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Expression of CDK6 in Salivary Gland Tumors

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To investigate the expression of CDK6 in salivary gland tumors. In this study, 59 samples of salivary gland tumors from Khalili Hospital pathology archive, including 19 cases of pleomorphic Adenoma, 14 cases of Mucoepidermoid Carcinoma and 19 cases of Adenoid Cystic Carcinoma, as well as 10 cases of normal salivary gland tissue, were reviewed by Immunohistochemistry (IHC) for CDK6 staining. CDK6 expression in normal salivary gland was limited to the cytoplasm of ductal cells, but in tumoral tissues was both nuclear and cytoplasmic. Mean percentage of CDK6 staining in the tumoral group (17.38±21.25) was significantly higher than the normal group (1.1±0.99). So, we recognized a higher expression of this marker in tumoral lesions than in normal tissues ($p = 0.021$). But there wasn't any statistically significant difference between expression of CDK6 in different types of tumors ($p = 0.2$). This study demonstrated that over expression of nuclear CDK6 and the dysregulation of PRb pathway play a role in the oncogenesis of salivary gland tumors.

Key words: Cyclin dependent kinase 6, salivary gland tumors, cell cycle, immunohistochemistry

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INTRODUCTION

Tumor cell proliferation provides insights into tumor biology and is correlated to the progression and prognosis of a variety of malignant tumors (Morgan, 1995).

The changes of different components of the mechanism for cell-cycle regulation lead to the development of numerous types of human cancer because these components regulate the progression of cells from a quiescent to a growing state (Santamaria and Ortega, 2006).

The proliferation of eukaryotic cells, especially of mammalian cells, is controlled by cyclins, cyclin-dependent protein kinases (CDKs) and CDK inhibitors at specific points in the cell cycle, particularly at the G1 to S and the G2 to M transitions (Wang *et al*., 2004).

CDKs are a family of kinases' proteins that were initially identified by their regulative role in the cell cycle. Cyclin-dependent Kinases (CDKs) are small proteins with a molecular weight of 34 to 40 kDa (Morgan, 2007). CDKs bind to protein regulators called cyclin. CDKs have a slight kinasic activity and only cyclin-CDK complexes have an active kinasic role (Satyanarayana and Kalid, 2009).

Passing from the G1 to the S phase is controlled by complex mechanisms, in which at least three types of CDKs and their regulators including CDK2, CDK4 and CDK6 have an important role (Malumbres and Barbacid, 2005; Sherr and Roberts, 1999). At first, mitogenic signals cause Cyclin-D synthesis and folding and transporting of CDK4 or CDK6 to the nucleus (Mendrzyk *et al*., 2005).

Cyclin D/ CDK4-6 complex drive phosphorylation and subsequent inactivation of the retinoblastoma tumor-suppressor gene product, pRb and pRb-related proteins p107 and pRb2/p130 (MacLachlan *et al*., 1995); this inactivation by cyclin D/CDK4-6 complex leads to the release of the E2F transcription factors that trigger progression into the S phase (Morgan, 2007). Any disturbance in regulation of Rb pathway, triggers carcinogenesis and leads to cancer (Gladden and Diehl, 2003).

The salivary gland tumors consist 2.0-6.5% of all neoplasms of the head and neck (Medema *et al*., 1995). To establish novel treatment strategies, a better understanding of salivary gland biology is necessary. Very few investigations have described salivary gland tumors at the molecular or cell- proliferation levels. The aim of the present study was to evaluate the expression of CDK6 in salivary gland tumors immunohistochemically.

MATERIALS AND METHODS

In this cross sectional study, 52 samples of salivary gland tumors from Khalili Hospital pathology archive including 19 cases of pleomorphic adenoma (PA), 14 cases of Mucoepidermoid Carcinoma (MEC) and 19 cases of Adenoid Cystic Carcinoma (AdCC) and 10 cases of normal salivary gland tissue were reviewed. Firstly, H and E slides of available blocks were reviewed and then the 52 cases with definitive diagnosis and adequate cellular tissue were selected for Immunohistochemical Staining (IHC). 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 4 μ 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 min. Internal Peroxidase activity was inhibited by 3% H_2O_2.

