Ameloblastoma and adenomatoid odontogenic tumor: the role of α2β1, α3β1, and α5β1 integrins in local invasiveness and architectural characteristics

Emanuel Sávio Souza Andrade, PhD a, Mârcia Cristina da Costa Miguel, PhD b, Leão Pereira Pinto, PhD b, Lévia Batista de Souza, PhD b,*

a Department of Oral Pathology, Dentistry School, University of Pernambuco, 55050-540 Pernambuco, Brazil
b Department of Oral Pathology, Dentistry School, Federal University of Rio Grande do Norte, CEP-59056-000 Rio Grande do Norte, Brazil

Abstract

Ameloblastoma is an odontogenic neoplasm characterized by local invasiveness and a tendency toward recurrence, whereas adenomatoid odontogenic tumor (AOT) is an indolent neoplasm. The objective of the present study was to immunohistochemically analyze the role of α2β1, α3β1, and α5β1 integrins in the cellular events and cell-matrix interactions that occur in these tumors and their consequent repercussions on the architectural arrangement and biologic behavior of these lesions. Paraffin-embedded specimens from 30 ameloblastomas (20 solid and 10 unicystic tumors) and 12 AOTs were submitted to immunohistochemistry using the catalyzed signal amplification system. A difference in the pattern of integrin expression was observed between the various histologic types of ameloblastoma. No significant difference in labeling intensity was observed between unicystic and solid ameloblastomas, but comparison between ameloblastomas and AOT showed a significantly stronger expression of α5β1 integrin in the former (P < .05). Our findings suggest an important role of the integrins studied in the architectural characteristics of ameloblastomas and AOTs and a possible participation of α5β1 integrin in the mechanism of local invasion of ameloblastomas.

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1. Introduction

Odontogenic tumors comprise a complex group of lesions characterized by a diverse biologic behavior and different histologic types and, like normal odontogenesis, show variable inductive interactions between the epithelium and ectomesenchyme [1].

Ameloblastoma is the most common epithelial odontogenic neoplasm [2] and is the second most frequent type among odontogenic tumors [3]. Based on their clinical behavior and prognosis, 3 types of ameloblastoma can be distinguished: solid or multicystic ameloblastoma, unicystic ameloblastoma, and peripheral ameloblastoma [4]. Among the intraosseous types, unicystic ameloblastoma shows a better biologic behavior and a more favorable prognosis than the solid type [5]. Adenomatoid odontogenic tumor (AOT) is the third most frequent odontogenic tumor [3,6].

Extracellular matrix (ECM) proteins have been shown to play an important role in epithelial-mesenchymal interactions in odontogenic tumors [7,8]. Many types of cells are unable to proliferate in the absence of anchorage to an extracellular substrate [9]. This anchorage to ECM proteins, including neoplasias, is mediated by integrins [10], and some studies have demonstrated a correlation between integrin expression and the biologic behavior of some tumors [11-13].

Integrins are transmembrane receptors consisting of 2 noncovalently linked glycoprotein subunits (1 α and 1 β
subunit that modulate cell-cell and cell-matrix binding and are implicated in growth, adhesion, migration, proliferation, apoptosis, and cellular morphology [14,15].

The objective of the present study was to analyze the expression of $\alpha 2\beta 1$, $\alpha 3\beta 1$, and $\alpha 5\beta 1$ integrins in ameloblastomas and AOTs by immunohistochemistry to

![Fig. 1](image-url)
better understand the role of these integrins in the cellular events and cell-matrix interactions that occur in these tumors.

2. Materials and methods

Thirty ameloblastomas (20 solid and 10 unicystic tumors) and 12 AOTs were selected from the archives of the Discipline of Oral Pathology, Federal University of Rio Grande do Norte (Brazil). Among the 20 solid ameloblastomas, the predominant histologic types were plexiform (6 cases), follicular (4 cases), basal cell (1 case), desmoplastic (1 case), and cases concomitantly exhibiting more than 1 histologic pattern: follicular and acanthomatous (3 cases), desmoplastic and plexiform (1 case), desmoplastic and follicular (1 case), follicular and granular cell (1 case), plexiform and follicular (1 case), and plexiform, acanthomatous, and basal cell (1 case). The study was approved by the research ethics committee of the Federal University of Rio Grande do Norte.

The paraffin-embedded specimens were cut into 3-μm-thick sections, and the sections were submitted to immunohistochemistry by the optimized streptavidin-biotin-peroxidase method using the Dako (Carpinteria, CA, USA) catalyzed signal amplification system for mouse primary antibodies. The following anti-integrin antibodies were used: α2β1 (BHA2,1 clone; Chemicon, Temeluca, CA, USA) diluted 1:1000 in Tris-HCl, α3β1 (M-KD102 clone, Chemicon) diluted 1:500 in Tris-HCl, and α5β1 (JBS5 clone, Chemicon) diluted 1:1000 in Tris-HCl, with an incubation time of 60 minutes.

