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Original Article

Morphological heterogeneity of oral salivary gland carcinomas: A clinicopathologic study of 41 cases with long term follow-up emphasizing the overlapping spectrum of adenoid cystic carcinoma and polymorphous low-grade adenocarcinoma

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Abstract: We analyzed 41 oral salivary gland carcinomas from consecutive 290 salivary gland carcinoma database (14%) with emphasis on the histological spectrum and clinical outcome of adenoid cystic carcinoma (ACC) and polymorphous low-grade adenocarcinoma (PLGA). The cohort included 14 ACCs, 14 mucoepidermoid carcinomas (MECs), 8 PLGAs, 3 adenocarcinomas, not otherwise specified and 2 acinic cell carcinomas. Mean age was 48, 58 and 61 yrs for ACC, MEC and PLGA, respectively. Eight patients (19.5%) died of tumor at a mean interval of 66.5 months. ACC and PLGA showed similar mean age, gender distribution, predominant palatal localization, nodal metastasis, perineural invasion and MIB-1 index. However, ACC tended to show higher tumor stage and residual tumor (R1/R2) more frequently than PLGA, but this was statistically not significant. ACC and PLGA showed overlapping architectural patterns. However, ACCs displayed well organized basal-luminal differentiation, highlighted by CK5/CK7 immunostaining. In contrast, PLGA showed a disorganized histological and immunohistological pattern. C-Kit expression (CD117) was common in ACC, generally mirroring that of CK7 and virtually lacking in PLGA. Kaplan-Meier analysis demonstrated a similar clinical course for ACC and PLGA with 5 years survivals of 87% and 80%, respectively. Fluorescence in situ hybridization (FISH) performed on all 290 salivary carcinomas confirmed the specificity of the translocation t (11;19) for MEC and its absence in all other carcinomas including ACC and PLGA. Our results emphasize the diversity of oral salivary gland carcinomas and the overlapping clinicopathological features of ACC and PLGA.

Keywords: Oral salivary gland carcinoma; adenoid cystic carcinoma, polymorphous low-grade adenocarcinoma; acinic cell carcinoma.

Introduction

Malignant tumors of oral minor salivary gland origin are rare, but constitute an important area in the field of oral pathology as they are mostly considered to behave clinically better than squamous cell carcinoma, the most frequent malignant tumor of the oral cavity [1]. Oral salivary carcinomas account for 2-3% of all malignant neoplasms of the upper aerodigestive tract and up to 20% of all salivary gland tumors [2]. Mucoepidermoid carcinoma (MEC) represented the most common oral carcinoma of salivary gland type in previous series (50%), followed by adenoid cystic carcinoma (ACC, 25%) and polymorphous low-grade adenocarcinoma (PLGA, 20%), whereas acinic cell carcinomas, adenocarcinomas, not otherwise specified and myoepithelial carcinomas were rare [3-7]. In particular, only a few anecdotal case reports exist on acinic cell carcinomas of oral minor salivary gland origin [8-10].

Following its initial recognition in 1983 by Freedman and Lumerman [11] and Batsakis et al. [12] as “lobular carcinoma” and “terminal

duct carcinoma", respectively, the term "polymorphous low-grade adenocarcinoma" was introduced by Evans and Batsakis in 1984 [13] and represents the current nomenclature in the WHO series [14]. PLGA has received much attention in the recent literature with most studies focusing on features that may help to differentiate PLGA from ACC as both entities share an intercalated duct origin and are morphologically characterized by a variety of growth patterns [15, 16]. Architectural differences could be demonstrated using immunohistochemical staining against various cytokeratins of luminal or basal type as well as against structures of the epithelial-mesenchymal interface, e.g. collagen IV, laminin, and integrin ligands [17-19], often with conflicting results [20] (reviewed in Darling et al., 2002 [21]). A recently published hierarchical cluster analysis revealed a distinction of both entities based on the expression of basal cell markers [22] although the application of one basal cell marker (p63) alone was not sufficient to differentiate these entities [23]. Other markers studied comprise galectin-3, beta-catenin, cyclin D1, and CD43, mostly with equivocal results [24, 25]. A comprehensive analysis of the literature has been published by Beltran et al. [26]. One of the most reliable markers distinguishing ACC and PLGA is c-Kit (CD117). As demonstrated by several authors [27-29] c-Kit is consistently expressed in ACC and mostly negative in PLGA. In addition to its diagnostic value, c-Kit was found to have a prognostic relevance in a subset of salivary gland carcinomas [30].

Due to the rarity of PLGA only one large clinicopathological study comprising 40 cases with long-term follow up has been published to date [31]. In that study, all patients survived a period of 10 years, apparently doing better than patients with ACC, although a study comparing the clinical course of patients with these two tumors is still lacking.

The objective of the present study was to correlate the clinical course of 41 oral salivary gland carcinomas with the histologic tumor type and to evaluate the morphological diversity of these tumors. Here, we focused on the similarities and differences in the architectural and immunohistochemical staining patterns for basal cytokeratin (CK5) and luminal cytokeratin (CK7) in PLGA and ACC as well as on the relevance of c-Kit and MIB-1 in the distinction of these two entities.

