HHS Public Access

Author manuscript

Laryngoscope. Author manuscript; available in PMC 2016 September 01.

Published in final edited form as:

Laryngoscope. 2015 September ; 125(9): E292–E299. doi:10.1002/lary.25356.

MYB rearrangement and clinicopathologic characteristics in head and neck adenoid cystic carcinoma

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Abstract

Objectives—Salivary gland adenoid cystic carcinoma (ACC) is rare, aggressive, and challenging to treat. Many ACCs have a t(6;9) chromosomal translocation resulting in a *MYB-NFIB* fusion gene, but the clinical significance is unclear. The purposes of this study were to describe the clinicopathologic factors impacting survival and to determine the prevalence and clinical significance of *MYB-NFIB* fusion.

Study Design—Case series.

Methods—Medical records of patients treated for ACC of the head and neck from 1974 to 2011 were reviewed and clinicopathologic data recorded. FISH was used to detect *MYB* rearrangement in archival tumor tissue as a marker of *MYB-NFIB* fusion.

Results—158 patients were included with median follow-up 75.1 months. Median overall survival was 171.5 months (95%CI=131.9–191.6) and median disease-free survival was 112.0 months (95%CI=88.7–180.4). Advanced stage was associated with decreased overall survival (adjusted $p_{trend}<0.001$), and positive margins were associated with decreased disease-free survival (adjusted HR=8.80, 95%CI=1.25–62.12, p=0.029). 91 tumors were evaluable using FISH, and 59 (65%) had evidence of a *MYB-NFIB* fusion. *MYB-NFIB*-positive tumors were more likely than *MYB-NFIB*-negative tumors to originate in minor salivary glands (adjusted PR=1.51,

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Conflict of interest: The authors report no conflict of interest.

The research in this manuscript was presented at the Combined Sections Meeting of the Triological Society at Coronado Island, CA, USA, January 22–24, 2015.

Supplementary Items

Supplementary Table 1: Clinical and pathologic characteristics of cases evaluated for MYB-NFIB status compared with those not evaluated.

95%CI=1.07–2.12, p=0.019). *MYB-NFIB* tumor status was not significantly associated with disease-free or overall survival (HR=1.53, 95% CI=0.77–3.02, p=0.22 and HR=0.91, 95% CI=0.46–1.83, p=0.80, respectively, for *MYB-NFIB*-positive compared with *MYB-NFIB* negative tumors).

Conclusion—Stage and margin status were important prognostic factors for ACC. Tumors with evidence of *MYB-NFIB* fusion were more likely to originate in minor salivary glands, but *MYB-NFIB* tumor status was not significantly associated with prognosis.

Keywords

Adenoid cystic carcinoma; salivary gland neoplasms; *MYB*; *MYB-NFIB* fusion gene; minor salivary glands; survival; disease-free survival

Introduction

Adenoid cystic carcinoma (ACC) of the salivary glands is a rare malignancy comprising 10% of salivary gland neoplasms.[1] ACC is slow-growing but aggressive, with high rates of late recurrence and distant metastasis.[2–4] Treatment for ACC is extremely challenging, with current therapy limited to surgery and/or radiation, and no reliable chemotherapeutic options available for long-term disease control.[1,4] Previously identified determinants of outcomes for ACC include stage, cervical lymph node metastases, margin status, perineural invasion, minor salivary gland site of origin, and histological pattern.[3,5–9]

MYB is a transcription factor with important roles in the regulation of cell proliferation, survival and differentiation.[10] Overactivation of the *MYB* oncogene has recently been described as a hallmark of ACC, noted in over 80% of ACCs but not in other salivary gland neoplasms.[11–13] *MYB* over-activation is often, but not always, the result of a chromosomal translocation, t(6;9)(q22–23;p23–24), resulting in fusion of the *MYB* oncogene with the transcription factor gene *NFIB*. This translocation is found in 29–86% of ACCs.[11,13–17] The MYB-NFIB fusion protein may represent an important new therapeutic target for ACC; however, the clinical and prognostic significance of the *MYB-NFIB* fusion gene is unclear.

The purposes of this study were to describe our institution’s experience with ACC and determine the clinicopathologic factors that impact survival, and to assess the prevalence and clinical significance of *MYB-NFIB* fusion in ACCs treated at our institution.

Materials and methods

This study was approved by the Johns Hopkins School of Medicine Institutional Review Board. Patients treated for ACC of the head and neck at Johns Hopkins Medical Institutions from 1974 to 2011 were included. The medical records for these patients were retrospectively reviewed and demographic and disease-related data were recorded.

