ORIGINAL ARTICLE

Immunohistochemical expression of cyclin D1 in ameloblastomas and adenomatoid odontogenic tumors

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ABSTRACT

Background: Cyclin D1, a member of G1 cyclins, controls the cell-cycle transit from the G1 to S phase. The deregulation and overexpression of cyclin D1 has been revealed in many tumors of diverse histogenesis. Ameloblastoma is the most frequently encountered odontogenic tumor known for its local invasiveness and a high tendency to recur. The adenomatoid odontogenic tumor is a benign, nonaggressive tumor with a limited growth and no tendency to recur. **Aim:** The aim was to investigate whether the immunohistochemical expression of cyclin D1 as a proliferation marker in ameloblastoma and adenomatoid odontogenic tumor correlates with the known clinical behavior of these two benign neoplasms. **Materials and Methods:** Ameloblastoma cases consisted of follicular, plexiform, and unicystic subtypes. The positive staining was assessed based on intensity of staining, localization of staining, and in different cell types in both the tumors. Two cases of follicular ameloblastoma and one case of plexiform ameloblastoma showed intense staining, but the predominant staining intensity was overall mild in both ameloblastoma and adenomatoid odontogenic tumors. The immunoreactivity was found both in nucleus and cytoplasm in ameloblastoma and only nuclear in adenomatoid odontogenic tumors. The peripheral columnar and central stellate reticulum-like cells of ameloblastoma showed immunoreactivity with squamous and granular cells being negative. In adenomatoid odontogenic tumors, the whorls showed predominant localization of staining. Statistical comparison with a Mann–Whitney *U*-test showed no significant difference in staining intensities between different histologic subtypes of ameloblastomas and also between ameloblastoma and adenomatoid odontogenic tumors ($P>0.005$). **Conclusion:** The marked expression of cyclin D1 in these tumors suggested its participation in proliferation of both the tumors and its expression patterns were irrespective of their known biologic behavior.

Key words: Adenomatoid odontogenic tumor, ameloblastoma, cell cycle, Cyclin D1, G1 cyclins

INTRODUCTION

Cell proliferation is the foundation of tumor development. Normally it follows an orderly progression through the cell cycle, which is controlled at specific checkpoints by a complex regulatory molecular system composed of cyclins and cyclin-dependent kinases (CDKs). One of the important checkpoints is the restriction point at the G1/S phase, at which the cell commits itself to another round of DNA synthesis and replication or remains in the resting phase. Among the various cyclins, cyclin D1 is implicated more in tumorigenesis.[1-3]

Cyclin D1, a 45 kd protein, a member of G1 cyclins, encoded by the CCND1gene on chromosome 11q13, controls the cell-cycle transit from the G1 to S phase. It acts by forming a complex with either CDK4 or CDK6. This complex leads to phosphorylation of the pRb tumor suppressor protein which in turn releases members of the E2F family of transcription factors leading to unhindered progression of the cell to the S phase. Thus cyclin D1 is an essential molecule for the dividing cell to enter the DNA synthesis phase.[2]

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The deregulation and overexpression of cyclin D1 has been revealed in many tumors of diverse histogenesis and has been correlated with rapid growth and increased proliferative activity,[4] histologic aggressiveness,[5] tumor invasiveness, and poor prognosis,[6] lymph node metastasis.[7]

Ameloblastoma is the most frequently encountered odontogenic tumor that exhibits different histologic patterns. It is known for its local invasiveness and a high tendency to recur. On the contrary, adenomatoid odontogenic tumor, a relatively rare tumor, is a benign, nonaggressive tumor with a limited growth and no tendency to recur.[8]

It has been showed that **c**yclin D1 participates in cell proliferation both in normal and neoplastic odontogenic epithelium.[9] We assessed the immunohistochemical expression of cyclin D1 as a proliferation marker to investigate whether the expression of this marker in ameloblastoma and adenomatoid odontogenic tumor correlates with the known behavior of these two benign neoplasms.

