บทวิทยาการ

การแสดงออกของพี 53 ในถุงน้ำชนิดที่มีต้นกำเนิดเกี่ยวข้องกับฟัน และในเนื้องอกอะมีโลบลาสโทมา

p53 expression in odontogenic cysts and ameloblastomas

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Abstract

Objectives : To assess p53 expression in odontogenic cysts and ameloblastomas and to establish whether there was a relationship between the aggressiveness of the lesions and p53 expression.

Materials and methods : 15 cases of radicular cysts, 15 cases of dentigerous cysts, 15 cases of odontogenic keratocysts, 12 cases of ameloblastomas, 12 cases of recurrent ameloblastomas and 4 cases of malignant ameloblastomas were stained with immunoperoxidase technique using monoclonal antibody to p53 as primary antibody. Immunoreactivity was evaluated by counting of cells. Data obtained were analyzed by descriptive statistics.

Results : All odontogenic cysts and ameloblastomas studied expressed p53 protein, but in different proportions; 1 from 15 cases (6.7%) in radicular cysts, 2 from 15 cases (13.3%) in dentigerous cysts, 9 from 15 cases (60.0%) in odontogenic keratocysts, 4 from 12 cases (33.3%) in ameloblastomas, 9 from 12 cases (75.0%) in recurrent ameloblastomas and 4 from 4 cases (100.0%) in malignant ameloblastomas.

Conclusion : Odontogenic cysts studied elicited a low number of p53 expression except odontogenic keratocyst while the ameloblastoma group exhibited a greater number of p53 expression, especially malignant ameloblastoma. P53 expression in odontogenic cysts and ameloblastomas correlated well with the clinical behavior of these lesions.

Key words : P53, odontogenic cyst, ameloblastoma

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การแสดงออกของพี53 ในถุงน้ำชนิดที่มีต้นกำเนิดเกี่ยวข้องกับฟันและในเนื้องอกอะมีโลบลาสโทมา

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บทคัดย่อ

วัตถุประสงค์ เพื่อศึกษาถึงการแสดงออกของพี53 ในถุงน้ำชนิดที่มีต้นกำเนิดเกี่ยวข้องกับฟันและในเนื้องอกอะมีโลบลาสโทมาและศึกษาถึงความสัมพันธ์ระหว่างความรุนแรงของรอยโรคกับการแสดงออกของพี53 **วัสดุและวิธีการ** การศึกษาครั้งนี้ใช้ถุงน้ำชนิดเรดิคูลาร์ ถุงน้ำชนิด เด็นติเจอรัส และถุงน้ำชนิดโอดอนโตเจนิกเคอราโทซิสต์ อย่างละ 15 ตัวอย่าง เนื้องอกอะมีโลบลาสโทมา 12 ตัวอย่าง เนื้องอกอะมีโลบลาสโทมาชนิดที่กลับเป็นซ้ำ 12 ตัวอย่างและอะมีโลบลาสโทมาชนิดร้ายแรง 4 ตัวอย่าง โดยนำตัวอย่างทั้งหมดมาย้อมด้วยเทคนิกทางอิมมูโนฮิสโตเคมีและใช้แอนติบอดีที่เฉพาะเจาะจงกับพี53 จากนั้นศึกษาโดยการนับเซลล์ที่ให้ผลบวกต่อการย้อมและวิเคราะห์ข้อมูลด้วยสถิติเชิงพรรณนา

