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Fatty Acid Binding Protein 7 Is a Molecular Marker in Adenoid Cystic Carcinoma of the Salivary Glands: Implications for Clinical Significance[1,2]

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Abstract

Adenoid cystic carcinoma (ACC) is an aggressive malignant neoplasm of the salivary glands. Its diagnosis is difficult due to overlapping features with other salivary tumors. Gene expression analysis may complement traditional diagnostic methods. We searched gene expression patterns in the Gene Expression Omnibus (GEO) database and in our tumor and normal samples. The biologic and prognostic potential of the identified genes was analyzed. The GEO data set of primary xenografted ACCs revealed that expression of five genes, *engrailed homeobox 1* (*EN1*), *fatty acid binding protein 7* (*FABP7*), *hemoglobin epsilon 1*, *MYB*, and *versican* (*VCAN*), was dramatically increased. mRNA expression of *EN1*, *FABP7*, *MYB*, and *VCAN* distinguished our sporadic ACCs from normal tissues and benign tumors. *FABP7* expression appeared to be regulated differently from *EN1* and *MYB* and was crossly correlated with poor prognosis in our ACC cohort. Immunohistochemistry showed that FABP7 protein was predominantly expressed in the nucleus of myoepithelial cells of both tubular and cribriform subtypes. In contrast, in the solid subtype, which is often associated with a lower survival rate, FABP7 protein was uniformly expressed in cancerous cells. One case with cribriform architecture and the highest level of *FABP7* mRNA showed strong FABP7 staining in both duct-type epithelial and myoepithelial cells, suggesting that diffuse expression of FABP7 protein might be related to aggressive tumor behavior and poor prognosis. We propose FABP7 as a novel biomarker in ACC. The molecule may be useful in diagnosis and for identifying more effective therapies targeting this protein or upstream molecules that regulate it.

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Introduction

Adenoid cystic carcinoma (ACC) is a high-grade malignant neoplasm of the salivary glands with unique histology and variable clinical behavior [1–5]. ACC has a propensity to metastasize extensively and the long-term prognosis is not favorable. Distant metastases can develop despite local and regional tumor control and can be delayed, sometimes occurring 10 to 20 years after diagnosis. Unfortunately, therapeutic options for ACC are limited and usually consist of surgery and postoperative radiation therapy. These interventions, however, have failed to affect long-term outcomes in ACC.

The diagnosis of ACC is another challenge. Existing imaging methods, including ultrasonography, computed tomography, magnetic resonance imaging (MRI), and radionuclide scanning, do not

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provide a definitive diagnosis [6,7]. Evaluation of fine needle aspiration biopsy material is not always reliable diagnostically, due to the overlapping microscopic features between ACC and other salivary gland neoplasms [8–10]. Accurate diagnosis, however, is important to guide proper surgery and adjuvant treatment [1–5]. Gene expression analysis will likely be an important complement to traditional diagnostic methods in the diagnosis of ACC [11,12]. As an example, immunohistochemical staining for c-Kit is often used in conjunction with histology to aid in diagnosis of ACC. c-Kit, a proto-oncogene, is overexpressed in almost all ACCs but seldom increased in other head and neck tumors [3–5].

Other potential diagnostic markers for ACC have been reported. For example, t(6;9) chromosomal translocations involving genes encoding transcription factors *MYB* and *nuclear factor I/B* have been found in roughly half of ACCs [13,14]. In addition, a gene expression profile of ACC found elevated expression of a variety of extracellular matrix gene products, including *versican (VCAN)* [15]. More recently, *engrailed homeobox 1 (EN1)* was reported as a biomarker for ACC [16]. However, it is not clear whether these molecules were increased specifically in ACC or to what extent they contribute to its malignant growth, metastasis, and prognosis. The objective of this study was to identify a diagnostic molecular marker for ACC, which would be a predictor of the prognosis and a possible therapeutic target. With a biomarker, advances in ACC management may be possible [2].

We searched the Gene Expression Omnibus (GEO) database for potential diagnostic biomarkers of ACC. Expression microarrays of 11 primary xenografted ACCs revealed that levels of *EN1*, *fatty acid binding protein 7* (*FABP7*; also known as *brain lipid binding protein*), *hemoglobin epsilon 1* (*HBE1*), *MYB*, and *VCAN* were elevated compared to normal salivary tissues and were the five most elevated genes [17]. Similar findings were obtained from 27 tumor samples of sporadic ACCs in our archives. We found that expression of *EN1*, *FABP7*, *MYB*, and *VCAN* were considerably elevated. Expression of these genes distinguished ACCs from normal salivary tissues and benign tumors, including basal cell adenomas (BCAs) and pleomorphic adenomas (PAs). *HBE1* was not detectable with our extracts. We also observed a correlation between *FABP7* expression and overall survival in ACC, suggesting that *FABP7* might have prognostic value in patients with ACC.