Material and methods

Patients and tumor samples

Forty-one salivary gland carcinomas were retrieved from a consecutive collection of well characterized primary salivary gland carcinomas of both major and minor salivary glands (total n=290) with complete follow-up treated at the Universities of Erlangen and Regensburg, Germany, and at the Nuremberg Hospital, Germany, between 1990 and 2007. Clinical information was obtained from the clinical tumor registries of Erlangen-Nuremberg and Regensburg as well as from the salivary gland carcinoma registry of Erlangen. The registries and the related translational research activities are covered by ethical vota of the medical faculties of the Universities of Regensburg and Erlangen-Nuremberg. All patients underwent surgery of the primary tumor, complemented by neck dissection in cases with suspicion of lymph node metastasis and/or high grade morphology. Radiotherapy was applied to patients with advanced tumor stage and/or presence of residual tumor (R1).

Histology and classification

On the basis of Hematoxylin and Eosin (H&E) and periodic acid-Schiff (PAS) stained slides from at least subtotally embedded tumors, all of the 41 tumors have been independently reviewed by two pathologists experienced in salivary gland tumor pathology (S.S. & A.A.) without knowledge of initial histological diagnosis or clinical outcome. All carcinomas were diagnosed according to the well established WHO criteria [14, 32-35]. The study cohort comprised 14 ACCs, 14 MECs, 8 PLGAs, 3 high-grade adenocarcinomas, not otherwise specified and 2 acinic cell carcinomas. The histological and the clinicopathological features of the 14 MECs have been included in a recent study on MEC of both major and minor salivary gland origin [36] and are hence not further analyzed in the current study. Due to the infrequency of acinic cell carcinomas (n=2) and adenocarcinoma, not otherwise specified (n=3), these cases were included to complete the morphological spectrum, but have been excluded from further analysis within this study.

Pattern analysis of adenoid cystic carcinoma and polymorphous low-grade adenocarcinoma

To evaluate the morphologic heterogeneity of

ACC and PLGA eight architectural patterns were assessed (for detailed description, see result section and **Figure 1**): cribriform, tubular, solid-basophilic, solid-clear cell, trabecular, reticular (corded), invasive lobular carcinoma (ILC)-like, and papillary pattern. The quantities of the patterns of each tumor were evaluated. For the purpose of this study, arbitrary cut-off values of ≥10% and <10% were used to define major and minor patterns respectively. A major tumor pattern comprising ≥50% of the tumor was considered a predominant pattern. Additionally, the presence of perineural invasion was noted. As a reliable grading system for ACC and PLGA does not exist so far, all tumors were classified low-grade, unless dedifferentiated areas indicating a high-grade tumor were present. Immunohistochemical staining for CK5 and CK7 was applied to characterize and quantify basal and luminal differentiations, respectively. A so-called "inverted epithelial-myoepithelial pattern" was applied in this study if basally differentiated (CK5-positive) cells were located centrally surrounding open lumina. CK5 antibody was purchased from Zytomed systems Ltd., Berlin, Germany, CK7 antibody from DCS Innovative Diagnostik-Systeme Dr. Christian Sartori Ltd., Hamburg, Germany. Immunostaining was performed on 5 µm sections according to the manufacturer's instructions. Antibodies against c-Kit (CD117) and MIB-1 (Ki-67) were from DAKO Deutschland Ltd., Hamburg, Germany, and applied according to the manufacturer's instructions.

For molecular analysis, fluorescence *in situ* hybridization (FISH) was performed on 5 µm sections of the TMAs encompassing the 290 primary salivary carcinomas using commercially available, directly labeled DNA break-apart probes to detect the translocation t(11;19) (ZytoVision Ltd., Bremerhaven, Germany). FISH scoring was performed by counting fluorescence signals in 50 malignant, non-overlapping cell nuclei for each case by two independent investigators (S.S., M.M.). A tumor was considered positive if >50% of the cells harbored the translocation.

Statistical analyses

All clinicopathologic data were analyzed with SPSS for Windows, version 15.0 (SPSS, Erkrath, Germany). Overall survival (= primary outcome measure) was calculated as the time from the date of diagnosis to death from any cause or the date the patient was last known to be alive. Patients lost to follow-up were treated as censored cases based on the date they were last known to be alive. Survival curves were generated using the Kaplan-Meier method, and log-rank tests compared the distributions between groups. Here, the follow-up period was limited to 120 months. The results of the MIB-1 and c-Kit staining were visualized by box-plot analyses. The significance of mean differences was evaluated by double-sided t-test. Fisher exact test was applied to contingency tables irrespective of the number of expected cases per cell.

Results

Frequency and clinical features of the different types of oral salivary gland carcinomas

