TÜBİTAK

Turkish Journal of Medical Sciences

http://journals.tubitak.gov.tr/medical/

Research Article

Turk J Med Sci
(2014) 44: 875-882
© TÜBİTAK
doi:10.3906/sag-1304-136

Expression and clinical implications of P53, P63, and P73 protein in malignant tumor of the parotid gland

Mei YONG[1], Ling YANG[2], Qimuge SUYILA[2], Wenyan HAN[2], Hongwei YUAN[3], Cheng ZHAO[1,*], Xiulan SU[2,*]

[1]Department of Stomatology, Affiliated Hospital of Inner Mongolia Medical University, Hohhot, Inner Mongolia, P.R. China
[2]Clinical Medical Research Center of the Affiliated Hospital of Inner Mongolia Medical University, Hohhot, Inner Mongolia, P.R. China
[3]Department of Pathology, Affiliated Hospital of Inner Mongolia Medical University, Hohhot, Inner Mongolia, P.R. China

Received: 30.04.2013 • **Accepted:** 08.10.2013 • **Published Online:** 15.08.2014 • **Printed:** 12.09.2014

Background/aim: To investigate the expression of P53, P63, and P73 proteins in malignant parotid gland tumors and adjacent nonneoplastic tissues and the association between the 3 proteins and their clinical characteristics.

Materials and methods: A total of 40 pairs of paraffin-embedded malignant parotid gland tumors and adjacent nonneoplastic tissues were collected. We detected P53, P63, and P73 protein expression by immunohistochemistry. Statistical analysis was performed by using the chi-square test.

Results: P53, P63, and P73 protein expression in malignant parotid gland tumors was higher than their expression in adjacent nonneoplastic tissue ($P = 0.030$, 0.001, and 0.001, respectively). Expression of P53, P63, and P73 proteins was not associated with age, sex, or lymph node metastasis ($P > 0.05$). Expression of P53 and P73 proteins, instead of the P63 protein, was correlated to the degree of malignancy ($P = 0.026$ and 0.018, respectively). There was no significant difference among the P53, P73, and P63 proteins in malignant parotid gland tumors ($P > 0.05$). In the follow-up, only one patient died of colon cancer.

Conclusion: Our results suggest that the P53, P63, and P73 proteins may play a role in the development of malignant parotid gland tumors and provide data for their diagnosis.

Key words: P53/P63/P73 gene, malignant parotid gland tumor, immunohistochemistry

1. Introduction

Salivary gland tumors, occupying 22.7% of all oral and maxillofacial tumors, are relatively common in oral and maxillofacial tumors (1) and contain various histologic types (2). Epithelium-derived salivary gland tumors make up the majority, while tumors derived from other tissues (such as mesenchymal tissue) are rare. The parotid gland is the largest gland in the salivary gland, a site susceptible to developing tumors or tumor-like lesions. The incidence of parotid gland tumors accounts for about 80% of all salivary gland tumors. The majority of parotid gland tumors are benign (about 75%); malignant tumors only make up about 25% of the total. The pathology of parotid gland tumors is complex, while their clinical manifestations, treatment, and prognosis are quite different (3). Malignant parotid gland tumors mostly derive from epithelial cells of parotid glands or gland ducts. Mucoepidermoid tumors, malignant mixed tumors, adenoid cystic carcinoma, and adenocarcinoma are common types, accounting for 80%–90% of malignant parotid gland tumors. Modern molecular biological studies have demonstrated that the activation of oncogenes and the loss of function of tumor-suppressor genes play a crucial role in carcinogenesis. Currently, there is no molecular biomarker for malignant parotid gland tumors in clinical treatment. Therefore, it is important to study the pathogenesis and the early diagnosis of malignant parotid gland tumors.

