Immuno-histochemical Expression of p53 Protein and iNOS in Odontogenic Cysts

Sopee Poomsawat DDS, MSc, PhD*,
Jirapa Punyasingh DDS, MS*, Paisarn Vejchapipat MD, PhD*

** Department of Oral Pathology, Faculty of Dentistry, Mahidol University, Bangkok, Thailand*
*** Department of Surgery, Faculty of Medicine, Chulalongkorn University, Bangkok., Thailand*

Objective: *The present study investigated the expression of p53 protein and iNOS in odontogenic keratocysts (OKCs), dentigerous cysts (DCs) and radicular cysts (RCs) and determined the correlation of these 2 proteins within each cyst type.*
Material and Method: *p53 and iNOS in epithelial lining cells were analyzed in 20 OKCs, 20 DCs and 20 RCs using immuno-histochemistry. The percentage of positive cells was assessed and presented as mean ± SD. The correlation between the expressions of these 2 proteins in each cyst was carried out using Spearman correlation analysis.*
Results: *p53 was found in 95% of OKCs, 50% of DCs and 65% of RCs. p53-positive cells in OKCs (12.2 ± 8.8%) were significantly higher than those in DCs (1.4 ± 3.2%) and RCs (1.3 ± 2.4%). iNOS was found in all examined lesions. Statistically, iNOS-positive cells in RCs (86.9 ± 9.9%) were lower than those in OKCs (94.8 ± 3.5%) and DCs (96.1 ± 4.4%). No correlation between the expression of p53 and iNOS was found in each cyst.*
Conclusion: *p53 was over expressed in OKCs compared with other lesions. RCs showed the lowest expression of iNOS.*

Keywords: *Dentigerous cyst, Immuno-histochemistry, Nitric oxide synthase type II, Odontogenic cysts, Radicular cyst, Tumor suppressor protein p53*

J Med Assoc Thai 2009; 92 (7): 952-60
Full text. e-Journal: *http://www.mat.or.th/journal*

Odontogenic cyst is a cyst of jaws originated from odontogenic apparatus or its remnants[1]. Being the second most common lesions in the oral and maxillofacial specimens[2], it has an important role in oral and maxillofacial pathology. There are three common odontogenic cysts including odontogenic keratocysts (OKCs), dentigerous cysts (DCs) and radicular cysts (RCs)[2]. OKCs and DCs are developmental cysts whereas RCs are the result of inflammation[1]. Of these three cysts, OKC has clinical importance of aggressive behavior, recurrence risk, and malignant potential[3]. Recently, OKC has been classified as a benign odontogenic tumor and designated as keratocystic odontogenic tumor in the new WHO classification[4]. However, the neoplastic nature of OKC is still controversial. Evidence supporting its neoplastic nature derived from its clinical aggressive behavior and also from its high expression of proliferation activities of the lining epithelium. The expression of proliferating cell nuclear antigen and ki67 was higher and stronger in OKCs than in DCs and RCs[5-8].

p53 gene is a tumor suppressor gene and plays an important role in the regulation of cell proliferation[9]. Mutations in the p53 gene yield a p53 protein which has an increased half-life, thus allowing this mutated protein to be detected immuno-histochemically[10,11]. However, the wild type p53 protein can also be observed in case of overproduction or stabilization of this protein[12,13]. Over expression of p53 protein in the lining epithelium of OKCs compared to DCs and

Correspondence to: Poomsawat S, Department of Oral Pathology, Faculty of Dentistry, Mahidol University, Yothi Street, Bangkok 10400, Thailand. Phone: 0-2644-8648, Fax: 0-2354-8510, E-mail: spoomsawat@yahoo.com

RCs has been documented in a few articles[6,7,14,15]. Expression of p53 protein in OKCs was correlated well with the expression of ki67, a proliferation marker, suggesting that p53 protein is related to cell proliferation activities in OKCs[7].

Besides the association of the tumor suppressor gene p53 with cellular proliferation activity, a relationship between p53 gene and iNOS (inducible nitric oxide synthase) has also been demonstrated[16-18]. i NOS is one of three different NOS isoforms namely neuronal, endothelial and inducible NOS[19]. Both neuronal and endothelial NOS are expressed constitutively, whereas iNOS is activated in some circumstances[19,20]. Under inductive conditions, iNOS produces NO, which contributes to a variety of pathological phenomena associated with inflammatory processes and cancer formation[19]. The expression of iNOS is increased in p53 knockout mice. The knockout mice died early because of multiple tumor formation[17]. Thus, it is hypothesized that p53 may induce cellular apoptosis in order to minimize NO-induced DNA damage[16,21]. In oral epithelial dysplasias, the expression of iNOS correlated with the expression of p53 protein[18]. Because a few articles have addressed an over expression of p53 protein in OKCs compared with DCs and RCs[6,7,14,15], it is interesting to examine the relationship between p53 protein and iNOS in OKCs. Additionally, this correlation may contribute to the aggressive behavior in OKCs and may involve in the pathogenesis of OKCs. In the English literature, the expression of iNOS has been studied only in RCs[22-24]. There are no articles in the literature studying the association between p53 and iNOS expression in odontogenic cysts. The present study investigated the expression of p53 protein and iNOS in OKCs, DCs and RCs and determined whether there was an association between these 2 proteins in each cyst type.

