MYB immunohistochemistry as a predictor of *MYB::NFIB* fusion in the diagnosis of adenoid cystic carcinoma of the head and neck

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Objectives. Diagnosing adenoid cystic carcinoma (AdCC) is challenging due to histopathological variability and similarities with other tumors. In AdCC pathogenesis, the cellular myeloblastosis gene (c-MYB) often exhibits a *MYB::NFIB* fusion from a reciprocal translocation. This study aimed to assess the predictive accuracy of MYB immunohistochemistry for detecting this translocation compared to fluorescence in situ hybridization (FISH).

Study design. This study included 110 AdCC patients (1999-2017) from two Dutch head and neck centers using tissue microarrays and full slides. Median MYB expression levels by immunohistochemistry were compared based on translocation status by FISH, and differences within clinicopathological parameters were examined. An immunohistochemical cut-off was established to estimate the translocation.

Results. MYB immunohistochemistry was available in 90/110 patients, with a median expression of 27%. FISH was interpretable in 79/108 tumors, identifying *MYB*::*NFIB* fusion in 44 (56%). Among 62 patients with both MYB expression and translocation data, the fusion was present in 38 (61%). These tumors had higher MYB expression (30%) than nontranslocated tumors (6%); $P = .02$. A 60% MYB expression cut-off yielded 100% specificity for detecting the translocation but had no prognostic value.

Conclusions. Although MYB protein expression alone lacks diagnostic precision, protein expression >60% predicted the *MYB::NFIB* fusion in all tumors. (Oral Surg Oral Med Oral Pathol Oral Radiol 2024;138:772−780)

Adenoid cystic carcinoma (AdCC) is a rare epithelial malignancy of the secretory glands. It comprises around 20% to 35% of all salivary gland malignancies in the head and neck region, with an annual incidence of 2 to 3 cases per 1.000.000 inhabitants. Its incidence peaks in the fifth and sixth decade, but it arises in all age groups with a slight predominance in women.[1-4] It consists of ductal (luminal) and basal/myoepithelial (abluminal) cells arranged in a glandular (cribriform), tubular, or solid growth pattern.[5] Histopathological diagnosis of salivary gland cancers, in general, is challenging on small biopsies and cytological specimens. In case of AdCC, the cribriform pattern is well recognized, while other patterns are less clear, reflected by the high reclassification rate of 14% to 29% on the surgical specimens after definitive treatment.[1]

The predominant gene mutated in recurrent or metastatic AdCC is *NOTCH1*, with an incidence of 26.3%, contrasting to 8.5% in nonrecurrent tumors, and is associated with a solid growth pattern.[6,7]

Ten-year rates of disease-free survival have significantly increased over the last decades, from approximately 16% in older studies to 48% in more recent publications.[2,3] Local recurrences are difficult to cure due to previous surgical procedures and radiotherapy limitations. Negative prognosticators are advanced tumor stage, AdCC originating from the minor salivary glands, inadequate resection margins, solid growth pattern, and (peri)neural invasion.[1-4,8-10]

The cellular myeloblastosis gene (*c-MYB* or *MYB*) is a proto-oncogene that encodes a transcription factor involved in cellular differentiation and proliferation. It

Statement of Clinical Relevance

A correlation was found between MYB protein expression by immunohistochemistry and *MYB::NFIB* fusion status by FISH, a known driver in AdCC. Translocated tumors exhibited substantially higher MYB expression. A 60% MYB expression cut-off yielded 100% specificity for detecting the translocation.

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functions as an oncogene in a variety of cancers, including leukaemia, breast-, colorectal- and pancreatic cancer, seminoma, and thymoma.[11-13] Fusion of MYB to the transcription factor gene NFIB by a translocation t(6;9)(q22-23;p23-24) is present in the majority of AdCC cases. This *MYB*::*NFIB* fusion results in the upregulation of a fusion protein that contains the aminoterminal of 90% of the MYB protein, which is believed to be an oncogenic driver of this tumor.[1,12] However, overexpression of the MYB protein is also observed in fusion-negative AdCC and, to a lesser extent, in non-AdCC salivary gland tumors, indicating the existence of additional mechanisms for MYB overexpression.[12] Nonetheless, the *MYB*::*NFIB* translocation, or rearrangements such as multiple variant fusions, have consistently emerged as AdCC-specific abnormalities, distinguishing it from other salivary gland tumors.[14,15] Disease-specific survival is not affected by the *MYB::NFIB* fusion specifically, while MYB rearrangements are frequently found to negatively influence prognosis.[16]

