Immunohistochemical and molecular genetic analysis of p 53 in oral squamous cell carcinoma (scc) in Hospital University Science Malaysia: a preliminary study

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Abstract
The role of p53 mutation in oral cancer cases has not been studied in Malaysia. This study was designed to focus on over expression of p53 nuclear protein and gene mutation in oral squamous cell carcinoma (SCC) cases in Hospital University Science Malaysia (HUSM).
Twenty randomly selected cases previously diagnosed as oral SCC lesions in HUSM since 2002 were included in this study. Patient's folders were reviewed to study clinical history and tumor staging. Extracted DNA amplified using PCR. Gene sequencing had done to detect genetic mutation. Protein overexpression detected using the Streptavidin-biotin Peroxidase technique. P53 gene mutations, analyzed within exons 5- 8, were observed in 10 out of 20 cases (50%) of oral SCC. The positive nuclear staining by immunohistochemical was observed in 18 cases (18/20, 90%). These data indicate the highly incidence of p53 protein overexpression as well as specific mutations in oral SCC patients in Malaysia. However this preliminary study had been based on analysis of relatively few cases of oral squamous cell carcinoma, so further study require to including large number of cases from different states in Malaysia.

Key words:
p53, SCC, immunohistochemical, mutation, PCR

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Introduction

Oral cancer is the 11th most common cancer in the world[1,2] and more than 90% are oral squamous cell carcinoma[3,4]. Human cells become malignant through the activation of oncogenes, which stimulate cell proliferation, inactivation of tumour suppressor genes. DNA repair genes lead to progression genetic instability resulted in uncontrolled growth, and loss of differentiation, invasion and metastasis[1-5]. p53 tumor suppressor gene (TSG), as the guardian of the genome[6] plays two major important roles as a "gatekeeper" in control the pathways of cell proliferation and 'caretaker' in DNA repair and maintain genetic stability. TSG are typically inactivated by gene mutations in one allele, followed by loss of the intact allele during cell replication lead to loss of suppressor function[1]. Thus, p53 mutation appears to be the most frequent genetic event[7,8]. According to the p53 mutation database R10 (July, 2005), 21,587 somatic mutations and 283 germline mutations have been reported[9]. As far as we are aware, there are no documented publications available for p53 mutation in the oral carcinogenesis in Malaysia. The aim of this study is to detect the p53 genetic alteration in the conserved region of the P53 gene in OSCC patients by polymerase chain reaction (PCR) and direct sequencing as well as to evaluate the over expression of p53 in oral SCC using immunohistochemical test.

Materials and Methods

Patients and study design

Twenty cases were randomly selected from the list of oral cancer patient in the DNA library /Human Genome Center/ University Science Malaysia (USM). For each case, a pair of tumor and normal adjacent non-tumor tissue samples were surgically dissected into small pieces, frozen immediately in liquid nitrogen and stored at -80 ^{0}C. Whole surgically removed samples were sent to the department of pathology for histopathological diagnosis. Histopathological changes were categorized as well differentiated squamous cell carcinoma, moderate differentiated squamous cell carcinoma and poor differentiated squamous cell carcinoma. The inclusive criteria were primary tumor diagnosed as Oral Squamous Cell Carcinoma under International Classification of Disease, ICD-10 (C00-C04) or ICD-9 (140.0-141.9, 143.0-144.9). On the other hand, the exclusive criteria were past history of cancer at other site of the body. This study was approved by the Ethical and Research Committee of University Science Malaysia. Patients already signed inform consent form prepared previously by the Malaysian National Oral Cancer Group. Data collection was done from the patient folders at record unit, Hospital USM.

Immunohistochemical staining

Immunohistochemical staining was performed according to strept-avidin-biotin peroxidase complex (ABC) method[10]. Briefly, 4 ìm sections from paraffin embedded specimens were deparafffinized with xylene and rehydrated with graded ethanol. Epitope retrieval (antigen retrieval) was done by incubating the sections in 10mM sodium citrate buffer (pH 6.0) for 20 minutes in a conventional microwave-oven. The endogenous peroxidase activity was blocked by immersing the sections in methanol with 0.3% hydrogen peroxide. Mouse Anti-Human p53 Protein (DO-7) (Dakocytomation, Cat# M7001) diluted 1:300 in primary antibody dilution buffer. Sections were incubated with diluted primary antibody overnight at 4 ^{0}C, followed by incubation with a biotinylated anti-mouse secondary antibody and streptoavidin-biotin-peroxidase complex (labeled Streptavidin Biotin Kit, Dako). The peroxidase reaction was developed using diaminobenzidine (DAB) as a chromogen. The sections were counter-stained with hematoxylin solution. For negative controls, primary antibody was replaced with 0.05M Tris - HCL buffer solution (PH 7.6). The slides were then viewed using a ZEISS microscope with an Image Analyzer. Immunostaining for p53 positive cases were graded as negative (-) no nuclear staining, mild positive (+) if fewer than 10% of nuclear were stained, or moderate positive (++) if more than 10% of nuclear were stained and strong positive (+++) if more than 50% of the nuclear were stained.