ผลการศึกษา ในทุกตัวอย่างที่นำมาศึกษาพบมีการแสดงออกของพี53 ในอัตราส่วนที่แตกต่างกัน คือ พบการแสดงออกของพี53 สำหรับถุงน้ำชนิดเรดิคูลาร์ จำนวน 1 ตัวอย่างจาก 15 ตัวอย่าง (6.7%) ถุงน้ำชนิดเด็นติเจอรัส 2 ตัวอย่างจาก 15 ตัวอย่าง (13.3%) ถุงน้ำชนิดโอดอนโตเจนิกเคอราโทซิสต์ 9 ตัวอย่างจาก 15 ตัวอย่าง (60.0%) เนื้องอกอะมีโลบลาสโทมา 4 ตัวอย่างจาก 12 ตัวอย่าง (33.3%) เนื้องอกอะมีโลบลาสโทมาชนิดที่กลับเป็นซ้ำ 9 ตัวอย่างจาก 12 ตัวอย่าง (75%) และอะมีโลบลาสโทมาชนิดร้ายแรง 4 ตัวอย่างจาก 4 ตัวอย่าง (100%) **สรุปผลการศึกษา** ในถุงน้ำชนิดที่มีต้นกำเนิดเกี่ยวข้องกับฟันที่นำมาศึกษาทั้งหมดพบว่าเกือบทุกชนิดมีการแสดงออกของพี53ที่น้อยยกเว้นในถุงน้ำชนิดโอดอนโตเจนิกเคอราโทซิสต์ โดยในกลุ่มของเนื้องอกอะมีโลบลาสโทมาพบว่ามีการแสดงออกของพี53 ที่มากกว่าโดยเฉพาะในอะมีโลบลาสโทมาชนิดร้ายแรงพบมีการแสดงออกของพี53 อย่างมาก ดังนั้นจึงได้ข้อสรุปว่าการแสดงออกของพี53 ในถุงน้ำชนิดที่มีต้นกำเนิดเกี่ยวข้องกับฟันและอะมีโลบลาสโทมา มีความสัมพันธ์กับพฤติกรรมทางคลินิกของรอยโรคเหล่านี้

คำสำคัญ : พี53, ถุงน้ำชนิดที่มีต้นกำเนิดเกี่ยวข้องกับฟัน, อะมีโลบลาสโทมา

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p53 expression in odontogenic cysts and ameloblastomas

Introduction

P53, also known as TP53 (tumor protein 53) is a 53 kD protein consisting of 393 amino acids encoded by a tumor suppressor gene which is located on the short arm of chromosome 17.[1] Wild-type P53 is an oligomeric DNA-binding protein which functions as a molecular policeman by blocking the entry of a DNA-damaged cell from the G_1 phase to the S phase in order that the damaged DNA has sufficient time to undergo reparative processes.[2] If the reparative processes fail, P53 may trigger cell suicide by means of apoptosis through a down-regulation of bcl2.[3] P53 also binds specifically to DNA at its N-terminus leading to activation of genes in the vicinity of P53-binding site. This activation can negatively control growth and/or invasion.[4]

It is gĕnerally accepted that increased cell proliferation plays a role in the development of odontogenic cysts and tumors. The cause of increased cell proliferation may stem from a mutation of the p53 gene.[5]

Mutations of p53 gene are the most frequent molecular events in human cancers. Approximately 60% of the head and neck squamous cell carcinoma demonstrated detectable P53 immunohistochemically suggesting the presence of p53 gene mutation.[6-8] These mutations produce abnormal p53 gene products which have extended half-life compared to the wild-type proteins. Normally, wild-type p53 protein can not be detected immunohistochemically in tissue section due to its short half-life. On the other hand, mutant p53 gene product can be observed by immunohistochemical methods in tissue section because of its prolonged half-life.[9-11]

The objectives of the present study were to assess p53 expression in odontogenic cysts and tumors in order to establish whether p53 contributed to the development of these lesions and whether there was a relationship between the aggressiveness of the lesions and p53 expression.

Materials and methods

In the present study, 15 cases of radicular cysts, 15 cases of dentigerous cysts, 15 cases of odontogenic keratocysts, 12 cases of ameloblastomas, 12 cases of recurrent ameloblastomas and 4 cases of malignant ameloblastomas were retrieved from the archives of the Department of Oral Pathology, Faculty of Dentistry, Chulalongkorn University. The deparaffi-nized tissue sections were autoclaved in 10mM citrate buffer pH 6.0 for antigen retrieval. The tissue sections were then immersed in 0.3% hydrogen peroxide for 10 minutes to block endogenous activity. Subsequently, the tissue sections were incubated with mouse anti-human P53 monoclonal antibody (DO-7; DAKO, Denmark) diluted with 1% bovine serum albumin in tris buffer saline solution at the dilution of 1:100 at 4°c overnight. After rinsing and washing in tris buffer saline solution, the tissue sections were incubated with peroxidase-labeled goat anti-mouse antibody (DAKO, Denmark) for 1 hour at room

temperature. Bound peroxidase was visualized by adding 3,3'-diaminobenzidine tetrahydrochloride (Sigma, USA). To ensure the consistency of the P53 staining, squamous cell carcinoma served as positive controls, while negative controls were achieved by substituting tris buffer saline for primary antibody. Data collected were analysed by nonparametric statistics.