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

Normal salivary gland tissue samples were stained with the same amount of antibody used for staining tumoral tissues. Omission of the primary antibody was considered as negative control, while gastric epithelium was used as positive control for CDK6.

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

Immunohistochemical results were interpreted by two pathologists. Immunoreactivity was expressed by determining the percentage of positive tumor cells. Briefly, at least 1000 neoplastic cells counted at five areas with X400 magnification.

Mann-Whitney and Kruskal Wallis tests were used to compare the results.

RESULTS

Patients with salivary gland tumors included 31 females (59.6%) and 21 males (40.4%) with a mean age of 49.1 years. In our study, both nuclear and cytoplasmic expression of CDK6 was observed. In normal tissue it was only cytoplasmic, but in tumoral tissues both patterns of expression was seen. CDK6 expression in normal salivary gland was limited to the cytoplasm of ductal cells (Fig. 1). Frequency of positive cytoplasmic CDK6 in normal and tumoral tissue, were recorded as 70% (7 cases) and 71% (37 cases) respectively which didn't show a statistically significant difference ($p = 0.4$). Positive nuclear CDK6 was

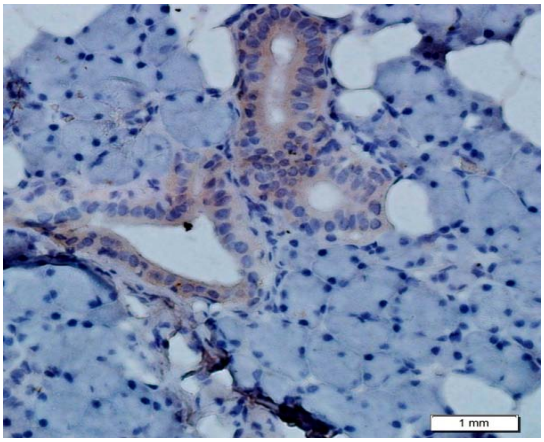

Fig. 1: Cytoplasmic expression of CDK6 in duct of normal salivary glands (x400)

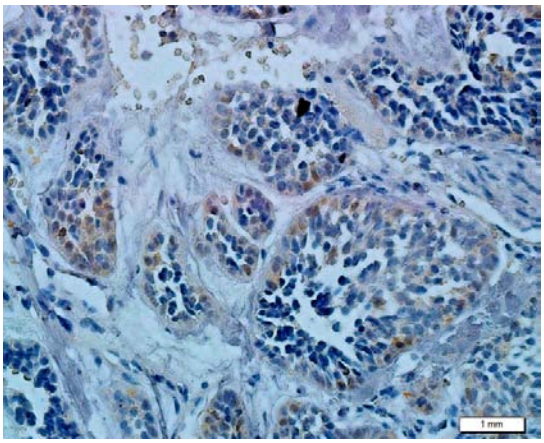

Fig. 4: Nuclear and cytoplasmic expression of CDK6 in AdCC (x400)

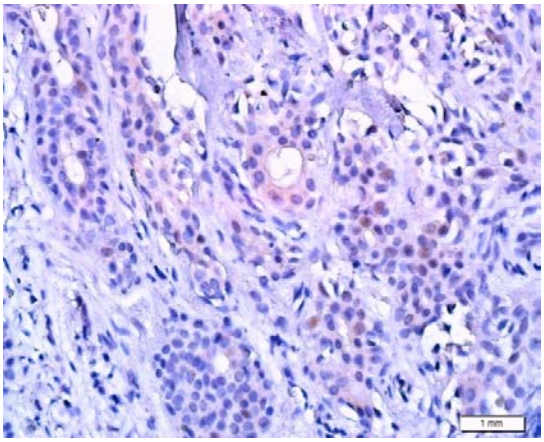

Fig. 2: Cytoplasmic expression of CDK6 in PA (x400)

