P53/MDM2 CO-EXPRESSION CORRELATES WITH THE TUMOUR DIFFERENTIATION IN ORAL SQUAMOUS CELL CARCINOMA – A RETROSPECTIVE STUDY AND A SYSTEMATIC REVIEW

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Original Article

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ABSTRACT

Background: MDM2 and p53 are involved in a negative feedback loop where p53 regulates *MDM2* at the transcriptional level. MDM2, in turn, down-regulates p53. This co-ordinated interaction between these proteins is set to play an important role in the regulation of cell cycle progression following DNA damage to cells. The over-expression of both p53 and MDM2 has been reported in various cancers. However there are only few studies discussing the co-expression of MDM2 with p53 in oral squamous cell carcinoma

Aim: The purpose of this study was to determine the correlation of co-expression of p53, MDM2, and Ki-67 proteins with clinico-pathological factors in oral squamous cell carcinoma (OSCC) and to conduct a systematic review of the co-expression of p53/MDM2.

Method: This is a retrospective descriptive study and a systematic review. Formalin-fixed paraffin-embedded tissues from 45 OSCC cases were stained by immunohistochemistry (IHC) for p53, MDM2, and Ki-67 proteins.

Results: Immuno-reactivity for p53, MDM2, and Ki-67 was seen in 75.6%, 97.8%, and 62.2% cases of OSCC respectively. The co-expression of p53 and MDM2 (p53/MDM2) was detected in 97.1%, however there was no significant correlation between p53 and MDM2 expression. Notably, p53/MDM2 co-expression was significantly associated with tumour differentiation (*p*-value = 0.045). The Ki-67LI was not significantly associated with neither MDM2 nor p53/MDM2 co-expression (*p*-value = 0.268, 0.916 respectively).

Conclusion: The expression of MDM2 was not significantly associated with p53 expression suggesting that MDM2 expression is mediated by p53-independent pathways or mutated p53 could not induce the expression of MDM2 in this set of OSCCs. The only clinico-pathological parameter that correlates significantly with co-expression of p53/MDM2 is tumour differentiation where it is suggestive that the co-expression of these 2 proteins is indicative of aggressive tumour behavior.

Key words: Oral squamous cell carcinoma, MDM2, p53, Ki-67, immunohistochemistry, systematic review

INTRODUCTION

The National Cancer Registry (NCR 2006) in Peninsula Malaysia recorded oral cancers separately into three different segments that include the lip, mouth and tongue cancers. Among the males, tongue and mouth cancers were ranked 21st and 26th while among the females they were ranked 25th and 19th of all cancers respectively. However, when both mouth and tongue cancers were grouped together, cancer of the oral cavity made up about 1.8% of all cancers and was the 17th most common cancers among males and 15th most common cancers among the females (1).

p53 is a tumour suppressor gene (TSG) which plays a key role in the control of cell cycle, maintenance of genomic stability, cellular differentiation and apoptosis (2). In the case of DNA damage, p53 blocks cell cycle to allow for DNA repair (3). The expression of wild-type p53 protein is undetectable due to its short half-life. However, once mutated; its half-life increases and the protein can be detected by immunohistochemistry (IHC). Therefore, it was suggested that over-expression of p53 protein is indicative of the presence of p53 mutation (4).

The p53 protein can induce the transcription of the human homologue of murine double minute 2 oncogene (*MDM2*) (5). MDM2 and p53 are involved in a negative feedback loop where p53 regulates *MDM2* at the transcriptional level (5, 6). MDM2, in turn, down-regulates p53. This co-ordinated interaction between these proteins is set to play an important role in regulation of cell cycle progression following DNA damage to cells (6, 7). MDM2 can binds to both wild-

type and mutated p53 to form a complex, thus inhibiting the transcriptional activation function of p53 (8, 9). MDM2 inhibits wild-type p53-mediated G1 arrest and apoptosis (10, 11) and also plays a role in promoting S phase entry in the p53-independent pathway (12) thus driving cellular proliferation.

The over-expression of both p53 and MDM2 has been reported in various cancers such as melanomas (13), bladder cancer (14), osteosarcomas (15) and oral squamous cell carcinomas (OSCC) (2, 16-18).

