Review

Polymorphous adenocarcinoma of the salivary glands: an overview of immunohistochemical features and insights on molecular pathology

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Summary. Polymorphous adenocarcinoma (PAC), represents a common minor salivary gland tumor (SGT) characterized by local growth, low metastatic potential and non-aggressive biologic behavior. Due to the clinical aggressiveness noted in a subset of such tumors, the former term polymorphous low-grade adenocarcinoma (PLGA) was recently revised. PAC's clinical features and histological diversity result in clinical overlap of this entity with several other SGTs including mainly adenoid cystic carcinoma (AdCC). Differential diagnosis among the entities is crucial, in terms of tumor management and patients' prognosis.

The aim of the present review is to summarize the histological, cytological, immunohistochemical and molecular features of PAC.

Histopathological examination is usually adequate for PAC differential diagnosis from other SGTs, except of AdCC. Several immunohistochemical markers including c-Kit, S-100/ MG, Mcm-2 and Integrin β-1, -3, -4, are reported to be useful diagnostic aids in borderline cases. Limitations in sample numbers and study methodology issues of the immunohistochemical PAC studies complicate the identification and selection of appropriate markers useful in the differential diagnosis. Additionally, molecular analyses of PAC specimens indicate that the PAC spectrum phenotypes result from different genotypes (protein kinase D positive; PRKD(+) and PRKD(-) tumors). PAC pathogenesis remains to be determined in each particular genotype while the convergence issue should be addressed in future studies.

Key words: Polymorphous adenocarcinoma, Salivary gland tumors, Histology, Immunohistochemistry, Cytology, Review

Abbreviations. ACC, Acinic cell carcinoma; aCGH, Array comparative genomic hybridization; AdCC, Adenoid cystic carcinoma; CA, Canalicular adenoma; CAd, Cribriform adenocarcinoma; CD, Cluster of differentiation; CD44, Cluster of differentiation 44; CEA, Carcinoembryonic antigen; CK, Cytokeratin; CMT, Cellular mixed tymor; DDR, DNA Damage Repair; EMA, Epithelial membrane antigen; EGFR, Epithelial growing factor receptor; FGF, Fibroblast growing factor; FGFR, Fibroblast growing factor receptor; FNAc, Fine needle aspiration cytopathology; GCDFP-15, Gross cystic disease fluid protein-15; GF, Growing factor; GFAP, Glial fibrillary acidic protein; GFR, Growing factor receptor; HA, Hyaluronic Acid; HHF-35, Anti-muscle actin antibody; IHC, Immunohistochemical; MASC, Mammary analog secretory carcinoma; Mcm-2, minichromosome maintenance complex component 2; MEC, Mucoepidermoid carcinoma; MG, Mammaglobin; MGG, May-Grunwald Giemsa stain; MMP-1, Matrix metalloproteinase-1; NR, Not Reported; OPN, Osteopontin; PA, Polymorphous adenoma; PAC, Polymorphous adenocarcinoma; PDGF, Platelet-derived growing factor; PDGFR, Platelet derived growing factor receptor; PLGA, Polymorphous low grade adenocarcinoma; PLUNC, Palate, lung and nasal epithelial clone protein; PRKD, Protein kinase D; RNA seq., RNA sequencing; SGT, Salivary gland tumors; SMA, Smooth muscle actin; WGS, Whole genome sequencing.

Introduction

Polymorphous Adenocarcinoma (PAC) is a common malignant salivary gland tumor (SGT), predominantly affecting minor salivary glands (Klijanienko and Viehl, 2000; Gnepp et al., 2009; Brandwein-Gensler et al., 2017). It was first described in 1983 by the groups of Freedman and Lumerman (1983) and Batsakis et al. (1983). The tumor was initially described under the terms "lobular carcinoma" and "terminal duct carcinoma" but the term "Polymorphous Low-Grade Adenocarcinoma", first proposed in 1984 (Evans and Batsakis, 1984) was preferred until recently, by the official nomenclature (Brandwein-Gensler et al., 2017). However, a subset of tumors with aggressive behavior has been described over the years (Kumar et al., 2004; Arora et al., 2013; Thennavan et al., 2013) and thus the term "low-grade" was removed from the latest WHO Head and Neck tumor classification in order to describe more accurately the full spectrum of this entity (Brandwein-Gensler et al., 2017). Recently published large cohorts provided further insights into PLGA, allowing better understanding of the tumor's behavior and more accurate patient survival estimations (Seethala et al., 2010; Patel et al., 2015; Elhakim et al., 2016).

More than 1000 cases have been reported until today (Klijanienko and Viehl, 2000; Gnepp et al., 2009; Seethala et al., 2010; Patel et al., 2015; Elhakim et al., 2016). PAC accounts for 26% of intraoral salivary glands malignancies, considered as the second most common minor salivary gland malignancy after muco-epidermoid carcinoma (MEC) (Klijanienko and Viehl, 2000; Gnepp et al., 2009; Brandwein-Gensler et al., 2017). Noteworthy, only a few cases affect the major salivary glands (Klijanienko and Viehl, 1998; Nagao et al., 2004; O'Rourke et al., 2006; Krishnamurthy et al., 2011; Patel et al., 2015; Elhakim et al., 2016) and other uncommon locations including nasal cavity, nasopharynx and lacrimal glands (Wenig et al., 1989; Suster and Young, 1994; Young et al., 2003; Lee et al., 2004; Selva et al., 2004; Asioli et al., 2006; Kimple et al., 2014; Patel et al., 2015). PAC presents significant inter-racial variability and is most prevalent among Africans and South Americans compared to Asians (Araujo at al., 2013; Patel et al., 2015). A predominance of female patients has been reported, with the female proportion ranging from two-thirds to three quarters of total patients (Patel et al., 2015; Elhakim et al., 2016; Brandwein-Gensler et al., 2017). PAC usually occurs during the sixth decade with the mean age varying from 55.2 to 61.3 years old and the age ranges from 16 to 94 years old (Klijanienko and Viehl, 2000; Gnepp et al., 2009; Brandwein-Gensler et al., 2017). Until today pediatric PAC remains extremely rare and most patients diagnosed are older than 50 years old (Tsang et al., 1991; Seethala et al., 2010; Patel et al., 2015; Elhakim et al., 2016). Most data are derived by monocentric case series or case reports and present large inter-study variability regarding the tumor's epidemiologic trends and incidence.