This study proposes that FABP7 is a biomarker that can be used to diagnose ACC, aid tumor screening, help delineate surgical margins, predict prognosis, monitor patients in remission, and is a starting point for more effective therapeutic options. We discuss a potential role of FABP7 in ACC based on its subcellular distribution and cell type–specific expression.

Materials and Methods

Tumor and Normal Samples

We obtained 27 ACCs, 4 BCAs, and 5 PAs, as well as 5 normal salivary tissue samples from the University of California, San Francisco (UCSF) Anatomic Pathology archives. Institutional review board (IRB) approval was obtained and UCSF guidelines for handling human tissue were followed. Representative normal salivary tissues were additionally chosen from ACC patients whose tumor samples were included in this study. Slides were reviewed to determine tissue suitability for gene expression analysis.

TaqMan Quantitative Polymerase Chain Reaction Assay

Gene expression was analyzed in triplicate with TaqMan quantitative polymerase chain reaction (qPCR). Total RNA was isolated using RNAeasy kits (Qiagen, Valencia, CA) from formalin-fixed, paraffin-embedded tumor tissue sections composed of at least 70% tumor cells. cDNA from 500 ng of total RNA was synthesized with an RT first-strand kit (Life Technologies, Carlsbad, CA). cDNA (5 ng) was mixed with transcriptase (RT) qPCR master mixes, and aliquots were placed with gene-specific primer sets. The following TaqMan assays (all from Life Technologies) were used: *EN1* (Hs00154977_m1), *FABP7* (Hs00361426_m1), *HBE1* (Hs00362216_m1), *VCAN* (Hs00171642_m1), and *MYB* (Hs00920554_m1). Expression levels normalized to endogenous *glyceraldehyde-3-phosphate dehydrogenase (GAPDH)* were determined by real-time PCR and analyzed at the UCSF Comprehensive Cancer Center Genome Analysis Core Facility. Fold changes in gene expression were calculated as the ratio of expression in each sample to average expression in controls. Statistical analyses and graphs were made using Microsoft Office Excel and XLSTAT (Addinsoft, New York, NY). Statistical comparisons between data sets were made with two-tailed Student's *t* tests and log rank tests, and $P < .05$ was considered significant.

Immunohistochemistry

Immunohistochemistry was performed on unstained sections using antibody-based staining kits for FABP7 (AF3166; R&D Systems, Minneapolis, MN) or goat isotype control (AB-108-C; R&D Systems) at the UCSF Comprehensive Cancer Center Immunohistochemistry and Molecular Pathology Core Facility. The detailed procedure for staining has been described [3]. FABP7 staining was visually estimated by a head and neck pathologist (A.v.Z.).

Results

Tumor Characteristics

A total of 41 samples of salivary tissues from the UCSF archives was included in this study: 27 cases of ACC, 4 BCAs, 5 PAs, and 5 normal salivary gland samples. We included BCAs and PAs in the study because the microscopic features of these tumors often overlap with those of ACC, and distinguishing them is important for appropriate management of patients with salivary gland tumors. All tumors had arisen sporadically. Sixteen ACC tumors occurred in women. The median age at presentation was 58 years (range, 33-91 years). Tumors arose at the following sites: maxillary sinus (nine tumors), submandibular gland (six tumors), parotid gland (five tumors), sublingual gland (two tumors), and one each in the nasal cavity, mandibular mucosa, nasopharynx, base of tongue, and tongue. Tumors were classified by morphologic subtype: tubular (4 cases), cribriform (3), solid (1), combined cribriform and tubular (10), combined solid and tubular (8), and combined cribriform and solid (1). In the BCAs, all four tumors occurred in women and arose in the parotid gland. Median age at presentation was 60 years (range, 40-73 years). In the PAs, three tumors occurred in women. Median age at presentation was 46 years (range, 20-67 years), and four tumors arose in the parotid gland with one in the submandibular gland. Two of five normal salivary tissue specimens were from women. Median age at presentation was 62 years (range, 33-91 years). Three samples represented tissue excised from the submandibular gland; two were from the parotid gland.

Expression of EN1, FABP7, MYB, and VCAN Distinguishes ACCs from Normal Tissues and Benign Tumors in the Salivary Glands

In our search of the GEO database, we obtained gene expression profiles of 11 primary xenografted ACCs and 3 normal salivary gland