MATERIALS AND METHODS

Tissues

Previously diagnosed tissues of ameloblastoma and adenomatoid odontogenic tumors were obtained from the files of the department of Oral and Maxillofacial Pathology, SDM college of Dental sciences and hospital, Dharwad. There were 39 ameloblastomas, and 11 adenomatoid odontogenic tumors. Among 39 cases of ameloblastoma, 25 were follicular, 10 were plexiform, and 4 were of unicystic types. A total of 22 cases of follicular ameloblastoma showed focal squamous metaplastic changes. Focal granular cell changes were observed in three cases of follicular ameloblastoma. All cases were reviewed histologically using hematoxylin and eosin staining to confirm the diagnosis. A total of 10 cases of poorly differentiated oral squamous cell carcinoma served as positive controls and were taken for each batch of the staining procedure.

Immunohistochemistry

Immunohistochemical staining was performed using labeled streptavidin–-biotin technique. Five micrometer sections were made from formalin-fixed paraffin embedded tissues blocks and taken onto silanized slides (SIGMA, USA). The sections were dewaxed in xylene and rehydrated in graded alcohols. Antigen retrieval was done by the pressure cooker method in a 10 mM citrate buffer (pH 6.0) for 2 minutes. Endogenous peroxidase activity was blocked by covering the tissue sections by 3% hydrogen peroxide for 15 minutes. Then the sections were incubated for 1 hour in a humidifying chamber with monoclonal anticyclin D1 antibody (clone DCS6, DAKO, Denmark) diluted to 1:50 in Tris buffered saline (TBS).

The sections were incubated with biotinylated link (secondary) antibody for 45 minutes. This is followed by incubation with streptavidin peroxidase for 30 minutes. The antigen antibody reactions were visualized with the chromogen DAB. Tris buffered saline was used instead of primary antibody in negative control tissue sections. The sections were washed and then lightly counterstained with Harris's hematoxylin, dehydrated, and mounted with DPX.

Interpretation of staining

The presence of brown-colored end-product at the site of target antigen indicated positive staining. Immunostaining was further graded as 1 when there was mild staining, 2 for moderate, and 3 for intense staining. The interpretation was similarly used by Mate *et al*.[10]

The localization of staining and cell types which showed immunostaining in both ameloblastoma and adenomatoid odontogenic tumors were also noted accordingly. All these observations were carried out by three observers to eliminate interobserver bias.

Statistical analysis

A rank sum two-sample test (Mann–Whitney test) was used for the statistical interpretation of the intensity of staining results in different types of ameloblastomas and also between ameloblastoma and adenomatoid odontogenic tumors. A P value of less than 0.05 was taken as statistically significant.

RESULTS

Most of the ameloblastoma cases (79.5%) and adenomatoid odontogenic tumor (63.3%) were positive for cyclin D1 expression [Table 1]. The immunoreactivity was predominantly concentrated in both nucleus and cytoplasm in ameloblastomas [Table 2] and only nuclear in adenomatoid odontogenic tumors.

In ameloblastomas, 3 cases showed intense staining (grade 3) [Figure 1], 12 cases showed moderate staining (grade 2) [Figure 2], and 16 cases showed mild staining (grade 1) [Figure 3] while in adenomatoid odontogenic tumors, 2 cases were moderately immunoreactive (grade2) [Figure 4], and 5 were mild (grade 1).

Cyclin D1 staining was observed both in peripheral columnar or cuboidal cells and in central stellate reticulum-like cells in most of the ameloblastoma cases. The squamous metaplastic cells and granular cells were negative for cyclin D1 expression [Figure 5]. In adenomatoid odontogenic tumors, the immunoreaction was found more commonly in whorls.

The rank sum two-sample test (Mann–Whitney test) between staining intensities in different histologic groups of ameloblastomas and also between ameloblastoma and adenomatoid odontogenic tumors showed nonsignificant P value (P>0.005) [Tables 3 and 4].