Ki-67 antibody recognizes a cell nucleus antigen which is expressed maximally in the G2 and M phases of cell cycle and thus reflects proliferation (19). Therefore Ki-67 is used as a cellular proliferation marker.

Although there are many studies about p53 expression and OSCC, there are only few studies discussing the co-expression of MDM2 with p53 or Ki-67. Therefore, in this study, IHC was used to investigate the co-expression of p53 and MDM2 along with Ki-67 labelling index (Ki-67LI) in OSCC, and further evaluated the correlation between the over-expression of these proteins either individually or in combination, (p53/MDM2/Ki-67LI) with the demographic and clinico-pathological characteristics in OSCC patients.

Further, we conducted a systematic review of the English language literature to review all studies that have evaluated the correlation between the co-expression of p53/MDM2 with demographic and clinico-pathological characteristics in OSCC.

MATERIALS AND METHODS

Samples:

Forty-five formalin-fixed, paraffin embedded (FFPE) tissue samples were retrieved from the archives of the Department of Oral Pathology, Oral Medicine and Periodontology, Faculty of Dentistry, University of Malaya (UM). The demographic and clinico-pathologic details for all cases were obtained from biopsy reports and the patients' clinical charts/folders. Ethical approval for this study was obtained from the Medical Ethics Committee, Faculty of Dentistry, UM [Medical Ethics Committee approval number: DFOP0301/0001(P)]. For all cases, hematoxylin and eosin stained slides were prepared and the diagnosis of OSCC was re-confirmed by two authors (RBZ and CYF).

Immunohistochemistry:

For each case, 4µm sections were cut from FFPE tissue blocks and dewaxed in xylene, rehydrated through alcohol and then treated with 3% hydrogen peroxide to block endogenous peroxidase activity. For antigen retrieval, the sections were soaked in 10mM citrate buffer (pH6) and processed in a microwave oven at 95ºC for 20 minutes.

IHC was performed using the streptavidin-biotin complex method for p53 and Ki-67 whereas the En Vision® detection system was used for MDM2. The following primary antibodies were used: (1) p53 (clone DO-7, DAKO, 1:100 dilution for 45 min), (2) MDM2 (Clone 1B10, Novacastra, 1:150 dilution for 30 min) and (3) Ki-67 (clone MIB-1, DAKO, 1:100 dilution for 2 hrs).

OSCC tissues which were known to have over-expression of p53, MDM2 and Ki-67 were used as positive controls. Negative controls were performed by replacing the primary antibodies with Tris-buffered saline. The peroxidase reaction was developed using diaminobenzidine (DAB) as the chromogen and Herlich's hematoxylin was used for counter staining. The IHC staining was evaluated by two authors (RBZ and CYF) in 3 chosen fields at the invasive front for each case. A uniform scoring system was devised for positive nuclear/cytoplasm staining with p53 and MDM2 in the malignant epithelia as follows: 1+: <20% of cells; 2+: 20 – 50%; 3+:>50%. The Ki-67LI was calculated using the ratio of Ki-67 positive nuclei to the total number of the cells in the 3 chosen fields and was expressed in percentage.

Statistical Analysis:

Statistical analysis of the data was carried out using SPSS version 12 to determine the correlation between demographic and clinico-pathologic parameters with the expression of p53, MDM2, Ki-67, either individually or in combination. Pearson's Chi-square and Fisher's exact were employed for data analysis of categorical data and Mann-Whitney U test was used for analyzing continuous data. A *p*-value of less than 0.05 is considered significant.

Systematic Review:

Search Strategy:

PubMed Medline and Elsevier Science Direct were systematically searched for relevant articles for the past 20 years from 1990 to 2010 by one of the authors (WN). The starting date of 1990 was chosen as the first suggestion that *MDM2* is an oncogene was reported in 1991 by Fakharzadeh and colleagues. The search strategy is as follows:

PubMed Medline:

1 – "Mouth Neoplasms" [Mesh] OR "oral cancer" OR "mouth cancer" OR "oral carcinoma" OR "oral malignancy" OR "oral tumor" OR "oral tumour" OR "oral squamous cell carcinoma"
2 – p53 OR Mdm2 OR MDM2 OR Ki67 OR Ki67LI OR P53
3 – 1 AND 2

Elsevier Science Direct:
"oral cancer" OR "mouth cancer" OR "oral carcinoma" OR "oral malignancy" OR "oral tumour" OR "oral

squamous cell carcinoma" and p53 OR P53 OR MDM2 OR Mdm2 OR Ki67 OR Ki67LI.

