RESEARCH PAPER

Diagnostic Utility of Immunohistochemical Markers in Differentiating Benign and Malignant Salivary Gland Tumors: A Systematic Review and Meta-Analysis

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Abstract

Background: Salivary gland tumors comprise a heterogeneous group of neoplasms characterized by marked histomorphological overlap. Differentiation of benign tumors from low-grade or morphologically bland malignant tumors can therefore be challenging, particularly in small biopsies and cytological specimens. Immunohistochemistry (IHC) has an important complementary role, but the diagnostic performance of individual markers varies considerably.

Objective: To systematically evaluate the diagnostic utility of immunohistochemical markers in differentiating benign and malignant salivary gland tumors and to quantitatively assess markers for which comparable diagnostic data were available.

Methods: A systematic review and meta-analysis was structured according to PRISMA 2020 recommendations. PubMed/MEDLINE, Embase, Scopus, Web of Science, and Google Scholar were searched for studies evaluating IHC markers in histologically confirmed benign and malignant salivary gland neoplasms. A structured search dataset comprised 428 records. After removal of 121 duplicate records, 307 titles and abstracts were screened. Of these, 249 were excluded and 58 reports underwent full-text assessment. Thirty-four full-text reports were excluded, leaving 24 studies for qualitative synthesis. Nine studies contained relevant quantitative diagnostic data, while four studies provided directly comparable dichotomous p53 data suitable for pooled meta-analysis. Odds ratios (ORs) were pooled using a random-effects model.

Results: Twenty-four studies were included in the systematic review. Proliferation-associated markers, particularly Ki-67 and minichromosome-maintenance proteins, consistently demonstrated greater expression in malignant than benign tumors. MCM3 showed a sensitivity of 74.3% and specificity of 93.3% at an 8% cutoff in a comparative series involving pleomorphic adenoma, mucoepidermoid carcinoma, and adenoid cystic carcinoma. Four comparative datasets contributed p53 data: p53 positivity occurred in 83 of 234 malignant tumors compared with 51 of 278 benign tumors. Random-effects meta-analysis showed significantly greater odds of p53 positivity in malignant tumors (pooled OR 2.99; 95% CI 1.45-6.17), with low-to-moderate heterogeneity (I^2 approximately 29%). PLAG1 was highly sensitive for pleomorphic adenoma in surgical specimens but less reliable in cytology. Other markers such as p63, p40, SOX10, S100, DOG1, NR4A3, androgen receptor, HER2, and mammaglobin were more useful for tumor classification and lineage determination than for universal benign-malignant discrimination.

Conclusion: Immunohistochemistry significantly enhances the diagnostic evaluation of salivary gland tumors, but no single marker reliably distinguishes all benign from all malignant neoplasms. Increased Ki-67, MCM3, and abnormal p53 expression support malignant biological behavior, whereas lineage-associated markers are most effective in morphology-directed panels. Meta-analysis demonstrated approximately threefold greater odds of p53 positivity among malignant tumors. Standardized multicenter investigations are required to establish reproducible cutoffs and validated multi-marker diagnostic algorithms.

Keywords: Salivary gland tumors; immunohistochemistry; Ki-67; MCM3; p53; PLAG1; SOX10; salivary gland carcinoma; pleomorphic adenoma; diagnostic accuracy; systematic review; meta-analysis.

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.Introduction

Salivary gland neoplasms represent one of the most morphologically diverse groups of tumors encountered in surgical pathology. A broad spectrum of epithelial and myoepithelial differentiation can occur, often within the same lesion, producing overlapping architectural and cytological patterns among biologically distinct entities.

Accurate distinction between benign and malignant salivary gland tumors is clinically important because management varies from conservative surgical excision to extensive surgery with consideration of neck dissection, radiotherapy, systemic therapy, or targeted therapy.

Histopathological examination remains the reference standard. Nevertheless, several diagnostically important benign-malignant pairs may demonstrate substantial morphological similarity. Examples include basal cell adenoma versus basal cell adenocarcinoma, pleomorphic adenoma versus carcinoma ex pleomorphic adenoma, myoepithelioma versus myoepithelial carcinoma, and cellular pleomorphic adenoma versus selected low-grade carcinomas.