Results

All odontogenic cysts studied revealed positive P53 staining, but varying in the number of cases in each cystic type. The number of cases with positive P53 staining in radicular cyst and dentigerous cyst was small; 1 in 15 (6.7%) and 2 in 15 (13.3%), respectively. In odontogenic keratocyst, 9 out of 15 (60.0%) showed positive P53 staining. The positively stained cells localized in both the basal and parabasal areas (Fig.1). Among the odontogenic cyst group, odontogenic keratocyst demonstrated the most intense and largest number of P53 positive cells (Table.1). However, the staining intensity was weak compared to that of squamous cell carcinoma which was used as positive control. In ameloblastoma, 4 out of 12 (33.3%) demonstrated positive P53 staining.

All of the positively stained cells were

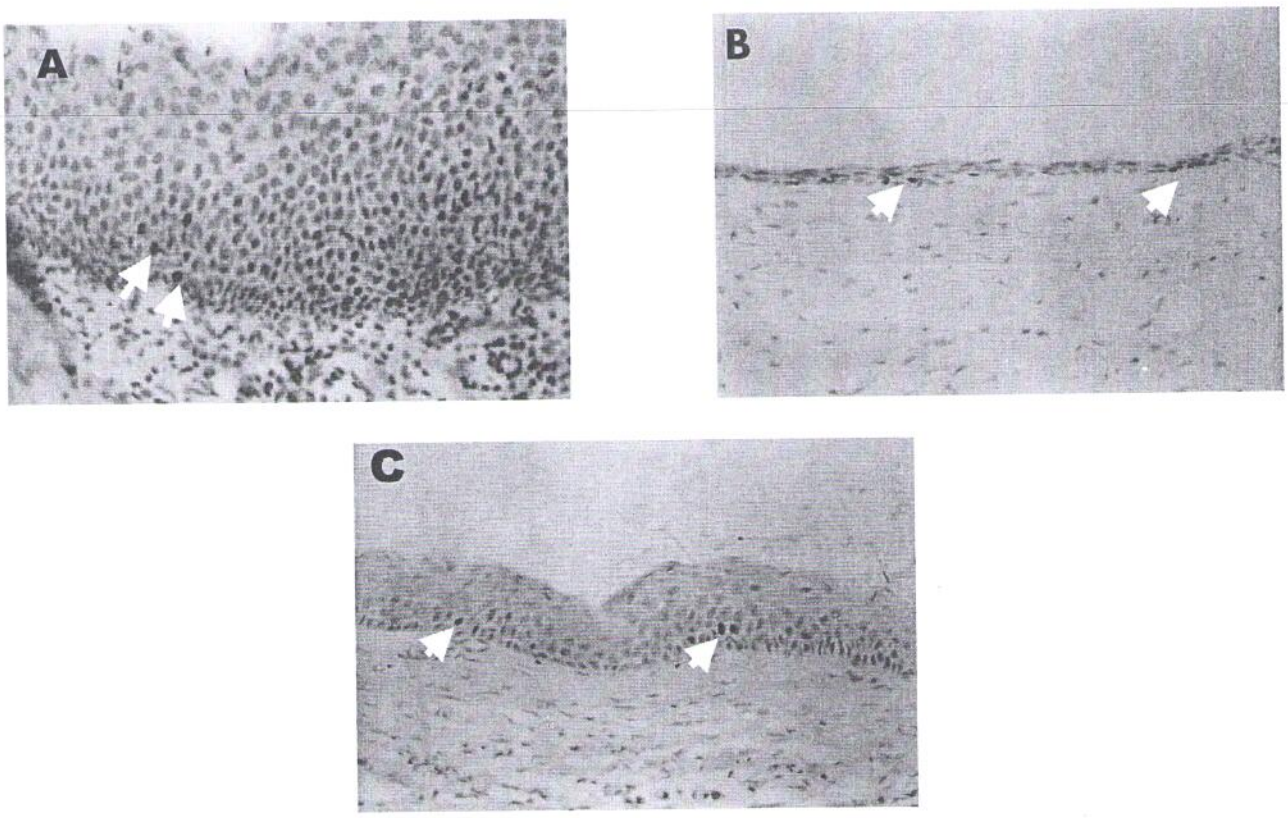

Fig.1 Photomicrographs showing P53+ cells (arrows) in
(A) Radicular cyst
(B) Dentigerous cyst
(C) Odontogenic keratocyst
(Immunoperoxidase staining,counterstained with hematoxylin, original magnification 100x).

