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Predictors of Outcome in Adenoid Cystic Carcinoma of Salivary Glands: a Clinicopathologic Study with Correlation between *MYB* Fusion and Protein Expression

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Abstract

Adenoid cystic carcinoma (ACC) is the second most common salivary gland malignancy and it has a high rate of recurrences and a poor long term prognosis. Our aim was to assess the prognostic factors in ACC and study *MYB-NFIB* fusion and MYB protein expression in a large retrospective cohort of 135 patients with a median follow up of 6.3 years. The 5-year and 10-year local recurrence-free survival rate of 94% and 78%, 5-year and 10-year distant metastasis survival rate of 77% and 58%, and 5-year and 10-year recurrence free survival (RFS) of 66% and 44%. The following features were identified as adverse prognostic factors of RFS on univariate analysis: large tumor size, solid growth pattern, increased mitoses, positive margin, AJCC clinical staging, high grade transformation, vascular invasion, nuclear atypia, open chromatin, prominent nucleoli, and tumor necrosis. However, on multivariate analysis, only increased mitoses (5/10 high power fields), any solid growth pattern, and advanced AJCC TNM staging were independent adverse predictors for RFS. MYB immuno-expression and *MYB-NFIB* translocation were common findings in ACC, occurring in 72% and 59% of the tested ACCs, respectively. The sensitivity and specificity of MYB immunohistochemistry in detecting *MYB-NFIB* fusion was relatively low at 78% sensitivity and 50% specificity. The high prevalence of alterations leading to high expression of the MYB transcription factor family suggests that targeted approaches developed to suppress the expression of these oncogenic transcription factors and/or the transcriptional activity of these proteins would be a rational therapeutic approach to investigate in ACC.

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Conflicts of Interest

The authors have disclosed that they have no significant relationships with, or financial interest in any commercial companies pertaining to this article.

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Keywords

adenoid cystic carcinoma; prognosis; histology; MYB immunohistochemistry; *MYB-NFIB* fusion

Introduction

Adenoid cystic carcinoma (ACC) is the second most common primary salivary gland epithelial malignancy, comprising approximately 10% of all salivary gland neoplasms and 0.6% of all head and neck cancers (1, 2). ACC may arise in major salivary glands (e.g. parotid, submandibular, sublingual, and lacrimal glands) or minor salivary glands distributed throughout the upper aerodigestive tract, especially those located within sinonasal tract, oral cavity, and oropharynx, and more rarely from other sites (1, 3–6). ACC is characterized by relentless clinical course and poor long term prognosis. The 5-year survival rate is about 55% – 89% (3, 7–12), while the 15 or 20 year survival is poorer, being approximately 23 – 40% (5, 11, 12). Clinically, ACC is known for its high risk of recurrence, especially late recurrence. The rate of local recurrence and distant metastasis (DM) is 16–67% and 8–46% respectively (1, 9, 11, 13–15). The reported independent prognostic factors include tumor site, tumor stage, nodal status, margin status, solid pattern, lymphovascular invasion, and intraneural invasion (3, 5, 14–19). However, to date, only a handful of published studies have investigated the prognostic factors in ACC using multivariate analysis. A detailed clinico-pathologic study investigating the prognostic impacts of various histologic features is still needed.

At the molecular level, multiple studies have reported that *MYB-NFIB* fusion is the genetic hallmark and key oncogenic event of ACC, affecting more than 50% of tumors (20–24). The fusion leads to elevated expression levels of MYB transcripts and protein which can be demonstrated by immunohistochemistry (IHC) for MYB (20, 21, 25). However, the existing evidence has shown that the sensitivity and specificity of MYB IHC is relatively low, being 82% and 86% respectively in detecting ACC (21), and 85% and 39% in detecting *MYB-NFIB* fusion (25). Moreover, it remains controversial whether the fusion carries any weight in predicting disease outcome (26, 27).

The current study describes the clinicopathologic characteristics of ACC through a meticulous review of 135 patients treated at a single tertiary cancer center. Its aims are to identify independent clinical or histologic prognostic predictors, and correlate *MYB* fusion and immunoexpression with clinical outcome.