Fluorescence in situ hybridization (FISH) is applied to detect this translocation using a break-apart probe that hybridizes respectively the 5' and 3' end of the *MYB* gene, labelled with different fluorophores.[17] The MYB::NFIB fusion is present in AdCCs irrespective of their derived tumor site, i.e., the major and minor salivary glands, lacrimal glands, ceruminous glands, or breasts.[14] In challenging diagnostic cases where salivary gland adenocarcinomas cannot be further specified histologically, FISH can be used to visualize MYB rearrangements pathognomonic for AdCC. Immunohistochemistry is deemed faster and more cost-effective than FISH.[18] This study aims to investigate MYB protein expression levels in AdCC, depending on the presence of the *MYB::NFIB* translocation by FISH, and to correlate these levels with clinicopathological parameters and patient survival. Additionally, it aims to assess the diagnostic value of MYB immunohistochemistry in the predictability of the *MYB::NFIB* translocation.

MATERIALS AND METHODS

Patient selection

A previously fabricated Tissue Microarray (TMA) and tissue blocks of the resection specimens of patients diagnosed with AdCC of the head and neck between 1990 and 2017 in the University Medical Center Utrecht and Antoni van Leeuwenhoek Hospital/The Netherlands Cancer Institute were used.[9]

The following clinicopathological parameters were available from the matching data files: histopathological diagnosis, sex, age at diagnosis, tumor site, treatment regimen, (time to) recurrence or metastasis, date of last follow-up, survival status, type and diameter of the tumor, pathological T- and N-stage (AJCC Cancer Staging Manual 7th edition), histopathological growth pattern and associated grade according to the differentiation of Perzin et al.,[19] surgical resection margins, and the presence of perineural, vascular, and bone invasion. All data were handled according to the European Union General Data Protection Regulation. Studies on AdCC residual tissue did not require formal consent. Approval was received from both institutional Medical and Biobank Research Ethics Committees, protocol numbers UMCU 16-564 and 17-073, respectively. The study is in accordance with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

MYB translocation analysis by FISH

The Zytolight *MYB* break-apart FISH probe, a mixture of two directly labelled probes hybridizing to the 6q23.2-q23.3 band, was used to determine *MYB* gene rearrangements. The orange fluorochrome direct labelled probe hybridizes distal, and the green fluorochrome direct labelled probe hybridizes proximal to the *MYB* breakpoint cluster region. TMA slides (4 μm) were deparaffinized and pretreated with citrate and protease buffers. Next, they were dehydrated and hybridized with 15-μl FISH probes in a ThermoBrite System (Abbott Laboratories, Chicago, Ill., USA) at 37˚C overnight. The next day, slides were washed in saline-sodium citrate buffers, counterstained and mounted with Vectashield containing 4',6-diamidino-2-phenylindole (DAPI) dihydrochloride. *MYB* probe signals were analysed in 100 random tumor cells from different areas at a 100x magnification using a Leica DM5500 B microscope system with Application Suite Advanced Fluorescence Software (Leica Microsystems, Rijswijk, NL).

A tumor was defined as translocated when a break apart signal was seen in >10% of the tumor cells of at least two arrayed cores or a whole slide. The mean percentage of cells showing the translocation was noted, as well as other *MYB* rearrangements that were seen within the tumor.

MYB expression analysis by immunohistochemistry

The slides were deparaffinized and rehydrated. Heat-induced antigen retrieval was applied by boiling the sections in a sodium citrate buffer (10 mM, pH 6.0) for 20 minutes. After cooling down for 15 minutes, the sections were washed in PBS-Tween twice. An UltraVision protein block buffer was added to the sections prior to primary antibody administration (Clone EP769Y (Abcam, Cambridge, UK)), dilution 1:200 in a BSA blocking buffer PBS with sodium azide. Sections were incubated overnight. Before adding the secondary antibody (BrightVision Poly-HRP-Anti-Rabbit

(ImmunoLogic, Duiven, NL)), slides were incubated in hydrogen peroxidase 0.3% in PBS for 15 minutes. Finally, the slides were counterstained using haematoxylin, dehydrated, and fixed.

