

# Salivary Mucinous Adenocarcinoma Is a Histologically Diverse Single Entity With Recurrent *AKT1* E17K Mutations

## *Clinicopathologic and Molecular Characterization With Proposal for a Unified Classification*

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**Abstract:** Mucin-producing salivary adenocarcinomas were historically divided into separate colloid carcinoma, papillary cystadenocarcinoma, and signet ring cell carcinoma diagnoses based on histologic pattern, but have recently been grouped together in the adenocarcinoma not otherwise specified category. It is currently unclear if these tumors represent 1 or more distinct entities and how they are related to well-circumscribed papillary mucinous lesions with recurrent *AKT1* E17K mutations that were recently described as salivary intraductal papillary mucinous neoplasm. Here, we sought to evaluate the clinicopathologic and molecular features of salivary mucinous adenocarcinomas to clarify their classification. We identified 17 invasive mucin-producing salivary adenocarcinomas, 10 with a single histologic pattern, and 7 with mixed patterns. While most tumors demonstrated papillary growth (n=15), it was frequently intermixed with colloid (n=6) and signet ring (n=3) architecture with obvious transitions between patterns. All were cytokeratin 7 positive (100%) and cytokeratin 20 negative (0%). Next-generation sequencing performed on a subset demonstrated recurrent *AKT1* E17K mutations in 8 cases (100%) and *TP53* alterations in 7 cases (88%). Of 12 cases with clinical follow-up (median: 17 mo), 4 developed cervical lymph node metastases, all of which had colloid or

signet ring components. Overall, overlapping histologic and immunohistochemical features coupled with recurrent *AKT1* E17K mutations across patterns suggests that mucin-producing salivary adenocarcinomas represent a histologically diverse single entity that is closely related to tumors described as salivary intraductal papillary mucinous neoplasm. We propose a unified mucinous adenocarcinoma category subdivided into papillary, colloid, signet ring, and mixed subtypes to facilitate better recognition and classification of these tumors.

**Key Words:** salivary gland neoplasms, adenocarcinoma not otherwise specified, mucinous adenocarcinoma, papillary cystadenocarcinoma, signet ring carcinoma, *AKT1*, salivary intraductal papillary mucinous neoplasm

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Salivary gland adenocarcinomas that demonstrate prominent mucinous differentiation but do not fit into more well-established diagnoses such as mucoepidermoid carcinoma or mucin-rich variant of salivary duct carcinoma are rare, poorly understood, and incompletely classified. Historically, these tumors have largely been characterized in the literature in single case reports and small series, which divided them into several categories based on distinctive histologic patterns. Tumors with colloid architecture, defined by malignant cells floating in lakes of extracellular mucin, were recognized as a standalone entity under the name mucinous adenocarcinoma in the 2005 World Health Organization (WHO) Classification of Head and Neck Tumours.<sup>1–13</sup> Invasive tumors composed of papillary mucinous epithelium, alternately described as papillary cystadenocarcinomas or mucus-producing adenopapillary carcinomas, also were included as a significant component of the cystadenocarcinoma category in the 2005 WHO.<sup>13–19</sup> Signet ring carcinomas composed of discohesive cells with intracellular mucin vacuoles have also rarely been described.<sup>20–22</sup> However, the 2017 WHO Classification of Head and Neck Tumours grouped all of these, along with many entirely dissimilar tumors, into the broad adenocarcinoma not otherwise specified (NOS) category with the rationale that mucinous differentiation was a nonspecific feature.<sup>23,24</sup> In light of these changing and somewhat contradictory classifications and sparse literature systematically

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characterizing these tumor types, it is currently unclear whether these various patterns of mucin-producing salivary adenocarcinoma are related to each other and if they merit recognition as 1 or more discrete diagnostic entities.

Recently, the novel entity intraductal papillary mucinous neoplasm (IPMN) of salivary glands has expanded the clinicopathologic and molecular spectrum of mucin-producing salivary tumors. Initially described by Agaimy et al<sup>25</sup> in 2018, salivary IPMN consists of a florid papillary proliferation lined by a single layer of columnar mucinous cells with a gastric foveolar-type appearance and variable cytologic atypia and mitotic activity. Eleven of 12 salivary IPMNs sequenced to date have demonstrated activating *AKT1* E17K mutations, with concomitant or alternate *HRAS* Q61R mutations in 4 cases.<sup>25,26</sup> Although they lack a surrounding myoepithelial cell layer, IPMNs tend to be well-circumscribed and follow a ductal distribution, leading to presumptive classification as a noninvasive, intraductal papillary lesion. However, a few cases have been reported to include areas of clearly invasive growth, with infiltrative micropapillary and mucinous nests involving stroma and lymphatics adjacent to the papillary proliferation.<sup>26,27</sup> The presence of associated stromal invasion raises the possibility that salivary IPMNs may be related to adenocarcinomas with mucinous differentiation. However, the relationship between these tumor types has never been assessed, and genetic alterations in mucin-producing adenocarcinomas have not been documented to facilitate this comparison. In this study, we sought to evaluate the clinicopathologic characteristics and molecular underpinnings of a broad series of invasive salivary adenocarcinomas with mucinous differentiation to more comprehensively evaluate the boundaries of this diagnostic category and explore their relationship to salivary IPMNs.

## MATERIALS AND METHODS

### Case Selection

After institutional review board approval, we identified salivary gland adenocarcinomas that showed prominent mucinous differentiation from the pathology archives at The Johns Hopkins Hospital, the University of Minnesota School of Dentistry, and the authors' consultation files. To avoid confusion, for the purposes of this study we will use the terms mucinous adenocarcinoma or mucin-producing adenocarcinoma to broadly refer to any salivary carcinomas that have diffuse mucinous differentiation and reserve the term colloid carcinoma to specifically describe tumors with cells floating in lakes of mucin. For inclusion in this study, we required tumors to (1) demonstrate unequivocally invasive growth, (2) have nonfocal (>10%) extracellular and/or intracellular mucin production, and (3) lack diagnostic features of other mucin-producing salivary carcinoma types or variants. Seventeen cases were identified that met these inclusion criteria. Four of the cases were previously included in a series of salivary adenocarcinoma NOS.<sup>28</sup> All available hematoxylin and eosin sections from each case were reviewed, and the histologic features, including the presence of papillary, colloid, or signet ring architectural patterns, were tabulated. Clinical and

demographic information, including any available follow-up data, was documented.

