative for GFAP, calponin, chromogranin.
	5	64 F	Maxillary tuberosity	NS	96 months: NED		Positive for CAM 5.2, SMA, s100, HHF-35, p63. Negative for GFAP, calponin, chromogranin.
	6	65 F	Palate	"Large"	lost to follow-up		Positive for CAM 5.2, SMA, GFAP, s100, HHF-35, p63. Negative for calponin, chromogranin.
	7	46 M	Palate	NS	None		Positive for CAM 5.2, SMA, GFAP, s100, HHF-35, p63. Negative for calponin.
Singh [21]	1	38 F	Parotid	3.0 cm	6 months: NED	Tumor is encapsulated with focal invasion. Solid sheets, islands and trabeculae of large cells with intracytoplasmic mucinous vacuoles in a hyalinized stroma.	Intra-cellular mucicarmine. Positive for CK & SMA. Negative for s100, CK20, A-fetoprotein, thyroglobulin.
Esteve [9]	1	69 M	Parotid	NS	NS	Encapsulated, sheets of closely packed nests and trabeculae of plasmacytoid to polygonal cells with abundant eosinophilic to foamy grayish-blue cytoplasm and mild nuclear pleomorphism.	Positive for CK, SMA, S100, GFAP. Intracytoplasmic staining with Mucicarmine and PASD
	2	75 F	Hard palate	NS	NS	Multinodular, well-circumscribed and focally invasive. Nests and sheets of cells with moderate nuclear pleomorphism and abundant lightly eosinophilic cytoplasm with mucinous-type material and focally prominent plasmacytoid configuration. Focal areas of extracellular mucinous material.	Positive for Calponin, CK, S100, CK7, CEA & GFAP. Intracytoplasmic and stromal staining with PASD.
Bastaki	1	18 M	Buccal mucosa	4.5 cm	24 months: NED	Infiltrative tightly packed islands and cords of vacuolated cells in a hyalinized and focally myxoid stroma.	See Table 3
	2	81 F	Parotid	2.0 cm	39 months: NED	Well-circumscribed, tightly packed islands and cords of predominantly plasmacytoid cells with pale eosinophilic cytoplasm with scattered vacuolated cells in a hyalinized stroma.	
	3	61 M	Buccal mucosa	NS	52 months: NED	Well-circumscribed tumor with round pushing borders. Composed of tightly packed islands and cored of vacuolated cells in a myxoid stroma.	
	4	65 M	soft palate	1.5 cm	lost to follow-up	Trabeculae and small islands of vacuolated cells in pools of mucin.	

NS not stated, NED no evidence of disease

lymph node metastasis at presentation. One possibility here is that the lack of myoepithelial immunophenotype in this particular case could be technically secondary to poor specimen preservation and loss of antigenicity of the specimen sample tested. Alternatively, this may truly represent a trans-differentiation towards a more secretory phenotype. The latter scenario may raise opposition to designating a tumor such as this one as ‘myoepithelial’ since ultrastructural analysis is often not feasible in daily practice. However, we felt that this tumor still did belong in the same morphologic spectrum. From a practical standpoint, it is reasonable to reserve the term ‘secretory myoepithelial carcinoma’ for cases where the myoepithelial phenotype can be demonstrated, and to be more descriptive in cases such as this one, perhaps even retaining the term ‘SRCA.’ In the future, a more formal separation may be warranted, particularly if cases such as this one are indeed more aggressive.

As expected, the differential diagnosis for SRCA includes metastatic signet ring carcinomas (gastrointestinal, breast and lung) to the head and neck from distant sites [18–20]. While clinical history is useful, such neoplasms can usually be differentiated from SRCA by immunohistochemical findings as well since none of our SRCA express TTF-1, CDX-2, CK20 or hormone receptors. Moreover, they express membranous E-Cadherin staining in 100 % of the cases (Table 3) while E-Cadherin expression is infrequent or variable in breast, gastric and colonic signet ring carcinomas [21].

Despite some level of morphologic similarity, SRCA do not show a biologic link to *ALK* gene rearranged lung adenocarcinomas with signet ring features [22, 23], MASC [8] or soft tissue type myoepitheliomas [6]. Of note, one case of a parotid myoepithelial carcinoma harboring the *ALK* gene rearrangement was presented in the 2012 College of American Pathologists (CAP) annual meeting [24]. However, the reported case was composed predominately of plasmacytoid myoepithelial cells with occasional spindled myoepithelial cells and lacked the characteristic signet ring or vacuolated cells seen in SRCA.

In summary, SRCA is confirmed to show secretory and myoepithelial differentiation, and is more aptly designated as a ‘secretory myoepithelial carcinoma.’ Our extensive survey fails to show any molecular link with MASC, *ALK* positive lung adenocarcinomas, or soft tissue type myoepithelial tumors. We confirm that SRCA behaves as a low grade malignancy.

Acknowledgements We would like to thank the Electron Microscopy, Immunohistochemistry and In-situ Hybridization Laboratories for their excellent technical support. We would also like to thank Dr. Kathleen Vergona, at the University of Pittsburgh School of Dental Medicine Department of Oral Biology, for her assistance with the ultrastructural assessment of our cases.

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