Original Article

Immunohistochemical expression of podoplanin as a myoepithelial cell marker in pleomorphic adenoma

Aanchal Adwani[1], Supriya Kheur[1], Mohit Kheur[2], Pratiksha Mahajan[1]

[1]Department of Oral Pathology and Microbiology and Dr. D. Y. Patil Dental College and Hospital, Dr. D. Y. Patil Vidyapeeth, [2]Department of Prosthodontics, M. A. Rangoonwala College of Dental Sciences, Pune, Maharashtra, India

Abstract

Purpose: Pleomorphic adenoma (PA) is the most common benign salivary gland tumor with salivary gland excision as the treatment of choice; however, recurrences after parotidectomy have been reported. The current study is aimed at evaluating podoplanin expression in PA as a myoepithelial (ME) cell marker and its role in estimating the prognosis and outcome of the tumors by correlating it with various clinical and histological parameters.

Materials and Methods: A total of 10 paraffin-embedded specimens of histologically diagnosed PA with clinical records of the patients were retrieved and the slides were then stained using hematoxylin and eosin staining and were then immunohistochemically stained with podoplanin.

Results: The study revealed the specificity of podoplanin as a differential marker for ME cells.

Conclusion: Although the study revealed the specificity of podoplanin as a differential marker for ME cells, additional markers to overcome the limitations of podoplanin which will predict the biologic behavior (biologic behavior of the tumor) the tumor with respect to parameters such as age, gender and site of the tumor are required. Furthermore, a larger sample size is required to validate the findings in our data.

Keywords: Myoepithelial cell marker, myoepithelial cells, pleomorphic adenoma, Podoplanin

Address for correspondence: Dr. Supriya Kheur, Department of Oral Pathology and Microbiology, Dr. D. Y. Patil Dental College and Hospital, Dr. D. Y. Patil Vidyapeeth, Pune, Maharashtra, India.
E-mail: supriya.kheur@dpu.edu.in

Submitted: 30-Nov-2019, **Revised:** 02-Jul-2020, **Accepted:** 05-Aug-2020, **Published:** 09-Jan-2021

INTRODUCTION

Salivary gland tumors (SGTs) account for 3%–6% of all tumors of the head-and-neck region.[1] These tumors show varied histopathological characteristics with different clinical presentation making the diagnosis difficult for the pathologist. A better understanding of the cellular components of these tumors, therefore, becomes necessary to determine the prognosis and render effective treatment.[2]

Pleomorphic adenoma (PA) accounts for 45%–74% of all the SGTs, making it the commonest benign SGT.[3] It is a mixed tumor containing both epithelial and mesenchymal components (chondromyxoid area). Histologically, it is classified into four types: principally myxoid, myxoid and cellular, predominantly cellular, extremely cellular.[4] The treatment of choice for PA is parotidectomy. However, recurrent lesions have also been reported. Reoperating the recurrent lesions poses a greater risk of injury to the facial nerve.[5]

Many theories have been proposed for the histogenesis of PA, including bicellular theory, which states that PA arises

Access this article online

Quick Response Code:

Website: www.jomfp.in

DOI: 10.4103/jomfp.JOMFP_337_19

How to cite this article: Adwani A, Kheur S, Kheur M, Mahajan P. Immunohistochemical expression of podoplanin as a myoepithelial cell marker in pleomorphic adenoma. J Oral Maxillofac Pathol 2020;24:579.

from intercalated stem cells. Recent studies have shown that myoepithelial cells (ME cells), which are the primary proliferating cells in PA, also play an important role in the pathogenesis of tumor.[6] Neoplastic ME cells (NMECs) have the capacity to differentiate into epithelial as well as mesenchymal components, as a result of which they produce different architectural patterns in a tumor. The NMEC can be spindle-shaped, plasmacytoid, epithelial type and clear.[7] The plasmacytoid cells followed by spindle-shaped cells are most commonly associated with PA. The extracellular matrix produced by these cells is myxoid, chondroid, myxo-chondroid, fibrous and osteoid. These cells are thus capable of producing different histological appearances in tumors.[8]