Material and Methods

Inclusion criteria and pathology review

After obtaining Institutional Review Board approval, the pathology database was searched for cases of adenoid cystic carcinoma diagnosed and operated at Memorial Sloan Kettering Cancer Center (MSKCC, New York, NY, United States) between 1985 and 2012. A total of 135 patients were identified and included in the present study. The cases were subsequently subjected to detailed histopathologic analyses. The following histomorphologic features of

the primary tumors were reviewed and documented: tumor size (1 tumor section was examined per 1 cm), mitotic index, atypical mitosis, tumor necrosis, prominent nucleoli, severe nuclear atypia, apoptosis, the most common architectural pattern (e.g. tubular, cribriform and solid patterns, Figure 1A/B/C), the presence and percentage of solid pattern, high grade transformation, squamous metaplasia, vascular invasion (VI), and perineural invasion (PNI). Mitotic index was determined by counting 10 high-power fields (HPFs, 400X, total field size 2.4 mm²) with an Olympus microscope (U-DO model BX41, Olympus America Inc., Center Valley, PA, United States) in the areas of highest concentration of mitotic figures. Microscopic margins were categorized as positive (tumor at the inked margin), close (tumor within 1mm from the inked margin), or negative (tumor > 1mm from the inked margin). High grade transformation was defined as tumors with severe nuclear atypia, elevated mitotic index, prominent necrosis, and loss of biphasic ductal-myoepithelial differentiation as previously described (28). Solid growth was defined as lobulated aggregates of tumor cells arranged in small to large nests/sheets without luminal spaces. Frequent mitotic figures, tumor necrosis and cellular atypia are often a feature seen in the solid pattern compared to the cribriform and tubular patterns. The percentage of solid pattern was calculated using the total volume of solid pattern divided by the total tumor volume. Among the eighty-three patients who underwent lymph node sampling/dissection at the time of the initial surgery, the total number of lymph nodes sampled, size of the largest lymph node with metastasis, as well as the presence of extranodal extension were documented.

Clinical review

All but one patient were followed clinically at MSKCC. The patients' charts were reviewed for age at diagnosis, gender, site of primary tumor, clinical American Joint Committee on Cancer (AJCC) staging (7th edition) (29), as well as adjuvant radiation, chemotherapy, and target therapy. Recurrence was determined on the basis of clinical, radiologic, and pathologic examination, and was further classified as local recurrence and distant metastasis.

MYB immunohistochemistry and fluorescence in situ hybridization

Immunohistochemical stain for MYB was performed on 79 ACCs, using a rabbit monoclonal antibody to v-MYB and c-MYB (clone EP769Y, dilution 1:500, ABCAM, Cambridge, MA, United States) and the Ventana system (Ventana Medical Systems Inc., Tucson, AZ, United States) according to the manufacturer recommendations. A tumor was considered as MYB-positive when ≥ 5% of tumor cells exhibited nuclear staining. The percentage and intensity of MYB IHC were documented.

Additionally, MYB IHC was performed on 56 control cases from different head and neck sites (5 oropharyngeal HPV related squamous cell carcinoma (SCC), 2 basaloid SCC of upper aerodigestive tract, 8 pleomorphic adenomas, 9 salivary duct carcinomas, 6 polymorphous low grade adenocarcinoma, 4 myoepithelial carcinoma, 3 mucoepidermoid carcinoma, 3 acinic cell carcinoma, 1 basal cell adenoma, 1 basal cell adenocarcinoma, 3 sinonasal adenocarcinoma, 2 nasopharyngeal carcinoma, 1 sinonasal rhabdomyosarcoma, 1 sinonasal high grade neuroendocrine carcinoma, 2 metastatic carcinoma to the cervical nodes).

Thirty four of the ACC were previously subjected to Fluorescence *in situ* hybridization (FISH) using a tree-color probe mixed consisting of BACs for 5' *MYB* (RP11-614H6, RP11-104D9, green) and 3' *NFIB* (RP11-413D24, RP11-589C16, red) (BACPAC Resources and Wellcome trust Sanger Institute, Cambridge, United Kingdom) (23). The FISH results from these cases were previously reported by our group (23).

Statistics

All statistical analyses were performed using R Version 3.3. The association between different clinico-pathologic characteristics and the risk of lymph node metastasis were investigated with Fisher's exact test. Disease specific survival (DSS) and recurrence free survival (RFS) were calculated from the date of diagnosis. Survival data was available for all but one patient. Cases with distant metastasis and/or local recurrence diagnosed within the first three months of the initial surgery (n=10) were considered as occurring at presentation and thus excluded from the analysis of RFS. Prognostic value of each clinico-pathologic feature with respect to DSS/RFS was determined using the Log rank test. Variables that were significant on univariate analyses for RFS were subsequently subjected to multivariate analyses using the Cox proportional hazards model. As there were only 17 disease-related deaths in our cohort, multivariate analysis for DSS was not performed. P values less than 0.05 were considered to be statistically significant.

