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Mutational Landscape of Lacrimal Gland Carcinomas and Implications for Treatment

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Abstract

Purpose—Lacrimal gland carcinomas are rare. Identification of molecular abnormalities underlying lacrimal gland carcinogenesis is critical to the development of new targeted therapies for lacrimal gland carcinomas. The purpose of our study was to look for mutations that can be targeted as new treatments for lacrimal gland carcinomas.

Methods—Genomic DNA from patients with lacrimal gland epithelial neoplasms was analyzed. The Sequenom MALDI TOF mass ARRAY platform was used to profile 168 common oncogenic point mutations in 40 genes. Mutation frequency was assessed overall and by histologic diagnosis. These genetic mutations were then correlated with clinical outcomes in the patients.

Results—The study included 14 males and 10 females with a median age of 45 years (range, 17–75 years). The histologic diagnoses were as follows: adenoid cystic carcinoma (n=16), low-grade carcinoma ex pleomorphic adenoma (n=2), high-grade carcinoma ex pleomorphic adenoma (n=2), squamous carcinoma (n=1), and pleomorphic adenoma (n=3). Analysis revealed 18 oncogenic mutations in 13 patients: *KRAS* mutations in 10 patients (46%), *NRAS* mutations in 2 patients (8%), *MET* mutations in 3 patients (13%), *PIK3CA* mutation in 1 patient (4%), and *BRAF* mutation in no patients. About half of the patients with adenoid cystic carcinoma had oncogenic mutations (7 out of 16, 44%). Of the 16 patients with adenoid cystic carcinoma, 5 had *KRAS* mutations, 1 had *MET* mutations, and 1 had an *NRAS* mutation.

Conclusions—*KRAS*, *NRAS*, and *MET* mutations are frequent in epithelial neoplasms of the lacrimal gland, with the highest rate of mutations found in adenoid cystic carcinoma. Therapies targeting these genes may be effective treatments for lacrimal gland carcinomas.

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INTRODUCTION

Lacrimal gland lesions represent approximately 9% of all orbital lesions. The estimated incidence of lacrimal gland lesions is 1.3 cases per 1,000,000 individuals per year.^{1,2} Epithelial neoplasms account for 22% to 45% of lacrimal gland lesions; the remainder of such lesions are lymphoproliferative or inflammatory processes.^{1,3} A large review by Shields et al found that among lacrimal gland epithelial lesions, 21.6% were pleomorphic adenomas, 27.4% were adenoid cystic carcinomas, and 9% were carcinoma ex pleomorphic adenomas.¹ Benign epithelial lesions, such as pleomorphic adenomas, account for up to 65% of primary epithelial neoplasms of the lacrimal gland.⁴

Lacrimal gland neoplasms are thought to be closely related to their more common counterparts in the major salivary glands. Therefore, the World Health Organization's classification of salivary gland tumors has been adapted to lacrimal gland neoplasms.¹ Despite advances in our understanding of the relationship between histologic subtypes of lacrimal gland carcinoma and biologic behavior,⁵ the survival outcomes for patients with aggressive forms of lacrimal gland carcinoma, such as adenoid cystic carcinoma, remain poor: reported 5-year survival rates for patients with adenoid cystic carcinoma of lacrimal gland are as low as 50%, and reported 15-year survival rates are as low as 15%.⁵⁻⁹ The identification of molecular abnormalities underlying lacrimal gland carcinogenesis is critical to the potential development of specific new targeted therapies.

In this study, we investigated the molecular profiles of tumor tissues in a cohort of patients with lacrimal gland epithelial neoplasms using a platform that probes 168 potentially targetable common oncogenic point mutations.

METHODS

Patients

The Institutional Review Board at The University of Texas MD Anderson Cancer Center approved this study and waived the requirement for informed consent. The medical records of all patients with a diagnosis of epithelial neoplasm of lacrimal gland according to the World Health Organization classification¹⁰ treated at our institution during the period from November 1, 1997, through December 1, 2012, were identified through a search of the Ophthalmology Database at MD Anderson. For each patient, the following data were collected from the medical record: age, gender, histologic subtype of lacrimal gland neoplasm, size of tumor at presentation, American Joint Committee on Cancer (AJCC), 7th edition, T category, initial treatment, follow-up time after treatment completed, and patient status at last contact.

