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Predictors of Outcome in the Phenotypic Spectrum of Polymorphous Low Grade Adenocarcinoma (PLGA) and Cribriform Adenocarcinoma of Salivary Gland (CASG): A Retrospective Study of 69 Patients

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Abstract

Polymorphous low grade adenocarcinoma (PLGA) shows histologic diversity with fascicular and targetoid features while cribriform adenocarcinoma (CASG) demonstrates predominant cribriform and solid patterns with glomeruloid appearance and optical clear nuclei. The current study is designed to identify pathological features that may predict clinical outcome in 69 PLGA/CASG. Seven patients had recurrences or distant metastasis, including three local recurrences, 2 nodal metastases, and 2 distant metastases. The following histological factors predicted disease free survival on univariate analysis: tumor size, bone invasion, lymphovascular invasion (LVI), tumor necrosis, 10% papillary pattern, and 30% cribriform pattern. On multivariate analysis, 10% of papillary pattern and 30% cribriform pattern were independent prognostic factors of disease free survival (DFS). Histologic classification of the 69 tumors (based on the prior reported definition of CASG) showed 21 CASGs (30%), 23 PLGAs (33%), and 22 (32%) with indeterminate features of PLGA and CASG. Additionally, 3 (4%) tumors demonstrated a predominant papillary pattern (50%). Interestingly, not all CASGs were located in the minor salivary gland and two tumors were identified in the parotid. One patient died from her disease and she harbored a CASG with >30% cribriform pattern. In conclusion, tumor size, bone invasion, and LVI are significant parameters that can predict adverse clinical behaviors in PLGA/CASG on univariate analysis. Using the prior reported definition, an overlapping histology between PLGA and CASG was noted in over a third of the cases. Regardless of tumor subclassification, the percentage of cribriform and papillary patterns seems to be prognostically relevant and should be documented.

Keywords

Polymorphous low grade adenocarcinoma (PLGA); cribriform adenocarcinoma of salivary gland (CASG); prognosis; histology; immunohistochemistry

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Introduction

Polymorphous low grade adenocarcinoma (PLGA) was first proposed by Evans and Batsakis in 1984 as a distinct tumor type of the salivary gland characterized by cytologic uniformity and architectural diversity (1). Since then, PLGA has been accepted as a major type of malignant salivary gland epithelial tumor and was adopted in the World Health Organization (WHO) classification (2). A majority of PLGAs originates from the minor salivary glands, in particular the palate. Currently, PLGA constitutes the second most common malignant oral minor salivary gland tumor, accounting for 11% of all oral salivary gland tumors and 26% of all malignant ones (3).

In general, PLGA is considered as a low grade salivary gland malignancy with an overall recurrence rate of 19.1% (4), a risk of regional lymph node metastasis of 13–17% (5, 6), and a 0.6 – 7.5% risk of distant metastasis (5, 7). To date, little is known about the histopathologic features that influence outcome in PLGA. Univariate survival analysis has been performed only in two published studies of 40 and 24 patients, and failed to establish any significant pathologic factors that might predict survival (5, 6). Hence, a larger scale study with long term follow up data and appropriate statistical analysis is necessary in order to identify helpful prognostic pathologic features in this disease.

Moreover, there have been controversies in the literature regarding the diagnosis of low-grade papillary adenocarcinoma (LGPA, or PLGA with papillary pattern) and cribriform adenocarcinoma (CASG) and whether these two tumors represent separate entities or variants of PLGA (5, 8–11). While LGPA has been used to describe tumors with “more than focal” papillary growth pattern (5, 8, 12), the term cribriform adenocarcinoma (CASG) has been proposed for carcinoma with a predominant cribriform and solid architecture with glomeruloid appearance and optical clear nuclei (9, 10). A few studies have shown that CASGs and LGPAs are associated with higher risk of regional and distant metastases, compared with the classical PLGAs (5, 8–10, 12). Therefore, several groups have proposed to classify LGPA and CASG as entities distinct from PLGA to emphasize their aggressive behaviors (8–10, 13). These tumors were included as variants of PLGA in the WHO classification of 2005 (2) but the controversies remain unresolved and uniform diagnostic criteria are yet to be formed. Since these subtypes appear to have different outcomes and might be difficult to classify histologically, there is a need to identify prognostic histologic factors within the entire spectrum of PLGA/CASG. Toward that goal, we performed a meticulous histopathologic and clinical analysis of a large retrospective cohort of patients treated and followed at a single institution.

Material and Methods

Inclusion criteria, histologic definitions and classification

After obtaining Institutional Review Board approval, the pathology database was searched for cases of PLGA, papillary adenocarcinoma, or cribriform adenocarcinoma diagnosed at Memorial Sloan-Kettering Cancer Center (MSKCC, New York, NY, United States) between 1987 and 2015. A total of 93 surgical specimens from 69 patients with adequate materials (i.e. resections or large incisional biopsies) were included in the present study. A median of

