Int J Clin Exp Pathol 2020;13(8):2100-2105
www.ijcep.com /ISSN:1936-2625/IJCEP0116026

Original Article

SKA1 expression in oral squamous cell carcinoma and its relationship to P53 and clinicopathologic features

Lijuan Zhao[1*], Bin Zhang[3*], Kun Li[2], Peng Sun[2]

Departments of [1]Oral and Maxillofacial Surgery, [2]Orthodontics, Yantai Stomatological Hospital Affiliated to Binzhou Medical College, Yantai 264001, China; [3]Department of Oral and Maxillofacial Surgery, Liaocheng People's Hospital Affiliated to Taishan Medical University, Liaocheng 252000, China. [*]Equal contributors.

Received June 11, 2020; Accepted July 20, 2020; Epub August 1, 2020; Published August 15, 2020

Abstract: In this study, 57 paraffin-embedded tissue specimens from patients with oral squamous cell carcinoma (OSCC) were collected and analyzed. Spindle and kinetochore-associated complex subunit 1 (SKA1) and P53 protein expression in selected samples was detected by immunohistochemistry. The positive expression rate of SKA1 and P53 was significantly higher in oral squamous cell carcinoma tissues than in normal controls. The expression of SKA1 protein was significantly associated with tumor-node-metastasis (TNM) stage, and p53 expression was significantly correlated with pathologic differentiation grade in oral squamous cell carcinoma tissues. There was a significant correlation between SKA1 and p53 protein expression in oral squamous cell carcinoma tissues. Our results indicate that the SKA1 gene might be involved in the development of oral squamous cell carcinoma and might predict its prognosis. SKA1 is expected to be a new molecular target for oral squamous cell carcinoma.

Keywords: Spindle and kinetochore-associated complex subunit 1, P53, oral squamous cell carcinoma, prognostic

Introduction

It has been reported that 90% of oral cancers histologically originate from squamous cells, and this is defined as oral squamous cell carcinoma (OSCC) [1]. In worldwide reports, cancers of all regions of the oral cavity and pharynx are grouped and collectively represent the sixth most common cancer in the world [2]. Despite research progress and enhanced novel therapies, survival has not improved significantly in recent decades. There are no specific biomarkers for OSCC, representing a continuing challenge for biomedical science [3].

The cell division cycle is a highly ordered process that is tightly regulated by intracellular intrinsic cell cycle checkpoints to ensure faithful replication of cells [4]. Checkpoint defects may cause genetic mutations and chromosomal structural abnormalities, leading to cell proliferation and tumorigenesis [5].

Spindle and kinetochore-associated complex subunit 1 (SKA1), a microtubule-binding protein, is localized to the spindle microtubule and outer kinetochore interface during mitosis [6-11]. SKA1 mediates the binding of the SKA complex (SKA1, 2 and 3) to the microtubule, which is essential for stabilizing kinetochore-spindle microtubule attachment during mitosis. Our previous study found that depletion of SKA1 in CAL-27 cells led to G2/M phase cell cycle arrest and apoptosis [12]. As one of the most frequently studied genes, research has confirmed that p53 plays an important role in the cell cycle and apoptosis of tumor cells [13-16]. P53 is also a common tumor biomarker in squamous cell carcinoma (SCC) [17]. Therefore, we postulated that there may be a correlation between SKA1 and P53 related to their influence on the cell cycle and apoptosis. In the present study, we investigated the expression and correlation of SKA1 and P53 protein in oral squamous cell carcinoma to further clarify the roles of SKA1.

To the best of our knowledge, the correlations between SKA1 expression and the clinicopathologic features of patients with OSCC, as well as the correlation between SKA1 and p53, have not been well reported. The present study aimed to investigate the expression of SKA1 and p53 in OSCC and in adjacent oral epitheli-

um tissue. The results of the present study may provide new insight into the diagnosis, treatment, and prognosis of OSCC.