Material and Method

The sample in the present study consisted of 20 OKCs, 20 DCs and 20 RCs from the files of the Department of Oral Pathology, Faculty of Dentistry. All tissues were obtained with the approval of the Committee on Human Rights Related to Human Experimentation, Mahidol University. Hematoxylin and eosin-stained sections of these cysts were reviewed by two oral pathologists to confirm the diagnoses based on the criteria in the World Health Organization Classification of Tumors. Pathology and Genetics of Head and Neck Tumors[4]. New serial sections of 4 μm thickness were cut from the formalin-fixed paraffin embedded blocks and mounted on glass slides coated by amino-propyltriethoxysilane (APES; Sigma Chemical Co., St Louise, MO, USA).

Sections were deparaffinized and rehydrated. Endogenous peroxidase activity was blocked with 10-minute incubation in 3% H_2O_2. Antigen retrieval was done by immersing the sections in 0.4% pepsin (Sigma Chemical Co) in 0.01 M HCL at 37°C, for one hour for antibody against iNOS and by heating the sections in a microwave at approximately 850 W for 5 minutes and 300 W for 10 minutes in 10mM citrate buffer pH 6.0 for antibody against p53. After washing with 0.1% Tween 20 (MERCK-Schuchardt, Hohanbrunn, Germany) in phosphate buffered saline (PBS), the sections were treated with 2% bovine serum albumin (BSA; Sigma Chemical Co) in PBS for 30 minutes and then treated with primary antibodies for two hours at room temperature. The primary antibodies used in the present study were against iNOS (clone 2D2-B2, R&D systems, Minneapolis, MN, USA) diluted at 1:200 and p53 (clone DO-7, DakoCytomation Denmark A/S, Glostrup, Denmark) diluted at 1:50. Both are monoclonal antibodies. The primary antibodies were diluted in PBS. After thorough washing in 0.1% Tween 20 in PBS, labeled polymer (Dako Envision System, Dako Corporation, Carpinteria, CA, USA) was applied to the sections for 30 minutes, followed by three washes of 0.1% Tween 20 in PBS. Color was developed in freshly made diaminobenzidine (Sigma Chemical Co). Sections were washed briefly in running tap water and lightly stained with Mayer's hematoxylin.

Negative controls for each sample were done by omission of the relevant primary antibody and were confirmed to be unstained. Sections of salivary gland tissue were stained at the same run as positive controls for antibody against iNOS. The ducts of salivary gland have been shown to stain intensely for iNOS[25]. Additionally, the positivity of the endothelial cells of the blood vessels served as an internal positive control. In order to avoid the degradation of antigen due to the age of the specimens, only cases that showed good internal positive control were included in the present study. Additionally, to reduce the differences of the expression caused by the differences in the ages of the specimens, each cyst type was retrieved between the same period of time (2001-2005). Sections of an oral squamous cell carcinoma (OSCC) known to have nuclear staining for p53, were stained at the same run as positive controls for antibody against p53. The staining intensity of these OSCC cells varied from weak to strong. The weak staining intensity was found in a

large number of tumor cells, indicating that even a small amount of p53 protein could be detected. Thus, the chances of false negative decreased.

Evaluation of tissue sections

The immuno-reactivity of iNOS and p53 in epithelial lining cells was assessed as the percentage of positive cells. A total of at least 1,000 cells per case were randomly counted using a grid to avoid repetition of counted cells. The counting was performed under the optical microscope at X400 magnification. Regardless of staining intensity, nuclear and cytoplasmic stains were considered to be positive for the p53 and iNOS antigen, respectively. Sections were examined independently by 2 qualified oral pathologists. The average of the percentage of positive cells was recorded.

Staining intensity of each case was visually evaluated and assigned to one of the following categories: weak intensity (W), moderate intensity (M) and strong intensity (S).

Statistical analysis

Statistical analysis was performed using Kruskal-Wallis tests with Dunn's multiple comparisons. p-value of less than 0.05 was considered as statistical significance. Spearman correlation analysis of p53 and iNOS expression was carried out for each lesion (OKCs, DCs, RCs). GraphPad Prism version 3.02 was used for all statistical analyses. The percentage of positive cells is presented as mean and standard deviation (SD).