Results

Clinico-pathologic characteristics of the study cohort

The clinico-pathologic features of the study cohort are summarized in Table 1. The median age at diagnosis was 54 (range 21 – 93, interquartile range ITR 46 – 68). There was a female predominance with a female to male ratio of 1.9:1. The majority of the tumors (63%) originated from the minor salivary glands of oral cavity (n = 72), sinonasal tract (n = 9), nasopharynx (n = 1), and trachea (n = 3), while the remaining cases were located in the major salivary glands, including submandibular gland (n = 24, 18%), parotid gland (n = 22, 16%), lacrimal gland (n = 2, 1%), and sublingual gland (n = 2, 1%). The median tumor size was 2.5 cm (range 0.70 – 12 cm, IQR 1.7 – 3.4 cm). The tumors were staged according to the AJCC (7th edition) (29) as follows: stage I in 32/130 (25%); stage II in 29/130 (22%); stage III in 26/130 (20%); and stage IV in 43/130 cases (33%). The majority of the patients (82%) were treated in addition to surgery with adjuvant radiation therapy. Additionally, 18% of the patients had chemotherapy including three received tyrosine kinase inhibitors.

Histologically, cribriform growth pattern was the most common pattern seen in the tumors (72%). Solid growth pattern (Figure 1C), was present in 33 ACCs (24%). Among these 33 tumors, 23 contained at least 30% solid area and 17 had solid pattern as the most common architecture. High grade transformation (HGT) was noted in 16 tumors (12%). Tumors with HGT showed comedo-type tumor necrosis, brisk mitotic activity with an elevated mitotic index of 5 per 10 HPFs (median: 15/10 HPFs, mean: 20/10 HPFs, range 5–60/10 HPFs), and severe nuclear atypia (Figure 1D/E). All but one tumor with HGT had solid pattern, and 15 had 30% solid growth. Five tumors with HGT exhibited focal squamous metaplasia.

Perineural invasion and positive margin were common findings in ACCs, affecting 88% and 50% of the patients, receptively. Additionally, elevated mitotic index (5/10 HPFs), tumor necrosis, prominent nucleoli, and vascular invasion were noted in 44%, 24%, 13%, and 10% of the primary tumors.

MYB immunohistochemistry and FISH results

MYB expression was identified in 57/79 (72%) of tested ACCs (Table 1 and Figure 1F). MYB immunostaining was categorized as follows: 5–49% tumor cells staining (n = 28, 35%), and 50% tumor cells labeling (n = 29, 37%). The intensity of the positive immunostaining was strong in 23 (40%) tumors, moderate in 27 (47%), and weak in 7 (12%) cases. The MYB IHC showed predominant staining in the tumor's periphery in 22/57 (39%) cases. The immunostaining positivity did not differ among different growth patterns (Fisher's exact test, $p = 0.919$). In ACCs with a tubular or cribriform pattern, in which dual epithelial and myoepithelial components could be appreciated, the MYB IHC was positive mainly in the myoepithelial cells. Five out of 56 (9%) control tumors showed positive MYB immunostaining (two oropharyngeal HPV SCC, one basaloid SCC, one nasopharyngeal carcinoma, and one sinonasal rhabdomyosarcoma).

Among the 34 ACCs tested for *MYB-NFIB* FISH, 20 (59%) were positive and 14 (41%) were negative for the translocation. The fusion status did not appear to be related to the tumor growth pattern or the site of origin (Fisher's exact test, $p = 0.454$ and 0.477 respectively). 11/20 (55%) fusion-positive and 10/14 (71%) fusion-negative tumors originated from the minor salivary glands. The MYB IHC was performed on all 34 tested with FISH. Compared to the gold standard *FISH* test, the sensitivity and specificity of MYB IHC were 78% and 50%, respectively.

Seven tumors were positive for MYB IHC but negative for *MYB-NFIB* translocation, while four were negative for MYB IHC but positive for the translocation (Table 5).

Prognosis and predictors of adverse outcomes

134 patients were followed at MKSCC with a median follow up of 6.3 years (range 0.01 – 29.5 years). Among the 83 patients who had lymph node sampling at the time of the initial surgery, 21 (25%) had metastasis to at least one regional lymph node. One lymph node showed tumor involvement by direct extension. No clinical/histologic features (e.g. tumor size, growth patterns, solid pattern, high grade transformation, nuclear polymorphism, tumor necrosis, and elevated mitotic activity) predicted lymph node status (Fisher's exact test, $p > 0.05$, Table 2).

Seventeen patients died of their disease (DOD). The 5-year, 10-year, and 20-year disease specific survival was 96% (.95 CI 92 %, 100%), 80% (.95 CI 71%, 91%), and 74 % (.95 CI 63%, 88%) respectively. Thirteen of these 17 patients developed distant metastases (DM) either at the time of presentation (n = 2) or during the course of their disease (n = 9). The other four patients died of unresectable local recurrence. The tumor in nine of the fatal cases contained solid pattern; five showed HGT of the primary tumor. Univariate analysis using log rank test showed that tumor size, elevated mitotic index, solid growth pattern, HGT,

vascular invasion, severe nuclear atypia, prominent nucleoli, and tumor necrosis were significant predictors for DSS (Table 3).

