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CORRELATION OF *CRTC1/3-MAML2* FUSION STATUS, GRADE AND SURVIVAL IN MUCOEPIDERMOID CARCINOMA

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Abstract

Objective—Mucoepidermoid carcinoma (MEC) is the most common malignant tumor of the salivary glands. Tumor stage and grade have historically been important predictors of survival. An oncogenic *CRTC1-* or *CRTC3-MAML2* gene fusion has been identified in a number of MECs. Historically, these gene fusions have been associated with lower grade tumors and better survival. However, reported gene fusion rates and prognosis varies widely across studies, and have not controlled for tumor grade. We sought to identify gene fusion rates and outcomes in our cohort of MEC patients.

Materials and Methods—An IRB-approved retrospective cohort of patients with MEC was identified at the University of Michigan. Clinical, histologic, and outcome data was collected from medical records. RNA was isolated from formalin fixed paraffin-embedded tumor sections, and qRT-PCR was performed to identify *CRTC1/3-MAML2* gene fusions. Sanger sequencing of qRT-PCR products was used to confirm gene fusions.

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Results—Overall, 90 patient MEC tumors were collected (58 low-grade, 25 intermediate-grade, and 7 high-grade). Gene fusions were identified in 59% (53/90) of tumors. On univariate and bivariate analysis, fusion status did not significantly associate with grade or survival.

Conclusion—We have identified a high rate of *CRTC1/3-MAML2* gene fusions in a large cohort of MEC. We do not identify any correlation between fusion status with tumor grade or survival. These findings suggest further characterization of MECs is needed before considering the *CRTC1/3-MAML2* gene fusion as a prognostic biomarker. Additional genetic drivers may account for survival and grade in MECs.

Keywords

Head and Neck Cancer; Mucoepidermoid Carcinoma; *CRTC1-MAML2*; *CRTC3-MAML2*; *CRTC1/3-MAML2*; Gene Fusion

Introduction

Mucoepidermoid carcinoma (MEC) is the most common malignant tumor of the salivary glands. Historically, tumor stage and grade have been important predictors of survival. Treatment for this disease has been primarily surgical, with adjuvant radiation reserved for advanced disease. Overall, survival is favorable in specific cases, particularly in tumors with low grade, early T stage, and absence of nodal disease[1]. Conversely, high grade and advanced stage tumors have worse survival. It is unclear if any genetic factors may play a role in prognosis in these tumors.

More recently, oncogenic *CRTC1-* and *CRTC3-MAML2* gene fusions have been identified in a high proportion of MECs, and has been proposed to be a putative driver mutation in tumors displaying this alteration[2, 3]. *MAML2* is a transcription factor that regulates the *NOTCH* pathway, which has been demonstrated to be aberrantly regulated in a variety of cancers [4]. Historically, *CRTC1/3-MAML2* gene fusions have been associated with lower grade tumors and better survival rates[3, 5]. However, reported rates for the *CRTC1/3-MAML2* gene fusion and associated patient prognosis have varied widely across subsequent studies, with some recent analyses suggesting these gene fusions may not be prognostic of grade or survival[6].

Precision medicine protocols, which seek to identify the specific patients who are most likely to respond to particular treatment regimens, may be useful in MECs. An increasing number of studies are considering these protocols in various cancers, including head and neck cancers[7–9], with promising success in improving clinical outcomes[10]. These protocols have the potential to group patients based on clinical, epidemiologic and genetic features and to use these distinguishing characteristics to predict the most effective therapies on an individual patient basis[11–14]. Since *CRTC1/3-MAML2* gene fusion status, considered here, is a candidate biomarker for patient prognosis in MEC, it may serve as a useful tool to predict the optimal treatments in patients with this cancer type. The association of gene fusion with clinical outcomes might indicate which individuals should receive more aggressive therapies and which might be able to avoid the negative side effects of these more toxic regimens. Here, we evaluate the rates of fusion gene status in a large cohort of MEC.

Additionally, we assess for any contribution of fusion gene status to survival, when controlling for other prognostic variables for survival.