Materials and methods

Patients and specimens

The protocol of the study was approved by the Institutional Ethics Committee of the Liaocheng People's Hospital Affiliated to Taishan Medical University (Liaocheng, China). All 57 paraffin-embedded tissue specimens analyzed were selected from patients with complete clinical data in the Department of Pathology, Liaocheng People's Hospital (Liaocheng Clinical School of Taishan Medical University) of China, between May 2004 and June 2013. The criteria for study enrollment were as follows: patients (19 females, 38 males) with primary oral squamous cell carcinoma (OSCC) who underwent resection without preoperative chemotherapy, hormone therapy, radiotherapy, or other anticancer treatment. All cases were assessed by pathologic examination after surgery and confirmed to be squamous cell carcinoma (cancer) with a clear presence of lymph node metastasis and nerve invasion. Two independent observers reviewed all of the original hematoxylin and eosin-stained sections and chose the most representative slide from each case to perform immunohistochemical staining. According to the seventh edition of the AJCC (American Joint Committee on Cancer) Cancer Staging Manual (tumor-node-metastasis: TNM), 33 patients (57.9%) had stage I or II disease, and 24 patients (42.1%) had stage III or IV disease. Three histologic grades were established according to the International Classification of Tumors of the WHO: well differentiated tumors, moderately differentiated tumors and poorly or undifferentiated tumors. Other clinical and pathologic features of the enrolled patients are summarized in **Table 1**.

Immunohistochemical staining

The sections were placed in 3% H_2O_2 in distilled water for 15 min to block endogenous peroxidase activity after being dewaxed in xylene and hydrated in a graded ethanol series. Then, the sections were subjected to antigen retrieval by heating the slides in an autoclave at 120°C for 3 min in 0.1 M citric acid buffer (pH 6.0). Following antigen retrieval, the sections were blocked with BSA and then probed with rabbit anti-SKA1 (Sigma-Aldrich, 1:800) and mouse anti-p53 (Maixin, 1:300) at 4°C overnight. After rinsing with PBS, the sections were incubated with biotinylated goat anti-rabbit immunoglobulins and goat anti-mouse immunoglobulins at 37°C for 30 min and visualized using peroxidase-conjugated streptavidin and diaminobenzidine (DAB), followed by counterstaining with Mayer's hematoxylin. The negative controls were obtained by using PBS instead of primary antibodies.

Evaluation of immunohistochemical staining

The results were evaluated independently by two pathologists blinded to all clinical data. Immunohistochemical staining for SKA1 and p53 was evaluated according to intensity and proportion. Immunopositivity of SKA1 and p53 was scored according to the percentage of positive cells in four distinct categories: 0 for 0-10%, 1 for 11-30%, 2 for 31-70%, and 3 for 71-100%. The staining intensity was then scored as 0 for bright yellow staining, 1 for yellow staining, 2 for brown-yellow staining, and 3 for red-brown staining. Both scores were multiplied together, resulting in a final score: 0, negative (-); 1, weakly positive (-~+); 2-4, positive (+); and 6~9, strong positive (++).

Statistical methods

Correlations were separately evaluated between SKA1 and p53 expression and several clinicopathologic variables according to the Pearson chi-square test and Fisher's exact test. McNemar's test was used to evaluate the relationship between SKA1 and p53 expression. A P value <0.05 was considered significant.

Results

SKA1 expression and its correlations with clinicopathologic variables

Positive expression of SKA1 was mainly observed in the cytoplasm and nucleus. A small number of SKA1-positive cells had reactivity present in the cytoplasm. Immunostaining results suggested significantly higher SKA1 expression in OSCC tissues than in adjacent normal tissues (**Figure 1**). Among 57 tumor samples, 46 (80.7%) showed high SKA1 expression, which was significantly higher than the

Table 1. Relationship between SKA1 and p53 expression and clinicopathologic features of oral squamous cell carcinoma (OSCC)

Factors	No. of cases	SKA1		P	P53		P
		+ (n)	- (n)		+ (n)	- (n)	
Sex							
Female	19	15	4	>0.05	12	7	>0.05
Male	38	31	7		27	11	
Age, years							
≤60	24	20	4	>0.05	18	6	>0.05
>60	33	26	7		21	12	
Tumor size							
≤2 cm	29	19	9	>0.05	21	8	>0.05
>2 cm	28	21	7		18	10	
TNM stage							
I-II	33	23	10	<0.05	20	13	>0.05
III-IV	24	23	1		19	5	
Differentiation grade							
Well/severe differentiated	42	34	8	>0.05	24	18	<0.05
Poorly differentiated	15	12	3		15	0	
Perineural invasion							
Yes	6	4	2	>0.05	5	1	>0.05
No	51	42	9		34	17	
Lymphatic metastasis							
Yes	10	9	1	>0.05	6	4	>0.05
No	47	37	10		33	14	
History of alcohol/tobacco use							
Yes	23	19	4	>0.05	15	8	>0.05
No	34	27	7		24	10	

normal tissue adjacent to the carcinoma (14.3%, P<0.001). The correlations between SKA1 and clinical variables are listed in **Table 1**. The expression of SKA1 in tumor-node-metastasis (TNM) stage III~IV was significantly higher than that in stage I~II (P<0.05). SKA1 expression was not significantly associated with patient sex, age, history of alcohol or tobacco use, pathologic differentiation grade, lymph node metastasis, tumor size, or nerve invasion (P>0.05).