Palate is the most common PAC location, but all sites containing minor salivary glands, in the upper aero-digestive system are potential tumor locations. Occasionally the tumor can be identified in major salivary glands, and particularly parotid. It presents as a slowly-growing submucosal mass, with a 2.2 cm average maximum diameter. It is commonly presented as a painless, yellowish, lobulated mass but painful, ulcerated cases have been described as well (Klijanienko and Viehl, 2000; Gnepp et al., 2009). Due to the tumor growth rate, by the time of diagnosis, the mass has a varying duration, ranging from weeks to years (Klijanienko and Viehl, 2000; Gnepp et al., 2009; Sedassari et al., 2016). Development of cervical lymph node metastases is reported in 0%, 3.5%, 9% and 15% of patients in different studies (total: 868 patients) (Klijanienko and Viehl, 2000; Gnepp et al., 2009). Distant metastases, despite being rare, have been reported at late disease stage, in lungs, skin, ileum and other regions (Klijanienko and Viehl, 2000; Kumar et al., 2004; Thennavan et al., 2013; Arora et al., 2013).

Cytological uniformity and architectural diversity, local tumor growth and low metastatic potential are eminent characteristics of PAC (Sedassari et al., 2016). The tumor's pathogenesis although not fully understood today, has been studied thoroughly, under the prism of novel molecular techniques (RNA sequencing- RNA seq., Whole genome sequencing- WGS etc.) (Ellis and Auclair, 2008; Weinreb et al., 2014). The aim of this review is to provide a comprehensive overview of the clinical and particularly histological, cytological, immunohistochemical, molecular and genetic characteristics reported in the current literature. PAC pathogenesis and diagnostic approach are also discussed.

Histological and cytological features

PAC is a circumscribed, non-capsulated, malignant tumor characterized by a locally infiltrative growth pattern in the salivary gland parenchyma and surrounding structures (Gnepp et al., 2009; Brandwein-Gensler et al., 2017). Tumor growth expands in adjacent soft tissues and in the underlying osseous structures, especially when located in the palate (Klijanienko and Viehl, 2000; Gnepp et al., 2009). Entrapped, non-neoplastic salivary gland tissue may be observed (Ellis and Auclair, 2008; Sedassari et al., 2016). Neurotropism and perivascular infiltration are commonly present during PAC progression and evolvement but vascular and lymphatic invasion is considered rather uncommon (Araujo et al., 1999; Ellis and Auclair, 2008; Araujo et al., 2013). Histologic diversity is an eminent feature of the tumor (Fig. 1a-f), although its central region is typically solid or lobulated and the periphery infiltrates the adjacent tissues and presents variable growth patterns: single malignant cells, peripheral single file rows of malignant cells (Indian files), solid islets, cribriform patterns, cystic areas, trabecular,

interconnecting cord-like structures and ducts (Araujo et al., 1999; Klijanienko and Viehl, 1998, 2000; Ellis and Auclair, 2008; Gnepp et al., 2009; Brandwein-Gensler et al., 2017). More than one of the major forms is usually observed in PAC specimens: lobular-acinar (Fig. 1a), papillary (Fig. 1b) or papillary-cystic (Fig. 1c), cribriform (Fig. 1d) and trabecular with single cyboidal-cell lined (Fig. 1e) and scant duct like structures (Fig. 1f) (Brandwein-Gensler et al., 2017). Intervening stroma is minimal and may present mucoid, hyaline (Fig. 2a), mucohyaline or desmoplastic appearance (Fig. 2b) (Klijanienko and Viehl, 2000; Ellis and Auclair, 2008; Gnepp et al., 2009). Epithelial hyperplasia, despite being observed in a small subset of PAC specimens (approximately 30%) constitutes a strong PAC indicator (Chi and Neville, 2015). Noteworthy, some authors have raised concerns on whether the cribriform pattern, which has been described in minor salivary glands and posterior tongue (Michal et al., 1999; Skalova et al., 2011; Xu et al., 2016) should be considered within the spectrum of PAC or if it should form a distinct entity (Brandwein-Gensler et al., 2017).

Tumor cells despite being arranged in several histologic patterns, present significant uniformity and innocuous appearance (Klijanienko and Viehl, 2000; Gnepp et al., 2009). They are small-to-medium sized, with oval clarified (Fig. 3) nuclei, occasionally with slightly enlarged nucleoli, surrounded by abundant cytoplasm with a clear-to- eosinophilic hue (Ellis and Auclair, 2008). Oncocytic cell foci and areas of squamous or mucinous metaplasia have been also described. Mitotic figures are rare and atypical mitoses are usually not observed (Klijanienko and Viehl, 2000; Gnepp et al., 2009; Brandwein-Gensler et al., 2017). Necrosis is typically absent or associated with overlying epithelium ulceration (Ellis and Auclair, 2008; Brandwein-Gensler et al., 2017). However, such features commonly appear in other benign and malignant SGTs, including pleomorphic adenoma (PA), canalicular adenoma (CA), cribriform adenocarcinoma (CAd), mammary analog secretory carcinoma (MASC) and adenoid cystic carcinoma (AdCC) (Nagao et al., 2004; Ellis and Auclair, 2008). Most commonly, diagnostic problems arise between AdCC and PAC due to their common histogenesis from intercalated duct cells and their architectural diversity (Gnepp et al., 2009; Brandwein-Gensler et al., 2017). Atypical presentation or small biopsy specimens may prevent the identification of the characteristic architectural patterns and combined with the overlapping features of PAC and other SGTs may result to diagnostic dilemmas and inappropriate therapeutic approaches.

Cytologically, PAC is composed of epithelial cells exhibiting clarified and elongated nuclei (Fig. 3a- c). Cytoplasm is grayish using MGG and slightly granulated using Papanicolaou stains. Chromatin is dusty. Cells are isolated or clustered in tubular or papillary structures. Pinkish connective tissue is usually present in the background (Fig 3d). In some cases, hyaline globules similar to AdCC are also present (Ellis and Auclair,
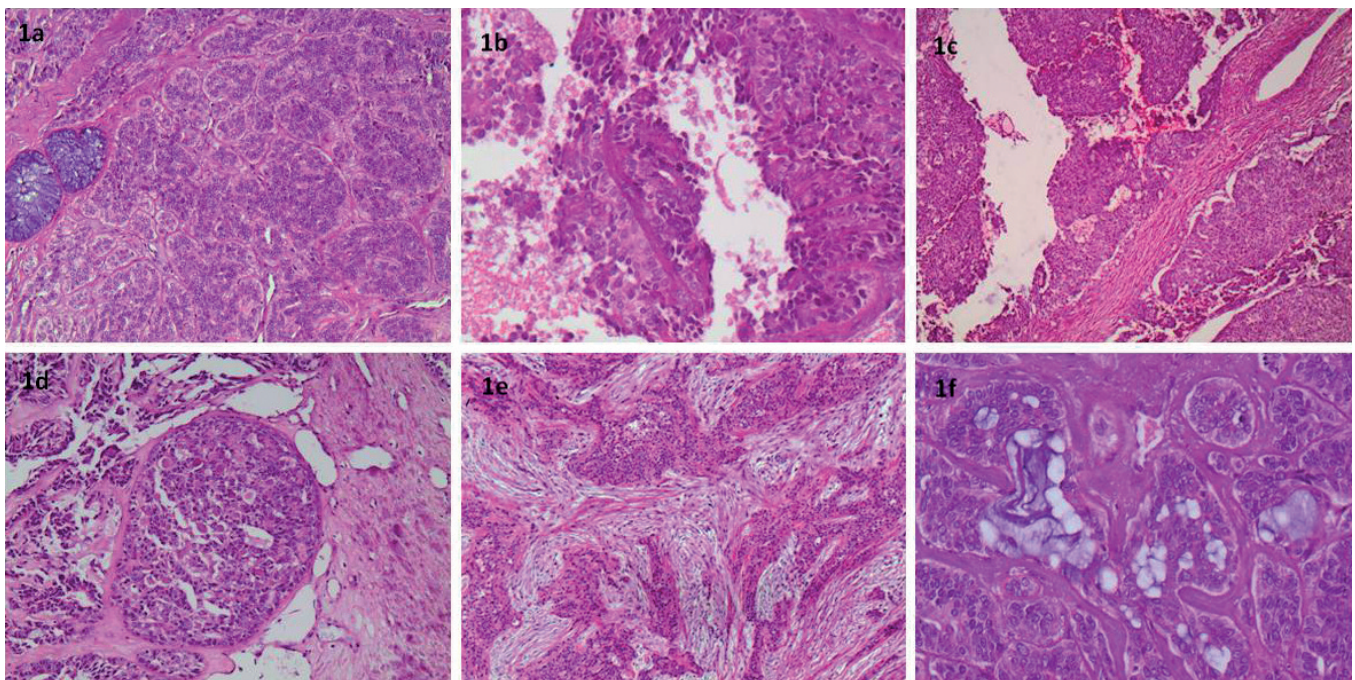