Materials and Methods

Collection of Clinical Specimens

An IRB-approved retrospective cohort of patients with MEC was identified at the University of Michigan. Clinical, histologic, and outcome data was collected from medical records. Death was documented for electronic medical record notes and the Social Security Death Index. We identified 90 MEC patients in this cohort, collected from 1998–2015.

Detection of CRTC1/3-MAML2

Detection of gene fusion status was performed in a two-step process. For a positive control, RNA was isolated from human MEC cell line (HMC-3A), which is known to contain *CRTC1-MAML2*. Total RNA was isolated from formalin-fixed paraffin embedded clinical samples of MEC tumors with a matching H&E stained slide, highlighting the tumor region. Using an 18-gage needle, 2–4 cores were extracted and processed (Qiagen AllPrep). Concentrations were determined using Qubit and samples normalized to a final concentration of 2 ng/μL. For the first step of the nested PCR, 10 ng of RNA was used in a one-tube RT-PCR (Invitrogen SuperScript® III One-Step RT-PCR System with Platinum® Taq DNA Polymerase) for each outer primer pair (*CRTC1-MAML2* and *CRTC3-MAML2*). Products were diluted in water and qPCR was performed with Qiagen's QuantiTech kit with a melt curve performed to monitor number of products formed. As an internal control GAPDH was run alongside each gene fusion primer pair. Primers used were designed as previously described [15].

Survival Analysis

Kaplan-Meier survival curves for *CRTC1/3-MAML2* fusion gene status and clinical factors were calculated using SPSS version 22 software (IBM; Armonk, NY). Disease-free survival (DFS) was calculated from date of diagnosis to biopsy proven recurrence. Disease specific survival (DSS) was calculated from date of diagnosis to date of death from disease. Overall survival (OS) was calculated from date of diagnosis to date of death from any cause. Bivariate survival analyses were performed to control for tumor grade, as this has been the most significant prognostic factor in MEC in previous studies.

Results

Fusion Rates of Mucoepidermoid Carcinomas

CRTC1/3-MAML2 fusions were identified in 59% of MECs. *CRTC1/3-MAML2* fusion positive tumors tended to be non-high grade in comparison to fusion negative tumors (98% non-high grade for fusion positive tumors vs. 84%, for fusion negative tumors $p = 0.02$; Table I). Fusion positive tumors and fusion negative tumors had similar rates of low grade, overall stage, T stage, and nodal stage (Table I). A significant proportion of African-American patients were identified to have fusion gene positive status (92%; $p = 0.006$). No other clinical or histologic variables were correlated with fusion status.

Survival Trends in Mucoepidermoid Carcinomas

We next examined patient survival by MEC fusion status. With univariate analysis, we observed no significant difference in regards to fusion status and survival for MEC OS (p = 0.31), DSS (p = 0.13), or DFS (p = 0.13) (Figure 1).

Given the strong survival impact of grade on MEC status, we next controlled for grade in calculating the effect of fusion status on survival. When grade was controlled, there was no statistically significant effect of fusion status on OS (p = 0.95; Figure 2), DSS (p =0.60), or DFS (p = 0.44). Similarly, there was no significant effect when controlling for other established drivers of MEC survival, namely overall pathologic stage (OS p = 0.31; DSS p = 0.16; DFS p = 0.19), tumor stage (OS p = 0.70; DSS p = 0.34; DFS p = 0.28) and nodal status (OS p = 0.21; DSS p = 0.04; DFS p = 0.08).

Discussion

We have identified a high rate of *CRTC1/3-MAML2* gene fusions in salivary gland MECs in our 90 patient cohort. Notably, we do not identify any correlation between fusion status with tumor grade or survival. This finding is in contrast to initial studies on fusion gene status in MECs and is an importantly shows no difference when controlling for known prognostic variables [3, 5,16–18].

Initial studies on the effect of *CRTC1/3-MAML2* gene fusions in MECs correlated positive fusion status with better survival. These studies, however, did not account for the confounding effect of grade on survival and no bivariate analysis was previously performed. We are the first group to demonstrate that when controlling for grade, fusion status does not appear to play a prognostic role in MECs. Likely, earlier studies, in which high-grade MECs had a low fusion gene rate, were confounded by the deleterious effects of high-grade MECs driving the difference in survival for fusion-negative MECs.

