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CDK6 expression in dentigerous cyst, odontogenic keratocyst and ameloblastoma

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ABSTRACT

To investigate the expression of CDK6 in odontogenic lesions. In this study 77 odontogenic lesions consist of 19 unicystic ameloblastoma (UA), 18 solid ameloblastoma (SA), 20 odontogenic keratocysts (OKC), and 20 dentigerous cysts were reviewed by immunohistochemistry (IHC) for CDK6 staining. Frequency of positive cytoplasmic CDK6 expression in ameloblastoma (solid and unicystic) and DCs, were recorded as, 100% and 15% respectively which show a statistically significant different (p=0.000). Positive nuclear CDK6 in SAs and UAs was 100% and 94.7% respectively which was significantly higher than DCs and OKCs (p=0.000). Mean percentage of both cytoplasmic (72.71 ± 10.0) and nuclear (45.0 ± 10.7) CDK6 staining in SAs was significantly higher than other lesions (p=0.000). Our study demonstrated that overexpression of the nuclear CDK6 and dysregulation of the PRb pathway play a role in the oncogenesis of ameloblastoma but not DCs and OKCs and might be one of the reasons for aggressive behavior of ameloblastoma.

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Introduction

Regulation of the cell cycle and control of the cell proliferation is important in development and disease. A key step in cell cycle regulation is the control of the G1/S and G2/M transition.[1]

Abnormality of the cell cycle at those mentioned steps may allow uncontrolled cell proliferation and carcinogenesis.[2]

The proliferation of eukaryotic cells, especially of mammalian cells, is controlled by cyclins, CDKs and CDK inhibitors.[3]

CDKs are low molecular weight proteins (34 to 40 KDa) which have a minor kinasic activity. CDKs can bind to regulative protein called cyclin and this complex has active kinasic role.[4]

Transition from G1 to S phase is regulated by complex mechanisms, and different CDKs including CDK2, CDK4 and CDK6 have an important role in these mechanisms .Mitogenic signals can include cyclin-D synthesis and transporting of CDK4 or CDK6 to the nuclear.[5]

Phosphorylation and subsequent inactivation of retinoblastoma tumor suppressor gene product, by these complexes, leads to the release of E2F transcription factors.[6]

Releases of these factors are important for s-phase entry.[7] Thus CDK6 control the cell cycle of G1 to S transition phase, and uncontrolled cell proliferation caused by changes of these molecules, have been identified in various tumors.[8-10]

Different types of odontogenic lesions originate from rests of dental lamina. The potential for additional proliferation of these epithelial remnants during lesion formation is different and thus cause variations in their biological behavior .[11]

Some cysts like odontogenic keratocysts have high recurrence rate and some tumors such as ameloblastoma have aggressive behavior despite of benign histopathologic features.

These different features insist the need of further studies about the nature and behavior of this lesion.

To our Knowledge, cell-cycle regulatory factors have not been sufficiently studies in odontogenic lesions so we investigated the presence of CDK6 in these lesions.

Material and Method:

Materials:

In this study 77 odontogenic lesions consist of 19 unicystic ameloblastoma (UA), 18 solid ameloblastoma (SA) , 20 odontogenic keratocysts(OKC), and 20 dentigerous cysts were studied.

Firstly, H & E slides of available blocks were reviewed and then cases with definite diagnosis and adequate tissue were selected for immunohistochemical staining (IHC). Cases with severe inflammation were excluded from study.

IHC staining and analysis:

IHC staining was performed by using Envsion Labled Peroxidase System (DAKO, Carpentaria, CA, USA). All the samples were fixed in 10% buffered formalin and were embedded in paraffin. Sections with 4µ thickness were prepared, deparaffinized in xylene,rehydrated in graded alcohol and were washed with distilled water. Antigen retrieval was performed by using DAKO cytomation target retrieval solution with PH = 9, for 20 minutes. Internal Peroxidase activity was inhibited by 3% H2O2.

Tissue sections were then incubated for 30 minutes with the anti-CDK6 antibody (Santa Cruz Biotechnology, SD-7961) at a 1/50 dilution.

Omission of the primary antibody was considered as negative control, while gastric epithelium was used as positive control for CDK6.

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Brown nuclear and cytoplasmic staining for CDK6 was considered as positive.

Immunoreactivity was expressed by determining the percentage of positive cells. Briefly, at least 1000 cells counted at five areas with X400 magnification and the percentage of both nuclear and cytoplasmic staining was calculated.

Statistical analysis:

Mann-Whitney and Kruskal Wallis tests were used to compare results. The level of significance was set at 0.05.