Fifty-six patients developed recurrence during clinical follow-up (Table 4), including 28 with DM only, 19 with local recurrence only, and 9 with both local and distant recurrences. The 5-year, 10-year, and 20-year recurrence free survival was 66% (0.95 CI 57%, 75%), 44% (0.95 CI 34%, 57%), and 36% (0.95 CI 26%, 51%), respectively. This cohort showed a 5-year and 10-year local recurrence-free survival rate of 94% and 78%, 5-year and 10-year distant metastasis survival rate of 77% and 58%. One patient with ACC showing a predominant cribriform pattern without solid pattern or HGT developed local recurrence 26.2 years after the initial surgery. The following features were identified as adverse prognostic factors using the log rank test of recurrence free survival: large tumor size, the presence and percentage of solid pattern, elevated mitotic index, positive margin, advanced AJCC clinical staging, HGT, vascular invasion, open chromatin, severe nuclear atypia, prominent nucleoli, and tumor necrosis (Table 4). On multivariate analysis using Cox proportional hazards model, the presence of solid pattern, elevated mitotic index of $\geq 5/10$ HPFs and AJCC clinical staging independently predicted shorter RFS (solid pattern: hazard ratio HR = 2.36, 95% confidence interval CI = 1.31 – 4.23, $p = 0.004$; mitotic index: HR = 3.03, 95% CI = 1.65 – 5.56, $p < 0.001$; and stage: HR = 1.63, 95% CI = 1.24 – 2.15, $p < 0.001$; Figure 2), while the other features failed to reach significance ($p > 0.05$). The prognosis did not differ significantly between ACCs with 1–30% of solid growth pattern and those with $>30\%$ solid area ($p > 0.05$, Figure 3). A positive MYB IHC or the presence of *MYB-NFIB* fusion did not predict lymph node metastasis, DSS or RFS (Tables 2, 3 and 4).

Discussion

Adenoid cystic carcinoma is the second most common salivary gland malignancy with an annual incidence of 1,200 cases in the United States (2, 30). Clinically, the tumor is characterized by a propensity for perineural spread, relentless slow growth, high frequency of local and distal recurrence as well as poor long term survival (1, 5, 9, 11–15, 17, 18).

Overall, ACC has been reported to have a 5-year survival rate of 55% – 89% (3, 7–12), 15 or 20-year survival of 23 – 40% (5, 11, 12), local recurrence rate of 16– 67%, and DM rate of 8 – 46% (1, 9, 11, 13–15). The 5-year and 20-year recurrence free survival in our cohort was 66% and 36% respectively, emphasizing the tendency of ACC to develop late recurrence. The most frequent sites of ACC were reported to be in minor salivary glands of the larynx, pharynx, and oral cavity, in particular the palate (7, 13, 18, 26, 31). However, ACC of the major salivary glands do occur at a frequency of 29% to 43% (18, 26). These findings have been confirmed by the results of this study.

To date, only a handful of multivariate analyses have been published investigating the prognostic impacts of various clinico-pathologic factors in ACC (3, 5, 14–19). The most consistent independent predictors repeatedly identified in these studies appeared to be AJCC TNM staging and margin status (3, 5, 14–16, 18). Additional independent adverse prognostic factors reported in a single multivariate study include female gender, older age, sinonasal or paranasal sinus location, solid pattern, LVI, and intraneural invasion (3, 14–17).

Similar to prior studies, we found TNM staging to be an independent predictor for RFS. In contrast to prior reported studies (14, 16), positive margin was not identified as an independent adverse prognostic factor in our study; this could be related to the infiltrative nature of ACC and its propensity to grow along the nerves which makes it difficult to secure a true negative margin even when it appears histologically negative.

In the study herein, lymph node metastasis was found in a significant number of patients, being 15% in the entire cohort and 25% in patients who had lymph node sampling; this seems to be higher than the reported lymph metastasis rate in typical adenoid cystic carcinoma (1, 15, 16, 28, 32), and is likely to be related to selection bias in performing lymph node sampling and referral bias as the study cohort included patients from a tertiary cancer center. Among the 21 patients who harbored nodal metastasis, the majority (13/21, 62%) were patients with intraoral or oropharyngeal ACC. None of the oropharyngeal ACC was positive for p16 and/or high risk human papillomavirus. In our cohort, it appeared that intraoral/oropharyngeal ACCs were associated with higher risk (38%) of regional metastasis compared to those from other sites (14%), which was in agreement with results from previous studies (16, 32).