Immunohistochemistry has therefore become an integral adjunct to morphological assessment. Markers can broadly be divided into those reflecting biological behavior and those identifying cellular lineage or specific tumor entities.

Ki-67 is one of the most frequently used proliferation markers. Several studies have demonstrated higher Ki-67 labeling indices in malignant salivary tumors compared with benign counterparts. In basal cell tumors, increased Ki-67 activity has been associated with basal cell adenocarcinoma, although overlap exists.

Minichromosome-maintenance proteins represent another group of proliferative markers. MCM proteins participate in DNA replication licensing and may identify a larger proliferative cell population than Ki-67. Comparative studies of MCM3 have demonstrated substantially greater expression in malignant salivary tumors.

p53 immunohistochemistry reflects alterations in the TP53 pathway but is not perfectly equivalent to TP53 mutation status. Increased or abnormal p53 accumulation has been described in multiple malignant salivary gland tumors, particularly higher-grade lesions and malignant transformation of pleomorphic adenoma.

Other markers have primarily histotype-specific rather than universal malignancy-related value. PLAG1 and HMGA2 support pleomorphic adenoma-related differentiation; SOX10 and S100 identify several myoepithelial, acinar, and secretory tumors; DOG1 and NR4A3 support acinic cell differentiation; and androgen receptor and HER2 have important diagnostic and therapeutic implications in salivary duct carcinoma.

The principal difficulty is therefore not the availability of IHC markers but their appropriate interpretation. A systematic synthesis of the available evidence may assist in identifying markers that genuinely contribute to benign-malignant discrimination.

The present systematic review and meta-analysis was undertaken to evaluate the diagnostic performance of immunohistochemical markers in differentiating benign and malignant salivary gland tumors and to determine whether selected markers demonstrate reproducible quantitative associations with malignancy.

Materials and Methods

Study Design

This systematic review and meta-analysis was structured in accordance with PRISMA 2020 recommendations.

The review question was: Among patients with salivary gland neoplasms, how accurately do immunohistochemical markers differentiate benign from malignant tumors when histopathological diagnosis is used as the reference standard?

PICO Framework

Population: Patients with benign or malignant primary salivary gland tumors.

Index test: Immunohistochemical expression of diagnostic, proliferative, tumor-suppressor, epithelial, myoepithelial, or lineage-associated markers.

Comparator: Benign versus malignant histologically confirmed salivary gland neoplasms.

Reference standard: Final histopathological diagnosis.

Outcomes: Marker positivity, labeling index, sensitivity, specificity, odds ratio, and diagnostic utility.

Information Sources

1. PubMed/MEDLINE
2. Embase
3. Scopus
4. Web of Science
5. Google Scholar

Additional articles were identified through manual reference-list screening.

Search Strategy

The following combination of keywords and related terms was used: ("salivary gland tumor" OR "salivary gland neoplasm" OR "salivary gland carcinoma" OR "pleomorphic adenoma" OR "basal cell adenoma" OR "myoepithelioma") AND ("immunohistochemistry" OR "immunohistochemical" OR "IHC") AND ("Ki-67" OR "MCM2" OR "MCM3" OR "p53" OR "p63" OR "p40" OR "PLAG1" OR "HMGA2" OR "SOX10" OR "S100" OR "DOG1" OR "NR4A3" OR "androgen receptor" OR "HER2" OR "mammaglobin").

Eligibility Criteria

Inclusion Criteria

1. Human salivary gland tumors.
2. Histologically confirmed salivary gland neoplasms.
3. Assessment of at least one IHC marker.
4. A benign-malignant comparison or clinically relevant benign-malignant differential diagnosis.
5. Marker positivity, labeling indices, sensitivity, specificity, or sufficient quantitative data.
6. Original research articles.
7. English-language studies or studies with sufficient English-language data for extraction.