Result

p53 staining

The staining of p53 in the nuclei of squamous carcinoma cells of the authors' positive control varied from weak to intense. Nuclear staining of p53 was found in the lining epithelial cells of 19 cases (95%) of OKCs, 10 cases (50%) of DCs and 13 cases (65%) of RCs (Fig. 1-3 and Table 1). The percentage of positive cells for p53 in OKCs was statistically significant higher than those in DCs and RCs. In DCs, there was 1 case with a high percentage of positive cells (13.5%). The epithelial lining in this case was composed of 4-5 layers of epithelial cells (Fig. 2). The epithelial lining of this cyst had more epithelial cells than the other examined DCs, showing a thickness of 2-3 cells. The staining intensity in each cyst type varied from weak to strong.

In OKCs, the majority of positive cells was located in the prickle cell layer (Fig. 1). However, p53 positive cells were also observed in the basal cell layer but were rarely seen in the surface layer. Like OKCs, the majority of positive cells in DCs and RCs were found in the prickle cell layer (Fig. 2, 3).

iNOS staining

In salivary gland tissue which was the authors' positive control sample, intense cytoplasmic staining was found in the ducts with the absence of staining in the acini. This staining pattern was similar to previous studies[18,25]. Additionally, the vascular endothelial cells present in the fibrous wall of each cyst showed positive reaction. Thus, these staining patterns confirm the accuracy of the methods. Cytoplasmic staining of the lining epithelial cells was observed in all examined cysts (Fig. 4-6 and Table 2). However, nuclear staining was also detected. Some of this nuclear staining was likely to be the over layer of the very strong intensity of the cytoplasm. Despite the high expression of iNOS in RCs, the percentage of iNOS positive cells in RCs was statistically significant lower than those of OKCs and DCs (Table 2). Although there was a variation of the staining intensity in each lesion, most OKCs and DCs showed strong intensity, while a high proportion of RCs demonstrated weak intensity.

Table 1. The percentage of p53- positive cells in OKCs, DCs and RCs

Lesion (n)	Mean ± SD (range)	No. of cases according to % of positive cells			
		0%	0.1-9.9%	10-19.9%	> 20%
OKCs (20)	12.2 ± 8.8 (0.6-34.0)	1	8	8	3
DCs (20)	1.4 ± 3.2* (0.1-13.5)	10	9	1	0
RCs (20)	1.3 ± 2.4* (0.1-8.6)	7	13	0	0

OKCs: odontogenic keratocysts; DCs: dentigerous cysts; RCs: radicular cysts; SD: standard deviation
* $p < 0.05$ compared to OKCs

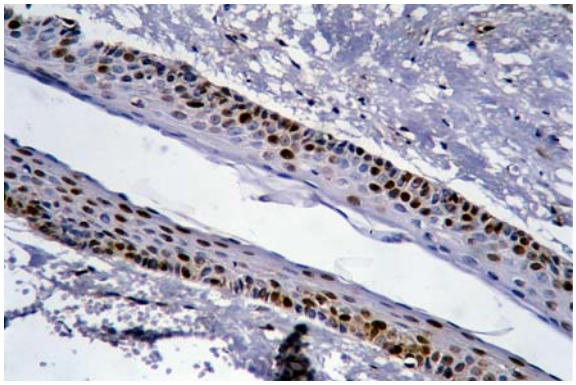

Fig. 1 Strong nuclear staining for p53 in epithelial lining cells of a representative of OKC. Expression is mainly found in the suprabasal layers (Immunostaining, original magnification: x400)

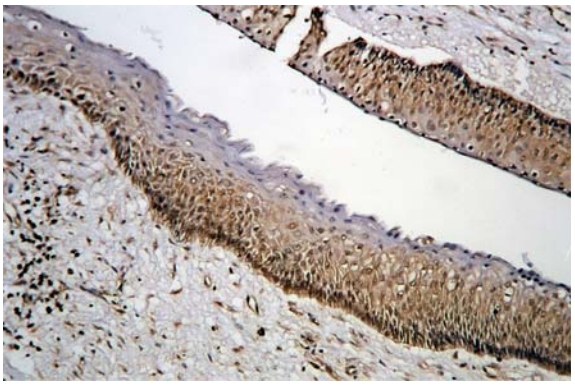

Fig. 4 In a representative of OKC, iNOS is strongly expressed in a large number of epithelial lining cells. Note that keratinized cells are devoid of expression (Immunostaining, original magnification: x200)

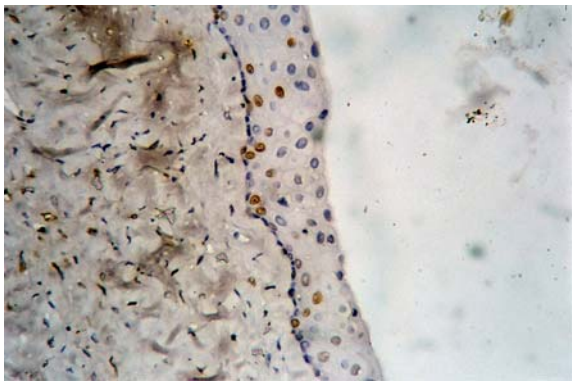

Fig. 2 Strong nuclear staining for p53 in epithelial lining cells of a representative of DC. Expression is mainly found in the suprabasal layers (Immunostaining, original magnification: x400)