Table 1 - Description of the primers used for p53 exons 4, 5-6, 7 and 8

Exon	Codon	Sequence of primers (5'-3')	Amplimer size (base pairs)	Melting Temperature (T_m) (^{o}C)
4	33-125	Forward: TGACTGCTCTTTTCACCCAT Reverse: GGAAGCCAGCCCCTCAGGGC	332	64
5-6	126-186, 187-224	Forward: AACTCTGTCTCCTTCCTCTT Reverse: GGAGGGCCACTGACAACCA	466	60
7	225-260	Forward: GTGTTATCTCCTAGGTTGGC Reverse: GTGTGCAGGGTGGCAAGTGG	152	63
8	261-306	Forward: AGGACCTGATTTCCTTACTG Reverse: TCCACCGCTTCTTGTCCTGCT	216	62

DNA Extraction

Single-strand conformation polymorphism analysis was used to analyze all tumor samples for mutations within exons 5-8 of the p53 gene. Cases, which were displaying an altered electrophoretic mobility, were re-amplified in another separate reaction. High molecular weight DNA was purified by digestion with proteinase K and extraction in phenol-chloroform. The primers used for exon 5-8of p53 showed in Table 1. T_m was calculated using the formula, T_m = [4(G+C)+2(A+T)] ^{0C}. DNA samples (100 ng) were subjected to PCR in a mixture (10 ìl) using two appropriate oligonucleotides as primers as described previously. The PCR mixture heated to 95°C with an equal volume of formamide dye mixture (95% formamide, 0.05% bromophenol blue, 0.05% xylene cyanol, 20mM EDTA); 2ìl of the preparation was applied to a 6% polyacrylamide gel, both with and without 10% glycerol. Electrophoresis was performed at 70W for 1.5 hour. The gel was analysed under UV light using Chemilmager Analyzer 4400 (AlphaInnotech Corp., USA). DNA purification was done using Wizard® SV Gel and PCR Clean-up System (Promega, USA) according the protocol provided by the manufacturer. It involves three main steps, which are binding of DNA, Washing and Elution. The purified PCR product (DNA) in the collection tube was stored at -20°C. The concentration of purified PCR product measured at 260nm using spectrophotometer (Eppendorf BioPhotometer, USA). Direct DNA sequencing was done. The PCR was treated with 1 ì g of genomic DNA, 200 ng of each primer, 200 ìM dNTPs, 1 X PCR reaction buffer, 2.5 U Taq polymerase. Aliquots of PCR amplified mixtures diluted with 2ml of distilled water and spun in a centricon 30 micro-concentrator to remove the excess primers and dNTPs. DNA was then resuspended in 50 ìl of 10 mM Tris pH 8.0 and 1 mM EDTA and direct sequencing were done followed the instructions of the Promega fmol ™ DNA sequencing system technical manual. The sequencing results were analysed. Obtained results were BLAST by align each sequence with the p53 sequence (code 35213) at the gene bank at http://www.ncbi.nlm.nih.gov/BLAST/Blast.cgi, Align two sequences. The results were further analyzed using DNA software Chromas Version 1.62 (32-bit). The codon and amino acid was check by referred to the p53 CODING SEQUENCE from IARC TP53 DATABASE - TP53 coding sequence, www.iarc.fr/p53. The presented mutation was confirmed by comparing with the International Agency for Research on Cancer (IARC) TP53 Database which is a database consisting of the p53 mutation reported in the world. The reference to other cancer was done when no cases was found to be compatible with the result in head and neck cancer.

Results

Population characteristic and clinical parameters

Archival representative blocks from the primary tumours were available for 20 patients included in this study. The demographic data and review of all patients' folders are shown in Table 2**.** The study included 14 male and 6 female. The patient races were 14 Malay, 4 Chinese and 2 Indian. The mean age (rang 41- 81 years) is 60.8±13.1 years. 90% of the patients had exposed to at least 1 of the risk factors for oral cancer**.** 80% (16/20) of the patients smoked, 15% (3/20) chewed betel quid and 5 % (1/20) were alcohol user. The patients included in this study showed considerable variation in histopathological appearances of oral SCC, according to their histopathological features as follow: ten patients showed typical features of well differentiated squamous cell carcinoma, eight patients showed histopathological features of Moderate differentiated squamous cell carcinoma and only two patients showed histopathological features of poor differentiated squamous cell carcinoma.