Fig. 1. Patterns of polymorphous low-grade adenocarcinoma. **1a.** Acinar pattern. **1b.** Papillary pattern. **1c.** Papillary-cystic pattern. **1d.** Cribriform pattern. **1e.** Presence of trabecular cyboidal cell-lined structures. **1f.** Presence of duct-like structures. (HE). 1a, 1c-1e, x 200; 1b, 1f, x 400.
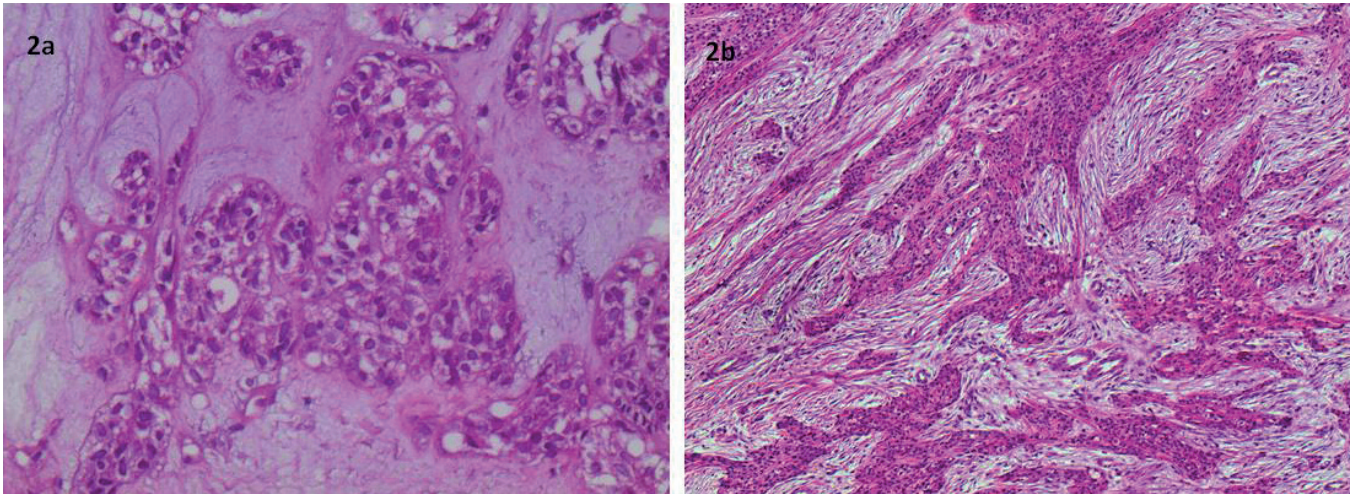
Fig. 2. Stromal patterns of polymorphous low-grade adenocarcinoma. **2a.** Hyaline appearance of stroma. **2b.** Desmoplastic appearance of stroma in Polymorphous Low-Grade Adenocarcinoma. (HE). 2a, x 400; 2b, x 100.

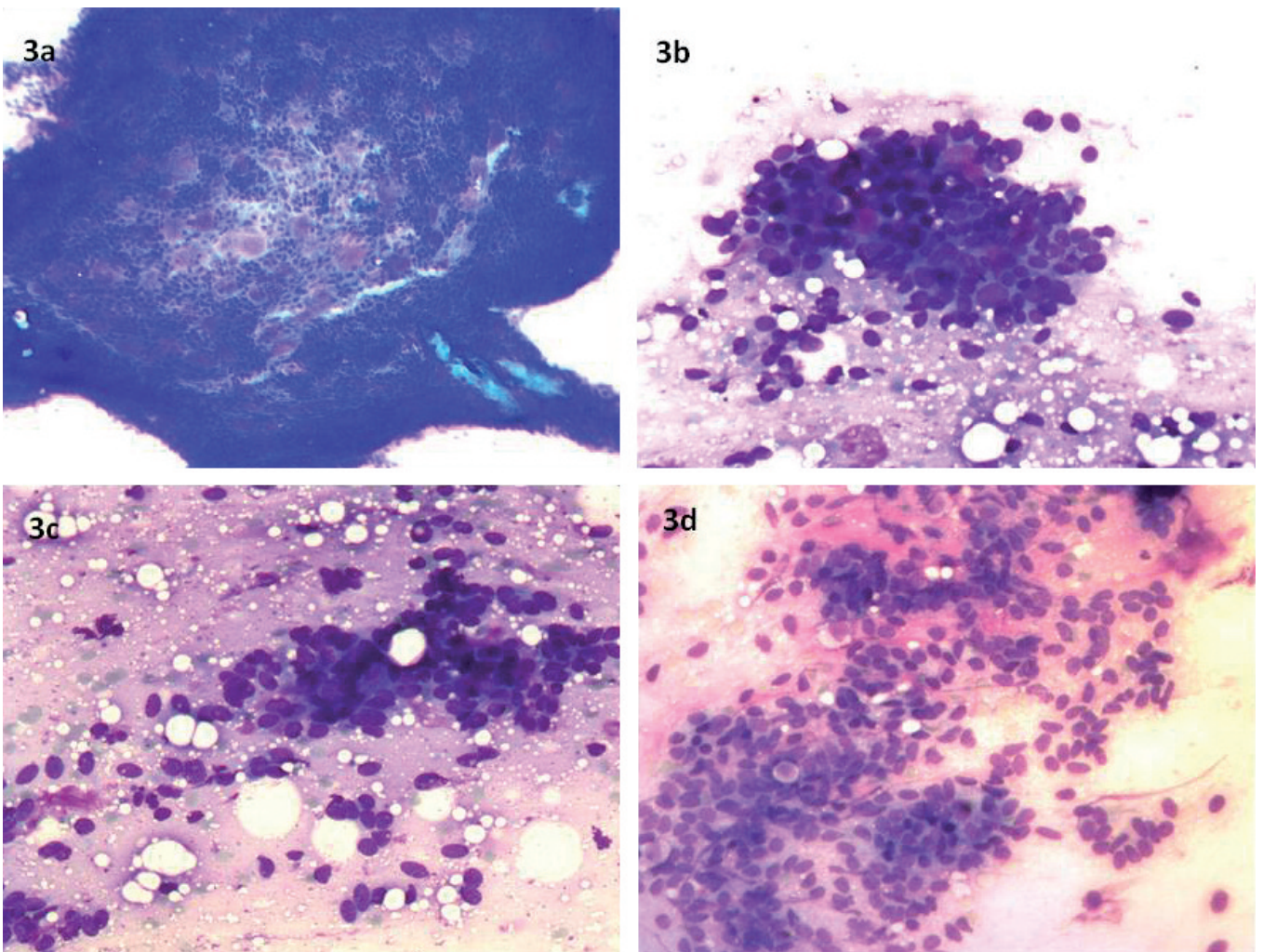**Fig. 3.** Cytopathological features in polymorphous low-grade adenocarcinoma. **3a.** Large clusters of monomorphic cancer cells around hyaline material (Diff Quick). **3b.** Monomorphous cancer cell population and hyaline material (May-Grünwald Giemsa MGG). **3c.** Dispersed cells and mucinous background (MGG). **3d.** Clarified and oval spindle nuclei with delicate chromatin (MGG). 3a, x 100; 3b-3d, x 200.