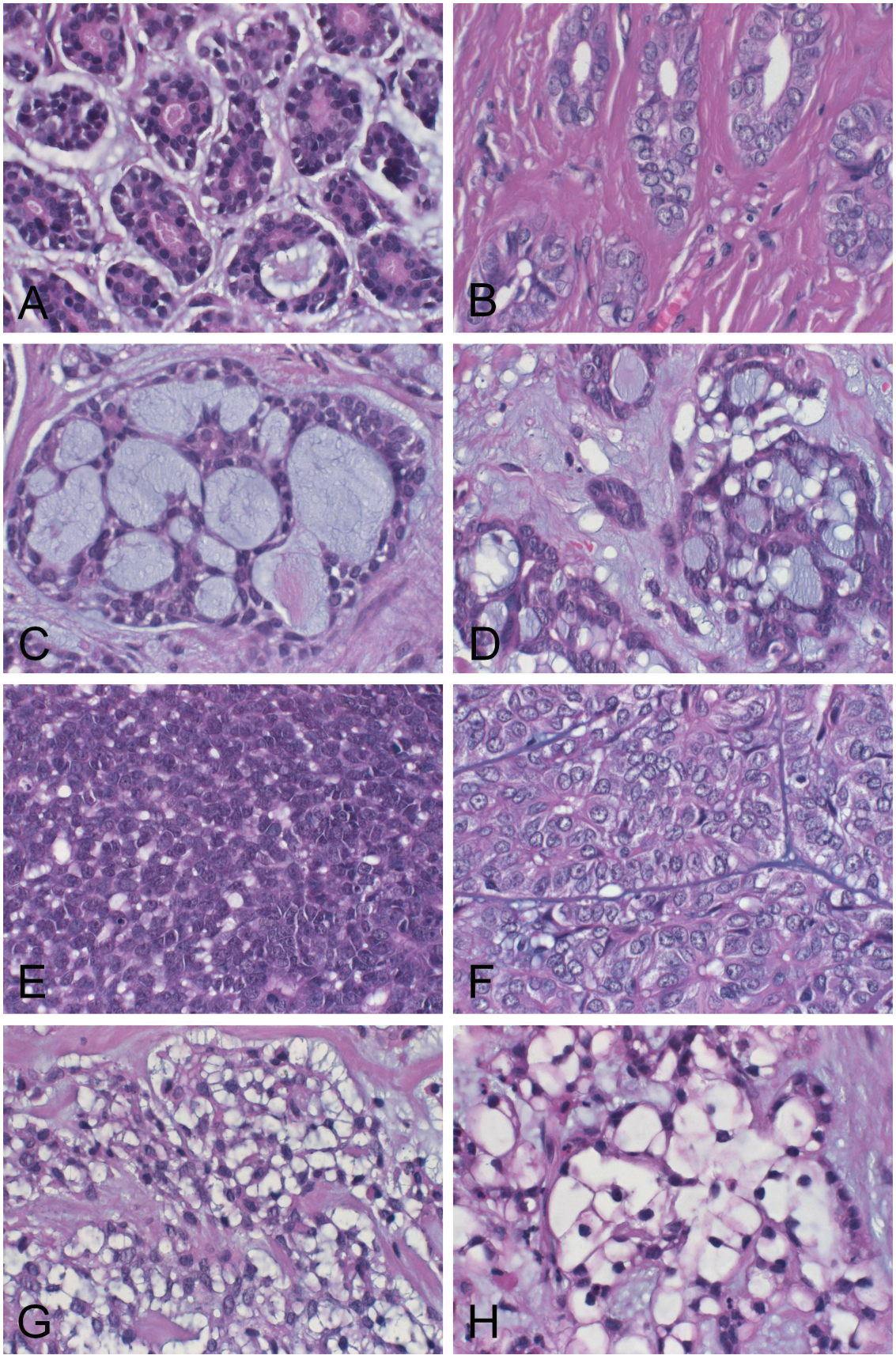
The 41 tumors were classified into ACC (n=14), MEC (n=14), PLGA (n=8), high-grade adenocarcinoma, not otherwise specified (n=3), and acinic cell carcinoma (n=2). Patients were 20 males and 21 females with a mean age of 56.2 years (range, 24 to 98 yrs). Mean age was 48, 58 and 61 years for MEC, ACC and PLGA, respectively. Mean follow-up was 80.1 months (range, 5 to 249 months). Eight patients (19.5%) died of their tumors at a mean interval of 66.5 months (range, 5 to 238 months).

Comparison of clinical features of ACC and PLGA

The pertinent clinical features are summarized in **Tables 1** and **2**. Both tumor types shared similar mean age, gender distribution, common location in the palate, similar low frequency of nodal spread and frequent perineural invasion. However, ACC tended to be more frequently than PLGA associated with high stage disease, residual tumor (R1/R2) and local tumor relapse, although statistical analyses did not reveal significant differences.