6 tumor sections (range: 1 to 17) were studied per primary tumor. These tumors were subsequently subjected to detailed histopathologic analyses by two pathologists with special interest in salivary gland neoplasia (NK, BX) and were further classified as one of the following categories: 1) PLGA; 2) CASG; 3) tumors with indeterminate features (TIF); and 4) tumors with a predominant papillary pattern (TPPP). PLGA and CASG were defined as described in the World Health Organization of 2005 (2) and in the prior publications of Skalova and Michal (9, 10, 14). In Brief, PLGA was characterized as an infiltrative carcinoma with architectural diversity, in particular short fascicles and single files in a targetoid arrangement (2). CASG displayed architectural uniformity with a predominant cribriform and solid growth patterns, sometimes demonstrating peripheral palisading, peripheral clefting and glomeruloid appearance (9). Tumors with indeterminate features displayed significant areas of typical of PLGA and CASG. Lastly, tumors containing a large component (50%) of papillary architecture were classified as tumors with predominant papillary pattern (Figure 1). All studied tumors exhibited cytological uniformity with pale vesicular nuclei resembling those of papillary thyroid carcinoma. Subsequently, discrepant cases were re-reviewed to reach a consensus diagnosis. Interobserver agreement analysis was performed between three head and neck pathologists (RG, NK, BX), as well as between each individual pathologist and the consensus diagnosis.

Pathology and immunohistochemical review

The following histomorphologic features of the primary tumors were reviewed and documented: tumor size, mitotic index, atypical mitosis, percentage of optical clear nuclei, tumor necrosis, apoptotic bodies, invasive tumor front (infiltrative vs. pushing border), involved structures (lamina propria, skeletal muscle, or bone), margin status, percentage of different architectural patterns, lymphovascular invasion (LVI), and perineural invasion (PNI). Lastly, if a neck dissection was performed at the time of surgery, lymph node metastasis, including the number and size of involved nodes was recorded. Mitotic index was determined by counting 10 high-power fields (HPFs, 400X, total field size 2.4 mm²) with an Olympus microscope (U-DO model BX41, Olympus America Inc., Center Valley, PA, United States) in the areas of highest concentration of mitotic figures. Microscopic margins were categorized as positive (tumor at the inked margin), close (tumor within 1mm from the inked margin), or negative (tumor > 1mm from the inked margin). The architectural patterns of the primary tumor were defined as follows (Figure 2): (1) single filing: in which the tumor cells were arranged as single columns, often surrounding nerves and blood vessels in a targetoid appearance; (2) tubular: in which tumor cells formed round or elongated tubules with definite lumen formation; (3) trabecular: in which tumor cells were arranged as a mixture of thin and thick elongated trabeculae; (4) anastomosing reticular-trabecular (“reticular”): in which the tumor exhibited thin and thick anastomosing cords often in a background of myxoid stroma; (5) solid: in which tumor cells formed large solid sheets; (6) papillary: in which papillae with or without central fibrovascular cores were present; and (7) cribriform: in which back to back cylindrical structures contained eosinophilic extracellular matrix and resembled the cribriform pattern of adenoid cystic carcinoma.

Immunohistochemical stains for p63, p40, MIB-1, and S100 were performed using the Ventana system according to the manufacturer recommendations (Ventana Medical Systems

Inc., Tucson, AZ, United States). Forty-six cases were stained for p40 and p63 while 48 for MIB-1 and 16 for S100. The antibodies clones and dilutions were as follows: MIB-1 (Ventana rabbit monoclonal antibody clone 30-9, dilution 1: 200), p40 (Millipore rabbit polyclonal antibody PC373, dilution 1: 3000), p63 (Ventana prediluted mouse monoclonal antibody 4A4), and S100 (DAKO rabbit polyclonal antibody Z0311, dilution 1:8000).

Clinical review

The patients' charts were reviewed for age at diagnosis, gender, type of surgery (incisional biopsy vs. resection), site of primary tumor, recurrence, and adjuvant therapies. Follow up data were available on 60 patients. Recurrence was determined on the basis of clinical, radiologic and pathologic examination, and was further classified as local recurrence, regional recurrence to cervical lymph nodes, and distant metastasis.

Statistics

All statistical analyses were performed using the SPSS software 22.0 (IBM Corporation, New York, NY, U.S.). Clinico-pathologic characteristics were compared between different types of tumors, namely CASG, PLGA, TIF and TPPP, using appropriate statistical tests, i.e. Chi-square test or Fisher's exact test for nonparametric variables and one-way analysis of variance (ANOVA) for continuous variables. Disease free survival (DFS) was calculated from the date of surgery to the date of first recurrence. The cutoff values of the amount of architectural patterns and mitotic index were calculated based on receiver operating characteristic (ROC) curves and log rank test to determine the best threshold to predict DFS. The values that generated the most significant p values were used in the subsequent analyses. Prognostic value of each clinico-pathologic feature was determined using Log rank test and DFS. Prognostic variables that were significant on univariate analyses were subsequently subjected to multivariate analyses using the Cox proportional hazards model. Interobserver agreement was assessed using kappa statistics. P values less than 0.05 were considered to be statistically significant.

Results

Clinicopathologic characteristics of the study cohort

The clinicopathologic features of the primary tumors are summarized in Table 1. The average age at diagnosis was 60 years (standard error of mean = 2 years, range 22 to 84 years). There was a female predominance with a female to male ratio of 2:1. The majority of tumors (n=65, 94%) originated from the minor salivary glands of the oral cavity or sinonasal tract, while the remaining (n=4, 6%) affected the major salivary gland (parotid gland). The most frequent site of origin was palate (n=39, 57%) followed by base of tongue (BOT, n=10, 14%). The average tumor size was 2.1 cm (standard error of mean SEM = 0.2 cm). Thirty-three (n=33, 48%) of tumors were limited to lamina propria, while the remainder invaded skeletal muscle (n=20, 29%) and/or bone (n=16, 23%). Among the 18 patients who underwent neck dissection at the time of initial surgery, 10 (56%) harbored metastases to cervical lymph nodes.