Results:

In our study, both nuclear and cytoplasmic expression of CDK6 was observed. In DCs it was only cytoplasmic, but in ameloblastoma (solid & unicystic), both patterns was seen. CDK6 expression was not seen in OKCs.(Figure 1-3)

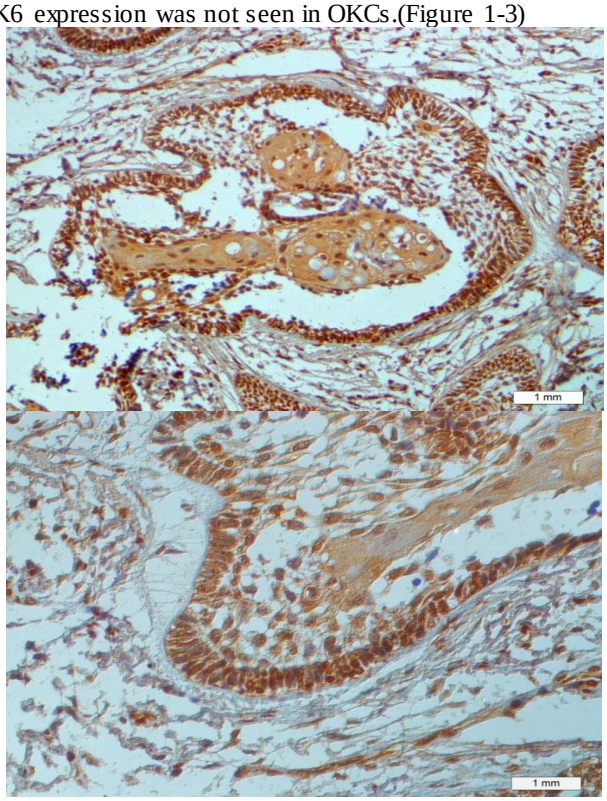

Figure 1: Cytoplasmic and nuclear staining of CDK6 in SA. Cytoplasmic CDK6 expression was also seen in keratinizing cells in acamthomatous ameloblastoma.

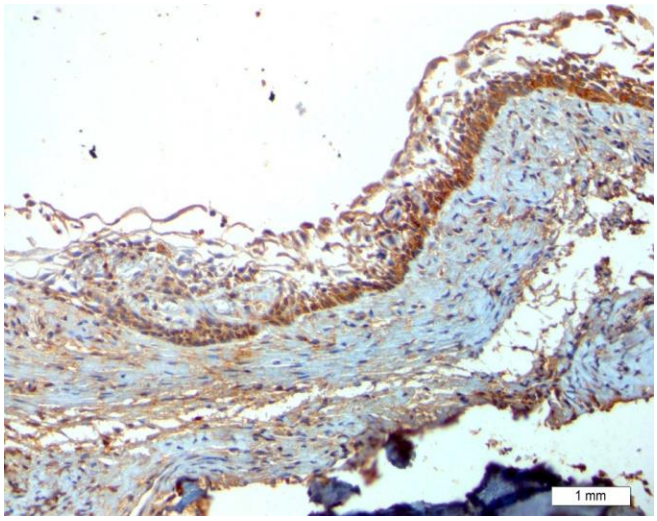

Figure 2: Cytoplasmic and nuclear staining of CDK6 in UA

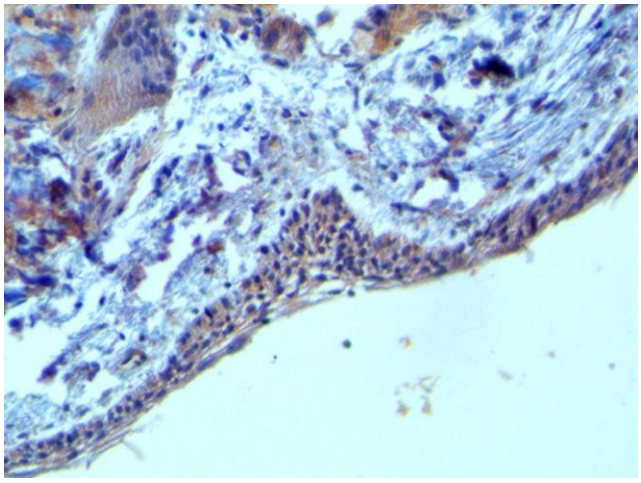

Figure 3: Cytoplasmic staining of CDK6 in *DC*

Frequency of positive cytoplasmic CDK6 expression in ameloblastoma (solid and unicystic) and DCs, were recorded as, 100% and 15% respectively which show a statistically significant different (p=0.000). Positive nuclear CDK6 in SAs and UAs was 100% and 94.7% respectively which was significantly higher than DCs and OKCs (p=0.000) (Table1).

Mean percentage of cytoplasmic and nuclear CDK6 staining in different lesions is show in Table2.

Mean percentage of both cytoplasmic (72.71 ± 10.0) and nuclear (45.0 ± 10.7) CDK6 staining in SAs was significantly higher than other lesions (p=0.000) (Table2). But there wasn't any statistically significant difference between CDK6 expression in DCs and OKCs (p=0.4).