### Immunohistochemistry

All existing stains performed at the time of diagnosis were tabulated for all tumors. As tissue availability permitted, additional immunohistochemistry was performed on a subset of cases using mouse monoclonal antibodies for cytokeratin (CK) 7 (clone ov-tl, 1:500; Dako, Carpinteria, CA), CK20 (clone Ks20.8, prediluted; Dako), CDX2 (clone EPR2764Y, prediluted; Dako), p63 (clone 4a4, prediluted; BioCare Medical, Pacheco, CA), p40 (clone BC28, 1:100; BioCare Medical), S100 protein (clone 4C4.9, prediluted; Ventana Medical Systems, Tucson AZ), smooth muscle actin (SMA; clone 1A4, prediluted; Ventana Medical Systems), calponin (clone M3556, 1:500; Dako), thyroid transcription factor 1 (TTF-1; clone 8G7G3, prediluted; Ventana Medical Systems), androgen receptor (AR; clone SP107, prediluted; CellMarque/Sigma-Aldrich, St. Louis, MO), and beta-catenin (clone 14, 1:1000; BD Biosciences, Franklin Lakes, NJ). In the majority of cases, staining was performed using standardized automated protocols on Ventana BenchMark Ultra autostainers (Ventana Medical Systems) in the presence of appropriate controls, and signals were visualized using the ultraView polymer detection kit (Ventana Medical Systems).

### Next-generation Sequencing

We also selected the 10 most recent cases with sufficient tissue available for next-generation sequencing (NGS). NGS was attempted on 9 of these cases at the University of Texas Southwestern Medical Center as described in detail elsewhere.<sup>29</sup> In short, Qiagen AllPrep kits (Qiagen, Germantown, MD) were used to isolate DNA, custom NimbleGen probes (Roche, Indianapolis, IN) were used to create an enriched library containing all exons from >1425 cancer-related genes, and sequencing was performed on the NextSeq 550 (Illumina, San Diego, CA) with a median target exon coverage of 900×. NGS was also performed on 1 case at The Johns Hopkins Hospital as previously described.<sup>30,31</sup> Briefly, the automated Siemens Tissue Preparation System (Siemens Healthcare Diagnostics, Tarrytown, NY) was used to isolate DNA, the SureSelect XT Target Enrichment System (Agilent Technologies, Santa Clara, CA) was used to create libraries containing the full coding regions of 644 cancer-associated genes, and sequencing was performed on the HiSeq 2500 platform (Illumina) to an average 500 to 1000× read depth. For all cases, variants were reviewed using the Integrated Genomics Viewer (Broad Institute, Cambridge, MA) and annotated using the gnomAD and dbSNP databases.

## RESULTS

### Clinical Information

Clinical and demographic information is summarized in Table 1. The 17 tumors arose in 9 women and 8 men with a median age of 77 years (range: 60 to 94 y). Among 12 patients who had detailed clinical history available, 2 patients had a history of breast carcinoma, 1 of whom also had renal cell carcinoma, and 1 patient had prostate carcinoma, but none of

TABLE 1. Clinical and Demographic Information

Case No.	Age (y)	Sex	Site	Size (cm)	Original Diagnosis	Treatment	Recurrence/Metastasis	Follow-up (mo)	Status
1	79	Male	Buccal	1.2	Papillary cystadenocarcinoma	Surgery (negative margins)	None	12	NED
2	60	Male	Upper lip	1.5	Papillary cystadenocarcinoma with atypical cells	Surgery (negative margins)	None	60	NED
3	70	Male	Palate	2	Colloid carcinoma	Surgery (positive margins)	Cervical LN metastasis at presentation	6	DOC
4	84	Female	Floor of mouth	1.2	Mucinous adenocarcinoma	Surgery (positive margins)	None	2	NED
5	78	Male	Palate	1.8	Mucinous adenocarcinoma	Surgery (positive margins)/XRT; Chemo at recurrence	Cervical LN metastasis at presentation; recurrence in cervical LN at 37 mo	141	NED
6	74	Female	Base of tongue	4	Mucinous adenocarcinoma	NA	NA	NA	NA
7	75	Male	Oral tongue	2.4	Invasive adenocarcinoma with papillary and mucinous features	Surgery (negative margins)/XRT/chemo	Cervical LN metastasis at presentation	4	NED
8	71	Male	Buccal	NA	Neoplastic mucinous cyst	NA	NA	NA	NA
9	77	Female	Buccal	1	Adenocarcinoma NOS with signet ring features	Surgery (negative margins)/XRT; chemo for metastasis	Cervical LN metastasis at 9 mo	15	NED
10	84	Male	Buccal	1.9	Mucinous adenocarcinoma	NA	NA	NA	NA
11	94	Female	Base of tongue	3.9	Mucinous cystadenocarcinoma	Surgery (negative margins)	None	9	NED
12	94	Female	Oral tongue	NA	Mucinous adenocarcinoma	NA	NA	NA	NA
13	90	Male	Upper lip	1.7	Mucin-rich adenocarcinoma	Surgery (negative margins)	None	18	NED
14	85	Female	Buccal	1.3	Papillary cystadenocarcinoma	Surgery (negative margins)	None	20	NED
15	67	Female	Palate	1.7	Papillary cystadenocarcinoma	Surgery (negative margins)	None	28	NED
16	75	Female	Sublingual and submandibular	5.8	Mucinous cystadenocarcinoma	NA	NA	NA	NA
17	67	Female	Buccal	3.5	Papillary cystadenocarcinoma	Surgery (negative margins)	None	60	NED

chemo indicates chemotherapy; DOC, dead of other causes; LN, lymph node; NA, not available; NED, no evidence of disease; XRT, external-beam radiation therapy.

these tumors were known to have mucinous differentiation; patients also had no evidence of concurrent gastrointestinal, pancreaticobiliary, or lung carcinoma on comprehensive clinical and radiographic evaluation. Fifteen patients presented with an intraoral mass, 1 complained of painful neck lymphadenopathy, and 1 had a submandibular swelling thought to be a plunging ranula. The vast majority of tumors arose in minor salivary glands, including the buccal mucosa (n=6), palate (n=3), base of tongue (n=2), oral tongue (n=2), upper lip (n=2), and floor of mouth (n=1), while a single tumor affected both the sublingual and submandibular glands (n=1). The median tumor size was 2.3 cm (range: 1 to 5.8 cm). Tumors received a variety of original diagnoses, as also listed in Table 1, with the most common being mucinous adenocarcinoma (n=5), papillary cystadenocarcinoma (n=4), or mucinous cystadenocarcinoma (n=2).