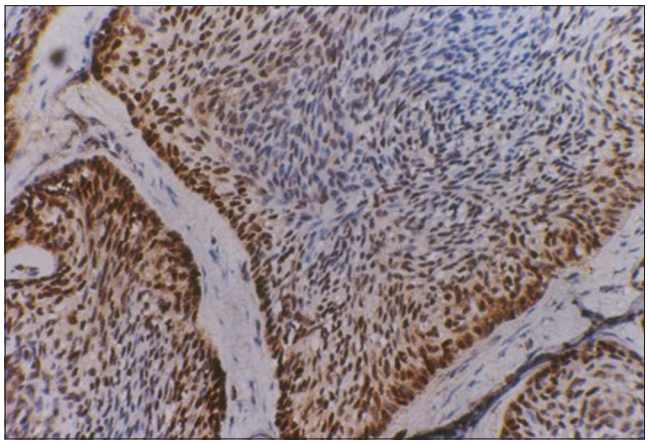

Figure 1: Photomicrograph of positive cyclin D1 expression showing intense nuclear staining of both basal and stellate reticulum-like cells in follicular ameloblastoma (250×)

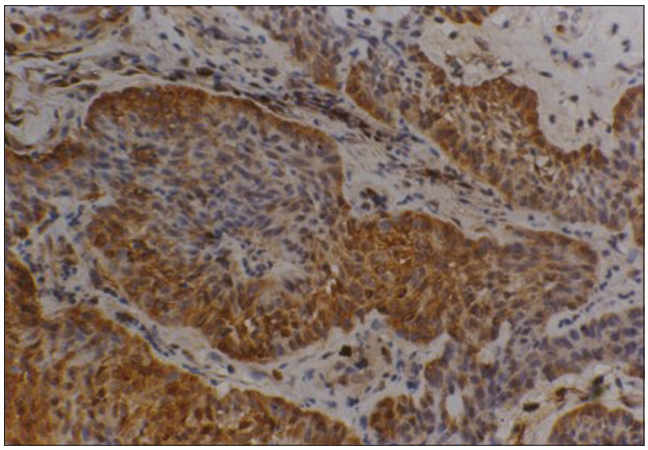

Figure 2: Photomicrograph of positive cyclin D1 expression showing moderate cytoplasmic staining of both basal and stellate reticulum-like cells in plexiform ameloblastoma (250×)

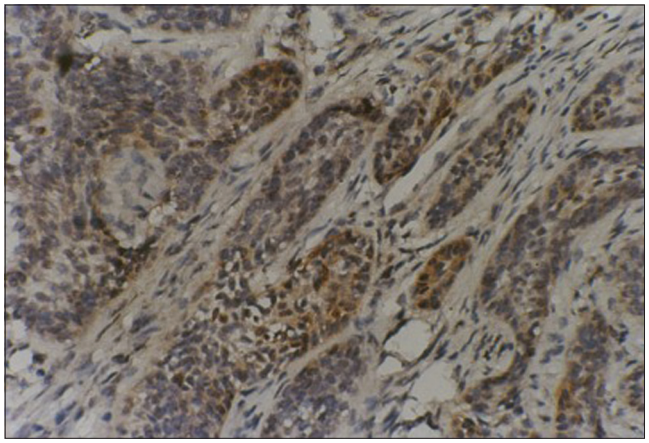

Figure 3: Photomicrograph of positive cyclin D1 expression showing mild cytoplasmic staining of both basal and stellate reticulum-like cells in follicular ameloblastoma (250×)

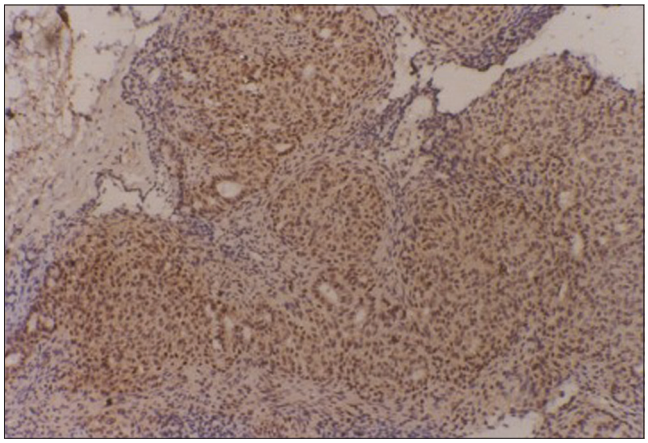

Figure 4: Photomicrograph of positive cyclin D1 expression showing predominantly moderate staining intensity in the nuclei of whorls, ducts, and sheets in the adenomatoid odontogenic tumor (100×)

