Diagnostic Cytopathology

WILEY

ORIGINAL ARTICLE OPEN ACCESS

Utility of Proliferation Markers Ki-67 and PHH3 in Predicting Malignancy in Basaloid Salivary Gland Neoplasms: A Study Using Digital Image Analysis of Cytology Cell Blocks

Yanki Yarman[1] | Robert Post[2] | Zachary Breslin[1] | Charalambos C. Solomides[2] | Stacey M. Gargano[2]

[1]Sidney Kimmel Medical College at Thomas Jefferson University, Philadelphia, Pennsylvania, USA | [2]Department of Pathology and Genomic Medicine, Thomas Jefferson University Hospital, Philadelphia, Pennsylvania, USA

Correspondence: Stacey M. Gargano (stacey.gargano@jefferson.edu)

Received: 30 October 2024 | **Revised:** 3 February 2025 | **Accepted:** 19 February 2025

Funding: The authors received no specific funding for this work.

Keywords: basaloid | cytopathology | digital image analysis | fine-needle aspiration | immunohistochemistry | proliferation markers | salivary gland neoplasms

ABSTRACT

Introduction: Basaloid salivary gland neoplasms (BSNs) are notoriously difficult to classify in fine needle aspiration (FNA) specimens due to the morphologic overlap of benign and malignant entities. Adenoid cystic carcinoma (AdCC) represents a particular diagnostic challenge, as it typically shows low-grade cytologic features despite its aggressive clinical behavior. We examined whether the proliferation markers Ki-67 and PHH3 could help predict malignancy in BSNs.

Methods: A retrospective search was conducted to identify FNA cases of BSNs that had adequate tumor cellularity in the cell block and a subsequent excision specimen. Ki-67 and PHH3 immunohistochemical stains were performed. Aperio (Leica Biosystems) was used to calculate the percentage of tumor cell nuclear expression. Proliferation scores and final histopathologic diagnoses were correlated using a two-sided *p*-value test.

Results: Ten benign and 14 malignant basaloid neoplasms were analyzed. Benign cases showed low mean percentages of tumor cell staining for Ki-67 (1.14%) and PHH3 (0.84%), while malignant cases showed significantly higher mean percentages, especially with Ki-67 (19% for low-grade malignancies and 25.5% for high-grade malignancies). The difference in proliferation marker scores between the benign and low-grade malignant cases showed statistical significance for both Ki-67 ($p = 0.0041$) and PHH3 ($p = 0.00397$). The difference between benign entities and AdCC was also statistically significant for Ki-67 ($p = 0.0013$) and PHH3 ($p = 0.002$).

Conclusion: Ki-67 and PHH3 analysis in cell block material may help predict malignancy in a cytologic specimen from a BSN, offering a valuable ancillary tool for cases with cytomorphologic ambiguity. In particular, the ability to suggest a sample is more likely to be AdCC rather than another morphologically similar low-grade BSN would be helpful for surgical planning.

1 | Introduction

Proper classification of salivary gland lesions in cytology samples is a challenging yet critically important task for the cytopathologist. The clinical management for salivary gland tumors ranges from conservative surgical excision for benign neoplasms and most low-grade malignancies to more extensive surgery for adenoid cystic carcinoma (AdCC) and high-grade

malignancies [1]. The Milan System for Reporting Salivary Gland Cytopathology (MSRSGC) is an evidence-based standardized reporting system that associates each diagnostic category with a malignancy risk and suggested management, thus providing clinical guidance for the diverse array of salivary gland neoplasms [2, 3]. However, the inherent cellular morphology of certain salivary gland neoplasms presents limitations to their classification, best seen with the category of salivary gland neoplasm of uncertain malignant potential (SUMP), which is further divided into subcategories based on whether the tumor cells show basaloid, oncocytoid, or clear cell features [2].

The term *basaloid* refers to a tumor containing cells with a high nuclear-to-cytoplasmic ratio, thus resembling primitive basal cells and imparting a "blue" appearance to the tumor at low power magnification [4]. Basaloid salivary gland neoplasms (BSN) are arguably the most diagnostically difficult salivary lesions to classify in cytology samples, as this category encompasses both benign (e.g., cellular pleomorphic adenoma (PA), basal cell adenoma (BCA), myoepithelioma) and malignant (e.g., AdCC, basal cell adenocarcinoma (BCAC), myoepithelial carcinoma and metastatic basaloid carcinoma) entities [5]. In a fine needle aspiration (FNA) specimen, many of these basaloid tumors will fall into the "cellular basaloid neoplasm" subcategory of SUMP. When the cytologic impression is suspicious or diagnostic of a malignancy, the MSRSGC encourages the cytopathologist to specify the nuclear grade (low or high). Previously, we identified certain cytomorphologic features, such as necrotic debris, mitotic activity, cellular discohesion, and anisonucleosis, which may help predict a high-grade basaloid salivary gland malignancy and help a clinician plan a more aggressive surgery [6]. However, this does not help solve the problem raised by AdCC, a BSN that typically exhibits low-grade nuclear cytomorphology (except for cases with high-grade transformation) despite its locally aggressive behavior. The ability to suggest this tumor on FNA would also be clinically helpful in addition to the delineation between benign/low-grade malignant and high-grade malignant tumors, yet this tumor remains notoriously difficult to identify in cytology specimens [7].