ภาพที่ 1 แสดงผลบวกของการติดสีเมื่อย้อมด้วยแอนติบอดีต่อพี53 (ศรชี้) สำหรับ
(A) ถุงน้ำชนิดเรดิคูลาร์
(B) ถุงน้ำชนิดเด็นติเจอรัส
(C) ถุงน้ำชนิดโอดอนโตเจนิกเคอราโทซิสต์
(ย้อมด้วยวิธีอิมมูโนเปอร์ออกซิเดส ย้อมทับด้วยฮีมาท็อกไซลิน กำลังขยาย 100 เท่า)

confined to the ameloblast-like cells. In recurrent ameloblastoma, 9 out of 12 (75.0%) elicited positive P53 staining. Likewise, all of the positively stained cells were confined to the ameloblast-like cells. In malignant ameloblastoma, all 4 (100.0%) showed positive P53 staining, but the positively stained cells were located in stellate reticulum-like and squamous metaplasia areas, not in the ameloblast-like area.(Fig.2) The results of P53 staining are summarized in Table 1.

Discussion

In the present study, P53 was rarely seen in radicular cysts and dentigerous cysts as compared to odontogenic keratocysts. These results agreed with the previous studies in which both radicular cysts and dentigerous cysts showed either no P53 positive staining[2,12] or a lower number of P53 positive cells,[13] or revealed a high proportion of P53 positive cells but the staining was very faint[5] when compared to odontogenic keratocyst. Even though the result of radicular