**Histologic Features**

The tumors in this series demonstrated a diverse range of histologic features, including 10 cases (59%) composed of a single growth pattern and 7 tumors (41%) that had a mix of architectural components. This included 9 tumors with pure papillary growth, 5 tumors with mixed papillary and colloid patterns, 1 tumor with mixed colloid and signet ring architecture, 1 tumor with a mixed signet ring and papillary components, and 1 pure signet ring tumor. The distribution of histologic patterns is documented in more detail in Table 2.

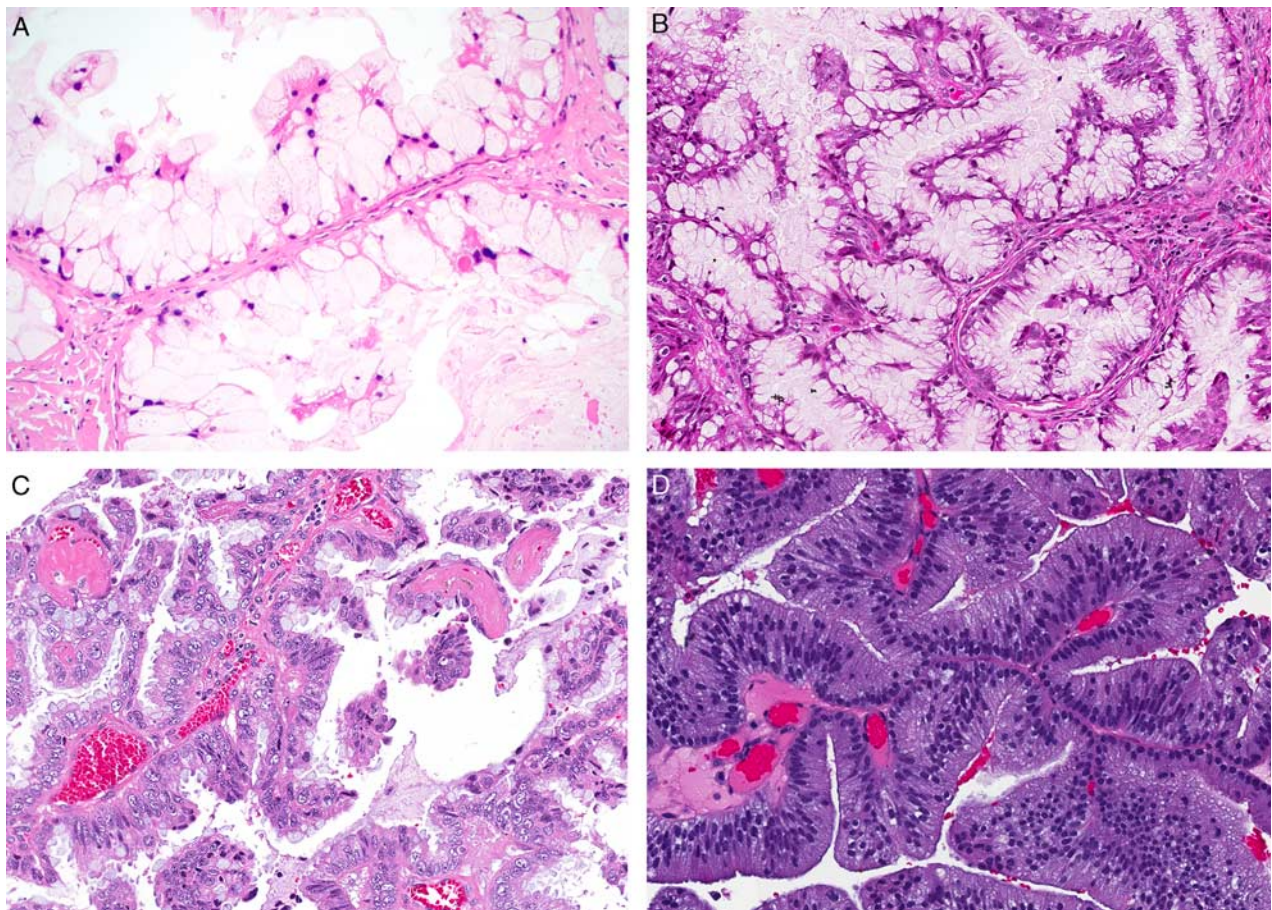
Papillary architecture was the most common pattern in this cohort, as seen in 15 tumors. These tumors harbored papillary projections and associated cysts lined by a single layer of columnar epithelial cells with variable degrees of tufting and nuclear stratification. Intracellular mucin was present in diverse forms in all of these cases, including diffuse intracytoplasmic accumulation (case 3, Fig. 1A), single large goblet cell-like vacuoles (case 6, Fig. 1B), apical caps (case 11, Fig. 1C), or innumerable gastric foveolar-type cytoplasmic droplets (case 17, Fig. 1D). Tumors showed a mix of complex arborizing papillary fronds (case 10, Fig. 2A), simple papillary projections into large cystic spaces (case 14, Fig. 2B), smaller papillary nests (case 1, Fig. 2C), or medium-sized cysts with occasional papillary excrescences (case 16, Fig. 2D). Although cases with pure papillary architecture demonstrated significant architectural and cytologic similarity to features previously reported in salivary IPMN, all of these tumors demonstrated unequivocal invasion by either small infiltrative papillary nests (case 15, Fig. 2E) or large irregular cysts (case 11, Fig. 2F) into surrounding stroma. Areas of papillary architecture were also present within lymph node metastases. No definitive well-circumscribed or intraductal papillary component was seen in any tumor.

