RESEARCH COMMUNICATION

Immunohistochemical Evaluation of p27 (kip1) in Pleomorphic Adenomas and Adenoid Cystic Carcinomas of the Minor Salivary Glands

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Abstract

Background: p27(kip1), a universal cyclin-dependent kinase inhibitor, is a useful marker for predicting clinical aggressiveness with various human tumors. In this study, p27 expression was investigated in pleomorphic adenomas (PAs) and adenoid cystic carcinomas (ACCs) of minor salivary glands to evaluate its utility for differentiation purposes. At the same time, the correlation between p27 and ACC grading was evaluated. Materials & Methods: Clinicopathological features of 22 patients (11 ACCs, 11 PAs), including age, sex and size of tumor were obtained from medical records. Immunohistochemical staining with p27(kip1) was performed for each specimen and p27 labelling indices were determined with a computer-assisted image-analyzing system (CAS 200). Pearson's correlation coefficient, Spearman's correlation coefficient, Students t-test, Kruskal-Wallis test and ANOVA were applied for statistical analyses using SPSS 11.5. Results: p27 LIs for all PAs were above 25% whereas for ACCs they were under 25% (except one case). p27 expression (LI and intensity) was significantly lower in ACCs than PAs. The correlation between p27 expression and ACC grading was not significant. Conclusion: Overall, these findings suggest that reduced expression of p27 might be correlated with the development of ACC and could be an indicator of malignant behavior.

Key Words: p27 (kip1) - Adenoid cystic carcinoma (ACC) - Pleomorphic adenoma (PA) - Salivary gland tumors

Asian Pacific J Cancer Prev, **6**, 527-530

Introduction

p27 is a family member of Cyclin-Dependent Kinase (CDK) inhibitors which can inhibit different CDKs and therefore control the cell cycle by balancing the activity of CDKs (Lloyd et al., 1999). Changes in the level of p27 (which can be shown by immunohistochemistry) can alter the normal progression of the cell cycle and its reduced expression has been shown in some tumors (Catzavelos et al., 1997; Sgambato et al., 1997; Fredersdorf et al., 1997; Guo et al., 1997; Kudo et al., 2000; Ohashi et al., 1999; Masciullo et al., 1999; Esposito et al., 1997; Tsihlias et al., 1998).

Pleomorphic adenoma (PA) and adenoid cystic carcinoma (ACC) are the two most common benign and malignant salivary gland tumors with different behaviors and their histopathologic differentiation may be difficult or even controversial in some cases (Gnepp, 1999; Regezi and Sciubba, 1999; Dardick, 1996). There are several studies which tried to differentiate these tumors by investigating the molecular agents involved in carcinogenesis but their results are still controversial (Ogawa et al., 2000; Lu et al., 2000; Devlin and Sloan, 2001).

As shown by Takata et al. reduced expression of p27(kip1) may correlate with the development and progression of salivary ACC and can be an indicator of its malignant behavior (Takata et al., 1999).

The purpose of this study was to evaluate and compare p27 expression in PA and ACC, and also investigate the correlation between p27 expression and ACC grading.

Materials and Methods

Tissue Sample Collection

Twenty two paraffin embedded blocks (11 PA and 11 ACC) were collected from the archives of the Oral Pathology Department, Dental School, Tehran University of Medical Sciences (TUMS), Amir Alam Hospital (TUMS-Tehran) and

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Cancer Institute (TUMS-Tehran). All tumors have been taken from minor salivary glands and the clinical features such as sex, age and size of tumor have been collected from the clinical records of patients.

In PA group, six patients were men and five were women (age range 25-72, mean 42.1) and in ACC group eight patients were men and three were women (age range 21-72, mean 50.6). ACC cases were also graded by Szanto et al. method (Szanto et al., 1984).

Immunohistochemistry (IHC)

The standard streptavidin-biotin peroxidase method was used for IHC. In brief, the 5 micro meter sections from the paraffin embedded blocks were dewaxed by xylene and dehydrated. The sections were autoclaved in citrate buffer (10mM) for 10 minutes at 121 degree of Centigrade, then placed in 0.3 percent H2o2 containing methanol for 15 minutes to inactivate endogenous peroxidase. Then they were incubated with monoclonal antibody of p27 (kip1, 1:50 dillution, Dako, Denmark) for 60 minutes at room temperature. They were rinsed with PBS and incubated in streptavidin-biotin peroxidase. Afterwards, the sections were incubated in DAB and counterstained with hematoxylin for 3 minutes. As negative control, the primary antibody was replaced by nonimmune mouse serum and as positive control, adenoid tissue was used (nuclei of lymphocytes are positive for p27). Positive or immunoreactive cells were those in which nuclei stained brown.

Staining was analyzed, using a computer-assisted image-analyzing system (CAS 200, Becton-Dickinson, Franklin Lakes, NJ, USA) and the percentage of positively stained nuclei (Computeral Labelling Index; CLI) in 1000 cells in 10 HPF and the intensity of staining were also determined and presented by percentage. The intensity of staining was scored; strong (>50%), moderate (25-50%), weak (5-25%) and negative (<5%).