As a supplement to morphologic evaluation, ancillary testing via immunohistochemistry (IHC) and molecular analysis may aid in the classification of BSNs. Because cellular proliferation is expected to be higher in malignancies than in benign tumors, the proliferation rate of tumor cells in a cytology sample is a potential predictor of biologic behavior. In this study, we applied two immunohistochemical markers of proliferation index, Ki-67 and PHH3, to cytology cell blocks from basaloid salivary gland neoplasms, in the hope of improving the diagnostic accuracy of this challenging area of cytopathology.

2 | Materials and Methods

The Institutional Review Board of Thomas Jefferson University Hospital approved the study (IRB #18D.521). A retrospective search was conducted for salivary gland FNA cases diagnosed at Thomas Jefferson University Hospital during a 10-year period (2013–2022). The search term basaloid was queried in combination with the MSRSGC diagnostic categories of benign neoplasm (BN), SUMP, suspicious for malignancy (SFM), and malignant or with any of the following older (before the implementation of MSRSGC) diagnostic categories: suspicious for neoplasm, positive for neoplasm, SFM, and positive for malignancy. Cases in which the sampled salivary gland lesion underwent surgical excision at our institution were selected for analysis. In addition, a query was conducted for surgical pathology cases of BSNs, including BCA, BCAC, and AdCC; for those with a corresponding previous FNA, the cytology slides were reviewed, and if basaloid morphology was present, these cases were also included in our cohort.

Two pathologists (S.G. and R.P.) performed a cytologic review of the FNA cases, which consisted of smears stained with Diff-Quik and Papanicolaou stains, to confirm the presence of basaloid morphology. Hematoxylin and eosin (H&E)-stained cell block slides were then reviewed to assess tumor cellularity, and cases with fewer than 50 tumor cells in the cell block were excluded from the analysis.

Immunohistochemical staining for Ki-67 (Roche Diagnostics, clone 30-9) and PHH3 (Cell Marque, polyclonal) was performed using 4 μm-thick, formalin-fixed, paraffin-embedded sections from the cell blocks. IHC slides were scanned using Aperio

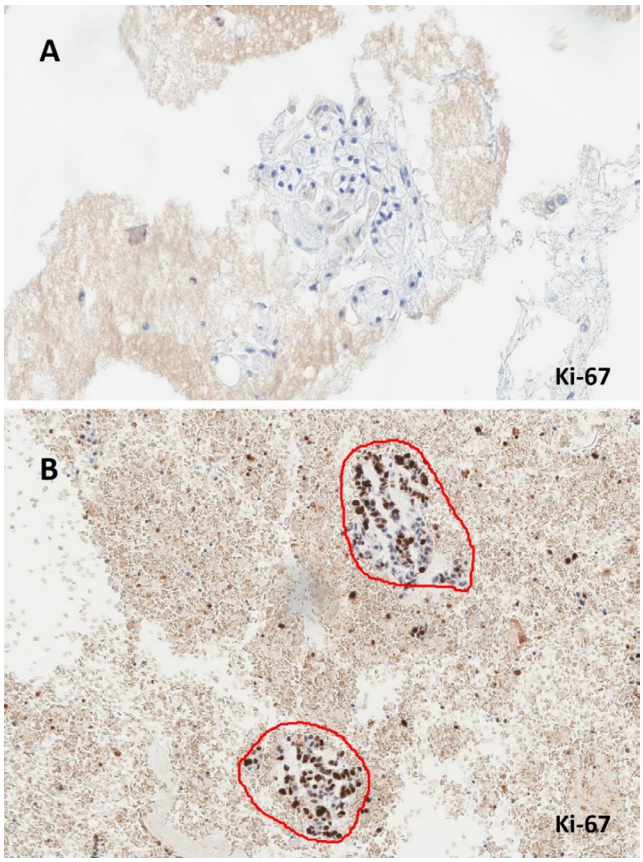

FIGURE 1 | Examples of regions excluded from digital analysis. (A) Non-neoplastic salivary tissue (shown) in a cell block which also contained fragments of pleomorphic adenoma (Ki-67, 20×). (B) Cell block from case of high grade myoepithelial carcinoma with extensive necrosis (Ki-67, 20×). Note that the regions circled for analysis include the intact tumor cell fragments and exclude the dispersed tumor cells within the necrotic background. Ki-67 proliferation index for this case was 45.83% (8196 total cells counted). [Color figure can be viewed at wileyonlinelibrary.com]

TABLE 1 | Pathologic diagnoses and proliferation marker results.

Case	Cytologic diagnosis	Surgical diagnosis	Ki-67 index (total % positive)	PHH3 index (total % positive)
1	Positive for neoplasm	Basal cell adenoma	1.91	0.17
2	Positive for neoplasm	Basal cell adenoma	0.78	1.30
3	Positive for neoplasm	Basal cell adenoma	0.32	1.17
4	Positive for neoplasm	Basal cell adenoma	0.99	0.58
5	Positive for neoplasm	Basal cell adenoma	0.15	0.06
6	Positive for neoplasm	Myoepithelioma	2.31	0.45
7	Positive for neoplasm	Pleomorphic adenoma	0.51	0.00
8	Positive for neoplasm	Pleomorphic adenoma	2.64	0.71
9	Positive for neoplasm	Pleomorphic adenoma	1.48	2.02
10	Positive for neoplasm	Pleomorphic adenoma	0.30	1.12
11	Positive for malignancy	Polymorphous adenocarcinoma, low-grade	1.42	2.93
12	Suspicious for neoplasm	Adenoid cystic carcinoma	8.82	3.93
13	Positive for neoplasm	Adenoid cystic carcinoma	27.14	1.39
14	Positive for neoplasm	Adenoid cystic carcinoma	29.93	10.17
15	Positive for neoplasm	Adenoid cystic carcinoma	10.43	4.52
16	Positive for neoplasm	Adenoid cystic carcinoma	5.92	3.17
17	Positive for neoplasm	Adenoid cystic carcinoma	12.36	2.71
18	Suspicious for malignancy	Adenoid cystic carcinoma	43.65	5.69
19	Positive for malignancy	Adenoid cystic carcinoma with high-grade transformation	12.64	0.68
20	Positive for neoplasm	Basal cell adenocarcinoma	31.30	12.34
21	Suspicious for malignancy	Myoepithelial carcinoma, high-grade	45.83	3.84
22	Positive for malignancy	Myoepithelial carcinoma with high-grade transformation	24.48	3.52
23	Positive for neoplasm	Myoepithelial carcinoma with high-grade transformation	12.97	1.51
24	Positive for neoplasm	Carcinoma ex pleomorphic adenoma with high-grade transformation	21.73	Unavailable

