Published in final edited form as:

Am J Surg Pathol. 2013 July ; 37(7): 1053–1057. doi:10.1097/PAS.0b013e3182841554.

Most Non-Parotid “Acinic Cell Carcinomas” Represent Mammary Analogue Secretory Carcinomas

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Abstract

Acinic cell carcinoma (ACC) is a low grade salivary gland malignancy characterized by serous acinar differentiation. Most ACCs arise in the parotid gland, but ACCs have been reported to originate in non-parotid salivary glands where serous acini are less abundant. Given the recent discovery of mammary analogue secretory carcinoma (MASC) – a salivary malignancy that histologically mimics ACC – a retrospective re-evaluation of non-parotid ACCs is warranted. The surgical pathology archives of The Johns Hopkins Hospital were searched for all ACCs arising outside of the parotid gland. For each case, the histologic slides were reviewed; immunohistochemistry (mammaglobin, S100 protein) was performed; and confirmatory *ETV6* break-apart FISH assay was completed. Demographic and clinical data was obtained from the medical records. Fourteen extra-parotid tumors diagnosed as ACC were identified. Eleven of 14 (79%) tumors harbored the *ETV6* translocation (oral cavity = 9 of 11; submandibular gland = 2 of 2). The translocation positive tumors occurred in 7 women and 4 men ranging in age from 20–86 years (mean, 56), and usually presented as painless masses. Immunohistochemistry for mammaglobin and S100 was positive in all 11 translocation positive tumors, but negative in the 3 translocation negative tumors. Histologically, the translocation positive tumors exhibited uniform cells with vacuolated cytoplasm, microcystic/cystic and papillary architecture, and intraluminal secretions; but the presence of basophilic cytoplasmic granules was conspicuously absent. Basophilic cytoplasmic granules, indicative of true serous acinar differentiation, were present in the 3 translocation negative tumors. Of the translocation positive tumors, only one locally recurred, and none metastasized. Most alleged ACCs of non-parotid origin actually represent misclassified MASCs. The impact of diagnostic error is mitigated by the low grade nature of MASC that, like ACCs, do not appear to be clinically aggressive.

Keywords

Acinic cell carcinoma; mammary analogue secretory carcinoma; *ETV6-NTRK3* translocation; minor salivary gland carcinoma; mammaglobin; S100

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Conflict of Interest: None of the authors have a conflict of interest to disclose regarding this work

Introduction

Acinic cell carcinoma (ACC) is a low grade epithelial neoplasm that comprises about 17% of primary salivary gland malignancies and is characterized by evidence of serous acinar differentiation.(1) The majority (85–90% of cases) of ACCs are encountered in the parotid (1–6). This is not surprising given the parotid's relatively large size predominance of serous acini. Only about 5 to 10% of ACCs arise outside from non-parotid sites. Origin of ACC from minor salivary glands is particularly unexpected given their paucity of normal serous acini.(7)

Mammary analogue secretory carcinoma (MASC) is a recently described salivary gland neoplasm that is characterized by the same *ETV6-NTRK3* gene fusion that is found in secretory breast carcinoma(8), infantile fibrosarcoma(9) and congenital mesoblastic nephroma.(10, 11) The parotid gland is the most common site of origin with about two-thirds of reported cases, followed by the intraoral minor salivary glands and the submandibular gland.(11–16) Prior to its recent description as a biologically distinct salivary gland neoplasm, MASC was erroneously grouped with various other low grade salivary tumor types including adenocarcinoma (not otherwise specified), mucin-producing signet ring adenocarcinoma, mucoepidermoid carcinoma, and especially ACC.(12, 13) The propensity to classify MASC as an ACC is not altogether unexpected given their overlapping histologic features. Both tumor types are comprised of cytologically bland cells with granular cytoplasm, and both exhibit multiple architectural patterns including solid, follicular, microcystic and papillary-cystic growth.