Table 2 - Clinical parameters of patients with OSCC (n =20)

Clinical parameters	Criteria's
Age (Mean±SD)(Year)	60.8±13.1
Age Rang	41-81
Sex (n%)	
Male	14 (70%)
Female	6 (30%)
Site of the primary tumour (n%)	
Tongue	8 (40%)
Buccal mucosa	8 (40%)
Floor of the mouth(FOM)	4 (20%)
Clinical staging (n %)	
Stag IV	20 (100%)
Risk Factors (n %)	
Cigarette smoker	16 (80%)
Betal Quid chewer	3 (15%)
Alcohol drinker	1 (5%)
Histopathological grading (n %)	
Well differentiated SCC	10 (50%)
Moderate differentiated SCC	8 (40%)
Poor differentiated SCC	2 (10%)

Immunohistochemical analysis

p53 positive immunoreactivity reaction was indicated by brown nuclear staining. Eighteen cases were revealed positive over expression reaction for p53 (Fig. 1 A, B). Thirteen patients of these cases revealed mild positive reaction for p53 and only two cases showed strong positive reaction, which appeared as dark, brownish nuclear stained. While three cases showed moderate reaction. Two cases were negative for p53. All negative control sections revealed no brown colour stain and the nucleus were stained with the haematoxylin counter stain. Clinico-pathological parameters in relation to the over expression of p53 are summarized in table 3.

Table 3 - Clinical parameters, histopathological (HP) and immunohistochemical (IHC) analysis of OSCC patients (FOM= floor of mouth, PD = poor differentiated, WD = well differentiated, MD = moderate differentiated), (Race: M = Malay, C = Chinese, I = Indian), Mutations occurring (MO) NO mutation detected (NMD)

Case No.	Sex	Race	Age/ year	smoking	BQ chewing	Alcohol drinker	Site	HP	IHC	MO
1	F	M	81	+	+	-	FOM	PD	+	Arg/Arg
2	F	I	41	+	+	-	FOM	WD	++	Arg/Arg
3	M	C	58	+	-	-	Tongue	MD	-	NMD
4	M	M	65	+	-	-	Tongue	MD	++	Arg/Arg
5	M	M	65	+	-	-	Tongue	WD	+	Arg/Arg
6	M	M	75	+	-	-	Tongue	WD	-	NMD
7	M	M	48	+	-	-	Gum	MD	+++	NMD
8	M	M	59	+	-	-	Gum	MD	+	Arg/Arg
9	F	C	81	+	-	-	FOM	PD	+	ND
10	M	C	59	-	-	+	Tongue	MD	+	Arg/Pro
11	M	M	48	+	-	-	Tongue	WD	+	Arg/Arg
12	M	M	75	+	-	-	Tongue	MD	+	Arg/Arg
13	M	M	41	+	-	-	Tongue	WD	+	NMD
14	M	I	48	+	+	-	Gum	WD	+	NMD
15	F	C	70	+	-	-	Gum	WD	++	NMD
16	F	M	70	+	-	-	Gum	MD	+	Arg/Arg
17	M	M	58	+	-	-	FOM	MD	+	NMD
18	F	M	41	-	-	-	Gum	WD	+++	Arg/Arg
19	M	M	70	-	-	-	Gum	WD	+	Arg/Arg
20	M	M	70	-	-	-	Gum	WD	+	NMD

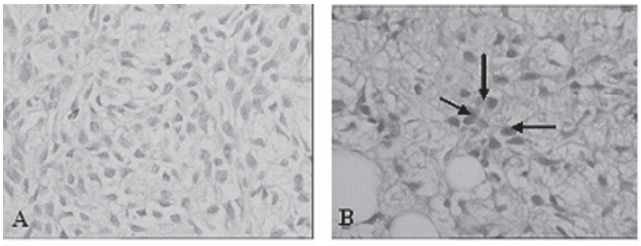

Fig. 1 - *Photomicrograph of p53 immunoreactivity in OSCC: (A) the negative control, No brown stain in the nuclei (X400); (B) moderate positive reaction (++) of the squamous cells stained with Peroxidase (arrows) (X400)*

Molecular genetic analysis

Eighteen p53 mutations were occurring in 10 OSCC patient's tumour tissue samples (50%) in exons 5-8. A total of 18 point mutations occurred in 10 out of 20 cases (50%). All the mutations were point mutation which presented as 11 missense mutation (61.11%), 5 nonsense mutation (27.77%) and 2 silent mutation (11.11%). Table 4showed the mutations occur in exon 5-8. Figure.2 shows an electropherogram for confirmation of the amplified PCR product (Amplier). BLAST result of case 1 represented in Figure.3 revealed point mutation at CPG site in codon 306, exon 8. Figure.4 represented the electropherogram of selected cases (7 and 8) showing a wild type genotype (relatively common genotype) of the same codon in case 7 and missense point mutation,in case 8 which showed a transition of C to T resulted in a change of amino acid from Proline (Pro) to Serine (Ser) at codon 278.