2008).

Immunohistochemical features

Tumor cytology and histology combined with clinical information are usually but not always adequate to establish a PAC diagnosis. Several studies report - more or less specific- immunohistochemichal markers that form several distinctive immunohistochemical profiles for PAC (Tables 1, 2) (Fig. 4a-f) and provide information regarding the patients' prognosis (Table 3).

Diagnostic biomarkers

The overlapping features of PAC and other SGTs result in a significant number of borderline cases, indicating the need for diagnostic biomarkers and tumor-specific immunohistochemical patterns for more accurate diagnoses in Fine needle aspiration cytopathology (FNAc) and biopsies (Tsang et al., 1991). Out of these entities, the differential diagnosis of PAC and AdCC in border cases is usually the most challenging.

Table 1. Diagnostic immunohistochemical markers in differential diagnosis of PAC and AdCC.

Name	Role	Number of (+) specimens	AdCC (+) specimens	Author
p63/p40	Myoepithelial cell marker; DDR system	5/5; 0/5	7/7; 7/7	Argyris et al., 2016
		11/11; 0/11	91/101 (90%)	Rooper et al., 2015
p63		4/5	10/10	Projetti et al., 2015
		3/3	3/4	Foschini et al., 2005
GCDFP-15	Glandular tissue marker	4/5	0/10	Projetti et al., 2015
MG	Glandular tissue marker	5/5	0/10	Projetti et al., 2015
		15/15	7/15	Patel et al., 2013
S-100	Inflammation-cancer linker, tumor progression	14/15	10/15	Patel et al., 2013
p53	Cell cycle regulator	10/17	13/17	Lazzaro and Cleveland, 2000
		1/13	5/56	Rosa et al., 1997
SMA	Myoepithelial cell marker	3/12	14/14	Epivatianos et al., 2007
		4/24	21/23	Prasad et al., 2008
Vimentin	Myoepithelial cell marker	12/12	14/14	Epivatianos et al., 2007
		4/4	7/8	Cavalcante et al., 2007
Calponin	Myoepithelial cell marker	21/23	5/24	Epivatianos et al., 2007
		4/4	8/8	Cavalcante et al., 2007
HHF-35	Myoepithelial cell marker	2/4	5/8	Cavalcante et al., 2007
CEA	Tumor marker (Cell adhesion molecule)	9/10	14/14	Epivatianos et al., 2005
EMA	Epithelial cell marker	8/10	14/14	Epivatianos et al., 2005
CD-43	Cell adhesion regulator	1/14	12/12	Woo et al., 2006
CK-5/CK-7	Epithelial cell marker	8/8; 8/8	14/14; 14/14	Schwartz et al., 2011
Galectin-3*	Neoplastic transformation, tumor progression	14/141	8/92	Penner et al., 2002
		8/83	12/124	El-Nagdy et al., 2013
Integrin-β1	Proliferation and cell migration regulator	5/5	5/5	Loducca et al., 2000
Integrin-β1/ -β3/ -β4		7/7	10/10	Loducca et al., 2003
Integrin-β6		14/18	7/19	
Tenascin-C	Stromal invasion and cell migration regulators	13/18	2/19	Westernoff et al., 2005
MMP-1		17/18	19/19	
Ki-67	Proliferation marker	8/8	14/14	Schwartz et al., 2011
		4/6	6/9	Do Prado et al., 2011
		14/17	15/17	Lazzaro and Cleveland, 2000
		9/10	13/13	Vargas et al., 2008a
c-Kit	Histogenesis marker	4/8		Schwartz et al., 2011
		9/14	9/9	Penner et al., 2002
		2/8	12/12	El-Nagdy et al., 2013
		7/14	11/14	Andreadis et al., 2006
		5/12	12/14	Epivatianos et al., 2007
		16/17	15/15	Edwards et al., 2003
Geminin	Proliferation marker	7/10	13/13	Vargas et al., 2008a
Mcm-2	Proliferation marker	9/10	13/13	Do Prado et al., 2011

*Galectin-3 immunostaining is also assessed in terms of extent. 1 PAC: >50% of (+) cells in all 14 cases. 2AdCC >50% of (+) cells in 7 cases and 25-50% in 1 case. 3 PAC: >50% of (+) cells in all 8 cases. 4AdCC: >50% of (+) cells in all 12 cases. IHC, Immunohistochemical; DDR, DNA Damage Repair; GCDFP-15, Gross cystic disease fluid protein-15; SMA, Smouth muscle actin; HHF-35, Anti-muscle actin antibody; CEA, Carcinoembryonic antigen; EMA, Epithelial membrane antigen; CD, Cluster of differentiation; CK, Cytokeratin; MMP-1, Matrix metalloproteinase.

Table 2. Diagnostic immunohistochemical markers in differential diagnosis of PAC and other SGTs.