Antigen retrieval was carried out with 0.5% pepsin, pH 1.8, at 37°C for 30 minutes. Before incubation with the

<table>
<thead>
<tr>
<th>Tumour</th>
<th>Integrin</th>
<th>n</th>
<th>Mean of ranks</th>
<th>Sum of ranks</th>
<th>Kruskal-Wallis KW</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Solid ameloblastoma</td>
<td>α2/1</td>
<td>20</td>
<td>31.75</td>
<td>635.0</td>
<td>4.216</td>
<td>.1215</td>
</tr>
<tr>
<td></td>
<td>α3/1</td>
<td>20</td>
<td>25.05</td>
<td>501.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>α5/1</td>
<td>20</td>
<td>34.70</td>
<td>694.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unicistic ameloblastoma</td>
<td>α2/1</td>
<td>10</td>
<td>17.50</td>
<td>175.0</td>
<td>8.136</td>
<td>.0171</td>
</tr>
<tr>
<td></td>
<td>α3/1</td>
<td>10</td>
<td>10.00</td>
<td>100.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>α5/1</td>
<td>10</td>
<td>19.00</td>
<td>190.0</td>
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<td></td>
</tr>
<tr>
<td>Adenomatoid odontogenic tumor</td>
<td>α2/1</td>
<td>12</td>
<td>18.00</td>
<td>216.0</td>
<td>4.595</td>
<td>.1005</td>
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<tr>
<td></td>
<td>α3/1</td>
<td>12</td>
<td>16.58</td>
<td>199.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>α5/1</td>
<td>12</td>
<td>20.92</td>
<td>251.0</td>
<td></td>
<td></td>
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</tbody>
</table>

Letters a and b indicate the results of Dunn’s post test, considering P = .05.
primary antibodies, the sections were immersed in 1% bovine serum albumin and 5% fetal calf serum in Tris-HCl, pH 7.4, for 60 minutes to block reactions with nonspecific tissue proteins. After incubation with the primary antibodies, the samples were washed twice in 1% Tween 20 for 5 minutes each and then incubated with the sequential reagents of the Dako catalyzed signal amplification kit for 15 minutes, with each step being intercalated with 2 washes in 1% Tween 20. The reaction was developed with diaminobenzidine (Sigma Chemical Company, St Louis, Mo) as chromogen, and counterstaining was performed with Mayer’s hematoxylin. Negative controls were obtained by replacing the primary antibody with 1% bovine serum albumin in Tris-HCl.

For analysis, the distribution, location, pattern, and intensity of immunohistochemical labeling were evaluated. Labeling intensity was analyzed using scores ranging from 0 to 2, where 0 corresponds to the absence of labeling, 1 to weak labeling, and 2 to strong labeling. The data were initially analyzed by the Kolmogorov-Smirnov test to determine whether they showed a normal distribution. Because no normal distribution was observed, the nonparametric Mann-Whitney U test and Kruskal-Wallis test, followed by Dunn’s post test, were used for statistical analysis, with the level of significance set at 95% (P < .05).

3. Results

In solid ameloblastomas, immunohistochemical labeling was detected in intercellular contacts and at the connective tissue interface. Diffuse labeling was observed for α2β1 and α5β1 integrins and focal labeling for α3β1 integrin, with the expression pattern being linear and granular (Fig. 1A-C). In the plexiform type, immunohistochemical labeling was more evident in epithelial sheets and cords where the cells were loosely arranged, resembling the stellate reticulum of the enamel organ; in the follicular type, the central and peripheral cells of the follicles were equally immunoreactive, with this type showing a strong tendency toward focal expression of α3β1 integrin. The acanthomatous type exhibited strong immunoreactivity, which was mainly observed for α5β1 integrin in cells undergoing squamous metaplasia. The desmoplastic and basal cell types exhibited predominantly strong labeling, both in the intercellular contact and at the epithelial-mesenchymal interface. In 1 tumor showing the granular cell pattern, strong immunoreactivity was observed for α2β1 and α5β1 integrins and weak labeling for α3β1 integrin, with the immunohistochemical labeling being more evident in cubic cells at the periphery of epithelial nests and scarcer in central granular cells. Analysis of solid ameloblastomas in general showed no significant difference in labeling intensity between the integrins studied (Table 1).

