Prognostic Impact of Notch1 Intracellular Domain, P63, and c-MYC in Lacrimal Gland Adenoid Cystic Carcinoma

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Purpose. We assessed whether NICD1 expression, c-MYC expression, and P63 expression by immunohistochemistry (IHC) correlate with prognosis and high-risk clinicopathological features in lacrimal gland adenoid cystic carcinoma (ACC).

Methods. Records of patients with lacrimal gland ACC who underwent surgery between 1998 to 2018 were reviewed. Clinicopathologic and treatment data were collected. Tumor tissues were subjected to light microscopy and IHC.

Results. Of 43 patients treated during the study period, 21 had archived tumor tissue available and were included. The median age at diagnosis was 47 years, and 13 patients (62%) were male. Thirteen patients (62%) had T2 disease, and none had nodal or distant metastasis at diagnosis. Tumors were positive for NICD1 expression in eight cases (38%), c-MYC expression in eight (38%), and P63 expression in 11 (52%). Positive NICD1 expression was associated with predominantly solid (vs. cribriform/tubular) pattern ($P < 0.001$), treatment with orbital exenteration (vs. eye-sparing surgery) ($P = 0.008$), local recurrence ($P = 0.047$), and death ($P = 0.012$). Negative P63 expression was associated with predominantly solid pattern ($P = 0.001$), local recurrence ($P = 0.012$), distant metastasis ($P = 0.001$), and death ($P = 0.035$). A higher percentage of tumor cells staining for c-MYC was associated with presence of perineural invasion ($P = 0.036$). Positive NICD1 expression was associated with worse disease-free survival (hazard ratio, 6.27; 95% CI, 1.29–30.46), whereas positive P63 expression was associated with better disease-free survival (hazard ratio, 0.03; 95% CI, 0.0002–0.26).

Conclusions. IHC for NICD1 and P63 should be considered in lacrimal gland ACC because of their prognostic value and potential as treatment targets.

Keywords: lacrimal gland adenoid cystic carcinoma, Notch1 intracellular domain, P63, c-MYC

Adenoid cystic carcinoma (ACC) accounts for less than 4% of all lacrimal gland lesions but is the most common epithelial malignancy of the lacrimal gland. ACC of the lacrimal gland is refractory to chemotherapy and associated with a poor prognosis.[1–3] There is no standard treatment for recurrent or metastatic lacrimal gland ACC.[3]

Previous studies of whole exome sequencing of mainly salivary gland ACC samples demonstrated that Notch pathway alterations were present in 11% to 29% of the samples.[4–6] Notch signaling is critical for angiogenesis, stem cell maintenance, cell fate specification, and cell proliferation.[7] *NOTCH1* mutations can be tumor activators or suppressors depending on the tumor type.[8–11] Most publications on Notch and its role in ACC have been generated from studies of salivary gland ACC. In a study of ACCs from the salivary glands and other disease sites, 91% of *NOTCH1* mutations were predominantly activating, and *NOTCH1* mutations characterized a subtype of ACC associated with solid subtype, distinct pattern of metastasis, advanced-stage disease, and worse prognosis.[12] In that study, *NOTCH1* mutations in ACC were significantly associated with Notch1 intracellular domain (NICD1) staining by immunohistochemistry (IHC), which is an established marker for Notch1 pathway activation.[12]

A recent proteogenomic analysis of salivary gland ACC identified two biologically distinct subtypes with different histologic characteristics and prognosis, independent of tumor stage.[13] The two subtypes can be differentiated on the basis of specific staining for NICD1, c-MYC, and P63 protein levels.

The purpose of this study was to assess whether NICD1 expression, c-MYC expression, and P63 expression by IHC correlate with prognosis in patients with ACC of the lacrimal gland, specifically local recurrence, distant metastasis, and

survival. In addition, we examined the relationship between the expression of these biomarkers and high-risk clinicopathological features of lacrimal gland ACC, including solid histopathological pattern, presence of perineural invasion, presence of bony invasion, and higher tumor stage.

Methods

Medical records of all patients with a diagnosis of lacrimal gland ACC who underwent surgery from January 1998 through February 2018 were retrospectively reviewed. The study was approved by the Institutional Review Board of The University of Texas MD Anderson Cancer Center. Data concerning patient characteristics, clinical and radiological findings, and treatment modalities were collected. All tissues obtained during surgery for lacrimal gland ACC were subjected to light microscopy and IHC studies. Cleaved Notch1 monoclonal antibody (D3B8; no. 4147; Cell Signaling, Danvers, MA, USA), P63 monoclonal antibody (4A4; CM163C; Biocare Medical, Pacheco, CA, USA), and c-MYC polyclonal antibody (790-4628; Roche, Basel, Switzerland) were used to detect NICD1, P63, and c-MYC expression, respectively. Protein expression was scored in terms of the percentage of cells with uniformly or diffusely strong nuclear staining and in a binary fashion. Data were categorized with a grading system similar to that used in a study by Ferrarotto et al.[13,14] For NICD1 and c-MYC, positive expression was defined as nuclear staining in ≥70% of tumor cells; negative expression was defined as nuclear staining in <70% of tumor cells. For P63, positive expression was defined as nuclear staining in ≥10% of tumor cells; negative expression was defined as nuclear staining in <10% of tumor cells.