P53, P63, and P73 are widely studied in tumorigenesis and highly expressed in various types of tumors. So far, P53 is the tumor-suppressor gene that is the most closely related with human tumors (4). It plays key roles in cell cycle regulation, cell growth inhibition, and apoptosis in tumor cells. More than 50% of human tumors contain a P53 gene mutation. P63, a homolog of P53, is a transcription factor. P63 plays an important role in the development, differentiation, and morphogenesis of epithelial tissue. Studies found that the P73 protein increased in many types of tumors including lung cancer (5), colon cancer (6), ovarian cancer (7), endometrial cancer (8), and breast cancer (9), while it decreased in pediatric acute

* Correspondence: kindba@163.com

lymphocytic leukemia (10). However, studies of these 3 factors in malignant parotid gland tumors are rarely reported. Our present study investigated P53, P63, and P73 expression in malignant parotid gland tumors using immunohistochemistry (IHC), and the relationship of their clinical characteristics, to provide information for diagnosis and individualized treatment for malignant parotid gland tumors.

2. Materials and methods

2.1. Specimens

A total of 40 pairs of paraffin-embedded parotid gland malignant tumors and adjacent nonneoplastic samples were collected from the Affiliated Hospital of Inner Mongolia Medical University and Qingdao Oral Medical College from 2006 to 2010, including 15 males and 25 females (average age: 51.5 years old, range: 21–78 years). No patients underwent preoperative chemoradiotherapy. Specimens were fixed in 10% formalin with paraffin embedded. All specimens were separately diagnosed by pathological experts and classified according to the World Health Organization's histological classification of salivary gland tumors (Table 1). Our study was approved by the Inner Mongolia Medical University Affiliated Hospital Ethics Committee.

2.2. Immunohistochemistry

Expression of P53, P63, and P73 protein was detected using an immunohistochemical S-P kit (MaiXin Bio, China) according to the manufacturer's instructions. Specific antibodies to P53 (MaiXin Bio), P63 (MaiXin Bio), and P73 (Bioss, China) were used as the first antibodies. The P53 antibody recognized the aa37-45 at the N-terminal of the whole P53. P63 and P73 antibodies also recognized the N-terminal of the P63 and P73 proteins. PBS buffer, instead of the first antibodies, was used as the negative control. Hematoxylin was used for counterstaining (in blue). Each section was observed in 10 random fields under 100× magnification. The overall IHC score (0, 1, 1.5–2, 2.5–3) was calculated by adding intensity (0, no staining; 1, light yellow; 2, orange-yellow; 3 brown) and proportion of positively stained cells (0, <10%; 1, 10%–50%; 2, 50%–75%; 3, >75%), and then dividing the sum by 2. Expression of P53, P63, and P73 was categorized as negative (IHC score = 0) or positive (IHC score > 0) for future analysis. Each slide was reviewed by 2 pathologists independently.

2.3. Statistical analysis

Statistical analysis was performed using SPSS 13.0. The chi-square test was used to compare P53, P63, and P73 protein expression and the clinical characteristics of malignant parotid gland tumors. Statistical significance was established at $P < 0.05$.

3. Results

We detected P53, P63, and P73 protein expression in 40 malignant parotid gland tumors and paired adjacent nonneoplastic tissues using IHC. Expressions of the 3 proteins in each subtype of tumors are listed in Table 1. We found that 28 (70%), 26 (65%), and 27 (67.5%) out of 40 tumor specimens expressed the P53, P63, and P73 proteins, respectively. On the contrary, 2 (5%), 2 (5%), and 0 (0%) adjacent nonneoplastic tissues expressed P53, P63, and P73 proteins ($P = 0.030$, 0.001, and 0.001, respectively; Table 2). Positive staining of the P53 protein was mainly located in the nuclei in adenoid cystic carcinoma, mucoepidermoid tumors, and adenocarcinoma (Figure 1, indicated by arrows). We found that the P63 protein also showed positive

Table 1. Histological classification of salivary gland tumors.

Histological classification	Number	P53	P63	P73	P53/P63/P73
Mucoepidermoid tumor	8	8	8	6	6
Acinic cell tumor	6	3	2	5	1
Carcinoma in pleomorphic adenoma	5	3	3	5	1
Basal cell adenocarcinoma	5	3	4	1	0
Poorly differentiated adenocarcinoma	3	3	1	2	1
Salivary duct carcinoma	2	1	2	1	0
Epidermoid carcinoma	2	2	2	1	1
Myoepithelial carcinoma	2	1	2	2	1
Adenoid cystic carcinoma	2	2	1	2	1
Adenocarcinoma (miscellaneous)	5	2	1	2	0

Table 2. Expression of P53, P63, and P73 proteins in malignant parotid gland tumors and adjacent normal tissues.