Despite the predominance of papillary architecture, colloid growth was also seen in 6 tumors and signet ring cells in 3 tumors. Colloid areas were composed of detached tubules, cords (case 3, Fig. 3A), papillae, nests, and sheets (case 13, Fig. 3B) of tumor cells suspended in stromal mucin pools of various sizes. Many of these cells also contained discrete intracytoplasmic mucin vacuoles. Not only were colloid zones closely intermixed with papillary elements in 5 cases (case 7, Fig. 3C), but in areas,

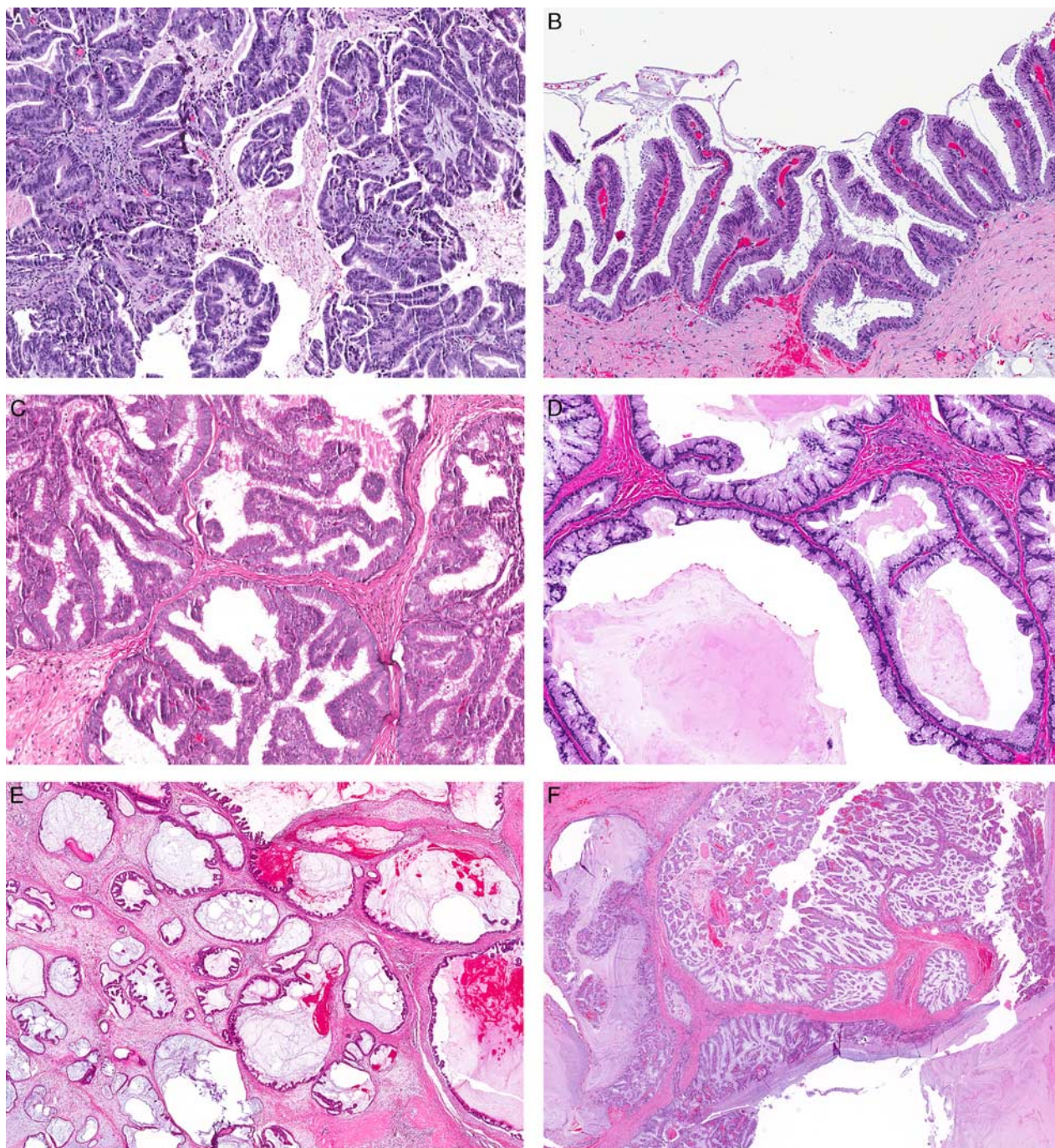
**TABLE 2.** Histologic and Immunohistochemical Features

Case No.	Papillary	Colloid	Signet Ring	CK7	CK20	CDX2	S100	p63/p40	Calponin	SMA	AR	TTF-1	Beta-catenin
1	Single	None	None	+	-	-	-	-	-	-	ND	-	Membranous
2	Single	None	None	+	-	-	-	-	-	-	ND	-	Membranous
3	Minor	Major	None	+	-	-	-	-	-	-	-	ND	Membranous
4	Minor	None	Major	F+	-	-	-	-	-	-	-	ND	Membranous
5	Major	Minor	None	+	-	-	-	-	-	-	-	ND	Membranous
6	Major	Minor	None	+	-	ND	ND	ND	ND	ND	ND	-	ND
7	Minor	Major	None	+	-	-	-	-	-	-	-	-	Membranous
8	None	Major	Minor	+	-	-	ND	-	ND	ND	ND	-	Membranous
9	None	None	Single	+	-	-	-	-	-	-	-	-	ND
10	Single	None	None	+	-	F+	-	-	ND	-	-	ND	ND
11	Single	None	None	+	-	-	-	-	-	-	-	-	Membranous
12	Single	None	None	+	-	-	ND	ND	ND	ND	ND	ND	ND
13	Major	Minor	None	+	-	-	ND	ND	ND	ND	ND	-	ND
14	Single	None	None	ND	ND	ND	-	-	ND	ND	ND	ND	ND
15	Single	None	None	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
16	Single	None	None	+	-	-	ND	ND	ND	ND	ND	ND	ND
17	Single	None	None	ND	-	-	ND	ND	ND	ND	ND	ND	ND

- indicates negative; +, positive; F+, focally positive; major, largest histologic pattern present; minor, additional histologic pattern; ND, not done; single, only histologic pattern present.