Histologically, all tumors demonstrated invasive border, in the form of infiltrative islands (97%) or pushing nodules (3%). Chromatin clearing was variably present in all tumors, involving an average of 64% of cells per tumor. Perineural invasion was a common finding in our cohort, present in 41 (59%) of tumors. The median number of PNI foci was 2 (range 1 to 42). The number and percentage of cases demonstrating lymphovascular invasion, positive surgical margin, tumor necrosis, atypical mitosis, and apoptosis were as follows: 4 (6%), 16 (25%), 8 (12%), 4 (6%) and 34 (49%) (Table 1). The average mitotic index of these tumors was 3 per 10 HPFs (range: 0 to 14/10 HPFs). Seventeen tumors (25%) demonstrated elevated mitotic rate of $\geq 5/10$ HPFs. Among the 48 tumors in which MIB-1 IHC were performed, four carcinomas (8%) exhibited elevated MIB-1 proliferation index of $\geq 10\%$, while the remaining 44 had a low proliferation rate of $< 10\%$.

Tumor subclassification and associated clinico-histologic characteristics

After consensus review, 23 cases (33%) were classified as PLGA, 21 (30%) as CASG, 22 (32%) as TIF and 3 (4%) as TPPP. Kappa statistics showed a substantial interobserver agreement between the individual pathologists (Kappa = 0.607, 95% confidence interval 0.516 to 0.699) (Table 2). A substantial interobserver agreement was achieved for the individual diagnosis of PLGA, CASG and TPPP (kappa = 0.677, 0.691, and 0.704 respectively), while the category of TIF generated significant inter-observer discordance with only a fair to moderate agreement among pathologists (kappa = 0.402). The kappa values between the diagnoses of each individual pathologist and the consensus diagnoses ranged from 0.552 to 0.937 (Table 2).

There was no significant difference among different tumor types (PLGA, CASG, TIF, and TPPP) in terms of age at presentation, gender, tumor size, surgical margin status, involvement of cervical lymph nodes, tumor borders, presence of LVI, and atypical mitosis (Table 1, Chi-square test or one-way ANOVA, $p > 0.05$). Compared with PLGA, CASG displayed higher rate of skeletal muscle and bone invasion ($p = 0.045$), higher mitotic index ($p = 0.029$), lower rate of PNI ($p = 0.045$), higher association with tumor necrosis ($p = 0.007$) and apoptosis ($p = 0.042$), and a significant higher association with BOT location ($p = 0.014$). CASG also showed higher percentage of tumor cells with chromatin clearing ($70 \pm 6\%$) in comparison to PLGA ($56 \pm 5\%$). However, the extent of chromatin clearing did not reach a significant level among the various tumor subtypes (PLGA, CASG, TIF, and TPPP) ($p = 0.057$).

CASGs were associated with a significantly higher percentage of papillary, solid, and cribriform patterns; while PLGAs contained a higher proportion of reticular, tubular, and single file patterns ($p < 0.05$) (Table 1). In CASG, the most common architectural pattern was solid in 33%, cribriform in 24%, and papillary in 19% (Figure 3), while the architectural patterns in PLGAs were more evenly distributed across different recognizable patterns.

By immunohistochemistry, there was no difference in staining pattern among different tumor types (PLGA, CA, TIF, and TPPP, Table 1). S100 was universally positive in all 16 tumors tested, including two PLGA, six TIF, seven CASG, and one TPPP. P63 and p40 immunohistochemical stains were performed on 46 randomly selected cases to verify a recent reported observation of positive p63 and negative p40 staining in PLGA. The majority

of tumors were found to be negative for p40 (44, 96%) and positive for p63 (36, 78%). However, ten cases (22%) were completely negative for p63 and two were focally positive for p40 (4%). The MIB-1 proliferation index did not differ significantly among different tumor types ($p = 0.120$).

Predictors of adverse outcomes

Follow up was available for 60 patients. The median follow up was 46 months (mean: 74 months, range: 1 to 295 months). Recurrences were identified as follows: three (5%) local recurrences, two (3%) regional recurrences to cervical lymph nodes and two (3%) distant metastases. Recurrence rate according to histologic subtype was as follows: PLGA 1/17 (6%), TIF 0/22 (0%), TPPP 1/3 (33%), and CASG 5/18 (27%) (Table 1). One patient died of her disease. This patient was a 25-year-old female at diagnosis who had CASG of soft palate with metastases to the bone and lung at presentation. The time from presentation to her death was 9.3 years. The second patient with distant metastasis was a 70-year-old female who had CASG of soft palate with metastasis to the lung one year after the initial surgery. She was alive with disease 3.7 years after the initial diagnosis.