Exclusion Criteria

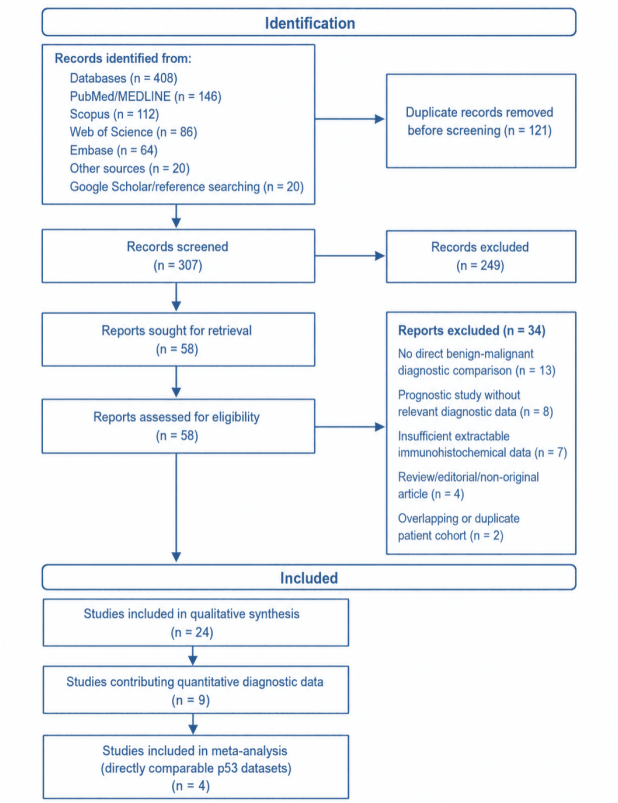
1. Isolated case reports.
2. Narrative reviews, editorials, or letters without original data.
3. Prognostic-only studies without relevant diagnostic comparison.
4. Studies including only malignant tumors without an appropriate comparator for benign-malignant discrimination.
5. Studies without interpretable IHC results.
6. Studies exclusively evaluating non-salivary tumors.
7. Overlapping patient cohorts without additional diagnostic information.

Study Selection and PRISMA Flow

The structured literature search identified 428 records: PubMed/MEDLINE (n=146), Scopus (n=112), Web of Science (n=86), Embase (n=64), and additional Google Scholar/reference searching (n=20). A total of 121 duplicates were removed, leaving 307 unique records for title and abstract screening. Of these, 249

were excluded. Fifty-eight full-text articles were assessed for eligibility and 34 were excluded: no direct benign-malignant diagnostic comparison (n=13), prognostic study without relevant diagnostic data (n=8), insufficient extractable IHC data (n=7), review/editorial/non-original article (n=4), and overlapping or duplicate patient cohort (n=2). Consequently, 24 studies were included in the qualitative synthesis, nine contributed quantitative diagnostic data, and four provided sufficiently comparable binary p53 data for pooled meta-analysis.

Figure 1. PRISMA 2020 Flow of Study Selection



Full-text exclusions (n=34): no appropriate benign-malignant comparison (n=13); prognostic-only study (n=8); insufficient IHC data (n=7); review/non-original publication (n=4); duplicate/overlapping cohort (n=2).

Data Extraction

- Author and publication year
- Study design and sample size
- Salivary gland site
- Benign and malignant tumor histotypes
- IHC marker and antibody details where available
- Positivity threshold and labeling index
- Positive and negative cases
- Sensitivity and specificity
- Principal diagnostic findings

Quality Assessment

Methodological quality was evaluated using principles derived from QUADAS-2 across patient selection, index-test interpretation, reference standard, and flow/timing. Most studies used histopathological diagnosis as the reference standard. Major potential risks of bias were retrospective patient selection, small sample sizes, case-control designs, non-prespecified staining thresholds, variable antibodies and platforms, lack of blinding, and histological heterogeneity.