The samples were also evaluated by an individual pathologist (F.S.) in a blind test under light microscopy who counted stained cells in 1000 cells and determined the Observational Labelling Index (OLI).

Statistical Analysis

SPSS 11.5 was used for statistical analyses. The relationship between p27 CLI, p27 OLI, p27 staining intensity were analyzed by t-student test in both groups. The association between p27 expression and ACC grades was also assessed with non-parametric Kruskal-Wallis analysis. Statistical correlation between age, size of tumor and p27 expression was also tested by Pearson's correlation coefficient and Spearman's correlation coefficient, respectively. A p-value less than 0.05 was considered statistically significant.

Results

In normal salivary glands adjacent to tumors, p27 were positive in the nuclei of cells surrounding acinic cells and the nuclei of ductal cells. p27 was also positive in the nuclei of inflammatory cells and fibroblasts. Positive staining of p27 was observed in tumoral part of all twenty two cases.

PA

In tubular portion of PA (which consisted of two cell layers) p27 staining intensity was higher in outer layer and all of the outer layer cells were strong to moderately positive (Figure 1). The nucleous of cells with plasmacytoid pattern were also strongly positive (Figure 2). In chondroid, mucoid and hyalinized portions of stroma, staining was moderate to strong, but in myxoid portion, the positive staining was weak to negative. The average of CLI was 53.52% (range 22.97 to 81.92%), OLI was 75.93% (range 46.36 to 97.26%) and staining intensity was 77.63% (range 55.60 to 94.30%).

ACC

p27 staining intensity in tubular areas of ACC was strong (Figure 3), in cribriform areas was moderate (Figure 4) and in solid areas was weak to negative. The average of CLI was 11.50% (range 0.02 to 20.32%), OLI was 27.51% (range 2.80 to 46.10%) and staining intensity was 24.18% (range

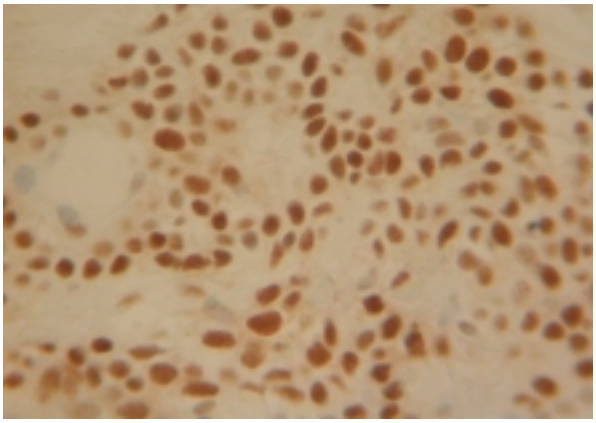

Figure 1. Photomicrograph Depicting Proliferating Cells Immunostained with Anti-p27(kip1) Antibody in Plasmacytoid Pattern of PA. (x 400)

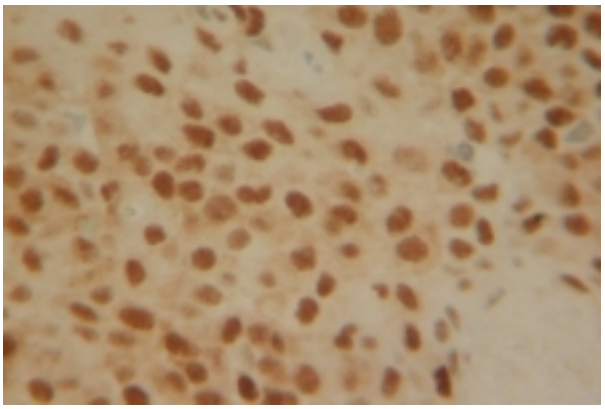

Figure 2. Photomicrograph Depicting Proliferating Cells Immunostained with Anti-p27(kip1) Antibody in Plasmacytoid Pattern of PA. (x 400)

0.10 to 38.69 %).

The p27 CLI, OLI and staining intensity was significantly different between PA and ACC but p27 expression was not significantly associated with ACC grading. Furthermore, p27 expression was not correlated with age and sex but p27 CLI was associated with the tumor size. There was also a positive correlation between p27 OLI and CLI. The negative controls for p27 staining showed no immunoreactivity.

Discussion

p27 is a CDK inhibitor that causes cell cycle arrest in G1 to S phase and it was suggested that may play an important role in carcinogenesis (Mineta et al., 1999).

Our results showed that p27 expression is different in PA and ACC and it can be used for differentiating these tumors. We found that p27 immunoreactivity in ACC was significantly lower than PA and it may show that high level of p27 expression is noted in benign tumors or tumors with quiescent clinical behavior and decreasing of its expression is associated with increased cell proliferation or malignant behavior. According to previous studies, it seems that decreased p27 expression is a late event in ACC progression which may be due to destruction of p27 by ubiquitin-proteasome system rathar than altered gene expression (Takata et al., 1999; Szanto et al., 1984; Mineta et al., 1999).