Tissue Specimens

Surgically removed, formalin-fixed tumor samples from the identified patients with a diagnosis of lacrimal gland epithelial neoplasm¹⁰ were retrieved from our institution's tissue bank.

Clinical and Histologic Data

Twenty-four patients with a diagnosis of lacrimal gland epithelial neoplasm were identified. Patients' demographic and clinicopathologic characteristics are described in Table 1. There were 14 males and 10 females. The median age was 45 years (range, 17-75 years). Sixteen patients had adenoid cystic carcinoma, 2 had low-grade myoepithelial carcinoma ex pleomorphic adenoma (patients 12, 13), 2 had high-grade "salivary duct-like" carcinoma ex pleomorphic adenoma (patients 16, 21), 1 had squamous carcinoma, and 3 had pleomorphic adenoma. The AJCC, 7th edition, T category at diagnosis was Tx in 1 patient; T1 in 2 patients; T2 in 4 patients; T4a in 4 patients; T4b in 8 patients; and T4c in 2 patients.

All patients underwent surgery. Twelve patients received postoperative radiation therapy as the only postoperative adjuvant therapy. Eight patients received chemotherapy (Table 2). Three patients received induction chemotherapy, including 1 patient who received intra-arterial chemotherapy at an outside institution. Four patients received postoperative combination chemoradiation therapy. One patient with a HER2-positive adenocarcinoma received postoperative adjuvant external beam radiation therapy followed by chemotherapy with carboplatin, paclitaxel, and trastuzumab. Concurrent chemoradiation was avoided in this patient because of concerns about temporal radionecrosis based on radiotherapy fields and the degree of temporalis/temporal bone resection.

Patients were followed for a median of 33 months after completion of therapy (range, 0.25-186 months). At last follow-up, 17 patients were alive without disease, 3 patients were alive with progressive disease, and 4 patients had died of disease (Table 1). The patient with only 1 week of follow-up was a man with a pleomorphic adenoma who returned for his 1-week postoperative visit but whom we have not been able to reach since that appointment.

Mutational Analysis

Genomic DNA was isolated from 10- μ m-thick paraffin sections using the Epicentre Master pure DNA and RNA isolation kit (Illumina Biotechnologies) according to the manufacturer's protocol following de-paraffinization and proteinase K treatment. The Sequenom MALDI TOF mass ARRAY platform was used to profile 168 common oncogenic point mutations in 40 genes. One microgram of genomic DNA per sample was submitted to the Sequencing and Microarray Facility at our institution. Each specimen was tested in duplicate for every mutation in the Sequenom panel.

RESULTS

Mutations

Results of mutation analysis are summarized in Table 3.

We found *KRAS* mutations in 10 patients (46%), *NRAS* mutations in 2 patients (8%), *MET* mutations in 3 patients (13%), a *PIK3CA* mutation in 1 patient (4%), and no *BRAF* mutations.

Half of the patients with adenoid cystic carcinoma had oncogenic mutations (8 mutations in 7 patients with adenoid cystic carcinoma). Of the 16 patients with adenoid cystic carcinoma,

5 had *KRAS* mutations, 1 had *MET* mutations, and 1 had an *NRAS* mutation. In 1 benign mixed tumor, a *KRAS* mutation was found along with *NRAS* and *PIK3CA* mutations; in another benign mixed tumor, two *KRAS* mutations were found. One high-grade carcinoma had both *KRAS* and *MET* mutations.