Blinded semiquantitative scoring of the tumor cores or whole slides was done until a consensus was reached by a head and neck pathologist and a head and neck surgeon (S.W. and T.K.N.). Per core or whole slide, the percentage of MYB-positive tumor cells was scored in increments of five percent. For the arrayed cores, MYB expression was defined by the mean percentage of MYB-positive tumor cells in the cores that contained >5% tumor tissue. When less than two adequate cores were available, a whole slide was subsequently stained and scored.

Statistical analysis

A two-way random model intraclass correlation coefficient (ICC) was employed to validate the consistency of a single core's MYB expression within the arrayed cores per tumor by assessing the degree of resemblance among different quantitative measurements.[20] The median MYB expression with interquartile range (IQR) within the clinical and pathological characteristics was compared using the Independent Samples Kruskal-Wallis (KW) test. An optimal cut-off value for MYB tissue expression to estimate a *MYB::NFIB* fusion was computed by plotting a Receiver Operating Characteristic (ROC)-curve, and sensitivity, specificity, predictive values, and likelihood ratios were calculated for the selected cut-off points. Pearson Chi-Square or Fisher's exact test (N<5) was used to analyze the number of translocated tumors within the dichotomized parameters. Survival rates were determined and compared across the groups using Fisher's exact test, and the univariate prognostic value of MYB expression was analyzed by the Log Rank test for overall, disease-specific, disease-free, recurrence-free, and metastasis-free survival.

RESULTS

The medical records of 110 patients diagnosed with adenoid cystic carcinoma (AdCC) of the secretory glands, who underwent surgery between 1990 and 2017 at the head and neck oncology departments of the University Medical Center Utrecht or the Antoni van Leeuwenhoek Hospital / The Netherlands Cancer Institute, were available. The clinical and pathological data are outlined in Table I.

Fluorescence in situ hybridization

Due to the retrospective nature of the study, it was not possible to gather additional tissue from two patients. As a result, FISH was carried out on 108 tumor specimens, of which 79 (73%) could be successfully interpreted for technical reasons. A *MYB::NFIB* fusion was identified in 44 of these 79 tumors (56%) and not detected in 26 tumors (33%). *MYB* rearrangements, other than a break apart signal, were present in the other 9 tumors (11%) and are of unclear significance. This led to exclusion from further analysis: one inversion, one single red signal, and seven specimens depicted a single green signal.

MYB immunohistochemistry

The average percentage of MYB positive tumor cells by immunohistochemistry could be successfully scored in the primary tumor samples of 90 out of all 110 patients. Core biopsies of 77 of these were previously incorporated in TMAs: results were based on three cores of 49, and on two cores of 12 tumors. Sixteen tumors were excluded due to insufficient (i.e., <2) cores, however whole slides could be obtained from these 16 and another 13 tumors. From the 20 remaining patients, tumor tissue could not be requested from different (referring) hospital archives, or there was insufficient tissue left for reliable diagnostics. Staining and scoring were done according to the study protocol in the UMC Utrecht pathology laboratory.

MYB expression was predominantly nuclear, tended to concentrate in the abluminal cells, and was homogenously distributed as reflected by a substantial single measurement ICC of .68 ($P < .01$). Eighty out of the 90 tumors (89%) showed positive MYB expression with a median expression of 27% (IQR) 8%-46%).

MYB immunohistochemistry vs FISH

Ultimately, both MYB expression and translocation status were accessible for 62 primary tumors. Clinical and pathological parameters of these patients were extracted from medical records and outlined in Table I; Figure 1 illustrates different immunostaining and fluorescence patterns. Out of these 62 patients, a *MYB::NFIB* fusion was detected in 38 tumors (61%). Four out of 62 tumors (6%) were negative for MYB expression on immunohistochemistry. The median MYB expression was 25% (IQR 5%-42%). In the 38 translocated tumors, 37 showed MYB expression with a median staining of 30% (IQR 12%-56%), significantly higher ($P = .02$) than the median 6% (IQR 4%-30%) in nontranslocated tumors.