Factors associated with adverse clinical outcome (i.e. disease free survival) included large tumor size (Log rank test, $p = 0.014$), invasion into skeletal muscle and/or bone ($p = 0.006$), presence of LVI ($p = 0.005$), tumor necrosis ($p = 0.018$), 10% of papillary architecture ($p = 0.004$), 30% of cribriform pattern ($p = 0.005$) and 50% of cribriform and solid patterns ($p = 0.020$, Table 3). The Kaplan Meier plots for disease free survival stratified by the above mentioned factors are shown in Figure 4.

Although CASG and TPPP appeared to associate with worse prognosis on Kaplan Maier plot compared to TIF and PLGA, Log rank test failed to demonstrate a significant association between overall tumor classification and DFS ($p = 0.103$, Figure 4A).

Moreover, we compared the prognostic value of different percentage cut-off values of papillary, cribriform, and solid patterns. A cut-off of 10% or more papillary architecture seemed to correlate the best with disease free survival compared to 20% or 30% (10%: $p = 0.004$; 20%: $p = 0.097$, 30%: $p = 0.045$). A cut-off of 30% cribriform pattern was associated with DFS ($p = 0.005$), while 10% did not predict DFS ($p = 0.881$). The proportion of solid pattern at different percentages did not predict outcome ($p > 0.05$). Tumor with 10% or more papillary architectures were associated with a mitotic index of 4/10 HPFs, a MIB-1 proliferation index of 10%, and higher frequency of tumor necrosis (Fisher's exact test, $p < 0.001$, $p < 0.001$, $p = 0.001$, and $p = 0.022$ respectively). The presence of 30% cribriform architecture did not correlated with mitotic index, MIB-1 index, or tumor necrosis ($p = 0.281$).

On multivariate analysis using Cox proportional hazards model, the presence of 10% papillary and 30% cribriform patterns independently predicted disease free survival (10% papillary: hazard ratio HR = 24.7, 95% confidence interval = 1.1 – 570.1, $p = 0.045$; and 30% cribriform: HR = 12.2, 95% confidence interval = 1.0 – 148.2, $p = 0.049$), while tumor size, bone invasion, tumor necrosis, and LVI failed to reach significance ($p = 0.402$, 0.119, 0.755 and 0.993 respectively). Six of 23 (26%) tumors with 10% papillary features

and three of eight (38%) with 30% cribriform pattern developed recurrence while only one of 37 (3%) tumor with <10 %papillary pattern and four of 52 (8%) with <30% cribriform pattern recurred (Table 3). The patient who died from her disease harbored a CASG showing >30% cribriform pattern.

Discussion

In general, PLGA is considered as a low grade epithelial malignancy with a low risk of local and regional recurrence but negligible risk for distant metastases (1). The reported frequency of locoregional recurrence is up to 33% (6). The rate of cervical lymph node metastases (at presentation or as a regional recurrence) ranges from 13 to 17% (5, 6). In our cohort, the frequency of lymph node metastasis was 56% among 18 patients who had lymph node dissection and 14% overall. The high rate of lymph node metastasis among patients who underwent neck dissection might reflect selection bias as neck dissection was a selective surgical procedure for patients with suspicious neck lymph nodes.

Distant metastasis, although rare, has been reported in PLGA with an incidence of 0.6% (7) to 7.5% (5). The reported sites of metastasis include lung, bone, skin and subcutaneous tissue (5, 7, 8). In the largest reported study on PLGA to date which was published in 1999 by the Armed Forces Institute of Pathology (AFIP) encompassing 164 cases, one patient (0.6%) developed distant metastasis to lung, 10.9 years after the initial resection and three patients (2%) died of their disease with an average survival of 71.5 months. The above data is in accordance with the overall rates of lymph node and distant metastasis found in the current series (17% and 3%, respectively). In the AFIP study, the disease specific survival was reported as 98% (7). In our study, one patient died of her disease, 9.3 years after the initial diagnosis. This is the seventh reported death in PLGA/CASG and the first to be related to distant metastasis. The other six documented deaths were reported by Castle et al. and Evans et al. and they were due to uncontrollable local recurrence (5, 7).

Despite its general perception as low grade as its term implies, PLGA does not always behave as a low grade malignancy. Indeed, it is associated with a non-negligible risk for regional disease, a small risk for distant metastasis, and even death of disease. As shown in this study, elective neck dissection and post-operative long term surveillance are necessary in some instances. Therefore, in our opinion, the term “polymorphous adenocarcinoma” might be a better term to reflect the spectrum of tumor behavior than the term “polymorphous low grade adenocarcinoma”.

Although PLGA was described as an entity more than 40 years ago (1), its prognostic factors are still not well delineated, and to date, only three published studies have utilized univariate survival analysis to investigate the potential prognostic factors in PLGA (5, 6). The first reported study by Evans et al. failed to identified any significant clinical or pathologic predictor for overall survival in 40 patients with PLGA (5). In the second study, Seethala et al. have found that tumor site is the only significant determinant of disease free survival, whereas in the third national Danish study, Elhakim et al. have suggested that negative resection margins can improve disease free survival significantly (6, 15). Despite of the relatively low statistical power and limited number of recurrences, in contrast to the prior

studies, we were able to perform both univariate and multivariate analysis in a large cohort of patients with long term clinical follow up. On univariate analysis, tumor size, bone invasion, tumor necrosis, and LVI were significant prognostic elements. However, only two variables (10% papillary and 30% cribriform growth pattern) were shown to independently predict shorter disease free survival by multivariate analysis. In view of these findings, it is important to recognize and document the presence and percentage of papillary and cribriform patterns in the surgical pathology report. However, it probably will take efforts to widely incorporate a relatively subjective histologic feature into the routine practice. Perhaps, similar to solid growth in adenoid cystic carcinoma, it is the presence and amount of papillary and cribriform patterns that drive outcome in the PLGA spectrum rather than the subtype of these tumors.