Continuous data were summarized using median and range. Categorical data were summarized using frequencies and percentages. Continuous covariates of interest were compared using the Wilcoxon rank-sum test, and categorical covariates of interest were compared using Fisher's exact test. Linear regression was used to explore the relationship between study covariates and biomarker expression considered as a continuous outcome. Disease-free survival (DFS) was defined as the time from diagnosis to local recurrence, distance metastasis, or death, whichever occurred first. Patients who did not experience a DFS event were censored at their date of last contact. Overall survival (OS) was defined as the time from diagnosis to death from any cause. Patients not experiencing death were censored at their date of last contact. Distributions of DFS and OS were estimated using the Kaplan-Meier method. Univariate Cox regression was used to explore associations between survival outcomes and study covariates of interest. Firth's method was used to provide hazard ratio (HR) estimates when convergence issues occurred. $P < 0.05$ was considered statistically significant.

Results

Clinicopathological Characteristics

Of 43 patients with ACC of the lacrimal gland treated during the study period, 21 had archived tumor tissue available for IHC studies and were included. Patient characteristics are summarized in Table 1. The median age at diagnosis was 47 years. The study included more men than women. Fourteen patients were White, three were Hispanic, two were Asian, and two were Black. According to the eighth edition of the *American Joint Committee on Cancer Staging Manual*, the most common T categories at diagnosis were T2 ($n = 13$) and T4 ($n = 5$); no patient had nodal or distant metastasis at initial presentation.

Near equal numbers of patients underwent eye-sparing surgery ($n = 11$) and orbital exenteration ($n = 10$). On histopathological analysis, a predominantly solid pattern

Table 1. Clinicopathological Characteristics of Patients With Lacrimal Gland ACC ($N = 21$)

Characteristic	
Age at diagnosis (yr), median (range)	47 (29–69)
Sex	
Male	13 (62%)
Female	8 (38%)
Race and ethnicity	
Asian	2 (10%)
Black	2 (10%)
Hispanic	3 (14%)
White	14 (67%)
AJCC (8th ed) tumor category at diagnosis	
T1	2 (10%)
T2	13 (62%)
T3	1 (5%)
T4	5 (24%)
AJCC (8th ed) nodal category at diagnosis	
N0	21 (100%)
N1	0 (0%)
AJCC (8th ed) metastasis status at diagnosis	
M0	21 (100%)
M1	0 (0%)
Type of surgery	
Eye-sparing	11 (52%)
Exenteration	10 (48%)
Histopathological pattern	
Predominantly cribriform and tubular	14 (67%)
Predominantly solid	7 (33%)
Perineural invasion	
No	3 (14%)
Yes	18 (86%)
Bone invasion	
No	12 (57%)
Yes	9 (43%)
Adjuvant radiation therapy	
No	0 (0%)
Yes	21 (100%)
Concurrent adjuvant chemotherapy	
No	8 (38%)
Yes	13 (62%)
Local recurrence during follow-up	
No	16 (76%)
Yes	5 (24%)
Distant metastasis during follow-up	
No	14 (67%)
Yes	7 (33%)
NICD1 expression	
Positive (staining in ≥70% of tumor cells)	8 (38%)
Negative	13 (62%)
c-MYC expression	
Positive (staining in ≥70% of tumor cells)	8 (38%)
Negative	13 (62%)
P63 expression	
Positive (staining in ≥10% of tumor cells)	11 (52%)
Negative	10 (48%)

AJCC (8th ed), eight edition of the *AJCC Cancer Staging Manual*.