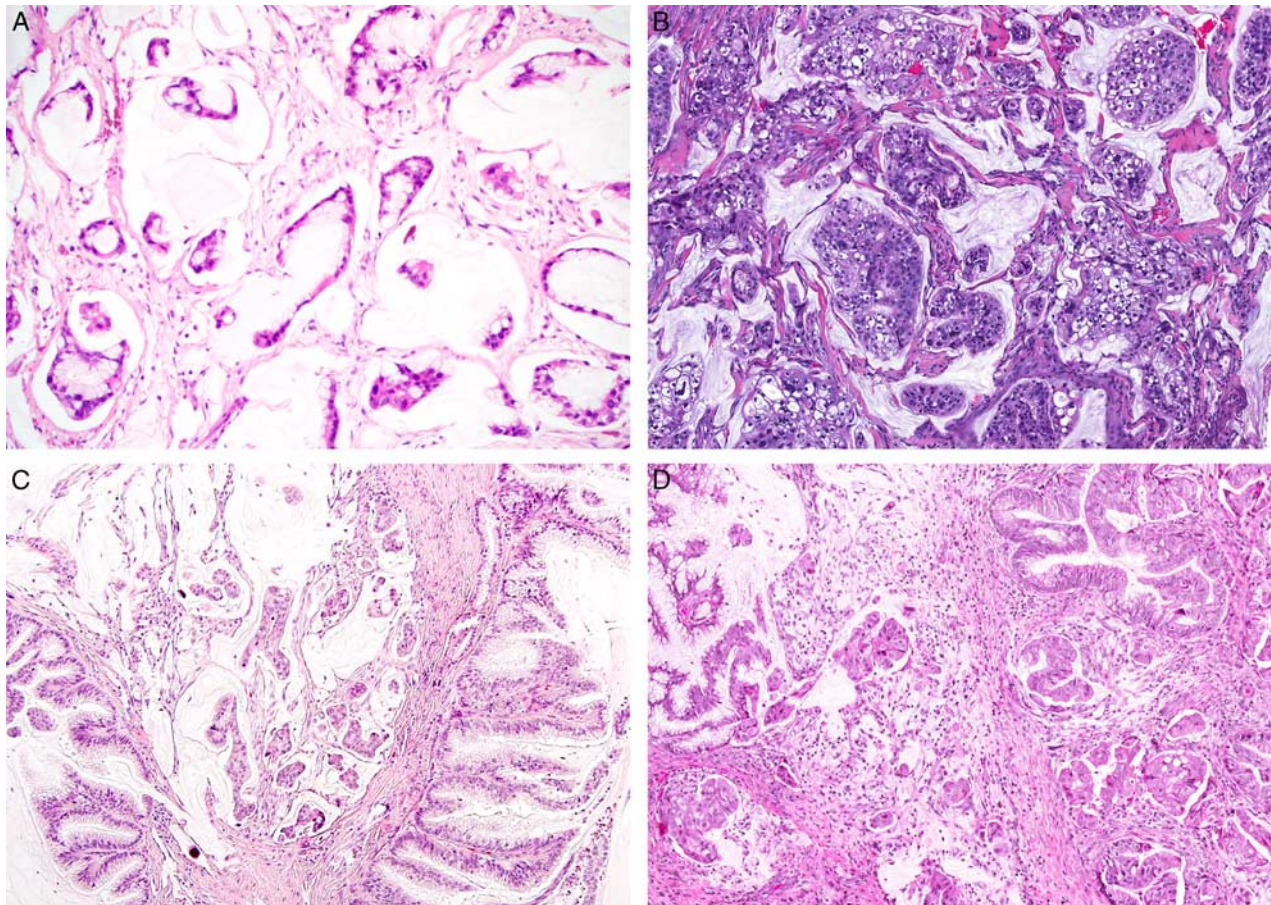
**FIGURE 1.** All papillary tumors demonstrated columnar epithelium with intracellular mucin in a variety of patterns, including diffuse intracytoplasmic mucin (case 3; A), well-formed mucin vacuoles (case 6; B), apical mucin caps (case 11; C), or innumerable cytoplasmic droplets similar to gastric foveolar epithelium (case 17; D).



**FIGURE 2.** Tumors with papillary architecture were variably composed of complex arborizing papillary fronds (case 10; A), simple papillary cyst lining (case 14; B), small papillary nests (case 1; C), or medium-sized cysts with occasional papillary excrescences (case 16; D). Although they showed architectural and cytologic overlap with salivary IPMN, they demonstrated invasive growth of small papillary nests (case 15; E) or large irregular cysts (case 11; F) into surrounding stroma.

there was an obvious transition between compressed papillary nests and micropapillary fragments free-floating in smaller mucin pools (case 6, Fig. 3D) or between papillary cyst lining and detached epithelial cords. The signet ring tumors were composed of discohesive epithelioid cells with discrete intracellular mucin vacuoles. These vacuoles occupied the

entire cytoplasm in 2 cases, including 1 where the signet ring cells were floating in mucin alongside colloid elements (case 8, Fig. 4A) and 1 where sheets of signet ring cells surrounded intact papillary structures (case 4, Fig. 4B). The single tumor that demonstrated only signet ring morphology had more eccentric eosinophilic cytoplasm with smaller but distinct



**FIGURE 3.** Tumors with colloid architecture demonstrated detached tubules, cords (case 3; A), papillae, nests, and sheets (case 13; B) of tumor cells floating in variably sized stromal mucin pools. The papillary and colloid elements were closely intermixed (case 7; C) with obvious areas of transition between small papillary nests and floating micropapillary fragments (case 6; D).

mucin vacuoles in a subset of cells that were best visualized on mucicarmine stain (case 9, Figs. 4C, D).

Across all histologic patterns, tumors demonstrated a wide range of cytologic atypia. While most tumors had relatively uniform medium-sized oval to elongated nuclei with vesicular chromatin and variably prominent nucleoli, there were occasional cases with larger nuclei that showed pronounced pleomorphism and hyperchromasia. Likewise, although most cases only contained occasional mitotic figures, a few showed an increased mitotic rate with atypical mitotic forms. Patchy tumor necrosis was seen in 2 cases.