Inclusion criteria:

The results from these searches were reviewed by title and abstract by 2 independent reviewers (AR and HA). Only the English language literature was included. The full manuscripts of appropriate studies were scrutinized and the studies were deemed "relevant" if they met the following inclusion criteria:

1. Samples used in the study were primary SCC of the oral cavity which were diagnosed histologically
2. Studies which assessed p53 and MDM2 (both markers) by IHC and compared the expression with demographic and clinico-pathologic parameters.

Exclusion criteria:

1. Other SCC of head and neck region such as oropharynx, larynx, recurrent SCC of the oral cavity and salivary gland tumours.
2. Studies which assessed p53 with molecular markers other than MDM2.

These inclusion and exclusion criteria were strictly adhered to allow a more meaningful systematic review.

Data Extraction:

Full-text published articles were obtained and reviewed by 2 independent reviewers (AR and HA). The data were extracted from these studies and collated in a Microsoft Excel worksheet. Clinical data such as age, gender, site and pathological data such as tumour size, lymph node metastasis, TNM staging, tumour grading and pattern of invasion were all recorded. Demographic data on ethnicity were recorded where available. IHC data were also recorded.

RESULTS

The demographic and clinico-pathologic details for 45 cases are presented in Table 1. The details of tumour size, lymph node metastasis and TNM staging were available for only 35 cases (Table 1).

p53 protein expression:

The p53 immuno-positive OSCC cases mostly showed intense nuclear staining in tumour cells and were distributed along the basal and suprabasal layer at the tumour invasive front (Figure 1A). Thirty-four cases out of 45 cases (75.6%) showed p53 positive immuno staining (Table 2) of which 5 cases (11.1%), 8 (17.8%) and 21 (46.7%) with score 1+, 2+ and 3+ respectively. Eleven cases (24.4%) were immune-negative for p53. The p53 expression does not

Table 1. Demographic and clinic-pathologic details of 45 OSCC cases

	Category	Number of patients n (%)
Age (Median=59 years old)	<59 years old	22 (48.9)
	≥59 years old	23 (51.1)
Sex	Male	25 (55.6)
	Female	20 (44.4)
Race	Indian	31 (68.9)
	Non-Indian[a]	14 (31.1)
Site	Buccal	19 (42.2)
	Tongue	12 (26.7)
	Lip	3 (6.7)
	Others	11 (24.4)
Tumor size[b]	T1 and T2	16 (45.7)
	T3 and T4	19 (54.3)
Lymph node metastasis[b]	N0	15 (42.9)
	N1 and N2	20 (57.1)
TNM staging[b]	I/II	11 (31.4)
	III/IV	24 (68.6)
Broder's Grading	Well differentiated (Group I)	19 (42.2)
	Moderately differentiated (Group II)	25 (55.6)
	Poorly differentiated (Group III)	1 (2.2)
Pattern of invasion	Cohesive (I and II)	14 (31.1)
	Non-cohesive (III and IV)	31 (68.9)

[a]Non-Indian – Chinese (10) and Malay (4)
[b]Numbers do not add up to the total due to missing data

correlate significantly with any of the clinico-pathologic characteristics.

MDM2 protein expression:

The immune-positivity of MDM2 was seen as intense nuclear staining in the basal and suprabasal layer at the tumour invasive front. The keratinized layer also showed intense nuclear staining (Figure 1B). MDM2 immuno-reactivity was seen in 44/45 cases (97.8%; Table 2) and all cases recorded a score of 3+. MDM2 expression does not correlate with any of the clinico-pathologic parameters.

Ki-67LI:

The proliferating antigen MIB1 was detected in 28/45 cases (62.2%). The intensity of the staining ranged from highly intense to faint and was localized in the nuclei of the basal layer of the tumour islands and also the basal and suprabasal layers of the tumour epithelium at the invasive front (Figure 1C). The Ki-67LI ranged from 15.9% to 70.2% with a mean of 23.4% ± SD 22.85 (median 19.4%; data not shown). The Ki-67 expression does not correlate significantly with any of the clinico-pathologic characteristics.