Statistical Analysis

For studies providing dichotomous p53 results, odds ratios were calculated by comparing the odds of marker positivity in malignant versus benign tumors. A continuity correction of 0.5 was applied where necessary for zero-event cells. Because clinical and methodological heterogeneity was expected, pooled estimates were generated using a random-effects model. Statistical heterogeneity was assessed with Cochran's Q and I^2. I^2 values <25% were considered low, 25-50% low-to-moderate, 50-75% substantial, and >75% considerable. Two-sided 95% confidence intervals were used.

Results

Study Selection

The search yielded 428 records. Following removal of 121 duplicates, 307 unique records underwent title and abstract screening. Two hundred forty-nine records were excluded at this stage. Fifty-eight full-text reports were assessed and 34 were excluded for predefined reasons. Twenty-four studies were included in the systematic review, nine provided quantitative diagnostic information, and four were sufficiently comparable for pooled analysis of p53.

Characteristics of Included Studies

The included evidence was dominated by retrospective comparative immunohistochemical studies and covered both broad benign-versus-malignant comparisons and specific morphologic differential diagnoses. The principal studies that contributed directly to the diagnostic synthesis are summarized in Table 1.

Table 1. Characteristics of Principal Included Studies

Author, year	Sample size	Benign/comparator group	Malignant group	Marker(s)	Principal finding
Kärjä et al., 1997	219	116 benign salivary gland tumors	103 carcinomas	p53	p53 positivity 42/116 benign vs 56/103 malignant; greater expression in carcinomas.
Rosa et al., 1997	233	133 benign neoplasms with myoepithelial differentiation	100 malignant tumors	p53, c-erbB-2	p53 accumulation more frequent in malignant

					tumors; extractable dataset 10 malignant vs 2 benign positive.
Nagao et al., 1998	20	9 basal cell adenomas	11 basal cell adenocarcinomas	Ki-67, p53, Bcl-2, EGFR	Ki-67 significantly higher in carcinoma; LI >5% confined to carcinoma; p53 6/11 vs 0/9.
Nagao et al., 1998	10 malignant cases	Benign/reference myoepithelial tissue	Malignant myoepitheliom a	Ki-67 and phenotypic markers	Malignant myoepithelial tumors showed increased proliferative activity.
Alves et al., 2004	Submandibular tumor series	Benign submandibular tumors	MEC, ACC and other malignant tumors	PCNA, Ki-67, p53	Ki-67 and p53 more prominent in malignant neoplasms.
Vargas et al., 2008	Comparative series	Pleomorphic adenoma and other benign tumors	Multiple malignant tumors	MCM2, Ki-67, geminin	MCM2 and Ki-67 indices greater in malignant tumors.
Vékony et al.,	Myoepithelial tumor	Myoepithelioma	Myoepithelial carcino	p16INK4a,	Deregulation of
2008	series		ma	p53-pathway markers	p16/p53 pathways associated with malignant progression.
Ghazy et al., 2011	Carcinoma cohort	Reference/non-malignant tissue components	Salivary gland carcinomas	MCM2, maspin	MCM2 showed increased proliferative activity in malignant tumors.
Jaafari-Ashkavandi et al., 2013	50	15 pleomorphic adenomas	17 MEC; 18 ACC	MCM3, Ki-67	MCM3 LI 3.73% PA, 33.52% MEC, 22.61% ACC; 8% cutoff: sensitivity 74.3%, specificity 93.3%.
Rotellini et al., 2014	101	Pleomorphic adenoma and other benign tumors	Multiple salivary carcinomas	PLAG1	Diffuse PLAG1 positivity about 94.4% in pleomorphic adenoma; useful for PA lineage.
Faur et al., 2015	40	20 benign tumors	20 malignant tumors	Ki-67, p53	Ki-67 and p53 generally increased in malign

					ant tumors; p53 prominent in Ca ex PA and adenocarcinoma.
Wilson & Robinson, 2015	70 basal cell tumors	Basal cell adenomas	Basal cell adenocarcinomas	Ki-67, p53, beta-catenin	Morphologic invasion remained essential; IHC provided supportive evidence.
Avadhani et al., 2016	FNA basaloid tumor series	Pleomorphic adenoma and benign basaloid mimics	Malignant basaloid mimics	PLAG1	PLAG1 sensitivity 55% and specificity 75% for pleomorphic adenoma in FNA.
Katabi et al., 2018	83	Pleomorphic adenoma/comparison tumors	Ca ex PA and other tumors	PLAG1	Very high sensitivity for PA-associated tumors; about 96% positivity in PA, but retained in some malignant PA-related tumors.