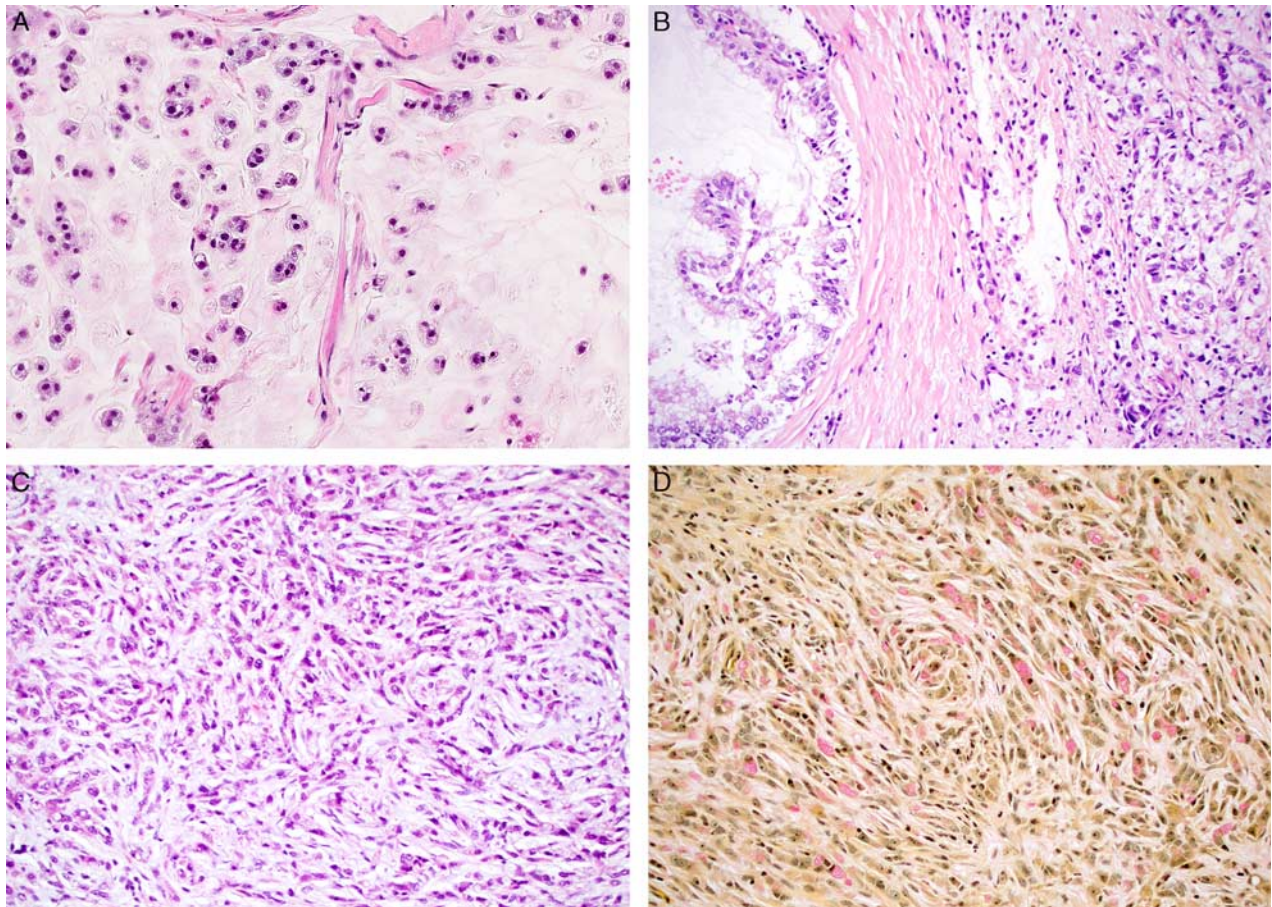
### Immunohistochemistry

The results of immunohistochemistry are also summarized in Table 2. All 14 tumors that underwent staining were positive for CK7 (100%), with diffuse expression in 13 cases (93%) and focal staining in 1 case (7%). They were uniformly negative for CK20 (0%). The vast majority of tumors were also negative for CDX2, with focal expression in only 1 of 14 tumors stained (7%). In addition, all tumors tested were negative for p63 or p40 (0/11), S100 protein (0/10), SMA (0/9), calponin (0/8), thyroid transcription factor 1 (0/8), and AR (0/7) with no nuclear localization of beta-catenin (0/8). No

myoepithelial cell layer was identified in the 11 tumors that underwent staining with p63, p40, S100, SMA, or calponin.

### Next-generation Sequencing

The results of NGS are summarized in Figure 5. Nucleic acids were successfully amplified for NGS in 8 of 10 cases where sequencing was attempted. NGS demonstrated *AKT1* E17K hotspot oncogene mutations in all 8 tumors (100%), including 5 tumors with pure papillary architecture, 2 tumors with mixed papillary and colloid growth, and 1 tumor with a pure signet ring pattern. Additional *TP53* tumor suppressor gene alterations, including both missense and nonsense mutations and copy number loss, were seen in 7 of 8 tumors (88%). A subset of tumors also demonstrated a variety of other genetic mutations or alterations, including *RBI* (n=3, 38%), *MTOR* (n=2, 25%), *BRCA2* (n=1, 13%), *CDHI* (n=1, 13%), *PALB2* (n=1, 13%), and *NF2* (n=1, 13%). Of note, the mutational pattern in the single tumor with pure signet ring histology, which included *BRCA2*, *PALB2*, and *CDHI* mutations raised consideration of metastatic breast carcinoma, but this patient had no history of breast carcinoma and no breast tumors were identified on mammography or positron emission tomography scan.



**FIGURE 4.** Tumors with signet ring architecture were defined by discohesive cells with prominent intracytoplasmic mucin, which were seen floating in mucin pools alongside colloid elements (case 8; A) and adjacent to papillary structures (case 4; B). One tumor showed only signet ring growth with small but distinct mucin vacuoles (case 9; C) that were best visualized on mucicarmine stain (case 9; D).

