• Basic Research •

Immunohistochemical features of carcinoma ex pleomorphic adenoma and pleomorphic adenoma in the lacrimal gland

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Abstract

• AIM: To investigate C-myc, Ki-67, pan-cytokeratin, and vimentin immunohistochemical features of carcinoma ex pleomorphic adenoma (Ca-ex-PA) and pleomorphic adenoma (PA) in the lacrimal gland in order to find some clues in the differential diagnosis between them.

• METHODS: We reviewed microscopic slides and clinical records of 64 cases of PA and 15 cases of Ca-ex-PA in the lacrimal gland. Immunohistochemical antibodies for C-myc, Ki-67, pan-cytokeratin, and vimentin were employed.

• RESULTS: Median age of PA was 43.2y (from 21 to 75). The 35 patients (54.7%) were male and 29 patients (45.3%) were female. For the PAs, the average positivity of C-myc was 4.6%; the average proliferation index of Ki-67 was 3.2%; pan-cytokeratin was positive in ductal cells, and vimentin was positive in myoepithelial cells. Median age of Ca-ex-PA was 54.3y (from 26 to 76). There were 7 male patients (46.7%) and 8 female patients (53.3%). Among 15 Ca-ex-PAs, there were 6 myoepithelial carcinomas, 4 adenocarcinomas, 3 epithelial-myoepithelial carcinomas, and 2 squamous cell carcinomas. For the Ca-ex-PAs, the average positivity of C-myc was 36.4%; the average proliferation index of Ki-67 was 29.2%; pan-cytokeratin was positive in all cases, and vimentin was positive in myoepithelial carcinomas.

• CONCLUSION: PA has a lower positivity of C-myc and Ki-67, while Ca-ex-PA had a higher positivity of these two biomarkers. These four biomarkers as a set could provide valuable clues in the differential diagnosis between Ca-ex-PA and PA. Our results indicate that the activation of C-myc could play an important role in the pathogenesis of Ca-ex-PA and PA.

• KEYWORDS: carcinoma ex pleomorphic adenoma; pleomorphic adenoma; C-myc; immunohistochemistry

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INTRODUCTION

Pleomorphic adenoma (PA, also called mixed tumor) is the most common tumor in the lacrimal gland, consisting 50% of epithelial lacrimal gland tumors[1-2]. Although lacrimal PA is benign, it is inclined to recur after incomplete surgical resection, and has the possibility to transform into carcinoma ex pleomorphic adenoma (Ca-ex-PA) with a poor prognosis[3]. Ca-ex-PA is a kind of infiltrative carcinoma arising in a PA.

Ki-67 is a marker for showing cells DNA synthesis before mitosis. Numerous studies have demonstrated that malignancies usually have high ki-67 expression related to high cellular proliferation. C-myc is a key protein in cell cycle regulation. A nuclear phosphoprotein encoded by MYC gene works as a kind of DNA-binding factor which activate or repress the transcription of a great quantity of genes. The aberrations of MYC result in its constitutive activation in many tumors[4].

In order to identify diagnostic factors for Ca-ex-PA and PA, we evaluated the expression of intermediate filaments vimentin, pan-cytokeratin, C-myc protein, as well as a proliferation marker Ki67 antigen in Ca-ex-PA and PA. The study is intended to find some immunohistochemical biomarkers that could provide assistance in the differential diagnosis between Ca-ex-PA and PA.

Although many markers have been researched for their expressions in salivary gland tumors, only several literatures were found about C-myc expression in Ca-ex-PA and PA[5-7]. We chose pan-cytokeratin, vimentin, Ki-67 and C-myc to test their capability to describe useful general diagnostic differences between PA and Ca-ex-PA in the lacrimal gland. This paper has a purpose to set up a baseline of some immunohistochemical markers which could provide aid in the diagnosis of controversial or difficult cases in some circumstances.

SUBJECTS AND METHODS

Ethical Approval This was a retrospective, noninterventional study, which was performed on the basis of the principles of the Declaration of Helsinki. Informed consent was waived due to the retrospective nature of the study.

Tissues PA tissues were collected from the archives of Zhongshan ophthalmic center in the period 2015-2018. Ca-ex-PA tissues were collected in the period 2012-2018. We obtained the clinical information from the medical records. Sections were cut from the formalin-fixed, paraffin-embedded specimens and were stained with hematoxylin and eosin.

