Annals of Diagnostic Pathology 22 (2016) 12–17

Contents lists available at ScienceDirect

Annals of Diagnostic Pathology

Myoepithelial differentiation in cribriform, tubular and solid pattern of adenoid cystic carcinoma: A potential involvement in histological grading and prognosis

Fei Du, MDS, Chuan-Xiang Zhou, DDS, PhD *, Yan Gao, DDS, PhD *

Department of Oral Pathology, Peking University School and Hospital of Stomatology, 22 South Avenue Zhongguancun, Haidian District, Beijing 100081, PR China

ARTICLE INFO

Keywords:
adenoid cystic carcinoma
solid pattern
myoepithelial differentiation
histograding
prognosis

ABSTRACT

Adenoid cystic carcinoma (AdCC) is known as a biphasic tumor composed of ductal and myoepithelial cells. The present study aimed to evaluate the amount and distribution of the myoepithelial cells in cribriform, tubular and solid subtypes of AdCC and analyze their relationship with histological grading and prognosis. A panel of myoepithelial markers including CK5/6, p63, p40, D2–40, calponin, α-SMA, S-100, and vimentin, together with a luminal cell marker CK7, and Ki-67 were used for immunohistochemical study in 109 AdCCs that included 38 cribriform, 36 tubular and 35 solid subtypes. The myoepithelial cells were labeled and found lined cystic-like paces, located at the periphery of the cribriform arrangements, and presented at the nonluminal cells of the two-layered tubular structures, while absent or dispersed in the solid pattern. Meantime, the solid subtype presented a higher proliferation rate assessed by mitotic count and Ki-67 labeling index, followed by poorer overall survival and recurrent-free survival. Furthermore, CK7 expression was found higher in solid pattern than in cribriform-tubular subtype, which showed negative correlation with the myoepithelial markers including D2–40, Calponin, α-SMA, p63, p40 and vimentin. The solid pattern of AdCC showed gland differentiation but loss of myoepithelial differentiation with a higher proliferation and more aggressiveness as well as poorer prognosis compared with the cribriform-tubular subtypes, which implies that loss of MEC differentiation might contribute to the poor prognosis of the solid subtype of AdCC. However, further studies are required to clarify its exact role in AdCC progression.

1. Introduction

Adenoid cystic carcinoma (AdCC) of the salivary glands is one of the most common salivary gland malignancies that accounts for 10–15% of all salivary neoplasms [1]. It is characterized by slow growth, high incidence of perineural invasion, local recurrence, and distant metastasis [2]. Histopathologically, AdCC is identified as a tumor with biphasic differentiation of epithelial and myoepithelial cell (MEC), which often shows mixed patterns of cribriform, tubular and solid types. It is usually classified according to the predominant growth pattern [3].The cribriform pattern is the most frequent, showing nests of cells with microcystic spaces, and the tubular pattern consists of tubules with central lumina lined by inner epithelial and outer MECs. Generally, the tubular and cribriform patterns are known for their indolent behavior, while the solid subtype, formed of sheets of basaloid cells lacking tubular or microcystic formation, is considered high grade with more aggressiveness and poorer prognosis [4]. Although AdCC is known as a biphasic tumor composed of ductal and MECs, the amount and distribution of the MECs in different subtypes and their relation to the grading and prognosis remain controversial.

MECs differentiate and form different morphological cell types in salivary gland tumors, such as basaloid, clear and spindle cells. Identification of modified MECs is valuable for the accurate diagnosis of various salivary gland tumors. However, neoplastic MECs are difficult to be defined in routine sections due to its morphological and phenotypic alterations, and immunohistochemistry is helpful [5]. A number of MEC markers have been commonly used, including smooth muscle actin, S-100, calponin, and p63, with varying sensitivity and specificity. Recently it has been suggested that D2–40 and p40 can be used to label MECs [6–8].

In the present study, we propose to investigate the amount and distribution of the MECs in cribriform, tubular and solid subtypes of AdCC

* Corresponding authors at: Peking University School and Hospital of Stomatology, 22 South Avenue Zhongguancun, Haidian District, Beijing, 100081, PR China. Tel.: +86 10 82195221; fax: + 86 10 62173402.
E-mail addresses: zhoucx2008@126.com (C.-X. Zhou), gaoyan0988@163.com (Y. Gao).

http://dx.doi.org/10.1016/j.anndiagpath.2016.03.001
1092-9134/

with the use of a panel of MEC markers and analyze the relationship between myoepithelial differentiation and the histological grading and prognosis of AdCC.

2. Materials and methods

2.1. Patients and tissue samples

Cases diagnosed as AdCC were reviewed from the files of Peking University School and Hospital of Stomatology during 2000–2014 and a total of 109 AdCCs were confirmed and selected for this study. Patients' follow-up data were obtained by clinical interviews or reviewing the medical records after surgery. The corresponding 109 paraffin-embedded samples were derived from the archives of the Department of Oral Pathology, Peking University School of Stomatology following the approval of the University Institutional Ethics Committee. The histological subtypes of the samples were decided based on the World Health Organization's histological classification of salivary gland tumors.