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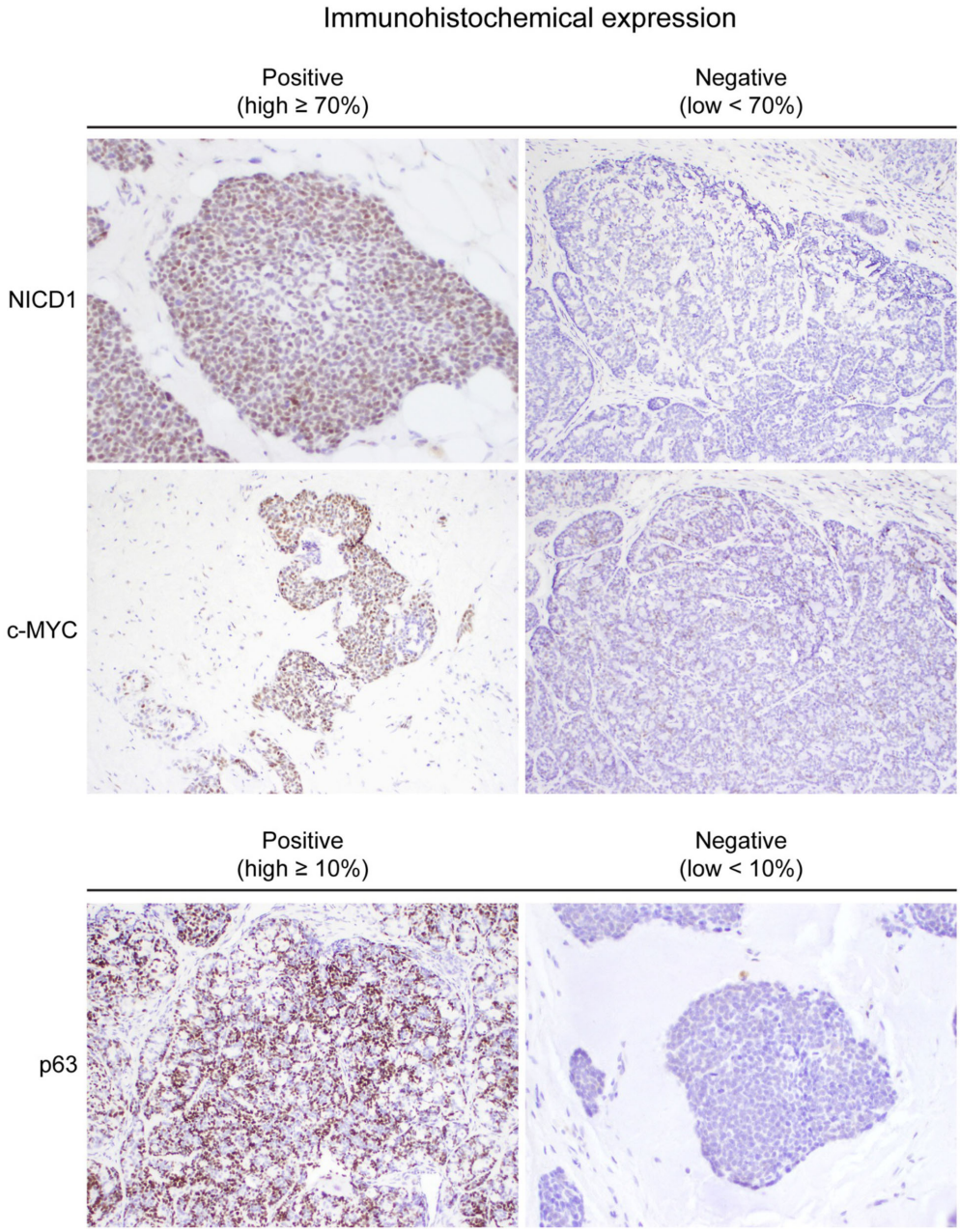

Figure 1. Representative immunohistochemistry staining of positive and negative expression of NICD1, c-MYC and P63 in lacrimal gland ACC tumor samples. Positive NICD1 and c-MYC staining were seen in tumors with a predominantly solid pattern, whereas negative NICD1 and c-MYC staining were seen in tumors with a predominantly cribriform pattern. In contrast, positive p63 staining was found in tumors with a predominantly cribriform pattern, whereas negative p63 staining was found in tumors with a predominantly solid pattern.

was detected in seven cases, whereas a predominantly cribriform and tubular pattern was detected in 14. Perineural invasion was present in 18 cases, and bone invasion was present in nine. All patients received adjuvant radiation therapy, and the median dose was 60 Gy (range 60–66). Thirteen patients also received concurrent chemotherapy. The median follow-up time was 5.8 years (range 0.5–11.6). During follow-up, local recurrence was detected in five patients, and distant metastasis was detected in seven. The sites of distant metastasis included lung ($n = 5$), bone ($n = 4$), liver ($n = 3$), retroperitoneal space ($n = 1$), brain ($n = 1$), adrenal gland ($n = 1$), and mediastinal/hilar lymph nodes ($n = 1$).

Positive NICD1 expression was found in 8 cases, positive c-MYC expression was found in eight cases, and positive P63 expression was found in 11 cases. Representative images of IHC staining for NICD1, c-MYC, and P63 are shown in Figure 1.

