Dental Research Journal

Original Article

Immunohistochemical comparison of cyclin D1 and P16 in odontogenic keratocyst and unicystic ameloblastoma

Seyed Mohammad Razavi[1], Hamid Poursadeghi[2], Atousa Aminzadeh[3]

[1]Torabinejad Dental Research Center, Department of Oral and Maxillofacial Pathology, School of Dentistry, Isfahan University of Medical Sciences, [2]Oral and Maxillofacial Pathologist, Private Practice, [3]Department of Oral and Maxillofacial Pathology, School of Dentistry, Khorasgan (Isfahan) Branch, Islamic Azad University, Isfahan, Iran

Received: July 2012
Accepted: January 2013

Address for correspondence:
Dr. Atousa Aminzadeh,
Department of Oral Pathology,
School of Dentistry,
Khorasgan (Isfahan) Branch,
Islamic Azad University,
Isfahan, Iran.
E-mail: A.Aminzadeh@khuisf.ac.ir

ABSTRACT

Background: The different growth mechanism and biologic behavior of the odontogenic keratocyst (OKC) compared to other odontogenic cysts might be related to the proliferating capacity of its epithelium. In this study, the aim was to evaluate and compare the distribution and staining intensity of P16 and cyclin D1 in OKC and unicystic ameloblastoma (UA).

Materials and Methods: In this descriptive analytic study, hematoxylin- and eosin-stained slides of OKCs and UAs available from the archives of the oral pathology laboratory of the Esfahan School of Dentistry were examined. Twenty-five noninflamed solitary odontogenic keratocysts and 25 unicystic ameloblastomas (of either type) were selected and stained immunohistochemically. Distribution and staining intensity score (SID score) for P16- and cyclin D1-positive cells was calculated in both groups. Results were analyzed statistically with Wilcoxon, Friedman, and Mann-Whitney tests; $P < 0.05$ was considered significant.

Results: The highest expression of Cyclin D1-positive cells was seen in the suprabasal layer of keratocysts ($P < 0.05$) and in the peripheral layer of UAs ($P < 0.05$). Likewise, the highest expression of P16-positive cells was observed in the basal and suprabasal layers of keratocysts ($P > 0.05$) and central portions of UAs ($P > 0.05$). Expression of Cyclin D1 was higher in UAs compared to keratocyts ($P < 0.05$), although P16 did not show a significant difference between the two study groups ($P > 0.05$).

Conclusion: Cyclin D1 did show a higher staining intensity in UAs compared to the keratocysts, although the expression of P16 was similar in the studied groups. The invasive growth of OKC might be related to the state of expression of cyclin D1 and P16 in the epithelium of this cyst.

Key Words: Cyclin D1, keratocyst, odontogenic cysts, P16, unicystic ameloblastoma

INTRODUCTION

Keratocyst or odontogenic keratocyst (OKC) is a distinctive form of developmental odontogenic cyst which due to its specific histopathologic features and clinical behavior, is different from other odontogenic cysts and deserves special consideration. Accordingly in the latest World Health organization (WHO) classification of odontogenic tumors, the name keratocystic odontogenic tumor has been given to this lesion.[1]

Toller (1967) and Ahlfors (1984) were the first to suggest the basis for regarding OKC as a benign neoplasm. Since then, this cyst has been the center of interest and research; nevertheless, a widespread confusion regarding the nature and nomenclature of this cyst exists among professionals.[1,2]

Cell proliferation, cell death, and expression of apoptosis related proteins of the epithelium of solitary OKCs have been compared to odontogenic cysts and tumors such as radicular cyst, dentigerous cyst, and ameloblastoma.[2-7]

Access this article online

Website: http//:drj.mui.ac.ir

Progression through the cell cycle from the presynthetic growth phase 1 (G1) is regulated by cyclins. More than 15 cyclins have been identified that appear sequentially during the cell cycle. Among them, cyclin D1 is thought to have a greater role in tumorigenesis.[8] Mishaps in the expression of cyclin seem to be a common event in neoplastic transformation. In contrast, P16 or CDKN2A is a tumor suppressor that has an inhibitory effect on retinoblastoma protein phosphorylation by blocking the cyclin D-CDK4 complex.[9,10]

It is believed that epithelial cells of OKCs have high proliferation activity.[7,11] In 2009, Razavi and Ardakani compared the antiapoptotic and proliferative markers, bcl-2 and Ki67, using TUNEL method (TUNEL: Terminal deoxynucleotidyl transferase dUTP nick end labeling) in OKC and solid ameloblastoma. They concluded that an equilibrium exists between these two markers in the epithelial layers of OKC, which in turn prohibits the progression of OKC toward a complete tumoral growth.[12]

The proliferative rate of odontogenic epithelium is under control by the P16 protein. In the study of Artese and co-workers, P16 showed a high expression in the basal and suprabasal layers of dentigerous cysts and radicular cysts. Such stainability was not observed in the epithelial layers of OKCs.[13]

In this study, unicystic ameloblastoma (UA), an odontogenic tumor with benign clinical behavior similar to odontogenic cysts, was compared to keratocyst, an odontogenic cyst with aggressive, tumor-like behavior. The aim of the present study was to compare the intensity of stainability and distribution of P16, a tumor suppressor and cyclin-D1, a cell-proliferation regulator in the epithelial layers of keratocysts and UAs.