Fluorescence in situ hybridization

Fluorescence in situ hybridization (FISH) was performed on formalin-fixed paraffin-embedded (FFPE) sections in a tissue microarray (TMA) using a commercially available

MYB dual-color break apart probe (ZTV-Z-2143–200, ZytoVision, Germany). The probe is designed to detect translocations involving the chromosomal region 6q23.3 harboring the *MYB* gene, and has two components: a probe labeled with a green fluorochrome that hybridizes proximal to and covers the 5' end of the MYB gene, and a probe labeled with an orange fluorochrome that hybridizes ~180kb distal to the 3' end of the MYB gene. Prior to hybridization, the TMA slide was deparaffinized using a VP 2000 processor (Abbott Molecular, Des Plains, IL). The slides and the *MYB* probe were co-denatured at 80 °C for 7 minutes and allowed to anneal over night at 37 °C in a humidified atmosphere. At the end of the incubation the slides were washed in 2 X SSC/0.3% NP-40 for 2 min at 72 °C and for 2 min at room temperature in 2 X SSC. The slides were counterstained with DAPI, and a cover slip was applied using Vectashield mounting medium (H-1000, Vector Laboratories, Inc.). A fluorescence microscope was used to evaluate the probe pattern. Interphase nuclei with two fusion signals of one orange and one green fluorochrome lack rearrangement at band 6q23.3 and are scored as normal (*MYB-NFIB* negative). A signal pattern consisting of one orange/green fusion signal and one orange and one green signal at distance from each other indicates one normal 6q23.3 locus and one locus affected by a translocation. This pattern was considered a marker of *MYB-NFIB* translocation (*MYB-NFIB* positive).

In several cases, signal patterns different from the normal (two fusion signals) and the typical rearranged (one fusion, one green, one orange signal) patterns were observed (e.g., one fusion and one green signal, one fusion and one red signal, or amplification of the green signal). These cases were re-evaluated using an in-house designed *MYB-NFIB* fusion probe. The probe identifies the fusion gene on the derivative chromosome 6 that results from a translocation t(6;9)(q22–23;p23–24). The probe covering the *MYB* gene consists of two BAC clones: RP11–104D9 and RP11–614H6. The BAC DNA is labeled by nick translation with Green-dUTP (Abbott Molecular). The probe is approximately 360 kb long, covers the *MYB* gene, and extends beyond the 5' end of the gene. The *NFIB* probe consists also of two BAC clones: RP11–589C16 and RP11–413D24. The BAC DNA is labeled by nick translation with Orange-dUTP (Abbott Molecular). The probe is approximately 370 kb long and covers the 3' end of the NFIB gene. The signal pattern in a normal cell consists of two green signals (chromosomes 6) and two orange signals (chromosomes 9). In the case of a rearrangement leading to a *MYB-NFIB* fusion the predicted signal pattern is: one green signal (chromosome 6), one orange signal (chromosome 9) and one green/orange fusion signal (derivative chromosome 6) (*MYB-NFIB* positive). Of the 17 cases that could not conclusively be interpreted with the *MYB* break apart probe and were re-evaluated with the *MYB-NFIB* fusion probe, only two did not show the gene fusion. These two cases were considered indeterminate.

Statistical analysis

Descriptive variables were summarized with frequencies and proportions for categorical variables, and medians with 95% confidence intervals (95%CI) or ranges for continuous variables. The association between clinical variables of interest and *MYB-NFIB* status was evaluated using a generalized linear model with extension to the binomial family[18,19] in both univariate and bivariate models, and reported as unadjusted and adjusted prevalence ratios (PR and aPR). Survival rates were estimated using the Kaplan-Meier method[20].

Overall survival (OS) was defined as time from diagnosis to time of death from any cause. Disease-free survival (DFS) was defined as time from diagnosis to time of local, regional or distant recurrence. Patients with distant metastases at the time of diagnosis, or who died of primary disease without recurrence, were considered to not have received definitive treatment and were excluded from the DFS analysis. Follow-up time was calculated from diagnosis to event of interest or the last known-follow up. Survival curves were compared using the log-rank test for equality of survival functions. Unadjusted and adjusted hazard ratios (HR and aHR) were estimated using the Cox regression model. This retrospective study had 80% power to detect a 2.9-fold or greater difference in survival by *MYB-NFIB* tumor status. Data analysis was performed using STATA 11.2 (College Station, TX, 2012). A p-value ≤ 0.05 was considered statistically significant.

Results

Clinical and pathologic features

The study population was comprised of 158 patients with ACC. Median age at the time of diagnosis was 51.5 years (range, 17–94 years). Clinical and pathologic characteristics are displayed in Table 1. The most common tumor site was the parotid gland (N=38, 25%). A greater proportion of tumors arose from minor (N=85, 57%) than major salivary glands (N=65, 43%). Histologically, most tumors were observed to have a cribriform pattern (N=78, 70%) and perineural invasion (N=68, 83%). Tumors with positive margins (N=73, 66%) were significantly more likely to have perineural invasion than those with negative or close margins (78% compared with 22%, p=0.026), and were also more likely to have local recurrence (29% compared with 8%, p=0.017).