	Tumors	Adjacent normal tissues	χ^2	P
P53				
Positive	28	2		
Negative	12	38	8.658	0.030
P63				
Positive	26	2		
Negative	14	38	11.250	0.001
P73				
Positive	27	0		
Negative	13	40	15.522	0.001

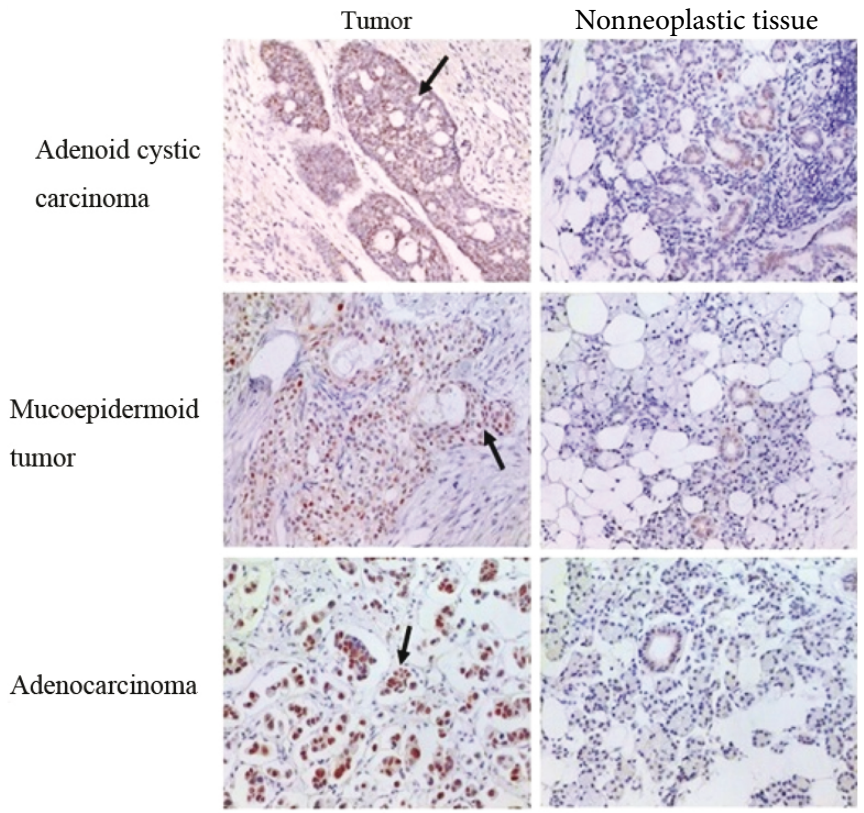

Figure 1. P53 expression in tumors and nonneoplastic tissues of adenoid cystic carcinoma, adenocarcinoma, and mucoepidermoid tumors (100×).

staining in the nuclei of adenoid cystic carcinoma and mucoepidermoid tumors, instead of in adenocarcinoma (Figure 2, indicated by arrows). P63 expression was observed in the nuclei of myoepithelium in the glands of nonneoplastic tissue of adenocarcinoma (Figure 2, indicated by arrow). The P73 protein was mainly located in the nuclei, with a little in the cytoplasm, and was diffusely stained in adenoid cystic carcinoma, mucoepidermoid tumors, and adenocarcinoma (Figure 3, indicated by arrows).