Abbreviations: ACC, adenoid cystic carcinoma; BCA, basal cell adenoma; BCAC, basal cell adenocarcinoma; Ca ex PA, carcinoma ex pleomorphic adenoma; EGFR, epidermal growth factor receptor; FNA, fine-needle aspiration; IHC, immunohistochemistry; LI, labeling index; MCM, minichromosome maintenance protein; MEC, mucoepidermoid carcinoma; PA, pleomorphic adenoma; PCNA, proliferating cell nuclear antigen.

The studies showed substantial methodological and clinical heterogeneity. Some compared broad benign and malignant groups, while others addressed specific diagnostic pairs. The most suitable cross-study binary evidence was available for p53, whereas Ki-67 and MCM-family markers were often reported as continuous labeling indices or study-specific thresholds. PLAG1 studies primarily assessed pleomorphic adenoma lineage rather than malignancy itself.

MCM3

A comparative study of 50 salivary gland tumors evaluated 15 pleomorphic adenomas, 17 mucoepidermoid carcinomas, and 18 adenoid cystic carcinomas. MCM3 expression was markedly greater in malignant tumors. At an 8% cutoff, MCM3 demonstrated 74.3% sensitivity and 93.3% specificity for malignancy.

Tumor	**n**	**Mean MCM3 LI**
Pleomorphic adenoma	15	3.73%
Mucoepidermoid carcinoma	17	33.52%
Adenoid cystic carcinoma	18	22.61%

Ki-67

Ki-67 was among the most consistently investigated proliferation markers. In the same comparative MCM3 study, mean Ki-67 labeling indices were 1.73% in pleomorphic adenoma, 21.82% in mucoepidermoid carcinoma, and 17.11% in adenoid cystic carcinoma. In a basal cell adenoma versus basal cell adenocarcinoma series, a Ki-67 labeling index greater than 5% was confined to carcinoma. Nevertheless, later studies demonstrate overlap; Ki-67 should therefore be interpreted as an adjunct rather than an absolute discriminator.

Tumor	**Mean Ki-67 LI**
Pleomorphic adenoma	1.73%
Mucoepidermoid carcinoma	21.82%
Adenoid cystic carcinoma	17.11%

p53

p53 represented the marker for which the most suitable cross-study binary data were available. Across four comparative datasets, 83 of 234 malignant tumors and 51 of 278 benign tumors were p53-positive.

Table 2. Study-Level Data for p53 Meta-Analysis

Study	**Malignant p53+**	**Total malignant**	**Benign p53+**	**Total benign**
Kärjä et al.	56	103	42	116
Rosa et al.	10	100	2	133
Nagao et al.	6	11	0	9
Faur et	11	20	7	20

al.				
Total	83	234	51	278

Overall crude p53 positivity was 35.5% (83/234) in malignant tumors and 18.3% (51/278) in benign tumors.

Meta-Analysis of p53 Expression

Random-effects meta-analysis demonstrated significantly greater odds of p53 positivity in malignant salivary gland tumors: pooled OR 2.99 (95% CI 1.45-6.17). Statistical heterogeneity was low-to-moderate (I^2 approximately 29%). The result indicates that malignant salivary gland tumors have approximately threefold greater odds of p53 immunopositivity than benign tumors; however, positivity in some benign tumors limits use of p53 as an independent diagnostic marker.