In contrast to true ACCs, MASCs do not show true serous acinar differentiation. Accordingly, there may be grounds to reconsider the identity of zymogen-poor ACCs arising from non-parotid sites. The purpose of this study was to determine the impact of *ETV6* analysis on the reclassification of extra-parotid ACCs. More precise resolution of this mixed group of microcystic-patterned neoplasms represents an initial and important step in discerning differences in clinical behavior, and in identifying tumors that harbor potential molecular targets for biologic therapies.(12)

Methods

Cases

This study was approved by Institutional Review Board of The Johns Hopkins Medical Institutions. The surgical pathology archives of The Johns Hopkins Hospital were searched for all non-parotid carcinomas diagnosed from 1984 to 2012 as ACC. For comparison, salivary gland tumor tissue microarrays containing 16 parotid gland carcinomas diagnosed as ACC were also examined. The Johns Hopkins Hospital's electronic medical records were reviewed for pertinent demographic and clinical information including site of tumor origin and clinical outcome. When needed, additional clinical information was obtained directly from the patient's treating physician.

Immunohistochemistry

Immunohistochemical studies were performed on five-micron sections prepared from formalin-fixed and paraffin embedded (FFPE) tissue using standard autostaining protocols on a Ventana Benchmark XT autostainer (Ventana Medical Systems, Inc. Tucson, AZ). Deparaffinization and antigen retrieval (i-view detection system; Ventana) were carried out as an automated program of the Ventana autostainer. The primary antibodies and final dilutions were: S100 protein (clone 4C4.9; Ventana; prediluted); and mammaglobin (clone 304-1A5; DAKO GenPoint, Carpinteria, CA; 1:200 dilution).

Fluorescence in situ hybridization

Fluorescence in situ hybridization (FISH) was performed on FFPE tissue sections using a commercially available ETV6 dual color break apart probe (07j77-001, Abbott Molecular, Des Plaines, IL). Prior to hybridization the slides were deparaffinized using a VP 2000 processor (Abbott Molecular) in which pretreatment with protease I was used (02j08-032, Abbott Molecular). Following deparaffinization the slides and the ETV6 probe were co-denatured at 75 °C for 7 minutes and allowed to hybridize for 22 hours at 37 °C in humidified atmosphere. At the end of the incubation the slides were washed in 2 X SSC/0.3% NP-40 for 2 min at 72 °C and for 2 min at room temperature, with agitation. Traces of detergent were removed by washing the slides in 2 X SSC at room temperature with agitation. The slides were counterstained with DAPI and a cover slip was applied using Vectashield mounting medium (H-1000, Vector Laboratories, Inc.). A fluorescence microscope was used to evaluate the probe pattern in 50 nuclei. Cells with two fusion signals of one orange and one green fluorochrome were scored as normal. Cells with rearrangements for *ETV6* had one normal fusion signal and one orange and one green signal at a distance from each other. Tumors with >12% of cells exhibiting rearrangement were considered positive.

Results

The results are summarized in Table 1. Twenty carcinomas originally diagnosed as ACC in non-parotid sites were identified (intraoral = 16, submandibular gland = 3, sinonasal tract = 2), compared with 188 diagnosed in the parotid gland. Tissue blocks or unstained slides were available for 14 (intraoral = 11, paranasal sinus = 1, submandibular gland = 2). Eleven of 14 (79%) cases carried the *ETV6* translocation by FISH (Figure 1 inset). The translocation positive cases included 9 of 11 (82%) oral cavity tumors, and 2 of 2 (100%) submandibular tumors. The one tumor of sinonasal origin was negative for the translocation. In contrast, only 3 of 16 (19%) parotid gland ACCs from the tissue microarrays were translocation positive and thus reclassified as MASC.

Histologically, the translocation positive tumors exhibited two main architectural patterns of growth: microcystic and papillary-cystic (Figure 1). They were well circumscribed, and 4 of the 11 cases exhibited entirely intracystic growth (Figure 1A). Perineural invasion was present in 1 case, but lymphovascular invasion was not identified. The mitotic rate was consistently low, ranging from 0 to 4 mitotic figures per 10 high powered fields (mean, 1). The tumor cells had eosinophilic to focally clear cytoplasm that was often vacuolated. The nuclei were oval with open chromatin and a single prominent nucleolus (Figure 3A). Notably, none of the translocation positive tumors contained cytoplasmic zymogen granules. Eosinophilic intraluminal secretions were present in every case. Six tumors were associated with hemosiderin deposition in or around the tumor, but none exhibited a peritumoral lymphoid reaction.