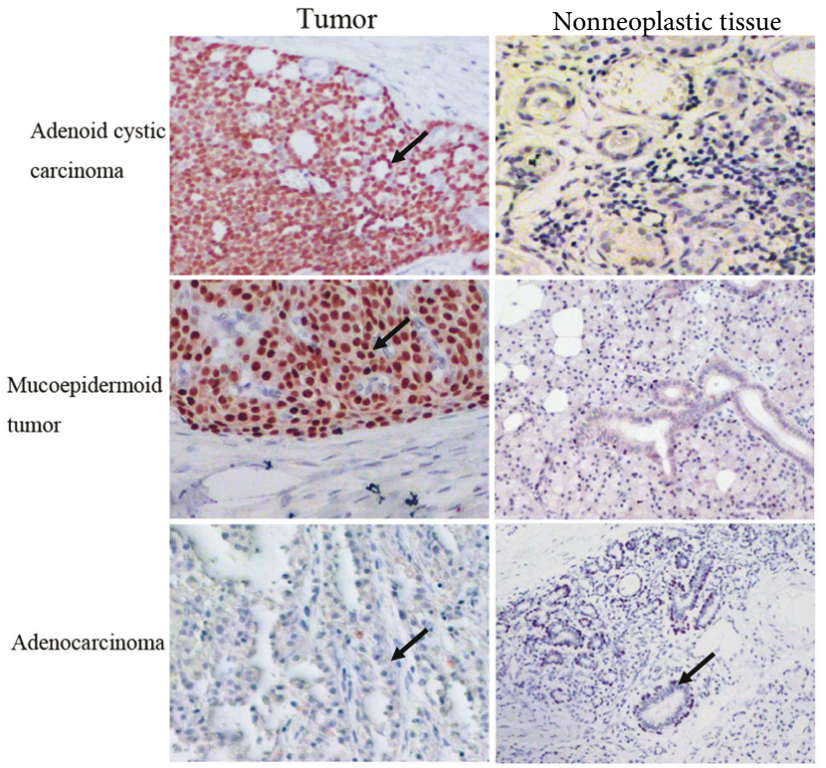

Figure 2. P63 expression in tumors and nonneoplastic tissues of adenoid cystic carcinoma, mucoepidermoid tumors, and adenocarcinoma (100×).

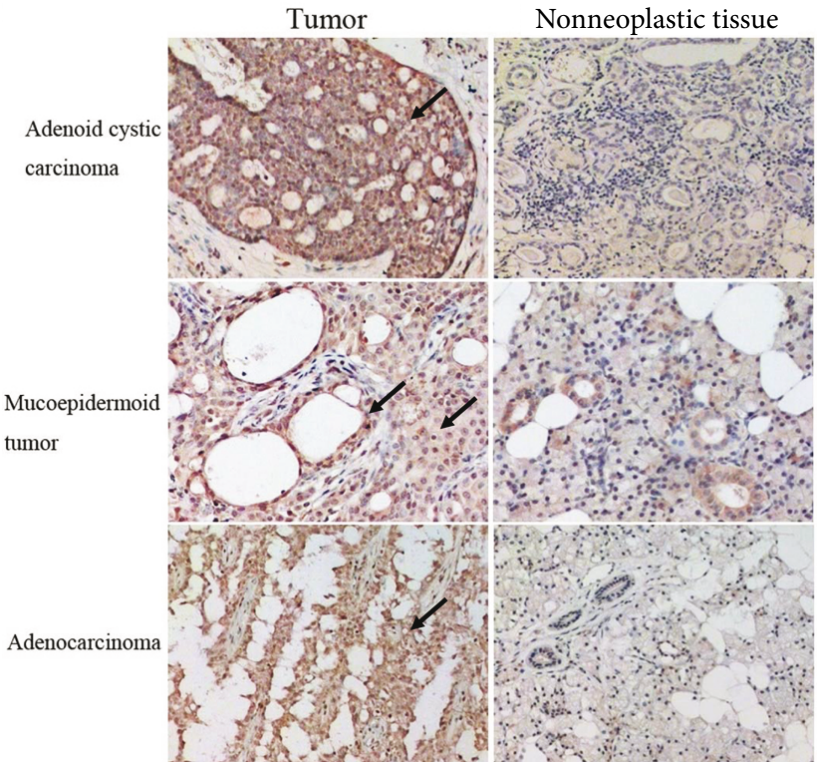

Figure 3. P73 expression in tumors and nonneoplastic tissues of adenoid cystic carcinoma, adenocarcinoma, and mucoepidermoid tumors (100×).