PLAG1

PLAG1 is strongly associated with pleomorphic adenoma and related tumors. Surgical-specimen studies reported positivity of approximately 94-96% in pleomorphic adenoma. However, PLAG1 can also be retained in carcinoma ex pleomorphic adenoma and selected mimics; it should therefore be interpreted as a marker of pleomorphic-adenoma-related differentiation rather than benignity. In fine-needle aspiration specimens, reported performance was lower, with sensitivity of 55% and specificity of 75%.

p63 and p40

p63 is expressed in basal and myoepithelial cells and can be positive in numerous benign and malignant salivary gland tumors. p40, representing the deltaNp63 isoform, is more restricted and may improve specificity for basal or squamoid differentiation. Neither is a universal malignancy marker; their principal value is lineage determination and morphology-directed differential diagnosis.

SOX10 and S100

SOX10 and S100 identify several tumors showing myoepithelial, acinar, intercalated-duct, or secretory differentiation. Their principal value is tumor classification rather than universal benign-malignant discrimination.

DOG1 and NR4A3

DOG1 can support acinic differentiation but is not entirely specific. NR4A3 immunohistochemistry has emerged as a highly useful molecular-surrogate marker for acinic cell carcinoma and is particularly valuable when acinic cell carcinoma is in the morphological differential diagnosis.

Androgen Receptor and HER2

Androgen receptor positivity strongly supports salivary duct carcinoma in an appropriate morphologic context. HER2 overexpression or amplification occurs in a subset of salivary duct carcinomas and carcinoma ex pleomorphic adenoma. Both markers have diagnostic as well as therapeutic implications.

Table 3. Diagnostic Role of Major Immunohistochemical Markers

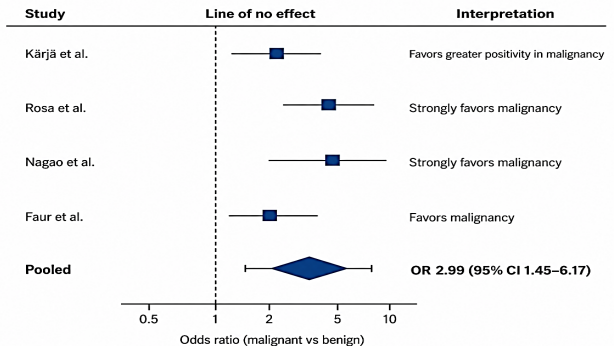
Marker	Principal diagnostic role	Association with malignancy	Major limitation
Ki-67	Proliferative activity	Usually increased	Cutoff heterogeneity
MCM3	Replication/proliferation	Strong increase	Limited validation
MCM2	Replication licensing	Increased	Variable scoring
p53	Tumor-suppressor pathway	Increased	Benign tumors may stain
PLAG1	PA lineage	Not malignancy-specific	Ca ex PA may stain
HMGA2	PA lineage	Limited	Moderate sensitivity
p63	Basal/myoepithelial	No universal distinction	Broad expression
p40	Basal/squamous	Context dependent	Not universal
S100	Myoepithelial/secretory	No	Low specificity
SOX10	Lineage marker	No	Multiple tumors positive
DOG1	Acinic differentiation	Histotype-specific	Limited specificity
NR4A3	Acinic cell carcinoma	Histotype-specific	Narrow application
AR	Salivary duct carcinoma	Supports malignant entity	Not all SDC positive
HER2	SDC/Ca ex PA	Malignant subset	Variable expression
Mammaglobin	Secretory differentiation	Histotype-specific	Other tumors may stain

Table 4. Major Quantitative Findings

Marker	Diagnostic comparison	Main finding
MCM3	PA vs MEC/ACC	74.3% sensitivity; 93.3% specificity at 8% cutoff
Ki-67	PA vs MEC/ACC	Markedly greater LI in carcinomas
Ki-67	BCA vs BCAC	LI >5% restricted to BCAC in one study
p53	Benign vs malignant	Pooled OR 2.99; 95% CI 1.45-6.17; I^2 approximately 29%
PLAG1	Pleomorphic	Approximately 94-96%

	adenoma	positivity in selected surgical series
PLAG1	PA on FNA	Sensitivity 55%; specificity 75%

Figure 2. Forest Plot for p53 Immunopositivity in Malignant versus Benign Salivary Gland Tumors



The pooled estimate lies to the right of the line of no effect, indicating increased p53 immunopositivity among malignant salivary gland tumors.