2.2. Immunohistochemistry

Immunohistochemistry was performed using the ChemMate Envision™ system as described previously [9]. Immunostaining was performed by adding the primary antibodies, incubated overnight at 4 °C and followed by 30 min incubation with the secondary antibody. The primary antibody was replaced by phosphate-buffered saline in negative control sections. The immunocomplexes were visualized using liquid DAB + substrate + chromogen system (Zymed), and slides were counterstained with Hematoxylin. The details of the primary antibodies used are listed in Table 1.

2.3. Evaluation of immunostainings

The staining intensity and pattern were assessed separately by two independent pathologists who were blinded for the information of each patient. Reactivity was determined according to the percentage of positive cells: up to 1% positive cells were scored as 0, 2–25% as 1, 26–50% as 2, 51–75% as 3, and over 75% as 4. Intensity was graded as follows: negative (no staining); 1+ (weakly positive); 2+ (moderately positive); and 3+ (strongly positive). A total score of 0–12 was finally calculated and graded as negative (−; score: 0–1), weak (+; 2–4), moderate (++; 5–8), and strong (+++; 9–12) [10]. For Ki-67, approximately 1000 nuclei were counted on each slide. The proliferative activity was assessed as the percentage of Ki-67-stained nuclei (Ki-67 labeling index). The median value of the Ki-67 labeling index was calculated.

2.4. Statistical analysis

All statistical analyses were performed using SPSS Version 20.0. Chi-squared or Fisher's exact tests and T tests were used to compare the variables between groups. Kaplan–Meier analysis was used to assess the prognostic of different variants for overall survival and recurrent-free survival, and survival differences were analyzed by the log-rank test. Differences at $P < .050$ were considered significant.

3. Results

There were 35 solid, 38 cribriform and 36 tubular subtypes of AdCCs. Patients' follow-up data from 20 solid and 44 cribriform-tubular variants were obtained and analyzed. The solid variants were found correlated with poorer overall survival and recurrent-free survival compared with cribriform-tubular variants (Fig. 1). The clinicopathologic characteristics of 109 AdCCs are summarized in Table 2.

MECs were labeled with a panel of markers including p63, p40, calponin, α-SMA, S-100, vimentin, CK5/6 and D2–40. The results of immunostaining are summarized in Table 3. In general, MECs were found lined cystic-like paces, located at the periphery of the cribriform arrangements and present at the nonluminal cells of the two-layered tubular structures, while absent or dispersed in the solid pattern. Intense staining for D2–40, calponin, α-SMA and vimentin were observed in the cytoplasm and/or membrane of tumor cells in cribriform and tubular subtypes, while negative or weak expression was detected in solid pattern (Fig. 2). The same is true of p63 and p40 expression profiles, while they were nuclear staining (Fig. 3). Meantime, p40 seems more specific than p63 for MECs' identification. S-100 expression was found in both nonluminal and luminal cells and not related to other MEC markers, showing no obvious difference among three subtypes (Fig. 3, Table 3). CK5/6 staining was also detected in luminal and nonluminal cells (Fig. 4). CK7 expression was found higher in solid pattern than in cribriform-tubular subtype and negatively correlated with MEC markers (Fig. 4). The mean Ki-67 proliferation index was 14.28 ± 11.34% in cribriform-tubular patterns while 41.86 ± 18.45% in solid variants, showing evident difference (Fig. 4).

4. Discussion

In the present study, we investigated the amount and distribution of myoepithelial cell (MEC) in tubular, cribriform, and solid growth patterns of AdCC using a panel of MEC markers including p63, p40, calponin, α-SMA, S-100, vimentin, CK5/6 and D2–40. Compared with cribriform and tubular subtypes, the solid pattern showed much less MECs with a higher proliferation rate assessed by mitotic count and Ki-67 labeling index. Futhermore, these tumor cells were mostly intensely stained for CK7, a commonly used luminal cell marker. Taken together, we demonstrated that the cribriform-tubular subtype of AdCC is a biphasic tumor with MEC differentiation, while the tumor cells in the solid growth pattern show gland differentiation with loss of MEC differentiation.

AdCCs often exhibit mixed patterns of cribriform, tubular and solid types, classified according to the predominant pattern. Compared with other two types, the solid subtype showed more aggressive behavior and relatively poorer short-term survival, which is considered as a high grade lesion. As early as 1958, Patey and Thackray [11] proposed

Table 1
Detail information of primary antibodies.

Antibody	Clone	Company	Dilution	Pretreatment
D2–40	D2–40	Dako,USA	1:100	Tris–EDTA, pH 8.9
Calponin	Calp	LabVision,UK	1:100	Citrate 0.01 M; pH 6.0
CK5/6	CK5/6.007	CellMarque,California,USA	1:100	Tris–EDTA, pH 8.9
α-SMA	EPR5368	Abcam,UK	1:100	Citrate 0.01 M; pH 6.0
S-100	polyclonal	Novocastra,UK	1:100	Citrate 0.01 M; pH 6.0
P63	A4A	GeneTech,China	1:100	Citrate 0.01 M; pH 6.0
P40	BC28	BiocareMedical, California,USA	1:70	Tris–EDTA, pH 8.9
Vimentin	V9	Invitrogen,California,USA	1:100	Tris–EDTA, pH 8.9
CK7	RN7	CellMarque,California,USA	1:100	Tris–EDTA, pH 8.9
Ki-67	MIB1	Origene, Maryland, USA	1:100	Citrate 0.01 M; pH 6.0

a-SMA, a-smooth muscle actin; CK, cytokeratin.