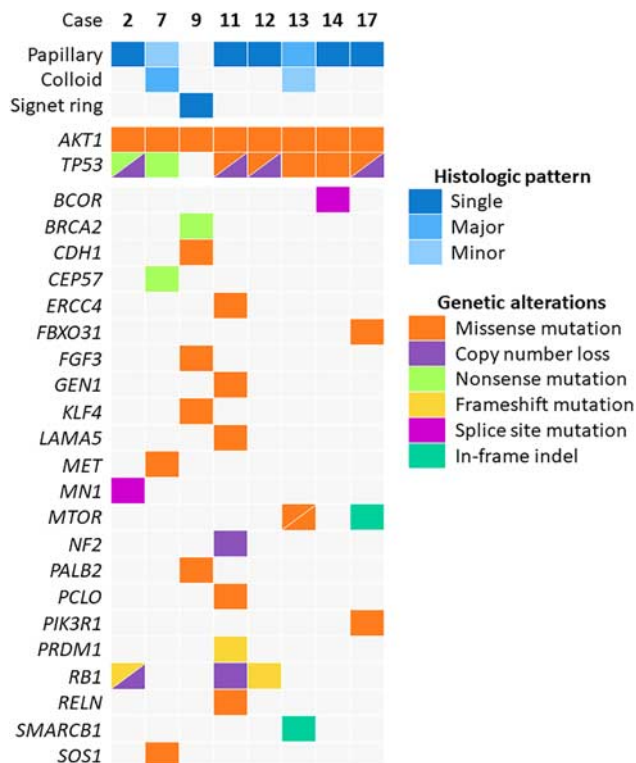
### Follow-up

There were 12 patients with detailed follow-up information available, with a median duration of 17 months (range: 2 to 141 mo). All patients underwent primary surgical resection, with negative margins in 9 cases and positive margins in 3 cases. There were 3 patients who had cervical lymph node metastasis at presentation, of whom 2 were also treated with adjuvant external-beam radiation and 1 received both adjuvant radiation and chemotherapy. One of these patients recurred in the cervical nodes at 37 months and 1 additional patient developed cervical lymph node metastases 9 months after primary surgical resection. Both of these patients were treated with additional surgical resection, external-beam radiation, and chemotherapy. Notably, all patients who developed lymph node metastasis at presentation or recurrence had colloid or signet ring components to their tumor. No patients developed distant metastases. At last follow-up, 11 patients had no evidence of disease and 1 patient was dead of unrelated causes.

### DISCUSSION

Mucin-producing adenocarcinomas of the salivary glands are a rare, ill-defined, and poorly understood group

of tumors that largely have been documented in the literature in case reports and small series. They were historically divided into discrete colloid carcinoma, papillary cystadenocarcinoma, and signet ring carcinoma categories based on their dominant histologic pattern. However, the 2017 WHO Classification of Head and Neck Tumours grouped all of these tumors with various other dissimilar, unclassified carcinomas under the broad adenocarcinoma NOS designation.<sup>23</sup> It is currently unclear whether they belong in this heterogeneous group or truly represent 1 or more distinct entities. This classification has been further complicated by the recent description of salivary IPMN, a well-circumscribed papillary mucinous tumor that is presumed to be benign but is sometimes associated with invasive growth.<sup>25–27</sup> Although IPMN are defined by recurrent *AKT1* E17K mutations, the molecular underpinnings of mucin-producing adenocarcinomas have not been established to permit a detailed comparison between these entities. In this study, we performed clinicopathologic and molecular analysis of a broad group of salivary mucinous adenocarcinomas with the aim of clarifying their classification and relationship to IPMN.



**FIGURE 5.** NGS highlighted recurrent *AKT1* E17K oncogene mutations in all 8 tumors that successfully underwent NGS, including tumors with papillary, colloid, and signet ring pattern. There were concomitant *TP53* tumor suppressor gene alterations in 7 cases as well as a range of other mutations.

Our findings suggest that mucin-producing salivary adenocarcinomas encompass a continuous spectrum of histologic patterns rather than discrete categories. While the majority of cases in this series demonstrated a predominance of papillary architecture, a significant subset also included colloid or signet ring components closely intermixed with the papillary elements with obvious areas of transition between growth patterns. The various patterns all had a consistent CK7-positive/CK20-negative immunophenotype. This histologic and immunohistochemical overlap belies the historical assumption that colloid carcinoma, papillary cystadenocarcinoma, and signet ring carcinoma comprise entirely separate entities. Although a few authors have previously acknowledged that mucin-producing salivary adenocarcinomas could show mixed morphologies, with particular similarities between papillary and colloid growth patterns,<sup>5,11,13,32</sup> virtually all observers have continued drawing firm lines between these categories. Our data suggest that overt mucin production, rather than architectural pattern, is the defining feature of these tumors that should drive a unified classification. Importantly, growth pattern may still be a prognostic indicator within the mucinous adenocarcinoma category. In this cohort, progressive disease exclusively occurred among patients whose tumors included colloid or signet ring patterns and was not seen with pure papillary tumors—findings that mirror the behavior of similar tumors previously reported as colloid

carcinoma, signet ring carcinoma, and papillary cystadenocarcinoma, respectively.<sup>6,11,13,14,18,20</sup> Although identification of these high-risk elements may be helpful for prognostication, recognition that the various mucinous adenocarcinoma patterns are all related and can show histologic overlap may actually allow for a more nuanced classification of these rare tumors.