Histologically, adenoid cystic carcinoma is a biphasic tumor composed of ductal and myoepithelial cells, forming tubular, cribriform, and solid growth patterns (1, 2). Two early studies using univariate analysis from 1978 and 1984 have shown that tumors containing solid pattern, especially those with >30% of solid area, were associated with an increased risk of recurrence and a decreased overall survival (OS) (12, 33). Since then, a histologic grade scheme has been proposed for ACC based on the presence and percentage of solid pattern, i.e. grade 1 – tumors with no solid growth; grade 2 – 1 to 30% of solid growth; and grade 3 – 30% solid growth. However, the exact cut off percentage of solid pattern and the prognostic value varied in the subsequent studies. For example, Spiro and Huvos utilized a grading system of 0%, <50%, and > 50% of solid pattern and found that grading was not a significant predictor for DSS and local recurrence in a cohort of 184 ACCs (34). In contrast, two recent multivariate analyses on 218 and 78 ACCs respectively have shown that the presence of any solid pattern could independently predict decreased DSS (15, 19). In accordance with these recent studies, we found that solid architecture was an independent adverse prognostic factor for shortened RFS. However, in our cohort, the percentage of solid growth (30% or > 30%) did not carry any additional significant impact on clinical outcome. Despite the prognostic significance of solid growth pattern, in our opinion, grading ACC should not be based on the percentage of solid pattern alone for multiple reasons. First, ACC is an extremely locally aggressive tumor and seems to act at the least as an intermediate grade malignancy even in the absence of solid pattern. This is especially true when compared to other types of salivary gland malignancies. Hence the concept of a low grade adenoid cystic carcinoma seems questionable. Second, in the current study, the percentage of solid growth pattern did not seem to impact tumor prognosis and any solid growth was found to be significant. Third, the criteria in most histologic grading systems include multiple parameters (proliferative activity, growth patterns/architecture, cytology, and tumor necrosis) and are not limited to tumor growth pattern solely. Interestingly, the current study is the first to show that increased mitotic activity is an independent prognostic

parameter in ACC on multivariate analysis. Hence, it is important to document mitotic count in the pathology report.

Perineural invasion is notoriously common in ACC, being reported in 29 to 63% of cases (6, 13, 17, 18, 35), and in 88% in our cohort. However, with the exception of one study showing an association of PNI with the extent of local disease and local recurrence, multiple previous studies have failed to reveal a significant association between perineural invasion and clinical outcomes, e.g. OS, DSS, regional or distant metastasis (6, 13, 17, 18, 35). Similarly, in our cohort, the presence of PNI did not predict OS, DSS, nodal and distant metastasis in ACC.

High grade transformation (or dedifferentiation), defined as severe nuclear atypia, brisk mitotic activity, prominent necrosis, and loss of the biphasic ductal-myoepithelial differentiation, has been reported in ACC (28, 36, 37). Although, distinguishing solid ACC from ACC with HGT might be sometimes challenging, the aggressive histologic features of solid ACC in general appear to be extensive and exaggerated in ACC with HGT compared to solid ACC. The largest series reported of ACC with HGT was by Seethala et al. in 2007, which included 11 cases (28). In the latter study, the mitotic index in the HGT component ranged from 5 to 67 per 10 HPFs with a mean of 17/10 HPFs and a median of 11/10 HPFs, which was similar to that reported in our series (mean: 20/10 HPFs, median: 15/10 HPFs, range: 5–60/10 HPFs). Seethala et al. have reported a highly aggressive clinical course and a high propensity to lymph node metastasis (over 50%) in HGT (28). To our knowledge, our cohort which included 16 ACCs with HGT is the largest reported series of ACC with HGT to date. In contrast to Seethala's study, we failed to identify any clinical significance for HGT in predicting lymph node metastasis. While ACCs with HGT were associated with an increased risk of disease-specific death and recurrence on univariate analysis, on multivariate analysis, HGT was not an independent predictor of outcome in RFS. Rather, it was the presence of elevated mitotic activity and solid pattern that independently drove clinical behavior in ACC. The fact that HGT was not an independent predictor for RFS may be explained by the small number of the cases with HGT. There were not enough disease-specific deaths to perform multivariate analysis for DSS.

Fusions involving the *MYB* family of oncogenes are the genetic hallmark and key oncogenic event in the majority of ACC (20–24, 27, 38). The reported chromosomal translocations in ACC include t(6,9) *MYB-NFIB*, t(8,9) *MYBL1-NFIB*, and t(8,14) *MYBL1-RAD51B*. These fusions result in high expression of MYB or MYBL1 proteins, which act on similar downstream target genes associated with apoptosis, cell cycle control, cell adhesion, angiogenesis, and differentiation (30). The *MYB-NFIB* fusion, in particular, has been identified in 57% to 86% of ACC (20–23). Moreover, MYB overexpression detected on IHC studies can be seen in fusion-positive ACCs. However, existing evidence shows that MYB immunostain has a relatively low sensitivity and specificity for *MYB* fusion (85% and 38% respectively) (25), and for a diagnosis of ACC (82% and 86% respectively) (21). Such findings were confirmed by the results in our cohort, which showed 78% sensitivity and 50% specificity of MYB IHC in detecting *MYB-NFIB* fusion. As a diagnostic marker, MYB IHC does not seem to be specific in the differential diagnosis of ACC, as around 30% of ACCs are negative and other tumor types such as squamous cell carcinoma can be positive