The introduction of immunohistochemistry (IHC) along with hematoxylin and eosin (H&E) staining has improved the accuracy of the diagnosis of these NMEC. The various markers used to identify these cells include actin, myosin, S-100 protein, calponin, vimentin, p63.[9]

Alpha-smooth muscle actin (α-SMA) is a known ME cell marker, which is a protein for cell motility. It also acts as a marker for actin containing myofibroblasts. The expression of this protein is known to be associated with morphologic differentiation in SGTs.[10] S-100 marker is a protein that shows solubility in 100% saturated $(NH_4)_2SO_4$. S-100 positivity is seen in various SGTs like polymorphous low-grade adenocarcinoma (PLGA), epithelial- ME carcinoma and undifferentiated carcinoma.[11] Calponin, a calcium-binding protein, is a marker smooth muscle cells and myofibroblasts and has shown positivity for ME cell differentiation.[12] Vimentin is an epithelial-mesenchymal transition (EMT) marker known to promote tumor invasion and metastasis. It is a marker for neoplastic ME differentiation.[12,13] Although these markers have been widely used, and the results are controversial with low specificity. Hence, the identification of newer markers with enhanced reliability and specificity becomes necessary.

Podoplanin, a transmembrane glycoprotein, is used as a lymphatic endothelium marker in various kinds of cancer. The expression of podoplanin has been studied in oral squamous cell carcinoma and other tumors of the central nervous system, cervix. Its expression has been correlated with patient survival in these cancers.[14] Recent studies have noted the immunoexpression of podoplanin in the ME cells of normal salivary glands in mouse.[15] It is known to maintain homeostasis in normal ME cells. The contribution of these cells in pathology is also striking, making the diagnosis of these cells by IHC important.

Hence, the current study is aimed at evaluating the podoplanin expression in PA as a ME cell marker and its role in estimating the prognosis and outcome of the tumors by correlating it with various clinical and histological parameters.

MATERIALS AND METHODS

Tissue samples

A total of 10 paraffin-embedded specimens of histologically diagnosed PA with clinical records of the patients were retrieved from the archives of the Department of Oral Pathology and Microbiology, D. Y. Patil Dental College and Hospital, Pimpri, Pune, after the necessary approval. Three mm sections were cut from the tissue blocks, and the slide was prepared. The slide were then stained using H&E staining and were then immunohistochemically stained with podoplanin. The slides were observed under light microscope.

Immunohistochemistry

The tissue sections were deparaffinized on a slide warmer for 20 min and treated in 2 changes of xylene for 3 min each followed by two changes of alcohol for 3 min. After distilled water washing for 10 min, the specimen was immersed in the citric acid buffer (pH-6) and antigen retrieval was done using a pressure cooker at 90°C for three whistles. The section was allowed to cool for 30 min. Washing of the sections with distilled water was carried out. The tissue was then treated with hydrogen peroxide block (0.3%) to inhibit the activation of endogenous peroxidase for 10 min. After phosphate-buffered saline (PBS) wash, the tissue was then covered with monoclonal mouse anti-human D2-40 primary antibody and incubated overnight at 4°C. This was followed by phosphate buffer saline washing. The slide was then covered with horseradish peroxidase secondary antibody and incubated for 40 min at room temperature, followed by PBS washing and then treated with Diaminobenzidine to visualize the reaction products for 10 min. The slides were then washed under tap water. Counterstaining of the sections was carried out with Harris hematoxylin for 2 min, dehydrated, followed by clearing with xylene and mounted. The slide was then observed under a light microscope for IHC grading.

The evaluation was based on the percentage of positive cells. The intensity was scored as follows: 0: Negavtive, 1+ : Mild, 2+ : Moderate and 3+ : Strong.

Statistical analysis

In the statistical analysis, Epi Info 7 software, developed by Centers for Disease Control and Prevention (CDC) in Atlanta, Georgia (US) was used.