Co-expression of p53 and MDM2 proteins (p53/MDM2):

Thirty-three out of 45 cases (97.1%) showed concordant over expression of both p53 and MDM2 proteins. Eleven cases (24.4%) showed p53-/MDM2+ and 1 (2.2%) was p53+/MDM2-. p53 expression did not show any significant correlation with MDM2 expression (*p*-value = 1.000).

Relationship of Ki-67LI with p53, MDM2 and p53/MDM2 expression:

The Ki-67 immuno-reactivity was not seen in the one case that was also negative for MDM2 but it was positive for p53. The p53 immuno-negative cases showed higher Ki-67LI (26.12 ± 24.42) than p53 immuno-positive cases (22.48 ± 22.64) but this was not statistically significant (*p*-value = 0.625; Table 2). However, the Ki-67LI showed no significant correlation with p53 immuno-reactivity. Ki-67LI was also not significant associated with MDM2 or p53/MDM2 expression (Table 2).

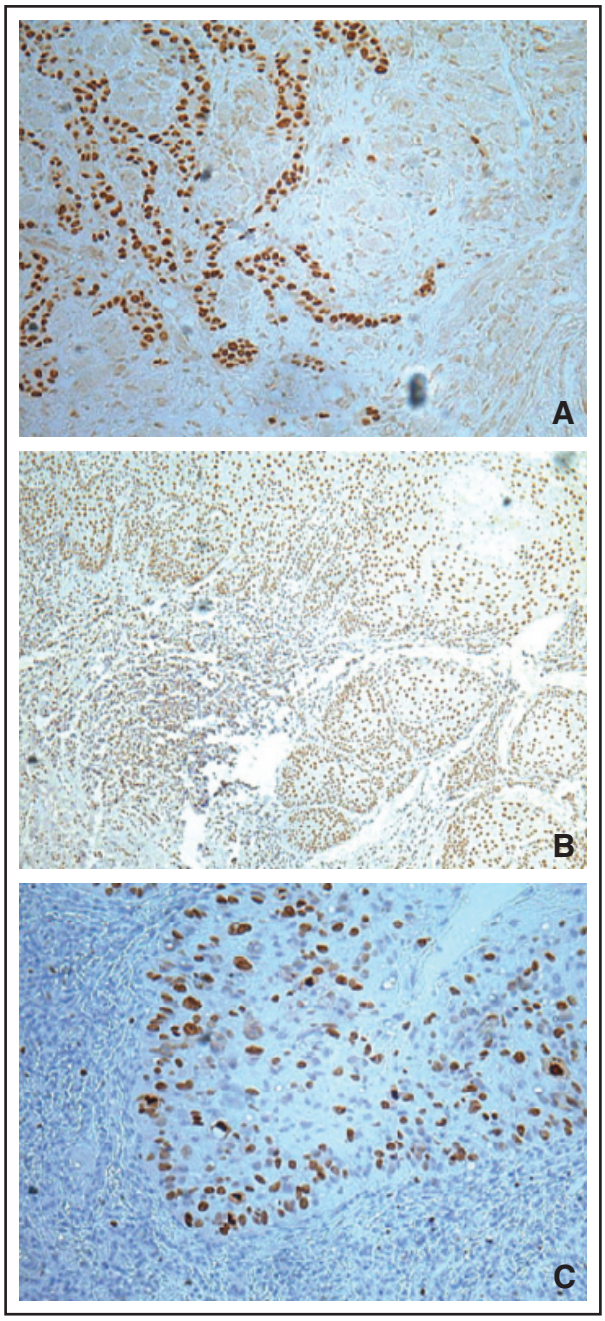

Figure 1. Expression of p53, MDM2 and Ki-67 in OSCC. Photomicrography shows A. Nuclear staining of p53 at the periphery of the tumor island (Original Magnification 100x). B. Nuclear staining of MDM2 protein in all layers of the tumor epithelium (Original Magnification 40x). C. Nuclear staining of Ki-67 protein in the basal and suprabasal layers at the tumor invasive front (Original Magnification 100x).