P53 expression and its correlation with clinicopathologic variables

The positive expression of p53 protein was located in the nuclei of the tumor cells, demonstrated by a brown color (**Figure 2**). The rate of positive p53 expression in OSCC (68.4%) was significantly higher than that in normal tissue adjacent to the carcinoma (9.5%, P<0.001). Correlations between p53 and clinical variables are listed in **Table 1**. P53 expression in OSCC was significantly associated with pathologic differentiation grade (P=0.001), and its expression in OSCC was not significantly associated with tumor size, TNM stage, age, sex, history of alcohol or tobacco use, lymph node involvement, or nerve invasion (P>0.05).

Correlation between SKA1 and p53 protein expression

As shown in **Table 2**, of 46 tumor tissues with positive SKA1 expression, 8 were negative and 38 were positive for p53 expression. By contrast, of 11 tumor tissues with negative SKA1 expression, 10 were negative and 1 was positive for p53 expression. McNemar's test reflected that there was a significant correlation between SKA1 and p53 expression in OSCC (P<0.05). The expression of SKA1 and p53 showed similar trends in patients grouped by sex, age, tumor size and TNM stage.

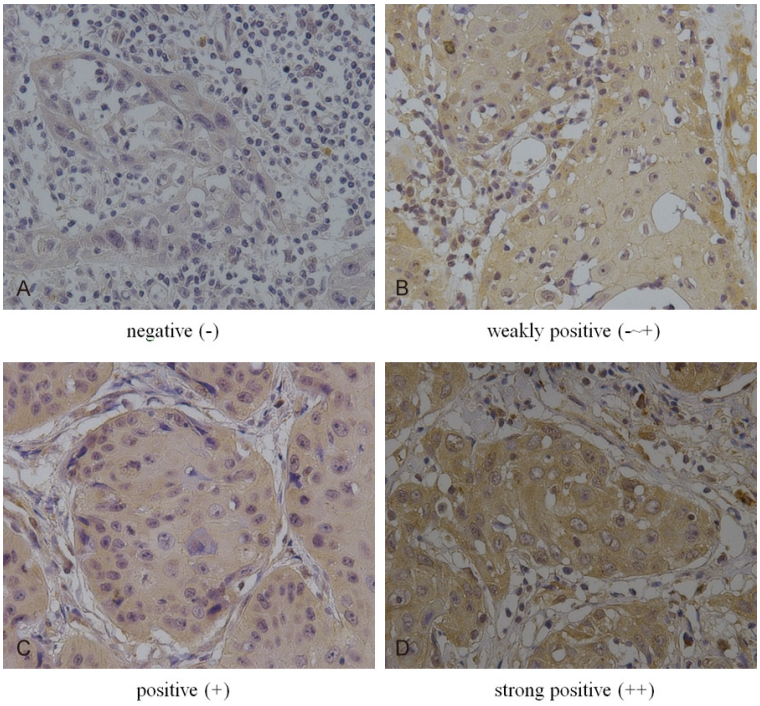

Figure 1. SKA1 expression in OSCC tissues, × 400. A. SKA1 immunoreactivity was negative in OSCC tissue. B. SKA1 positive expression was observed in the cytoplasm and nucleus. C. SKA1 positive expression was mainly observed in the cytoplasm. D. SKA1 positive expression was observed in the cytoplasm and cell membrane.