Within the clinical and pathological parameters, there was an equal distribution of translocated and nontranslocated tumors. Although there was a rise in median MYB expression with increasing solid growth, no association was found between the growth pattern, MYB immunohistochemistry, or translocation status. This lack of association applied to all other differences within the clinicopathological (sub)groups as well.

Table I. Cohort characteristics

	N (%)	*Median % MYB*	*Translocated (%)*	*MYB ≤ 60%*	*MYB > 60%*
Patients	62	29%		53	9
Sex					
Male	19 (31%)	28%	12 (63%)	15 (79%)	4 (21%)
Female	43 (69%)	23%	26 (60%)	38 (88%)	5 (12%)
Age at diagnosis					
Median (IQR)	57 (44-67)			57 (43-67)	52 (47-65)
Range	20-89			20-83	37-89
Site and subsite					
Major salivary gland	38 (61%)	23%	23 (61%)	34 (89%)	4 (11%)
Minor salivary and seromucous gland	24 (39%)	30%	15 (63%)	19 (79%)	5 (21%)
Parotid gland	17	17%	9 (53%)	16 (94%)	1 (6%)
Submandibular gland	19	23%	12 (63%)	17 (90%)	2 (10%)
Sublingual gland	2	50%	2 (100%)	1 (50%)	1 (50%)
Oral cavity (lip/buccal mucosa/hard palate gingival)	8	5%	3 (38%)	7 (88%)	1 (12%)
Oropharynx (soft palate/base of tongue)	7	17%	6 (86%)	7 (100%)	0
Nasal cavity/nasopharynx/maxillary sinus	3	42%	3 (100%)	2 (67%)	1(33%)
Larynx/trachea	3	43%	1 (33%)	2 (67%)	1 (33%)
Lacrimal gland	1	77%	1 (100%)	0	1 (100%)
External auditory canal	2	55%	1 (50%)	1 (50%)	1 (50%)
Tumor					
pT-stage (TNM 7th ed.)					
pT1	23	25%	13 (57%)	19 (83%)	4 (17%)
pT2	23	23%	15 (65%)	21 (91%)	2 (9%)
pT3	3	30%	3 (100%)	3 (100%)	0
pT4a	9	30%	4 (44%)	7 (78%)	2 (22%)
pT4b	4	21%	3 (75%)	3 (75%)	1 (25%)
Nodal status					
pN0	56 (90%)	27%	36 (64%)	47 (84%)	9 (16%)
pN+	6 (10%)	12%	2 (33%)	6 (100%)	0
Distant metastasis					
cM0	61 (98%)	25%	37 (61%)	52 (85%)	9 (15%)
cM1	1 (2%)	1%	1 (100%)	1 (100%)	0
Resection margins					
Clear (>5 mm)	9 (15%)	17%	5 (56%)	8 (89%)	1 (11%)
Close or positive (<5 mm)	53 (85%)	25%	33 (62%)	45 (85%)	8 (15%)
Perineural growth					
Present	43 (69%)	30%	24 (56%)	39 (91%)	4 (9%)
Absent	18 (29%)	12%	14 (78%)	13 (72%)	5 (28%)
Vasoinvasive growth					
Present	9 (15%)	5%	5 (56%)	9 (100%)	0
Absent	52 (84%)	30%	33 (63%)	43 (83%)	9 (17%)
Bone invasion					
Present	10 (16%)	33%	7 (70%)	7 (70%)	3 (30%)
Absent	52 (84%)	23%	31 (60%)	46 (89%)	6 (11%)
Growth pattern (Perzin grade[19])					
Tubular (grade 1)	25 (40%)	17%	14 (56%)	23 (92%)	2 (8%)
Cribriform; < 30% solid (grade 2)	27 (44%)	25%	19 (70%)	22 (82%)	5 (18%)
Solid (grade 3)	10 (16%)	34%	5 (50%)	8 (80%)	2 (20%)
**MYB*::*NFIB* fusion					
Present	38 (61%)	*30%		†29 (76%)	9 (24%)
Absent	24 (39%)	6%		24 (100%)	0
Treatment					
Adjuvant radiotherapy					
Yes	60 (97%)		36 (58%)	51 (85%)	9 (15%)
No	2 (3%)		2 (100%)	2 (100%)	0

*Kruskal-Wallis analysis $P = .02$.
†Pearson Chi^2 test $P < .01$.