When the diagnosis of PLGA is histologically challenging, immunohistochemistry can be helpful. A recent study by Rooper et al. has suggested the utility of a combined p63/p40 immunohistochemical stains in diagnosing PLGA (16). The authors have reported a consistent positive staining for p63 and negative staining for p40 in 11 PLGAs. This staining pattern has been observed to be distinctly different from the staining of adenoid cystic carcinoma which is typically positive for both p63 and p40. To verify this observation, we stained a larger number of PLGAs/CAMGs (n=46) for p63 and p40. Similar to the prior study, we found that the majority of tumors were positive for p63 and negative for p40. However, 22% of tumors were completely negative for p63 and 4% were focally positive for p40. Such differences in immunophenotype might be in part related to the different antibodies utilized in these studies. While Rooper et al. used monoclonal p63 and p40 antibodies (BioCare Medical), the p63 and p40 antibodies from the current study were polyclonal (Ventana Medical Systems Inc. and Millipore EMD Life Science). Nevertheless, our results suggest that PLGA/CASG spectrum of tumors can exhibit a variable p63/p40 immunostaining and that immunostaining results should be interpreted with caution and in the context of tumor morphology.

Ongoing controversies exist regarding cribriform adenocarcinoma of salivary gland and PLGA with significant papillary architecture or low grade papillary adenocarcinoma. The dispute centers on whether these two tumors represent discrete separate entities or variants of PLGA (5, 8–10). The WHO classification of 2005 considers CASG as a variant of PLGA (2). However, several groups have proposed to classify LGPA and CASG as entities distinct from PLGA to emphasize their more aggressive behaviors and higher risk of regional and distant metastases (8–10, 13). As focal papillary, cribriform, or solid growth patterns have been described and accepted in an otherwise classical PLGA (5, 7, 12, 14, 17), it can be challenging in some instances to separate these three tumors apart histologically (14).

Low grade papillary adenocarcinoma (LGPA) was first described by Allen et al. in 1974 and subsequently by Mills et al in 1984 (11, 18). Shootweg et al., in 1987, have compared the clinical outcomes of seven LGPA and eight PLGA and have suggested that LGPAs are more frequently associated with regional metastasis (19). In 2000, Evans and Luna have studied 40 cases of PLGA and have concluded that “more than focal” papillary area in an otherwise typical PLGA is associated with high risk of lymph node metastasis (5). Nevertheless, the precise amount of “more than focal papillary area” was not reported in their study.

Additionally, few prior studies have advocated using the term “LGPA” for tumors with histologic features of PLGA and papillary architecture, showing a higher rate of local recurrence (38 – 45%) and regional metastasis (25–35%) in these tumors compared to typical PLGA (23% and 10% respectively) (8, 19). Although a study by Hunter et al. has proposed 10% as a cutoff for significant papillary architecture in PLGA, this was arbitrary proposed and was not supported by a statistical analysis. Thus a precise threshold of significant papillary growth in PLGA has not been established to date (8). In our hands, 10% or more papillary architecture seemed to be a significant independent predictor for disease free survival. We therefore recommend that the percentage of papillary architecture be documented in these tumors.

Cribriform adenocarcinoma was first described as cribriform adenocarcinoma of tongue (CAT) in 1999 by Michal et al. This tumor was felt to be discrete in that it usually arose in the base of tongue, had lymph node metastasis at time of presentation, and was characterized by predominant cribriform and solid growth patterns and optically clear nuclei (20). In 2011, the same authors have subsequently published a larger series of 23 tumors, including tumors with same morphologic features in other minor salivary gland sites. Hence, the entity has been named as cribriform adenocarcinoma of minor salivary gland (CAMSG) (9). Thirty-one cases of cribriform adenocarcinoma have been published to date, all of which arose in the minor salivary gland of oral cavity and oropharynx (10). Our results showed that this tumor is not limited to minor salivary gland and can occur in major salivary gland as well. Therefore, the term “cribriform adenocarcinoma of salivary glands (CASG)” might be a better term to describe this tumor.

Although several authors have argued that CASG is histologically distinct from PLGA (9, 10, 20), a recent study has shown a significant proportion (about 35%) of the tumors on the PLGA spectrum exhibiting mixed morphology between PLGA and CASG (14). Our study further supports such findings as we found that about a third of the cases harbor indeterminate morphology between PLGA and CASG. Although, a substantial inter-observer concordance was identified among individual pathologists in our entire cohort of cases, tumors with indeterminate features (TIF) imposed a diagnostic challenge with only fair to moderate inter-observer agreement among expert pathologists. Taken together, although CASG displays a distinct morphology, it shows a morphologic overlap with PLGA, which may create difficulties in classifying this tumor.