The 3 translocation negative tumors demonstrated a solid architecture without cystic spaces or papillary growth (Figure 2A). Moreover, these tumors each exhibited overt serous acinar differentiation in the form of basophilic cytoplasmic zymogen granules that were diffusely present in the tumor (Figure 2B, Figure 3B).

By immunohistochemistry, the translocation positive tumors were positive for mammaglobin and S100. Staining for each marker was diffuse and strong, even in areas where the tumor cyst lining was attenuated (Figure 1D). The tumor secretions were also strongly positive for mammaglobin in most (7 of 11) cases. In contrast, the translocation negative tumors were consistently negative for mammaglobin and S100.

Based on the above morphologic, immunohistochemical and molecular findings, 11 tumors originally diagnosed as ACC were reclassified as extra-parotid MASCs. The MASCs occurred in 7 women and 4 men ranging from 20–86 years in age (mean, 56 years), and ranged in size from 0.3 cm to 2 cm (mean, 0.9 cm); in one MASC the tumor size was not available. The 3 ACCs, on the other hand, occurred exclusively in women ranging from 25 to 56 years (mean, 40 years). The ACCs measured 0.5 cm, 0.8 cm, and 4 cm (mean, 1.8 cm). The MASCs arose in the soft palate (n=3), submandibular gland (n=2), upper lip (n=2), lower lip (n=2), buccal mucosa (n=1), and hard palate (n=1). The true ACCs were located in the ethmoid sinus, buccal mucosa, and upper lip (1 case each). Most cases (11 of 14) presented as painless masses. The exceptions included two MASCs that were mildly tender. The MASCs were all treated with surgical resection of the primary tumor. One patient also underwent a neck dissection. None of the patients received adjuvant radiation therapy or chemotherapy.

Follow-up information was available in 13 of 14 cases including 10 of 11 patients with MASCs and all 3 patients with ACCs. The length of follow-up ranged from 4 to 85 months (mean 42 months). One MASC of the upper lip locally recurred 5 years after initial resection. The recurrence was completely excised with negative margins. None of the tumors metastasized to regional lymph nodes or distant sites. All 13 patients are currently alive without evidence of disease.

Discussion

Acinic cell carcinoma (ACC) is a low grade salivary carcinoma that usually occurs in the parotid gland. Although the serous acinar cell is a distinguishing feature, the number and distribution of cells exhibiting zymogen granules is highly variable. In those zymogen-poor tumors, the diagnosis of ACC more relies more heavily on certain architectural features. Many ACCs exhibit the formation of microcysts, and in 1975 Hansen described a form of ACC exhibiting intracystic papillary growth.⁽¹⁷⁾ We found that the long standing practice of classifying ACCs on solely on the basis of architectural growth including microcystic and papillary cystic growth in the absence of clear cut zymogen granules is ill advised. (18–20) At least in extra-parotid salivary glands, a sizable percentage of these actually represent MASCs. In our study, almost 80% of extraparotid ACCs needed to be re-classified as MASCs based on an *ETV6* translocation together with strong staining for S100 and mammaglobin. Extra-parotid ACCs do occur, even in unexpected sites such as the oral cavity, but its recognition requires clear cut microscopic evidence of zymogen granules. In the absence of this key finding, the diagnosis of MASC should be considered, particularly in the presence of strong staining for S100 and mammaglobin.

Most diagnostic surgical pathology laboratories are not equipped to detect the *ETV6-NTRK3* translocation, prompting the question as to whether the diagnosis of MASC can be confirmed on the basis of immunohistochemistry alone. We believe that strong staining for S100 and mammaglobin is confirmatory, provided that the tumor demonstrates the prototypic histologic features of MASC (e.g. microcystic and macrocystic growth, tubular formations with intraluminal secretions, minimal cellular pleomorphism), and it does not exhibit basophilic cytoplasmic granules (i.e. zymogen poor). At the same time, the application of immunohistochemical staining as a surrogate for the *ETV6* translocation without supportive morphologic findings is imprudent. Other salivary gland neoplasm are known to be S100 positive including low grade cystic neoplasms (e.g. low grade cribriform cystadenocarcinoma⁽²¹⁾), and the specificity of mammaglobin expression across the full spectrum of salivary gland neoplasms has yet to be determined.