Discussion

Oncogenes alone are not sufficient to cause oral cancer and

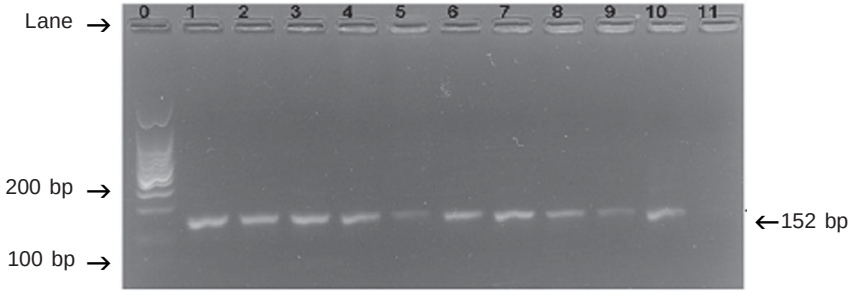

Fig. 2 - *Electropherogram for confirmation of the amplified PCR product (Amplier). Electrophoresis 2% agarose gel for PCR product of exon 7 with 152 basepairs. Lane 0 shoes the DNA ladder (DNA molecular weight marker). Lanes 1 to 10 are the PCR products from OSCC patient for exon 7. Lane 11 is a negative control, which the DNA template is replaced by nucleus free water preparation of master mix.*

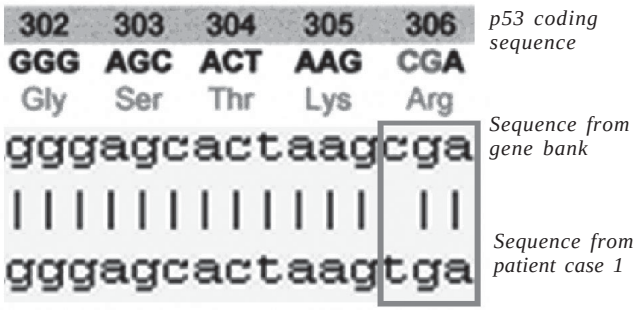

Fig. 3 - *Point mutation at CpG site in codon 306, exon 8. Blast result of case 1 showing point mutation at CpG site in codon 306, exon 8*

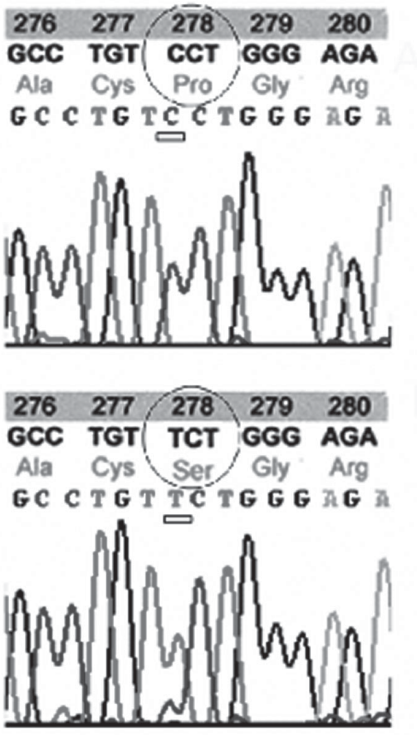

Fig. 4 - *Electropherograms showing the wild type and missense point mutation in codon 278: A – Electropherogram of case 7 showing a wild type genotype (relatively common genotype) of the same codon; B - Electropherogram of case 8 showing missense point mutation, which is a transition C'!T result in a change of amino acid from Proline (Pro) to Serine (Ser) at codon 278*