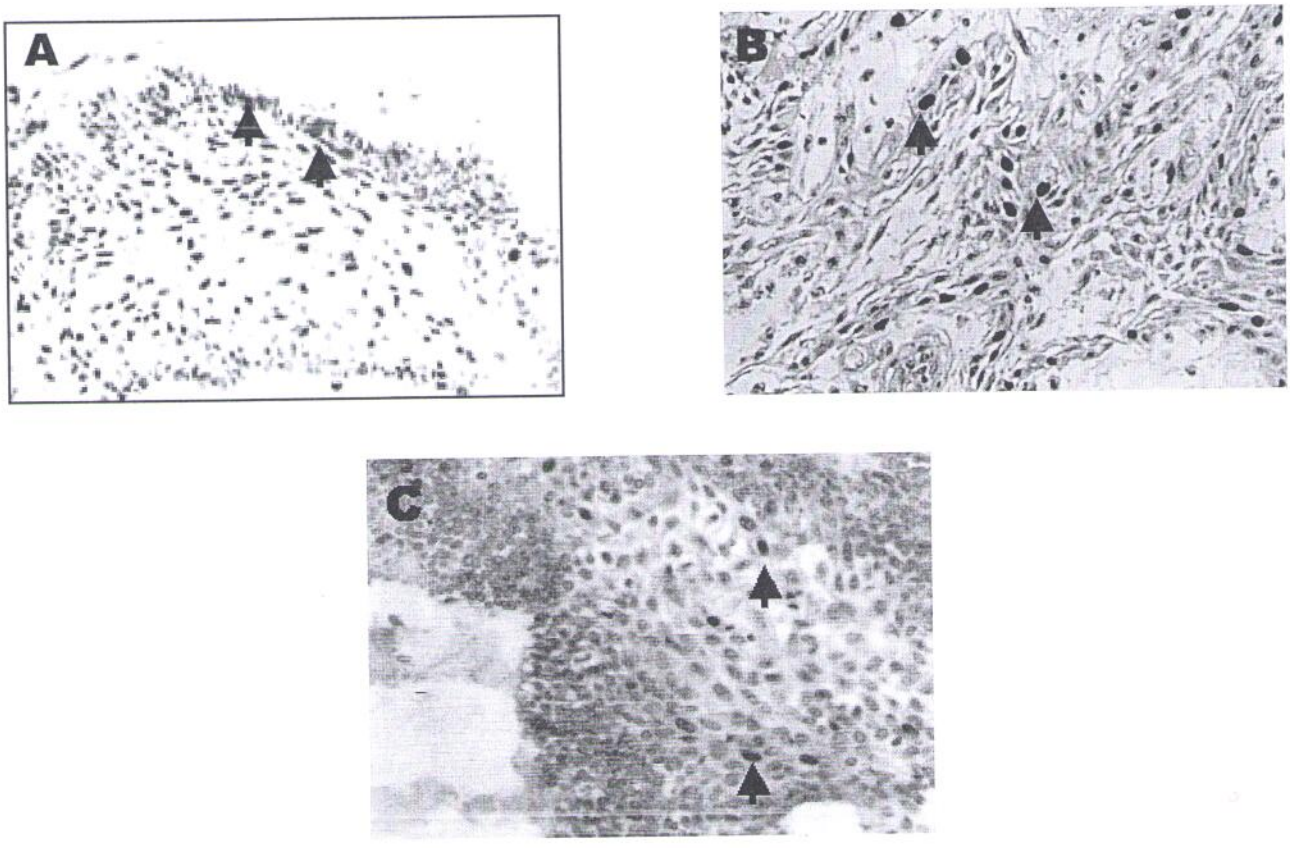

Fig.2 Photomicrographs showing P53+ cells (arrows) in
(A) Ameloblastoma
(B) Recurrent ameloblastoma
(C) Malignant ameloblastama
(Immunoperoxidase staining,counterstained with hematoxylin, original magnification 100x)

ภาพที่ 2 แสดงผลบวกของการติดสีเมื่อย้อมด้วยแอนติบอดีต่อพี 53 (ศรชี้) สำหรับ
(A) เนื้องอกอะมีโลบลาสโทมา
(B) เนื้องอกอะมีโลบลาสโทมาชนิดกลับเป็นซ้ำ
(C) มาลิกแนนท์อะมีโลบลาสโทมา
(ย้อมด้วยวิธีอิมมูโนเปอร์ออกซิเดส ย้อมทับด้วยฮีมาท็อกไซลิน กำลังขยาย 100 เท่า)

Table 1 P53 Staining of odontogenic cysts and ameloblastomas.

Lesions	No. of positive cases / total case	Percentage of positive cases
Radicular cyst	1 / 15	6.7
Dentigerous cyst	2 / 15	13.3
Odontogenic keratocyst	9 / 15	60.0
Ameloblastoma	4 / 12	33.3
Recurrent Ameloblastoma	9 /12	75.0
Malignant Ameloblastoma	4 / 4	100.0

cyst agreed with our results. Carvalhais et al[2] demonstrated the P53 negative immunostaining in odontogenic keratocyst which was different from our results as well as the aforementioned studies. Carvalhais et al[2] explained that the failure of P53 detection in their study might result from no mutation in p53 gene, deletion of the p53 gene or unstable p53 gene product in these lesions. In our study, odontogenic keratocyst elicited the highest P53 immunostaining among odontogenic cysts studied. The different results in P53 immunostaining may be due to several factors: antibody clones, antigen retrieval method, detection system and finally subjective judgment of positivity.

As P53 is rarely detected in radicular cysts and dentigerous cysts, it is unlikely to be the essential part of the development of these cysts. On the contrary, P53 was found in a significant number of cases in odontogenic keratocyst, ameloblastoma, recurrent ameloblastoma and all cases of malignant ameloblastoma. The latter lesions had been reported to have aggressive behavior. Therefore, p53 expression seems to play a part in the development of these lesions or to have an important role in their aggressive clinical behavior.

Since the expression of p53 gene was significantly related to cell proliferation and tumorigenesis, the presence of p53 gene product was extensively studied. The presence of p53 gene product could be either long-life mutated p53 protein or accumulation of wild type p53 protein.[1] The presence of wild type p53 protein usually resulted from overproduction or stabilization of p53 gene product.[5] The stabilization of wild type p53 protein could be the result of binding of p53 protein to intracellular or viral protein[14,16] or from inactivation of enzymatic pathways for the degradation of p53 protein.[17] In the previous report by Li et al[13], mutation of the p53 gene had not been detected in odontogenic keratocyst which advocated the notion that p53 protein detected in odontogenic keratocyst resulted from the accumulation of wild type p53 protein.[13] Therefore the p53 positive cases in radicular cysts and dentigerous cysts as well as in the odontogenic keratocyst in this study may reflect the presence of wild type p53 protein rather than the presence of p53 mutated gene product.

The concept that p53 expression is associated with neoplastic transformation is well attested to in the present study as ameloblastoma, recurrent ameloblastoma and malignant ameloblastoma exhibited a significant and increasing number of P53 positive cells, respectively. The explanation for P53 positivity in odontogenic keratocyst is that this cyst elicits aggressive clinical behaviour, high recurrent and

mitotic rate. These properties are comparable to those of benign tumor.[18] The more aggressive clinical behavior, the larger number of P53 positive cells. This trend is also appreciated in the study by Murti et al[19] which revealed overexpression of p53 protein in severe epithelial dysplasia more often than in mild epithelial dysplasia.