Name	Role	Number of (+) specimens	Other SGT (+) specimens	Author
CK7/ CK8/ CK13	Epithelial cell markers	10/10; NR; 0/10	CA: 5/ 5; 3/ 5; 3/ 5	Furuse et al., 2003
CK7/ CK20	Epithelial cell markers	11/11; 0/11	ACC: 4/4; 0/ 4 MEC: 26/26; 2/ 26	Nikitakis et al., 2004
SMA	Myoepithelial cell marker	0/10	CA: 0/ 5	Furuse et al., 2003
Vimentin	Myoepithelial cell marker	10/10	CA: 2/ 5	Furuse et al., 2003
S-100	Inflammation-cancer linker, tumor progression	14/15	MEC: 3/15	Patel et al., 2013
GFAP	NR	0/30	PA: 21/ 21 CA: 26 /27	Curran et al., 2007
GFAP	NR	2/12	CMT: 12/12	Gnepp et al., 1997
p63	Myoepithelial cell marker; DDR system	4/5 (nucl.; cyt.)	MASC: 5/10 (nucl); 6/10 (cyt.) ACC: 2/9 (nucl.) MEC: 10/10 (nucl.)	Projetti et al., 2015
GCDFP-15	Glandular tissue marker	4/5	MASC: 6/10 ACC: 7/9 MEC: 3/10	Projetti et al., 2015
MG	Glandular tissue marker; Tumor progression; ↓ chemosensitivity	5/5	MASC: 10/10 ACC: 1/9 MEC: 4/10	Projetti et al., 2015
SPLUNC-1/ -2	Mucous cell markers	0/10	PA: 0/10 ACC: 0/13 MEC: 0/ 10	Vargas et al., 2008b
LPLUNC-1	Mucous cell markers	0/10	PA: 0/ 10 ACC: 0/ 13 MEC: 10/ 10	Vargas et al., 2008b

IHC, Immunohistochemical; CK, Cytokeratin; NR, Not Reported; CA, Canalicular adenoma; CK, Cytokeratin; MEC, Mucoepidermoid carcinoma; ACC, Acinic cell carcinoma; SMA, Smooth muscle actin; GFAP, Glial fibrillary acidic protein; CMT, Cellular mixed tymor; nucl., Nuclear; cyt., cytoplasmic; GCDFP-15, Gross cystic disease fluid protein-15; MASC, Mammary analog secreting carcinoma; MG, Mammaglobin; PLUNC, Palate, lung and nasal epithelial clone protein.
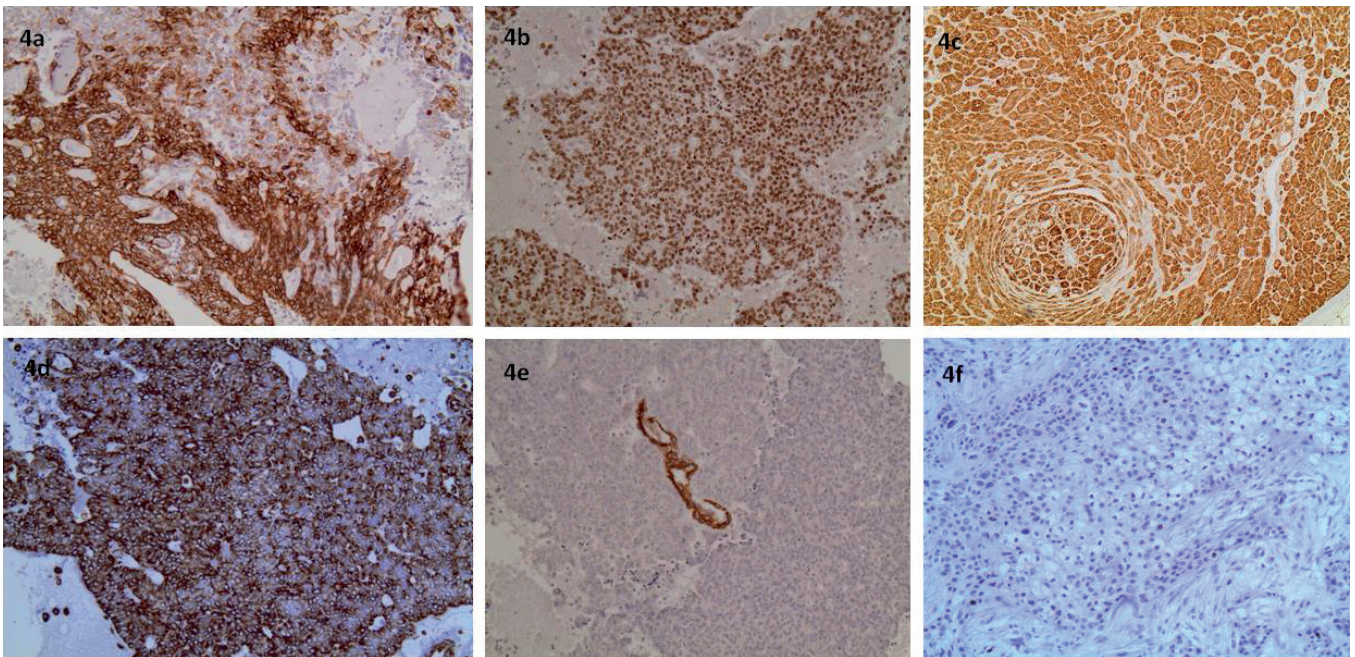

Fig. 4. Immunohistochemical features in polymorphous low-grade adenocarcinoma. Possitive immunoexpression of: CK5/6 **(4a)**, p63 **(4b)**, S100 **(4c)**, Vimentin **(4d)**. **4e.** Negative immunoexpression of a-SMA. **4f.** Low Ki-67 immunoexpression (Chromogen used DAB, counterstaining Harris Hematoxylin). 4a, 4c-4e, x 200; 4b, 4f, x 400.

AdCC

The fact that these neoplasms have a significantly different biologic behavior and AdCC presents higher recurrence rates and metastatic potential affects both treatment and prognosis and thus the establishment of an unequivocal diagnosis is of great importance for patients' management. The current literature contains studies investigating in-vivo the potential diagnostic utility of several genome abnormalities and products with known or still unknown roles in the carcinogenic pathways of PAC (Table 1). Structural chromosomal abnormalities reported in AdCC, including *MYB* rearrangement and *MYB/NFIB* fusion were considered as candidate diagnostic biomarkers that differentiate PLGA and AdCC. The presence of *MYB* rearrangement t(6;9) in AdCC and PAC was evaluated by fluorescent in-situ hybridization (FISH) in a study of 12 cases (Argyris et al., 2016). The translocation was exclusively identified in AdCC specimens, indicating a potential involvement of MYB rearrangement in AdCC pathogenesis. The same cohort was also evaluated immunohistochemically for p63/p40 expression and revealed two different immunoprofiles (PLGA: p63+/p40- and AdCC: p63+/p40+) (Argyris et al., 2016). These findings are in agreement with previous reports of the p63/p40 phenotype and p63 expression in PLGA and AdCC (Foschini et al., 2005; Rooper et al., 2015; Projetti et al., 2015). One of these studies evaluated also the expression of Gross cystic disease fluid protein- 15 (GCDFP-15) and mammaglobin (MG) (Projetti et al., 2015). The study reported that MG was able to discriminate AdCC and PLGA while GCDFP-15 expression was present in 50% of PLGA specimens and absent in AdCC. MG and S-100 presented significant immunoreactivity in terms of strength and extent in most of PAC cases of another study and in a small subset of AdCC specimen cases

indicating a potential role for these molecules in PAC diagnosis (Patel et al., 2013).