Histological patterns of ACC and PLGA

The major histological and immunohistochemical features are summarized in Table 3. When present the main histological architectural patterns were strikingly similar in both ACC and PLGA (**Figure 1**). The tubular pattern was characterized by tubules with open lumina (**Figures 1A** and **1B**). The cribriform pattern showed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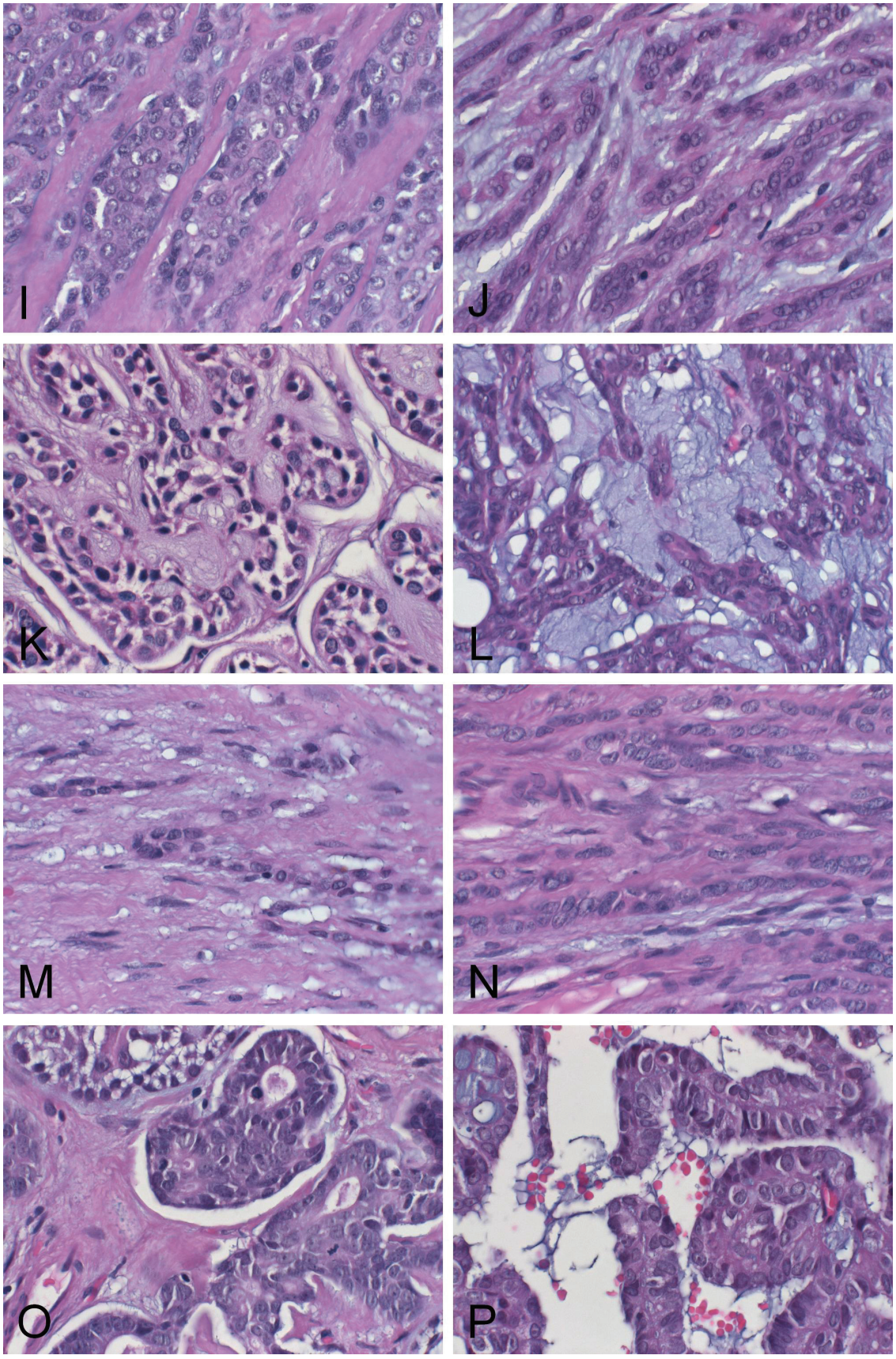
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Figure 1. Architectural patterns of adenoid cystic carcinoma (left column) and polymorphous low-grade adenocarcinoma (right column). **A/B** Tubular pattern. **C/D** Cribriform pattern. **E/F** Solid pattern of "basophilic type". **G/H** Solid pattern of "clear cell type". **I/J** Trabecular pattern. **K/L** Reticular pattern. **M/N** Pattern reminiscent of invasive lobular carcinoma (ILC) of the breast (ILC like pattern / indian file pattern). **O** Example of a dedifferentiated area (non specific pseudopapillary pattern) of ACC: high nuclear-cytoplasmic ratio, large nuclei and enhanced mitotic activity. **P** Papillary pattern of PLGA. *Magnification 630× with oil immersion, HE staining.*

Table 1. Clinical parameters of 14 adenoid cystic carcinomas (ACC) and 8 polymorphous low-grade adenocarcinomas (PLGA) of the oral cavity.

Case	Entity	Sex	Age	Localization	UICC stage	TNM stage	R-status	Relapse
1	ACC	f	98	right lower lip	I	pT1 N0 M0	R0	0
2	ACC	f	50	left lower lip	I	pT1 N0 M0	R0	0
3	ACC	m	72	right soft palate	I	pT1 N0 M0	R0	0
4	ACC	f	57	left palate	I	pT1 N0 M0	R0	0
5	ACC	f	41	right buccal mucosa	II	pT2 N0 M0	R0	0
6	ACC	m	67	right sublingual gland	II	pT2 N0 M0	R1	1
7	ACC	f	50	left palate / maxilla	II	pT2 N0 M0	R0	1
8	ACC	f	49	left palate / maxilla	III	pT3 N0 M0	R1	0
9	ACC	f	69	right palate / maxilla	IVa	pT4a N0 M0	R0	0
10	ACC	m	51	left palate / maxilla	IVa	pT4a N0 M0	R1	1
11	ACC	f	56	right palate / maxilla	IVa	pT4a N2c M0	R1	0
12	ACC	m	75	left palate / maxilla	IVb	pT4b N0 M0	R1	0
13	ACC	f	36	left soft palate	IVc	pT1 N1 M1	R1	1
14	ACC	m	47	left palate / maxilla	IVc	pT4a N2c M1	R2	1
15	PLGA	m	39	right buccal mucosa	I	pT1 N0 M0	R0	0
16	PLGA	f	99	left buccal mucosa	I	pT1 N0 M0	R0	0
17	PLGA	m	64	left palate	I	pT1 N0 M0	R1	0
18	PLGA	f	51	right palate	II	pT2 N0 M0	R0	0
19	PLGA	m	54	right upper lip	III	pT1 N1 M0	R1	0
20	PLGA	m	42	left palate	III	pT3 N0 M0	R0	1
21	PLGA	m	64	left oropharynx	III	pT3 N1 M0	R1	0
22	PLGA	f	75	right palate	IVb	pT4b N0 M0	R0	1