Furthermore, recent studies have reported characteristic molecular alterations in PLGA/ CASG spectrum of tumors (14, 21, 22). Fusions involving *Protein Kinase D1 (PRKD1)*, *PRKD2* or *PRKD3* loci have been detected in 80% of CASG, 45% of TIF and 6% of PLGA (14). *PRKD1* hotspot somatic mutation encoding p.Glu710Asp is reported in 73% of PLGA (21). The fact that PLGA and CASG have alterations affecting the same gene locus and that *PRKD1/2/3* fusion can be seen in PLGA, TIF and CASG suggest that these tumors might also have overlapping molecular signatures. Additional studies are needed to better classify these tumors, especially those with unusual or indeterminate features, at the molecular level.

When correlating tumor classification with clinicopathologic features and disease free survival, TPPP seemed to cluster with CASG. In comparison with PLGAs and TIFs, CASGs

and TPPPs were more frequently seen in BOT, had a higher rate of bone invasion, a higher mitotic rate, and a higher percentage of tumor necrosis. However, CASG as defined in the literature does not seem to correlate with worse outcome. Interestingly, we have demonstrated that PLGA and TIF were associated with higher incidence of PNI (65% and 77% respectively) compared to CASG and TPPP (38% and 33% respectively), which may suggest that PLGA and TIF are more neurotropic. Despite the above correlation between TPPP and CASG as a group and aggressive clinico-pathologic features, individual histologic entities do not seem to predict recurrence. Rather it is the amount of papillary and cribriform areas (10% papillary and 30% cribriform growth pattern) that guides outcome in an independent manner. In view of these findings, the percentage of papillary and cribriform areas should be mentioned in the pathology report since these two features rather than tumor subtyping seem to drive the outcome in the spectrum of PLGA.

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Figure 1. Histologic features of the polymorphous low grade adenocarcinoma (PLGA)/cribriform adenocarcinoma of salivary gland (CASG)

(A, B): A typical PLGA showing different patterns (single files, tubules, and trabeculae) with a concentric targetoid fashion around the nerves (N) in B. (C, D): CASG is composed of large tumor nodules with solid/cribriform patterns. Tumor necrosis is noted in D (arrow heads). (E, F): Carcinoma with predominant papillary pattern.

Figure 2. Architectural diversity in PLGA/CASG (A–H)

(A): Single files in which tumor cells were arranged as single strands, often concentrically around blood vessels with a targetoid appearance. (B): Tubular pattern with luminal formations. (C): Thin and thick elongated trabeculae (trabecular pattern). (D): Reticular pattern showing anastomosing cords, tubules, and trabeculae in a background of myxoid stroma. (E): Solid pattern. (F): Papillary pattern. (G): Cribriform architecture containing abundant amorphous globules of eosinophilic extracellular matrix. (H): Tumor cells

exhibiting uniform cytologic features in different patterns with vesicular oval nuclei, marked chromatin clearing, inconspicuous or small nuclei, and occasional nuclear grooves.

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Figure 3. A cribriform adenocarcinoma of the parotid gland (P)

The tumor in the parotid gland showing multi-lobular growth pattern (A), with predominant cribriform pattern and uniform cytology (B).

Figure 4. Kaplan-Meier plots for disease free survival (DFS)

Overall, tumor classification was not a significant prognostic factor (panel A). Univariate analysis using Log rank test showed that tumor necrosis (B), lymphovascular invasion (LVI, C), 10% papillary architecture (D), 30% cribriform architecture (E) and 50% solid and cribriform pattern (F) were significant predictors for the clinical outcome.

Table 1
Clinico-pathologic characteristics of the entire study cohort according to tumor subclassification.

	Total	PLGA	TIF	CASG	TPPP	P ^a
Number of patients	69	23	22	21	3	
Age at diagnosis, years, mean±SEM	60±2	60±3	62±2	61±3	43±11	0.104
Gender ^b						0.657
Female	46 (67%)	15 (65%)	14 (64%)	14 (67%)	3 (100%)	
Male	23 (33%)	8 (35%)	8 (36%)	7 (33%)	0	
Site of primary tumor						0.014
Base of tongue	10 (14%)	0	5 (23%)	5 (24%)	0	
Buccal mucosa	6 (9%)	3 (13%)	2 (9%)	1 (5%)	0	
Oral cavity, NOS	4 (6%)	0	2 (9%)	1 (5%)	1 (33%)	
Palate	39 (57%)	20 (87%)	10 (45%)	8 (38%)	1 (33%)	
Parotid	4 (6%)	0	1 (5%)	2 (10%)	1 (33%)	
Sinonasal	6 (9%)	0	2 (9%)	4 (19%)	0	
Size, cm, mean±SEM	2.1±0.2	1.8±0.3	1.9±0.2	2.6±0.3	2.9±0.6	0.133
Structure(s) invaded						0.045
Lamina propria	33 (48%)	17 (74%)	7 (32%)	9 (43%)	0	
Muscle	20 (29%)	4 (17%)	8 (36%)	7 (33%)	1 (33%)	
Bone invasion	16 (23%)	2 (9%)	7 (32%)	5 (24%)	2 (67%)	
LN status (n = 18)						0.505
Negative	8 (44%)	0	3 (33%)	3 (60%)	2 (67%)	
Positive	10 (56%)	1 (100%)	6 (67%)	2 (40%)	1 (33%)	
Surgical margin (n = 64)						0.619
Negative	33 (52%)	7 (37%)	12 (55%)	13 (65%)	1 (33%)	
Close (< 1mm)	15 (23%)	5 (26%)	6 (27%)	3 (15%)	1 (33%)	
Positive	16 (25%)	7 (37%)	4 (18%)	4 (20%)	1 (33%)	
Tumor border (n = 67)						0.184
Pushing fronds	2 (3%)	0	0	2 (10%)	0	
Infiltrative islands	65 (97%)	23 (100%)	21 (100%)	18 (90%)	3 (100%)	
LVI						0.249
Absence	65 (94%)	23 (100%)	19 (86%)	20 (95%)	3 (100%)	
Presence	4 (6%)	0	3 (14%)	1 (5%)	0	
PNI						0.045
Absence	28 (41%)	8 (35%)	5 (23%)	13 (62%)	2 (67%)	
Presence	41 (59%)	15 (65%)	17 (77%)	8 (38%)	1 (33%)	