ScanScope XT (Leica Biosystems). Tumor cells were demarcated by a pathologist, with the exclusion of benign salivary elements and necrotic areas (Figure 1). The selected areas were analyzed using Aperio ImageScope software and the Aperio IHC Nuclear Algorithm, which scores nuclear IHC expression in the tumor cells as negative (0), weak (1+), moderate (2+) or strong (3+). The proliferation index for each stain was defined as the total number of tumor cells staining with any intensity (1+, 2+ or 3+) divided by the total number of tumor cells counted by the algorithm.

Statistical analysis was performed using GraphPad Prism to correlate proliferation scores and final histopathologic diagnosis. One-way ANOVA test was performed to compare benign, low-grade malignancies, and high-grade malignancies, and an unpaired *t*-test was used to compare benign to AdCC. The alpha level was set at $p \leq 0.05$.

3 | Results

Our search query yielded 98 salivary gland FNA cases, of which 69 underwent surgical excision at our institution, and 24 of these cases met the tumor cellularity criteria stated above. The diagnoses and proliferation indices of the 24 cases included in this study are summarized in Table 1. Specimen sources included

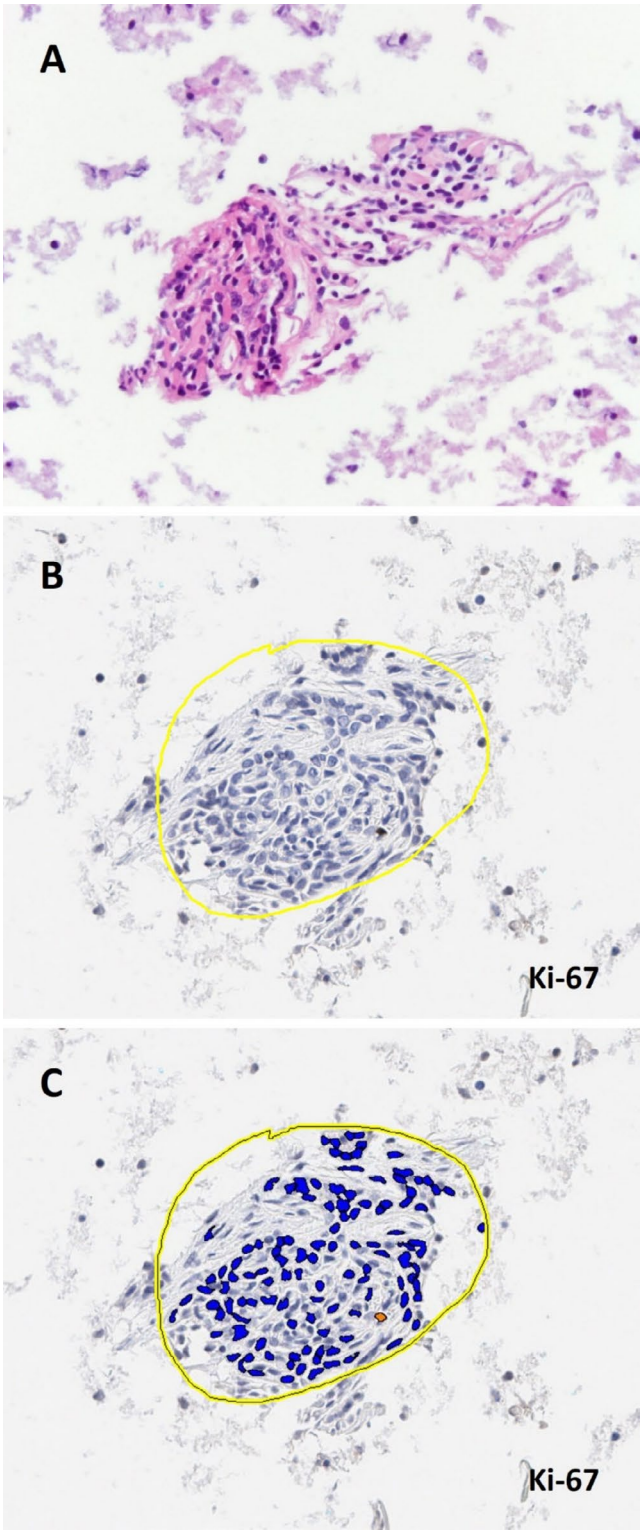

FIGURE 2 | Digital analysis of case of basal cell adenoma. (A) Small tumor fragment in the cell block (H&E, 20×). (B) Tumor fragment circled prior to digital analysis with one tumor cell showing nuclear positivity of moderate intensity (Ki-67, 20×) (C) Aperio algorithm identified the one positive (2+, indicated by orange color) tumor cell in this tumor fragment (Ki-67, 20×). The total Ki-67 index was 0.32%, with 934 total cells counted on the slide. PHH3 stain appeared similar. [Color figure can be viewed at wileyonlinelibrary.com]

parotid ($n = 17$), submandibular gland ($n = 4$), oral cavity ($n = 2$) and anterior neck ($n = 1$). Of the malignant cases, nine were low-grade, and five had high-grade transformation. Examples of IHC scoring of select cases using the Aperio digital analysis software are shown in Figures 2–4. The total number of tumor cells counted by the Aperio algorithm for each stain was similar (Ki-67: mean 5870, range 204–36,673; PHH3: mean 5433, range 246–36,900).