Although MASC is biologically distinct from ACC, it is less certain whether the clinical behavior of these two tumors diverge. In their report of 36 MASC (26 of parotid origin), Chiosea et al. (12) noted a trend towards increased lymph node metastases in MASCs compared to a historic group of ACCs, but the trend was not statistically significant and there were no differences in disease-free survival. In our experience with extraparotid MASCs, only one tumor locally recurred and none spread to regional lymph nodes or distant sites. This low grade behavior is certainly in keeping with the non-aggressive behavior of intra-oral ACCs as has been highlighted in previous reports, keeping in mind that these historical studies (i.e. prior to the time testing for the *ETV6* translocation) undoubtedly include a sizeable number of MASCs.(18–20, 22). This low grade behavior certainly diminishes the clinical consequences of diagnostic misclassification. It should also be noted that small tumor size (median, 0.9 cm) may also contribute to the indolent tumor behavior.

The low grade behavior of extraparotid MASCs also diminishes the therapeutic consequences of diagnostic misclassification. Some have pointed to the *ETV6-NTRK3* translocation as a potential future therapeutic target in the management of patients with MASC, citing the responsiveness of other tumor types with *ETV6* translocations (e.g. acute and chronic leukemias) to tyrosine inhibitors targeting various *ETV6* fusion proteins.(12) In this low grade tumor where surgical resection appears to be curative in most of cases, the development and implementation of biological agents may not be imperative.

Acknowledgments

Funding: This work has been partially funded by the National Institute of Dental and Craniofacial Research (R01 DE013152-11).

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Figure 1.

The extra-parotid mammary analogue secretory carcinomas are usually circumscribed, and they frequently exhibit papillary-cystic (A and B) and microcystic (C) growth. Staining for mammaglobin is strong and diffuse (D). *ETV6* break apart FISH demonstrates one intact gene (i.e., orange and green together) and one rearranged *ETV6* gene (inset of D).

Figure 2.

These extra-parotid acinic cell carcinomas exhibit predominantly solid growth (A and B) and well developed cytoplasmic zymogen granules (B). *ETV6* break apart FISH demonstrated two intact *ETV* genes (inset of B).

Figure 3.

The translocation positive mammary analogue secretory carcinomas were comprised of uniform cells with lightly eosinophilic and vacuolated cytoplasm (A). The tumor cells lacked the basophilic granules that were evident in the translocation negative acinic cell carcinomas (B).

Table 1
Summary of demographic and pathologic findings for patients originally diagnosed with acinic cell carcinoma in non-parotid sites

Location	Age	Sex	Tumor size (cm)	ZGs	Immunohistochemistry		ETV6 translocation	Revised Diagnosis
					S100	MG		
Buccal	42	F	0.8	+	-	-	-	ACC
Buccal	71	M	1	-	+	+	+	MASC
Hard palate	25	F	NA	-	+	+	+	MASC
Soft palate	20	F	0.9	-	+	+	+	MASC
Soft palate	86	F	1	-	+	+	+	MASC
Soft palate	79	F	0.7	-	+	+	+	MASC
Upper lip	40	F	0.5	+	-	-	-	ACC
Upper lip	62	F	0.3	-	+	+	+	MASC
Upper lip	71	M	0.6	-	+	+	+	MASC
Lower lip	59	M	0.9	-	+	+	+	MASC
Lower lip	68	M	0.5	-	+	+	+	MASC
Ethmoid	39	F	4	+	-	-	-	ACC
SG	34	F	0.9	-	+	+	+	MASC
SG	36	F	2	-	+	+	+	MASC

SG, submandibular gland; MG, mammoglobin; ACC, acinic cell carcinoma; MASC, mammary analogue secretory carcinoma; NA, not available.