MATERIALS AND METHODS

In this descriptive analytic study, hematoxylin- and eosin-stained slides of OKCs and UAs available from the archives of the oral pathology laboratory of the Esfahan School of Dentistry from 2000 to 2010 were examined. Twenty-five noninflamed solitary OKCs and 25 UAs of either type were selected.

4 μm sections prepared from selected paraffin blocks were deparaffinized and rehydrated. Immunohistochemical staining was performed by biotin-streptavidin Novolink Polymer Detection System (Novocastra, Germany). After antigen retrieval, ready-to-use mouse monoclonal antibody cyclin D1 (Novocastra RTU-CYCLIN-D1-GM) and Novocastra monoclonal mouse antibody P16 (clone 6H12) were used for immunostaining. Negative and positive controls (breast cancer) were used for both staining procedures. P16- and cyclin D1-positive cell count was performed blinded by two pathologists by light microscopy (Olympus BX41TF, Tokyo, Japan) in a randomly selected successive high – power (×400 magnification) field.

Both cytoplasmic and nuclei stainability was observed but researchers only included nuclei-positive cells in cell count described as:[13,14]

1. No stainability (negative: 0)
2. Stainability less than 25% (light: 1)
3. Stainability between 25 and 50% (moderate: 2)
4. Stainability more than 50% (high: 3).

To evaluate the distribution of cyclin D1- and P16-positive cells, the epithelium was divided into basal, suprabasal, and superficial layers in OKCs and peripheral and central layers in UAs. Staining intensity distribution (SID) score for each layer of the epithelium was calculated as the multiplication of distribution (proportion of stained cells) in staining intensity.[15] The results were analyzed with Wilcoxon, Friedman, and Mann-Whitney statistical tests.

RESULTS

Expression of cyclin D1 in OKCs and UAs

As shown in Table 1, the expression of cyclin D1 in the suprabasal layers of OKCs was significantly

Table 1: SID score for cyclin D1 in OKCs and UAs

Group (layer (SID)	1 (%)	2 (%)	3 (%)	4 (%)	6 (%)	9 (%)	*P* value	*P* value OKC vs. UA
OKC								
Basal	28	48	4	12	8	0	Basal layer vs. superficial layer $P>0.05$	<0.05
Suprabasal	0	0	0	32	36	32	Suprabasal layer vs. basal and superficial layer $P<0.05$	
Superficial	56	24	4	8	8	0		
UAs								
Peripheral	0	20	0	44	24	12	<0.05	
Central	0	36	4	12	8	0		

SID: Distribution and staining intensity score; OKC: Odontogenic keratocyst; UA: Unicystic ameloblastoma

higher than in the basal and superficial layers ($P < 0.001$). In UAs, cyclin D1 showed a significantly higher expression in the peripheral layers rather than in the central layers ($P < 0.001$) [Table 1, Figure 1].

Expression of P16 in OKCs and UAs

P16 was expressed more in the basal and suprabasal layers of OKCs than superficial layers but this difference was not significant ($P > 0.05$) as is shown in Table 2. In UAs, the expression of P16 was higher in the central portions of the epithelial lining than peripheral layers, although this difference was not statistically significant ($P = 0.058$) [Table 2, Figure 2].

Table 2: Frequency distribution of SID score for P16 in OKC and UA

Group (layer) SID	0 (%)	1 (%)	2 (%)	*P* value	*P* value OKC vs. UA
OKC					
Basal	24	72	4	>0.05	>0.05
Suprabasal	8	88	4		
Superficial	20	80	0		
UAs					
Peripheral	28	72	0	>0.05	
Central	12	80	8		

SID: Distribution and staining intensity score; OKCs: Odontogenic keratocyst; UA: Unicystic ameloblastoma