appear to be initiators of the process. The crucial event in the transformation of a premalignant cell to a malignant cell is inactivation of cellular negative regulators—tumor suppressor genes—and is regarded to be a major event leading to the development of malignancy. Tumor suppressor genes are most often inactivated by point mutations, deletions, and rearrangements in both gene copies[11,12]. The importance of the p53 tumor suppressor gene in the process of carcinogenesis is well established. The p53 protein blocks cell division at the G1 to S boundary, stimulates DNA repair after DNA damage, and also induces apoptosis. These functions are achieved by the ability of p53 to modulate the expression of several genes[13,14]. The p53 protein transcriptionally activates the production of the p21 protein, encoded by the WAF1/CIP gene, p21 being an inhibitor of cyclin and cyclin dependant kinase complexes[15]. p21 transcription is activated by wild-type p53 but not mutant p53[16]. However, WAF1/CIP expression is also induced by p53 independent pathways such as growth factors, including platelet derived growth factor, fibroblast growth factor, and transforming growth factor ß[17]. Wild-type p53 has a very short half life (four to five minutes)[18], whereas mutant forms of protein are more stable, with a six hour half life[19].

In the developed world, about 30% of all oral cancer affects the lip and has a more clearly defined aetiology (mainly exposure to sunlight) and a better prognosis than intra-oral cancers. In this study, the entire patient's lesion is from intra oral, which include tongue, buccal mucosa and floor of the mouth. All patients presented with advance clinical staging (Stage IV). This clinical presentation for the patient in this study is compatible with previous observation described by other researchers. They found that most mouth cancers are asymptomatic or presented with minimal pain at early stages[20], which lead to delay in seeking professional care[4]. In this study 18/20 patients were presented with nodal metastasis, which revealed higher frequency than previous studies reported in literature[20].The results of this study showed that patients had exposed to at least 1 of the risk factors for oral cancer. 80% (16/20) of the patients smoked, 15% (3/20) chewed betal quid and 5% (1/20) were user of alcohol. These suggest that smoking and betal quid chewed play an important role in the development of oral cancer. The results of this study are compatible with others. Other studies explained that specific risk factors, i.e. tobacco, betel quid and alcohol may contain or are metabolized to several known carcinogens, which are extremely important in oral mutagenesis[3]. Other study found that the primary cause of high incidence in Asians is the widespread habit of chewing betel quid, deficiency of antioxidant micronutrients such as P carotene and vitamin C and some viruses, such as HPV (types 16 & 18)[20-48]. Most population of this study showed low prevalence of drinking habit due to religion reason.

There are totally eighteen p53 mutations occurred in 10 OSCC patient's tumour tissue, (50%) (10/20patients) with point mutation, occur in exon5-8, with predominance in exon 8. This percentage (50%) of mutation is relatively compatible with the results found previously by other researcher[21], they found p53 mutations in 42% of the patients (54 of 129). All the mutations have been reported by other studies. Eight out of 18 mutations have been observed in previous studies in head and neck cancer[22-34]. The other 4 was observing in studies of gliomas, keratosis, ulcerative colitis and acitinic keratoses[35]. This result suggests that the mutation occur is site specific. The most prevalent mutation found in this study was C to T transition, which occurred in 6 of 18 mutations

Table 4 - Mutation of p53 gene from OSCC tumour samples

Cases No	Codon72 polymorphism	Exon	Codon	WT Codon	MUT Codon	Event	Type	CPG	WT AA	MUT AA	Types of AA Changes
1	Arg/Arg	7	233	CAC	AAC	C?A	Tv	No	His	ASN	MS
		8	306	CGA	TGA	C?T	TS	Yes	Arg	Stop	NS
2	Arg/Arg	6	198	GAA	TAA	C?T	Tv	No	GLU	Stop	NS
		8	301	CCA	TAA	C?T	Ts	No	Pro	SER	MS
4	Arg/Arg	8	262	GCT	GGC	T? C	Ts	No	GLY	GLY	SM
5	Arg/Arg	5	134	TTT	ATT	T?A	Tv	No	Phe	LIE	MS
		8	266	GAA	TAA	G ?T	Tv	No	GLU	Stop	NS
8	Arg/Arg	5	134	CCT	ATT	T?A	Tv	No	Phe	Lie	MS
		8	278		TCT	C?T	TS	No	Pro	Ser	MS
11	Arg/Pro	5	141	CAG	CCG	A?C	Tv	No	Cys	Phe	MS
12	Arg/Arg	6	196	GAA	TAA	G?T	Tv	No	Glu	Stop	NS
		8	273	CCA	TCA	G?T	Ts	No	Pro	Ser	MS
16	Arg/Arg	8	273	CGT	TGT	C?T	TS	No	Arg	Cys	SM
		5	144	TTT	ATT	T?A	Tv	No	Phe	LIe	MS
18	Arg/Arg	5	134	TTT	ATT	T?A	Tv	No	Phe	LIe	MS
		8	278	CCT	TCT	C?T	TS	No	Pro	Ser	MS
19	Arg/Arg	5	154	TTT	ATT	T?A	Tv	No	Phe	LIE	MS
		8	266	GGA	TTA	G ?T	Tv	No	Glu	Stop	NS