fused glands with multiple lumina filled with mucoid or myxohyaline material (**Figures 1C** and **1D**). The solid pattern was characterized by large sheets of closely packed tumor cells, usually more than 5 cells thick and separated by variable collagenized stroma. The cell morphology in the solid areas was either basophilic (high nuclear-cytoplasmic ratio, large nucleus with condensed chromatin) (**Figures 1E** and **1F**) or clear (low nuclear-cytoplasmic ratio with cytoplasmic vacuoles/clearing (**Figures 1G** and **1H**). The trabecular pattern was characterized by fused tubules and thus represented the architectural transition from the tubular to the solid pattern (**Figures. 1I** and **1J**). The reticular pattern consisted of branching communicating narrow trabeculae (**Figures 1K** and **1L**). The ILC-like or Indian-file pattern was very reminiscent of ILC of the breast and was mainly seen at the infiltrating tumor border or at the periphery of tumor lobules (**Figures 1M** and **1N**). The papillary pattern displayed true papillae with vascularized central fibrous cores (**Figure 1O** and **1P**).

For ACCs, 3 major patterns were observed in 3 tumors and 2 patterns in 6 cases. The remainder showed a single major pattern in combination with diverse minor patterns. Predominant

Table 2. Comparison of clinicopathologic parameters of 14 adenoid-cystic carcinomas (ACC) and 8 polymorphous low grade adenocarcinomas (PLGA) of the oral cavity

	ACC (n=14)	PLGA (n=8)
medium age	58.4 yrs	61 yrs
sex ratio (m:f)	1:1.8	1:1.6
predominant localization frequency	palate 10/14 (71%)	palate 4/8 (50%)
frequency of pT4	5/14 (36%)	1/8 (12.5%)
nodal metastasis	3/14 (21%)	2/8 (25%)
distant metastasis	1/14 (7%)	0/8 (0%)
residual tumor	7/14 (50%)	3/8 (37.5%)
relapse	5/14 (36%)	2/8 (25%)
perineural infiltration	8/14 (57%)	5/8 (62.5%)
C-kit staining in >10% cells	12/14 (86%)	0/8 (0%)
Mib-1 staining in <5%	2/13 (15%)	3/8 (37.5%)

patterns were cribriform (n=10), tubular (n=2) and solid-basophilic (n=2). A single file pattern (ILC-like pattern) was seen as a minor pattern in 3 out of the 14 cases. Perineural invasion was seen in 8 cases.

Using the same cut-off values as for ACC, 3 or more major architectural patterns were seen in 4 of the 8 PLGAs. The remaining 4 tumors revealed 2 or one major pattern combined with minor other patterns. A papillary pattern was seen in only 2 tumors. The solid-basophilic pattern was the most commonly encountered major pattern (seen in 5 cases) followed by the tubular and trabecular (4 cases each) and the solid-clear cell (3 cases) pattern. An ILC pattern was seen in 4 out of the 8 cases.

Immunohistochemical findings in ACCs and PLGAs

As illustrated in **Figure 2** and **Table 3**, ACCs showed well organized dual differentiation throughout with CK5-positive basal and CK7 positive luminal cells reminiscent of the normal salivary gland tissue (**Figure 2A-D**). However, the spatial arrangement of these two cell types varied from tumor to tumor and within the same tumor. Generally, basally differentiated cells tended to cluster at the inner portion of the tumor sheets thus often adopting an "inverted epithelial-myoepithelial pattern" (**Figure 2E**). Some of these inner circle cells coexpressed CK5 and CK7. On the other hand, CK5-positive cells were less prominent and less organized in PLGA (**Figure 2F**).

In contrast to ACCs (**Figure 2H**), a less well organized staining pattern was seen in PLGAs characterized by more diffuse and prominent CK7 staining and by under-representation of CK5 positive basal cells (**Figure 2F, I**). As already seen on close inspection in ACC, some cells seemed to co-express both markers.

The pattern of c-Kit expression in ACC varied between tumors and within the same tumor, but showed generally a more organized pattern that tended to mirror that of CK7 (**Figure 2K**). 12 of 14 ACCs expressed c-Kit in more than 10% of cells. In the case of PLGAs, c-Kit expression was strikingly less with complete lacking in 4 tumors and focal weak staining in 5-10% tumor cells in the remainder ($p<0.001$, comparing ACC and PLGA by Fisher exact test) (**Figure 2L**). As shown in **Figure 3**, the mean percentage of c-Kit positive cells was significantly higher in ACC than in PLGA (37.9% vs. 3.1%, $p<0.001$). The mean MIB-1 index tended to be lower for PLGAs than for ACCs (4.4% vs. 7.1%) but results were largely overlapping for both tumors ($p=0.12$) (**Figure 2N**, O). In particular, a MIB-1 index of ≤5% was seen in 9 of 13 ACCs compared to a similar value in 7 of 8 PLGA ($p=0.61$, Fisher exact test).