Table 2. Correlation of p53 and MDM2 expression with Ki-67LI in OSCC

Immunohistochemical phenotype	Number of OSCC cases n (%)	Ki-67LI (Mean ± SD)	*p*-value
p53 immunopositive cases	34 (75.6)	22.48 ± 22.64	0.625
p53 immunonegative cases	11 (24.4)	26.12 ± 24.42	
MDM2 immunopositive cases	44 (97.8)	23.90 ± 22.84	0.268
MDM2 immunonegative cases	1 (2.2)	–	
Combined p53/MDM2	33 (73.3)	23.16 ± 22.64	0.916
Lack of combined p53/MDM2	12 (26.7)	23.94 ± 24.47	

Analysis was done using Mann-Whitney test

Relationship of demographic and clinico-pathological parameters and expression of p53, MDM2, Ki-67 and p53/MDM2:

The expression of p53, MDM2 and Ki-67 did not show any correlation with demographic and clinico-pathological parameters (Table 3). The co-expression of p53 and MDM2 showed significance only with the tumour grading (p-value = 0.045) whereas the demographic and all other clinico-pathologic parameters were not significant (Table 4).

Systematic search:

The search in PubMed Medline and Elsevier Science Direct yielded a total of 983 and 3483 articles respectively; of these 935 and 3468 articles were excluded by title/abstract review. After removing duplicated studies from the two search engines; 50 articles remained for full review, of these only 13 articles met the inclusion criteria for our study. We were not able to obtain the full article by Matsumura *et al* (1996) so only 12 articles were fully reviewed.

Out of the 12 articles reviewed, the number of cases with individual expression of p53 and MDM2 was obtained from all articles (Table 5a) whereas the numbers of cases with and without co-expression of p53/MDM2 were obtained in 9 articles (Table 5b).

DISCUSSION

The first suggestion that MDM2 is an oncogene came from the genomic cloning of MDM2 that was amplified in rodent cells and the high tumourigenic potential it conferred in nude mice (20). Oliner et al in 1992

Table 3. Relationship of demographic and clinic-pathological characteristics and expression of p53, MDM2, Ki-67 in OSCC case

Demographic/ clinicopathological characteristic	Total (n)	Immmunohistochemical expression of markers, p-value								
		p53+ n (%)	p53- n (%)	*p*-value	MDM2+ n (%)	MDM2- n (%)	*p*-value	Ki-67+ n (%)	Ki-67- n (%)	*p*-value
Age										
<59 years old	22	16 (47.1)	6 (54.5)	0.666[a]	22 (50.0)	0 (0.0)	1.000[b]	16 (57.1)	6 (35.3)	0.155[a]
≥59 years old	23	18 (52.9)	5 (45.5)		22 (50.0)	1 (0.0)		12 (42.9)	11 (64.7)	
Sex										
Male	25	16 (47.1)	9 (81.8)	0.079[b]	25 (56.8)	0 (0.0)	0.444[b]	17 (60.7)	8 (47.1)	0.371[a]
Female	20	18 (52.9)	2 (18.2)		19 (43.2)	1 (100.0)		11 (39.3)	9 (52.9)	
Race										
Indian	31	25 (73.5)	6 (54.5)	0.277[b]	30 (68.2)	1 (100.0)	1.000[b]	20 (71.4)	11 (64.7)	0.637[a]
Non-Indian	14	9 (26.5)	5 (45.5)		14 (31.8)	0 (0.0)		8 (28.6)	6 (35.3)	
Site										
Others	12	27 (79.4)	6 (54.5)	0.131[b]	32 (72.7)	1 (100.0)	1.000[b]	21 (75.0)	12 (70.6)	0.743[b]
Tongue	33	7 (20.6)	5 (45.5)		12 (27.3)	0 (0.0)		7 (25.0)	5 (29.4)	
Tumor Size[c]										
T1 and T2	16	11 (40.7)	5 (62.5)	0.424[b]	16 (47.1)	0 (0.0)	1.000[b]	9 (40.9)	7 (53.8)	0.458[a]
T3 and T4	19	16 (59.3)	3 (37.5)		18 (52.9)	1 (100.0)		13 (59.1)	6 (46.2)	
Lymph node metastasis[c]										
N0	15	10 (37.0)	5 (62.5)	0.246[b]	14 (41.2)	1 (100.0)	0.429[b]	9 (40.9)	6 (46.2)	0.762[a]
N1 and N2	20	17 (63.0)	3 (37.5)		20 (58.8)	0 (0.0)		13 (59.1)	7 (53.8)	
TNM staging[c]										
Stage I and II	11	7 (25.9)	4 (50.0)	0.226[b]	11 (32.4)	0 (0.0)	1.000[b]	7 (31.8)	4 (30.8)	1.000[b]
Stage III and IV	24	20 (74.1)	4 (50.0)		23 (67.6)	1 (100.0)		15 (68.2)	9 (69.2)	
Broders' grading										
Well differentiated	19	12 (35.3)	7 (63.6)	0.160[b]	18 (40.9)	1 (100.0)	0.422[b]	11 (39.3)	8 (47.1)	0.609[a]
Moderately + Poorly differentiated	26	22 (64.7)	4 (36.4)		26 (59.1)	0 (0.0)		17 (60.7)	9 (52.9)	
Pattern of invasion										
Cohesive	14	10 (29.4)	4 (36.4)	0.717[b]	14 (31.8)	0 (0.0)	1.000[b]	9 (32.1)	5 (29.4)	0.848[a]
Non-cohesive	31	24 (70.6)	7 (63.6)		30 (68.2)	1 (100.0)		19 (67.9)	12 (70.6)	