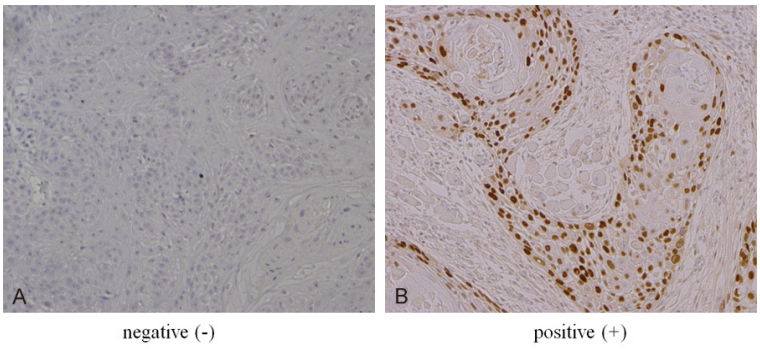

Figure 2. p53 expression in OSCC tissues. Magnification, × 200. A. p53 immunoreactivity was negative in OSCC tissue. B. Positive expression of p53 protein was located in the nucleus of the tumor cells.

Table 2. Relationship between SKA1 and p53 expression in oral squamous cell carcinoma (OSCC)

		P53		Total (n)	P
		+ (n)	- (n)		
SKA1	+ (n)	38	8	46	<0.05
	- (n)	1	10	11	
Total (n)		39	18	57	

Discussion

To date, there are no specific biomarkers for oral cancer. To predict long-term prognosis and define individual treatment modalities for patients with OSCC, extensive studies have focused on the identification of useful biologic and molecular markers in the diagnosis and treatment of OSCC [12, 18, 19]. There is no study demonstrating the altered expression of SKA1 in oral cancer.

Spindle and kinetochore-associated complex subunit 1 (SKA1), a newly discovered gene associated with mitosis [20], has been found to silence the spindle checkpoint [6]. SKA1 is a subtype of the SKA complex that causes spindle microtubules to attach firmly to the kinetochore in mitosis [6, 21, 22]. Overexpression of SKA1 has been found in the malignant progression of several human cancers, such as hepatocellular carcinoma, gastric cancer, prostate cancer, bladder cancer, glioblastoma, and non-small-cell lung cancer [23-28], indicating that SKA1 may be associated with the occurrence and development of oral cancer.

The present study shows that SKA1 is overexpressed in OSCC and is associated with the clinicopathologic features of OSCC. In OSCC, the prognosis largely depends on factors such as smoking, alcohol consumption, medical comorbidity, and in particular, tumor stage [29]. Our study of primary OSCC samples found that patients with higher SKA1 expression were typically in the advanced stage, suggesting that SKA1 may be a new immunohistochemical prognostic marker for human OSCC. The results of our study were consistent with those of previous research [30]. Therefore,

SKA1 assessment may provide a theoretical basis for diagnosis and staging and help to predict the prognosis of oral cancer. In addition, our study found that SKA1 expression was not significantly related to alcohol and tobacco use.

Among the genes related to oral cancer, p53 has been one of the most frequently studied. The corresponding relationship between P53 expression in OSCC and clinicopathologic data remains controversial [31-33]. More than 60% of our OSCC samples were p53 positive which is consistent with the results described in the literature [34]. Our results also clearly showed that p53 expression is significantly correlated with the differentiation grade of OSCC but not with tumor stage or lymph node metastasis. Patients with high p53 expression had poorly differentiated tumors, indicating that p53 may be considered as a marker for the grade of human OSCC.

SKA1 expression correlates with abnormal cell cycle distribution in a variety of tumor cells [23, 26, 28]. Research has also confirmed that p53 plays an important role in tumor cell cycle and apoptosis [13-16]. We found that the protein expression of SKA1 and p53 showed the similar trends in patients grouped by sex, age, tumor size, and TNM stage. There was a significant correlation between SKA1 and p53 expression in OSCC, suggesting that the carcinogenic potential of SKA1 may be related to p53 molecular pathways.

In conclusion, the present study indicates that the SKA1 gene might be involved in the development of OSCC. In future studies, we will increase the sample size and verify whether there is a synergistic carcinogenic mechanism between SKA1 and p53 by molecular biology experiments. With further research, SKA1 may become a molecular target in oral squamous cell carcinoma.

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Peng Sun, Department of Orthodontics, Yantai Stomatological Hospital, No. 142, Beida Street, Zhifu District, Yantai 264001, Shandong, China. Tel: +86-0535-621-7096; E-mail: drsun1983@163.com

References

[1] Khurshid Z, Zafar MS, Khan RS, Najeeb S, Slowey PD and Rehman IU. Chapter two-role of salivary biomarkers in oral cancer detection. In: Makowski GS, editor. Advances in Clinical Chemistry. Elsevier; 2018. pp. 23-70.