t(11;19) status in oral salivary gland carcinomas

The t(11;19) translocation was detected in 67% of oral MEC and in 62% of all MEC in our data-

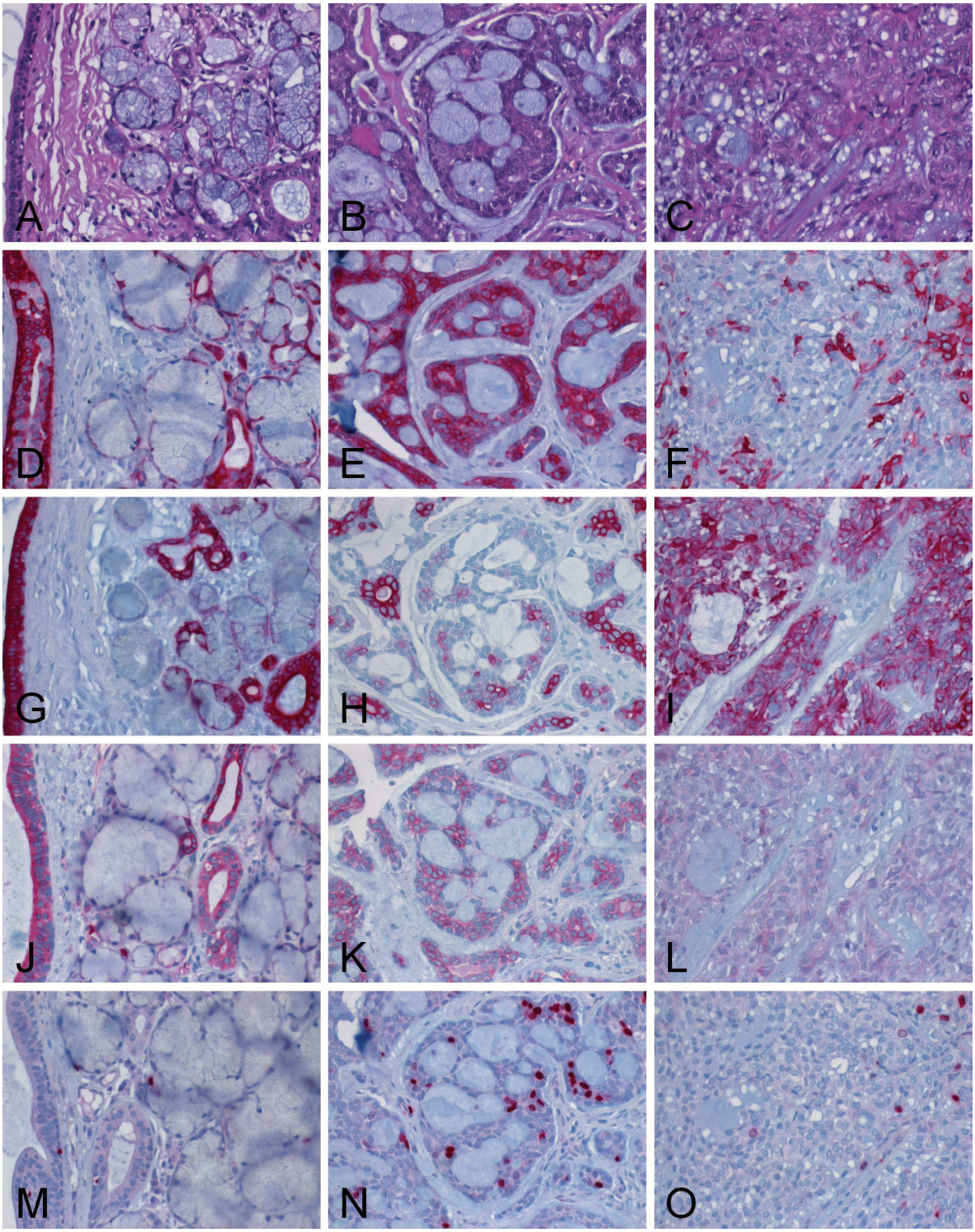

Figure 2. Different expression patterns of CK5, CK7, C-Kit and MIB-1 in normal salivary gland tissue (left column), adenoid cystic carcinoma (middle column) and polymorphous low-grade adenocarcinoma (right column). **A/B/C** HE staining. **D/E/F** Cytokeratin 5 staining. **G/H/I** Cytokeratin 7 staining. **J/K/L** C-Kit staining. **M/N/O** MIB-1 staining. *Magnification 400×*.

Table 3. Pathological features of 14 adenoid cystic carcinomas (ACC) and 8 polymorphous low-grade adenocarcinomas (PLGA) of the oral cavity with respect to the percentage of different growth patterns and to immunohistochemical marker expression.