[a]Analysis was done using chi-square test
[b]Analysis was done using Fisher exact test
[c]Numbers do not add up to total due to missing data

Table 4. Relationship between demographic and clinico-pathological characteristic and co-expression of p53/MDM2 in OSCC

Demographic/ clinico-pathological characteristic	Total (n)	Co-expression of p53 and MDM2 Yes n (%)	Co-expression of p53 and MDM2 No n (%)	*p*-value
Age				
<59 years old	22	16 (48.5)	6 (50.0)	0.928[a]
≥59 years old	23	17 (51.5)	6 (50.0)	
Sex				
Male	25	16 (48.5)	9 (75.0)	0.113[a]
Female	20	17 (51.5)	3 (25.0)	
Race				
Indian	31	24 (72.7)	7 (58.3)	0.470[b]
Non-Indian	14	9 (27.3)	5 (41.7)	
Site				
Others	33	26 (78.8)	7 (58.3)	0.254[b]
Tongue	12	7 (21.2)	5 (41.7)	
Tumor Size[c]				
T1 and T2	16	11 (42.3)	5 (55.6)	0.700[b]
T3 and T4	19	15 (57.7)	4 (44.4)	
Lymph node metastasis[c]				
N0	15	9 (34.6)	6 (66.7)	0.129[b]
N1 and N2	20	17 (65.4)	3 (33.3)	
TNM staging[c]				
Stage I and II	11	7 (26.9)	4 (44.4)	0.416[b]
Stage III and IV	24	19 (73.1)	5 (55.6)	
Broders' grading				
Well differentiated	19	11 (33.3)	8 (66.7)	0.045[a]
Moderately + Poorly differentiated	26	22 (66.7)	4 (33.3)	
Pattern of invasion				
Cohesive	14	10 (30.3)	4 (33.3)	1.000[b]
Non-cohesive	31	23 (69.7)	8 (66.7)	

[a]Analysis was done using chi-square test
[b]Analysis was done using Fisher exact test
[c]Numbers do not add up to total due to missing data

Table 5a. Over-expression of p53 and MDM2 in all studies reviewed including the present study

Study	Over-expression of p53 n (%)	Over-expression of MDM2 n (%)
Stoll *et al* (1998)[22]	54/107 (50.5%)	78/107 (72.9%)
Regezi *et al* (1999)[23]	42/72 (58.3%)	14/71 (19.7%)
Agarwal *et al* (1999)[16]	45/65 (69.2%)	51/65 (78%)
Ng *et al* (1999)[17]	58/84 (69%)	22/84 (26.2%)
Partridge *et al* (1999)[24]	25/45 (55.6%)	43/45 (95.6%)
Ralhan *et al* (2000)[25]	31/46 (67.4%)	71/100 (71%)
Li *et al* (2000)[26]	29/38 (76%)	19/38 (50%)
Sano *et al* (2000)[27]	13/37 (35.1%)	10/37 (27.0%)
Shwe *et al* (2001)[18]	30/40 (75%)	29/40 (72.5)
Yanamoto *et al* (2002)[2]	44/69 (63.8%)	25/69 (36.2%)
Pande *et al* (2002)[28]	69/105 (66%)	72/105 (69%)
Liu *et al* (2004)[29]	81%	88%
Present study (2011)	34 (75.6%)	44 (97.8)