Survival

Median follow-up time was 75.1 months (range, 0.5–360.0 months). Five-year OS was 80% (95%CI=71–86%) and median OS was 171.5 months (95%CI=131.9–190.6). At the time of last follow-up, 78 (53%) of patients were alive. In univariate analysis, factors associated with decreased OS included older age (HR=1.04, 95%CI=1.02–1.06, p<0.001 for each year of age), nodal metastases at the time of neck dissection (HR=3.09, 95%CI=1.20–7.98, p=0.020), and more advanced overall stage (p_{trend}<0.001) (Table 2). OS was also significantly decreased among patients treated with single modality therapy (surgery or radiation) compared to those treated with surgery and adjuvant radiation (HR=3.01, 95%CI=1.35–6.72, p=0.007). In multivariate analysis, more advanced overall stage (adjusted p_{trend}<0.001) and older age at diagnosis (aHR=1.03, 95%CI=1.01–1.05, p=0.001) were independently associated with OS (Table 2).

Five-year DFS was 65% (95%CI=55–73%) and median DFS was 112.0 months (95%CI=88.7–180.4). More advanced overall stage was associated with decreased DFS (p_{trend}=0.014), as were receipt of single modality therapy compared with surgery and adjuvant radiation (HR=3.31, 95%CI=1.47–6.63, p=0.003) and close or positive surgical margins (HR=5.98, 95%CI=1.30–29.86, p=0.029 for close and HR=6.93, 95%CI=1.64–29.24, p=0.008 for positive compared with negative margins). In multivariate analysis, margin status was the only independent predictor of DFS; positive margins were

independently associated with an eight-fold decrease in DFS (aHR=8.80, 95%CI=1.25–62.12, p=0.029) (Table 2).

MYB-NFIB rearrangement

FISH was used to determine *MYB* rearrangement as a marker of *MYB-NFIB* fusion tumor status for 93 ACC specimens, of which 91 tumors were considered evaluable for analysis. Of these, 59 (65%) tumors were *MYB-NFIB* positive and 32 (35%) were *MYB-NFIB* negative (Figure 1).

There were no significant differences in the clinical and pathologic characteristics of cases with *MYB-NFIB* tumor status available compared with cases that did not have *MYB-NFIB* tumor status available (Supplementary Table 1).

Clinicopathologic factors associated with *MYB-NFIB* positive tumor status were considered (Table 3). Tumors arising from minor salivary glands were more likely to be *MYB-NFIB* positive (PR=1.52, 95%CI=1.06–2.18, p=0.023), and female gender was significantly associated with *MYB-NFIB* positive tumor status (PR=1.44, 95%CI=1.01–2.05, p=0.041). Tumors that recurred or had nodal metastases at the time of neck dissection were more likely to be *MYB-NFIB* positive, although these differences were not statistically significant (p=0.15 and p=0.14). Age, gender, smoking history, stage and perineural invasion were not associated with *MYB-NFIB* status (Table 3). In bivariate analysis, both minor salivary gland origin (aPR=1.51, 95%CI=1.07–2.12, p=0.019) and female gender (aPR=1.49, 95%CI=1.05–2.11, p=0.026) remained significantly associated with *MYB-NFIB* positive tumor status.

MYB-NFIB and survival

Median follow-up time for patients evaluated for *MYB-NFIB* tumor status was 62.6 months (range, 1.0–357.4 months). Five-year OS was 84% (95%CI=70–92%) for *MYB-NFIB* positive compared with 68% (95%CI=45–82%) for *MYB-NFIB* negative tumors, and median OS was 170.3 months (95%CI=92.6–237.3) for *MYB-NFIB* positive compared with 163.7 months (95%CI=57.0–270.5) for *MYB-NFIB* negative tumors. There was no difference in OS by *MYB-NFIB* tumor status (HR=0.91, 95% CI=0.46–1.83, p=0.80).

In contrast, *MYB-NFIB* positive tumors exhibited a trend toward decreased DFS. Five-year DFS was 54% (95%CI=37–68%) for *MYB-NFIB* positive compared with 70% (95%CI=44–85%) for *MYB-NFIB* negative tumors, and median DFS was 88.7 months (95%CI=42.0–127.8) for *MYB-NFIB* positive compared with 187.2 months (95%CI=55.4–318.9) for *MYB-NFIB* negative tumors (Figure 2). The association of *MYB-NFIB* tumor status with decreased DFS was not, however, statistically significant (HR=1.53, 95% CI 0.77–3.02, p=0.22), and was further attenuated after adjustment for minor versus major salivary gland site of origin (adjusted HR=1.18, 95%CI 0.51–2.71, p=0.70).