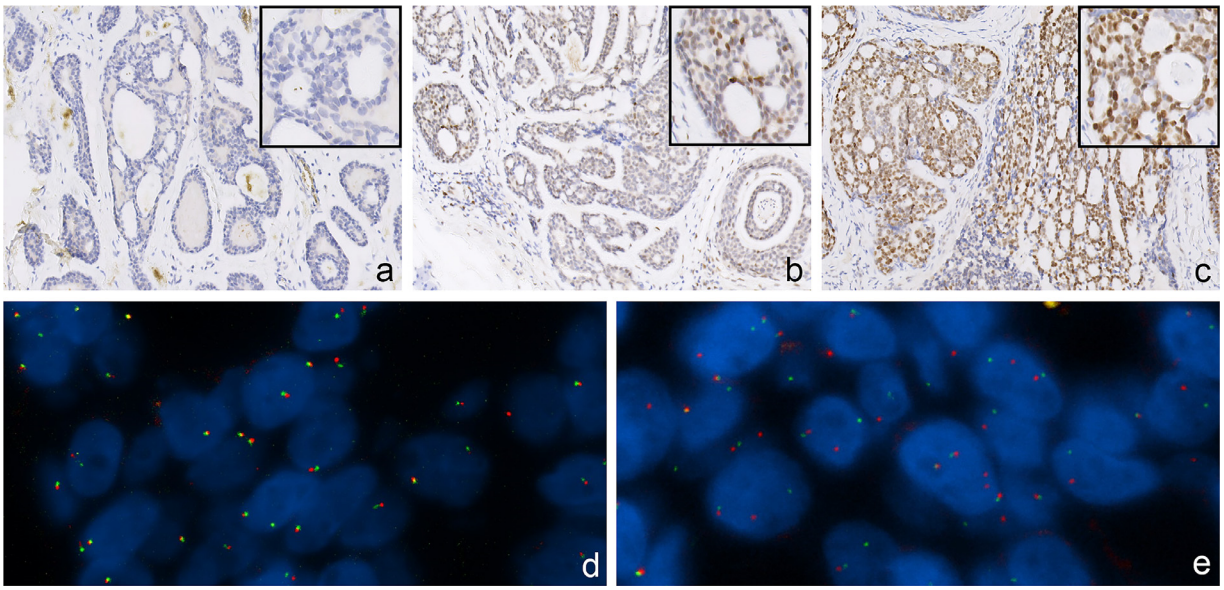

Figure 1. MYB immunohistochemistry and MYB FISH patterns. *MYB immunohistochemistry*. (A) 2% expression; (B) 40% expression; (C) 70% expression. Magnification: 100x, with insets at 200x. *MYB FISH*. (D) no translocation; (E) *MYB::NFIB* translocation >10% break apart signal.

The area under the ROC-curve was 0.71 (95% confidence interval 0.58-0.84). Given the moderate diagnostic predictability of the *MYB::NFIB* fusion by MYB expression, the optimal cut-off value of 5% has no clinical relevance for daily practice. It was determined that MYB expression of 60% or higher accurately indicates the presence of the translocation in this cohort (N=9; specificity 100%; positive predictive value 100%). Conversely, the absence of MYB expression indicates the absence of the translocation (N = 4; sensitivity 97%; negative predictive value 75%), as shown in Table II. A proposed diagnostic workflow based on these findings is presented in Figure 2. Table III presents the five and ten-year survival rates regarding both *MYB::NFIB* fusion status and dichotomized MYB expression. Disease recurrence occurred with equal frequency in all groups; no statistical difference was found. Furthermore, the current data revealed no univariate prognostic value for either variable in terms of overall survival, disease-specific survival, disease-free survival, recurrence-free survival, and metastasis-free survival (data not shown).