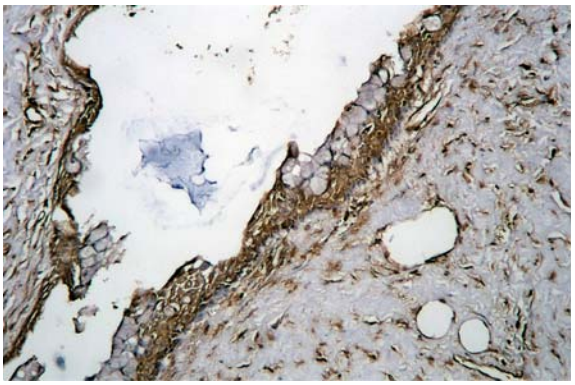

Fig. 5 In a representative of DC, iNOS is strongly expressed in all epithelial lining cells, except for mucous cells (Immunostaining, original magnification: x200)

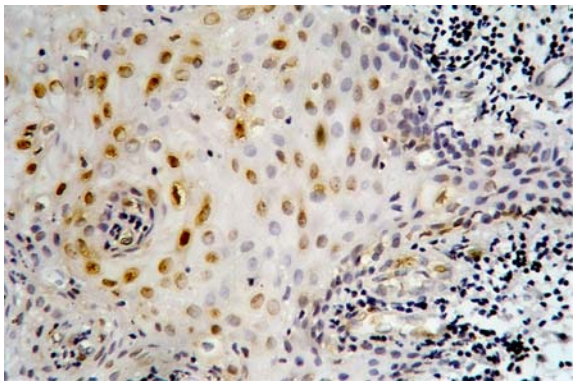

Fig. 3 Strong nuclear staining for p53 in epithelial lining cells of a representative of RC. Note: The acanthosis of the epithelial lining and the positive prickle cells (Immunostaining, original magnification: x400)

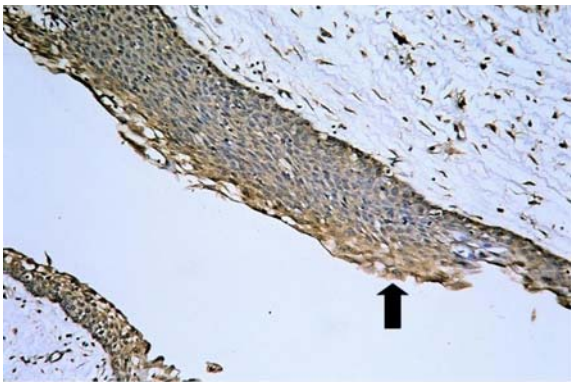

Fig. 6 In a representative of RC, iNOS is not uniformly present in the epithelial lining cells. Note that a group of positive cells (arrow) is seen adjacent to negative cells (Immunostaining, original magnification: x200)

Table 2. The percentage of iNOS- positive cells in OKCs, DCs and RCs

Lesion (n)	Mean ± SD (range)	No. of cases according to % of positive cells		
		< 80%	80-89.9%	90-100%
OKCs (20)	94.8 ± 3.5* (87.3-99.0)	0	4	16
DCs (20)	96.1 ± 4.4* (83.7-100)	0	2	18
RCs (20)	86.9 ± 9.9 (56.7-99.8)	3	11	6

OKCs: odontogenic keratocysts; DCs: dentigerous cysts; RCs: radicular cysts; SD: standard deviation
* $p < 0.05$ compared to RCs

Besides the expression of iNOS in the epithelial lining cells, the reactivity of iNOS was detected in many cells of the fibrous connective tissue walls. These include fibroblasts, endothelial cells of blood vessels, macrophages and some plasma cells.

The correlation between the expression of p53 and iNOS immuno-reactivities

Statistically, there was no correlation between the expression of p53 and iNOS in any lesions.

Discussion

The present study found that p53 protein was expressed in OKCs, DCs and RCs with differences in terms of the number of positive cases and the number of positive cells. The finding that p53 was present in these 3 odontogenic lesions generally corresponds to previous reports[6,7,15,26,27]. However, Carvalhais et al[28] studied the expression of p53 in OKCs and RCs and found that there was no positivity of p53 in all studied cysts. Ogden et al[14] showed that p53 was expressed only in OKCs, but not in all examined DCs and RCs. These discrepancies may largely be due to the variations in immuno-histochemical technique. Additionally, there was also a dissimilarity of evaluation methods among studies examining the expression of p53 in these 3 odontogenic lesions[7,15,26,27,29,30]. Therefore, the interpretation and discussion of these results are complicated.