Discussion

ACC of the head and neck is uncommon and difficult to treat due to its high propensity for late recurrence and distant metastasis, sometimes many years after initial diagnosis and

treatment.[1,3,4] In this study, we have elucidated important prognostic factors for both overall and disease-free survival, and explored clinicopathologic correlates of the *MYB-NFIB* fusion gene.

Clinical outcomes

Similar to previous studies,[3,5] we found that ACCs were frequently of minor salivary gland origin (57%) and had a high rate of perineural invasion (83%). Recurrences were largely local (31%) or distant (29%), with regional recurrence in only 5% of cases, consistent with the known patterns of ACC recurrence.[3] Our five-year rates of OS (80%) and DFS (65%) are also consistent with previous reports.[3,5,21]

Advanced overall stage was the strongest independent predictor of poor OS in our study, and was also significantly associated with decreased DFS. Stage was therefore a more important prognostic factor than histologic pattern, perineural invasion or site of origin. Indeed, overall stage is known to correlate with outcomes for ACC.[3,6–8,21]

Margin status was the only factor independently associated with DFS, with a significant trend toward decreasing DFS with closer proximity of disease to the surgical margin. Positive margins were also associated with greater risk of local failure, consistent with previous reports.[22,23] These associations are not surprising, especially given that in this and other studies,[3] tumors with positive margins were also significantly more likely to demonstrate perineural invasion. Tumors that are challenging to surgically extirpate appear to have a more aggressive biological phenotype and higher likelihood of recurrence. The current treatment paradigm for ACC with positive margins is surgical resection followed by adjuvant radiation therapy,[4,24,25] and our results support the indication for adjuvant therapy in these cases.

MYB-NFIB tumor status

The *MYB* oncogene is overexpressed in 89–97% ACCs of the head and neck.[11,13] A subset of these (29–86%) are characterized by an activating *MYB-NFIB* fusion resulting from a variable balanced t(6;9)(q22–23;p23–24) translocation event that is not found in other salivary gland cancers.[11,13,15–17] Overexpression of *MYB* RNA and the Myb transcription factor protein are associated with the *MYB-NFIB* fusion, but also occur independent of the translocation, suggesting additional mechanisms of *MYB* dysregulation.[11,13,15,16]

The combination of an initial *MYB* break-apart probe with a confirmatory *MYB-NFIB* fusion probe that was used for the FISH assay in this study has not previously been used in ACC of the head and neck. The *MYB* break-apart probe alone detects a break in the 6q23.2–q23.3 locus containing the *MYB* gene that most likely results in a *MYB-NFIB* fusion gene, as has been well described,[13,14,17] and our use of a confirmatory *MYB-NFIB* fusion probe ensured that indeterminate cases were properly categorized. The prevalence of *MYB-NFIB* positive tumors in this study was 65%, which is similar to previous FISH studies in formalin-fixed tissue.[11,15,17]

The clinical significance, if any, of the *MYB-NFIB* fusion gene has not been well established. We demonstrated a higher prevalence of the *MYB-NFIB* fusion in tumors from

minor salivary glands and among females, and a trend toward higher likelihood of recurrence and decreased DFS with *MYB-NFIB* positive tumor status, although there was no association with OS. The significance of these results is unclear. Importantly, our sample size was underpowered to detect with certainty the observed difference in DFS by *MYB-NFIB* status (HR=1.53, 95% CI 0.77–3.02, p=0.22), which was further attenuated after adjusting for the fact that *MYB-NFIB* positive tumors were predominantly from minor salivary glands. However, several previous studies have also reported borderline associations of the *MYB-NFIB* fusion in ACC with poor prognostic parameters, including perineural invasion[15], local recurrence[15], and decreased overall survival.[16] Taken together with our study, this suggests a possible trend towards more aggressive disease with *MYB-NFIB* positive tumor status. Larger studies are necessary to determine with certainty whether the *MYB-NFIB* fusion is of prognostic significance, or is simply a biomarker of ACC carcinogenesis and potential therapeutic target.

It may be the case that *MYB* overexpression, independent of the mechanism by which it occurs, is a key event in ACC pathogenesis. Indeed, current evidence indicates that *MYB* is dysregulated by diverse and complex mechanisms in ACC,[13,17] and overexpression of the *MYB* transcript 5' end was found in one study to be independently associated with decreased overall survival.[16] In this scenario, the weak associations of *MYB-NFIB* tumor status with prognosis found in ours and other studies may simply reflect a greater importance of *MYB* overexpression than the *MYB-NFIB* fusion *per se* in influencing outcomes. Sequencing studies have suggested a possible separate role for *NFIB* as well.[17,26] Further research is required to describe the role of *MYB* or *NFIB* dysregulation individually, and in the context of the *MYB-NFIB* fusion.