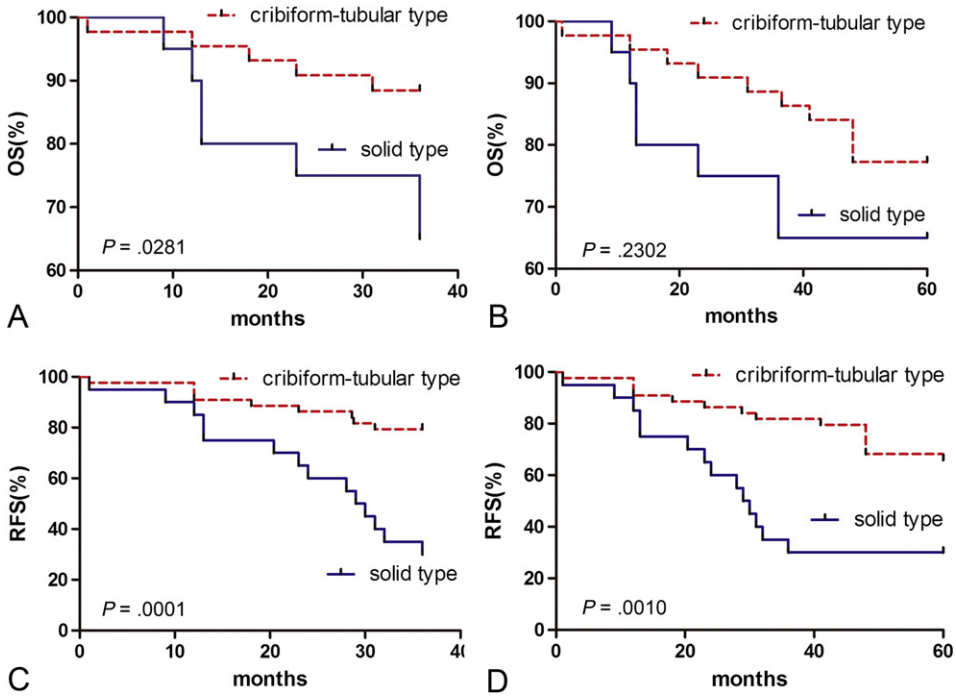

Fig. 1. Kaplan–Meier curves of 3-year and 5-year overall survival (A and B) as well as 3-year and 5-year recurrent-free survival (C and D) between solid type and cribriform-tubular type AdCC. *P*-values were calculated by the log-rank test.

that a solid growth pattern reveals a poor prognosis. Subsequently, grading of this tumor has evolved into stratification into 3 grades with increasing aggressiveness based on predominant growth pattern: tubular as Grade I, cribriform as Grade II, and solid type as Grade III. Regarding the histopathological grading of AdCC, several systems have been proposed. In Perzin/Szanto system, AdCC is considered high grade if a solid component represents more than 30% of the tumor, while Spiro recommended only when the solid parts account for more than 50% of the tumor it is considered high grade [12–14]. Recently, van Weert et al. proposed the presence of a solid pattern regardless of its quantity is a poor prognosticator. Anyway, the solid growth pattern is related to more aggressiveness, poorer short-term survival and probably higher risk of nodal metastasis and early distant metastasis [15–18]. In this study, all the 35 cases of solid subtypes have a solid component more than 30% of the tumor. We found although the cribriform and tubular subtypes of AdCC consist of biphasic differentiation with MECs, the solid growth pattern showed gland

Table 2
Clinicopathologic characteristics of 109 adenoid cystic carcinomas.

Patient Characteristics (N = 109)	N (%)
Age	
<55 years	59(54)
≥55 years	50(46)
Gender	
Male	42(39)
Female	67(61)
Salivary gland type	
Major	39(36)
Minor	70(64)
Primary site	
Parotid	10(9)
Submandibulargland	18(17)
palate	43(39)
Other	38(35)
Histological subtype	
Tubular	38(35)
Cribriform	36(33)
Solid	35(32)
Perineural invasion	
Yes	72(66)
No	37(34)

N, number of cases.

Table 3
Immunostaining of myoepithelial markers and CK7 in different histological subtypes.