Benign cases showed a low mean percentage of tumor cell staining for Ki-67 (1.14%) and PHH3 (0.84%), while malignant cases showed significantly higher mean percentages, especially with Ki-67 (19% for low-grade malignancies and 25.5% for high-grade malignancies) (Figure 5). The difference in proliferation marker scores between the benign and low-grade malignant cases showed statistical significance for both Ki-67 ($p = 0.0041$) and PHH3 ($p = 0.00397$). In addition, the difference in proliferation marker scores between the benign and high-grade malignant cases showed statistical significance only for Ki-67 ($p = 0.0026$). There was no significant difference in proliferation marker scores between the low-grade and high-grade malignant cases. The difference between benign entities and AdCC (excluding the case with high-grade transformation) was statistically significant for both Ki-67 ($p = 0.0013$) and PHH3 ($p = 0.002$) (Figure 6).

4 | Discussion

We hypothesized that IHC for cell proliferation markers may be helpful in predicting malignancy in FNA samples from BSNs, as cellular proliferation is expected to be higher in a malignant tumor compared to a benign one [8]. Several biomarkers of cellular proliferation are available, as they are utilized for grading and prognosis of certain tumor types [9–11]. One of the most widely studied is Ki-67 antigen, a non-histone nuclear protein present in all active phases of the cell cycle. Assessment of the percentage of cells staining positive with antibody against Ki-67 allows for determination of the overall growth fraction of a tumor [11, 12]. Antibody against phosphorylated histone H3 (PHH3) is a relatively newer immunostain against a protein that is expressed only during chromatin condensation in the mitotic phase of the cell cycle, rendering it a specific marker for cells undergoing mitosis. Because it does not stain apoptotic tumor cells, PHH3 holds a significant advantage over Ki-67 in its utility as an alternative to manually counting mitoses. As a result, PHH3 has been studied in several tumor types for which mitotic count is routinely performed for grading, such as meningiomas and neuroendocrine tumors of the pancreas and lung [10, 13]. This marker has also been validated as an alternative to manual mitotic count in breast carcinoma, with PHH3 scoring performed either manually or by digital image analysis [9, 14]. To our knowledge, there have been no previous studies on PHH3 expression in salivary gland tumors.

Our study suggests that Ki-67 and PHH3 analysis in cell block material may help predict malignancy in a cytologic