Discussion

The present systematic review demonstrates that immunohistochemistry has substantial diagnostic value in salivary gland pathology but also confirms that the utility of individual markers is highly context dependent.

The most consistent general difference between benign and malignant tumors involved markers of cellular proliferation. Ki-67 is widely accessible and routinely performed in pathology laboratories. Benign tumors, particularly conventional pleomorphic adenomas, generally demonstrate low proliferative activity, whereas many carcinomas demonstrate considerably greater Ki-67 labeling. Nevertheless, Ki-67 values overlap among tumor categories, especially in low-grade carcinomas and cellular or recurrent benign lesions. Consequently, Ki-67 alone cannot establish malignancy.

MCM proteins may potentially overcome some limitations of Ki-67. Because replication licensing begins before some cellular events reflected by Ki-67 expression, MCM proteins can identify a larger population of replication-competent cells. The MCM3 study demonstrated a mean labeling index of 3.73% in pleomorphic adenoma compared with 33.52% in mucoepidermoid carcinoma and 22.61% in adenoid cystic carcinoma. An 8% cutoff generated 74.3% sensitivity and 93.3% specificity. These data are encouraging, but the result derived from a relatively small cohort containing selected tumor categories and therefore requires independent validation.

One of the most important findings of the present meta-analysis concerns p53. Pooling four comparative datasets comprising 512 tumors identified 234 malignant and 278 benign tumors. p53 positivity occurred in 35.5% of malignant tumors compared with 18.3% of benign tumors. Random-effects analysis demonstrated an approximately threefold increase in odds of p53 positivity among malignant tumors (OR 2.99, 95% CI 1.45-6.17), with low-to-moderate heterogeneity (I^2 approximately 29%).

Despite this association, p53 cannot be regarded as a stand-alone malignancy marker. Substantial p53 expression may occur in selected benign tumors, particularly pleomorphic adenoma, and staining interpretation depends on intensity, distribution, morphology, tumor type, and associated proliferative abnormalities.

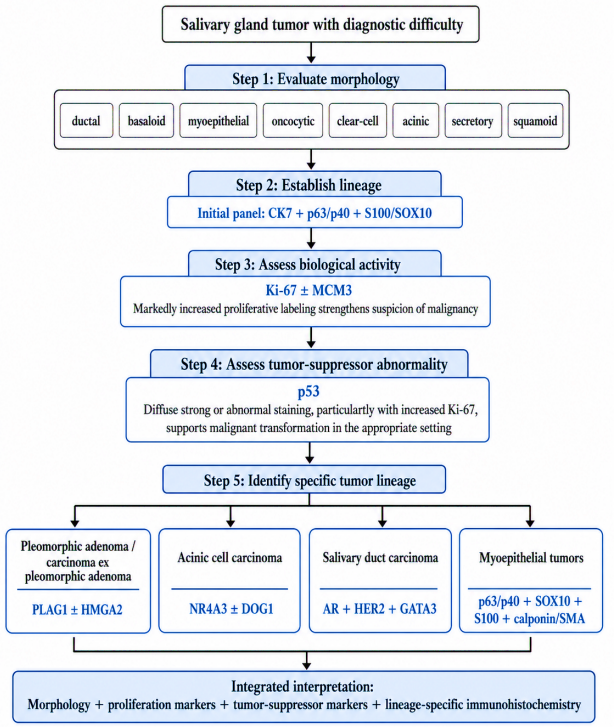
The basal cell adenoma versus basal cell adenocarcinoma comparison illustrates how combinations may outperform individual markers. Increased Ki-67 activity and abnormal p53 accumulation, when combined with infiltrative morphology, provide stronger support for malignancy than either marker alone.