The expression of P53, P63, and P73 protein was not associated with age or sex of patients or lymph node metastasis of malignant parotid gland tumors (P > 0.05, Table 3). Expression of the P53 and P73 protein, but not the P63 protein, was correlated to degree of malignancy (P = 0.026 and 0.018, respectively; Table 3). In total, 2, 2, 1, and 4 high malignant tumors were observed to have P53/P63, P53/P73, and P63/P73 double-positive staining and P53/P63/P73 triple-positive staining, respectively (Table 4). Lymph node metastasis was found in P53/P73 and P63/P73 double-positive and P53/P63/P73 triple-positive samples (Table 4). There was no significant difference among the expressions of P53, P73, and P63 proteins in malignant parotid gland tumors (P > 0.05, Table 5). In the follow-up, ranging from 5 months to 3 years, only 1 patient died of colon cancer, which indicates that expression of the P53, P63, and P73 proteins may not be involved in the prognosis of malignant parotid gland tumors.

Table 3. The relationships between P53, P63, and P73 protein expression and clinical characteristics of malignant parotid gland tumors.

Clinical characteristics	No.	P53 Positive	Negative	P	P63 Positive	Negative	P	P73 Positive	Negative	P
Sex										
Male	15	11	4		10	5		10	5	
Female	25	17	8	0.722	16	9	0.864	17	18	0.931
Age (years)										
≤60	30	22	8		20	10		19	11	
>60	10	6	4	0.426	6	4	0.702	8	2	0.330
Malignancy degree										
High	9	9	0		8	1		9	0	
Moderate to low	31	19	12	0.026*	18	13	0.088	18	13	0.018*
Lymph node metastasis										
No	37	26	11		24	13		24	13	
Yes	3	2	1	0.896	2	1	0.950	3	0	0.211

*: P < 0.05 indicates significant differences.

Table 4. Degree of malignancy and lymph node metastasis in malignant parotid gland tumors with double- and triple-positive or negative staining of P53, P63, and P73.

Clinical characteristics	No.	P53/P63 Positive	P53/P63 Negative	P53/P73 Positive	P53/P73 Negative	P63/P73 Positive	P63/P73 Negative	P53/P63/P73 Positive	P53/P63/P73 Negative
Malignancy degree									
High	9	2	0	2	0	1	0	4	0
Moderate to low	31	6	3	5	1	4	1	8	3
Lymph node metastasis									
No	37	8	3	6	1	4	1	11	3
Yes	3	0	0	1	0	1	0	1	0

Table 5. The relationships among the expressions of P63, P53, and P73 proteins in malignant parotid gland tumors.

P53/P73	P63		χ^2	P
	Positive	Negative		
P53				
Positive	20	8		
Negative	6	6	1.695	0.193
P73				
Positive	17	10		
Negative	9	4	0.152	0.697

4. Discussion

High expression of P53 protein represents a high degree of malignancy and poor prognosis in oral tumors (11). Expression of the P53 protein increased in malignant salivary gland tumors (12–14) and was correlated with poor differentiation, high degrees of malignancy, and poor prognosis (15). The mutation rate of the P53 gene varies in oral and maxillofacial tumors (12). Studies suggest that the conformation change of mutant P53 facilitates malignant cells escaping from regulation and immortalization. Ki-67 is a nuclear protein that indicates cellular proliferation, and it is expressed in most proliferative cells. It is reported that the expression of Ki-67 and P53 coexists in malignant salivary gland tumors (16), indicating that dysregulation of proliferative genes and loss of function of tumor-suppressor genes interacts in concert in the development of salivary gland tumors. We detected P53 protein expression in malignant parotid gland tumors and adjacent nonneoplastic tissues using IHC and found that high expressions of the P53 protein in tumor tissues compared to adjacent nonneoplastic tissues correlated with degree of malignancy. P53 may be an important biomarker for degree of malignancy and prognosis of malignant parotid gland tumors.

Different splicing and promoters produce 6 isoforms of P63, including ΔN and TAp63. ΔNP63 is essential for the development, differentiation, and homeostasis of the oral squamous epithelium (17). In oral tumors, ΔNP63 binds to the DNA through its DNA binding domain or directly interacts with P53 or TAP63, which makes ΔNP63 inhibit cell apoptosis and perform the function of an oncogene in the occurrence of oral tumors (18,19). P63 also plays an important role in oral squamous cell carcinoma (20,21). P63 was involved in the physiopathology of the oral mucosa (20). Low P63 gene mutation rates but increased expression of ΔNP63α was found in oral squamous cell carcinoma (21).