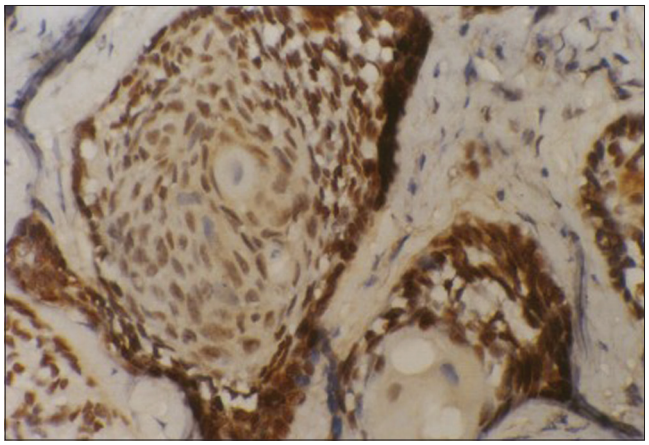

Figure 5: Photomicrograph of positive cyclin D1 expression showing negative staining of squamous metaplastic cells in follicular ameloblastoma (400×)

In positive cases, the staining pattern was heterogeneous; the unstained cells formed a minor fraction and were admixed with the stained cells which formed a major fraction of the sections studied. In negative cases all the cells were unstained.

Table 1: Staining results of ameloblastoma and adenomatoid odontogenic tumor

	Total number of cases	Number of positive cases (%)	Number of negative cases (%)
Ameloblastoma			
Follicular	25	19	06
Plexiform	10	09	01
Unicyctic	04	03	01
Total	39	31 (79.5)	08 (20.5)
Adenomatoid odontogenic tumor	11	07 (63.3)	04 (37.7)

Table 2: Total number of ameloblastoma cases showing the cyclin D1 immunolocalization

Localization	Total
Nuclear	2
Nuclear and cytoplasmic	19
Cytoplasmic	10
Total	31

Table 3: Statistical comparison of cyclin D1 staining intensity between follicular, plexiform, and unicystic ameloblastomas Mann-Whitney test

Types	Groups compared	*P* value
Follicular (group I)	Groups I and II	0.3628
Plexiform (group II)	Groups II and III	0.4054
Unicystic (group III)	Groups III and I	0.7741

Table 4: Overall staining results and statistical comparison of staining intensity of both ameloblastomas and adenomatoid odontogenic tumors

	Intense	Moderate	Mild	Negative	Mann-Whitney test *P* value
Ameloblastoma (39 cases)	3	12	16	8	0.1744
Adenomatoid odontogenic tumor (11 cases)	0	2	5	4	

Hence the staining reaction analyzed was based on the majority of stained cells and the predominant staining intensity and localization were chosen in both the lesions.

DISCUSSION

Cyclins and CDKs play a central role in cell-cycle control and any alteration in them may lead to oncogenesis.[1-3,11] Among the potentially oncogenic elements of the cell-cycle machinery, cyclin D1 is strongly implicated in oncogenesis. Its fundamental role is to integrate mitogenic signals with the cell-cycle regulating system.[2]

In various studies, immunohistochemical expression of cyclin D1 in different tissue specimens is seen to be positively correlating with other proliferation markers such as Ki-67,[10] PCNA,[12] Topo II alpha, and histone H3 mRNA[9] and other cell-cycle regulatory proteins such as CDK4, p21, E2F1,[12] proapoptotic protein p53[13], and inversely correlating with expression of tumor suppressor pRb protein[14], and bcl-2.[12] These studies supported the role of cyclin D1 as a potential marker of proliferation and oncogenesis.