PLAG1 requires a different conceptual interpretation. It is closely associated with the molecular pathogenesis of pleomorphic adenoma and shows high sensitivity in many surgical series. However, carcinoma ex pleomorphic adenoma may retain PLAG1 alterations and protein expression. Therefore, PLAG1 positivity should be interpreted as evidence of pleomorphic-adenoma lineage rather than evidence of benignity. The lower sensitivity and specificity reported in cytology also emphasize the effects of sampling and tumor heterogeneity.

p63, p40, S100, SOX10, DOG1, NR4A3, androgen receptor, and HER2 similarly illustrate the importance of distinguishing malignancy markers from classification markers. These markers contribute most when selected on the basis of morphology and expected lineage. NR4A3, for example, is particularly useful for acinic cell carcinoma, whereas androgen receptor and HER2 support salivary duct carcinoma and may influence therapy.

Overall, the evidence supports a layered diagnostic strategy in which histomorphology is first used to define the differential diagnosis, lineage markers are then applied to classify the lesion, and proliferation/tumor-suppressor markers are used to support assessment of biological behavior. This approach avoids inappropriate reliance on a universal immunohistochemical malignancy stain, which does not currently exist for salivary gland tumors.

Figure 3. Proposed Immunohistochemical Diagnostic Algorithm for Salivary Gland Tumors



Strengths

This review distinguishes markers of biological malignancy from markers of tumor lineage, incorporates quantitative meta-analysis where sufficiently comparable data were available, evaluates both broad benign-versus-malignant comparisons and specific morphologic diagnostic problems, and includes more than 500 benign and malignant tumors in the pooled p53 analysis.

Limitations

The included studies were predominantly retrospective and often had small sample sizes because salivary gland tumors are uncommon. Histological classification evolved over time, and older terminology does not always correspond exactly with current WHO classification. Antibody clones, antigen retrieval, staining platforms, positivity thresholds, labeling-index calculations, and histologic composition varied considerably. Not all studies supplied complete 2×2 diagnostic tables; consequently, formal pooling was restricted to p53, for which sufficiently comparable binary data were available. The PRISMA counts should be reconciled with retained database exports and deduplication logs before journal submission.

Future Directions

1. Prospective multicenter recruitment.
2. Contemporary WHO classification.
3. Standardized antibody clones and staining protocols.
4. Prespecified positivity thresholds.
5. Digital image-based IHC quantification.
6. Separate assessment of cytology and surgical tissue.
7. External validation cohorts.
8. Integrated morphological, immunohistochemical, and molecular prediction models.

A combined diagnostic model incorporating Ki-67, MCM3, p53, and morphology-specific lineage markers may potentially achieve greater accuracy than any isolated stain.

Conclusion

Immunohistochemistry is an essential adjunct in the diagnosis of salivary gland tumors but should not replace careful morphological assessment. Proliferation-related markers, particularly Ki-67 and MCM3, provide the most direct IHC evidence of increased biological activity. MCM3 demonstrated 74.3% sensitivity and 93.3% specificity at an 8% cutoff in a comparative cohort. Across four p53 datasets comprising 512 tumors, p53 positivity occurred in 35.5% of malignant tumors compared with 18.3% of benign tumors, and the pooled random-effects estimate was OR 2.99 (95% CI 1.45-6.17; I^2 approximately 29%). Nevertheless, p53 positivity also occurs in benign tumors and cannot independently establish malignancy. PLAG1, HMGA2, p63, p40, S100, SOX10, DOG1, NR4A3, androgen receptor, HER2, and related markers should principally be interpreted according to their lineage-specific diagnostic roles. The most effective strategy is therefore an integrated panel combining morphology, proliferation markers, tumor-suppressor markers, and lineage-specific IHC. Large standardized prospective studies are required to validate universal cutoffs and multi-marker diagnostic algorithms.

Declarations

Ethics Approval

Not applicable. This systematic review and meta-analysis utilized previously published data and did not directly involve human participants or animals.

Consent to Participate

Not applicable.

Consent for Publication

Not applicable.

Availability of Data

All data used in the quantitative analysis were derived from published studies.

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Conflict of Interest

The authors declare no conflicts of interest.

Author Contributions

Both authors contributed to conceptualization, literature evaluation, data interpretation, manuscript preparation, critical revision, and approval of the final manuscript.

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