Given the apparent lack of effect on *CRTC1/3-MAML2* gene fusion status and survival, new biomarkers are necessary to identify prognostic markers and genetic drivers of MECs, for both fusion-positive and fusion-negative tumors. Further investigation into alternate genetic drivers and prognostic biomarkers will be important in this cohort. Characterization of novel driver mutations and biomarkers could lead to treatment stratification paradigms and targeted therapy options to improve patient survival. As *CRTC1* and *CRTC3* are involved with cell cycle, and *MAML2* is involved with cell differentiation, further analysis of mutations in cell cycle and cell differentiation pathways by next generation sequencing techniques may highlight new, and novel driver mutations in MECs[19–22]. Previous studies have identified some genetic differences between low-grade MECs, and higher grade MECS, including copy number variations in *SMAD4*, *CDKN2A*, *DCC*, and *LYN*, all cancer-associated genes[23]. Additional studies will be necessary learn more about the potential role of these pathways in MEC, the dependence of their function on fusion status, and their potential correlation with patient outcomes. Overall, this research could lead to biomarkers that might be useful in optimizing and personalizing MEC treatment options.

The development of precision medicine protocols has been greatly accelerated by studies utilizing next-generation sequencing and other molecular techniques, as these methods more easily and more accurately display the detailed and individualized features of each individual sample [24, 25]. These methods also have the potential to identify additional mutations that drive survival as a “second hit” in some fusion gene positive MECs that do poorly [26]. Moreover, no studies have been able to identify the genetic drivers behind fusion-negative MECs, which historically have been proposed to be more aggressive and have worse survival. Further work is necessary to explain survival differences both within and between fusion-negative and fusion-positive disease.

Our study has limitations. Specifically, we have a low number of high-grade MECs in our cohort. Additionally, we had relatively few deaths in our cohort. Increasing the number of high-grade MECs will be important in order to validate effects of grade on survival. Nevertheless, we have a high number of low and intermediate grade tumors with which we can examine fusion status in MEC.

In sum, we have identified a high rate of *CRTC1/3-MAML2* gene fusions in MECs. Notably, we do not see any correlation between MEC fusion status and survival in our cohort, in contrast to previous studies. Previous studies did not control for grade while assessing for survival, with likely confounding effects. Thus, further investigation into additional genetic drivers in MECs that may have an effect on tumor grade and survival is warranted.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights

- We calculated gene fusion rates in patients with mucoepidermoid carcinoma
- This is among the largest mucoepidermoid carcinoma cohorts to date
- Fusion status did not significantly associate with tumor grade or survival
- In previous studies, fusion status prognosis was likely confounded by tumor grade
- Further characterization of additional mutational drivers is needed

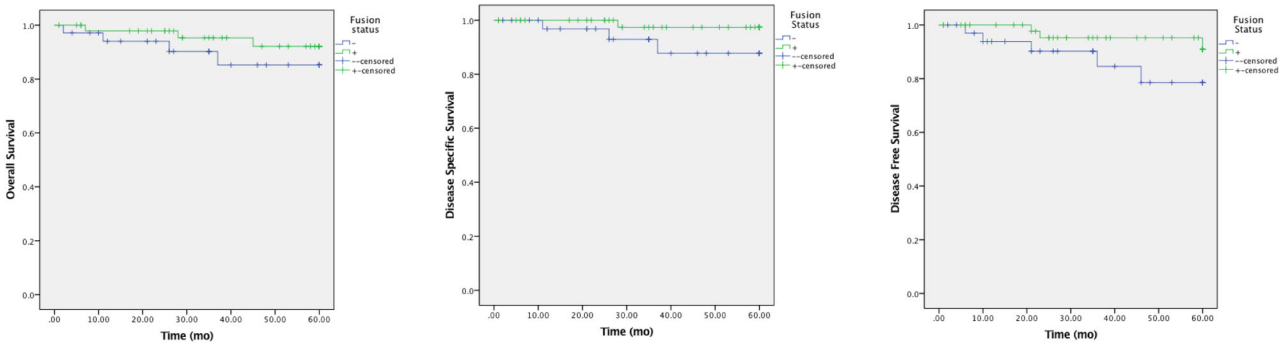

Figure 1. Survival Analysis for MEC Patients by *CRTC1/3-MAML2* Fusion Status

For the 90 MEC patients in our cohort, individuals with and without *CRTC1/3-MAML2* gene fusions did not exhibit any significant difference in OS ($p = 0.31$), DSS ($p = 0.13$), or DFS ($p = 0.13$).