This study also demonstrates the remarkably consistent presence of *AKT1* E17K mutations in tumors across this histologic spectrum. All 8 mucin-producing salivary adenocarcinomas that underwent NGS, including cases with papillary, colloid, and signet ring patterns, demonstrated *AKT1* E17K oncogene mutations, with concomitant alterations in tumor suppressor genes including *TP53* in 7 cases. *AKT1* is a serine/threonine kinase that serves as a key intermediary between upstream regulatory proteins and downstream signaling molecules in the phosphatidylinositol-3-OH kinase pathway. Hotspot G > A point mutations at nucleotide 49 lead to the substitution of lysine for glutamic acid in amino acid 17, causing localization to the cell membrane and constitutive upregulation of signaling.<sup>33</sup> *AKT1* mutations are most commonly seen in breast cancer, where they have been implicated in up to 8% of cases, but are also rarely reported in colon, lung, ovarian, endometrial, and prostate carcinomas.<sup>33–39</sup> They also have been identified in benign lesions including breast intraductal papilloma and adenomyoepithelioma, ciliated muconodular papillary tumor, and sclerosing pneumocytoma of the lung, cutaneous hidradenoma papilliferum, and meningioma.<sup>40–50</sup> Notably, emerging pan-*AKT* kinase inhibitors have shown promising activity against tumors with *AKT1* E17K mutations in early clinical trials.<sup>51,52</sup> While *AKT1* E17K mutations were recently reported as the defining feature of salivary IPMN, documentation of the same mutations in salivary mucinous adenocarcinomas here represents the first recurrent role for this gene in salivary malignancies. Only rare *AKT1* mutations have previously been reported in epithelial-myoeplithelial carcinoma, salivary duct carcinoma, adenoid cystic carcinoma, and adenocarcinoma NOS, where they occur interchangeably with other common oncogenic mutations such as *PIK3CA*, *HRAS*, *NRAS*, and *BRAF*.<sup>53–56</sup> Detailed histologic characterization of these *AKT1*-mutant cases is not available, so it is unclear whether any of these tumors might overlap with mucin-producing adenocarcinomas described in this series. While validation of this finding is certainly needed in a larger group of tumors with pure colloid or signet ring patterns, the consistent presence of *AKT1* E17K mutations in a diverse group of mucinous adenocarcinoma provides strong evidence that they represent a single entity.

Of course, the presence of both papillary mucinous morphology and recurrent *AKT1* E17K mutations also suggests that salivary mucinous adenocarcinomas are closely related to the recently described salivary IPMN, though the nature of this relationship remains speculative. One possibility is that IPMN represents an intraductal precursor to invasive mucinous adenocarcinoma. Several well-circumscribed papillary lesions reported as salivary



IPMNs with adjacent foci of overtly infiltrative growth would certainly support this theory.<sup>26,27</sup> Likewise, the absence of *TP53* mutations in 3 IPMN cases analyzed by a limited NGS panel<sup>25</sup> raises the possibility that *AKT1* E17K oncogene mutations define the neoplastic mucinous phenotype while additional tumor suppressor gene mutations are necessary for malignant transformation. However, we did not identify any clearly noninvasive areas in the 17 cases in this series to confirm the concept of IPMN as precursor lesion. Furthermore, IPMNs have not undergone comprehensive sequencing using a broad NGS panel to rule out the presence of other tumor suppressor gene mutations as seen in case 9 in this study. An alternate possibility is that salivary IPMN represents the extreme low-grade end of the mucinous adenocarcinoma spectrum— analogous to well-circumscribed mucoepidermoid or acinic cell carcinomas that nevertheless have the diagnostic histologic and molecular features of those entities. Salivary IPMN was originally defined as a benign papillary lesion because of its tendency to follow a ductal distribution, even though it lacks a surrounding myoepithelial layer.<sup>25</sup> While the understanding of how myoepithelial cells contribute to various presumed intraductal tumors is evolving,<sup>57</sup> it is difficult to confirm the noninvasive nature of salivary IPMN in the absence of myoepithelial cells. Moreover, we found many papillary areas that were histologically identical to tumors reported as salivary IPMN except for the presence of unequivocal invasive growth; these areas were even present in lymph node metastasis. Overall, given the histologic and molecular similarities between salivary IPMN and mucin-producing adenocarcinomas and the absence of objective features to differentiate these entities, we favor they both fall within the mucinous adenocarcinoma spectrum.

Importantly, these mucinous adenocarcinomas can clearly be distinguished from other salivary tumor types that have similar features. Papillary architecture and focal extracellular mucin can be seen in several other tumors that overlap with the historical cystadenocarcinoma category, including secretory carcinoma, cribriform adenocarcinoma variant of polymorphous adenocarcinoma, and intercalated duct-like type of intraductal carcinoma. However, these tumors all lack significant intracellular mucin production and generally show diffuse S100 protein reactivity.<sup>58</sup> Tumors with a colloid pattern can mimic mucoepidermoid carcinoma, which occasionally produces abundant extracellular mucin, or the mucin-rich variant of salivary duct carcinoma, which is also defined by nests of cells floating in pools of mucin. Fortunately, mucoepidermoid carcinoma consistently demonstrates p63 and p40 expression<sup>59</sup> and salivary duct carcinoma is generally positive for AR,<sup>60,61</sup> both of which are absent in pure mucinous adenocarcinomas. Signet ring architecture has also been documented in the recently described variant of salivary duct carcinoma with rhabdoid features and secretory myoepithelial carcinomas—a significant subset of which were previously classified as signet ring carcinoma.<sup>62</sup> These tumors can also be differentiated by identification of AR positivity in salivary duct carcinoma with rhabdoid features<sup>63</sup> and myoepithelial markers including S100, calponin, p63, p40, and SMA in secretory myoepithelial carcinoma.<sup>62,64,65</sup> Of course, mucin-producing

adenocarcinomas are currently classified as adenocarcinoma NOS—a heterogenous category regarded as a diagnosis of exclusion for tumors that do not meet criteria for more specific entities. However, mucin production is an uncommon feature in other adenocarcinoma NOS, and the characteristic papillary, colloid, and signet ring architecture in tumors with diffuse mucinous differentiation also makes them histologically distinct from the remainder of that group.<sup>28</sup>

In summary, the presence of recurrent and distinctive histologic, immunohistochemical, and molecular findings strongly suggests that these mucin-producing salivary adenocarcinomas, including the group previously reported as salivary IPMN, should be regarded as a single diagnostic entity rather than being split into multiple tumor types or lumped into the larger adenocarcinoma NOS category. We propose that the familiar mucinous adenocarcinoma label be applied broadly to all tumors with prominent mucin production rather than just those with a colloid pattern, similar to how the terminology is used in lung adenocarcinomas. This group could then be subclassified into a papillary, colloid, signet ring, and mixed subtypes to account for possible differences in clinical behavior across architectural patterns (eg, mucinous adenocarcinoma, mixed papillary and colloid type). Specific recognition of mucinous adenocarcinoma as a single entity and documentation of its spectrum of histologic appearance and clinical behavior will allow for a better understanding of these rare but distinctive tumors.

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