		−	+	++	+++	X^2	P-value
D2–40	Cribriform(n = 38)	3	24	7	4	51.910*	0.000
	Tubular(n = 36)	6	24	5	1		
	Solid(n = 35)	28	4	3	0		
Calponin	Cribriform(n = 38)	11	18	4	5	37.188*	0.000
	Tubular(n = 36)	6	24	5	1		
	Solid(n = 35)	28	7	0	0		
CK5/6	Cribriform(n = 38)	0	13	19	6	18.377*	0.003
	Tubular(n = 36)	1	8	20	7		
	Solid(n = 35)	7	11	17	0		
α-SMA	Cribriform(n = 38)	1	18	14	5	55.726*	0.000
	Tubular(n = 36)	3	24	5	4		
	Solid(n = 35)	24	10	1	0		
S-100	Cribriform(n = 38)	12	15	10	1	5.453*	0.460
	Tubular(n = 36)	6	18	12	0		
	Solid(n = 35)	7	20	7	1		
P63	Cribriform(n = 38)	0	2	15	21	79.538α	0.000
	Tubular(n = 36)	1	9	21	5		
	Solid(n = 35)	22	6	7	0		
P40	Cribriform(n = 38)	1	6	29	2	67.295*	0.000
	Tubular(n = 36)	1	16	19	0		
	Solid(n = 35)	25	5	5	0		
Vimentin	Cribriform(n = 38)	0	13	25	0	28.257*	0.000
	Tubular(n = 36)	3	21	12	0		
	Solid(n = 35)	8	23	4	0		
CK7	Cribriform(n = 38)	1	22	13	2	18.341*	0.001
	Tubular(n = 36)	1	14	19	1		
	Solid(n = 35)	0	5	28	2		

negative (−; score: 0–1), weak (+; 2–4), moderate (++; 5–8), and strong (+++; 9–12).
α Pearson Chi-Square.
* Fisher's Exact Test.

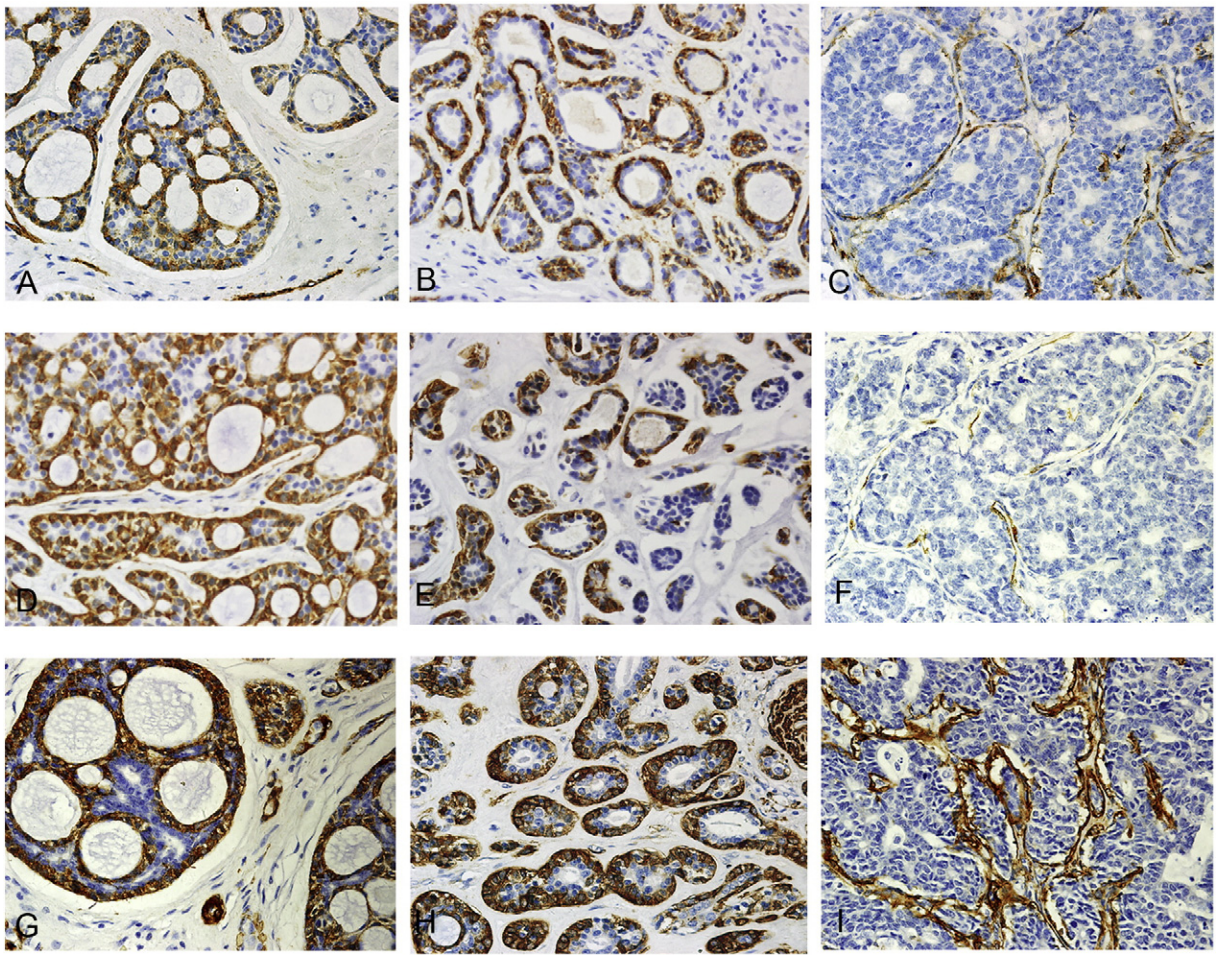

Fig. 2. D2–40 immunostaining in cribriform (A), tubular (B) and solid pattern (C). Calponin immunostaining in cribriform (D), tubular (E) and solid pattern (F). Immunoreactivity for a -smooth muscle actin in cribriform (G), tubular (H) and solid pattern (I). (All images, original magnifications × 400).