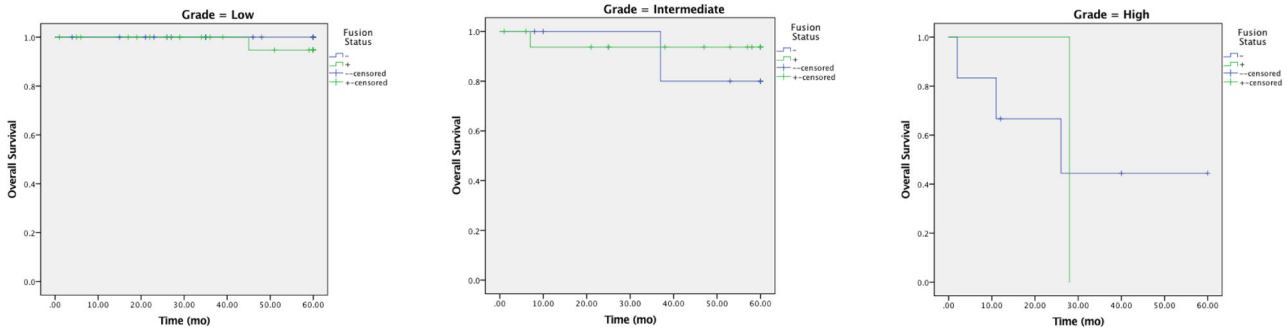

Figure 2. Overall Survival Analysis for MEC Patients by *CRTC1/3-MAML2* Fusion Status and Grade

Controlling for grade, a known predictor of survival, *CRTC1/3-MAML2* fusion status does not exhibit any survival differences in OS (p=0.95). Similarly, fusion status does not exhibit any survival difference for DSS (p =0.60), or DFS (p = 0.44).

Table I
Clinical and Histologic Characteristics by Fusion Status

Demographic and clinical staging is listed in the columns. Overall % is calculated within respective fusion positive and fusion negative groups.

	Fusion Positive n = 53 (%)	Fusion Negative n = 37 (%)	p-value
Grade			
Low	34 (64%)	24 (65%)	
Intermediate	18 (32%)	7 (19%)	**0.02**
High	1 (2%)	6 (13%)	
Site			
Parotid	24 (45%)	16 (43%)	0.85
Minor salivary gland/Other	29 (62%)	21 (57%)	
Pathologic Stage			
I	31 (58%)	17 (46%)	
II	8 (15%)	8 (22%)	0.55
III	7 (13%)	3 (8%)	
IV	7 (13%)	7 (19%)	
unk	0 (0%)	2 (5%)	
Nodal Status			
Neg	44 (83%)	34 (92%)	0.22
Pos	9 (17%)	3 (8%)	
Tumor Stage			
1	38 (72%)	17 (46%)	
2	8 (15%)	8 (22%)	0.17
3	3 (6%)	4 (11%)	
4	4 (8%)	6 (16%)	
unk	0 (0%)	2 (5%)	
Perineural Invasion			
Yes	6 (11%)	6 (16%)	0.48
No	45 (85%)	29 (78%)	
unk	2 (4%)	2 (5%)	
Tumor Size (cm)	2.12	2.47	0.30
Gender			
Male	26 (49%)	18 (49%)	0.97
Female	27 (51%)	19 (51%)	
Ethnicity			
Caucasian	40 (75%)	29 (78%)	**0.006**
Black	11 (21%)	1 (3%)	

	Fusion Positive n = 53 (%)	Fusion Negative n = 37 (%)	p-value
Other/unk	2 (4%)	7 (19%)	
Smoking status			
Current	8 (15%)	4 (11%)	0.83
Former	13 (25%)	9 (24%)	
Never	32 (60%)	24 (65%)	
Age (yrs)	49.3	53.2	0.31