The gatekeeper of the cell cycle, p53, was also evaluated as a diagnostic biomarker in 2 studies. Both case series did not present differential expression between AdCC and PAC and both SGTs exhibited low p53 accumulation in both PAC and AdCC (Rosa et al., 1997; Lazzaro and Cleveland, 2000).

Myoepithelial cell presence in ductular and tubular patterns of PAC was considered a feature with potential diagnostic utility and thus, several myoepithelial cell markers have been studied (Epivatianos et al., 2007; Prasad et al., 2008). Alpha-SMA (α-SMA) and Vimentin are both expressed in AdCC and PAC specimens but α-SMA presents a significantly different percentage of positivity between the two entities suggesting a potential role in differential diagnosis. Notably, α-SMA IHC as an adjunct to histopathologic examination is reported as being equally efficient at discriminating PAC from AdCC as multiple IHC tests (Prasad et al., 2008). Other myoepithelial cell markers including Vimentin, Calponin and HHF-35 presented inconclusive results, regarding their expression in AdCC and PAC (Epivatianos et al., 2007; Cavalcante et al., 2007). IHC positivity for these markers is observed in both tumors and the proportions of positive cells are not always significantly different.

Membrane glycoproteins CEA, EMA and CD43 have been also evaluated as PAC candidate diagnostic markers (Gnepp et al., 1988; Epivatianos et al., 2005; Woo et al., 2006; Schwartz et al., 2011). EMA and CEA did not present different IHC patterns in a study of 24 specimens (Epivatianos et al., 2005) while a previous study of a smaller sample suggested a potential role in AdCC and PAC differential diagnosis (Gnepp et al., 1997). Cytokeratins and Galectin-3 were also assessed but no differential expression was reported between PAC and AdCC (Penner et al., 2002; Schwartz et al., 2011;

Table 3. Prognostic immunohistochemical markers in PAC.

Name	Role in the carcinogenesis of PAC	Number of (+) specimens	Author
Mcm-2	Proliferation marker	9/10	Vargas et al., 2008a
FGF-2/ PDGF-A/ PDGF-B/ PDGFR-α/ FGFR-1/ FGFR-2/ EGFR	Proliferation and tumor progression markers	24/24	Rosa et al., 2016
OPN	Tumor progression, Invasion, Metastasis	12/12	Darling et al., 2006
		16/16	Fok et al., 2013
HA	Tumor invasion, Metastasis	1/9	Xing et al., 1998
CD44	OPN pathway	8/10	
		16/16	Fok et al., 2013
Integrin avβ3	Tumor invasion	1/10	Fok et al., 2013
Integrin-β1/ -β3/ -β4	Proliferation and cell migration regulators	7/7	Loducca et al., 2003
Bcl-2	Apoptosis	31/31	Soares et al., 2017
		17/17	Perez-Ordonez et al., 1998
Integrin-β6		14/18	
Tenascin-C	Stromal invasion and cell migration regulators	13/18	Westernoff et al., 2005
MMP-1		17/18	

Bcl-2: B-cell lymphoma 2; IHC: Immunohistochemical; FGF: Fibroblast growing factor; PDGF: Platelet-derived growing factor; PDGFR: Platelet derived growing factor receptor; FGFR: Fibroblast growing factor receptor; EGFR: Epithelial growing factor receptor; OPN: Osteopontin; HA: Hyaluronic Acid; CD44: Cluster of differentiation 44; MMP-1: Matrix metalloproteinase-1.

El-Nagdy et al., 2013). Another family of proteins, Integrins, which have a well-demonstrated role in carcinogenesis (Seguin et al., 2015), has been evaluated for diagnostic purposes in SGT (Loducca et al., 2000, 2003; Westernoff et al., 2005). PAC specimens examined for integrin-β1, -β3, -β4 were all positive and a distinctive bipolar IHC staining pattern was reported in all tumor cells, confirming cellular uniformity and single-cell layers forming several histologic patterns (Loducca et al., 2003). It is of note though, that another study evaluating integrin-β6, tenascin-C and MMP-1 did not identified any PAC specific IHC signature that discriminates from other SGT (Westernoff et al., 2005).

The proliferation marker Ki-67 is a well-studied protein expressed particularly in phase M, which is overexpressed in several neoplasms and provides information about the tumor growth rate. In SGT, several studies have evaluated Ki-67 efficacy (Lazzaro and Cleveland, 2000; Vargas et al., 2008a; Schwartz et al., 2011; do Prado et al., 2011) and the protein is proposed by some authors as a useful diagnostic marker in the discrimination between AdCC and PAC (Vargas et al., 2008a; Schwartz et al., 2011). The expression levels vary among different studies. Ki-67 levels in AdCC were reported slightly higher but overlapping with PAC (Lazzaro and Cleveland, 2000) and significantly elevated in AdCC (Vargas et al., 2008a), in different case series. Noteworthy one study failed to correlate Ki-67 expression with PAC proliferation rate (do Prado et al., 2011). Other histogenesis markers studied in SGT include c-Kit/ CD117 and DNA replication regulators minichromosome maintenance complex component 2 (Mcm-2) and Geminin (Penner et al., 2002; Edwards et al., 2003; Andreadis et al., 2006; Epivatianos et al., 2007; Vargas et al., 2008a; do Prado et al., 2011; Schwartz et al., 2011; El-Nagdy et al., 2013). The proto-oncogene c-Kit is considered by some authors a promising marker in the differential diagnosis between AdCC and PAC, due to its characteristic immunostaining pattern in AdCC and its relatively low expression in PAC (Penner et al., 2002; Andreadis et al., 2006; Epivatianos et al., 2007; Schwartz et al., 2011; El-Nagdy et al., 2013). The anti-apoptotic factor Bcl-2 is also reported to be expressed both in PAC (Perez-Ordonez et al., 1998; Soares et al., 2017; Kikuchi et al., 2018) and AdCC (Carlinfante et al., 2005) and it is not considered to be useful in differential diagnosis between these two entities (Meer et al., 2011). However, other studies report that c-kit expression is not specific (Edwards et al., 2003) and at present there is no consensus regarding its clinical utility. The anti-apoptotic factor Bcl-2 is also reported to be expressed both in PAC (Perez-Ordonez et al., 1998; Soares et al., 2017; Kikuchi et al., 2018) and AdCC (Carlinfante et al., 2005) and it is not considered to be useful in differential diagnosis between these two entities (Meer et al., 2011) Geminin presented insignificant differences in expression among various SGT, but notably mcm-2 presented high sensitivity in discriminating PAC from AdCC (Vargas et al., 2008a).