for this protein. The prognostic values of the genetic alterations in ACC remain controversial. While results from Rettig et al. (2015) (26) and the present studies failed to established any significant impact of *MYB-NFIB* fusion status and MYB immuno-positivity on disease outcome; Brayer et al. (27) have shown that a combined high expression of MYB and MYB1 measured by RNA-seq correlated with advanced AJCC stage and poor outcome on univariate analysis. As only a portion of the study cohort (35/134, 25%) was tested for *MYB-NFIB* fusion and the utilized FISH probe did not recognize the other fusion variants, additional studies are needed to better characterize the utility of MYB IHC and the impacts of *MYB* fusion on disease progression. Currently, there is no standard therapy specifically targeting *MYB* oncogene families. However, a recent study has shown that some *MYB* rearrangements drive MYB expression by positioning super-enhancer elements to the *MYB* locus. The hypothesis that MYB targets gene are also regulated by super-enhancer elements suggests that bromodomain inhibitors may be a rational therapeutic approach for this disease, and the authors indeed demonstrated that a bromodomain inhibitor blocked and modestly suppressed MYB expression in low grade ACC preclinical models (39), suggesting that MYB and its transcriptional program may be viable therapeutic targets.

Conclusions

Increased mitoses, solid growth pattern, and advanced AJCC staging are independent adverse predictors for recurrence in adenoid cystic carcinoma. The mere presence of solid growth but not its percentage seems to be prognostically significant. Since the currently used “grading” system of ACC is solely based on the presence of >30% solid growth, consideration should be given to change the grading scheme by factoring in mitotic rate. Whatever one’s position is in regard to the controversies surrounding the grading system of ACC, pathologists need to document mitotic activity and any solid growth in their reports. MYB IHC can be positive in fusion positive and negative ACCs and does not seem to be specific. Although there is no correlation between *MYB-NFIB* translocation and adverse prognostic factors, the presence of the translocation in a significant proportion of adenoid cystic carcinoma (around 60%) makes it a prime target for therapy.

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Figure 1. Histologic features of adenoid cystic carcinoma

(A,B) An adenoid cystic carcinoma exhibited predominantly tubular (T) and cribriform (Cr) growth patterns (hematoxylin and eosin stain, magnification 100x in panel A and C, 200X in panels B, D, and F, and 400X in panel E). Myxoid and hyalinized basement membrane material is noted in the cribriform area. (C) Solid growth pattern (So) of adenoid cystic carcinoma. (D,E) High grade transformation of adenoid cystic carcinoma, showing comedo-type tumor necrosis (N), brisk mitotic activity (arrow heads), nuclear atypia and loss of biphasic ductal-myoepithelial differentiation. (F) MYB immunostain is strongly positive in myoepithelial cells.

Figure 2. Kaplan Meier curves for recurrence free survival

Univariate and multivariate analyses showed that solid growth pattern (A), an elevated mitotic index (B) their co-occurrence (C) and advanced AJCC clinical staging (D) were adverse prognostic factors in adenoid cystic carcinoma.

Figure 3. The prognostic significance of solid pattern (A/B) and mitotic index (C/D)
 Adenoid cystic carcinomas with any solid growth pattern were associated with significantly worse disease specific survival ($p < 0.001$, A) and recurrence free survival ($p < 0.001$, B). Tumors with $>30\%$ of solid architectures had similar prognosis compared to tumors with 1–30% of solid component ($p = 0.36$). (C/D) Mitotic index predicted DSS and RFS.

Table 1

Clinicopathologic features of 135 patients with adenoid cystic carcinoma

		N	Column%
Gender	Female	89	66%
	Male	46	34%
Age, median (range)		54 (21–93)	
Tumor size	< 2 cm	40	30%
	≥ 2 cm	95	70%
Tumor site	Minor salivary gland	85	63%
	Submandibular gland	24	18%
	Parotid gland	22	16%
	Lacrimal gland	2	1%
	Sublingual gland	2	1%
Tumor site	Sinonasal/nasopharynx	14	10%
	Oral cavity/oropharynx	66	49%
	Other sites	55	41%
AJCC clinical Stage (n = 130)	I	32	25%
	II	29	22%
	III	26	20%
	IV	43	33%
Lymph node status (n=83)	Negative	62	75%
	Positive	21	25%
Most common growth pattern	Tubular	21	16%
	Cribriform	97	72%
	Solid	17	13%
Solid pattern	Absent	102	76%
	Present	33	24%
30% solid pattern	Absent	112	83%
	Present	23	17%
High grade transformation	Absent	119	88%
	Present	16	12%
Vascular invasion	Absent	122	90%
	Present	13	10%
Perineural invasion	Absent	16	12%
	Present	119	88%
Severe nuclear atypia	Absent	115	85%
	Present	20	15%
Open chromatin	Absent	15	11%
	Present	120	89%
Prominent nucleoli	Absent	117	87%