Information in the English language literature on cyclin D1 expression in ameloblastoma is quite sparse. In a comparative study of cyclin D1 expression between odontogenic keratocyst, dentigerous cyst, radicular cyst, and ameloblastoma, Vicente DJC *et al*., found diffuse expression in ameloblastoma, nuclear staining in parabasal cells of odontogenic keratocyst and sporadic expression in dentigerous cyst and radicular cyst supporting the role of cyclin D1 in proliferation of these odontogenic lesions.[15] Lo Muzio *et al*. also found expression of cyclin D1 in odontogenic keratocyst associated with nevoid-basal cell carcinoma syndrome, supporting odontogenic keratocyst's neoplastic nature.[16] Kumamoto *et al*.[9] found sporadic expression of cyclin D1 in inner and outer enamel epithelium, stratum intermedium, stellate reticulum of tooth germs. Dental lamina showed little or no expression. Follicular and plexiform ameloblastomas showed cyclin D1 expression in many peripheral columnar or cuboidal cells and some central polyhedral cells.[9] Similar expression patterns of cyclin D1 in ameloblastoma were detected by Tanahashi *et al*., in their study of Wnt signaling molecules in this tumor.[17] In our study, the expression patterns of cyclin D1 in different histologic types of ameloblastomas were in line with these previous studies. However, the statistical analysis of intensity of staining between these different histologic types did not yield significant *P* values.

The predominant expression of cyclin D1 in both peripheral basal cells and central stellate reticulum-like cells suggests that basal cells posses a higher proliferative activity, supporting a finding by Stenmen *et al*.[18] Stellate reticulum-like cells unlike basal cells do not belong to the proliferative compartment. Hence cyclin D1 expression in these cells indicated its role in differentiation possibly by forming inactive complex with CDK2 and CDK5 helping cells to stabilize the differentiated state or to carry out cell-type-specific functions. Other possible explanation may be that the cyclin D1-CDK6 or CDK4 complex is inactivated by the simultaneous presence of a CDK inhibitor.[19] However, similar to Kumamoto *et al*.,[9] we did not find cyclin D1 expression in terminally differentiated cells-like granular cell and squamous metaplastic cells in some follicular ameloblastomas. Further investigations are required in this regard to establish the role of cyclin D1 in differentiating odontogenic cells.

A search in the English language literature with key words "cyclin D1," "adenomatoid odontogenic tumor," and "immunohistochemistry" revealed no studies of cyclin D1 in the adenomatoid odontogenic tumor so far. However, in a study of PCNA, a proliferative marker, in adenomatoid odontogenic tumors, its expression was observed more in solid areas consisting of adenomatoid and duct-like structures similar to our findings with cyclin D1, indicating more proliferative activity in these areas.[20]

Though cyclin D1 predominantly expresses in nucleus, we found its immunoreactivity both in nucleus and cytoplasm and a combination of two. As cyclin D1 plays a role in proliferation and differentiation and the shift between nucleus and cytoplasm is necessary to regulate finely the passage across different phases of the cell cycle, immunogold studies have indicated its transition between nucleus and cytoplasm through nuclear pores.[21] Other possible reason is that cyclin D1 or its related CDK may be inactivated by being bound to further substrate and as a consequence loses its nuclear localization.[22] Even glycogen synthase kinase-3β (GSK-3β) exports cyclin D1 to the cytoplasm by facilitating cyclin D1 and exportin interaction or it might phosphorylate cyclin D1

in cytoplasm preventing its association with proteins required for nuclear import.[23]

The heterogeneous staining pattern seen in our study in positive cases probably indicated that all cells might not be proliferating. The staining intensity also varied from mild to intense in the same section possibly due to variations in protein levels during cell-cycle progression.[1,5] Hence the predominant staining intensity was considered.

Though some cases of ameloblastoma showed intense and moderate staining indicating its distinctive clinical behavior compared to that in the adenomatoid odontogenic tumor, which predominantly showed mild staining, this did not reach statistical significance. Thus the marked expression of cyclin D1 suggested its participation in proliferation of both the tumors and its expression patterns were irrespective of their biologic behavior.

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