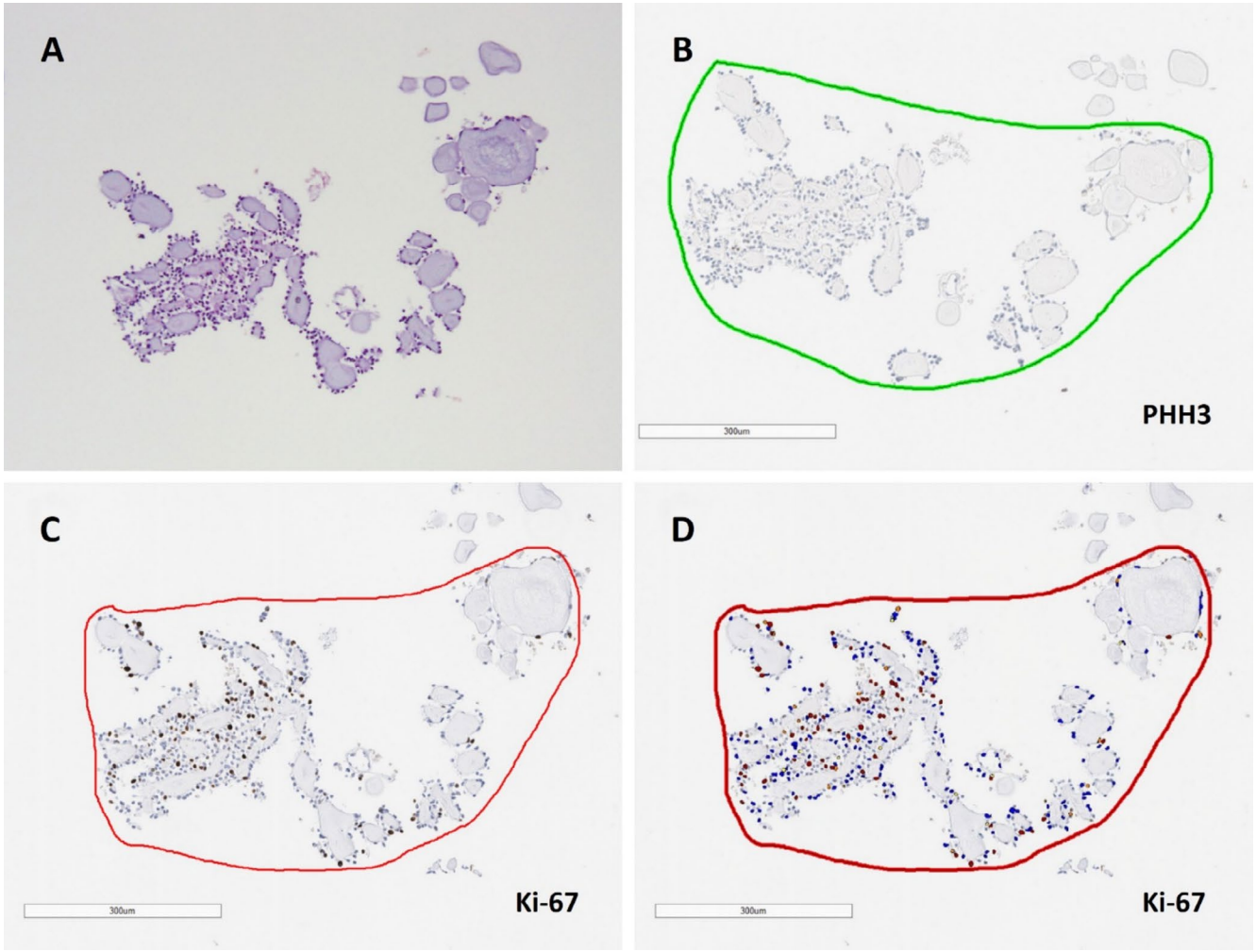

FIGURE 3 | Digital analysis of case of adenoid cystic carcinoma with tubular and cribriform patterns. (A) Tumor fragments in cell block (H&E, 20×). Same region stained with PHH3 (B) and Ki-67 (C and D, pre- and post-digital analysis, respectively). Proliferation indices were 1.39% with PHH3 (4541 total cells counted) and 27.14% with Ki-67 (7162 total cells counted). [Color figure can be viewed at wileyonlinelibrary.com]

specimen from a BSN, offering a valuable ancillary tool for cases with cytomorphologic ambiguity. Based on our cohort, a Ki-67 proliferation index of > 5% in an FNA sample from a BSN is strongly suggestive of malignancy, as all but one (polymorphous adenocarcinoma, low-grade) of our malignant cases had Ki-67 scores greater than 5%, and the benign cases showed values ranging from 0.15% to 2.64%. A specific cut-off value for PHH3 is more difficult to propose, as there was considerably more overlap in values between benign and malignant cases. While PHH3 values ranged from 0 to 2.02 for benign cases, three malignant tumors had values < 2%, including two cases with high-grade transformation. A possible explanation for this stems from the extensive necrosis in these tumors, which limited the number of viable cells available for scoring. Moreover, we found no significant difference in proliferation index between low-grade and high-grade basaloid malignancies, but this distinction can be reliably made on cytomorphologic grounds alone [6].

Under the MSRSGC, a salivary neoplasm with basaloid morphology may potentially fall under the categories of benign neoplasm, SUMP, suspicious for malignancy, and malignant. A definitive benign or malignant classification is most helpful to clinicians but can be rendered only in the presence of specific diagnostic features [15]. Given the morphologic overlap exhibited by benign and malignant BSNs in cytology specimens, several studies have looked at the utility of ancillary tools like IHC and molecular testing in achieving more specific cytologic diagnoses, particularly for AdCC, as its preoperative diagnosis is beneficial for surgical planning. For AdCC, C-kit (CD117) expression is sensitive but not specific, as positivity has been reported in PA, basal cell adenocarcinoma, and several other salivary tumors [16]. IHC for MYB overexpression is more specific than C-kit and may be helpful in separating AdCC and PA in cytology samples [17–19]. Foo et al. [20] suggest using an IHC panel including CD117, MYB, and PLAG1 to help facilitate the diagnosis of AdCC versus PA, as AdCC is expected to be negative for the latter marker. If there is sufficient tissue for testing, detection of MYB gene rearrangement by fluorescent in situ hybridization is the most specific option, though it is less sensitive than MYB IHC [17].

Studies on Ki-67 IHC in salivary gland tumors have focused on the diagnostic and prognostic value in surgical pathology