Case	Entity	tu	cr	sb	sc	tr	re	pa	ilc	no. p.	Pn	CK5 (%)	CK7 (%)	C-Kit (%)	MIB1 (%)	iemp
1	ACC	80	5	15	–	–	–	–	–	3	0	90	5	20	5	+
2	ACC	5	90	5	–	–	–	–	–	3	1	80	50	50	3	+
3	ACC	90	10	–	–	–	–	–	–	2	0	30	80	50	5	–
4	ACC	20	80	–	–	–	–	–	–	2	0	50	70	5	2	–
5	ACC	5	85	5	–	–	–	–	5	4	1	40	60	50	10	–
6	ACC	5	80	5	5	5	–	–	–	5	1	30	60	60	3-20*	+
7	ACC	5	90	–	–	–	–	–	5	3	0	80	25	20	5	–
8	ACC	–	10	90	–	–	–	–	–	2	1	30	90	90	20*	–
9	ACC	5	70	25	–	–	–	–	–	3	1	80	50	50	5	+
10	ACC	5	90	5	–	–	–	–	–	3	1	60	30	30	n.d.§	+
11	ACC	5	85	10	–	–	–	–	–	3	0	30	100	40	5	+
12	ACC	20	55	5	–	20	–	–	–	4	1	70	60	30	10	+
13	ACC	5	55	20	5	10	–	–	5	6	1	90	5	5	5	–
14	ACC	10	10	50	–	–	30	–	–	4	0	80	30	30	5	+
15	PLGA	10	–	60	5	20	–	–	5	5	1	20	50	10	2	–
16	PLGA	50	–	50	–	–	–	–	–	2	0	30	90	0	5	–
17	PLGA	10	–	50	25	10	–	–	5	5	1	20	50	5	5	+
18	PLGA	5	10	–	–	10	70	–	5	5	1	20	60	5	5	–
19	PLGA	–	–	75	5	–	–	20	–	3	0	50	10	0	10	–
20	PLGA	30	20	40	–	–	–	10	–	4	0	5	80	5	5	–
21	PLGA	5	–	–	95	–	–	–	–	2	1	70	50	0	1	–
22	PLGA	–	–	–	85	10	–	–	5	3	1	80	10	0	2	–

tu, tubular; cr, cribriform; sb, solid basophilic; sc, solid clear cell; tr, trabecular; re, reticular; pa, papillary; ilc, invasive lobular carcinoma-like; no. p., number of patterns; Pn0, no perineural invasion; Pn1, perineural invasion present; iemp, inverted epithelial-myoepithelial pattern; *dedifferentiated adenoid cystic carcinoma (high grade). §n.d., not determined due to aggressive decalcification of the specimen.

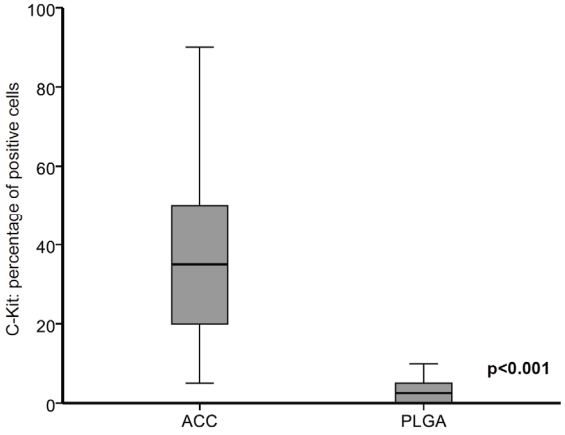

Figure 3. Expression of c-Kit in adenoid cystic carcinoma (ACC) and polymorphous low-grade adenocarcinoma (PLGA). Box plot analysis and double-sided t-test.

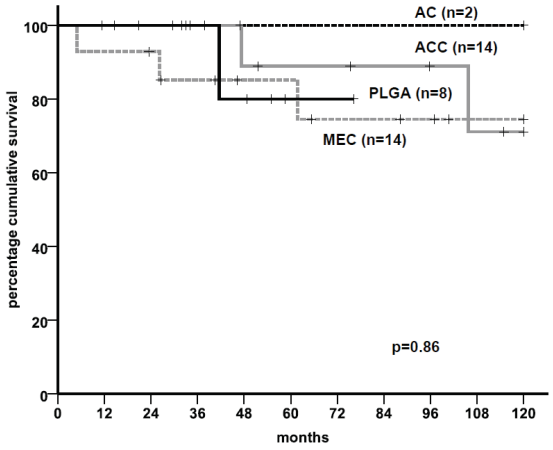

Figure 4. Kaplan-Meier survival analysis and log rank statistics of acinic cell carcinoma (AC), adenoid cystic carcinoma (ACC), polymorphous low-grade adenocarcinoma (PLGA) and mucoepidermoid carcinoma (MEC).

base. All of the non-MEC salivary carcinomas of oral and extra-oral origin were translocation-negative.

Prognostic significance of histologic subtyping in oral salivary gland carcinomas

To examine the prognostic significance of the histopathologic type in oral salivary gland carcinomas, Kaplan-Meier analysis was applied (**Figure 4**). The analysis was limited to the most frequent entities: ACC, MEC and PLGA. The two rare cases of acinic cell carcinoma arising in the oral cavity were added in order to demonstrate the excellent prognosis of this entity. None of the two patients with acinic cell carcinoma died of his tumor within the first 10 years after primary surgery although one case initially presented as pT4 tumor. However, the low case number of adenocarcinomas NOS, and acinic cell carcinoma did not allow for statistical analysis. The Kaplan-Meier analysis demonstrated a similar clinical course in ACC, MEC and PLGA (p=0.86). Excluding censored cases, the 5 year survival of patients with ACC was 87.5%, the 10 year survival was 60%. Patients with MEC had a 5 year survival of 80% and a 10 year survival of 50%. The 5 year survival of patients with PLGA was 80%; the 10 year survival was not available due to censored cases.