Other SGTs (Table 2)

Other SGTs occasionally mimic PAC histologic appearance and may result in diagnostic pitfalls, although less frequently than those between AdCC and PAC. MASC presents a similar IHC pattern to PAC regarding S-100 and MG expression, but the two entities can be discriminated using FISH for ETV6 re-arrangement, which is considered a pathognomonic feature for MASC (Bishop et al., 2013; Patel et al., 2013). It is of note though that in a case series of 62 SGTs, MG expression was able to differentiate MASC, ACC, AdCC and PAC (Projetti et al., 2015) while another study reported that p63 cytoplasmic expression was also significantly different in PAC and MASC specimens and thus should be further evaluated (Foschini et al., 2005).

Occasionally, CA and PAC can present overlapping histological features and thus several studies evaluate the ability of IHC to differentiate these entities. The evaluation of the role of cytokeratins (CK) CK7/CK8/CK20 and vimentin in a case series of 15 SGT (PAC and CA) reported that although CKs were present in both PAC and AdCC, vimentin was able to discriminate CA from PAC (Furuse et al., 2003; Nikitakis et al., 2004). In the same direction, another SGT case series revealed a distinct IHC pattern of Glial Fibrillary acidic protein (GFAP) that allows differentiation between CA and PAC (Curran et al., 2007). Interestingly, GFAP expression was also present in focal or diffuse pattern in cellular mixed tumors and thus, GFAP evaluation is also proposed in differential diagnosis of these cases with PAC (Gnepp et al., 1997).

Besides AdCC, structural chromosomal abnormalities have also been associated with other SGT (Schwartz et al., 2011; Bishop et al., 2013; Argyris et al., 2016). In this aspect, FISH for the t(11;19) translocation, which is highly specific for MEC, was negative in PAC specimens, indicating a distinctive feature between MEC and PAC (Schwartz et al., 2011).

The absence of mucous cells is another feature of PAC with potential utility in differential diagnosis with other SGT entities. In this aspect, the Palate, lung and nasal epithelial clone (PLUNC) family of proteins (SPLUNC-1, SPLUNC-2, LPLUNC-1) has been evaluated (Vargas et al., 2008a,b). As expected, PAC specimens were negative, while mucous cell-containing tumors presented strong positivity (Papillary, cystadenocarcinoma, MEC) indicating the need for further investigation of these molecules (Vargas et al., 2008a,b).

Prognostic biomarkers (Table 3)

The establishment of prognostic biomarkers for PAC could provide further information on tumor behavior and a more personalized approach based on each tumor's particular characteristics. Additionally, aggressive variants of PLGA, presenting rapid growth and high metastatic potential have been occasionally reported in

the literature (Simpson et al., 2002; Kumar et al., 2004; Arora et al., 2013; Thennavan et al., 2013). The identification of these subtypes has tremendous impact in both prognosis and therapeutic management. Despite the fact that several molecules, most of them from the pool of candidate diagnostic biomarkers, have been evaluated, until today there is no reported correlation between candidate markers and clinical outcomes. An overview of these results, despite being negative, might provide useful insights in PAC pathogenesis and contribute in future identification of prognostic biomarkers.

Mcm-2 and maspin, besides their role in PAC diagnosis, were evaluated for correlation with clinical parameters (metastatic potential, risk of recurrences etc.) but no associations were reported (Vargas et al., 2008a,b; Ghazy et al., 2011). Another study evaluated the expression of growth factors (GFs) and GF-receptors (GFR) and reported significantly increased levels compared to normal salivary gland tissues (Rosa et al., 2016). The authors proposed a potential role in tumor formation and progression, related to proliferation and decreased apoptosis. Notably, no further associations with tumor behavior or clinical parameters were demonstrated.

Osteopontin, which is aberrantly expressed in PAC, failed to correlate with other clinical and prognostic parameters (Darling et al., 2006; Fok et al., 2013). Absence of prognostic potential was also reported after evaluation of Hyaluronan and CD44, Integrin β1, β3, β4 and β6, tenascin-C and Matrix metalloproteinase-1 (MMP-1) (Xing et al., 1998; Andreadis et al., 2006; Vargas et al., 2008a; Fok et al., 2013). Another study in SGTs with myoepithelial differentiation (PAC, AdCC, PA etc.) evaluated p53 and c-ErbB-2 expression in terms of recurrence risk and reported the absence of prognostic correlation. According to these findings, despite their eminent role in several neoplasms, p53 and c-ErbB-2 are not involved in PAC formation and progression (Rosa et al., 1997).

Several studies report strong, uniform expression of Bcl-2 among PAC samples (Soares et al., 2017; Kikuchi et al., 2018; Perez-Ordonez et al., 1998). However, it is suggested that the ratio of pro- and anti-apoptotic factors is more indicative than the expression of a single factor (Wong, 2011). Besides its role in apoptosis, Bcl-2 has also beem associated with tumor cell invasion and migration (Um HD, 2016). At present it is not clear whether either Bcl-2 alone or in pro- / anti-apoptotic factor ratio can be associated with particular clinicopathological parameters of PAC.

Genetic features

Molecular and cytogenetic studies are limited in PAC and as a result, its molecular pathogenesis is poorly understood (Klijanienko and Viehl, 2000). Chromosome abnormalities have been reported in some cases, with most of them being located in chromosomes 8 (regions 8q12, 8q13-15) and 12 (regions 12q12- q13, 12q22 and 12p12.3) (Mark et al., 1991, 1992; Martins et al., 2001). It is of note that structural abnormalities in chromosome 12 are also reported in AdCC specimens (Martins et al., 2001). The authors proposed a theory of shared histogenesis between PAC and AdCC but the lack of functional data to support this statement combined with inconsistent future findings have not confirmed it. Other reported deviations include two cases of monosomy 22 and reciprocal t(6;9) (p21;p22) respectively (Mark et al., 1992; Dahlenfors et al., 1997). Small samples and inability to correlate these findings with cell products and procedures prevented for years an integrated approach in PAC pathogenesis.

However, novel molecular techniques (e.g. WGS and RNA seq.) provided further insights into the processes resulting in tumor formation and progression. Consistent with the observation that several malignant SGTs arise from particular somatic genetic alterations, recent findings suggested that the majority of a PAC series (8 /12 specimens) was driven by Protein kinase D1 (PRKD1) hotspot mutations (Weinreb et al., 2014). Another study on 13 PAC samples identified 11/13 PRKD1mut (+) specimens (Wysocki et al., 2017). Despite the fact that PRKD1 mutations are also observed in other malignancies, the particular PRKD1 mutations observed in PAC samples (c.2130A>T and c.2130A>C) were not reported in other tumors (Weinreb et al., 2014). Thus these single-nucleotide variants (SNV) were considered pathognomonic for PAC. Genomic rearrangements in the PRKD family were also observed in a subset of PAC tumors of a large cohort of SGTs (Piscuoglio et al., 2016).