Cytoplasmic CDK6 expression was also seen in keratinizing cells in a acamthomatous ameloblastoma. (Figure 1)

Nuclear CDK6 expression was predominantly seen in peripheral ameloblast like cells.

Discussion:

Cell cycle regulation is an essential process by which the cell controls its differentiation and growth during morphogenesis and embryonic development.[12]. Different regulatory factors, such as cyclines, CDKs and CDK inhibitors, have been expressed in tooth germ, propose that regulation of the cell cycle has a role in the development and fate of odontogenic cells.[13]. This study focused on CDK6 protein to demonstrate its role in odontogenic lesions. In this study overexpression of nuclear CDK6 was seen in both unicystic and solid ameloblastoma, but DCs and OKCs didn't express CDK6, which indicates that this marker and Rb pathway have a role in tumorogenesis. PRb phosphorylation at the G1/S transition is usually driven by CDK4 and CDK6, in complex with cyclin D1, leading to the release of associated proteins like E2F that can activate the genes necessary for cell progression through the G1 phase.[7]

Thus the increase expression of CDK6 in our samples suggests that this protein is probably involved in oncogenesis of ameloblastomas. Increased expression of CDK6 in SAs compare with UAs demonstrates the more aggressive behavior of SAs. In a study conducted by Kumamoto et al, it was shown that phosphorylated RB and active free E2F-1 are expressed in both normal odontogenic tissue and ameloblastoma. They concluded that expression of these two factors is involved in differentiation and proliferation of odontogenic epithelium via cell cycle regulation.[13]

Table1: Frequency of nuclear and cytoplasmic CDK6 expression in different groups

Type of lesion	Cytoplasmic expression n (%)	Nuclear expression n (%)
SA	18 (100)	18 (100)
UA	19 (100)	18 (94.7)
OKC	0 (0)	0 (0)
DC	3 (15)	0 (0)

Table 2: Mean percentage of nuclear and cytoplasmic CDK6 expression in different groups

Type of lesion	Cytoplasmic expression	Nuclear expression
SA	72.7 ± 10.0	45.0 ± 16.7
UA	59.3 ± 17.3	21.3 ± 18.7
OKC	0 ± 0.0	0 ± 0.0
DC	7.5 ± 1.4	0 ± 0.0

Overexpression of CDK6 in this study is in accordance with Kumamoto et al (2006) [13], and may be indicated that overexpression of this marker is implicated in dysregulation of the cell cycle and increased PRB and active free E2F-1.

Different genetic changes such as genetic rearrangement and point mutations in the gene of CDK6, can lead to tumor formation. In other words, these genetic changes in each step of DNA transcription can induce tumorogenesis and enhance the expression of this marker in tumors.

Another mechanism that can cause CDK6 expression in tumors,is the absence of P16 protein which is a kinase inhibitor and a negative regulator of the cell cycle. Its binding to CDK6 ,prevents the formation of CDK6-cycline –D complex and cell proliferation.[14] Finally, the results of these mechanism (Genetic variation in CDK6 and lack of P16 expression), are hyper-phosphorylation of Rb protein and passing through G1 phase.

But in Kumamoto et al(2001)[15] study it was suggested that alteration of the P16 gen was not occur in ameloblastoma hence CDK6 overexpression might be due to genetic variation although, future studies which investigate the mechanisms of the increasing express of this marker are recommended.

In our study CDK6 expression was seen in both nuclear and cytoplasm of tumoral tissues.

Cytoplasmic staining of CDK6 was reported in different study. But its function in terms of its cellular distribution is still poorly understood. However, some functions have been proposed for CDK6 in cytoplasm. Recently, it has been suggested that CDK6 has a role in cell differentiation.[16]

Slomainy (2006)[17], has suggested that cytoplasmic overexpression of CDK6, can increased the motility of rat astrocytes through changing the dynamics of actin filaments.

Therefore, according to CDK6 role as a regulator of cell proliferation and differentiation, it is expected that this protein may have an active function in both the nuclear and cytoplasm. It means that it is involved in cytoskeleton remodeling in cytoplasm and in regulation of transcription in the nucleus.[16]

So its cytoplasmic expression in ameloblastoma and DCs may be related to its role in the remodeling of cytoskeleton or differentiation and only nuclear overexpression has a role in oncogenesis and cell proliferation.

So cytoplasmic expression of CDK6 in keratinizing cells in acanthomatous ameloblastoma may be a reflection of its role in differentiation.

In the present study, expression of CDK6 was not seen in OKCs. So the dysregulation of PRb pathway didn't play a role in the pathogenesis and aggressive behavior of OKCs. This result was in accordance with Moreira et al (2009)[18] study which showed that methylation of the RB1 gene was not seen in OKCs.

Conclusion:

Our study demonstrated that overexpression of the nuclear CDK6 and dysregulation of the PRb pathway play a role in the oncogenesis of ameloblastoma but not DCs and OKCs and might be one of the reasons for aggressive behavior of ameloblastoma.

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