RESULTS

A total of 10 tumors of PA were analyzed in this study. The age range was 25–65 years with the mean age of 45 years. The study included 8 (80%) females and 2 (20%) males. The most common site of occurrence of PA was found to be palate (50%) followed by parotid gland tumors (20%), upper labial mucosa (20%) and one case (10%) where parotid lymph node was the site of the primary tumor. The demographic details are shown in Table 1.

Histopathological characteristics

The tumor was classified histopathologically based on Foote's and Frazell's histopathologic classification as: Type 1: Principally myxoid, type 2: Mixed (cellular and myxoid), type 3: Principally cellular, type 4: Extremely cellular.

Out of the 10 cases, 4 (40%) cases were principally myxoid stroma, 4 (40%) cases were mixed with an equal proportion of cells and myxoid stroma and 2 (20%) cases were of principally cellular type. NMEC differentiation was seen in all cases in histopathologic sections. Capsular involvement of tumor was seen in 2 (20%) out of 10 cases. Squamous metaplasia with keratin formation was seen in two cases.

Expression of podoplanin in pleomorphic adenoma

The immunohistochemical expression of podoplanin was seen in all 10 cases in the cytoplasm of tumor cells [Figure 1a and b]. Out of 10 cases, 2 (20%) cases showed strong expression (3+), 4 (40%) cases showed moderate expression and 4 (40%) cases showed weak expression of podoplanin. One (10%) case showed positive blood vessel reactivity with podoplanin.

Correlation of podoplanin expression with clinical and histologic parameters

Table 1: Demographic data and patient characteristics of pleomorphic adenoma

Patient characteristics	*n* (%)
Total	10
Age	
Range	25-65
Mean	45
Gender	
Male	2 (20)
Female	8 (80)
Location	
Palate	5 (50)
Parotid gland	2 (20)
Labial mucosa	2 (20)
Parotid lymph node	1 (10)

Correlation of podoplanin expression with age

Strong expression (3+) of podoplanin was seen in two cases with a mean age of 38 years. Moderate expression (2+) of podoplanin was seen in four cases with the mean age of 49.25 years and weak expression (1+) was seen in 4 cases with the mean age of 56.75 years. A *P* value of 0.25155 was obtained using analysis of variance test. On Chi-square analysis, statistically nonsignificant results were seen between the expression of podoplanin and age of the patients (Chi-square value: 1.45436, *P*: 0.48327, Pearson correlation showed an inverse relation (−0.56597) between expression and age of the patients, i.e., the younger age group showed increased expression of podoplanin in tumor cells.

Correlation with gender

Podoplanin expression was found to be moderate (2+) in both (100%) male cases. Strong expression (3+) was seen in 2 (25%) females, moderate expression (2+) was seen in 2 (25%) females and weak expression was seen in 4 (50%) females. On analysis with the Mann–Whitney test, a statistically nonsignigicant correlation was seen between the expression of podoplanin and gender of the patient ($P = 0.5762$).

Correlation of podoplanin expression with the location of tumor

Out of 5 cases of palatal mucosa, 3 (60%) cases showed weak podoplanin expression and 2 (40%) cases showed moderate expression. Both the cases of the parotid gland showed moderate to strong expression, whereas tumors on labial mucosa showed weak-to-moderate expression. The case of the primary tumor in the parotid lymph node revealed a strong podoplanin expression.

Correlation between histopathological classification and expression of podoplanin

Out of 4 cases of principally myxoid stroma, 2 cases showed weak expression and 2 cases showed moderate expression of podoplanin. Two out of 4 cases of mixed myxoid and cellular type showed weak expression and

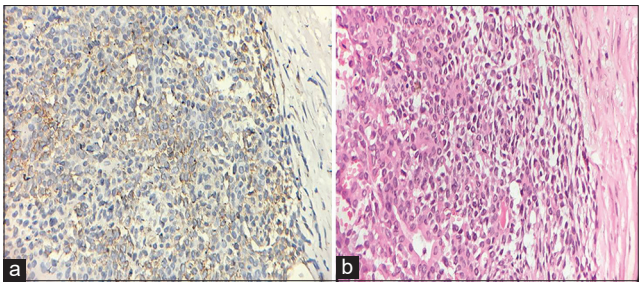

Figure 1: (a) Photomicrograph showing immunohistochemical expression of podoplanin in pleomorphic adenoma with positivity for myoepithelial cells in the cystoplasm with fibrous capsule (b) Photomicrograph with corresponding H&E stained section (×10 magnification) of pleomorphic adenoma with with fibrous capsule