NICD1, c-MYC, and P63 Expression and Correlation With Clinicopathological Features

The percentages of tumor cells staining for NICD1, c-MYC, and P63 for the cases in the cohort are shown in Figure 2. The percentage of tumor cells staining for NICD1 was higher in tumors with a predominantly solid pattern ($\beta = 42.50$,

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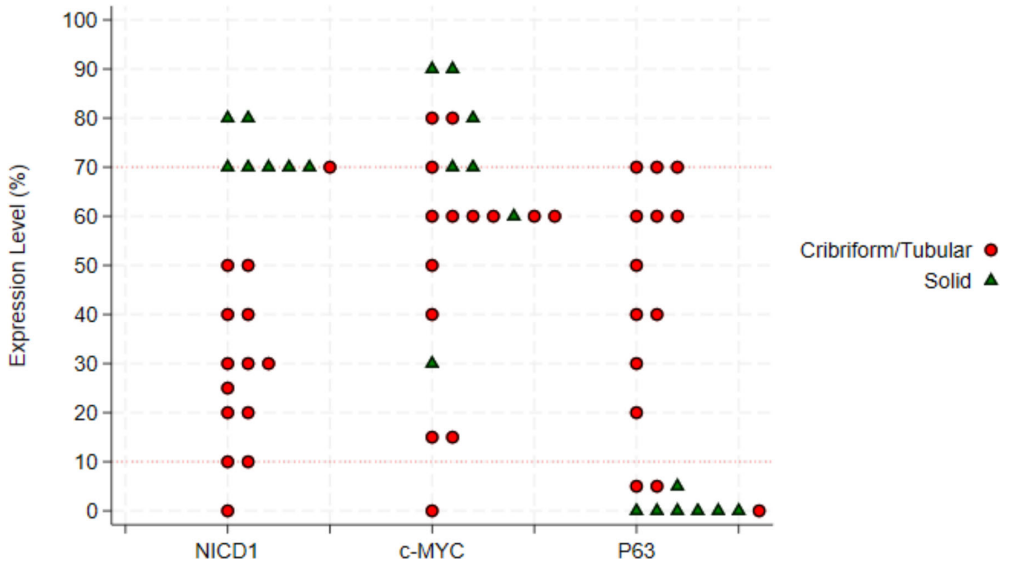

Figure 2. Percentages of tumor cells staining for NICD1, c-MYC, and P63 expression in lacrimal gland ACC. The top *dashed line* indicates 70%, at or above which cases were considered to have positive expression for NICD1 and c-MYC in the bivariate analysis. The bottom *dashed line* indicates 10%, at or above which cases were considered to have positive expression for P63.

Table 2. Bivariate Analysis of Relationships Between NICD1, P63, and MYC Expression and Clinicopathological Characteristics*

	NICD1 Expression			P63 Expression			MYC Expression		
Characteristic	**Negative (<70%)**	**Positive (≥70%)**	***P* Value**	**Negative (<10%)**	**Positive (≥10%)**	***P* Value**	**Negative (<70%)**	**Positive (≥70%)**	***P* Value**
Age at diagnosis, yr			0.277			0.078			0.744
Median (range)	50 (32–69)	40 (29–63)		39 (29–63)	52 (34–69)		47 (29–69)	43 (34–63)	
Type of surgery			**0.008**			0.086			1
Eye-sparing	10 (77)	1 (12)		3 (56)	8 (73)		7 (54)	4 (50)	
Exenteration	3 (23)	7 (88)		7 (44)	3 (27)		6 (46)	4 (50)	
AJCC (8th ed) T category			0.146			0.361			1
T1-T2	11 (85)	4 (50)		6 (60)	9 (82)		9 (69)	6 (75)	
T3-T4	2 (15)	4 (50)		4 (40)	2 (18)		4 (31)	2 (25)	
Histopathological pattern			**<0.001**			**0.001**			0.056
Predominantly solid	0 (0)	7 (12)		7 (70)	0 (0)		2 (15)	5 (62)	
Predominantly cribriform/tubular	13 (100)	1 (88)		3 (30)	11 (100)		11 (85)	3 (38)	
Perineural invasion			0.257			1.00			0.257
No	3 (23)	0 (0)		1 (10)	2 (18)		3 (23)	0 (0)	
Yes	10 (77)	8 (100)		9 (90)	9 (82)		10 (77)	8 (100)	
Bone invasion			1			0.387			0.367
No	7 (54)	5 (62)		7 (70)	5 (46)		6 (46)	6 (75)	
Yes	6 (46)	3 (38)		3 (30)	6 (54)		7 (54)	2 (25)	
Local recurrence			**0.047**			**0.012**			0.325
No	12 (92)	4 (50)		5 (50)	11 (100)		11 (85)	5 (62)	
Yes	1 (8)	4 (50)		5 (50)	0 (0)		2 (15)	3 (38)	
Distant metastasis			0.056			**0.001**			0.656
No	11 (85)	3 (38)		3 (30)	11 (100)		8 (62)	6 (75)	
Yes	2 (15)	5 (62)		7 (70)	0 (0)		5 (38)	2 (25)	
Patient status			**0.012**			**0.035**			0.618
Alive	13 (100)	4 (50)		6 (60)	11 (100)		11 (85)	6 (75)	
Dead	0 (0)	4 (50)		4 (40)	0 (0)		2 (15)	2 (25)	

AJCC (8th ed), eight edition of the *AJCC Cancer Staging Manual*.
Significant *P* values are in bold.
* For NICD1 and c-MYC, positive expression was defined as nuclear staining in ≥70% of tumor cells; for P63, positive expression was defined as nuclear staining in ≥10% of tumor cells.