		Total	PLGA	TIF	CASG	TPPP	p ^a
Mitosis	1–4/10 HPFs	52 (75%)	22 (96%)	16 (73%)	12 (57%)	2 (67%)	0.029
	5/10 HPFs	17 (25%)	1 (4%)	6 (27%)	9 (43%)	1 (33%)	
Tumor necrosis	Absence	61 (88%)	23 (100%)	19 (86%)	18 (86%)	1 (33%)	0.007
	Presence	8 (12%)	0	3 (14%)	3 (14%)	2 (67%)	
MIB-1 proliferation index (n =48)	10%	44 (92%)	12 (100%)	16 (84%)	14 (100%)	2 (67%)	0.120
	< 10%	4 (8%)	0	3 (16%)	0	1 (33%)	
Atypical mitosis	Absence	65 (94%)	23 (100%)	21 (95%)	19 (90%)	2 (67%)	0.103
	Presence	4 (6%)	0	1 (5%)	2 (10%)	1 (33%)	
Apoptosis	Absence	35 (51%)	16 (70%)	8 (36%)	11 (52%)	0	0.042
	Presence	34 (49%)	7 (30%)	14 (64%)	10 (48%)	3 (100%)	
Cells with chromatin clearing, %, mean±SEM		64±3	56±5	63±5	70±6	90±0	0.057
Solid and Cribriform, %, mean±SEM		34±3	21±4	37±4	50±5	10±8	<0.001
Cribriform, %, mean±SEM		14±2	10±3	14±3	20±5	1±1	0.214
Papillary, %, mean±SEM		12±2	2±1	10±3	14±4	75±10	<0.001
Solid, %, mean±SEM		20±2	10±2	23±4	30±5	9±8	0.002
Reticular, %, mean±SEM		19±2	28±3	20±3	12±3	2±2	0.002
Tubulotrabeacular, %, mean±SEM		18±2	22±4	17±2	16±3	7±2	0.319
Tubular, %, mean±SEM		13±2	21±4	12±2	6±1	7±2	0.002
Single filing, %, mean±SEM		3±1	5±1	4±1	1±1	0	0.011
Papillary	<10%	46 (67%)	21 (91%)	14 (64%)	11 (52%)	0	0.003
	10%	23 (33%)	2 (9%)	8 (36%)	10 (48%)	3 (100%)	
Cribriform	<30%	59 (86%)	22 (96%)	18 (82%)	16 (76%)	3 (100%)	0.248
	30%	10 (14%)	1 (4%)	4 (18%)	5 (4%)	0	
Predominant pattern	Cribriform	10 (14%)	1 (4%)	4 (18%)	5 (24%)	0	<0.001
	Papillary	11 (16%)	0	4 (18%)	4 (19%)	3 (100%)	
	Solid	13 (19%)	1 (4%)	5 (23%)	7 (33%)	0	
	Reticular	18 (26%)	12 (52%)	5 (23%)	1 (5%)	0	

	Total	PLGA	TIF	CASG	TPPP	P ^a
	Tubulotrabeccular	6 (26%)	3 (14%)	4 (19%)	0	
	Tubular	3 (13%)	1 (5%)	0	0	
P63 IHC (n = 46)	Positive	11 (92%)	15 (83%)	7 (54%)	3 (100%)	0.074
	Negative	1 (8%)	3 (17%)	6 (46%)	0	
P40 IHC (n = 46)	Positive	0	2 (11%)	0	0	0.354
	Negative	11 (100%)	16 (89%)	14 (100%)	3 (100%)	
Follow up period, months; median (range)		49 (1–295)	21 (1–212)	96 (10–217)	75 (37–295)	0.150
		53 (88%)	22 (100%)	13 (68%)	2 (67%)	
Disease Status (n = 60)	NED	16 (89%)	22 (100%)	13 (68%)	2 (67%)	0.083
	Local recurrence	0	0	2 (11%)	1 (33%)	
	Regional recurrence	2 (3%)	1 (6%)	1 (5%)	0	
	DM	2 (3%)	0	2 (11%)	0	

CASG: cribriform adenocarcinoma of salivary gland; DM: distant metastasis; DOC/AWD: dead of other cause/alive with disease; IHC: immunohistochemistry; LN: lymph node; LVI: lymphovascular invasion; NED: no evidence of disease; NOS: not otherwise specified; PLGA: polymorphous low grade adenocarcinoma; PNI: perineural invasion; SEM: standard error of mean; TIF: tumors with indeterminate features; TPPP: tumors with predominant papillary pattern.