(33.33%). The mutation in codon 306 was at the CpG site, where cytosines that were located next to a guanine (5'-CpG-3')[36]. C>T mutations occur frequently at CpG sites by endogenous mechanisms, which were hydrolytic deamination and enzymatic deamination. Other studies showed that many of the genes associated with methylated CpG islands could be reactivated in cell lines by experimental demethylation using 50-aza-20 deoxycytidine[36]. Codon 278 is one of the residual conserved areas for p53 mutation. There are no direct structural explanations for many of these, one can assume that they are conserved throughout evolution for a good reason and, in the case of surface residues; this is likely to be that the amino acid is critical for interactions with other proteins[37]. Regarding codon 72 polymorphism, the Arg/Arg, Arg/Pro and Pro/Pro genotype frequencies were 70%, 20 and 10% respectively. The Arg/Arg genotype frequency is high compare to previous studies reported as 52 % in SCC head and neck (SCCHN) patient and 52.6 % for the control group as reported previously[38] and 52% in SCCHN and 50% in control group. This might suggest that Arg/Arg have role in oral carcinogenesis in advance stage. This is contradicting with others[39]. This suggests that arginine allele appears to protect against head and neck cancer. However, several studies show that p53 Arg/Arg variant individuals were seven times more susceptible to develop human papilloma virus (HPV) associated cervical cancer but both confirm and contradicted by several groups[39]. Studies in OSCC or SCCHN suggested that there was no difference in the distributions of p53 codon 72 genotypes between cases and controls but Pro allele was associated with an early onset of cancer[38],

which is contradict with the pro allele patient in this study who have late onset of cancer.

p53 protein expression as detected by immunohistochemical does not always reflect the presence of mutant p53 protein, and neither does the absence of p53 staining preclude it . In this study 18 cases showed positive p53 immunoreactivity and only 2 cases were negative reaction. All cases with gene mutation showed p53 protein overexpression. This showed there is some good relationship between immunohistochemical detection of p53 protein and the presence of mutations within the p53 gene. On the other hand, 8 cases revealed positive reaction for p53 protein immunoperoxidase overexpression with no gene mutation detection. There is also controversy about the relation between p53 mutation and detection of the p53 protein by immunocytochemical. Some authors have suggested a high correlation between p53 expression and point missense mutation[40], whereas others have reported some discrepancy in oral cancer, with lack of expression demonstrated by immunocytochemical having been attributed to insensitive methods of detecting p53 mutation, or the existence of truncating mutations that result in the absence of protein[41-43]. However, stabilisation of p53 and detection by immunocytochemical might not necessarily be the result of mutation. In Li-Fraumeni syndrome, p53 is mutant but the protein is unstable, like the wild-type p53 protein, which suggests that some other event may be necessary for stabilization , and that stability of p53 is not intrinsic to the mutant p53 structure, but might vary in different cell backgrounds[43,44]. In this study 2 cases were negative for p53 immunoreactivity. It is used base on the principle that normal p53 protein only has half life of 15 to 20 min and the mutated p53 have a longer half life[45]. The short half life is control by feedback loop. The p53 protein bind to MDM2 gene to stimulate the MDM2 protein synthesis, and the MDM2 protein will bind to p53 protein and stimulates the addition of ubiquitin groups to the protein and cause it degradation[46]. This is the reason for p53 protein to undergo stabilization without any mutation after exposure to DNA damage agent or functional inactivation by the binding of the p53 protein to other viral or cellular proteins. Where the feedback loop is diminish and cause accumulation of p53 protein. In addition immunohistochemical has a number of disadvantages, including the fact that any mutations that abolish p53 expression (splicing signal mutations, nonsense mutations, insertions or deletions) do not produce the protein and therefore give a negative result. In conclusion this study revealed high prevalence of p53 mutation in the OSCC patient with advance clinical stage with good relationship to the immunohistochemical result, which revealed high prevalence of p53 protein overexpression.