Strengths and limitations

The strengths of this study are the report of clinicopathologic variables that impact outcomes in ACC, contributing to our understanding of prognostic determinants for this rare disease. In addition, the *MYB* break-apart and *MYB-NFIB* fusion FISH assay is a new tool that may be used in studying the role of the *MYB-NFIB* fusion and *MYB* alterations in ACC. However, this assay is limited in that it is possible that a subset of tumors with a *MYB* translocation pattern on FISH actually harbored an atypical translocation. In addition, detection of the *MYB-NFIB* translocation by RT-PCR has been shown to be lower in formalin-fixed compared with frozen tissue (44–57% compared with 86%)[11,13,15,17] so that the use of formalin-fixed tissue in the present study may lower the sensitivity of our assay for *MYB-NFIB* positive tumor status and limit the interpretation of clinicopathologic correlates. Other limitations of this study include the retrospective nature of the medical chart review and the relatively small sample size with FISH assay results available, resulting in inadequate power to detect significant survival differences by *MYB-NFIB* tumor status.

Conclusion

In summary, stage and margin status are important prognostic indicators for ACC. The *MYB-NFIB* fusion may be more prevalent in tumors with aggressive features, but was not significantly associated with prognosis in our study. Further research is indicated to

determine both the clinical significance and therapeutic targeting potential of the *MYB-NFIB* fusion gene.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Location of research: This work was performed in the Johns Hopkins University School of Medicine Department of Otolaryngology-Head and Neck Surgery (EMR, MT, SL, CF, PKH) and Johns Hopkins University School of Medicine Department of Pathology (RY, JAB).

Financial support: Research reported in this manuscript was supported by the National Institute of Dental and Craniofacial Research (NIDCR) and NIH grant R01- DE023227 (PKH), The University of Texas M. D. Anderson Cancer Center Career Development Award (JAB) and NIDCR grant 2T32DC000027-26 (EMR). The content is solely the responsibility of the authors and does not necessarily represent the official view of the National Institutes of Health.

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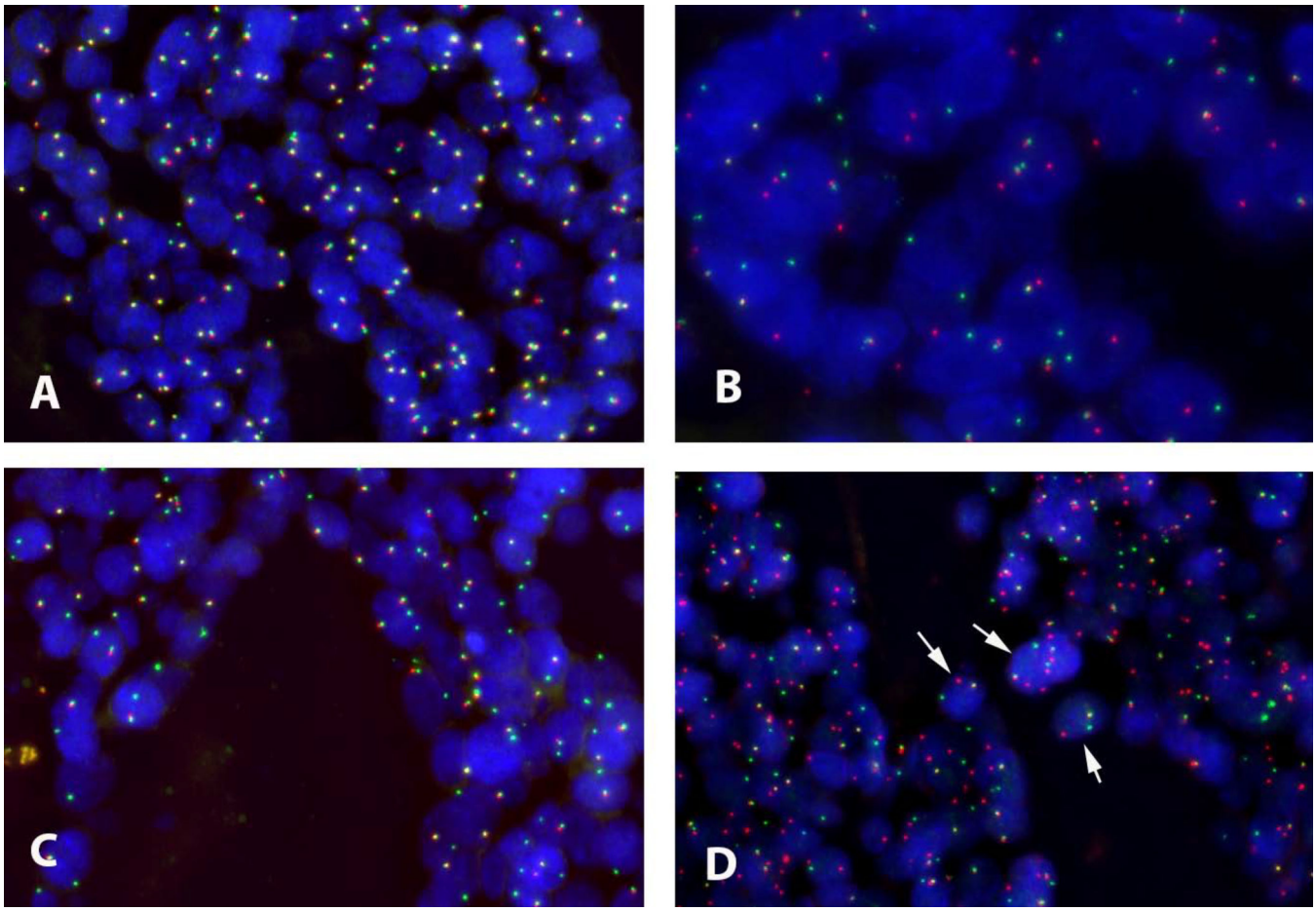