Another interesting point to note in the present study is that P53 positive cells in ameloblastoma and recurrent ameloblastoma were found in the ameloblast-like cells, while those of malignant ameloblastoma were encountered in stellate reticulum-like and squamous metaplasia areas, not in the ameloblast-like area. This change in staining pattern might represent the cellular genetic alteration from benign to malignant. The reason for the negative P53 staining in malignant ameloblast-like cells can possibly be accounted for by either deletion of p53 gene or expression of truncated p53 protein which is unrecognized by the primary antibody used in this study.

In conclusion, p53 expression correlates well with the clinical behavior, showing low detection rate in radicular cyst and dentigerous cyst and higher detection rate in odontogenic keratocyst, ameloblastoma and malignant ameloblastoma. Presently p53 has been shown to have some clinical utility as a tumor marker or prognostic tool for a high risk individual. It is of interest for the future treatment technology if the tumors have been early detected. It is possible that the low cost assay for detection of P53 could be of health benefit in developing countries.

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References

1. Chang F, Syrj_nen S, Tervahauta A, Syrj_nen K. Tumourigenesis associated with the p53 tumour suppressor gene. ***Br J cancer*** 1993;68:653-61.
2. Carvalhais JN, Aguiar MCF de, Araujo VC, Araujo NS, Gomez RS. p53 and MDM2 expression in odontogenic cysts and tumours. ***Oral Diseases*** 1999;5: 218-22.
3. Piattelli A, Rubini C, Fioroni M, Lezzi G, Santinelli A. Prevalence of p53, bcl-2, and Ki-67 immunoreactivity and apoptosis in normal oral epithelium and in premalignant and malignant lesions of the oral cavity. ***J Oral Maxillofac Surg*** 2002;60: 532-40.
4. Farmer G, Bargonetti J, Zhu H, Friedman P, Prywes R, Prives C. Wild- type p53 activates transcription in vitro. ***Nature*** 92;358:83-6.
5. Slootweg PJ. p53 protein and Ki-67 reactivity in epithelial odontogenic lesions. An immunohistochemical study. ***J Oral Pathol Med*** 1995; 24:393- 7.
6. Scully C, Field JK, Tanzawa H. Genetic aberrations in oral or head and neck squamous cell carcinoma 2: chromosomal aberrations. ***Oral Oncol*** 2000;36:311-27.
7. Field JK, Pavelic ZP, Spandidos DA, Stambrook PJ, Jones AS, Gluckman JL. The role of the p53 tumor suppressor gene in squamous cell carcinoma of the head and neck. ***Arch Otolaryngol Head Neck Surg*** 1993;119:1118-22.
8. Raybaud-Diogene H, Tetu B, Morency R, Fortin A, Monteil RA. p53 overexpression in head and neck squamous cell carcinoma: review of the literature. ***Eur J Cancer B Oral Oncol*** 1996;32B:143-9.
9. Soussi T.p53 Antibodies in the sera of patients with various types of cancer: a review. ***Cancer Res*** 2000;60:1777-88.
10. 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.
11. Ogden GR, Kiddie RA, Lunny DP, Lane DP. Assessment of p53 protein expression in normal, benign, and malignant oral mucosa. ***J Oral Pathol*** 1992 ;166:389-94.
12. 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.
13. Li TJ, Browne RM, Prime SS, Paterson IC, Matthews JB. p53 expression in odontogenic keratocyst epithelium. ***J Oral Pathol Med*** 1996;25:249-55.
14. Wynford-Thomas D. P53 in tumour pathology: can we trust immunocytochemistry? ***J Pathol*** 1992;166:329-30.
15. Hall PA, Lane DP. p53 in tumour pathology: can we trust immunohistochemistry?--Revisited! ***J Pathol*** 1994;172:1-4.
16. Battifora H. p53 immunohistochemistry: a word of caution. ***Hum Pathol*** 1994;25:435-7.
17. Bennett WP, Hollstein MC, He A, Zhu SM, Resau JH, Trump BF, et al. Archival analysis of p53 genetic and protein alterations in Chinese esophageal cancer. ***Oncogene*** 1991;6:1779-84.
18. 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.
19. Murti PR, Warnakulasuriya KA, Johnson NW, Bhonsle RB, Gupta PC, Daftary DK, et al. p53 expression in oral precancer as a marker for malignant potential. ***J Oral Pathol Med*** 1998;27:191-6.