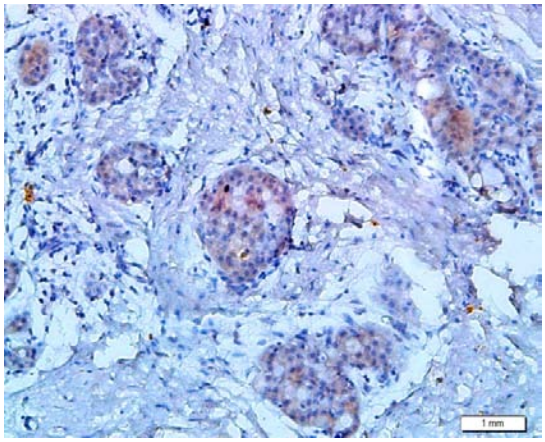

Fig. 5: Cytoplasmic expression of CDK6 in MEC (x200)

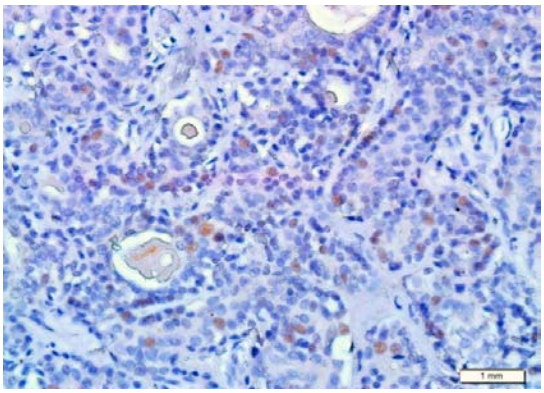

Fig. 3: Nuclear expression of CDK in PA (x400)

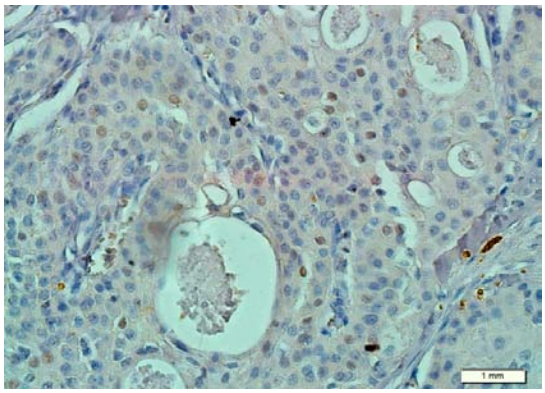

Fig. 6: Nuclear expression of CDK6 in MEC (x400)

not seen in the normal tissues but, 46.1% of tumor cases showed nuclear expression which revealed a statistically significant difference ($p = 0.01$) (Fig. 2-6). Cytoplasmic and nuclear expression of CDK6 in each lesion is shown in

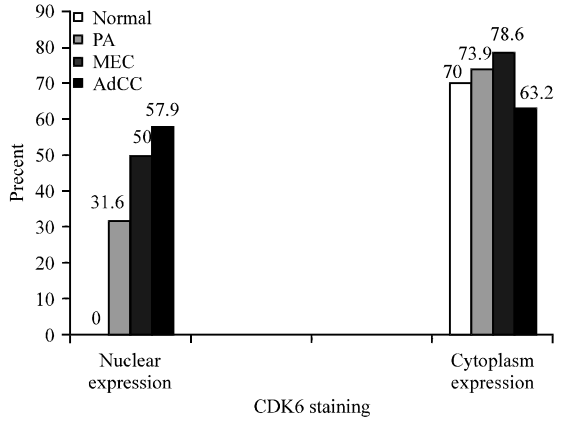

Fig. 7: Frequency of nuclear and cytoplasmic expression of CDK6 in different groups of lesions

Table 1: Mean percentage of CDK6 staining in different groups of lesions

Type of tissue	Mean ± SD	P.value
Normal tissue	1.1±0.9	Normal ≠ PA (p = 0.031)
PA	10.2±12.9	Normal ≠ MEC (p = 0.007)
		Normal ≠ AdCC (p = 0.045)
MEC	24.6±27	PA ≠ MEC (p = 0.123)
AdCC	19.2±23.7	PA ≠ AdCC (p = 0.418)
		MEC ≠ AdCC (p = 0.506)

(Fig. 7). 73/7% of PA and 69/7% of malignant tumors showed cytoplasmic expression of CDK6 which revealed no statistically significant difference (p = 0.9).

The 31.6% of PA and 54.5% of malignant tumors showed nuclear expression of CDK6 which revealed no statistically significant difference (p = 0.1).