Figure 1. Representative FISH assay results

A: *MYB* break apart assay using commercial *MYB* dual-color break apart probe. Normal pattern (*MYB*-rearrangement negative) with two fusion signals in each cell (5' *MYB* green fluorophore, 3' *MYB* orange fluorophore). **B:** *MYB* break-apart assay abnormal pattern (*MYB*-rearrangement positive) with one fusion signal, one 3' *MYB* orange signal, and one 5' *MYB* green signal. **C:** *MYB* break-apart assay atypical pattern with one fusion signal and one green signal but no orange, requiring clarification using the *MYB-NFIB* fusion probe shown in D. **D:** *MYB-NFIB* fusion assay using laboratory designed probe. The pattern of one fusion (5' *MYB* green fluorophore /3' *NFIB* orange fluorophore), one orange (3' *NFIB*) and one green (5' *MYB*) signal confirms *MYB-NFIB* positive tumor status for the tumor shown in C. Arrows indicate cells representative of *MYB-NFIB* fusion pattern.

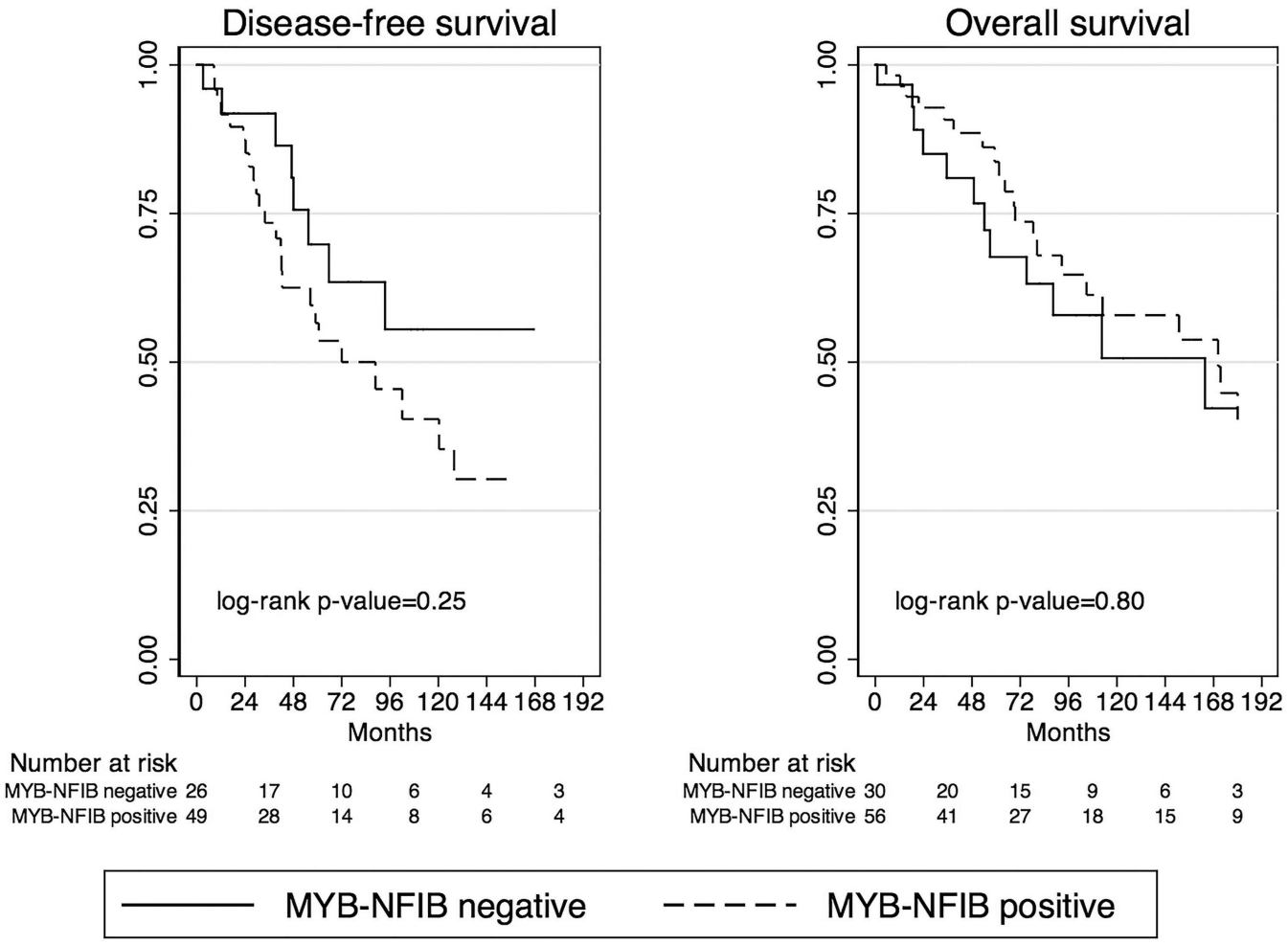

Figure 2. Disease-free and overall survival by *MYB-NFIB* tumor status

Table 1

Clinicopathologic characteristics of patients with adenoid cystic carcinoma at Johns Hopkins Medical Institutions from 1974 to 2011.

Patient Characteristics (N=158)	N (%)
Age	
<55 years	79 (53)
≥55 years	71 (47)
Gender	
Male	62 (39)
Female	96 (61)
Smoking history	
No	62 (52)
Yes	58 (48)
Salivary gland type	
Major	65 (43)
Minor	85 (57)
Primary site	
Parotid	38 (25)
Submandibular gland	23 (15)
Oral cavity	35 (23)
Nose & paranasal sinuses	31 (21)
Other	23 (15)
Predominant pattern	
Tubular	16 (14)
Cribriform	78 (70)
Solid	17 (15)
Perineural invasion	
No	14 (17)
Yes	68 (83)
Overall Stage	
I	21 (19)
II	21 (19)
III	18 (17)
IV	48 (44)

Patient Characteristics (N=158)	N (%)
Treatment	
Surgery and radiation	107 (84)
Surgery	15 (12)
Radiation[A]	6 (5)
Neck dissection	
No	57 (50)
Yes	56 (50)
Nodal metastases found on neck dissection	
No	41 (76)
Yes	13 (24)
Margin status	
Negative	21 (19)
Close	16 (15)