two cases showed moderate and strong expression of podoplanin, respectively. One out of 2 cases of principally cellular tumors showed moderate expression and one case showed strong podoplanin expression [Table 2]. Chi-square analysis showed no significant correlation between the expression of podoplanin and histological subtypes of PA (Chi-square: 0.60375, *P*: 0.73943) both cases with squamous metaplasia showed moderate expression of podoplanin.

DISCUSSION

In the current study, we analyzed the reliability of podoplanin in PA as a new ME cell marker. PA is a SGT which has a variable morphology. This diversity is attributed to the ME cells, which form a major component of this tumor.[16] The neoplastic differentiation of these cells contributes in the histogenesis and morphogenesis of PA. Hence, to assess the progression and behavior of the tumor, the identification of these cells becomes important. The use of IHC as a method to identify these NMECs has long been studied. Markers such as α-SMA, myosin, vimentin, calponin, S-100, p-63, etc., have been advocated for this purpose.

α-SMA is a known ME cell marker, which is a protein for cell motility.[10] The expression was, however, found to be restricted to the outer tubular cells only,[16] thereby limiting its specificity. S-100 positivity is seen in various SGTs like PLGA and epithelial-ME carcinoma and undifferentiated carcinoma.[11] However, reduced specificity for ME differentiation was seen in subsequent studies due to positivity for ductal cells.[16] similar limitations were seen with vimentin, which is an EMT marker.[12,13] Recent studies have recognized calponin as a more sensitive marker for ME cell differentiation.[12] The results, however, remain controversial, highlighting the need for more specific markers.

Podoplanin, a transmembrane glycoprotein, was initially found in glomerular podocytes and is now widely used as a lymphatic endothelial marker. It is known to have a role in tumor invasion and metastasis.[17] Studies have shown the expression of podoplanin in the normal salivary gland of mice in the ME cells,[9] thus paving way to its use as a ME cell differentiation marker in SGTs. Hence, the current study evaluated the expression of D2-40 in ME cells of PA and correlated the expression with various clinical and histologic parameters.

Table 2: Correlation of podoplanin expression with histological subtypes

Intensity of staining	Type 1	Type 2	Type 3	Type 4	Total
Strong	0	1	1	0	2
Moderate	2	1	1	0	4
Weak	2	2	0	0	4
Total	4	4	2	0	10

The study comprised 10 cases of PA. With a female predominance (80%), the average age of the patient was 45 years and the age ranged from 25 to 65 years. The palate was the most common site of occurrence of PA, followed by parotid gland and labial mucosa. These demographic findings were in accordance with the study carried out by Al-Khtoum *et al*.,[18] where the mean age of occurrence of PA was found to fourth decade and palate as the common minor salivary gland site for PA.

Histopathologically, the cases were classified according to Foote's and Frazell's criteria[19] as: Type 1: Principally myxoid, Type 2: Mixed (cellular and myxoid), Type 3: Principally cellular, Type 4: Extremely cellular. In this study, 40% of cases were identified to be stroma rich with principally myxoid stroma. This was in line with studies conducted by Ito *et al*.[8] and Stennert *et al*.[20] This finding was found to be associated with higher recurrences.[21] We found 40% of cases to be of the mixed type with an equal proportion of cells and myxoid stroma and 20% of cases of cellular type. Two out of 10 cases showed squamous metaplasia with keratin pearl formation, which is a rare finding and poses a diagnostic pitfall.[22]

In the present study, podoplanin was expressed in the cytoplasm of the ME cells with weak, moderate and strong intensity as the grading. All 10 cases showed positive podoplanin expression. The expression positivity was seen in nonluminal cells [Figure 2a and b], which showed ME cell differentiation. Among 10 cases, 2 (20%) cases demonstrated strong expression, 4 (40%) specimens showed moderate expression, whereas 4 (40%) of the specimens showed weak expression. Similar results were found in the study conducted by Kaur and Gupta[2] where all cases of