differentiation but loss of MEC differentiation, followed by higher aggressiveness and proliferation rate. Therefore, we hypothesized loss of MEC differentiation might contribute to the poor prognosis of the solid subtype of AdCC.

MEC is a normal component of the salivary acini and ducts, locates between the epithelial cells and the basement membrane. During embryonic development, MEC is involved in branching morphogenesis of developing salivary gland and promotion of epithelial cell differentiation. Recently, many lines of evidence suggest MECs are tumor suppressors as they can inhibit cell proliferation and invasiveness through secreting suppressor proteins [19]. Sternlicht et al. recommended myoepithelial cells might not only function as natural paracrine suppressors of invasion and metastasis but specifically inhibit the progression of precancerous disease states to invasive cancer [20]. Gudjonsson et al. suggested that MECs play a crucial role in maintenance of polarity in normal breast and act as structural tumor suppressors [21]. Farhanji et al. demonstrated the inhibitory effects of MECs on cell viability of cancerous cells [22]. It is accepted that MECs have an important regulatory role in breast cancer pathology via influencing epithelial and luminal compartments. Although myoepithelial proliferations in various salivary gland tumors have been reported, little is known on the role of MECs in tumor progression. Sternlicht et al. found MECs have a lower proliferation rate than basal type epithelial cells and secrete excess substances inhibiting invasion and metastasis in salivary gland tumors [23]. These accumulated myxoid ground substances and basement membrane components as well as numerous proteinase inhibitors contribute to an anti-invasive matrix for myoepithelial-rich salivary gland tumors. Actually, epithelial cells are known to be more susceptible to the transforming events leading to cancer than myoepithelial cells, and tumors comprised of myoepithelial cells are usually benign or low grade, exhibiting the property of accumulating an abundant extracellular matrix. Recently, high-grade transformation has been described in AdCC, and consistently devoid of myoepithelial differentiation was found in the transformed areas of AdCC. Taken together, it indicates loss of MEC differentiation in solid pattern of AdCC has a potential role in grading and poor prognosis.

To assess the participation of MECs in salivary gland neoplasia, it is pivotal to define neoplastic MECs. However, identification of neoplastic MECs on routine sections is difficult due to its histologic diversity, and immunohistochemistry is helpful. MECs possess a dual smooth muscle and epithelial phenotype with the potential of undergoing divergent differentiation. In the present study, classic MEC markers, calponin and α-SMA, were used as related to the smooth muscle apparatus of MECs. CK5/6 was used according to the epithelial property of MECs. S100 and p63 were also used as previously reported. Meantime, D2–40 and p40, the newly reported MEC markers were detected. D2–40 is a commercially available mouse monoclonal antibody directed against human podoplanin, a mucin-type transmemebrane protein [24]. D2–40 is often used for highlighting lymphatic and also found to stain MECs [25,26]. As a new antibody recognizing the p63 isoform ΔNp63, p40 is reported superior to p63 for squamous differentiation in the differential diagnosis of non-small cell lung cancer [27], which also appears to be similar to p63 as a MEC marker in breast carcinomas [28,29]. Herein, we compared p40 and p63 staining in MECs and found p40 seems more specific than p63 for identification of myoepithelial differentiation. D2–40 expression was observed in the cytoplasm of MECs in

