Human Pathology (2015) **46**, 94–103

ELSEVIER

Human PATHOLOGY

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Original contribution

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☆,☆☆

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Received 3 July 2014; revised 19 September 2014; accepted 24 September 2014

Keywords:
Mammary analogue secretory carcinoma;
Acinic cell carcinoma;
Cystadenocarcinoma;
Salivary gland;
ETV6-NTRK3;
Immunohistochemistry

Summary Mammary analogue secretory carcinoma (MASC) is a recently recognized salivary gland tumor harboring an *ETV6-NTRK3* translocation similar to secretory carcinoma of the breast. Histologically, MASC mimics papillary-cystic, microcystic, and follicular-type acinic cell carcinoma (AciCC) and low-grade cribriform cystadenocarcinoma (LGCCC) of the salivary gland. Using histology, immunohistochemistry (IHC), and molecular genetic techniques, we reevaluated 18 cases originally diagnosed as AciCC between 1993 and 2012. The last of these methods was used to detect the *ETV6-NTRK3* translocation. The results reconfirmed 6 cases as AciCC (3 men; average age, 63 years) and helped us reclassify 10 cases as MASC (6 men; mean age, 46 years) and 2 as LGCCC (2 women; mean age, 48 years). Using IHC, we identified the 3 histologic types according to the expression patterns of vimentin, high-molecular-weight cytokeratin, cytokeratin 19, S-100, mammaglobin, MUC1, GATA-binding protein 3, adipophilin, α-amylase, DOG-1, SOX-10, and p63. The number of tumors diagnosed as MASC indicates that AciCC includes bona fide MASC cases. Because differential diagnosis among zymogen granule–poor AciCC, MASC, and LGCCC tumors is challenging, we recommend using molecular genetic tests for *ETV6-NTRK3* for accurate diagnosis. Furthermore, detailed analyses of hematoxylin and eosin–stained tissues and IHC studies using the markers described here should be incorporated into routine practices.

1. Introduction

Acinic cell carcinoma (AciCC) is considered a distinct salivary gland tumor; however, accurate diagnosis of AciCC is not straightforward because of its diverse histologic and cytologic features [1]. Although AciCC may originate from exocrine acinar cells, intercalated duct cells, or both, pathologists

☆ Presented at the United States and Canadian Academy of Pathology, 103rd Annual Meeting, 2014, San Diego, CA.
☆☆ Competing interests: The authors have no conflicts of interest to disclose.
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http://dx.doi.org/10.1016/j.humpath.2014.09.012
0046-8177/

lack an unambiguous definition of its characteristics [2]. Low-grade cribriform cystadenocarcinoma (LGCCC), a novel noninvasive, low-grade salivary gland adenocarcinoma was first described as low-grade salivary duct carcinoma [3] and was controversially renamed by the World Health Organization in 2005 [4]. Histologically, it mimics papillary-cystic and follicular-type AciCC. Mammary analogue secretory carcinoma (MASC) [5] harbors a characteristic balanced chromosomal translocation, t(12;15)(p13;q25), that generates an *ETV6-NTRK3* fusion similar to secretory carcinoma (SC) of the breast [6]. Histologically, MASC closely resembles zymogen granule–poor AciCC, LGCCC, and adenocarcinoma not otherwise specified. Their differential diagnosis is challenging [7,8]. We describe 18 cases of salivary gland tumors originally diagnosed as AciCC. Histologic, immunohistochemical, and molecular genetic analyses distinguished these tumors from MASC and LGCCC, and some with the *ETV6-NTRK3* translocation were reclassified. We describe their clinicopathological, cytologic, and histologic characteristics useful for routine diagnostics. Furthermore, we describe immunohistochemical diagnostic markers and discuss therapy that targets *ETV6* rearrangements.

2. Material and methods

2.1. Patients

We reviewed 18 salivary gland tumors with detailed clinical data, originally diagnosed as AciCC, in 1993 to 2012 in the Department of Diagnostic Pathology, Fujita Health University.

2.2. Reverse transcription polymerase chain reaction

RNA was extracted from formalin-fixed, paraffin-embedded tissues [9] and converted to complementary DNA (cDNA) using 1 mL of random primers and 200 U of reverse transcriptase (SuperScript II; Life Technologies, Carlsbad, CA). Reverse transcription polymerase chain reaction (RT-PCR) was performed using primers to amplify the 2 *ETV6-NTRK3* transcripts' junction sequences [9]. Messenger RNA and cDNA encoding the TATA-box binding protein served to assess RNA and cDNA quality.

2.3. Fluorescence in situ hybridization

Fluorescence in situ hybridization (FISH) was performed using dual-color Break Apart Rearrangement Probes for *ETV6* (Abbott Molecular, Chicago, IL) [10]. The probes represent 1 fusion sequence, and 1 red and 1 green signal represent separate sequences. When 2 separate signals (a red and a green) were observed, *ETV6* was considered split. The cutoff value for the *ETV6* split was 10%.

2.4. DNA sequence analysis

PCR products were cloned into pCR2.1 (Invitrogen, San Diego, CA) using TA ligation and sequenced using an ALFexpress DNA Sequencer (Pharmacia Biotech, Uppsala, Sweden).

Table 1 Antibodies used for immunohistochemical study

Antibody	Source	Clone	Dilution
LMWK	Becton Dickinson (San Jose, CA, USA)	CAM5.2	1:40
HMWK	Dako (Glostrup, Denmark)	34βE12	1:200
CK14	Novocastra (Newcastle upon Tyne, UK)	LL002	1:50
CK19	Dako (Glostrup, Denmark)	RCK108	1:200
Vimentin	Dako (Glostrup, Denmark)	V9	1:200
S-100 protein	Dako (Glostrup, Denmark)	Polyclonal	1:1000
GCDFP-15	Biolegend (Dedham MA, USA)	D6	1:100
MMG	Dako (Glostrup, Denmark)	304-1A5	1:1
MUC1	Novocastra (Newcastle upon Tyne, UK)	Ma695	1:200
Adipophilin	Fitzgerald (Sudbury Road Acton, MA, USA)	Polyclonal	1:50
GATA3	Biocare Medical (Concord, CA, USA)	L50-823	1:100
AMY	Biomeda (Foster City, CA, USA)	Polyclonal	1:300
DOG1	Nichirei (Tokyo, Japan)	SP31	1:1
SOX10	Cell Marque (Rocklin, CA, USA)	Polyclonal	1:25
p63	Dako (Glostrup, Denmark)	4A4	1:100
Calponin	Dako (Glostrup, Denmark)	CALP1	1:100
CD10	Novocastra (Newcastle upon Tyne, UK)	56C6	1:100
IgA	Dako (Glostrup, Denmark)	Polyclonal	1:2000
Ki-67	Dako (Glostrup, Denmark)	MIB-1	1:200