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SE = 7.25, $P < 0.001$) and in patients who underwent orbital exenteration ($\beta = 21.91$, SE = 10.31, $P = 0.047$) or experienced local recurrence ($\beta = 28.19$, SE = 11.79, $P = 0.027$) or death ($\beta = 34.56$, SE = 12.25, $P = 0.011$). The percentage of tumor cells staining for P63 was higher in patients aged 50 years and older ($\beta = 25.14$, SE = 11.55, $P = 0.042$), whereas the percentage was lower in tumors with a predominantly solid pattern ($\beta = -40.71$, SE = 9.82, $P = 0.001$) and in patients who experienced local recurrence ($\beta = -35.25$, SE = 12.63, $P = 0.012$), distant metastasis ($\beta = -38.57$, SE = 10.26, $P = 0.001$), or death ($\beta = -34.41$, SE = 14.22, $P = 0.026$). A higher percentage of tumor cell staining for c-MYC was associated with presence of perineural invasion ($\beta = 31.67$, SE = 14.02, $P = 0.036$).

The relationships between the biomarkers scored as positive or negative and clinicopathological features are summarized in Table 2. Positive NICD1 expression was associated with predominantly solid histopathological pattern ($P < 0.001$), need for exenteration ($P = 0.008$), local recurrence ($P = 0.047$), and death ($P = 0.012$). Negative P63 expression was significantly associated with predominantly solid histopathological pattern ($P = 0.001$), local recurrence ($P = 0.012$), distant metastasis ($P = 0.001$), and death ($P = 0.035$). These findings for NICD1 and P63 were similar to ones from the linear regression model. For c-MYC, there was a trend toward an association between positive c-MYC expression and predominantly solid histopathological pattern ($P = 0.056$).

Examining the relationships between the biomarkers themselves revealed that NICD1 expression was positively correlated with c-MYC expression ($\rho = 0.497$; 95% CI, 0.083–0.764; $P = 0.022$) and negatively correlated with P63 expression ($\rho = -0.654$; 95% CI, −0.847 to −0.310; $P = 0.001$).

NICD1, c-MYC, and P63 Expression and Survival

The estimated OS rates at three, six, and nine years were 94.4%, 80.8%, and 80.8%, respectively. The four patients who died during the study period all had distant metastasis. On Kaplan-Meier survival analysis, T3-T4 disease ($P = 0.025$) and distant metastasis ($P = 0.006$) were associated with significantly poorer OS (Figs. 3A, 3B). Patients with positive NICD1 expression had poorer OS, with a P value approaching statistical significance ($P = 0.051$) (Fig. 3C). No statistically significant associations with OS were found for predominant histopathological pattern, perineural invasion, bone invasion, P63 expression, or c-MYC expression. By the end of the study period, four of the eight patients with positive NICD1 expression versus none of the 13 patients with negative NICD1 expression had died.

Nine DFS events were observed. The median DFS was 8.2 years. On Kaplan-Meier survival analysis, positive NICD1 expression ($P = 0.009$) (Fig. 4A), negative P63 expression ($P < 0.001$) (Fig. 4B), and predominantly solid histopathological pattern ($P = 0.031$) (Fig. 4C) were associated with significantly worse DFS. No statistically significant correla-