Relationship between Mutations and Histologic Subtype, T Category, and Outcome

We did not detect trends between the presence of an oncogenic mutation or specific oncogenic mutations and AJCC T category, specific histologic type, or patient outcome. Of the 7 patients who had progressive disease or died of their disease, 3 had no mutations, 1 had a *MET* mutation, 1 had an *NRAS* mutation, and 2 had *KRAS* mutations. Of the 13 patients in whom mutations were detected, 3 had pleomorphic adenomas and were not staged, 2 had T1 disease, 1 had T2 disease, 2 had T4a disease, 4 had T4b disease, and 1 had T4c disease. Overall, 7 of 14 patients with T4 disease had mutations. Eleven of 16 patients with adenoid cystic carcinoma had predominantly solid (basaloid) histology; 5 of these 11 had *KRAS*/*NRAS* mutations, and 6 did not.

DISCUSSION

We found that *KRAS*, *NRAS*, and *MET* mutations are frequently present in epithelial neoplasms of the lacrimal gland, with the highest rate of mutations found in adenoid cystic carcinoma. Furthermore, we found that *KRAS* mutation coexisted with *NRAS* and *PIK3CA* mutations in 1 pleomorphic adenoma; with an *NRAS* mutation in a second pleomorphic adenoma; and with a *MET* mutation in 1 high-grade carcinoma.

Detection of *RAS* mutations in lacrimal gland carcinomas opens the possibility of considering drugs that target the EGFR-RAS-RAF signaling cascade for treatment of such tumors.

Activation of the EGFR-RAS-RAF signaling cascade is an important pathway in cancer development and is considered a key target for therapeutic molecules. EGFR transmits signals to the nuclei, instructing cancer cells to proliferate and metastasize, and *KRAS* is one of the signaling molecules downstream of EGFR. Anti-EGFR therapies interrupt the cancer-triggering signaling cascade; however, if the *KRAS* gene is mutated, the Kras protein is locked into an active conformation, regardless of whether the EGFR is therapeutically blocked. Data about the correlation between EGFR expression levels and the response of salivary/lacrimal gland carcinoma to anti-EGFR therapy are rare. The mutation status of *EGFR* is known to be a molecular marker to identify patients most likely to benefit from EGFR tyrosine kinase inhibitor therapy.

The Kit, EGFR, NF-kB, and VEGF pathways have previously been implicated in salivary gland cancers (reviewed in^{11,12}).

Previous studies suggest that targeted therapy using EGFR inhibition may hold promise in the treatment of adenoid cystic carcinoma.¹³ Twenty-nine patients with incurable salivary gland cancers (19 of whom had adenoid cystic carcinoma) were accrued in a phase II trial of gefitinib, which is a Food and Drug Administration-approved EGFR inhibitor.^{14,15} Among

24 evaluable patients, 13 had stable disease and 11 had disease progression after 2 months of treatment. Gefitinib was associated with a 53% stable disease rate (10/19) in adenoid cystic carcinoma, which was maintained for at least 16 weeks in 26% (5/19) of the patients in this cohort.^{14,15} Another EGFR inhibitor, cetuximab (Erbix, C225), a human-murine chimeric monoclonal antibody to ErbB1, has been tested in a phase II study in 30 patients with recurrent and/or metastatic salivary gland tumors (23 adenoid cystic carcinoma, 2 mucoepidermoid carcinomas, 3 myoepithelial tumors, 1 cystadenocarcinoma, and 1 acinic cell carcinoma).^{14,15} Among 22 patients evaluable for response after at least 3 months of treatment, 11 patients had stable disease and 9 had progressive disease, while 2 patients refused to continue treatment after 1 month. Lapatinib (GW572016) is another orally active small molecule that reversibly inhibits ErbB1 and ErbB2 tyrosine kinases. A phase II study of lapatinib was conducted in 34 patients with progressive, recurrent, or metastatic adenoid cystic cancer expressing ErbB1 and/or ErbB2.^{14,15} Among 14 patients with adenoid cystic carcinoma evaluable for response, 9 (64%) had stable disease, 3 had progressive disease, and 2 died prior to cycle 2 of therapy. Lapatinib was well tolerated.

Copy number gain and amplification of c-MET, the cell surface receptor for hepatocyte growth factor, has been shown to enhance tumor growth and invasiveness and promote metastasis in certain tumor types. The relevance of its expression in adenoid cystic carcinoma of salivary gland origin was first described in 2003 in a study published by Suzuki et al.¹⁶ Although these findings were novel, the small number of patients in the study limited the impact of the study.