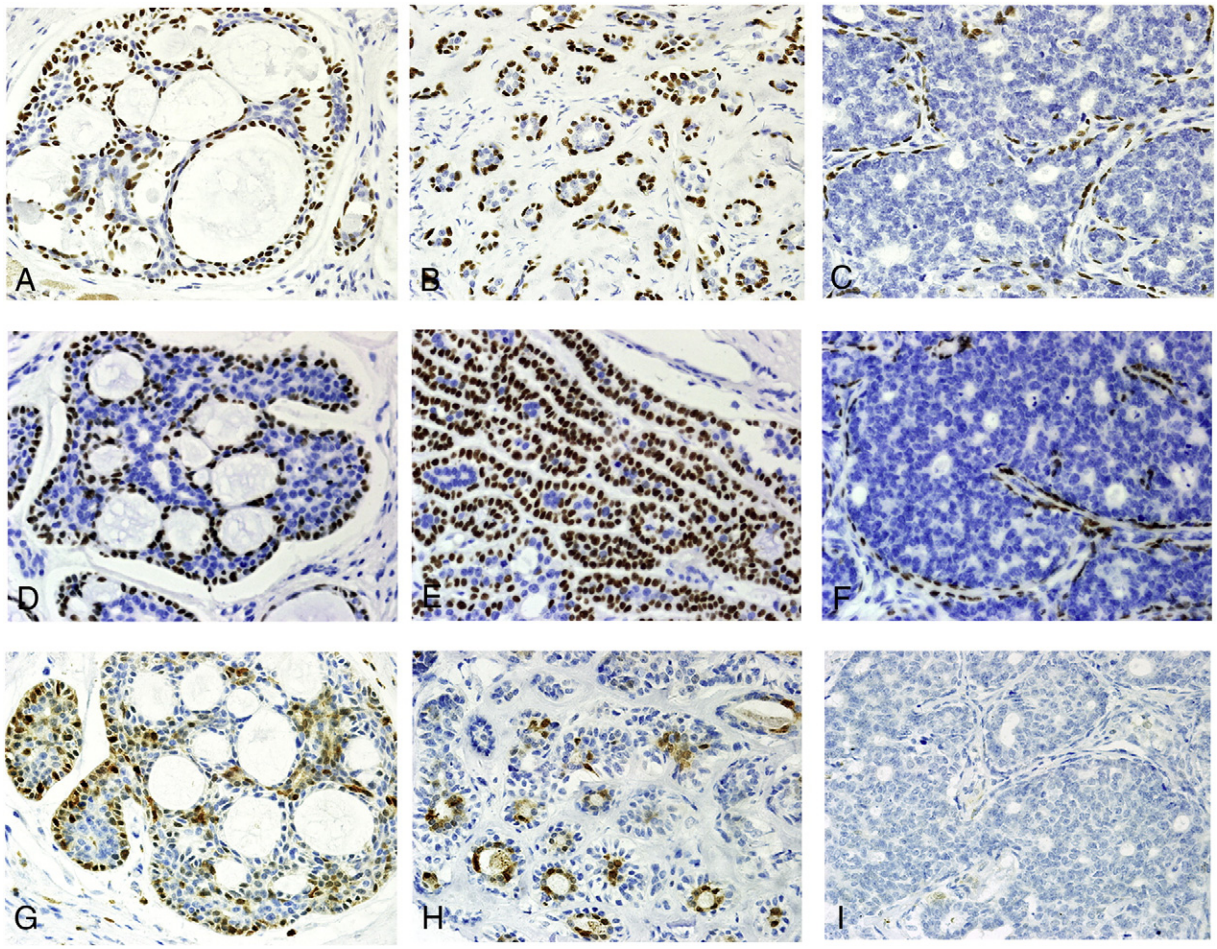

Fig. 3. P63 immunostaining in cribriform (A), tubular (B) and solid pattern (C). P40 immunostaining in cribriform (D), tubular (E) and solid pattern (F). Immunoreactivity for S-100 in cribriform (G), tubular (H) and solid pattern (I). (All images, original magnifications × 400).

cribriform-tubular patterns, but mostly negative in solid pattern. Although S-100 protein is considered as a sensitive marker for neoplastic MECs, the staining for S-100 has been reported variable for different cell types in salivary gland tumors, which is also present from the earliest stages of salivary gland maturation [30]. In this study, S-100 expression was detected in both myoepithelial and ductal cells, indicating it is not sufficiently specific [31]. Taken together, a panel of markers including CK5/6, p40, calponin, α-SMA, D2–40 and S-100 protein is recommended to be used for identification neoplastic myoepithelial differentiation.

In conclusion, we found the solid pattern of AdCC showed gland differentiation but loss of MEC differentiation with higher proliferation and aggressiveness compared with the cribriform-tubular types, which implies the potential involvement of MEC differentiation in the grading and prognosis of AdCCs. However, further studies are required to clarify the exact role of MECs in AdCC progression.

Conflict of interests

None.

Acknowledgment

This work was supported by research grants from the National Nature Science Foundation of China (303075838).