DISCUSSION

This study demonstrates that 58/62 tumor samples showed substantial homogenous MYB protein expression and that overexpression >60% corresponds to a *MYB::NFIB* fusion in all tested samples (9/9). Therefore, we suggest a 60% immunohistochemical expression of MYB as a reliable cut-off point to predict *MYB::NFIB* fusion in AdCC. Furthermore, negative

Table II. Predictability results for *the presence of MYB*::*NFIB* fusion/translocation (6;9)(q22-23;p23-24) using immunohistochemistry

	MYB cut-off 0%			*MYB cut-off > 60%*		
		FISH+	FISH-		FISH+	FISH-
N = 62	MYB > 0%	37	21	MYB > 60%	9	0
	MYB 0%	1	3	MYB ≤ 60%	29	24
Sensitivity		97.4%			23.7%	
Specificity		12.5%			100%	
Positive predictive value		63.8%			100%	
Negative predictive value		75.0%			45.3%	
Likelihood ratio +		1.11			∞	
Likelihood ratio -		0.03			0.76	

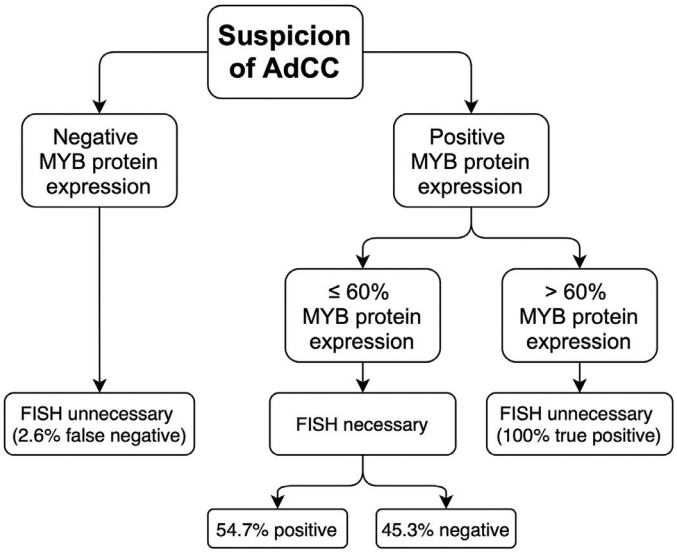

Figure 2. Proposed diagnostic workflow.

MYB expression by immunohistochemistry may be used to rule out the translocation as only one out of the 38 translocated tumors did not show any MYB expression, reflected by a high sensitivity of 97.4%. This then enables a shift from secretory gland translocation analysis, such as by FISH, towards immunohistochemistry in future diagnostic screening (see Figure 2). In this study, the test has proven to be reliably applicable in 13 patients (23%). It provides a fast and accurate diagnosis against reduced costs in 9 patients and raises a strong suspicion of the absence of AdCC in 4 cases.

Several studies on the same subject, as elaborated below, have concluded that *MYB::NFIB* fusion is absent in salivary gland neoplasms other than AdCC, thereby establishing its pathognomonic association with this specific entity. Furthermore, West et al.[12] suggest that *strong* MYB protein expression, defined by positivity in more than 50% of the tumor cells, is highly specific to AdCC, as all other salivary gland neoplasms in their study either stained negative or showed weak and focal expression at most.

Table III. Survival data

	MYB::NFIB Negative	*MYB::NFIB Positive*	*MYB ≤60%*	*MYB >60%*
Overall survival (OS)				
5-year OS	79%	84%	83%	78%
10-year OS	75%	82%	79%	78%
Disease-specific survival (DSS)				
5-year DSS	88%	87%	89%	78%
10-year DSS	75%	82%	79%	78%
Disease-free survival (DFS)				
5-year DFS	63%	68%	68%	56%
10-year DFS	50%	63%	59%	56%
Locoregional recurrence-free survival (RFS)				
5-year RFS	88%	76%	83%	67%
10-year RFS	79%	71%	76%	67%
Metastatis-free survival (MFS)				
5-year MFS	71%	79%	77%	67%
10-year MFS	63%	74%	70%	67%