Mean percentage of CDK6 staining in the tumoral group (17.38±21.25) was significantly higher than the normal group (1.1±0.99). So this study recognized a higher expression of this marker in tumoral lesions than in normal tissues (p = 0.021). But there wasn't any statistically significant difference between expression of CDK6 in different types of tumors (p = 0.2). Mean percentage of CDK6 staining of different groups of lesions is shown in Table 1.

DISCUSSION

Disorientation in cell-cycle regulation is a fundamental process in tumor growth, which is a common phenomenon in many types of tumors (Dobashi *et al*., 2004; Mendrzyk *et al*., 2005). This study focused on CDK6 protein to demonstrate its role in salivary gland tumors. In this study nuclear expression of CDK6 differ in normal salivary glands from both benign and malignant tumors. In our study over expression of nuclear CDK6 was observed in tumoral tissues, which indicates that this marker and Rb pathway have a role in tumorogenesis. pRb phosphorylation at the G1/S transition is usually driven by CDK-4 and CDK-6, in complexes with cyclin D1; leading to the release of associated proteins like E2F-1 that can activate the genes necessary for cell progression through the G1 phase (Morgan, 1995). Thus, the increased expression of CDK6 in our samples suggests that this protein is probably involved in salivary gland oncogenesis. In a study conducted by Etge's and others it was detected that there is a higher increase in CDK4 expression within the tumors compared to normal tissues. In this study like our study, there wasn't any difference between benign and malignant tumors regarding the CDK4 expression (Etges *et al*., 2004). CDK6 has been reviewed in many studies and many transformations including point mutations and genetic rearrangement was scrutinized in the gene of this marker, which could be involved in the process of tumorogenesis. In other words, these genetic changes during every stage of DNA transcription can induce tumorogenesis and increase the expression of these markers in tumors. Another mechanism that increases CDK6 activity in tumors is the absence of P16 protein expression in these cells. P16 is a kinase inhibitor which is a negative regulator of the cell cycle. Binding of this protein to CDK6, inhibits its binding to D-cyclin, blocking G1 phase that inhibits cell proliferation (Patel *et al*., 1997). 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. In this study, only the expression was studied. Therefore, future investigations to study the mechanisms of the increasing expression of this marker are recommended.

In our study, the expression of CDK6 marker was observed in both nucleus and cytoplasm of the tumoral tissues. Expression of this marker in normal tissue was only cytoplasmic. In several studies cytoplasmic staining of CDK6 has been reported, but the significance and function of this marker in terms of its cellular distribution is still poorly understood. Kohrt *et al*. (2009) demonstrate that some of CDK6's activity is regulated by breaking this kinase in the cytoplasm. This may indicate a new function of this marker which is not yet known. The first theory asserts that breaking of CDK6 in the cytoplasm is necessary to activate CDK6 in the nucleus. To confirm this theory, a study showed the presence of CDK6 in the cytoplasm and nucleus of T-cell but only nuclear CDK6 was active and showed the ability of Rb phosphorylation (Mahony *et al*., 1998). However, some functions have been proposed for CDK6 in the cytoplasm. For instance, in a study conducted by Slomiany *et al*. (2006), it was shown that the increased staining of CDK6 in the cytoplasm resulted in changes in the dynamics of actin filaments and increased the mobility of rat astrocytes (Slomiany *et al*., 2006). Moreover,

Fahraeus and Lane (1999) showed CDK6 in folded edges of fibroblast cells and confirmed its role in the proliferation and movement of these cells. Recently, the role of CDK6 in cell differentiation has been asserted. Therefore, according to CDK6's role as a coordinator in cell proliferation and differentiation, it is expected that this protein may have an active function in both the nucleus and cytoplasm. It means that CDK6 can be involved in regulating the transcription in the nucleus and also in the remodeling of the cytoskeleton in cytoplasm (Kohrt *et al*., 2009). So, its cytoplasmic expression in both normal and tumoral tissue may be a reflection of its role in differentiation or in the remodeling of cytoskeleton and only nuclear expression has a role in the cell proliferation and oncogenesis.

CONCLUSION

In conclusion, our study demonstrated that over expression of the nuclear CDK6 and the dysregulation of the PRb pathway play a role in the oncogenesis of salivary gland tumors.

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