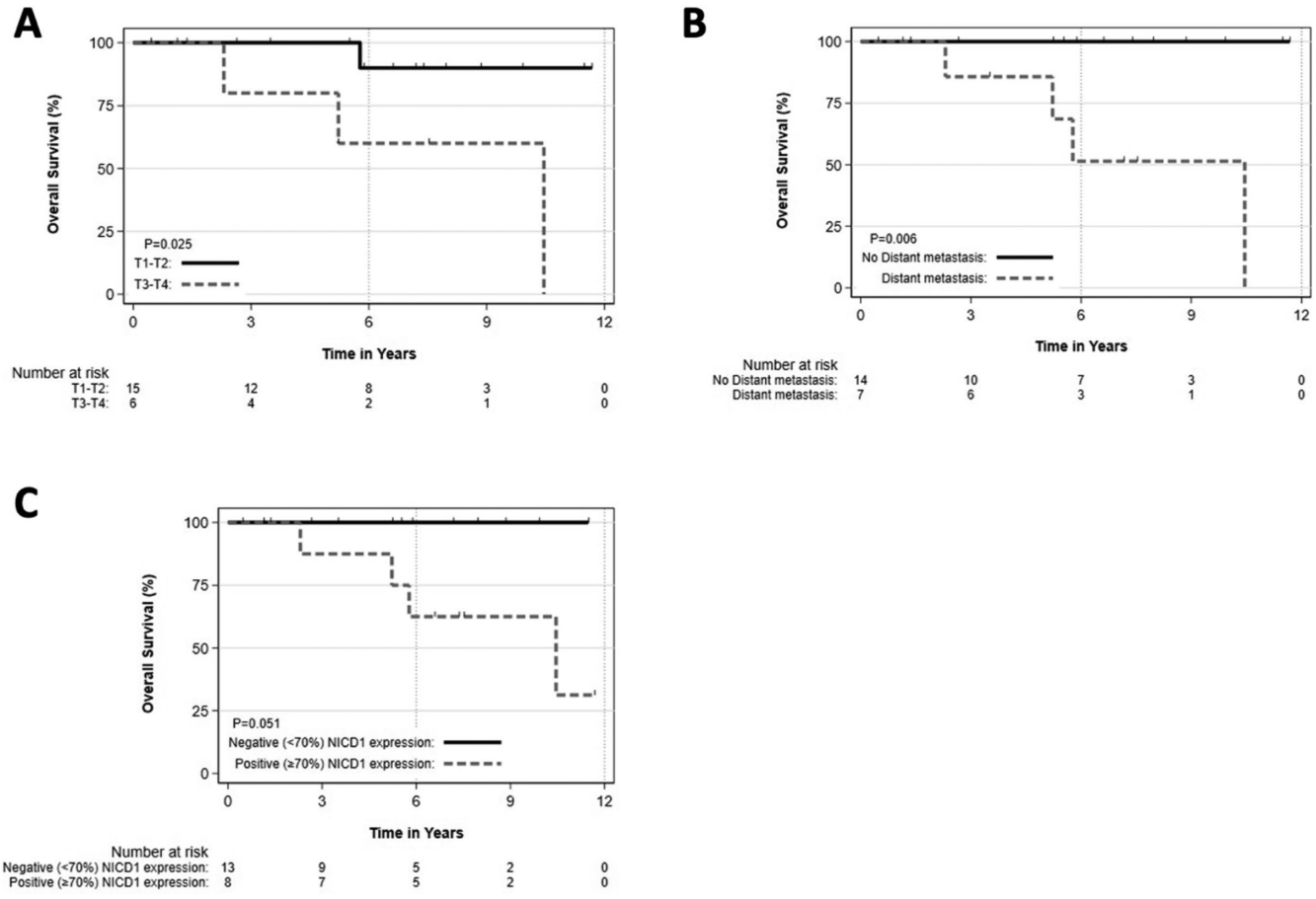

Figure 3. Kaplan-Meier curves for OS of patients with lacrimal gland ACC by (**A**) T category according to the eighth edition of the *AJCC Cancer Staging Manual*, (**B**) distant metastasis status, and (**C**) NICD1 expression.

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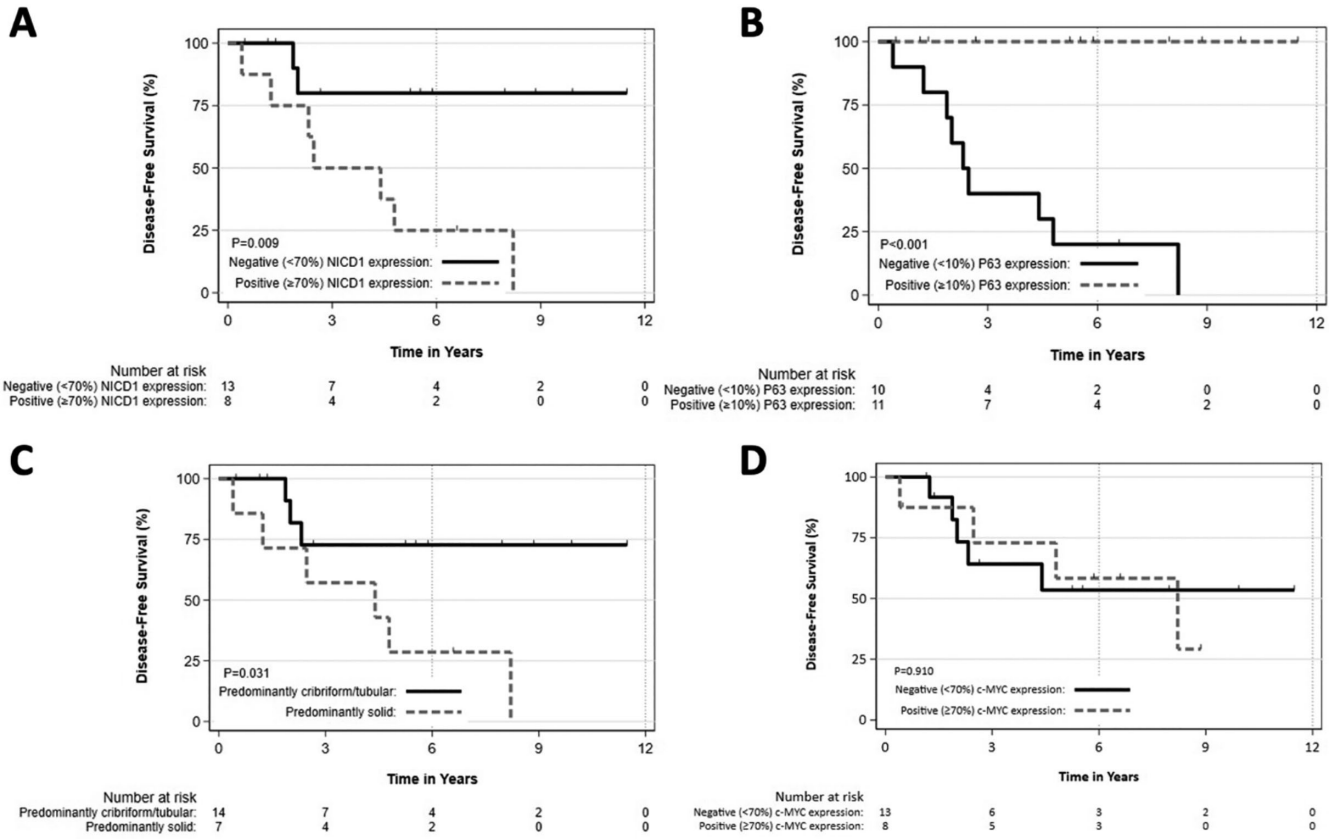