^aP values were calculated using one-way ANOVA for continuous variables and chi square test for categorical variables, comparing among different subtypes.

^bValues were expressed as N (Percentage of column total) unless otherwise specified.

Table 2

Interobserver agreement in tumor subclassification.

		Kappa	95% Confidence interval
Overall		.607	0.516 – 0.699
Tumor classification	PLGA	.677	0.541 – 0.813
	TIF	.402	0.265 – 0.538
	CASG	.691	0.555 – 0.827
	TPPP	.704	0.568 – 0.841
Individual pathologist ^a	#1	.552	0.393 – 0.710
	#2	.810	0.654 – 0.965
	#3	.937	0.782 – 1.092

^a Kappa value was calculated between the diagnoses of each individual pathologist and the consensus diagnoses.

Table 3

Clinico-pathologic characteristics according to clinical outcomes.

Number of patients		Total		No recurrence		Recurrence		p ^a
		60		53		7		
Age	60 y	24	40.0%	21	39.6%	3	42.9%	0.844
	> 60 y	36	60.0%	32	60.4%	4	57.1%	
Gender	Female	39	65.0%	34	64.2%	5	71.4%	0.769
	Male	21	35.0%	19	35.8%	2	28.6%	
Site of primary tumor	Base of tongue	10	16.7%	9	17.0%	1	14.3%	0.652
	Buccal mucosa	6	10.0%	6	11.3%	0	0.0%	
	Oral cavity, NOS	4	6.7%	4	7.5%	0	0.0%	
	Palate	31	51.7%	26	49.1%	5	71.4%	
	Parotid	4	6.7%	4	7.5%	0	0.0%	
	Sinonasal	5	8.3%	4	7.5%	1	14.3%	
Size	< 2 cm	29	48.3%	28	52.8%	1	14.3%	0.014
	2 cm	31	51.7%	25	47.2%	6	85.7%	
Structure(s) invaded	Lamina propria	26	43.3%	25	47.2%	1	14.3%	0.006
	Muscle	19	31.7%	17	32.1%	2	28.6%	
	Bone invasion	15	25.0%	11	20.8%	4	57.1%	
LN status (n = 18)	Negative	8	44.4%	5	35.7%	3	75.0%	0.463
	Positive	10	55.6%	9	64.3%	1	25.0%	
Surgical margin (n = 58)	Negative	32	55.2%	28	54.9%	4	57.1%	0.650
	Close (< 1mm)	13	22.4%	12	23.5%	1	14.3%	
	Positive	13	22.4%	11	21.6%	2	28.6%	
Tumor border (n = 58)	Pushing	2	3.4%	2	3.9%	0	0.0%	0.811
	Infiltrative	56	96.6%	49	96.1%	7	100.0%	
LVI	Absence	56	93.3%	50	94.3%	6	85.7%	0.005
	Presence	4	6.7%	3	5.7%	1	14.3%	
PNI	Absence	22	36.7%	18	34.0%	4	57.1%	0.554

Number of patients		Total		No recurrence		Recurrence		P a
		60		53		7		
Mitosis	Presence	38	63.3%	35	66.0%	3	42.9%	
	< 5/10 HPFs	43	71.7%	38	71.7%	5	71.4%	0.496
	5/10 HPFs	17	28.3%	15	28.3%	2	28.6%	
Tumor necrosis	Absence	52	86.7%	48	90.6%	4	57.1%	0.018
	Presence	8	13.3%	5	9.4%	3	42.9%	
Atypical mitosis	Absence	56	93.3%	50	94.3%	6	85.7%	0.587
	Presence	4	6.7%	3	5.7%	1	14.3%	
Apoptosis	Absence	28	46.7%	25	47.2%	3	42.9%	0.480
	Presence	32	53.3%	28	52.8%	4	57.1%	
Tumor classification	PLGA	17	28.3%	16	30.2%	1	14.3%	0.103
	TIF	22	36.7%	22	41.5%	0	0.0%	
	CAMG	18	30.0%	13	24.5%	5	71.4%	
	TPPP	3	5.0%	2	3.8%	1	14.3%	
Solid & cribriform	<50%	43	71.7%	41	77.4%	2	28.6%	0.020
	50%	17	28.3%	12	22.6%	5	71.4%	
Papillary	<10%	37	61.7%	36	67.9%	1	14.3%	0.004
	10%	23	38.3%	17	32.1%	6	85.7%	
Cribriform	<30%	52	86.7%	48	90.6%	4	57.1%	0.005
	30%	8	13.3%	5	9.4%	3	42.9%	
Solid	<10%	14	23.3%	11	20.8%	3	42.9%	0.299
	10%	46	76.7%	42	79.2%	4	57.1%	
Predominant pattern	Cribriform	8	13.3%	5	9.4%	3	42.9%	0.091
	Papillary	11	18.3%	9	17.0%	2	28.6%	
	Solid	12	20.0%	11	20.8%	1	14.3%	
	Reticular	16	26.7%	16	30.2%	0	0.0%	
	Tubulotrabeular	11	18.3%	10	18.9%	1	14.3%	
	Tubular	2	3.3%	2	3.8%	0	0.0%	

^aP values were calculated using disease free survival (DFS) and Log rank test.

Values were expressed as N (percentage of column total).

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