Immunohistochemistry Formalin-fixed paraffin-embedded specimens were cut at a thickness of 4 μm and mounted on coated slides for immunohistochemical staining. The following antibodies were utilized: C-myc (clone Y69; rabbit monoclonal; Abcam, prediluted), Ki-67 (clone 7B11; mouse monoclonal; Abcam, prediluted), pan-cytokeratin (clone AE1/AE3; mouse monoclonal; Abcam, prediluted), vimentin (clone V9; mouse monoclonal; Abcam, prediluted). The sections were processed using Leica Bond Max autostainer at our Department of Pathology. Positive controls and negative controls were carried out respectively. The negative controls were omitted the primary antibodies. The tissues were stained with chromogen diaminobenzidine and were counterstained with hematoxylin. The positive cells with brown nucleuses of Ki-67 and C-myc were counted in three representative high-power fields. Then the results were averaged.

Statistical Analysis The independent-samples *t* tests were conducted for analyzing data. SPSS software version 22 was used for the analyses. The statistical tests were two-sided. And a *P* value of 0.05 or less was considered statistically significant.

RESULTS

There were 64 cases of PA and 15 cases of Ca-ex-PA in the lacrimal gland in all. The mean age of patients with PA was 43.2y (range from 21 to 75). Among them 35 patients (54.7%) were male and 29 patients (45.3%) were female. And the mean age of patients with Ca-ex-PA was 54.3y (range from 26 to 76). Eight patients (53.3%) were female and seven patients (46.7%) were male. Among 15 cases of Ca-ex-PAs, there were 6 myoepithelial carcinomas, 4 adenocarcinomas, 3 epithelial-myoepithelial carcinomas, 2 squamous cell carcinomas.

Histologically, PA is benign neoplasm consisting of ductal cells (DCs) and myoepithelial cells (MECs) which are in a chondromyxoid stroma. All specimens of PA had a pseudocapsule of variably thick and were composed of lumens formed with double-layered cellular walls as well as myoepitheliomatous cells of spindle shape. The DCs are generally cuboidal epithelium cells lining a tubule. And that the MECs are generally spindle, oval, or polygonal with punctate nuclei chromatin which has no nucleolus or only has a minute one. The outer layer MECs in the ductular structures feathered into the stroma. The malignant components of the Ca-ex-PAs are myoepithelial carcinomas, adenocarcinomas, epithelial-myoepithelial carcinomas, squamous cell carcinomas respectively (Figure 1).

Immunohistochemically, in the Pas, the DCs displayed strong and diffuse positivity to cytokeratin. And the myoepithelial component showed positive to vimentin and few positive to pan-cytokeratin. While in Ca-ex-PAs, pan-cytokeratin was positive in all cases, and vimentin was positive in myoepithelial carcinomas (Figure 2).

The proliferation index of Ki-67 for the PAs was obviously lower with an average of 3.2%±1.3% (range of 1% to 6%). The average C-myc positivity in the PAs was 4.6%±1.5% (range of 2% to 8%). The proliferation index of Ki-67 in the Ca-ex-PAs was 29.2%±5.5% (range of 20% to 35%). The average C-myc positivity in the Ca-ex-PAs was 36.4%±5.8% (range of 25% to 45%; Figure 3). Whereas the immunohistochemistry expression results of Ki-67 and C-myc in the residual PA areas of Ca-ex-PAs are similar to the PAs. Compared with PA, Ca-ex-PAs showed higher C-myc and Ki67 expression ($P<0.01$, $P<0.01$, respectively; Figure 4).

DISCUSSION

Lacrimal PA is a kind of benign tumor with an ability to transform into Ca-ex-PA. Clinically, patients with PA generally present with a history of slowly increasing bulbar displacement painlessly. Patients with Ca-ex-PA usually present with rapidly increasing bulbar displacement with a poor prognosis that has a median survival of 3y[8-9].

Histologically, PA is a benign neoplasm consisting of DCs and spindle or polygonal MECs in a chondromyxoid stroma with a pseudoencapsule. The lumens usually contained eosinophilic, amorphous secretory material which was positive for Alcian blue and periodic acid Schiff. There is basophilic mucoid material around the ductlike units[8]. PA had double-layered, epitheliumlined glandular structures which have small to expanding lumens and the MECs of the outer layer of ductular structures feathering into the stroma[8].