In conclusion, although limited by looking only at mutation analysis, our study found novel genetic mutations in lacrimal gland tumors. While these mutations may play a role in development of lacrimal gland tumors and were more frequently seen in lacrimal gland adenoid cystic carcinomas, it is important to note that some of the mutations were found in both benign and malignant lacrimal gland lesions and thus these mutations may be earlier events in the development of lacrimal gland tumors and a direct oncogenic effect of these mutations cannot be concluded from the current study. Further investigating the molecular mechanisms and the mutation status of proteins downstream from the EGFR-RAS-RAF signaling cascade might identify additional new predictive markers for targeted therapy for lacrimal gland carcinomas.

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Dr Esmaeli and Dr Bell had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

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Precís

KRAS, NRAS and MET are frequent mutations in lacrimal gland carcinomas and may be a potential target for treatment for these rare cancers.

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Figure 1. Morphological features of low-grade lacrimal carcinoma ex pleomorphic carcinoma (a), high-grade lacrimal carcinoma ex pleomorphic carcinoma (b), and adenoid cystic carcinoma solid type (c).

Demographic and Clinicopathologic Characteristics of 24 Patients with Lacrimal Gland Tumors

Table 1

Patient	Sex	Age, y	Lesion Size, cm *	AJCC 7th Edition T Category	Histologic Type	Histologic Subtype	Recurrence	Metastasis	Follow-up Time, mo	Status at Last Contact
1	M	29	3.4	T4c	ACC	Solid	Yes	Yes	59	Alive with metastatic disease
2	M	47	2.0	T4c	ACC	n/a	No	No	13	Alive with NED
3	M	42	3.5	T4a	ACC	Solid	No	Yes	19	Died of disease
4	M	39	3.0	T2	ACC	Cribriform/tubular	No	No	42	Alive with NED
5	F	26	n/a	Tx	ACC	Solid	No	Yes	31	Died of disease
6	M	36	2.0	T2	ACC	Solid	No	Yes	64	Alive with metastatic disease
7	F	51	2.5	T4b	ACC	Solid	No	Yes	93	Alive with metastatic disease
8	M	48	3.0	T4b	ACC	Solid	Yes	Yes	62	Died of disease
9	F	38	3.5	T4a	ACC	Cribriform	No	No	186	Alive with NED
10	M	57	3.0	T4b	ACC	Solid	No	No	24	Alive with NED
11	F	69	2.5	T4a	ACC	Solid and cribriform	No	No	84	Alive with NED
12	F	24	3.2	T2	ACC	Cribriform, tubular	No	No	60	Alive with NED
13	F	43	3.5	T4b	ACC	Solid	No	Yes	23	Died of disease
14	F	65	2.9	T4b	ACC	Cribriform	No	No	3	Alive with NED
15	M	24	3.0	T4a	ACC	Solid	No	No	2	Alive with NED
16	M	54	4.8	T4b	ACC	Cribriform, tubular, and solid	No	No	13	Alive with NED
17	F	69	1.2	n/a	Pleomorphic adenoma	Myoepithelial	No	No	66	Alive with NED
18	F	29	2.5	n/a	Pleomorphic adenoma	n/a	No	No	17	Alive with NED
19	M	57	4.5	n/a	Pleomorphic adenoma	n/a	No	No	0.25	Alive with NED
20	F	40	1.3	T1	Carcinoma ex pleomorphic adenoma	Low-grade myoepithelial	Yes	No	35	Alive with NED
21	M	62	1.3	T1	Carcinoma ex pleomorphic adenoma	Low-grade myoepithelial	No	No	16	Alive with NED
22	M	17	4.0	T4b	Carcinoma ex pleomorphic adenoma	High-grade salivary duct-like	No	No	64	Alive with NED
23	M	61	4.3	T4b	Carcinoma ex pleomorphic adenoma	High-grade salivary duct-like	No	No	8	Alive with NED

Patient	Sex	Age, y	Lesion Size, cm [*]	AJCC 7th Edition T Category	Histologic Type	Histologic Subtype	Recurrence	Metastasis	Follow-up Time, mo	Status at Last Contact
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24	M	75	3.2	T2	Squamous carcinoma	n/a	No	No	36	Alive with NED
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Abbreviations: ACC, adenoid cystic carcinoma; AJCC, American Joint Committee on Cancer; NED, no evidence of disease.