Positive	73 (66)
Recurrence	
Local recurrence	
No	97 (69)
Yes	44 (31)
Regional recurrence	
No	132 (95)
Yes	7 (5)
New distant metastases	
No	99 (71)
Yes	41 (29)
Any recurrence after initial treatment[B]	
No	70 (52)
Yes	64 (48)
Last known vital status	
Alive	78 (53)
Expired	70 (47)

[A] Two patients receiving radiation also received chemotherapy and were excluded from further analysis.

[B] Any recurrence includes local or regional recurrence and new distant metastases.

Table 2

Clinicopathologic characteristics associated with overall and disease-free survival.

	Overall Survival		Disease-free Survival	
Groups compared	**Univariate HR; 95% CI (p-value)**	**Multivariate[A] aHR; 95% CI (p-value)**	**Univariate HR; 95% CI (p-value)**	**Multivariate[A] aHR; 95% CI (p-value)**
Age (years, continuous)				
	1.04; 1.02–1.06 (<0.001)	1.03; 1.01–1.05 (0.001)	0.99; 0.98–1.01 (0.52)	1.01; 0.99–1.04 (0.39)
Gender				
Male	REF		REF	
Female	0.86; 0.53–1.41 (0.55)		0.84; 0.50–1.40 (0.50)	
Smoking history				
No	REF		REF	
Yes	0.73; 0.41–1.28; 0.27		0.92; 0.52–1.63 (0.78)	
Salivary gland type				
Major	REF		REF	
Minor	0.81; 0.50–1.33 (0.41)		1.04; 0.63–1.70 (0.89)	
Predominant pattern				
Tubular	REF		REF	
Cribriform	0.60; 0.25–1.47 (0.27)		0.88; 0.33–2.30 (0.79)	
Solid	1.01; 0.36–2.86 (0.98)		1.46; 0.48–4.50 (0.51)	
Perineural invasion				
No	REF		REF	
Yes	1.91; 0.67–5.42 (0.23)		1.94; 0.58–6.49 (0.28)	
Overall Stage				
I	REF[B]	REF[B]	REF[C]	REF[C]
II	2.45; 0.83–7.24 (0.11)	1.92; 0.66–5.64 (0.23)	2.17; 0.73–6.43 (0.16)	3.40; 0.94–12.32 (0.062)
III	3.82; 1.35–10.85 (0.012)	3.94; 1.28–12.07 (0.017)	2.58; 0.76–8.80 (0.13)	3.20; 0.96–10.69 (0.059)
IV	5.07; 2.05–12.57 (<0.001)	5.15; 2.12–12.54 (<0.001)	3.20; 1.24–8.30 (0.017)	2.46; 0.88–6.85 (0.085)
Treatment				
Surgery *and* radiation	REF	REF	REF	REF
Surgery *or* radiation	3.01; 1.35–6.72 (0.007)	2.21; 0.92–5.32 (0.076)	3.13; 1.47–6.63 (0.003)	1.51; 0.41–5.59 (0.54)
Nodal metastases on neck dissection[D]				
No	REF		REF	