The present study found that 95% of OKCs were positive to p53 and the number of positive cells in OKCs was significantly higher than those in DCs and RCs. Furthermore, more than 50% of cases of OKCs showed percentage of positive cells more than 10%. Unlike OKCs, none of RCs contained positive cells more than 10%. These differences may be used as an adjunctive tool in the differential diagnosis between RCs and OKCs with inflammation. The histopathological features of inflamed OKCs are sometimes similar to those of RCs.

An over-expression of p53 protein in OKCs compared to DCs and RCs from this study was consistent with previous studies[6,7,14,15]. The correlation of the expression of p53 protein with ki67[7] together with the role of p53 in controlling cell replication indicates that p53 is involved in the proliferation activity of the epithelial lining cells in OKCs. Interestingly, the authors' previous study on the expression of basement membrane proteins in odontogenic cysts demonstrated that a continuous staining pattern for fibronectin at the basement membrane zone was found only in OKCs, but not in DCs and RCs[31]. The role of fibronectin in promote cell proliferation has been reported[32,33]. Therefore, the high proliferation activities of the epithelial lining in OKCs as demonstrated by the high expression of p53 and ki67 may be regulated by fibronectin via its receptor.

The immuno-histochemical detection of p53 protein in tissue can be due to the mutation of the p53 gene[10,11] and the binding of wild type protein with viral[12] or cellular proteins[13]. Although some tumors[10,11] showed a strong association between high levels of expression of p53 protein detected by immuno-histochemistry and the mutation of this gene, some tumors, including breast cancer[34] and colorectal cancer[35], did not have this relationship. OKCs have been reported to have a high expression of p53 protein without mutation of p53 gene within exons 5-9, as demonstrated by a combination of PCR and SSCP analysis[7]. The present result indicates that the elevated p53 protein expression presumably reflects over-expression and/or stabilization of wild type p53 protein. However, a loss of heterozygosity in the p53 gene was found in 17 cases of OKCs reported by 2 studies[36,37]. Thus, more cases of OKCs are needed to determine whether there is a mutation of p53 gene in

OKCs and whether this leads to the detection of p53 protein.

The present study, as well as a previous study[22-24], demonstrated that iNOS was expressed in the epithelial lining cells of RCs. However, this is the first time to demonstrate the quantitative data of the immuno-positive lining epithelial cells in RCs. The number of iNOS positive cells in RCs was as high as approximately 87%. Besides the lining epithelial cells of RCs, many other cells including fibroblasts, endothelial cells, macrophages, plasma cells and neutrophils, were also stained for iNOS[22-24]. Thus it appears that NO, the end product of iNOS, in RC is produced by a variety of cell types. Interestingly, co-expression of iNOS and interferon-γ was demonstrated in all iNOS producing cells of RCs, except for lining epithelial cells[23]. Since interferon-γ is one of an important inflammatory cytokines, it is likely that iNOS in the lining epithelial cells of RCs is activated by another potent mechanism, not by inflammatory process. It has been demonstrated that the epithelial lining cells of OKCs, DCs and RCs can produce their own cytokines such as interleukin-1α and interleukin-6[38]. Therefore, these cytokines may activate iNOS expression of these epithelial cells in an autocrine fashion. These findings are in line with the results of the present study showing that a large number of epithelial lining cells in OKCs and DCs, both are developmental cysts, showed strong intensity for iNOS.

The role of iNOS in these 3 odontogenic cysts remains to be answered. High expression of iNOS in these cysts suggests that iNOS contributes to the pathogenesis and/or pathology of these lesions. In RCs, iNOS may involve in the proliferation of Malassez epithelial rests and cystic formation. This hypothesis is based on the finding that the epithelial staining intensity of iNOS in RCs was greater than that in Malassez epithelial rests, the cells of origin of RCs and iNOS can be stimulated by bacterial lipo-polysaccharide and inflammatory cytokines such as interleukin-1β, tumor necrosis factor and interferon-γ[24]. NO may cause tissue destruction in RCs as it does in other inflammatory diseases such as rheumatoid arthritis[39] and periodontal disease[40]. High NO production in these three cysts may participate in bone resorption and cystic enlargement. This is because synthesis of matrix metalloproteinases (MMPs) can be activated by NO[41,42] and MMPs play a key role in destruction of bone matrix[43].

The expression of iNOS in these 3 odontogenic cysts and in some odontogenic tumors reported by another group[44] suggests that an iNOS inhibitor may play an important role in the treatment of odontogenic lesions. The iNOS inhibitor may be applied in root canals of teeth with RCs. iNOS inhibitor may be used to limit tumor or cystic growth. In an animal model, a specific antagonist to iNOS reduced tumor growth and muscular invasion in the EMT6 mammary tumor[45]. Further studies are needed to prove this therapeutic approach.