Table 5b. Co-expression of p53 and MDM2 in all studies reviewed including the present study

Study	Co-expression of p53/MDM2 n (%)	Lack of co-expression of p53/MDM2 n (%)	Total number of cases n (%)
Partridge *et al* (1999)[24]	24 (53.3)	21 (46.7)	45 (100.0)
Agarwal *et al* (1999)[16]	39 (60.0)	26 (40.0)	65 (100.0)
Ng *et al* (1999)[17]	17 (20.2)	67 (79.8)	84 (100.0)
Regezi *et al* (1999)[23]	12 (16.9)	59 (83.1)	71 (100.0)
Ralhan *et al* (2000)[25]	24 (52.2)	22 (47.8)	46 (100.0)
Li *et al* (2000)[26]	13 (34.2)	25 (65.8)	38 (100.0)
Sano *et al* (2000)[27]	0 (0.0)	37 (100)	37 (100.0)
Shwe *et al* (2001)[18]	25 (62.5)	15 (37.5)	40 (100.0)
Yanamoto *et al* (2002)[2]	20 (29.0)	49 (71.0)	69 (100.0)
Present study (2011)	33 (73.3)	12 (26.7)	45 (100.0)

mapped the human homologue of *MDM2* to chromosome 12q13-14 and also showed it to be amplified in approximately 30% of soft tissue tumours and osteosarcomas (8). Momand et al (1992) demonstrated that MDM2 is a 90 kDa phospho protein that co-immuno precipitate with p53 and further showed that this inactivates the transactivation activity of the p53 protein, thus elucidating the oncogenic property of MDM2 (9).

In this study, the over-expression of MDM2 was present in all but one case of OSCC (97.8%) which is one of the highest when compared to finding from other studies were the reported percentage may range from 19.7% (24) to 100% (30). The over-expression of MDM2 has been shown previously to be due to gene amplification, enhanced transcription or translation (25). The MDM2 expression was present in only 26.2% of cases studied by Ng et al (1999), and this observation led them to suggest that MDM2 may not be important in oral carcinogenesis which contradicts the findings of Agarwal et al (1999) and Ralhan et al (2000). Notably, Ralhan et al (2000) showed significant correlation between MDM2 protein over-expression and tumour stage and also loss of cell differentiation.

p53 acts as a checkpoint gene. It either blocks cell cycle progression or induces apoptosis in response to DNA damage and stress. During stress there is an increase in the level of p53 protein within 1 to 12 hours (1) which is due to the combination of an increase in p53 translation rate (31) and a decrease in p53 degradation rate (32). In addition, the level of p53 is also governed by MDM2 which binds to p53 directly to initiate p53 degradation (33). In the present study, the over-expression of p53 was seen in only 34 cases (75.6%). Most of the studies have shown MDM2 to be over-expressed in more number of cases studied than p53 except Ng et al (1999), Regezi et al (1999), Li et al (2000), Sano et al (2000) and Yanamoto et al (2002) where p53 was expressed in more number of cases (Table 5a). These discrepancies could be explained by the fact that, factor other than p53 regulates MDM2 (24). Other mechanisms such as chromosomal translocation or mutation could also increase the level of MDM2 protein (34-36).

In this study, the co-expression of p53/MDM2 was found in 73.3% of OSCC cases. The co-expression of p53 and MDM2 has been reported in a subset of osteosarcomas (15), melanomas (13) and 60% of OSCCs (16). The co-existence of p53 and MDM2 aberration is indicative of a 'gain of function' phenotype with more aggressive characteristics (16). When compared to other studies of the systematic review the co-expression of p53/MDM2 in the present study was the highest (73.3%). We hypothesed this could be due to the tobacco/betel quid chewing habits which is predominantly practiced by the ethnic Indian in Malaysia since majority of our samples were obtained from patients of Indian ethnicity.