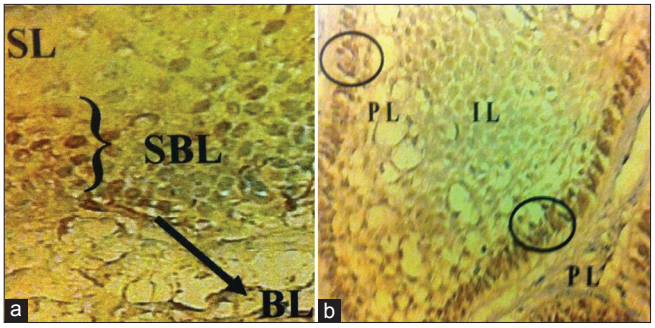

Figure 1: Cyclin D1-positive cells in (a) basal (BL) and suprabasal (SBL) layers of keratocyst, (b) peripheral layer of unicystic ameloblastoma (×400)

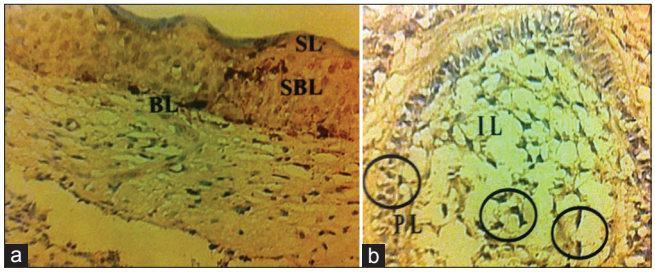

Figure 2: P16-positive cells in (a) three epithelial layers: Basal (BL), suprabasal (SBL), and superficial (SL) layers of keratocyst, (b) central (IL) and peripheral layers (PL) of unicystic ameloblastoma (×400)

Comparison of cyclin D1 in OKCs and UAs

Cyclin D1 showed a significantly higher score in the peripheral layers of UAs rather than basal layers of OKCs ($P < 0.001$). Expression of cyclin D1 in the suprabasal layers of OKCs was higher than in the central layers of UAs ($P < 0.001$) [Table 1].

Comparison of P16 in OKCs and UAs

Expression of P16 was higher in the peripheral layers of UAs compared to basal layers of OKCs, although this difference was not statistically significant ($P < 0.593$). The expression of P16 was higher in the central layers of UAs compared to the suprabasal layer of OKCs, although this difference was not statistically significant ($P < 0.988$) [Table 2].

DISCUSSION

In a study by Kimi *et al.* in 2001, a higher expression of cyclin D1 in the suprabasal and basal layers of keratocysts was shown and these layers were introduced as proliferative layers of OKCs.[16] Murtadi *et al.* revealed a higher expression of cytokeratin 13 in basal and superficial layers of OKCs compared to suprabasal layers, which means that basal and superficial layers of OKCs are more differentiated than suprabasal layers. Thus a lower rate of proliferation in basal and superficial layers of OKCs have to be expected.[17] In our study, cyclin D1 showed a significantly higher expression in the suprabasal layers than basal layers of OKCs which is in accordance with the study of Murtadi *et al*. As proliferation occurs in the basal layers of the epithelium, a lower expression of cyclin D1 in basal layers can explain the limited cell proliferation in OKCs.

In our study, cyclin D1 in UAs showed a significantly higher expression in peripheral layers, cells adjacent to the basement membrane, in accordance with the study of Kumamoto *et al.* and other studies with different proliferative markers which introduce peripheral cells as proliferative components of ameloblastomas.[18-20] However, Kumar *et al.* and Khalili *et al.* have shown similar expression of cyclin D1 in the peripheral and central layers of ameloblastomas ($P > 0.05$).[8,14] The difference between our study and similar studies might be related to the selection of sample, as in previous studies, samples were selected from both solid and UAs but in the present study, samples were selected only from one type: UAs.

In our study, P16 showed a higher expression in basal and suprabasal layers of OKCs although the difference

between the three layers was not significant, which is in accordance with the study of Kimi *et al*.[16] In UAs, between the two epithelial layers, a higher expression of p16 was observed in the central layer but the difference was not significant, which is in accordance with the study of Kumamoto *et al*.[20]

Expression of cyclin D1 in suprabasal layers of keratocysts can be related to its aggressive behavior, but expression of P16 in the basal, suprabasal, and superficial layers of OKCs shows that further proliferation toward a tumoral growth is inhibited.

Our study shows that the expression of P16, the tumor suppressor, was not significantly different in OKCs and UAs. Cyclin D1 was expressed significantly higher in UAs compared to OKCs. Khalili *et al.* also revealed a high expression of cyclin D1 in unicystic and solid ameloblastomas,[14] although Song *et al.* showed a significantly higher expression of proliferating cell nuclear antigen (PCNA) and Ki-67 in OKCs compared to UAs ($P < 0.05$).[7]

CONCLUSION

The invasive growth of OKC, an odontogenic cyst, and the cystic behavior of UA, an odontogenic tumor, might be related to state of expression of cyclin D1 and P16 in the epithelium of these lesions. The expression of cyclin D1 was higher in UAs compared to OKCs although P16 showed similar expression in UAs and OKCs.