Figure 4. Kaplan-Meier curves for DFS of patients with lacrimal gland ACC by (**A**) NICD1 expression, (**B**) P63 expression, (**C**) histopathological pattern, and (**D**) c-MYC expression.

tions with DFS were found for T category, perineural invasion, bone invasion, or c-MYC expression.

Predominantly solid histopathological pattern and positive NICD1 expression were found to be significant prognostic factors for worse DFS, with HRs of 4.11 (95% CI, 1.02–16.5; $P = 0.047$) and 6.27 (95% CI, 1.29–30.46; $P = 0.023$), respectively. Positive P63 expression was associated with better DFS, with a HR of 0.03 (95% CI, 0.0002–0.26; $P < 0.001$). For OS, T3-T4 disease trended toward significance, with a HR of 8.96 (95% CI, 0.91–88.48; $P = 0.06$).

Discussion

One important finding of our study was confirmation of the prognostic value of NICD1 expression and P63 expression in lacrimal gland ACC. High NICD1 expression and low P63 expression were correlated with worse prognosis. IHC studies of these biomarkers can be performed in any clinical pathology laboratory to help provide information on prognosis and stratify patients for possible Notch-directed targeted therapy. Overall, we found high expression of NICD1 in eight cases (38%), P63 expression in 11 (52%), and c-MYC expression in eight (38%).

NICD1 staining by IHC is a good marker for Notch1 pathway activation,[12] and our study suggests that NICD1 expression can be used as a prognostic factor for survival, specifically DFS, in patients with lacrimal gland ACC. High NICD1 expression was also associated with tumors with a predominantly solid pattern, local recurrence, and death. This is similar to findings in a study including mainly salivary gland ACCs by Ferrarotto et al.,[12] in which NICD-positive tumors were significantly associated with a solid pattern, liver metastasis, and shorter DFS, but NICD-positive and NICD-negative tumors did not differ with respect to OS. In a study that included mainly samples of salivary gland and upper airway ACC, Sajed et al.[15] also found that NICD1 positivity in ACC samples was associated with solid growth pattern and significantly worse OS (mean OS of 56 months for NICD1 positive vs 140 months for NICD1 negative). Our study included both linear regression (biomarker expression considered as a continuous variable described in terms of percentage of staining) and bivariate analysis (staining in ≥70% tumor cells categorized as positive), with both analyses supporting the same conclusions.

We also found that patients with positive NICD1 expression were more likely to need an orbital exenteration versus eye-sparing surgery. The clinical decision making was based on the extent of tumor involvement in the orbit and adjacent structures and was performed before clinicians knew the individual patient's NOTCH status. In our cohort, two of 13 (15%) patients with negative NICD1 expression had T category of T3-T4, compared to four of eight (50%) with positive NICD expression. This result did not reach statistical significance, although the overall sample size was small. In the literature on salivary gland ACC, we found no published studies assessing the relationship between NICD1 expression and morbidity of surgical resection of tumor, but Feeney et al.[16] reported that significantly fewer patients with

NOTCH-activated salivary gland ACC than without NOTCH pathway activation had operable disease.