		N	Column%
	Present	18	13%
Mitotic Index	< 5/10 HPFs	75	56%
	5/10 HPFs	60	44%
Apoptosis	Absent	10	7%
	Present	125	93%
Necrosis	Absent	102	76%
	Present	33	24%
Squamous metaplasia	Absent	130	96%
	Present	5	4%
Margin status (n = 131)	Negative	29	22%
	Close	36	28%
	Positive	66	50%
MYB IHC (n = 79)	Negative	22	28%
	Positive	57	72%
MYB-NFIB FISH (n = 34)	Negative	14	41%
	Positive	20	59%
Follow up time, years, median (range)		6.3 (0.01 – 29.5)	
Radiation therapy (n = 129)	Yes	106	82%
	No	23	18%
Chemotherapy and targeted therapy (n = 128)	Yes	23	18%
	No	105	82%

AJCC: American Joint Committee on Cancer, HPFs: high power fields, IHC: immunohistochemistry, FISH: fluorescence in situ hybridization.

Table 2

Predictors for regional metastasis in adenoid cystic carcinoma.

		Lymph Node Status						P value
		Total		Negative		Positive		
						N	column %	
Total			83	62	75%	21	25%	
Gender	Female		54	41	76%	13	24%	0.794
	Male		29	21	72%	8	28%	
Tumor Site	Lacrimal gland		0	0	0%	0	0%	0.422
	Minor salivary gland		44	29	66%	15	34%	
	Parotid gland		16	13	81%	3	19%	
	Sublingual gland		2	2	100%	0	0%	
	Submandibular gland		21	18	86%	3	14%	
	SN/NP		6	6	100%	0	0%	
Tumor site	Oral cavity/oropharynx		34	21	62%	13	38%	0.056
	Others		43	35	81%	8	19%	
	2cm		21	18	86%	3	14%	
Tumor size	2cm		62	44	71%	18	29%	0.243
	Tubular		13	10	77%	3	23%	
Most common architectural pattern	Cribriform		59	43	73%	16	27%	0.923
	Solid		11	9	82%	2	18%	
Solid pattern	Absent		60	47	78%	13	22%	0.263
	Present		23	15	65%	8	35%	
30% solid pattern	Absent		68	51	75%	17	25%	1.00
	Present		15	11	73%	4	27%	
High grade transformation	Absent		71	54	76%	17	24%	0.488
	Present		12	8	67%	4	33%	
vascular invasion	Absent		73	56	77%	17	23%	0.264
	Present		10	6	60%	4	40%	
Perineural invasion	Absent		9	9	100%	0	0%	0.103

		Lymph Node Status						P value
		Total		Negative		Positive		
						N	column %	
		N	N	N	column %	N	column %	
Severe nuclear atypia	Present	74	53	72%		21	28%	0.744
	Absent	69	52	75%		17	25%	
	Present	14	10	71%		4	29%	
Open chromatin	Absent	11	11	100%		0	0%	0.058
	Present	72	51	71%		21	29%	
Prominent nucleoli	Absent	72	56	78%		16	22%	0.135
	Present	11	6	55%		5	46%	
Atypical mitosis	Absent	65	48	74%		17	26%	1.000
	Present	18	14	78%		4	22%	
Mitotic Index	<5/10HPF	45	36	80%		9	20%	0.312
	5/10HPF	38	26	68%		12	32%	
Apoptosis	Absent	7	6	86%		1	14%	0.673
	Present	76	56	74%		20	26%	
Necrosis	Absent	62	47	76%		15	24%	0.773
	Present	21	15	71%		6	29%	
Calcifications	Absent	70	52	74%		18	26%	1.000
	Present	13	10	77%		3	23%	
Squamous metaplasia	Absent	80	60	75%		20	25%	1.000
	Present	3	2	67%		1	33%	
Margin status	Negative	17	15	88%		2	12%	0.166
	Close (<1mm)	21	17	81%		4	19%	
	Positive	44	29	66%		15	34%	
MYB IHC	Negative	14	13	93%		1	78%	0.081
	Positive	34	23	68%		11	32%	
MYB FISH	Negative	9	8	89%		1	11%	0.615
	Positive	15	11	73%		4	27%	