The gene fusion and immunostaining results presented in this paper align with the common findings of similar studies despite variations in staining protocols and interpretation thresholds. West et al.[12] reported a *MYB::NFIB* translocation in 49% of the 37 AdCCs out of a group of 149 salivary gland tumors. All 112 non-AdCC samples were fusion negative, although *MYB* rearrangements were seen in 16%. Negative FISH results in all tested non-AdCC salivary gland tumors were concordantly reported by Mitani et al.[15] (N = 34) and all non-AdCC lacrimal tumors by Holstein et al.[21] (N=19). In their AdCCs, the translocation was identified in 28% and 50% respectively. Xu et al.[22] identified the translocation in 59% of its AdCCs. The current study identified comparable results, with the translocation detected in 56% and rearrangements in 11% of the tumors. Additionally, immunohistochemistry results from these studies consistently demonstrate predominant nuclear expression of MYB.

West et al.[12] described positive (>5%) nuclear MYB expression in 24/37 (65%) of all AdCCs, 14/18 (78%) of the translocated AdCCs and 6/13 (46%) of the non-translocated AdCCs. The suggested trend of higher recurrence rates in translocated AdCCs was concordantly seen in our study, although it was not statistically significant. Increased perineural invasion related to the translocation was not observed (see Tables I and III). The immunohistochemical analysis by Mitani et al.[15] described positive nuclear expression (≥10%) in 85% of the translocated tumors (17/20), in contrast to 61% (25/41) of the nontranslocated tumors. They

found an association between translocation status and age above 50 years, which is not supported by our data. Lacrimal AdCCs all showed strong nuclear expression of the MYB protein.[21]

Xu et al.[22] reported positive (>5% nuclear staining) MYB expression in 72% of the samples (57/79), and peripheral staining was described in 39%. Five out of 56 (9%) of the nonsalivary gland tumors were MYB positive. Sensitivity and specificity indicating the translocation by immunostaining were 78% and 50% respectively.

MYB overexpression in *MYB::NFIB* negative tumors is probably caused by alternative genomic rearrangements, such as different fusion partners that involve the *MYB* locus or the loss of genetic materials at the same location, denoting the presence of a tumor suppressor gene. Furthermore, epigenetic modifications affecting the transcriptional activity of the *MYB* promoter can lead to a positive-feedback loop that upregulates the MYB protein expression.[11,12,15]

Xu et al.[23] investigated *MYB* gene expression by (m) RNA analysis. They concluded that high expression was significantly associated with solid growth pattern (known as a negative prognosticator) and lung metastases.[2] In contrast, the study by Park et al.[24] found a higher risk of developing distant metastases in tumors without MYB protein expression and argued that MYB acts protectively. Our results reflected neither of these findings.

A recent and large study unraveled the different *MYB* rearrangements and identified the specific loss of the 3'-part of the *MYB* gene to be associated with diminished overall survival in AdCC. It was more common in grade 3 tumors (>30% solid growth pattern) but remained a significant independent prognosticator in multivariate analysis. Again, *MYB* rearrangements demonstrated no association with age, sex, perineural invasion, or other clinicopathological characteristics, consistent with our findings.[25]

Patients with advanced recurrent or metastatic AdCC face limited treatment options, prompting research into targeted therapies.[26] The *MYB*::*NFIB* fusion in AdCC is regulated by the AKT-dependent IGF1R signaling pathway, and inhibitors like linsitinib demonstrate potential therapeutic effects.[27] The polyether ionophore monensin and proteasome inhibitor oprozomib exhibit MYB inhibition, but require further investigation for clinical use.[28] The proto-oncogene C-KIT, a target gene of MYB, shows increased expression in solid pattern AdCC, indicating its role in disease progression, although its inhibitor imatinib has limited efficacy. Various markers and immunotherapeutic agents are under study, emphasizing the need for a personalized approach based on patient-specific expression patterns.[29-31]

The histopathologic differential diagnosis for head and neck AdCC encompasses various salivary gland neoplasms that may show reactivity to the MYB antibody. Furthermore, interpreting MYB immunohistochemistry on cytology samples may raise a challenge due to the presence of strong lymphocyte positivity, although lymphocytes should not typically constitute the majority of the cells.[32] In addition to the AdCC cases, West et al.[12] conducted MYB immunohistochemistry on 112 other salivary gland neoplasms. Among these, 9% showed weak and focal expression: 2/4 myoepithelial carcinomas; 4/12 polymorphous adenocarcinomas; 1/5 myoepithelioma; 2/35 (cellular) pleomorphic adenomas; and 1/23 mucoepidermoid carcinoma. All negative were 9 basal cell adenomas; 9 oncocytomas; 5 adenosquamous carcinomas; 7 acinic cell carcinomas; and 3 salivary duct carcinomas.