High P63 expression was reported in salivary gland tumors (22,23). Bilal et al. (24) found strong expressions of P63 in myoepithelial cells, while weak expressions were seen in the cellular lumen in mucoepidermoid carcinoma. P63 was expressed in the basal cells of emunctories in normal salivary gland tissue, while in salivary gland tumors P63 was expressed in myoepithelial cells, and especially in the myoepithelial cells of malignant salivary gland tumors (25). The different expressions of P63 in normal and malignant salivary gland tissues may be applied to the diagnosis of salivary gland tumors. Our results found that expression of P63 protein in malignant parotid gland tumors was higher than that in adjacent nonneoplastic tissues, indicating that the overexpression of P63 protein was closely related to the development of malignant parotid gland tumors.

Loss of heterozygosity (LOH) and methylation of P73 was associated with tumorigenesis. Araki et al. (26) found LOH in 31 of 41 (73%) oral cancers, high methylation of the CpG island in the P73 gene promoter, and decreased expression of mRNA. In tumor tissue with P73 gene methylation, no or low transcriptional levels of P73 mRNA might lead to tumorigenesis. Expression of P73 increased in oral squamous cell carcinoma compared to that in normal oral mucosa (27,28). Elevated expression of the P73 protein was found in oral precancerous lesions and squamous cell carcinoma (29,30), indicating that P73 expression was involved in the carcinogenesis from normal oral mucosa to precancerous lesions and squamous cell carcinoma. P73 expression was positively correlated to lymph node metastasis in oral squamous cell carcinoma (28–30), suggesting that tumors with high P73 expression were more invasive than those with low or no expression of P73.

Although P73 has been widely studied in many types of tumors, the P73 gene's function has not been clarified (29); in particular, P73 expression in malignant parotid gland tumors and its correlation with degree of malignancy have

been rarely reported. Chen et al. (31) found that there was no significant difference between P73 expression in benign and malignant pleomorphic adenoma and in normal parotid gland tissue, suggesting that P73 expression failed to differentiate normal parotid gland tissue from benign and malignant pleomorphic adenoma. We found that P73 protein expression was higher in tumors than in adjacent nonneoplastic tissue, which was consistent with other reports (32), suggesting that overexpression of the P73 protein may play an important role in the development of malignant parotid gland tumors. Our results showed that expression of P73 protein was correlated with degree of malignancy but not with lymph node metastasis in malignant parotid gland tumors. To further confirm our results, it is necessary to do additional studies with larger samples.

Studies showed that P73 arrested cell growth and induced apoptosis by activating the specific target genes of P53 and that different splicing isoforms of P53 and P73 activated distinct target genes. Therefore, P53 and P73 signaling pathways were partially overlapped (33). Positive correlation between P53 protein and P73 protein was found in oral squamous carcinoma (28), gastric cancer (34), and osteosarcoma (35). Recent studies suggested that P73 might not be a tumor suppressor gene, but rather an analog of P53, which can simulate P53's function and therefore cause the P53 gene to lose function, subsequently leading to cellular malignancy (36,37). Our results showed that expression of P53 and P73 proteins was not significantly different in malignant parotid gland carcinoma ($P > 0.05$), which was consistent with other studies (38). Our results suggested that the function of P53 and P73 proteins might be independent from the development of malignant parotid gland tumors. At the same time, our results showed that there was no significant difference between the expression of P53 and P63 proteins and that of P73 and P63 proteins in malignant parotid gland carcinoma ($P > 0.05$).

We found a high expression of P53, P63, and P73 proteins in malignant tumors of the parotid gland, suggesting that P53, P63, and P73 proteins may play a role in the development of malignant parotid gland tumors. After further study of the functions and regulation mechanisms of P53, P63, and P73 genes, new insight will be revealed on the carcinogenesis of oral tumors and early molecular intervention will become possible.

Acknowledgment

Our research was supported by the National Natural Science Foundation of China (No. 81160254).