REFERENCES

1. Nevill B, Damm D, Allen C, Bouquot J. Oral and maxillofacial pathology. 3rd ed. Missouri: Saunders; 2009. p. 683-7.
2. Shear M, Speight P. Cysts of the oral and maxillofacial region. 4th ed. Munksgaard: Blackwell; 2007. p. 6-7.
3. Gunhan Ö, Yildiz E, Kaslioglu Y, Aydintug Y, Dogan N, Celasun B. Nuclear morphometric features of epithelial cells lining keratocysts. Anal Quant Cytol Histol 2003;25:85-9.
4. Slootweg P. p53 protein and Ki-67 reactivity in epithelial odontogenic lesions. An immunohistochemical study. J Oral Pathol Med 1995;24:393-7.
5. Hume W, Moore J, Main D. Differences in *in-vitro* growth of epithelium from inflammatory and developmental odontogenic cysts. Br J Oral Maxillofac Surg 1990;28:25-8.
6. Stenman G, Magnusson B, Lennartsson B, Juberg-Ode M. *In vitro* growth characteristics of human odontogenic keratocysts and dentigerous cysts. J Oral Pathol 1986;15:143-14.
7. Song M, Lee S, Lee J, Yi W, Heo M, Lee J, *et al*. Ralation of the radiologic findings and labelling index of ki-67, PCNA and cytokeratin in UAs, dentigerous cyst and odontogenic keratocyst. Korean J Oral Maxillo fac Radiol 2004;34:75-9.
8. Kumar H, Vandana R, Kumar GS. Immunohistochemical expression of cyclin D1 in ameloblastomas and adenomatoid odontogenic tumors. J Oral Maxillofac Pathol 2011;15:283-7.
9. Regezi J, Sciubba J, Jordan R. Oral Pathology clinical pathologic correlations. 5th ed. Philadelphia: Saunders; 2008. p. 237-40.
10. Stricker T, Kumar V. Neoplasia. In: Kumar V, Abbas A, Fausto N, Aster J, editors. Pathologic basis of disease. 8th ed. Philadelphia: Saunders; 2010. p. 284-6.
11. Takahashi H, Fujita S, Yamabe S, Moriishi T, Okabe H, Tajima Y, *et al*. Comparison of proliferating cell nuclear antigen expression in odontogenic keratocyst and ameloblastoma: An immunohistochemical study. Anal Cell Pathol 1998;16:185-92.
12. Razavi M, Ardakani H.A comparison of antigens bcl-2 and ki-67 expression alongside apoptosis between odontogenic keratocyts and amloblstomas. J Esfahan Dent Sch 2009;5:139-147.
13. Artese L, Iezzi G, Piattelli A, Rubini C, Goteri G, Pernotti V, *et al*. P16 Expression in Odontogenic Cysts. Dent Res J 2008;5:61-4.
14. Khalili M, Eslami M, Masoumi P. Immunohistochemical evaluation of p21 and cyclin-D1 expression in ameloblastoma of the jaws. J Tehran Univ Med Sci 2007;20:33-40.
15. Moreno G, Ruiz M, Sáenz D, Peláez D, Arroyave S. Cytomorphometric and immunohistochemical comparison between central and peripheral giant cell lesions of the jaws. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2008;105:625-32.
16. Kimi K, Kumamoto H, Ooya K, Motegi K. Immunohistochemical analysis of cell-cycle- and apoptosis-related factors in lining epithelium of odontogenic keratocysts. J Oral Pathol Med 2001;30:434-42.
17. Murtadi A, Grehan D, Toner M, McCartan B. Proliferating cell nuclear antigen staining in syndrome and nonsyndrome odontogenic keratocysts. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 1996;81:217-20.
18. Sandra F, Mitsuyasu T, Nakamura N, Shiratsuchi Y, Ohishi M. Immunohistochemical evaluation of PCNA and Ki-67 in ameloblastoma. Oral Oncol 2001;37:193-8.
19. Liu J, Wang S, Chen X, Li Y.Proliferating activity and differentiation between peripheral cells and central cells in ameloblastoma. Zhonghua Kou Qiang Yi Xue Za Zhi 2002;37:62-4.
20. Kumamoto H, Kimi K, Ooya K. Detection of cell cycle-related factors in ameloblastomas. J Oral Pathol Med 2001;30:309-15.

How to cite this article: Razavi SM, Poursadeghi H, Aminzadeh A. Immunohistochemical comparison of cyclin D1 and P16 in odontogenic keratocyst and unicystic ameloblastoma. Dent Res J 2013;10:180-3.

Source of Support: Nil. **Conflict of Interest:** None declared.