Original Article

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

Seyed Mohammad Razavi1, Hamid Poursadeghi2, Atousa Aminzadeh3

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

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 keratocysts (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.2,3

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.2,4-7
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. Mislaps 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.

It is believed that epithelial cells of OKCs have high proliferation activity. 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.

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.

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:

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.

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

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

<table>
<thead>
<tr>
<th>Group (layer (SID))</th>
<th>1 (%)</th>
<th>2 (%)</th>
<th>3 (%)</th>
<th>4 (%)</th>
<th>6 (%)</th>
<th>9 (%)</th>
<th>P value OKC vs. UA</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>OKC</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basal</td>
<td>28</td>
<td>48</td>
<td>4</td>
<td>12</td>
<td>8</td>
<td>0</td>
<td>Basal layer vs. superficial layer P&lt;0.05</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Suprabasal</td>
<td>0</td>
<td>0</td>
<td>32</td>
<td>36</td>
<td>32</td>
<td></td>
<td>Suprabasal layer vs. basal and superficial layer P&lt;0.05</td>
<td></td>
</tr>
<tr>
<td>Superficial</td>
<td>56</td>
<td>24</td>
<td>4</td>
<td>8</td>
<td>8</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>UAs</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Peripheral</td>
<td>0</td>
<td>20</td>
<td>0</td>
<td>44</td>
<td>24</td>
<td>12</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Central</td>
<td>0</td>
<td>36</td>
<td>4</td>
<td>12</td>
<td>8</td>
<td>0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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**

<table>
<thead>
<tr>
<th>Group (layer)</th>
<th>SID 0 (%)</th>
<th>SID 1 (%)</th>
<th>SID 2 (%)</th>
<th>$P$ value OKC vs. UA</th>
</tr>
</thead>
<tbody>
<tr>
<td>OKC</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basal</td>
<td>24</td>
<td>72</td>
<td>4</td>
<td>$&gt;0.05$</td>
</tr>
<tr>
<td>Suprabasal</td>
<td>8</td>
<td>88</td>
<td>4</td>
<td>$&gt;0.05$</td>
</tr>
<tr>
<td>Superficial</td>
<td>20</td>
<td>80</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>UAs</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peripheral</td>
<td>28</td>
<td>72</td>
<td>0</td>
<td>$&gt;0.05$</td>
</tr>
<tr>
<td>Central</td>
<td>12</td>
<td>80</td>
<td>8</td>
<td></td>
</tr>
</tbody>
</table>

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

**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.\textsuperscript{[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.\textsuperscript{[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.\textsuperscript{[13]} also revealed a high expression of cyclin D1 in unicystic and solid ameloblastomas,\textsuperscript{[14]} although Song et al.\textsuperscript{[17]} showed a significantly higher expression of proliferating cell nuclear antigen (PCNA) and Ki-67 in OKCs compared to UAs ($P < 0.05$).\textsuperscript{[17]}

**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**


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