In unicystic ameloblastomas, immunohistochemical labeling was diffuse and was detected at the epithelial-connective tissue interface and in the intercellular contacts in all layers of the epithelial component, showing a linear and granular pattern for α2β1 and α5β1 integrins and a focal and granular pattern for α3β1 integrin (Fig. 1D-F). In these ameloblastomas, a significant difference in the intensity of immunohistochemical expression was observed between the α3β1 and α5β1 integrin, with stronger labeling being noted for the latter (Table 1).

Adenomatoid odontogenic tumor specimens tended to show a focal labeling for α2β1 and α3β1 integrins and a diffuse labeling for α5β1 integrin. With respect to the location of integrin expression, in solid areas and cords, labeling was detected in intercellular contacts, conferring a reticular pattern. In ductlike structures, granular or linear expression was observed at the luminal pole of cells in some areas, whereas in others, expression tended to be bipolar (Fig. 1G-I). In this tumor, no significant difference in labeling intensity was observed between the integrins studied (Table 1).

Because there was no significant difference in the immunohistochemical expression of the integrins studied between solid and unicystic ameloblastomas (Table 2), these 2 clinical-pathologic types were pooled into a single group for comparison with AOTs. As shown in Table 3, ameloblastomas presented a stronger labeling for α5β1 integrin.

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Comparison of the immunohistochemical expression of integrins between the different types of ameloblastoma by the nonparametric Mann-Whitney U test</th>
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</thead>
<tbody>
<tr>
<td>Integrin</td>
<td>Types</td>
</tr>
<tr>
<td>α2β1</td>
<td>Solid</td>
</tr>
<tr>
<td>α3β1</td>
<td>Solid</td>
</tr>
<tr>
<td>α5β1</td>
<td>Solid</td>
</tr>
</tbody>
</table>

4. Discussion

The integrin-mediated binding of cells to ECM proteins can cause changes in cytoplasmic domains that are associated with different postbinding events such as cell differentiation, migration, proliferation, and gene induction [16]. In addition, it is an important requisite for the survival and proliferation of cells, with this binding playing an
important role in the promotion and progression through the cell cycle [17].

In the present study, we analyzed the expression of integrins involved in the binding of cells to the ECM of ameloblastomas and AOTs. Ameloblastomas were divided into solid and unicystic tumors because of the known large difference in their biologic behavior [4] with the solid type showing a locally invasive and infiltrative behavior [5], whereas the unicystic type has a more favorable prognosis. Adenomatoid odontogenic tumor is an odontogenic tumor with slow but progressive growth [18].

In the 2 types of ameloblastoma, immunohistochemical labeling of the integrins studied was similar in distribution, location, and expression pattern. A tendency toward a focal labeling of $\alpha_{3\beta1}$ integrin was observed for both solid and unicystic ameloblastomas. Because this integrin is known to be an important laminin receptor [19], our findings agree with studies analyzing ECM components in ameloblastomas that demonstrated focal expression of laminin in these tumors [7,20,21]. This focal expression of $\alpha_{3\beta1}$ integrin may lead to basement membrane disorganization in some regions, as demonstrated in animal studies [22], thus contributing to the infiltrative behavior of ameloblastomas.

Analysis of the intensity of integrin expression in ameloblastomas revealed a predominance of 1 integrin over the other only for unicystic ameloblastomas, with a stronger labeling being observed for $\alpha_{5\beta1}$ integrin compared with $\alpha_{3\beta1}$ integrin (Table 1). A decrease in $\alpha_{5\beta1}$ integrin expression results in the loss of matrix fibronectin, which is accompanied by an increase in the incidence of metastases [23]. In addition, the expression of this integrin has been shown to inactivate cell proliferation genes such as c-fos, c-jun, and junB, decelerating cell growth and reducing tumorigenicity [24]. In another study involving benign and malignant epithelial tumors, an association was observed between the increased suprabasal expression of $\alpha_{2\beta1}$, $\alpha_{3\beta1}$, and $\alpha_{6\beta4}$ integrins and early recurrence and tumor progression [11]. The predominant expression of $\alpha_{5\beta1}$ integrin compared with $\alpha_{3\beta1}$ integrin observed in the present study for unicystic ameloblastomas might be explained by the less aggressive behavior of these tumors compared with the solid type. However, this hypothesis cannot be completely confirmed because no significant difference in the intensity of integrin labeling was observed between solid and unicystic ameloblastomas (Table 2). We believe that, in this context, it is important to determine whether the detected integrin is bound to its ligand in the ECM. In unicystic ameloblastomas, $\alpha_{5\beta1}$ integrin was detected in all epithelial layers where little or no ECM is present. In contrast, in solid ameloblastomas, these integrins are probably bound to the ECM, as reported in another study [8], demonstrating the presence of ECM proteins among neoplastic cells. This fact might result in an increased expression and secretion of metalloproteinases that are involved in tumor invasion [24] and whose presence has been demonstrated in ameloblastomas [25,26].