[2] Das R, Kundu S, Laskar S, Choudhury Y and Ghosh SK. Assessment of DNA repair susceptibility genes identified by whole exome sequencing in head and neck cancer. DNA Repair (Amst) 2018; 66-67: 50-63.

[3] Rivera C. Essentials of oral cancer. Int J Clin Exp Pathol 2015; 8: 11884-11894.

[4] Chao HX, Poovey CE, Privette AA, Grant GD, Chao HY, Cook JG and Purvis JE. Orchestration of DNA damage checkpoint dynamics across the human cell cycle. Cell Syst 2017; 5: 445-4599, e5.

[5] Pietenpol JA and Stewart ZA. Cell cycle checkpoint signaling: cell cycle arrest versus apoptosis. Toxicology 2002; 181-182: 475-481.

[6] Hanisch A, Sillje HH and Nigg EA. Timely anaphase onset requires a novel spindle and kinetochore complex comprising Ska1 and Ska2. EMBO J 2006; 25: 5504-5515.

[7] Schmidt JC, Arthanari H, Boeszoermenyi A, Dashkevich NM, Wilson-Kubalek EM, Monnier N, Markus M, Oberer M, Milligan RA, Bathe M, Wagner G, Grishchuk EL and Cheeseman IM. The kinetochore-bound Ska1 complex tracks depolymerizing microtubules and binds to curved protofilaments. Dev Cell 2012; 23: 968-980.

[8] Jeyaprakash AA, Santamaria A, Jayachandran U, Chan YW, Benda C, Nigg EA and Conti E. Structural and functional organization of the Ska complex, a key component of the kinetochore-microtubule interface. Mol Cell 2012; 46: 274-286.

[9] Park JE, Song H, Kwon HJ and Jang CY. Ska1 cooperates with DDA3 for spindle dynamics and spindle attachment to kinetochore. Biochem Biophys Res Commun 2016; 470: 586-592.

[10] Monda JK, Whitney IP, Tarasovetc EV, Wilson-Kubalek E, Milligan RA, Grishchuk EL and Cheeseman IM. Microtubule tip tracking by the spindle and kinetochore protein Ska1 requires diverse tubulin-interacting surfaces. Curr Biol 2017; 27: 3666-3675, e6.

[11] Zhao LJ, Yang HL, Li KY, Gao YH, Dong K, Liu ZH, Wang LX and Zhang B. Knockdown of SKA1 gene inhibits cell proliferation and metastasis in human adenoid cystic carcinoma. Biomed Pharmacother 2017; 90: 8-14.

[12] Zhang B, Li KY, Chen HY, Pan SD, Jiang LC, Wu YP and Liu SW. Spindle and kinetochore asso-