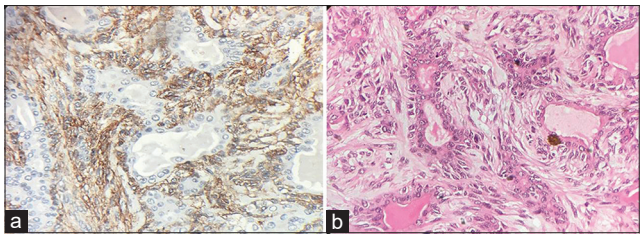

Figure 2: (a) Photomicrograph showing immunohistochemical expression of podoplanin in pleomorphic adenoma with positivity for myoepithelial cells in the cystoplasm (b) Photomicrograph with corresponding H&E stained section (×40 magnification) with myoepithelial cells in pleomorphic adenoma

PA showed positive podoplanin expression in ME cells.

In this study, an attempt was made to correlate the expression of podoplanin with clinical parameters such as age, gender and location of the tumor. Strong expression was seen in patients of PA with a mean age of 38 years, moderate expression with a mean age of 49.25 years and weak expression was seen with a mean age of 56.75 years. The results revealed an inverse correlation on statistical analysis with Pearson's test, between the expression intensity and age of the patient. The younger age group showed a strong expression of podoplanin while the older age group showed weaker expression. Thus, we can imply that younger patients show an increased ME cell number and hence an increased aggressiveness of the tumor. However, no literature evidence could be obtained to support our hypothesis. No significant statistical correlation was found between gender and location of tumor with the expression of podoplanin.

The statistical correlation was insignificant between the podoplanin expression and histopathologic grading of PA. Literature evidence on the correlation between histologic grading and IHC expression of podoplanin in PA is scarce. However, a study by Wu *et al*.[14] also showed no correlation of the expression status of podoplanin with age, gender and histologic grading in cases of salivary adenoid cystic carcinoma. The type 1 cases showed moderate-to-weak expression of podoplanin, whereas type 3 cases with cellular predominance showed moderate-to-strong expression depicting the reliability of podoplanin expression in NMECs.

Thus, our study revealed the specificity of podoplanin as a differential marker for ME cells. However, additional markers to overcome the limitations of podoplanin, which will predict the biologic behavior of the tumor with respect to parameters such as age, gender, site of the tumor are required. Furthermore, a larger sample size is required to validate the findings in our data. Lack of literature evidence on the role of podoplanin in SGT also acts as a drawback for this study.

However, with its role as a ME differential marker, podoplanin is an interesting diagnostic protein and an additional aid in protein targeted therapies.

CONCLUSION

The role of podoplanin as a ME cell differentiation marker has been studied by a lot of researchers. This study on similar lines with other studies proved the role of podoplanin as a ME marker and also correlated the histopathologic and clinical factors of PA with the immunohistochemical expression of podoplanin, thus making it unique. Although the results were nonsignificant, a larger sample size would help confirming the results. Other comprehensive researches would help to prove it as a therapeutic target in various SGTs.

Financial support and sponsorship
Nil.

Conflicts of interest
There are no conflicts of interest.