Because the expression of iNOS in OKCs was similar to those found in DCs, it is unlikely that iNOS participates in the clinical aggressive behavior in OKCs. Nevertheless, NO, produced by iNOS, may cause DNA damage. Generally, cells with DNA-damage could be arrested or apoptotic by the mediation of wild type p53 protein[16,21]. Since the mutation of p53 gene exists in some OKCs, not in DCs and RCs[36,37], it is, therefore, possible that OKCs with mutated p53 gene will have a chance to accumulate cells with DNA-damage induced by a high concentration of NO. The present result may contribute to the clinical aggressive behavior and neoplastic nature found in some OKCs.

In contrast to oral epithelial dysplasia[18], the present study found that there was no correlation between the expression of p53 and iNOS in OKCs, DCs and RCs. These results indicate that a feedback loop between p53 protein and iNOS is not present in these 3 odontogenic lesions.

In conclusion, the over expression of p53 protein in OKCs compared with DCs and RCs was demonstrated. However, the expression of iNOS was high in all lesions. These results suggest that p53, but not iNOS, contribute to the aggressive behavior in OKCs. In odontogenic lesions, iNOS play an important role in the pathology of both inflammatory and developmental lesions. There was no correlation between the expression of p53 and iNOS in these 3 odontogenic lesions.

Acknowledgements

The present study was supported by the Faculty of Dentistry, Mahidol University Research Grant. PV was supported by Thailand Research Fund. The authors wish to thank Mrs. Unruen Meesakul for her technical assistance and Dr. Justin Meiland for reviewing the manuscript.

References

1. Shear M. Developmental odontogenic cysts. An update. J Oral Pathol Med 1994; 23: 1-11.
2. Jones AV, Franklin CD. An analysis of oral and

maxillofacial pathology found in adults over a 30-year period. J Oral Pathol Med 2006; 35: 392-401.
3. Shear M. The aggressive nature of the odontogenic keratocyst: is it a benign cystic neoplasm? Part 1. Clinical and early experimental evidence of aggressive behaviour. Oral Oncol 2002; 38: 219-26.
4. Barns L, Eveson JW, Reichart PA, Sidransky D. World Health Organization Classification of Tumours. In: Pathology and genetics of head and neck tumours. Lyon: IARC Press; 2005: 283-314.
5. Li TJ, Browne RM, Matthews JB. Quantification of PCNA+ cells within odontogenic jaw cyst epithelium. J Oral Pathol Med 1994; 23: 184-9.
6. Slootweg PJ. p53 protein and Ki-67 reactivity in epithelial odontogenic lesions. An immunohistochemical study. J Oral Pathol Med 1995; 24: 393-7.
7. Li TJ, Browne RM, Prime SS, Paterson IC, Matthews JB. p53 expression in odontogenic keratocyst epithelium. J Oral Pathol Med 1996; 25: 249-55.
8. Piattelli A, Fioroni M, Santinelli A, Rubini C. Expression of proliferating cell nuclear antigen in ameloblastomas and odontogenic cysts. Oral Oncol 1998; 34: 408-12.
9. Finlay CA, Hinds PW, Levine AJ. The p53 proto-oncogene can act as a suppressor of transformation. Cell 1989; 57: 1083-93.
10. Iggo R, Gatter K, Bartek J, Lane D, Harris AL. Increased expression of mutant forms of p53 oncogene in primary lung cancer. Lancet 1990; 335: 675-9.
11. Greenblatt MS, Bennett WP, Hollstein M, Harris CC. Mutations in the p53 tumor suppressor gene: clues to cancer etiology and molecular pathogenesis. Cancer Res 1994; 54: 4855-78.
12. Werness BA, Levine AJ, Howley PM. Association of human papillomavirus types 16 and 18 E6 proteins with p53. Science 1990; 248: 76-9.
13. Wu X, Bayle JH, Olson D, Levine AJ. The p53-mdm-2 autoregulatory feedback loop. Genes Dev 1993; 7: 1126-32.
14. Ogden GR, Chisholm DM, Kiddie RA, Lane DP. p53 protein in odontogenic cysts: increased expression in some odontogenic keratocysts. J Clin Pathol 1992; 45: 1007-10.
15. Piattelli A, Fioroni M, Santinelli A, Rubini C. p53 protein expression in odontogenic cysts. J Endod 2001; 27: 459-61.
16. Forrester K, Ambs S, Lupold SE, Kapust RB, Spillare EA, Weinberg WC, et al. Nitric oxide-induced p53 accumulation and regulation of inducible nitric oxide synthase expression by wild-type p53. Proc Natl Acad Sci USA 1996; 93: 2442-7.
17. Ambs S, Ogunfusika MO, Merriam WG, Bennett WP, Billiar TR, Harris CC. Up-regulation of inducible nitric oxide synthase expression in cancer-prone p53 knockout mice. Proc Natl Acad Sci USA 1998; 95: 8823-8.
18. Brennan PA, Conroy B, Spedding AV. Expression of inducible nitric oxide synthase and p53 in oral epithelial dysplasia. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2000; 90: 624-9.
19. Nathan C, Xie QW. Nitric oxide synthases: roles, tolls, and controls. Cell 1994; 78: 915-8.
20. Knowles RG, Moncada S. Nitric oxide synthases in mammals. Biochem J 1994; 298 (Pt 2): 249-58.
21. Messmer UK, Ankarcrona M, Nicotera P, Brune B. p53 expression in nitric oxide-induced apoptosis. FEBS Lett 1994; 355: 23-6.
22. Takeichi O, Saito I, Hayashi M, Tsurumachi T, Saito T. Production of human-inducible nitric oxide synthase in radicular cysts. J Endod 1998; 24: 157-60.
23. Takeichi O, Hayashi M, Tsurumachi T, Tomita T, Ogihara H, Ogiso B, et al. Inducible nitric oxide synthase activity by interferon-gamma-producing cells in human radicular cysts. Int Endod J 1999; 32: 124-30.
24. Suzuki T, Kumamoto H, Ooya K, Motegi K. Expression of inducible nitric oxide synthase and heat shock proteins in periapical inflammatory lesions. J Oral Pathol Med 2002; 31: 488-93.
25. Brennan PA, Umar T, Buckley J, Zaki GA, Langdon JD, Spedding A, et al. Expression of nitric oxide synthase in pleomorphic adenomas of the parotid. Br J Oral Maxillofac Surg 2000; 38: 338-42.
26. Tosios KI, Kakarantza-Angelopoulou E, Kapranos N. Immunohistochemical study of bcl-2 protein, Ki-67 antigen and p53 protein in epithelium of glandular odontogenic cysts and dentigerous cysts. J Oral Pathol Med 2000; 29: 139-44.
27. Suzuki T, Kumamoto H, Kunimori K, Ooya K. Immunohistochemical analysis of apoptosis-related factors in lining epithelium of radicular cysts. J Oral Pathol Med 2005; 34: 46-52.
28. Carvalhais J, Aguiar M, Araujo V, Araujo N, Gomez R. p53 and MDM2 expression in odontogenic cysts and tumours. Oral Dis 1999; 5: 218-22.
29. Lo ML, Staibano S, Pannone G, Bucci P, Nocini PF, Bucci E, et al. Expression of cell cycle and apoptosis-related proteins in sporadic