In PA, both myoepithelial and luminal cells could transform into malignancy. But in most cases, the malignancy seems to occure from luminal cells[10]. Once carcinoma has arised, it could

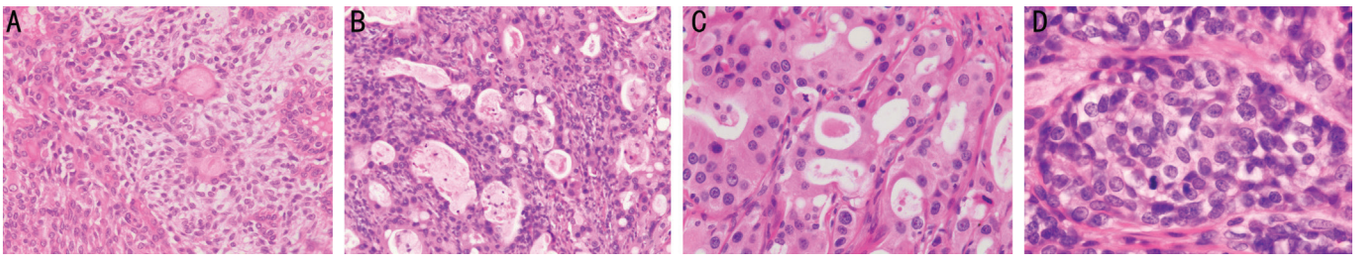

Figure 1 Histopathology of lacrimal gland Ca-ex-PA and PA A: PA is composed of MECs and DCs in a chondromyxoid stroma (HE×200); B: Epithelial-myoepithelial carcinoma displays DCs and MECs with atypical hyperchromatic nuclei (HE×200); C: Adenocarcinoma consist of cuboidal cells which have large hyperchromatic nuclei with prominent nucleoli (HE×200); D: Myoepithelial carcinoma is composed of clear tumour cells arranged in small lobules and sheets with hyperchromatic nuclei and mitosis (HE×400).

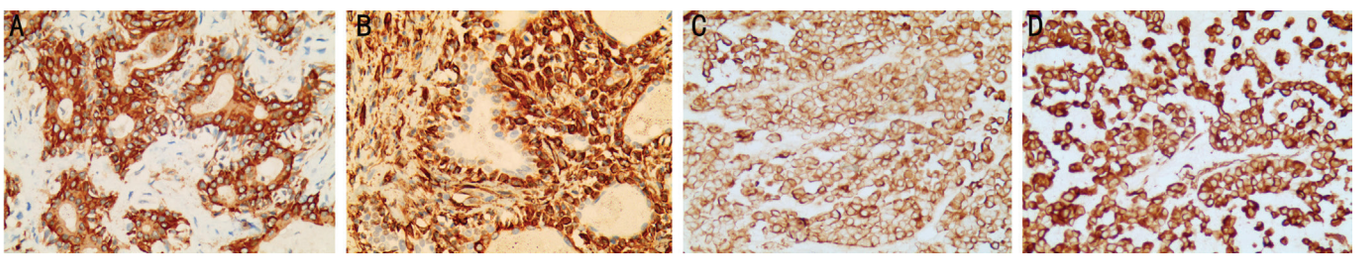

Figure 2 Immunohistochemical staining results of PA and myoepithelial carcinoma (×200**)** A: DCs in PA displayed strong and diffuse positivity for pan-cytokeratin; B: The myoepithelial of PA were positive to vimentin; C: The tumor cells of myoepithelial carcinoma showed positive to pan-cytokeratin; D: The tumor cells of myoepithelial carcinoma showed also positive to vimentin.

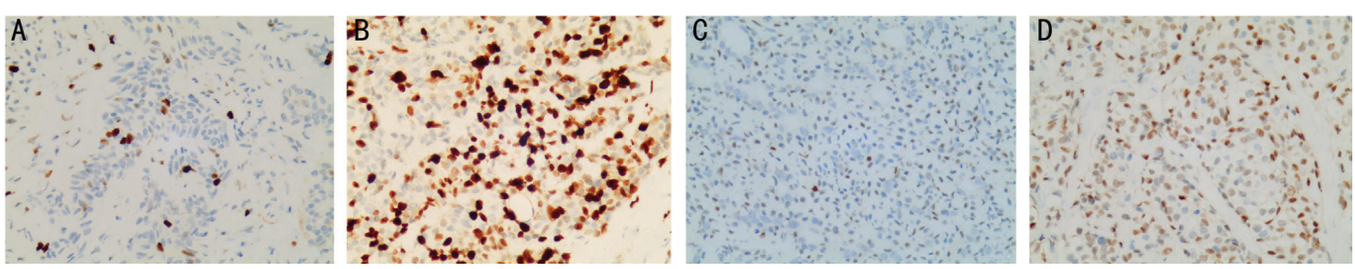

Figure 3 Immunohistochemical staining results of PA and Ca-ex-PA (×200**)** A: A few of Ki-67 positive cells in PA; B: A lot of Ki-67 positive cells in Ca-ex-PA; C: A few of C-myc positive cells in PA; D: A lot of C-myc positive cells in Ca-ex-PA.