In the present study, there was no statistical difference between the co-expression of p53/MDM2 and their lack of co-expression of p53/MDM2 (p value = 1.000) (Table 4). This is in contrast to the systematic reviewed studies by Agarwal et al (1999), Shwe et al (2001) and Yanamoto et al (2002) but similar to other studies by Ng et al (1999), Li et al (2000) and Liu et al (2004) that did not show any significant correlation between the co-expression and the lack of co-expression of p53/MDM2. These results suggest the existence of a p53-independent MDM2 regulatory pathway (29) and that these pathways may also be important in OSCC (26). Furthermore, it shows that the functional status of p53 is not the only factor that determines the expression of MDM2 (26). This is further supported by the study of Ralhan et al (2000), where three different isoforms of MDM2 proteins (i.e. p90, p76 and p57) were identified. The p90 and p57 proteins can bind to p53 protein and cause the accumulation of wild-type p53 in oral cancer. They also detected several alternatively spliced MDM2 transcripts in transformed cell lines/tumours which have lost the ability to bind to p53 proteins as they lack the p53 binding domain and thus are not able to regulate p53 transactivation function. They further

suggested that isoform p76 of MDM2 may contribute directly to oncogenic potential by alternative p53-independent pathways as MDM2 has the ability to activate E2F1/DP1 transcription factors (36) and interact with Rb protein (37). Notably, in 11 OSCCs (24.4%) where over-expression of MDM2 was observed, p53 expression was absent further supporting a p53-independent role for MDM2 in the oral carcinogenesis in these tumours (16).

Interestingly, we found that the co-expression of p53/MDM2 was significantly correlated with tumour differentiation (p-value =0.045) (Table 4). This result was similar to Pande et al (2002) and Shwe et al (2001) but in contrast to Yanamoto et al (2002). Pande et al (2002) also reported that the co-expression of p53/MDM2 was significantly related to the nodal involvement. In contrast, in the present study there was no significant association found (Table 4). The tumour differentiation and nodal involvement of tumour are parameters associated with aggressive tumour behavior and therefore the result of Pande et al (2002) suggests that co-expression of p53/MDM2 could be markers of aggressive tumours. However, our results did not directly support this given that the data indicated significant association with poor tumour differentiation but not with nodal metastasis.

Yanamoto et al (2002) suggested that MDM2 expression and the co-expression of p53/MDM2 were closely related to tumour proliferation. They used Ki-67LI as a marker for cellular proliferation and showed Ki-67LI of co-expression of p53/MDM2 was significantly higher than the lack of co-expression of these proteins. Consistently, 1 case in this series which was MDM2 immune-negative was also immune-negative for Ki-67 expression but positive for p53. However overall, Ki-67LI was not significant associated with MDM2 positive tumours (p-value=0.268) (Table 2). Ki-67LI was also not significantly associated with the co-expression of p53/MDM2 (p-value=0.916) (Table 2). Therefore the correlation suggested by Yanamoto et al (2002) failed to be proven in our study, and this could be due to a limited sample size.

CONCLUSION

In conclusion, the present study showed that the expression of MDM2 was not significantly associated with p53 expression, suggesting that MDM2 expression is mediated by p53-independent pathways or mutated p53 could not induce the expression of MDM2 in this set of OSCCs. The E2F1/DP1 transcription factors and Rb protein pathways have to be studied in this set of OSCC. Whereas, the systematic review has clearly shown that both p53-dependent and p53-independent pathways that regulate MDM2 play a role in oral carcinogenesis. The co-expression of p53/MDM2 was not significantly associated with Ki-67LI in the present study, again this need to be confirmed with larger sample size. The only clinico-pathological parameter that correlated significantly with co-expression of p53/MDM2 is the tumour differentiation which is suggestive of aggressive tumour behavior. The limitation of this study was that it did not take into account patient survival and disease recurrence; these factors could have been examined to determine the prognostic significance of co-expression of p53/MDM2, however given the lack of correlation between the co-expression of p53/MDM2 with existing clinico-pathological parameters, in particular lymph node metastasis, we think that the prognostic role for these markers would be minimal.

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