The different histologic types of solid ameloblastomas exhibited some variations in the location and intensity of integrin labeling, which reflect the various functions attributed to these molecules [15].

In the acanthomatous type, strong expression of $\alpha_{5\beta1}$ integrin was observed in cells undergoing squamous metaplasia. It has been suggested that the excessive production of this integrin, which causes it not to bind to the ECM, is a mechanism of tumor suppression and promotion of apoptosis induced by the p16 gene [27]. Studies on ameloblastomas have demonstrated the absence of labeling for cyclin D1 and p27 protein in cells undergoing squamous metaplasia and the presence of apoptotic bodies in these cells [28,29]. According to these studies, the presence of intense labeling for $\alpha_{5\beta1}$ integrin in central cells of the acanthomatous type observed in the present investigation suggests a mechanism of terminal differentiation or apoptosis of these cells. In support of this hypothesis, a significantly lower recurrence rate has been demonstrated for acanthomatous ameloblastomas [5], which might reflect a stage of terminal differentiation or apoptosis of cells of this histologic type.

Strong immunoreactivity to the integrins studied was observed for the desmoplastic type. Studies have demonstrated strong expression of collagens I and IV in the tumor stroma of this histologic type [8,30] and of transforming growth factor $\beta$, which seems to have an important function in the production of desmoplastic matrix [31]. Our findings suggest some role of integrins in the increased matrix production observed in these ameloblastomas.

An integrin-mediated signaling pattern in the promotion and progression through the cell cycle has also been demonstrated [17], and it has been reported that the interaction of integrins with growth factors regulates various steps in the transition from the G1 to the S phase of the cell cycle. Investigations using markers of cell proliferation have reported a higher proliferation rate for basal cell ameloblastomas [28,32,33], and poor reactivity of cells to apoptotic markers has been demonstrated for this histologic type [29]. Our findings regarding the expression of integrins in basal cell ameloblastoma suggest an important role of these molecules in the survival and cell proliferation in these tumors.

Regarding the granular cell type in which labeling of central granular cells was scarce, our findings again agree with studies demonstrating a weak reactivity of these cells to cell proliferation markers [28] and a strong presence of apoptotic markers [29,34].

In the ductlike structures of AOTs, integrin expression showed a linear or granular pattern at the luminal pole of the cells, or even a bipolar pattern. The presence of these integrins in cells surrounding the ductlike structures might be related to the regulation of cell polarization in these structures because the participation of $\beta1$ integrin in this regulation has been demonstrated in cell cultures [35]. In addition, the expression of these integrins at the luminal
pole of cells surrounding these ductlike structures may be necessary to activate the synthesis of ECM proteins or to anchor the cells to these proteins whose secretion by AOT cells, together with their retention in the lumen of ductlike structures, has been demonstrated [36].

Among the tumors studied, the labeling intensity for α5β1 integrin was significantly stronger in ameloblastomas. Integrin α5β1 is the classic receptor for fibronectin [23], a protein that plays an important role in the epithelial-mesenchymal interactions observed in odontogenic tumors [7,8]. Studies have shown that this integrin plays a crucial role in the migration and motility of both normal and neoplastic cells in fibronectin-rich matrix [37,38]. Stronger labeling of α5β1 integrin in the neoplastic cells of ameloblastomas may be associated with a greater migration capacity of these cells because large amounts of fibronectin have been detected in the stroma of these tumors [7,8]. Another role attributed to α5β1 integrin in the mechanism of tumor invasion is that its binding to fibronectin increases the secretion and expression of metalloproteinases. The presence of these enzymes in ameloblastomas has been reported [25,26], suggesting an interdependent mechanism involving metalloproteinases and cell proliferation in the local invasiveness of these tumors [26]. Therefore, a stronger expression of α5β1 integrins in ameloblastomas compared with AOTs suggests the participation of this molecule in the local invasiveness of the former.

Although no significant difference was observed in the expression of α2β1 and α3β1 integrins, immunohistochemical staining for all integrins studied tended to be stronger in ameloblastomas. This finding might be related to the higher proliferation rate of these tumors compared with AOT because integrin-mediated signaling is important in cell-cycle progression [27].

The present findings suggest the participation of integrins in the mechanism of invasion of ameloblastomas, with α5β1 integrin apparently playing a greater role. Agents that block this integrin might be an important target for the study and development of strategies for the treatment or control of the invasiveness of these tumors.

References