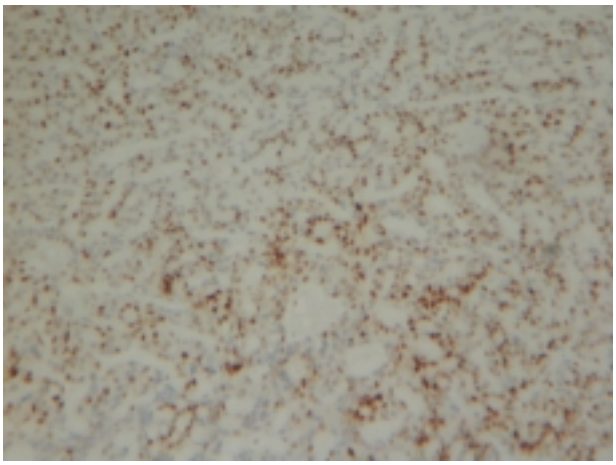

Figure 3. Photomicrograph Depicting Proliferating Cells Immunostained with Anti-p27(kip1) Antibody in Cribriform Area of ACC. (x 100)

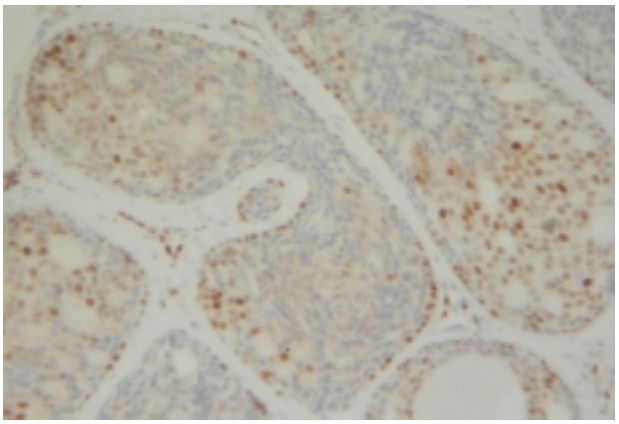

Figure 4. Photomicrograph Depicting Proliferating Cells Immunostained with Anti-p27(kip1) Antibody in Cribriform Area of ACC. (x 100)

It has been suggested that p27 gene mutations are extremely rare in human tumors and alteration of p27 may be related to its protein level and activity (Xiaohui and Zheng, 2001). Similar results were recently showed by Okabe et al. and Zhang et al. in comparison between mucoepidermoid carcinoma (MEC) and PA, which they also reported decreasing of p27 in MEC (Okabe et al., 2001; Zhang et al., 2001).

It must be considered that p27 can be induced or inhibited by different factors such as cell to cell contact , TGF-β and IL-2 (Polyak et al, 1994b; Polyak et al., 1994a; Nourse et al., 1994). Therefore, its alteration may just be a secondary event in carcinogenesis and can be influenced by multiple factors.

In our study, the meaningful score of p27 that indicates a difference between PA and ACC was 25% which means that all CLI of PAs were more than 25% and all CLI of ACCs (except one case) were less than 25%. Our OLI cut-off point was 50% which is similar to previous reports (Porter et al., 1997; Mori et al., 1997).

Among the histological subtypes of ACC, tubular and cribriform patterns seem to have better prognosis than solid form (Gnepp, 1999). In our study, staining intensity of p27 was increasingly higher in tubular, cribriform and solid pattern, respectively, but neither could we stablish a relation between ACC grading and p27 expression nor Takata et al. (Takata et al., 1999). As some other studies did not show a correlation between p27 expression and grading of tumors (Feakins et al., 2000), it seems that p27 just plays a role in final steps of carcinogenesis and is not active in all steps. We found a significant inverse relation between p27 expression and size of tumor which may indicate that decreased p27 is associated with cell proliferation and increased tumor size, but as numerous agents are involved in tumor growth, it is better to express this hypothesis by caution.

Differences of staining intensity in outer and inner layers of tumor tubules of PA may indicate that outer cell layer (which seems to be myoepithelial cells) can express p27, but inner cell layer (ductal cells) cannot. As plasmacytoid cells (which are also myoepithelial) express p27 intensely, it can be concluded that myoepithelial cells may have lower proliferative activity than ductal cells (Smith and Pereira-Smith,1996).

In summary, our study has revealed p27 expression is significantly lower in ACC than PA and it may have a role in late events of carcinogenesis of ACC.

Acknowledgments

We thank Dr I. Jahanzad and Dr A. Abdirad of the Cancer Institute, TUMS for advice on immunohistochemical

processes of this study, Dr MJ Kharrazi for statistical analysis and also A. Kazemian Amiri for his assistance.This research was supported by grants for scientific research of Tehran University of Medical Sciences.

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