References

1. Wang X. P53 gene and salivary gland tumors. Int J Stomatol 2006; 33: 460–462 (article in Chinese).
2. Renehan AG, Gleave EN, Slevin NJ, McGurk M. Clinico-pathological and treatment-related factors influencing survival in parotid cancer. Br J Cancer 1999; 80: 1296–1300.
3. Lei AP, Cheng ZG. The clinical analysis of 112 cases of parotid tumors. J Clin Stomatol 2009; 25: 296–297 (article in Chinese).
4. Freed-Pastor WA, Prives C. Mutant p53: one name, many proteins. Genes Dev 2012; 26: 1268–1286.
5. Tokuchi Y, Hashimoto T, Kobayashi Y, Hayashi M, Nishida K, Hayashi S, Imai K, Nakachi K, Ishikawa Y, Nakagawa K et al. The expression of P73 is increased in lung cancer independent of P53 gene alteration. Br J Cancer 1999; 80: 1623–1629.
6. Su XL, Ouyang XH, Yan MR, Liu GR. P73 expression and its clinical significance in colorectal cancer. Colorectal Dis 2009; 11: 960–963.
7. Zhang W, Fu FW, Wang QY. The expression of P73 in human epithelial ovarian carcinoma and its clinical significance. Pract Clin Med 2008; 29: 43–44 (article in Chinese).
8. Zhang J, Chu CJ, Zhang XL, Zhang LY. The expressions and clinical significance of c-erbB-2, p53 and p73 in endometrial cancer. Current Physician 2010; 26: 2–3 (article in Chinese).
9. Yang WG. The expressions and clinical significance of survivn and P73 in breast cancer tissues. J Med Forum 2008; 29: 43–44 (article in Chinese).
10. Liu ML, Li RS, Lin TX, Zhu GJ, Guo SC. A study of the abnormal expressions of P73 in children undergone therapy for acute lymphoblastic leukemia. Chin J Hematol 2002; 23: 239–242 (article in Chinese).
11. Feng SF. The research progress of the correlation between P53 gene mutation and oral neoplasms. Med Recapitulate 2008; 14: 2113–2115 (article in Chinese).
12. Alves FA, Pires FR, De Almeida OP, Lopes MA, Kowalski LP. PCNA, Ki-67 and P53 expressions in submandibular salivary gland tumours. Int J Oral Maxillofac Surg 2004; 33: 593–597.
13. Tarakji B, Kujan O, Nassani MZ. Immunohistochemical expression of P53 in pleomorphic adenoma and carcinoma ex pleomorphic adenoma. J Cancer Epidemiol 2010; 2010: 250606.
14. Liu W, Chen LL, Liu Y. The expression of P53 protein, vascular endothelial growth factor and microvessel density in salivary gland tumors. J Pract Stomatol 2009; 25: 66–70 (article in Chinese).
15. Liu F, Zhang JZ, Ma M, Han ES, Li GF. Scoring of P53, PCNA in salivary gland adenoid cystic carcinoma and Bd-2 expression and their correlation with the pathological types and recurrence. J Clin Stomatol 2004; 20: 653–655 (article in Chinese).

16. Bacsi A, Stanton GJ, Hughes TK, Kruze M, Boldogh I. Colostrinin-driven neurite outgrowth requires p53 activation in PC12 cells. Cell Mol Neurobiol 2005; 55: 1123–1139.

17. Tettamanti L, Caprioglio A, Tecco S, Barello G, Macchi A, Tagliabue A, Levrini L. Oral squamous cell carcinoma in the paediatric patient: a literature review. Eur J Paediatr Dent 2012; 13: 35–40.

18. Oncel S, Cosgul T, Calli A, Calli C, Pinar E. Evaluation of p53, p63, p21, p27, ki-67 in paranasal sinus squamous cell carcinoma and inverted papilloma. Indian J Otolaryngol Head Neck Surg 2011; 63: 172–177.

19. Nandini D, Subramanyam R. Nuclear features in oral squamous cell carcinoma: a computer-assisted microscopic study. J Oral Maxillofac Pathol 2011; 15: 177–181.

20. Vilas-Boas F, Magro F, Balhau R, Lopes JM, Beça F, Eloy C, Lopes S, Macedo G. Oral squamous cell carcinoma in a Crohn's disease patient taking azathioprine: case report and review of the literature. J Crohns Colitis 2012; 20: 845–849.

21. Rivetti di Val Cervo P, Lena AM, Nicoloso M, Rossi S, Mancini M, Zhou H, Saintigny G, Dellambra E, Odorisio T, Mahé C et al. P63-microRNA feedback in keratinocyte senescence. Proc Natl Acad Sci U S A 2012; 109: 1133–1138.