Similarly, Brill et al.[33] studied a cohort of 133 non-AdCC salivary gland neoplasms for both *MYB::NFIB* fusion status using PCR and immunohistochemical expression. Intense MYB expression in more than 5% of cell nuclei was defined as positive. They observed a total positive immunohistochemistry rate of 14%: 4/5 basaloid squamous cell carcinomas; 3/9 monomorphic/-basal cell adenomas; 2/15 polymorphous adenocarcinomas; 2/18 salivary duct carcinomas; and one of each acinic cell carcinoma, basal cell adenocarcinoma, mucoepidermoid carcinoma, epithelial-myoepithelial carcinoma, and pleomorphic adenoma. However, the specific percentage of positive cells was not provided despite the knowledge that MYB immunohistochemistry results do not strictly categorize as positive or negative. It is emphasized that it is crucial to assess both the quality and quantity of MYB protein expression.[32] Additionally, all non-AdCC samples that showed MYB protein expression in both studies were *MYB::NFIB* fusion negative.[12,33]

A diagnostic dilemma lies in distinguishing cribriform AdCC and cribriform basal cell adenomas and -carcinomas. A direct comparison analyzing MYB yielded incongruent results in protein expression between the studies by Tian et al. and the study by Rooney et al. Positive gene splitting was observed by Tian et al.[34] in 9/20 (45%) cases in the AdCC group, and positive MYB immunostaining in 11/20 (55%). All basal cell adenomas and carcinomas tested negative for both FISH and immunohistochemistry. In contrast, Rooney et al.[35] described MYB overexpression in 17/30 (57%) of basal cell adenomas and 9/17 (53%) of basal cell adenocarcinomas, utilizing the same protein. However, the specific percentage of MYB-positive nuclei was again not provided, except for the 10% threshold. Another histopathological diagnosis that shares similarities with AdCC and basal cell

adeno(carcino)mas is Human Papillomavirus-Related Multiphenotypic Sinonasal Carcinoma, which exhibits a salivary gland tumor-like appearance. This carcinoma demonstrates a variable spectrum of MYB expression on immunohistochemistry, ranging from moderate to strong intensity.[36]

It is challenging to conclude from the aforementioned studies whether a high percentage of diffuse MYB nuclear staining is restricted to AdCC, as several studies do not specifically mention the staining pattern. Relying solely on MYB immunohistochemistry in diagnosing AdCC can still pose a diagnostic pitfall. The sensitivity and specificity values provided by this study might be lower in typical clinical settings, where other entities besides AdCC are present.

In summary, despite consistent and substantially homogenous protein expression in AdCC, future investigations into the clinical significance of various *MYB* rearrangements and their impact on associated protein expression, clinicopathological prognosticators and survival will be relevant for a more comprehensive understanding in the context of daily clinical practice.

CONCLUSION

Identification of the *MYB::NFIB* fusion is relevant for diagnosing AdCC. This fusion was present in the majority of AdCC cases (61%), exhibiting significantly higher MYB expression compared to nontranslocated tumors. A MYB expression assessed by immunohistochemistry using a cut-off value of 60% accurately predicted the presence of *MYB::NFIB* fusion in all cases of this cohort. Negative MYB expression served as a strong indicator for the absence of the translocation. The study did not identify a correlation between MYB expression, prognostic parameters and survival. Further research is necessary to clarify whether this high percentage of MYB-positive cell nuclei is specific enough for diagnosing AdCC in routine clinical practice.

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DECLARATIONS OF INTEREST

None.

CREDIT AUTHORSHIP CONTRIBUTION STATEMENT

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