References

[1] van der Wal JE, Becking AG, Snow GB, van der Waal I. Distant metastases of adenoid cystic carcinoma of the salivary glands and the value of diagnostic examinations during follow-up. Head Neck 2002;24(8):779–83.
[2] Khafif A, Anavi Y, Haviv J, Fienmesser R, Calderon S, Marshak G. Adenoid cystic carcinoma of the salivary glands: a 20-year review with long-term follow-up. Ear Nose Throat J 2005;84(10):662 [664-7].
[3] Da CPD, de Abreu AF, Nobuko NI, de Almeida OP, Kowalski LP. Prognostic factors in head and neck adenoid cystic carcinoma. Oral Oncol 2006;42(2):139–46.
[4] Barrett AW, Speight PM. Perineural invasion in adenoid cystic carcinoma of the salivary glands: a valid prognostic indicator? Oral Oncol 2009;45(11):936–40.
[5] Zhao L, Yang X, Khan A, Kandil D. Diagnostic role of immunohistochemistry in the evaluation of breast pathology specimens. Arch Pathol Lab Med 2014;138(1):16–24.
[6] Ren S, Abuel-Haija M, Khurana JS, Zhang X. D2-40: an additional marker for myoepithelial cells of breast and the precaution in interpreting tumor lymphovascular invasion. Int J Clin Exp Pathol 2011;4(2):175–82.
[7] de Araujo VC, Altemani A, Furuse C, Martins MT, de Araujo NS. Immunoprofile of reactive salivary myoepithelial cells in intraductal areas of carcinoma ex-pleomorphic adenoma. Oral Oncol 2006;42(10):1011–6.
[8] Rabban JT, Chen YY. D2-40 expression by breast myoepithelium: potential pitfalls in distinguishing intralymphatic carcinoma from in situ carcinoma. Hum Pathol 2008;39(2):175–83.
[9] Li YY, Zhou CX, Gao Y. Snail regulates the motility of oral cancer cells via RhoA/Cdc42/p-ERM pathway. Biochem Biophys Res Commun 2014;452(3):490–6.
[10] Li YY, Zhou CX, Gao Y. Moesin regulates the motility of oral cancer cells via MT1-MMP and E-cadherin/p120-catenin adhesion complex. Oral Oncol 2015;51(10):935–43.
[11] Patey DH, Thackray AC. The treatment of parotid tumours in the light of a pathological study of parotidectomy material. Br J Surg 1958;45(193):477–87.
[12] Perzin KH, Gullane P, Clairmont AC. Adenoid cystic carcinomas arising in salivary glands: a correlation of histologic features and clinical course. Cancer Am Cancer Soc 1978;42(1):265–82.
[13] Szanto PA, Luna MA, Tortoledo ME, White RA. Histologic grading of adenoid cystic carcinoma of the salivary glands. Cancer Am Cancer Soc 1984;54(6):1062–9.