^{*}Maximal dimension of lesion.

Table 2**Clinical Profile of Patients Receiving Chemotherapy**

Age, y	Sex	Histologic Type	Histologic Subtype	AJCC T Category	Chemotherapy Type	Chemotherapy Agents	Metastasis	Status at Last Contact	Follow-up Time, mo
38	F	ACC	Cribriform	T4a	Induction	Taxol, ifosfamide, and cisplatin	No	Alive with NED	186
57	M	ACC	Solid	T4b	Induction/intra-arterial	Intra-arterial cisplatin, doxorubicin	No	Alive with NED	24
43	F	ACC	Solid	T4b	Induction	Vinorelbine and cisplatin	Yes	Died of disease	23
65	F	ACC	Cribriform	T4b	Concurrent	Cisplatin	No	Alive with NED	3
24	M	ACC	Solid	T4a	Concurrent	Cisplatin	No	Alive with NED	2
54	M	ACC	Cribriform, tubular, and solid	T4b	Concurrent	Cisplatin	No	Alive with NED	13
17	M	Carcinoma ex pleomorphic adenoma	High grade	T4b	Adjuvant	Carboplatin, paclitaxel, and trastuzumab	No	Alive with NED	64
61	M	Carcinoma ex pleomorphic adenoma	High grade	T4b	Concurrent	Cisplatin	No	Alive with NED	8

Abbreviations: ACC, adenoid cystic carcinoma; AJCC, American Joint Committee on Cancer; NED, no evidence of disease.

Table 3

Detailed Mutation Analysis of 24 Lacrimal Gland Tumors

Patient	Histology Type	Mutation(s)	Call
1	Adenoid cystic	NRAS_G12DAV_G35ACT	GA,GA
2	Adenoid cystic	None	
3	Adenoid cystic	KRAS_G12DAV_G35ACT	GA,GA
4	Adenoid cystic	None	
5	Adenoid cystic	None	
6	Adenoid cystic	None	
7	Adenoid cystic	None	
8	Adenoid cystic	MET_R988C_C2962T	CT,CT
9	Adenoid cystic	None	
10	Adenoid cystic	KRAS_G12DAV_G35ACT; KRAS_G13DAV_G38ACT	GA,GA
11	Adenoid cystic	KRAS_G13DAV_G38ACT	GA,GA
12	Adenoid cystic	KRAS_G13DAV_G38ACT	GA,GA
13	Adenoid cystic	KRAS_G12DAV_G35ACT	GA,GA
14	Adenoid cystic	None	
15	Adenoid cystic	None	
16	Adenoid cystic	None	
17	Pleomorphic adenoma	KRAS_G12DAV_G35ACT	GA,GA
18	Pleomorphic adenoma	KRAS_G12DAV_G35ACT; NRAS_G12DAV_G35ACT10/22/2012; PIK3CA_H1047RL_A3140GT_R	GA,GA
19	Pleomorphic adenoma	KRAS_G12DAV_G35ACT; KRAS_G13DAV_G38ACT	GA,GA
20	Carcinoma ex pleomorphic adenoma	MET_N375S_A1124G	AG,AG
21	Carcinoma ex pleomorphic adenoma	KRAS_G13DAV_G38ACT	GA,GA
22	Carcinoma ex pleomorphic adenoma	KRAS_G13DAV_G38ACT; MET_T1010I_C3029T	GA,GA; CT,CT
23	Carcinoma ex pleomorphic adenoma	None	
24	Squamous carcinoma	None	

Abbreviations: n/a, not applicable; NED, no evidence of disease.