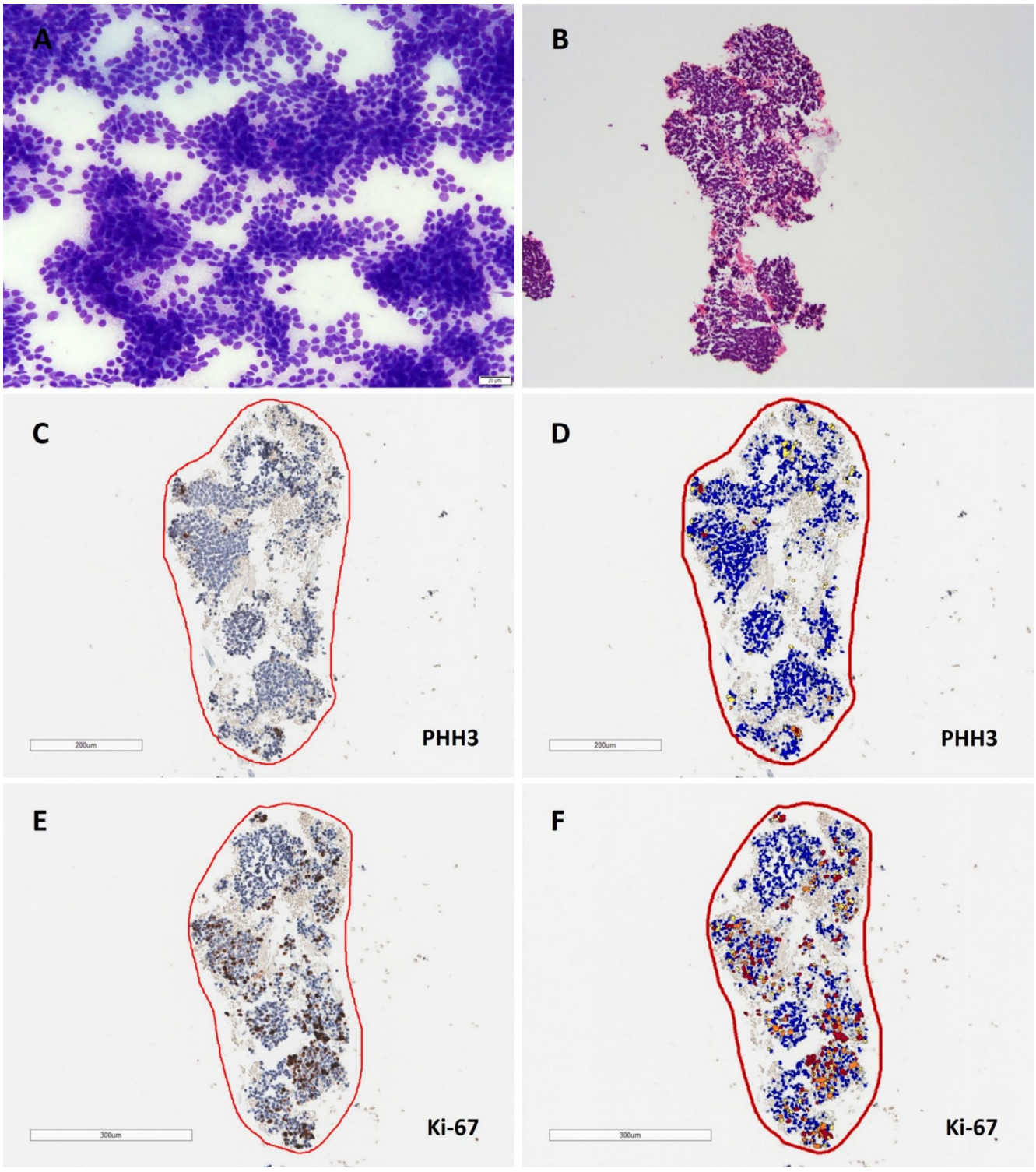

FIGURE 4 | Digital analysis of case of adenoid cystic carcinoma with predominantly solid pattern. Direct smear (A, Diff-Quik stain, 20×) and cell block (B, H&E, 20×) showing cellular basaloid neoplasm. Same tumor fragment in the cell block stained with PHH3 (C, D) and Ki-67 (E, F), pre- and post-digital analysis, respectively. Proliferation indices were 10.17% with PHH3 (13,836 total cells counted) and 29.93% with Ki-67 (11,282 total cells counted). [Color figure can be viewed at wileyonlinelibrary.com]

samples. Nagao et al. [21] demonstrated that the Ki-67 proliferation index is helpful in the differential diagnosis between myoepithelioma (< 10%) and myoepithelial carcinoma (> 10%). In combination with the results of other markers (p53, EGFR, bcl-2), a Ki-67 index of > 5% has been reported to be helpful in the diagnosis of BCAC over BCA [22]. These two situations highlight the diagnostic utility of Ki-67 in separating benign tumors from their malignant counterparts, otherwise distinguishable only by the presence of invasive features unlikely to be captured in a small biopsy or cytology sample. In terms of prognostic significance, the Ki-67 proliferation index has been shown to correlate with unfavorable clinical outcomes

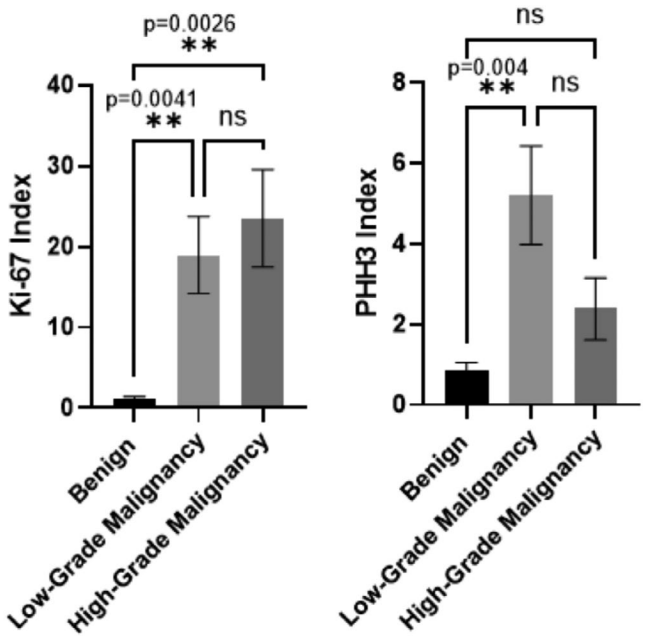

FIGURE 5 | Proliferation marker expression in benign vs. malignant basaloid neoplasms.