22. Cao Y. A study of the myoepithelial immunophenotype in salivary gland tumors. J Pract Stomatol 2005; 21: 329–331 (article in Chinese).

23. Yang YP, Cao Y, Qin YH. The expression of p63 protein in salivary gland tissue and tumor. Chin J Pathol 2003; 32: 261–262 (article in Chinese).

24. Bilal H, Handra-Luca A, Bertrand JC, Fouret PJ. P63 is expressed in basal and myoepithelial cells of human normal and tumor salivary gland tissues. J Histochem Cytochem 2003; 51: 133–139.

25. Lu YH, Gao Y. The expression of calponin and P63 in human submandibular glands. West China J Stomatol 2007; 25: 19–21 (article in Chinese).

26. Araki D, Uzawa K, Watanabe T, Shiiba M, Miyakawa A, Yokoe H, Tanzawa H. Frequent allelic losses on the short arm of chromosome 1 and decreased expression of the P73 gene at 1p36.3 in squamous cell carcinoma of the oral cavity. Int J Oncol 2002; 20: 355–360.

27. Ebrahimi M, Wahlin YB, Coates PJ, Wiik A, Roos G, Nylander K. Detection of antibodies against P63 and P73 isoforms in sera from patients diagnosed with oral lichen planus. J Oral Pathol Med 2007; 36: 93–98.

28. Cao X, Wang LH, Liu JT, Liu PY. The expression and significance of P73, P63 and P53 in oral squamous cell carcinomas. J Pract Med 2008; 24: 3864–3867 (article in Chinese).

29. Boominathan L. The guardians of the genome (p53, TA-p73, and TA-p63) are regulators of tumor suppressor miRNAs network. Cancer Metastasis Rev 2010; 29: 613–639.

30. Chen YK, Huse SS, Lin LM. Differential expression of P53, P63 and P73 protein and mRNA for DMBA-induced hamster buccal-pouch squamous cell carcinomas. Int J Exp Pathol 2004; 85: 97–104.

31. Chen L. The localization and expression of P73 and PCNA in benign and malignant superficial multiform adenoma. MSc, Tianjin Medical University, Tianjin, China, 2011 (thesis in Chinese).

32. Pluta A, Nyman U, Joseph B, Robak T, Zhivotovsky B, Smolewski P. The role of P73 in hematological malignancies. Leukemia 2006; 20: 757–766.

33. Li G, Sturgis EM, Wang LE, Chamberlain RM, Amos CI, Spitz MR, El-Naggar AK, Hong WK, Wei Q. Association of a P73 exon 2 G2C14-to-A4T14 polymorphism with risk of squamous cell carcinoma of the head and neck. Carcinogenesis 2004; 25: 1911–1916.

34. Di MJ. The expressions of P73 and P53 in gastric carcinoma tissue and their relevance. J Yunyang Med Coll 2008; 27: 230–232 (article in Chinese).

35. Hu D, Huang JW, Hu SP, Wang B, Gao LY. The expression of P53, P63 and P73 in human osteosarcoma and its relevant research. Chin J Bone Tumor Bone Dis 2004; 3: 138–141 (article in Chinese).

36. Ichimiya S, Nimura Y, Kageyama H, Takada N, Sunahara M, Shishikura T, Nakamura Y, Sakiyama S, Seki N, Ohira M et al. Genetic analysis of P73 localized at chromosome lP36.3 in primary neuroblastomas. Med Pediatr Oncol 2001; 36: 42–44.

37. Nicholson SA, Okby NT, Khan MA, Welsh JA, McMenamin MG, Travis WD, Jett JR, Tazelaar HD, Trastek V, Pairolero PC et al. Alteration of P14ARF, P53 and P73 genes involved in the E2F-1-mediated apoptotic pathways in non-small cell lung carcinoma. Cancer Res 2001; 61: 5636–5643.

38. Guan HW, Shang W, Yang XC. The expression of P73 and P53 in oral squamous cell carcinoma and its significance. Acta Academiae Medicinae Qingdao Universitatis 2009; 45: 94–96 (article in Chinese).