Discussion

In this study, we analyzed the full histological spectrum of oral salivary gland carcinomas in a consecutive database retrieved from three institutions. The study confirmed MEC, ACC and PLGA as the most common types of intraoral salivary gland carcinomas. Kaplan-Meier analysis revealed comparable prognosis for these three major entities with no significant difference in outcome.

Our study is the second larger study analyzing the spectrum of oral salivary gland carcinomas with long-term follow-up and the first one to critically compare the clinicopathological features of ACC and PLGA with the long-term clinical outcome. From a clinical point of view, ACC and PLGA show similar age and sex distribution, frequency of perineural invasion, limited regional lymph node metastasis and locoregional disease relapse. However, compared to PLGA, ACC tend to present as higher stage disease being associated with residual local disease (R1/R2) and give rise to rare distant metastasis. The latter two features of ACC might be responsible for the unfavorable outcome compared to PLGA reported in some previous studies [37].

Critical analysis of the different histological features of ACC and PLGA showed strikingly overlapping features between these two entities. The presence of a prominent cribriform pattern can be considered the hallmark of ACC. This pattern is usually combined with diverse major and minor patterns, most of which were also seen in cases of PLGA. In particular, occasional

areas in ACC were practically indistinguishable from PLGA including the presence of ILC-like single file pattern and solid or trabecular areas. These findings are in line with a common differentiation pathway in both tumors (intercalated duct-like differentiation). More important, these observations suggest that both tumor entities lay on a morphologic disease spectrum with the ACC showing the most organoid pattern featuring both luminal and basal differentiation as reflected by the typical expression of CK5, CK7 and c-Kit. On the other hand, PLGA displays a variable and, most strikingly, disorganized pattern with gradual mergence of the different patterns within the same tumor as also evidenced by the overlapping immunoprofile for CK5 and CK7. The varied historical nomenclature suggested for PLGA, even by the same working group [11-13], emphasizes the uncertainty regarding diagnostic criteria and differentiation of this tumor entity. Thus, it is not surprising that in a given PLGA different histological patterns may either dominate the histological picture alone or in different combinations in a manner reminiscent of the spectrum of pleomorphic adenoma. Given that the solid pattern was most dominant in our PLGAs, that (in our experience) it is seldom to observe the different tumor patterns in small biopsies, and the observation that both ACC and PLGA may reveal a combination of 2 or more major architectural patterns, it seems that the term "polymorphous" for PLGA is a misnomer and may be misleading for general pathologists with limited experience in salivary tumor pathology. As PLGA is histogenetically derived from the intercalated duct epithelium [16], the designation "intercalated duct carcinoma, not otherwise specified" would be more appropriate as already the original nomenclature "terminal duct carcinoma" and "lobular carcinoma" was [12]. The modifier "low-grade" is useful in discriminating PLGA from the entity of adenocarcinoma not otherwise specified, a high grade neoplasm that more likely derives from the interlobular (excretory) ducts.

By immunohistochemistry the absence of c-Kit expression in most PLGA as compared to a prominent staining in the majority of ACC represents the most striking and reproducible difference between these two tumors and suggests that this marker may be of great value for differentiating the two tumors in small biopsies.

Although our results showed that the proliferation fraction (estimated by the MIB-1 antibody) tends to be higher for ACC than for PLGA, it revealed largely overlapping values for both tumor groups. With a MIB-1 labeling index of ≤5% in most of ACCs and PLGAs, this marker remains of limited value in the distinction of these tumors. However, Skalova et al demonstrated a significantly higher MIB-1 index in ACC than PLGA with no overlap zone [38]. The differences with regards to the MIB-1 index in different studies might be biased by interobserver variability and/or examination of non-representative tumor specimens in some of the tumors.

From a differential diagnostic point of view, PLGA have to be, if possible and clinically relevant, distinguished from ACC and, most importantly, from pleomorphic adenoma. In well fixed and preserved tissue sections, the condensed hyperchromatic nuclei of ACC usually contrast with the bland-looking palely staining nuclei of PLGA, but this important feature may be largely impaired following frozen section examination in our experience. Likewise, the presence of a predominant cribriform pattern suggests ACC. Contrasting with pleomorphic adenoma PLGA usually lacks evidence of chondromyxoid mesenchymal differentiation. On the other hand, pleomorphic adenoma lacks evidence of peripheral invasion into surrounding tissue, perineural invasion and the characteristic ILC-like and onion-skin pattern commonly seen at the periphery of PLGA. Thus, it is of great importance to carefully examine the tumor periphery in excisional biopsies looking for evidence of infiltration into surrounding salivary gland and other normal tissue. A varied histological pattern in the absence of a chondromyxoid component should alert to the possibility of PLGA if one is dealing with a limited biopsy material from an intraoral neoplasm.

In summary, we analyzed 41 oral salivary gland carcinomas, demonstrating a high histological variation and greatly overlapping clinicopathological and immunohistochemical features of ACC and PLGA. Differentiating ACC and PLGA, particularly in limited preoperative biopsy material, might be impossible given their highly overlapping features. However, as both neoplasms are treated the same way and the clinical course is comparable for both, the preoperative distinction of the two entities seems not to be of clinical significance.

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