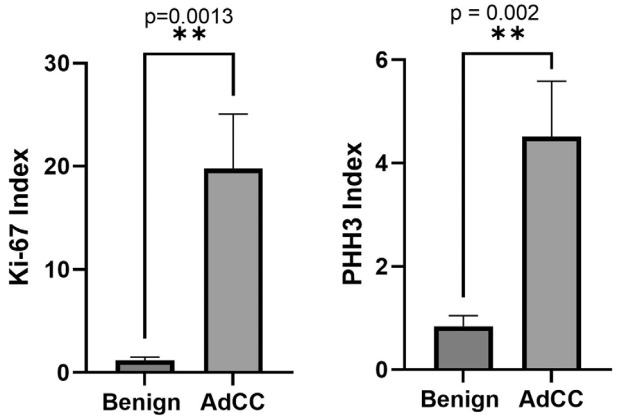

FIGURE 6 | Proliferation marker expression in benign basaloid neoplasms compared to adenoid cystic carcinoma (excluding the one case with high-grade transformation).

in patients with acinic cell carcinoma and mucoepidermoid carcinoma [12].

The application and interpretation of IHC in BSNs must take into consideration the tumor morphology. Some benign salivary gland tumors are difficult to separate from their malignant counterparts (e.g., BCA vs. BCAC; myoepithelioma vs. myoepithelial carcinoma), especially in cytology or biopsy samples where the ability to assess the interface between the tumor and salivary gland parenchyma is limited or absent. In other situations, benign tumors that deviate from the typical morphology may mimic a malignant tumor, as in the case of a PA with predominant cribriform architecture and hyaline matrix mimicking AdCC [15]. The preoperative ability to suggest a cytology sample is more likely to be AdCC rather than another morphologically similar low-grade BSN that would be particularly helpful to clinicians planning the extent of surgical management, as AdCC requires wider resection and consideration of elective neck dissection [23]. Our results suggest that the application of Ki-67 or PHH3 IHC to a cytology specimen morphologically suspicious for AdCC can help support or refute that diagnosis. Our analysis even omitted the case of AdCC with high grade transformation, lending further support to the valuable role of proliferation index in cases of AdCC without overt malignant (i.e., high-grade) cytologic features.

A potential limiting factor in the application of our study findings is specimen cellularity. In our original cohort, several cases were excluded due to inadequate cell block material, despite the fact that pathologist-performed FNAs at our institution routinely include a dedicated pass taken for the cell block. In addition to adequate cellularity, the confident distinction of lesional versus non-lesional (e.g., adjacent normal salivary gland tissue, inflammatory cells) is critical for the accurate assessment of tumor proliferation index in a cell block sample, necessitating the presence of at least some intact tissue fragments rather than only scattered isolated cells. Finally, tumor heterogeneity in a BSN, especially in instances of carcinoma ex pleomorphic adenoma, may interfere with an accurate evaluation of the proliferation index, again emphasizing the need for careful attention to morphology when choosing tumor foci to incorporate in the IHC analysis.

5 | Conclusions

In the present cohort, proliferation index IHC, especially using Ki-67, seems to be a reliable supplementary tool in the cytologic workup of a BSN. Specifically, a Ki-67 proliferation index of 5% or higher may be predictive of malignancy. However, further work with a greater sample size is necessary before implementing these findings into routine practice, and it would also help clarify the role of PHH3. If reproducibility is confirmed in larger cohorts, more definitive Ki-67/PHH3 cutoff values could be proposed for suggesting malignancy in a BSN sample. Our work also emphasizes the need for adequate tumor cellularity in the cell block, perhaps facilitated by the submission of additional dedicated FNA passes, as many of our cases had adequate diagnostic material in the smears but insufficient cellularity in the cell block for IHC.

Author Contributions

The study was designed and planned by S.M.G. Data was collected by S.M.G., R.P., and Y.Y. Statistical analysis was performed by Z.B. Resources were provided by C.C.S. The manuscript was written by S.M.G., R.P., Y.Y., and Z.B. and was reviewed by all authors.

Acknowledgments

The authors have nothing to report.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

References

1. J. L. Geiger, N. Ismaila, B. Beadle, et al., "Management of Salivary Gland Malignancy: ASCO Guideline," *Journal of Clinical Oncology* 39, no. 17 (2021): 1909–1941, https://doi.org/10.1200/JCO.21.00449.

2. W. C. Faquin, E. D. Rossi, Z. Baloch, et al., eds., *The Milan System for Reporting Salivary Gland Cytopathology*, 2nd ed. (Springer International Publishing, 2023).

3. M. Pusztaszeri, E. D. Rossi, Z. W. Baloch, and W. C. Faquin, "Salivary Gland Fine Needle Aspiration and Introduction of the Milan Reporting System," *Advances in Anatomic Pathology* 26 (2019): 84–92, https://doi.org/10.1097/PAP.0000000000000224.

4. R. R. Seethala, "Basaloid/Blue Salivary Gland Tumors," *Modern Pathology* 30, no. s1 (2017): S84–S95, https://doi.org/10.1038/modpathol.2016.190.

5. W. C. Faquin, "Diagnosis and Grading of Basaloid Salivary Gland Tumors Using the Milan System for Reporting Salivary Gland Cytopathology," *Cancer Cytopathology* 128, no. 2 (2020): 87–88, https://doi.org/10.1002/cncy.22207.

6. S. M. Gargano, C. Sebastiano, C. C. Solomides, C. C. Griffith, and K. HooKim, "Cytohistologic Correlation of Basaloid Salivary Gland Neoplasms: Can Cytomorphologic Classification Be Used to Diagnose and Grade These Tumors?," *Cancer Cytopathology* 128, no. 2 (2020): 92–99, https://doi.org/10.1002/cncy.22208.