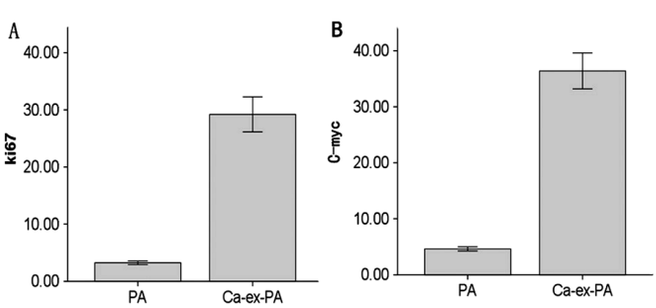

Figure 4 Immunohistochemical results of Ca-ex-Pas and PAs A: Compared with PA, Ca-ex-PAs showed higher Ki67 expression (P<0.01); B: Compared with PA, Ca-ex-PAs showed higher C-myc expression (P<0.01). Bars indicate standard deviation. PA, n=64; Ca-ex-PA, n=15.

present with multiple tumor phenotypes. The most common malignant component in Ca-ex-PA is adenocarcinoma. And the other malignant components are myoepithelial carcinoma, adenoid cystic carcinoma, epithelial-myoepithelial carcinoma, squamous cell carcinoma, and clear cell carcinoma, adenosquamous carcinoma, acinic cell carcinoma[11-12]. Katabi *et al*[13] reported that the salivary duct carcinoma and myoepithelial carcinomas are the most common subtypes of Ca-ex-PA. In our series of 15 cases of Ca-ex-PA there are 6 myoepithelial carcinomas, 4 adenocarcinomas, 3 epithelial-myoepithelial carcinomas, and 2 squamous cell carcinomas respectively. So in our study the most common malignant component in Ca-ex-PA is myoepithelial carcinoma. Maybe the types of lacrimal glandular Ca-ex-PAs are different with salivary Ca-ex-PAs, which needs to be further studied.

Sometimes it is difficult to differentiate between Ca-ex-PA and PA. So we need Immunohistochemical stain to help us to make a correct diagnosis. In this study, the DCs in the PA areas displayed strong and diffuse positive for pan-cytokeratin and negativefor vimentin. The myoepithelial components of PA were positive for vimentin and negative for pan-cytokeratin. These results were similar to the research reported by Sedassari *et al*[5]. Myoepithelial carcinoma of lacrimal gland is rare, and

there are only a few cases reported in the literature[14-15]. In our study the myoepithelial carcinoma displayed strong and diffuse positive to both pan-cytokeratin and vimentin which is similar to the case reported by Larbcharoensub *et al*[16].

Ki-67 is a marker showing DNA synthesis before mitosis. Numerous studies have demonstrated that malignancies usually have high ki-67 expression related to high cellular proliferation. This antibody recognizes a nuclear protein that is involved in the premitotic phases (G1, S, G2 and M) in the cell cycle. This nuclear protein can be used to estimate the growth status by showing the positive cells from all other present cells (Ki-67 proliferation index, or PI)[17-18]. Our results manifested an obviously low Ki-67 proliferation index in PAs (average 3.2%±1.3%, range of 2.1% to 5.2%), Whereas Ca-ex-PAs demonstrated much higher Ki-67 proliferation index (average 29.2%±5.5%; range of 20% to 35%). Whereas the immunohistochemistry expression results of Ki-67 in the residual PA areas of Ca-ex-PAs were similar to the PAs. These results are similar to the other researches[3,17,19].

C-myc is a key protein in cell cycle regulation. Encoded by MYC gene, a nuclear phosphoprotein can serve as a factor of DNA-binding which will activate or repress the transcription of a great quantity of genes such as P27, P21 and P15, that makes contribution to cell cycle progression in the phase of early and mid-G1[4,20-21]. Research shows that C-myc not only functions as a transcription factor that enhances many downstream genes to translate but also relate to regulating many cellular processes such as chromate instructure, mRNA translation, DNA replication and biogenesis of ribosomes[22-23]. MYC overexpressed in head and neck squamous cell carcinomas[24] and in gastric carcinomas[25]. Several researches show that C-myc overexpressed in salivary PA[6-7,26]. Our research found that the average C-myc positivity in Ca-ex-PAs was much higher than that in PAs, which helps to make a correct diagnosis in some confused situations.

In a conclusion, the DCs in the PA displayed positive for pan-cytokeratin and negative for vimentin. The myoepithelial component in the PA displayed positive for vimentin and negative for pan-cytokeratin. While the myoepithelial carcinoma showed positive to both pan-cytokeratin and vimentin. And the average Ki67 and C-myc positivity in Ca-ex-PAs was much higher than those in PAs. So the set of these four antibodies could help to provide clues in the differential diagnosis between Ca-ex-PA and PA of the lacrimal gland.

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