odontogenic keratocysts and odontogenic keratocysts associated with the nevoid basal cell carcinoma syndrome. J Dent Res 1999; 78: 1345-53.
30. Lombardi T, Odell EW, Morgan PR. p53 immunohistochemistry of odontogenic keratocysts in relation to recurrence, basal-cell budding and basal-cell naevus syndrome. Arch Oral Biol 1995; 40: 1081-4.
31. Poomsawat S, Punyasingh J, Weerapradist W. Expression of basement membrane components in odontogenic cysts. Oral Dis 2006; 12: 290-6.
32. Sottile J, Hocking DC, Swiatek PJ. Fibronectin matrix assembly enhances adhesion-dependent cell growth. J Cell Sci 1998; 111 (Pt 19): 2933-43.
33. Sechler JL, Schwarzbauer JE. Control of cell cycle progression by fibronectin matrix architecture. J Biol Chem 1998; 273: 25533-6.
34. Dunn JM, Hastrich DJ, Newcomb P, Webb JC, Maitland NJ, Farndon JR. Correlation between p53 mutations and antibody staining in breast carcinoma. Br J Surg 1993; 80: 1410-2.
35. Cripps KJ, Purdie CA, Carder PJ, White S, Komine K, Bird CC, et al. A study of stabilisation of p53 protein versus point mutation in colorectal carcinoma. Oncogene 1994; 9: 2739-43.
36. Agaram NP, Collins BM, Barnes L, Lomago D, Aldeeb D, Swalsky P, et al. Molecular analysis to demonstrate that odontogenic keratocysts are neoplastic. Arch Pathol Lab Med 2004; 128: 313-7.
37. Henley J, Summerlin DJ, Tomich C, Zhang S, Cheng L. Molecular evidence supporting the neoplastic nature of odontogenic keratocyst: a laser capture microdissection study of 15 cases. Histopathology 2005; 47: 582-6.
38. Meghji S, Qureshi W, Henderson B, Harris M. The role of endotoxin and cytokines in the pathogenesis of odontogenic cysts. Arch Oral Biol 1996; 41: 523-31.
39. Pozza M, Guerra M, Manzini E, Calza L. A histochemical study of the rheumatoid synovium: focus on nitric oxide, nerve growth factor high affinity receptor, and innervation. J Rheumatol 2000; 27: 1121-7.
40. Batista AC, Silva TA, Chun JH, Lara VS. Nitric oxide synthesis and severity of human periodontal disease. Oral Dis 2002; 8: 254-60.
41. Rodini CO, Batista AC, Dionisio TJ, Santos CF, Cunha FQ, Lara VS. Morphologic evaluation and expression of matrix metalloproteinases-2 and 9 and nitric oxide during experimental periodontal disease in rat. J Mol Histol 2008; 39: 275-82.
42. Okamoto T, Akaike T, Nagano T, Miyajima S, Suga M, Ando M, et al. Activation of human neutrophil procollagenase by nitrogen dioxide and peroxynitrite: a novel mechanism for procollagenase activation involving nitric oxide. Arch Biochem Biophys 1997; 342: 261-74.
43. Accorsi-Mendonca T, Zambuzzi WF, Silva Paiva KB, Pereira L Jr, Cestari TM, Taga R, et al. Expression of metalloproteinase 2 in the cell response to porous demineralized bovine bone matrix. J Mol Histol 2005; 36: 311-6.
44. Kumamoto H, Suzuki T, Ooya K. Immunohistochemical analysis of inducible nitric oxide synthase (iNOS) and heat shock proteins (HSPs) in ameloblastomas. J Oral Pathol Med 2002; 31: 605-11.
45. Thomsen LL, Scott JM, Topley P, Knowles RG, Keerie AJ, Frend AJ. Selective inhibition of inducible nitric oxide synthase inhibits tumor growth in vivo: studies with 1400W, a novel inhibitor. Cancer Res 1997; 57: 3300-4.