PRKD1 gene, located in in chromosome 14q12, encodes a kinase involved in cell adhesion and migration, affecting cell survival. c.2130A>T and c.2130A>C mutations result in a p.Glu710Asp alteration which affects PRKD1 catalytic region and thus disorganizes vital cell procedures (Weinreb et al., 2014; OMIM #605435.2016; Pisquoglio et al., 2016). PRKD family is a highly conserved region of the genome and includes besides PRKD1, PRKD2 and PRKD3 which present similar cellular activity (Weinreb et al., 2014; OMIM #605435.2016). It is of note though that a small subset of PACs is not driven by PRKD related alterations (somatic mutations or rearrangements), indicating that besides phenotypic variation, PACs also present significant genotypic heterogeneity (Piscuoglio et al., 2016). The evaluation of genomic imbalances in PAC, performing genome-wide, high-resolution array comparative genomic hybridization (aCGH) analysis revealed genetic stability and significantly fewer copy number alterations than AdCC (Persson et al., 2012). These data are in agreement with the tumors' clinical behavior: PAC usually presents low growth rate and metastatic potential while AdCC is far more aggressive, presenting high growth rate and metastatic potential (Klijanienko and Viehl, 2000; Gnepp et al., 2009). Interestingly, one of the PAC specimens was positive for

the MYB-NFIB1 fusion, which is a characteristic translocation of AdCC (Persson et al., 2012). The authors proposed that instead of PAC, this specimen could be a low grade AdCC variant.

Research limitations and future perspectives

Despite being described thirty-two years ago, until recently information on PAC was limited. The SGTs literature has dramatically expanded during the last decade and the knowledge tank has grown. Besides quantity, qualitative changes were also noted over the previous years. Larger series of patients obtained from one or more centers were reported (Seethala et al., 2010; Patel et al., 2015; Elhakim et al., 2016) allowing better study designs and methods compared to the past, when the majority of cases were reported as case reports/ case series. Large PAC cohorts shed light on tumor behavior and allowed more accurate evaluation of diagnostic and prognostic parameters reported (Seethala et al., 2010; Patel et al., 2015; Elhakim et al., 2016).

Additionally, novel molecular techniques provided a more integrated approach of PAC etiopathogenesis (Persson et al., 2012; Weinreb et al., 2014; Piscuoglio et al., 2016). However, PAC research has a long road to go until this knowledge can be applied to clinical practice. Most of the current information on the tumor's etiology and molecular pathologenesis is still derived from small cohorts and at present there are no qualitative synthesis studies (systematic reviews/ meta-analyses). Significant progress has been achieved in the identification of driver genes (PKRD family) however we do not have the full picture yet. PRKD region is linked to other genome regions and alterations in them could affect this oncogenic path or create an alternative path. As a result, the presence of mutations or other genetic abnormalities should be explored in relative regions and the role of epigenetic modifications on the affected regions should be examined (Weinreb et al., 2014; Piscuoglio et al., 2016)

Noteworthy, despite the fact that PAC has been studied in terms of histopathology, immunohisto-chemistry and recently molecular biology, no correlation between these features and the biological behavior has been established. Tumor heterogeneity affects histological architecture, immunohistochemical expression patterns and molecular profile, but classification of PACs under any of these categories fails to associate with prognostic or predictive parameters. Although a subset of aggressive PACs has been described there are no known features that permit the identification of this variant before the expression of its clinical behavior. Further investigation of these tumors is required in order to unravel their characteristics and identify markers that allow early detection.

It is also important to address the issue of the cribriform variant (Brandwein-Gensler et al., 2017). There is a discussion on whether these tumors should be classified as cribriform pattern of PAC or as a new entity

(Michal et al., 1999; Skalova et al., 2011; Chi et al., 2015; Brandwein-Gensler et al., 2017). To address this issue, more evidence is required on the cribriform variant's clinical behavior and histopathological features. This variant should be investigated for distinct patterns of tumor progression and if present, whether they result in a unique pattern of IHC expression of known PAC markers. Also the prognostic and predictive parameters of this entity should be examined and compared to the data of PAC. New studies emphasizing these features will guide us towards one direction or another in this inconclusive matter. Another issue to be faced is the genotype heterogeneity among tumors considered as PAC.

The progress of molecular techniques has changed tumor classification approaches from observational to genomic and molecular signature-based approaches (Ashworth et al., 2011; Weigelt and Reis-Filho, 2014). Several studies indicate distinct genotypes among PAC specimens (Mark et al., 1991, 1992; Dahlenfors et al., 1997; Martins et al., 2001; Piscuoglio et al., 2016). This fact combined with the well-demonstrated clinical and histological heterogeneity of the tumor (Thennavan et al., 2013; Patel et al., 2015) has led some authors to suggest that PAC constitutes a convergent phenotype (Piscuoglio et al., 2016). Further studies to identify the driver genes of PAC and the potential role of epigenetic modifications are demanded in order to conclude.

Conclusion

PAC is a rare SGT presenting clinical heterogeneity, cellular uniformity and several histological patterns. Normally, the tumor presents a non-aggressive clinical behavior but there is a subset of reported cases that present aggressive characteristics. This resulted in the revised term "polymorphous adenocarcinoma" instead of the former term "polymorphous low-grade adenocarcinoma". The tumor's clinical and microscopic features can overlap with other SGT and histological differential diagnosis from AdCC is occasionally quite a challenging procedure. The establishment of an unequivocal diagnosis of either entity is critical for treatment planning and prognosis. In this aspect, several IHC markers have been evaluated, with Ki-67, c-Kit, S-100/MG, Mcm-2 and Integrin β-1, -3, -4 being the most promising ones. However, current data are inconclusive and mostly derived from small sample series. Further investigation is demanded for identification of reliable IHC markers with a translational potential into laboratory practice. Overlapping features of PAC and other SGTs (e.g. MASC, MEC) resulting in diagnostic dilemmas are far less frequent and even in such cases, IHC patterns or FISH are adequate for diagnosis. At present, tumor behavior is not related with any known marker. Despite the relatively innocuous cytologic appearance, aggressive variants reported in the literature, highlight the necessity of prognostic markers

in PAC.

Several genetic abnormalities have been reported in tumor specimens suggesting potential players in PAC pathogenesis. However, only PRKD1 gene has been identified as driver gene in a subset of PAC samples. Other members of the same pathway including genetic abnormalities and epigenetic alterations and driver genes with a causative role in the PRKD1(-) tumors remain to be presumed. The emerging genetic variants of PAC and the possibility of distinct tumor profiles are questioning the integrity of the entity.

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