	Overall Survival		Disease-free Survival	
Groups compared	**Univariate HR; 95% CI (p-value)**	**Multivariate[A] aHR; 95% CI (p-value)**	**Univariate HR; 95% CI (p-value)**	**Multivariate[A] aHR; 95% CI (p-value)**
Yes	3.09; 1.20–7.98 (0.020)		1.28; 0.35–4.63 (0.70)	
Margin status				
Negative	REF		REF[E]	REF[E]
Close	1.16; 0.44–3.04 (0.76)		5.98; 1.30–29.86 (0.029)	7.04; 0.89–55.94 (0.065)
Positive	1.58; 0.75–3.30 (0.23)		6.93; 1.64–29.24 (0.008)	8.80; 1.25–62.12 (0.029)

A Adjusted models include all variables with adjusted hazard ratios reported

B $p_{trend}<0.001$ for univariate analysis and multivariate analysis.

C $p_{trend}=0.014$ for univariate analysis and $p_{trend}=0.23$ for multivariate analysis.

D Nodal metastases on neck dissection was not included in multivariate analysis because of the small number of patients undergoing neck dissection (N=56).

E $p_{trend}=0.0034$ for univariate analysis and $p_{trend}=0.039$ for multivariate analysis.

Abbreviations: HR, hazard ratio; aHR, adjusted hazard ratio

Table 3

Clinicopathologic characteristics compared with *MYB-NFIB* tumor status.

Characteristics	MYB-NFIB(−) N (%)	MYB-NFIB(+) N (%)	Prevalence ratio MYB-NFIB(+) vs. MYB-NFIB(−) PR (95% CI)	p-value
Total (N=91)				
	59 (63)	32 (34)		
Age				
<55 years	14 (34)	27 (66)	REF	
≥55 years	16 (36)	29 (64)	0.98 (0.72–1.33)	0.89
Gender				
Male	18 (48)	19 (52)	REF	
Female	14 (26)	40 (74)	1.44 (1.01–2.05)	0.041
Smoking history				
No	13 (38)	21 (62)	REF	
Yes	12 (31)	27 (69)	1.12 (0.80–1.57)	0.51
Salivary gland type				
Major	18 (50)	18 (50)	REF	
Minor	12 (24)	38 (76)	1.52 (1.06–2.18)	0.023
Predominant pattern				
Tubular	5 (45)	6 (54)	REF	
Cribriform	18 (30)	42 (70)	1.28 (0.73–2.26)	0.39
Solid	5 (45)	6 (54)	1.00 (0.46–2.15)	1.00
Margin status				
Negative	4 (36)	7 (64)	REF	
Close	4 (40)	6 (60)	0.94 (0.48–1.86)	0.86
Positive	16 (38)	26 (62)	0.97 (0.58–1.62)	0.92
Perineural invasion				
No	3 (33)	6 (67)	REF	
Yes	17 (40)	25 (60)	0.89 (0.53–1.52)	0.68
Overall Stage				
I	5 (63)	3 (38)	REF	
II	5 (31)	11 (69)	1.82 (0.70–4.79)	0.22
III	4 (29)	10 (71)	1.90 (0.73–4.98)	0.19
IV	9 (31)	20 (69)	1.84 (0.72–4.68)	0.20

Characteristics	MYB-NFIB(−) N (%)	MYB-NFIB(+) N (%)	Prevalence ratio MYB-NFIB(+) vs. MYB-NFIB(−) PR (95% CI)	p-value
Nodal metastases on neck dissection				
No	9 (45)	11 (55)	REF	
Yes	2 (20)	8 (80)	1.45 (0.88–2.41)	0.14
Any recurrence[A]				
No	16 (42)	22 (58)	REF	
Yes	10 (26)	28 (74)	1.27 (0.91–1.77)	0.15

Abbreviations: PR, prevalence ratio

[A] Any recurrence includes local or regional recurrence and new distant metastases.