7. Z. L. Tabatabai, M. Auger, D. F. Kurtycz, et al., "Performance Characteristics of Adenoid Cystic Carcinoma of the Salivary Glands in Fine-Needle Aspirates: Results From the College of American Pathologists Nongynecologic Cytology Program," *Archives of Pathology & Laboratory Medicine* 139 (2015): 1525–1530, https://doi.org/10.5858/arpa.2016-0138-LE.

8. M. Murakami, I. Ohtani, H. Hojo, and H. Wakasa, "Immunohistochemical Evaluation With Ki-67: An Application to Salivary Gland Tumours," *Journal of Laryngology and Otology* 106, no. 1 (1992): 35–38, https://doi.org/10.1017/s0022215100118535.

9. J. Y. Kim, H. S. Jeong, T. Chung, et al., "The Value of Phosphohistone H3 as a Proliferation Marker for Evaluating Invasive Breast Cancers: A Comparative Study With Ki67," *Oncotarget* 8, no. 39 (2017): 65064–65076, https://doi.org/10.18632/oncotarget.17775.

10. J. Tracht, K. Zhang, and D. Peker, "Grading and Prognostication of Neuroendocrine Tumors of the Pancreas: A Comparison Study of Ki67 and PHH3," *Journal of Histochemistry and Cytochemistry* 65, no. 7 (2017): 399–405, https://doi.org/10.1369/0022155417708186.

11. J. Gerdes, "Ki-67 and Other Proliferation Markers Useful for Immunohistological Diagnostic and Prognostic Evaluations in Human Malignancies," *Seminars in Cancer Biology* 1, no. 3 (1990): 199–206.

12. A. Skálová and I. Leivo, "Cell Proliferation in Salivary Gland Tumors," *General & Diagnostic Pathology* 142, no. 1 (1996): 7–16.

13. K. Tsuta, D. C. Liu, N. Kalhor, I. I. Wistuba, and C. A. Moran, "Using the Mitosis-Specific Marker Anti-Phosphohistone H3 to Assess Mitosis in Pulmonary Neuroendocrine Carcinomas," *American Journal of Clinical Pathology* 136, no. 2 (2011): 252–259, https://doi.org/10.1309/AJCPDXFOPXGEF0RP.

14. B. F. Dessauvagie, C. Thomas, C. Robinson, F. A. Frost, J. Harvey, and G. F. Sterrett, "Validation of Mitosis Counting by Automated Phosphohistone H3 (PHH3) Digital Image Analysis in a Breast Carcinoma Tissue Microarray," *Pathology* 47, no. 4 (2015): 329–334, https://doi.org/10.1097/PAT.0000000000000248.

15. R. L. Cantley, "Fine-Needle Aspiration Cytology of Cellular Basaloid Neoplasms of the Salivary Gland," *Archives of Pathology & Laboratory Medicine* 143, no. 11 (2019): 1338–1345, https://doi.org/10.5858/arpa.2019-0327-RA.

16. G. Vijayakumar, M. Kamboj, A. Narwal, and G. Sharma, "Diagnostic Reliability of c-KIT (CD117) in Salivary Gland Tumours—A Systematic Review and Meta-Analysis," *Journal of Oral and Maxillofacial Pathology* 28, no. 1 (2024): 11–20, https://doi.org/10.4103/jomfp.jomfp_70_24.

17. C. C. Griffith, M. T. Siddiqui, and A. C. Schmitt, "Ancillary Testing Strategies in Salivary Gland Aspiration Cytology: A Practical Pattern-Based Approach," *Diagnostic Cytopathology* 45, no. 9 (2017): 808–819, https://doi.org/10.1002/dc.23715.

18. A. Moon, C. Cohen, and M. T. Siddiqui, "MYB Expression: Potential Role in Separating Adenoid Cystic Carcinoma (ACC) From Pleomorphic Adenoma (PA)," *Diagnostic Cytopathology* 44, no. 10 (2016): 799–804, https://doi.org/10.1002/dc.23551.

19. M. P. Pusztaszeri, P. M. Sadow, A. Ushiku, P. Bordignon, T. A. McKee, and W. C. Faquin, "MYB Immunostaining Is a Useful Ancillary Test for Distinguishing Adenoid Cystic Carcinoma From Pleomorphic Adenoma in Fine-Needle Aspiration Biopsy Specimens," *Cancer Cytopathology* 122, no. 4 (2014): 257–265, https://doi.org/10.1002/cncy.21381.

20. W. C. Foo, V. Y. Jo, and J. F. Krane, "Usefulness of Translocation-Associated Immunohistochemical Stains in the Fine-Needle Aspiration Diagnosis of Salivary Gland Neoplasms," *Cancer Cytopathology* 124, no. 6 (2016): 397–405, https://doi.org/10.1002/cncy.21693.

21. T. Nagao, I. Sugano, Y. Ishida, et al., "Salivary Gland Malignant Myoepithelioma: A Clinicopathologic and Immunohistochemical Study of Ten Cases," *Cancer* 83, no. 7 (1998): 1292–1299.

22. T. Nagao, I. Sugano, Y. Ishida, et al., "Basal Cell Adenocarcinoma of the Salivary Glands: Comparison With Basal Cell Adenoma Through Assessment of Cell Proliferation, Apoptosis, and Expression of p53 and Bcl-2," *Cancer* 82, no. 3 (1998): 439–447.

23. R. Xiao, R. K. V. Sethi, A. L. Feng, J. B. Fontanarosa, and D. G. Deschler, "The Role of Elective Neck Dissection in Patients With Adenoid Cystic Carcinoma of the Head and Neck," *Laryngoscope* 129, no. 9 (2019): 2094–2104, https://doi.org/10.1002/lary.27814.