การแสดงออกของ p53 โปรตีน และ iNOS ในถุงน้ำที่มีต้นกำเนิดจากเนื้อเยื่อฟัน

โสภี ภูมิสวัสดิ์, จีราภา บุณยสิงห์, ไพศาล เวชชพิพัฒน์

วัตถุประสงค์: *การศึกษานี้สำรวจการแสดงออกของ p53 โปรตีนและ iNOS ในถุงน้ำโอดอนโทเจนิกเคอราโทซิสต์ ถุงน้ำเดนทิเจรัส และถุงน้ำแรดิคิวลาร์ และหาความสัมพันธ์ของการแสดงออกระหว่าง p53 โปรตีน และ iNOS ในถุงน้ำแต่ละชนิด*

วัสดุและวิธีการ: *การแสดงออกของ p53 โปรตีนและ iNOS ในเซลล์เยื่อบุผิวจะทำในถุงน้ำโอดอนโทเจนิกเคอราโทซิสต์ 20 ราย ถุงน้ำเดนทิเจรัส 20 ราย และถุงน้ำแรดิคิวลาร์ 20 ราย โดยใช้เทคนิค อิมมูโนฮิสโตเคมีสตรี ร้อยละของจำนวนเซลล์ที่ให้ผลบวก จะถูกประเมินและรายงานผลเป็นค่าเฉลี่ย ± ส่วนเบี่ยงเบนมาตรฐานความสัมพันธ์ของการแสดงออกระหว่าง p53 โปรตีนและ iNOS ในถุงน้ำแต่ละชนิดจะถูก วิเคราะห์โดยสถิติสหสัมพันธ์*

ผลการศึกษา: *พบการแสดงออกของ p53 โปรตีน ใน 95% ของถุงน้ำโอดอนโทเจนิกเคอราโทซิสต์ 50% ของถุงน้ำเดนทิเจรัสและ 65% ของถุงน้ำแรดิคิวลาร์ ร้อยละของจำนวนเซลล์ที่ให้ผลบวกต่อ p53 โปรตีน ในถุงน้ำโอดอนโทเจนิเคอราโทซิสต์ (12.2 ± 8.8%) จะสูงกว่าถุงน้ำเดนทิเจรัส (1.4 ± 3.2%) และถุงน้ำแรดิคิวลาร์ (1.3 ± 2.4%) อย่างมีนัยสำคัญทางสถิติ พบการแสดงออกของ iNOS ในถุงน้ำทั้งหมดที่ศึกษา ร้อยละของจำนวนเซลล์ที่ให้ผลบวกต่อ iNOS ในถุงน้ำแรดิคิวลาร์ (86.9 ± 9.9%) จะต่ำกว่าถุงน้ำโอดอนโทเจนิกเคอราโทซิสต์ (94.8 ± 3.5%) และถุงน้ำเดนทิเจรัส (96.1 ± 4.4%) อย่างมีนัยสำคัญทางสถิติ ไม่พบความสัมพันธ์ของการแสดงออกระหว่าง p53 โปรตีน และ iNOS ในถุงน้ำแต่ละชนิด*

สรุป: *ถุงน้ำโอดอนโทเจนิกเคอราโทซิสต์ มีการแสดงออกของ p53 โปรตีนมากกว่าถุงน้ำชนิดอื่น ถุงน้ำแรดิคิวลาร์มีการแสดงออกของ iNOS ต่ำที่สุด*