Due to the rarity of lacrimal gland ACC, most of the data on NOTCH mutations and other biomarkers have focused on salivary gland tumors of the head and neck region. Whole exome sequencing of lacrimal gland ACC has previously demonstrated functionally severe mutations in the Notch signaling pathway, including mutations in *NOTCH1* and *NOTCH2*.[17] Anjum et al.[18] examined expression of Notch1 and NICD1 by IHC and found Notch1 expression in 15 of 23 (63%) cases and NICD1 expression in 9 of 23 (39%). Anjum et al.[18] did not find any correlation between Notch1 or NICD1 expression and clinicopathological features, including histologic pattern, recurrence, and disease-related death. High Notch1 expression was a statistically significant predictor of poor DFS on Kaplan-Meier analysis but not on univariate Cox regression analysis. NICD1 was not found to have any prognostic value. In our study, we did not examine expression of Notch1 as NICD1 is a more direct indicator of Notch1 pathway activation. Our findings regarding NICD1 expression differed from those of Anjum et al. Our cohort and their cohort were very similar in terms of sample size, proportion of tumors with solid histopathological pattern, and percentage of patients with disease-related death. However, our cohort had higher percentages of patients with perineural invasion (86% vs. 39%) and distant metastasis (33% vs. 19%) and lower percentages of patients with T category of T3-T4 (29% vs. 43%) and local recurrence (24% vs 61%). In addition, our median length of follow-up was longer (70 months vs. 20 months). The longer follow-up in our study may explain the higher percentage of patients with distant metastasis in our cohort.

In addition to NICD1, we investigated two other biomarkers, P63 and c-MYC. P63 is a homologue of P53 with an essential role in morphogenesis of epidermis and limb and has been shown to be required for P53-dependent apoptosis in response to DNA damage.[19,20] P63 is also a selective immunohistochemical marker for basal stem cells of stratified epithelium and for myoepithelial cells.[21] The antagonism between *TP63* (gene for P63) and *NOTCH1* during cell differentiation and development is well established.[22,23] A study in salivary gland and upper airway ACC showed that P63-positive myoepithelial cells were generally NICD1 negative and that more aggressive specimens lacked P63 staining.[24] Our study similarly found a negative correlation between P63 expression and NICD1 expression in lacrimal gland ACC. Similar to positive NICD1 expression, negative P63 expression was significantly associated with predominantly solid histopathological pattern, local recurrence, distant metastasis, death, and worse DFS.

Prior studies demonstrated upregulation of MYC by activation of various pathways, including Notch1, MYB, and β-Catenin.[25–27] The Notch1/MYC axis also has an essential oncogenic role in T-cell acute lymphoblastic leukemia.[27] One study in lacrimal gland ACC revealed overexpression of MYC in 12 of 13 tumors.[28] Our study showed a positive correlation between c-MYC expression and NICD1 expression. Eight patients (38%) had positive expression of c-MYC. Higher percentage of tumor cells staining for c-MYC was associated with the presence of perineural invasion, but no relationship was found between percentage of tumors cells staining for c-MYC and histopathological pattern, local recurrence, distant metastasis, or survival.

A recent proteogenomic study in salivary gland ACC revealed two molecular subtypes.[14] ACC-I consisted of tumors with any solid component with enrichment of NOTCH-activating mutations and strong upregulation of c-MYC to promote tumorigenesis through prooncogenic c-MYC signaling. ACC-I was associated with worse prognosis with higher propensity to metastasize. ACC-II included more tumors of cribriform and tubular histology than of solid pattern; had upregulation of *TP63*, driving progrowth receptor tyrosine kinases in tumor proliferation; and had a less aggressive clinical course. The authors proposed rapid clinical subtyping of ACC by staining for only P63 and MYC. In this immunohistochemistry study, we also found two subtypes of lacrimal gland ACC, although with some differences to the proteogenomic study in salivary gland ACC. Tumors with positive NICD1 but negative P63 expression more commonly had a predominantly solid histopathological pattern with higher recurrence rate and worse prognosis. Tumors with positive P63 but negative NICD1 expression had a predominantly cribriform/tubular pattern, lower recurrence rate, and better prognosis. We did not find any relationship between DFS and T category, perineural invasion, bone invasion, and c-MYC expression.

Stratifying lacrimal gland ACC into distinct subtypes by IHC could facilitate counseling of patients regarding their prognosis. More importantly, it could allow tumor-specific targeting with different therapeutic agents depending on biomarker profile. The current standard treatment for lacrimal gland ACC is surgery, usually followed by radiotherapy with or without chemotherapy. There is no US Food and Drug Administration–approved systemic therapy for recurrent or metastatic ACC.[29] Several Notch-directed targeted therapies are currently in clinical trials, including γ-secretase inhibitor AL101 (BMS 906024) (NCT03691207) and pan-Notch inhibitor CB-103 (NCT03422679). Patients with lacrimal gland ACC who have positive expression of NICD1 can be considered for targeted treatments in some of these trials.

Limitations of this study include its retrospective and single-center design. Although 43 patients were identified over the 10-year study period, only 21 patients had archived tumor tissue available for light microscopy and IHC studies. The relatively small sample size precluded meaningful multivariate analysis to better understand the relationships among different variables. Given the rarity of lacrimal gland ACC, the number of samples included in our study was significant.

In conclusion, our study identified NICD1 and P63 as biomarkers that can help distinguish subtypes of lacrimal gland ACC with distinct clinicopathological features and prognosis. As more novel therapeutic agents are being developed, stratification of tumors based on their biomarker profile can help direct treatment decisions.

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