SN/NP: sinonasal/nasopharynx

Table 3

Correlation between clinico-pathologic characteristics and disease specific survival

		Total	Event (DOD)	P value ^a
		N	N	
Total		134	17	
Tumor size	< 2 cm	40	1	0.010
	≥ 2 cm	94	16	
Tumor site	Major salivary gland	50	5	0.871
	Minor salivary gland	84	12	
Tumor site	Sinonasal/ nasopharynx	14	3	0.523
	Oral cavity/ oropharynx	65	9	
	Other	55	5	
Most common growth pattern	Tubular	21	2	0.003
	Cribriform	96	10	
	Solid	17	5	
Solid pattern	Absent	101	8	<0.001
	Present	33	9	
30% solid pattern	Absent	111	11	0.002
	Present	23	6	
High grade transformation	Absent	118	12	<0.001
	Present	16	5	
Vascular invasion	Absent	121	13	0.001
	Present	13	4	
Perineural invasion	Absent	16	2	0.926
	Present	118	15	
Severe nuclear atypia	Absent	114	10	<0.001
	Present	20	7	
Open chromatin	Absent	15	0	0.162
	Present	119	17	
Prominent nucleoli	Absent	116	12	0.033
	Present	18	5	
Mitotic Index	< 5/10 HPFs	74	4	<0.001
	≥ 5/10 HPFs	60	13	
Apoptosis	Absent	10	0	0.361
	Present	124	17	
Necrosis	Absent	101	5	<0.001
	Present	33	12	
Squamous metaplasia	Absent	129	16	0.159
	Present	5	1	
Margin status (n = 130)	Negative	29	4	0.978

		Total	Event (DOD)	P value ^a
		N	N	
	Close	35	4	
	Positive	66	8	
Lymph node status (n=82)	Negative	61	7	0.736
	Positive	21	2	
MYB IHC (n = 78)	Negative	21	4	0.601
	Positive	57	6	
MYB-NFIB FISH (n = 34)	Negative	14	3	0.294
	Positive	20	1	
AJCC clinical Stage (n = 129)	I	32	0	0.077
	II	28	4	
	III	26	4	
	IV	43	9	

^aP value was calculated using Log rank test and disease specific survival.

DOD: dead of disease.

Table 4

Clinicopathologic predictors for recurrence free survival

		Total	Event	P value
		N	N	
Total		124	56	
Tumor size	< 2 cm	39	8	<0.001
	≥ 2 cm	85	48	
Tumor site	Major salivary gland	45	18	0.935
	Minor salivary gland	79	38	
Tumor site	Sinonasal/ nasopharynx	14	9	0.107
	Oral cavity/ oropharynx	60	28	
	Other	50	19	
Most common growth pattern	Tubular	21	5	<0.001
	Cribriform	87	40	
	Solid	16	11	
Solid pattern	Absent	94	33	<0.001
	Present	30	23	
30% solid pattern	Absent	102	40	<0.001
	Present	22	16	
HG transformation	Absent	109	46	<0.001
	Present	15	10	
Vascular invasion	Absent	113	49	0.005
	Present	11	7	
Perineural invasion	Absent	16	6	0.496
	Present	108	50	
Severe nuclear atypia	Absent	106	43	<0.001
	Present	18	13	
Open chromatin	Absent	14	2	0.030
	Present	110	54	
Prominent nucleoli	Absent	109	44	<0.001
	Present	15	12	
Mitotic Index	< 5/10HPF	71	22	<0.001
	≥ 5/10HPF	53	34	
Apoptosis	Absent	10	2	0.306
	Present	114	54	
Necrosis	Absent	94	33	<0.001
	Present	30	23	
Squamous metaplasia	Absent	119	53	0.084
	Present	5	3	
Margin status (n = 120)	Negative	28	11	0.030

		Total	Event	P value
		N	N	
	Close	32	8	
	Positive	60	34	
Lymph node status (n=76)	Negative	58	23	0.467
	Positive	18	10	
MYB IHC (n = 71)	Negative	20	11	0.270
	Positive	51	19	
<i>MYB-NFIB</i> FISH (n = 31)	Negative	12	7	0.479
	Positive	19	6	
AJCC clinical Stage (n = 129)	I	31	4	<0.001
	II	27	11	
	III	25	13	
	IV	37	26	

Table 5

Correlation between MYB immunohistochemistry (IHC) and *MYB-NFIB* fluorescence *in situ* hybridization (FISH) in adenoid cystic carcinoma

		<i>MYB-NFIB</i> FISH		
		Negative	Positive	Total
MYB IHC	Negative	7	4	11
	Positive	7	14	21
	Total	14	18	32