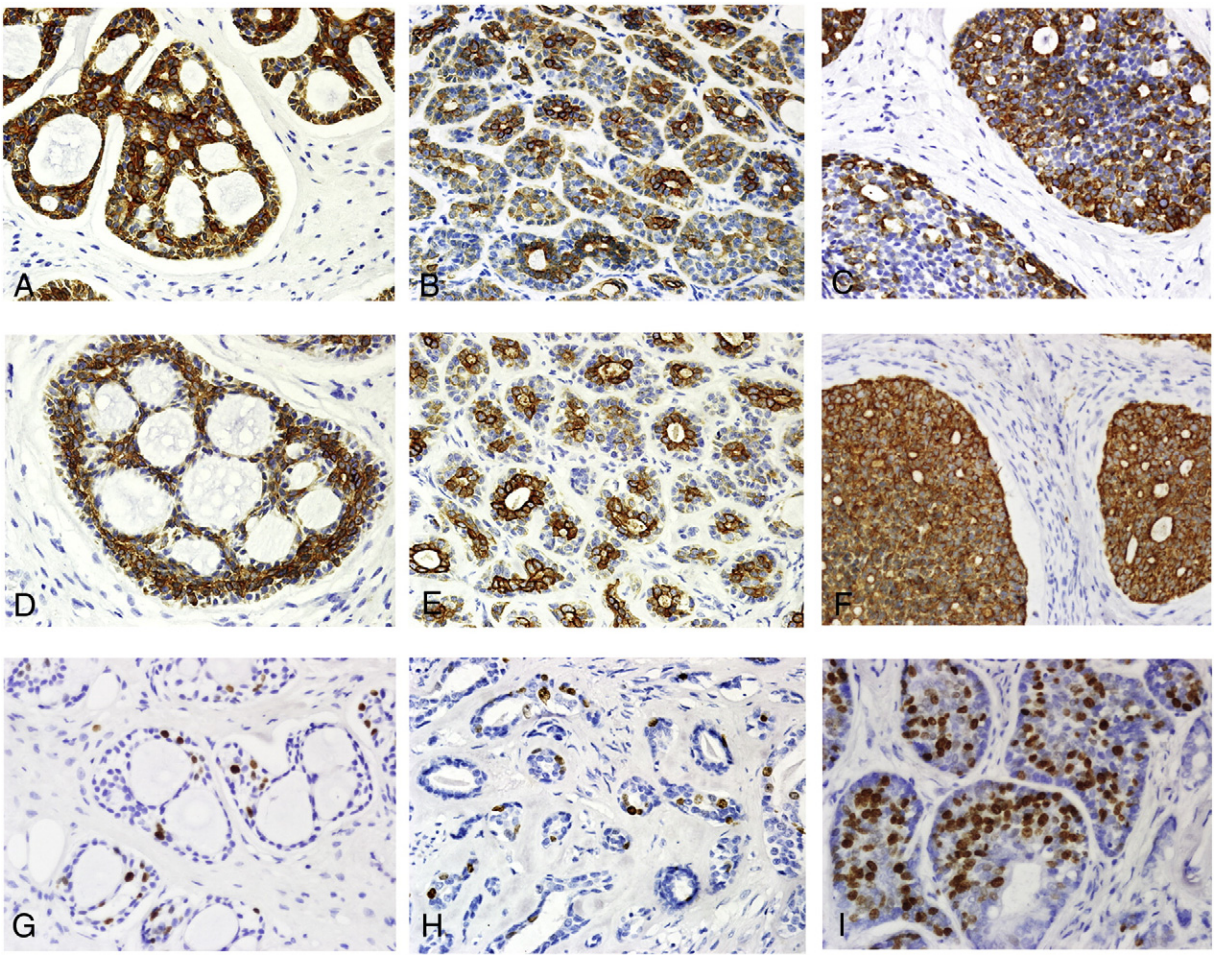

Fig. 4. CK5/6 immunostaining in cribriform (A), tubular (B) and solid pattern (C). CK7 immunostaining in cribriform (D), tubular (E) and solid pattern (F). Immunoreactivity for Ki-67 in cribriform (G), tubular (H) and solid pattern (I). (All images, original magnifications × 400).

[14] Spiro RH, Huvos AG, Strong EW. Adenoid cystic carcinoma of salivary origin. A clinicopathologic study of 242 cases. Am J Surg 1974;128(4):512–20.
[15] van Weert S, Bloemena E, van der Waal I, de Bree R, Rietveld DH, Kuik JD, et al. Adenoid cystic carcinoma of the head and neck: a single-center analysis of 105 consecutive cases over a 30-year period. Oral Oncol 2013;49(8):824–9.
[16] Khan AJ, DiGiovanna MP, Ross DA, Sasaki CT, Carter D, Son YH, et al. Adenoid cystic carcinoma: a retrospective clinical review. Int J Cancer 2001;96(3):149–58.
[17] Nascimento AG, Amaral AL, Prado LA, Kligerman J, Silveira TR. Adenoid cystic carcinoma of salivary glands. A study of 61 cases with clinicopathologic correlation. Cancer Am Cancer Soc 1986;57(2):312–9.
[18] Matsuba HM, Spector GJ, Thawley SE, Simpson JR, Mauney M, Pikul FJ. Adenoid cystic salivary gland carcinoma. A histopathologic review of treatment failure patterns. Cancer Am Cancer Soc 1986;57(3):519–24.
[19] Deugnier MA, Teuliere J, Faraldo MM, Thiery JP, Glukhova MA. The importance of being a myoepithelial cell. Breast Cancer Res 2002;4(6):224–30.
[20] Sternlicht MD, Kedeshian P, Shao ZM, Safarians S, Barsky SH. The human myoepithelial cell is a natural tumor suppressor. Clin Cancer Res 1997;3(11):1949–58.
[21] Gudjonsson T, Ronnov-Jessen L, Villadsen R, Rank F, Bissell MJ, Petersen OW. Normal and tumor-derived myoepithelial cells differ in their ability to interact with luminal breast epithelial cells for polarity and basement membrane deposition. J Cell Sci 2002;115(Pt 1):39–50.
[22] Farhanji B, Latifpour M, Alizadeh AM, Khodayari H, Khodayari S, Khaniki M, et al. Tumor suppression effects of myoepithelial cells on mice breast cancer. Eur J Pharmacol 2015;765:171–8.
[23] Sternlicht MD, Barsky SH. The myoepithelial defense: a host defense against cancer. Med Hypotheses 1997;48(1):37–46.
[24] Breiteneder-Geleff S, Soleiman A, Kowalski H, Horvat R, Amann G, Kriehuber E, et al. Angiosarcomas express mixed endothelial phenotypes of blood and lymphatic capillaries: podoplanin as a specific marker for lymphatic endothelium. Am J Pathol 1999;154(2):385–94.
[25] Schacht V, Dadras SS, Johnson LA, Jackson DG, Hong YK, Detmar M. Up-regulation of the lymphatic marker podoplanin, a mucin-type transmembrane glycoprotein, in human squamous cell carcinomas and germ cell tumors. Am J Patho 2005;166(3):913–21.
[26] Kanner WA, Galgano MT, Atkins KA. Podoplanin expression in basal and myoepithelial cells: utility and potential pitfalls. Appl Immunohistochem Mol Morphol 2010;18(3):226–30.
[27] Nonaka D. A study of DeltaNp63 expression in lung non-small cell carcinomas. Am J Surg Pathol 2012;36(6):895–9.
[28] Kovari B, Szasz AM, Kulka J, Marusic Z, Sarcevic B, Tiszlavicz L, et al. Evaluation of p40 as a myoepithelial marker in different breast lesions. Pathobiology 2015;82(3–4):166–71.
[29] Sarda R, Taylor J. p40 (DeltaNp63), a lung squamous cell marker, can also be used to label breast myoepithelial cells. Arch Pathol Lab Med 2014;138(5):584.
[30] Ianez RF, Buim ME, Coutinho-Camillo CM, Schultz R, Soares FA, Lourenco SV. Human salivary gland morphogenesis: myoepithelial cell maturation assessed by immunohistochemical markers. Histopathology 2010;57(3):410–7.
[31] Nagao T, Sato E, Inoue R, Oshiro H, T.R. H, Nagai T, et al. Immunohistochemical analysis of salivary gland tumors: application for surgical pathology practice. Acta Histochem Cytochem 2012;45(5):269–82.