REFERENCES

1. Mythily C, Vasahar A. P63 expression in pleomoorphic adenoma and mucoepidermoid carcinoma. Int J Pharm Bio Sci 2016;7:B 507-10.
2. Kaur H, Gupta S. An analysis of the expression of Bcl-2, podoplanin and lymph angiogenesis in benign and malignant salivary gland tumours. J Clin Exp Pathol 2013;3:145.
3. Gahine R, Sudarshan V, Hussain N, Krishnani C. Pleomorphic adenoma: A diagnostic pitfall in the diagnosis of salivary gland lesions on FNAC: Case reports with review of the literature. Cytojournal 2010;7:17.
4. Rajendran R. Tumors of the salivary glands. Rajendran R, Sivapathasundharam B, editors. Shafer's Textbook of Oral Pathology. 7th ed. India: Elsevier; 2012. p. 223-57.
5. Bijai LK, Mathew P, Rajathi M, Austin RD. Recurrent pleomorphic adenoma: A case report. IJDSR 2014;2:92-4.
6. Chitturi RT, Veeravarmal V, Nirmal RM, Reddy BV. Myoepithelial cells (MEC) of the salivary glands in health and tumours. J Clin Diagn Res 2015;9:ZE14-8.
7. Balachander N, Masthan KM, Aravindha N, Anbazhagan V. Myoepithelial cells in pathology. J Pharm Bioallied Sci 2015;7:S190-3.
8. Ito FA, Jorge J, Vargas PA, Lopes MA. Histopathological findings of pleomorphic adenomas of the salivary glands. Med Oral Patol Oral Cir Bucal 2009;14:E57-61.
9. Tsuneki M, Maruyama S, Yamazaki M, Essa A, Abé T, Babkair HA, *et al*. Podoplanin is a novel myoepithelial cell marker in pleomorphic adenoma and other salivary gland tumors with myoepithelial differentiation. Virchows Arch 2013;462:297-305.
10. Ravi M, Boaz K, Natarajan S, Lewis A, Prasad M, Yellapurkar S. Expression of α-smooth muscle actin in benign and malignant salivary gland tumors: An immunohistochemical study. Indian J Pathol Microbiol 2018;61:479.
11. Varghese SS, Mathew P, Jose J. Diagnostically relevant molecular markers in head and neck neoplasms. Article ID 873953, ISRN Biomarkers 2013;2013:1-6.
12. Nagao T, Sato E, Inoue R, Oshiro H, H Takahashi R, Nagai T, *et al*. Immunohistochemical analysis of salivary gland tumors: Application for surgical pathology practice. Acta Histochem Cytochem 2012;45:269-82.
13. Irani S, Jafari B. Expression of vimentin and CD44 in mucoepidermoid carcinoma: A role in tumor growth. Indian J Dent Res 2018;29:333-40.
14. Wu HM, Ren GX, Wang LZ, Zhang CY, Chen WT, Guo W. Expression of podoplanin in salivary gland adenoid cystic carcinoma and its association with distant metastasis and clinical outcomes. Mol Med Rep 2012;6:271-4.
15. Hata M, Ueki T, Sato A, Kojima H, Sawa Y. Expression of podoplanin in the mouse salivary glands. Arch Oral Biol 2008;53:835-41.
16. Langman G, Andrews CL, Weissferdt A. WT1 expression in salivary gland pleomorphic adenomas: A reliable marker of the neoplastic myoepithelium. Mod Pathol 2011;24:168-74.
17. Li W, Tamamura R, Wang B, Liu Q, Liu H, Lin T, *et al*. Expressions of ABCG2, CD133, and podoplanin in salivary gland adenoid cystic

carcinoma. Biomed Res Int 2014; 2014:132349.
18. Al-Khtoum N, Qubilat AR, Al-Zaidaneen S, Al Mefleh S, Al-Qudah A. Clinical characteristics of pleomorphic adenoma of salivary glands among Jordanian patients. J Pak Med Assoc 2013;63:358-60.
19. Foote FW Jr., Frazell EL. Atlas of tumor pathology, section IV, fascile 11. 1st ed. Washington DC: Armed Force Institute of Pathology; 1954.
20. Stennert E, Guntinas-Lichius O, Klussmann JP, Arnold G. Histopathology of pleomorphic adenoma in the parotid gland: A prospective unselected series of 100 cases. Laryngoscope 2001;111:2195-200.
21. Dulguerov P, Todic J, Pusztaszeri M, Alotaibi NH. Why do parotid pleomorphic adenomas recur? A systematic review of pathological and surgical variables. Front Surg 2017;4:26.
22. Sharma S, Mehendiratta M, Chaudhary N, Gupta V, Kohli M, Arora A. Squamous Metaplasia in Pleomorphic Adenoma: A Diagnostic and Prognostic Enigma. J Pathol Transl Med 2018;52:411-5.