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References

1. Parkin DM, Freddie Bray MD, Ferlay J, Paola Pisani. Global cancer statistics, 2002, CA Cancer J Clin. 2005; 55: 74–108.
2. Stewart BW, Kleihues P, editors. Head and neck cancer. World Cancer Report, International Agency for Research on Cancer, IARC Press. Lyon. 2003; 83-126, 172-174, 232-6.
3. Scully C, Newman L, Bagan JV. the role of the dental team in preventing and diagnosing cancer: 2. Oral cancer risk factors. Dent Update. 2005; 32: 261-76.
4. Neville BW, Damm DD, Allen CM, Bouquot JE. Epithelial pathology, squamous cell carcinoma. Oral Maxillof Pathol. 2002; 10: 356-67.
5. Vogelstein B, Lane D, Levine AJ. Surfing the p53 network. Nature. 2000; 408; 307-10.
6. Lane DP. p53, guardian of the genome. Nature. 1992; 358: 15-6.
7. Soussi T. p53 protein biological and clinical aspects. Encycl Ref Cancer. 2001 Springer; 669-75.
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. oral oncology. Eur J Cancer. 1996; 32B: 143-9.
9. Soussi T. The TP 53 website. 2005. Available from: URL: http://p53.free.fr [2007 Dec 15].
10. Mao C, Lu Y, Lai Q, Xia Y, Yang C. Expression of p53 gene in oral squamous cell carcinoma and its relation with clinical and pathological parameters and prognosis of patients. Chin Med Sci J. 1995; 10: 199-203.
11. Tang R, Wang PF, Wang JY, Hsieh LL. Mutations of p53 gene in human colorectal cancer: distinct frame shifts among populations. Int J Cancer. 2001; 91: 863-8.
12. Lee WH. Tumour suppressor genes—the hope. FASEB J. 1993; 7: 819.
13. Yokota J, Sugimura T. Multiple steps in carcinogenesis involving alterations of multiple tumour suppress genes. FASEB J. 1993; 7: 920-5.
14. Vogelstein B, Kinzier KW. The multistep nature of cancer. Trends Genet. 1993; 9: 138–41.
15. Harper JW, Adamy GR, Wei N, Keyomarsi K, Elledge SJ. The p21 cdk-interacting protein Cip1 is a potent inhibitor of G1 cyclin-dependent kinases. Cell. 1993; 75: 805–16.
16. El-Deiry WS, Tokino T, Velculesco VE, Levy DB, Parsons R, Trent JM, et al. WAF1. A potential mediator of p53 tumour suppression. Cell. 1993; 75: 817–25.
17. Datto MB, Li Y, Panus JF. Transforming growth factor induces the cyclin-dependent kinase inhibitor p21 through a p53-independent mechanism. Proc Natl Acad Sci USA. 1995; 92: 5545–9.
18. Thomas R, Kaplan L, Reich N, Lane DP, Levine AJ. Characterization of human p53 antigens employing primate specific monoclonal antibodies. Virology. 1983; 131: 502–17.
19. 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.
20. Warnakulasuriya S. Cancers of the oral cavity and pharynx. The genetic basic of human cancer. 2nd ed. Mc Graw Hill; 2002. p. 773-84.