ciated complex subunit 1 regulates the proliferation of oral adenosquamous carcinoma CAL-27 cells in vitro. Cancer Cell Int 2013; 13: 83.
[13] Saleem S, Azhar A, Hameed A, Khan MA, Abbasi ZA, Qureshi NR and Ajmal M. P53 (Pro-72Arg) polymorphism associated with the risk of oral squamous cell carcinoma in gutka, niswar and manpuri addicted patients of Pakistan. Oral Oncol 2013; 49: 818-823.
[14] Yeh YT, Yeh H, Su SH, Lin JS, Lee KJ, Shyu HW, Chen ZF, Huang SY and Su SJ. Phenethyl isothiocyanate induces DNA damage-associated G2/M arrest and subsequent apoptosis in oral cancer cells with varying p53 mutations. Free Radic Biol Med 2014; 74: 1-13.
[15] Hong A, Zhang X, Jones D, Veillard AS, Zhang M, Martin A, Lyons JG, Lee CS and Rose B. Relationships between p53 mutation, HPV status and outcome in oropharyngeal squamous cell carcinoma. Radiother Oncol 2016; 118: 342-349.
[16] Yao Y, Zhou WY and He RX. Down-regulation of JMJD5 suppresses metastasis and induces apoptosis in oral squamous cell carcinoma by regulating p53/NF-κB pathway. Biomed Pharmacother 2019; 109: 1994-2004.
[17] Soland TM and Brusevold IJ. Prognostic molecular markers in cancer-quo vadis? Histopathology 2013; 63: 297-308.
[18] Yang X, Song X, Chu W, Li L, Ma L and Wu Y. Clinicopathological characteristics and outcome predictors in squamous cell carcinoma of the maxillary gingiva and hard palate. J Oral Maxillofac Surg 2015; 73: 1429-1436.
[19] Zheng WY, Zhang DT, Yang SY and Li H. Elevated matrix metalloproteinase-9 expression correlates with advanced stages of oral cancer and is linked to poor clinical outcomes. J Oral Maxillofac Surg 2015; 73: 2334-2342.
[20] Sauer G, Korner R, Hanisch A, Ries A, Nigg EA and Sillje HH. Proteome analysis of the human mitotic spindle. Mol Cell Proteomics 2005; 4: 35-43.
[21] Ivashina MV, Arts MJ, Bij de Vaate JG, Bakker R, Iupikov O, Dekker J and van Ardenne A. An axi-symmetric segmented composite ska dish design: performance and production analysis. 2011.
[22] Joglekar AP and DeLuca JG. Chromosome segregation: Ndc80 can carry the load. Curr Biol 2009; 19: R404-R407.
[23] Qin X, Yuan B, Xu X, Huang H and Liu Y. Effects of short interfering RNA-mediated gene silencing of SKA1 on proliferation of hepatocellular carcinoma cells. Scand J Gastroenterol 2013; 48: 1324-1332.
[24] Sun W, Yao L, Jiang B, Guo L and Wang Q. Spindle and kinetochore-associated protein 1 is overexpressed in gastric cancer and modulates cell growth. Mol Cell Biochem 2014; 391: 167-174.
[25] Li J, Xuan JW, Khatamianfar V, Valiyeva F, Moussa M, Sadek A, Yang BB, Dong BJ, Huang YR and Gao WQ. SKA1 over-expression promotes centriole over-duplication, centrosome amplification and prostate tumourigenesis. J Pathol 2014; 234: 178-89.
[26] Tian F, Xing X, Xu F, Cheng W, Zhang Z, Gao J, Ge J and Xie H. Downregulation of SKA1 gene expression inhibits cell growth in human bladder cancer. Cancer Biother Radiopharm 2015; 30: 271-7.
[27] Shi X, Chen X, Peng H, Song E, Zhang T, Zhang J, Li J, Swa H, Li Y, Kim S, Liu X and Zhang C. Lentivirus-mediated silencing of spindle and kinetochore-associated protein 1 inhibits the proliferation and invasion of neuronal glioblastoma cells. Mol Med Rep 2015; 11: 3533-3538.
[28] Shen L, Yang M, Lin Q, Zhang Z, Miao C and Zhu B. SKA1 regulates the metastasis and cisplatin resistance of non-small cell lung cancer. Oncol Rep 2016; 35: 2561-2568.
[29] Sproll CK, Holtmann H, Schorn LK, Jansen TM, Reifenberger J, Boeck I, Rana M, Kübler NR and Lommen J. Mandible handling in surgical treatment of oral squamous cell carcinoma (OSCC): lessons from clinical results after marginal and segmental mandibulectomy. Oral Surg Oral Med Oral Pathol Oral Radiol 2020; 129: 556-564.
[30] Dong C, Wang XL and Ma BL. Expression of spindle and kinetochore-associated protein 1 is associated with poor prognosis in papillary thyroid carcinoma. Dis Markers 2015; 2015: 616541.
[31] Helal Tel A, Fadel MT, El-Thobbani AK and El-Sarhi AM. Immunoexpression of p53 and hMSH2 in oral squamous cell carcinoma and oral dysplastic lesions in Yemen: relationship to oral risk habits and prognostic factors. Oral Oncol 2012; 48: 120-124.
[32] Kang YH, Lee JS, Byun JH, Kim UK and Park BW. Positive expression of NANOG, mutant P53, and CD44 is directly associated with clinicopathological features and poor prognosis of oral squamous cell carcinoma. Int J Oral Maxillofac Surg 2015; 44: e86-e87.
[33] Karpathiou G, Monaya A, Forest F, Froudarakis M, Casteillo F, Marc Dumollard J, Prades JM and Peoc'h M. p16 and p53 expression status in head and neck squamous cell carcinoma: a correlation with histological, histoprognostic and clinical parameters. Pathology 2016; 48: 341-348.
[34] Abrahao AC, Bonelli BV, Nunes FD, Dias EP and Cabral MG. Immunohistochemical expression of p53, p16 and hTERT in oral squamous cell carcinoma and potentially malignant disorders. Braz Oral Res 2011; 25: 34-41.