21. Brennan JA, Boyle JO, Koch WM, Goodman SN, Hruban RH, Eby YJ, et al. Association between cigarette smoking and mutation of the p53 gene in squamous-cell carcinoma of the head and neck. New Engl J Med.1995; 332: 712-7.
22. Temam S, Flahault A, Perie S, Monceaux G, Coulet F, Callard P, et al. p53 gene status as a predictor of tumor response to induction chemotherapy of patients with loco regionally advanced squamous cell carcinomas of the head and neck. J Clin Oncol. 2000: 18; 385-94.
23. Balz V, Scheckenbach K, Gotte K, Bockmuhl U, Petersen I, Bier H. Is the p53 inactivation frequency in squamous cell carcinomas of the head and neck underestimated? Cancer Res. 2003; 63: 1188-91.
24. Boyle JO, Hakim J, Koch W, Vanderriet P, Hruban RH, Roa RA, et al. The incidence of p53 mutations increases with progression of head and neck cancer. Cancer Res. 1993: 53; 4477-80.
25. van Rensburg EJ, Engelbrecht S, vanHeerden WFP, Kotze MJ, Raubenheimer EJ. Detection of p53 gene mutations in oral squamous cell carcinomas of a black African population sample. Human Mutat. 1998; 11: 39-44.
26. Partridge M, Li SR, Pateromichelakis S, Francis R, Phillips E, Huang XH, et al. Detection of minimal residual cancer to investigate why oral tumors recur despite seemingly adequate treatment. Clin Cancer Res. 2000; 62: 718-25.
27. van Oijen MG, Leppers Vd Straat FG, Tilanus MG, Slootweg PJ. The origins of multiple squamous cell carcinomas in the aerodigestive tract. Cancer. 2000; 88: 884-93.
28. Kropveld A, van Mansfeld ADM, Nabben N, Hordijk GJ, Slootweg PJ. Discordance of p53 status in matched primary tumours and metastases in head and neck. oral oncology. Eur J Cancer. 19963; 2B: 388-93.
29. Cabelguenne A, Blons H, de Waziers I, Carnot F, Houllier AM, Soussi T, et al. p53 alterations predict tumor response to neoadjuvant chemotherapy in head and neck squamous cell carcinoma: a prospective series. J Clin Oncol. 2000; 18: 1465-73.
30. Nylander K, Schildt EB, Eriksson M, Magnusson A, Mehle C, Roos GA. Non-random deletion in the p53 gene in oral squamous cell carcinoma. Br J Cancer. 1996; 73: 1381-6.
31. Ibrahim SO, Vasstrand EN, Johannessen AC, Idris AM, Magnusson B, Nilsen R, et al. Mutations of the p53 gene in oral squamous-cell carcinomas from Sudanese dippers of nitrosamine-rich toombak and non- snuff-dippers from the Sudan and Scandinavia. Int J Cancer. 1999; 81: 527-34.
32. Hsieh LL, Wang PF, Chen IH, Liao CT, Wang HM, Chen MC, et al. Characteristics of mutations in the p53 gene in oral squamous cell carcinoma associated with betel quid chewing and cigarette smoking in Taiwanese. Carcinogenesis. 2001; 22: 1497-503.
33. Yamazaki Y, Chiba I, Hirai A, Notani K, Kashiwazaki H, Tei K, et al. Radioresistance in oral squamous cell carcinoma with p53 DNA contact mutation. Am J Clin Oncol. 2003; 26: 24-9.
34. Yamazaki Y, Chiba I, Hirai A, Sugiura C, Notani K, Kashiwazaki H, et al. Specific p53 mutations predict poor prognosis in oral squamous cell carcinoma. Oral Oncol. 2003; 39: 163-9.
35. Kyritsis A P, Bondy M L, Xiao M. Germline p53 gene mutations in subsets of glioma patients. J Natl Cancer Inst. 1994; 86: 344–9.
36. Plass C, Methylation. Encycl Ref Cancer. 2001 Springer; 561.
37. Andrew CR, Martin AMF, Alison L, Tina HB, Hainaut P, Janet MT. Integrating mutation data and structural analysis of the p53 tumour-suppressor protein. Human Mutat. 2002; 19: 149-64.
38. Shena H, Zhenga Y, Sturgisa EM, Spitza MR, Weia Q. P53 codon 72 polymorphism and risk of squamous cell carcinoma of the head and neck: a case-control study. Cancer Letters. 2002; 183: 123–30.
39. Cortezzi SS, Provazzi PJ, Sobrinho JS, Prado JCM, Reis PMP, Freitas SEN, et al. Analysis of human papillomavirus prevalence and TP53 polymorphism in head and neck squamous cell carcinoma. Cancer Genet Cytogenet. 2004; 150: 44-9.
40. Varley JM, Brammar WJ, Lane DP. Loss of chromosome 17p 13 sequences and mutation of p53 in human breast carcinomas. Oncogene. 1991; 6: 413–2
41. Ahomadegbe JC, Barrois M, Fogel S. High incidence of p53 alterations (mutation, deletion, overexpression) in head and neck primary tumours and metastases; absence of correlation with clinical outcome. Frequent protein overexpression in normal epithelium and in early non-invasive lesions. Oncogene. 1995; 10: 1217–27.
42. Xu L, Chen Y-T, Huvos AG. Overexpression of p53 in squamous cell carcinoma of head and neck without apparent gene mutations. Diagn Mol Pathol. 1994; 3: 83–92
43. Partridge M, Kiguwa S, Emilion G. New insights into p53 protein stabilisation in oral squamous cell carcinoma. Eur J Cancer B Oral Oncol. 1999; 35: 45–55.
44. Midgeley CA, Lane DP. p53 protein stability in tumour cells is not determined by mutation but is dependent on mdm2 binding. Oncogene. 1997; 15: 1179–89.
45. Soussi T. p53 protein, biological and clinical aspects. Encycl Ref Cancer. 2001 Springer; 669-75.
46. Vogelstein B, Lane D, Levine AJ. Surfing the p53 network. Nature. 2000; 408: 307-10.
47. Keohavonga P, Gaoa WM, Hussam HM, Amal KS, Mona FM. Analysis of p53 mutations in cells taken from paraffin-embedded tissue sections of ductal carcinoma in situ and atypical ductal hyperplasia of the breast. Cancer Letters. 2004; 212: 121–30.
48. Scully C. Clinical review, ABC of oral health: Oral cancer. Br Med J. 2000; 321: 97-100.