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Prognostic impact of p53 and p63 immunoexpression in oral squamous cell carcinoma

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BACKGROUND: The role of p53 and p63 proteins in the prognosis of oral squamous cell carcinoma (OSCC) is still debatable. Our aim here was to investigate the relationship between the immunoexpression of these proteins with some clinicopathologic parameters of prognostic significance in OSCC.

METHODS: Formalin-fixed paraffin-embedded sections from 106 patients were used for study together with the following data: primary site, histologic differentiation, recurrences, metastasis, disease-free survival and overall survival (OS).

RESULTS: In OSCCs, the positive rate for p63 protein immunoexpression (87.8%) was higher than p53 (52.8%). p53 expression correlated with metastasis. Tumors negative for p53 and with strong intensity for p63 expression had a significantly higher OS.

CONCLUSIONS: p53 overexpression is associated with a larger number of metastases and is correlated with a poor outcome as well as decreased intensity in p63 immunoexpression.

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Keywords: oral cancer; immunohistochemistry; oral squamous cell carcinoma; p53; p63; prognosis

Introduction

For the study of p53 protein expression, immunohistochemistry (IHC) is the method of choice, whereas for studies of the *TP53* gene status amplification by PCR followed by either sequencing or various kinds of gel analysis is commonly applied (1). Due to its short half-life (around 20 min), the wild-type p53 protein is difficult to detect in normal tissues (1). Nevertheless, the normal function of the p53 gene can be altered by mutations, defects in the degradation pathway, fusion with viral onco-proteins or fusion with the individual's own proteins (1, 2). These alterations likely lead to stabilization of this protein, which can be detected by IHC. Previous studies concluded that p53 mutations change its conformation making the molecule more stable, and are the principal mechanism for the tumor-specific accumulation of this protein (3). However, there have been consistent reports of p53 detection by IHC in the absence of mutation (4, 5).

The overexpression of p53 has been associated with either improved survival, no change in survival, or poor prognosis in patients with carcinomas of the upper aerodigestive tract (6–10). When looking at the whole group of squamous cell carcinoma of the head and neck (HNSCC), most investigations performed have not shown any correlation between p53 protein expression and prognosis (7, 8, 11). However, an association between p53 and poor prognosis was found for laryngeal carcinomas (12, 13). In another study of 16 patients diagnosed as end-stage HNSCC, a correlation between worse prognosis and p53 expression also was found (14). On the other hand, in other studies the overexpression of p53 was related with a better prognosis in squamous cell carcinomas of the tongue base (6).

Factors of prognostic significance in oral squamous cell carcinoma (OSCC) include the presence of regional lymph node metastasis and amplification of chromosome 3q21-29, where the *TP63* gene is located (15). The *TP63* gene is a member of the *TP53* gene family located at the 3q27-29 region and due to differential mRNA splicing and alternative promoter usage gives rise to an array of two different protein classes with three protein isoforms each: a class of three protein isoforms with the transactivating domain (TA), and other lack the N-terminal domain (ΔN). Several investigations have suggested that these isoforms exert opposite biologic properties (16). The TAp63 and ΔNp63 isoforms are known to be differentially expressed in keratinocyte differentiation systems. The TAp63 isoforms can decrease the growth capacity and increase the keratinocyte differentiation process, and the ΔNp63 isoforms can

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act to the opposite (17). In neoplastic cells, the ΔNp63 isoforms can promote growth and survival of neoplastic cells by competing for p53-binding sites. TAp63 isoforms possess a p53-like NH_2-terminal TA domain and can transactivate p53 target genes that also induce apoptosis (16, 18).

A considerable interest has been focused on p63, and because of its overexpression in epithelial cells and various solid tumors, it has been suggested to have an oncogenic role in the regulation of proliferation and differentiation in many neoplasms (19). Squamous cell carcinomas of different organs, including head and neck, cervix, and lung, as well as basal cell carcinomas of the skin, show strong nuclear p63 expression (20–22). Many studies show high variability in the established scores for p63 IHC evaluation. The majority of the studies only evaluated the quantity of cells stained for p63, and did not assess p63 intensity (19, 23–26).

The objective of this study was to investigate p53 and p63 immunoexpression in OSCC and their relationship with some clinicopathologic parameters of prognostic significance.

Materials and methods

Patients

The study protocol was approved by the local Ethics Committee. Cases of primary OSSCs ($n = 106$) diagnosed between 1990 and 2002 were retrieved from the medical files of Ribeirão Preto School of Medicine General Hospital, University of São Paulo, Brazil. Paraffin-embedded blocks corresponding to the biopsy or surgical resection of the selected cases were retrieved from the Department of Pathology's files of the same institution. Specimens had been routinely fixed in 4% neutral-buffered formalin. Tissue sampling and histological diagnosis were performed according to the guidelines recently published (27). The hematoxylin and eosin and immunostaining sections were reviewed by two medical pathologists (ARS and SZ). Tumors were classified as well, moderate or poorly differentiated according the World Health Organization classification of histologic differentiation (28). Medical files were analyzed and reviewed to collect information concerning age, gender, primary site, metastasis and tumoral recurrences, histological classification, disease-free survival (DFS) and overall survival (OS) of the patients. The patient clinical data are presented in Table 1.

Inclusion criteria and definitions

The inclusion criteria for this study were: (1) adequate clinicopathologic data; (2) availability of sufficient paraffin-embedded tumor material; (3) oral cavity cancer (including oral tongue, floor of mouth, gingiva, lips, buccal mucosa, hard palate, and retromolar trigone) (29); (4) no previous head and neck cancer; (5) no previous radio- or chemotherapy; (6) histologically proven squamous cell carcinoma; (7) a single lesion; and (8) absence of initial or distant metastasis. Patients with *in situ* and T4 tumors were also excluded as well as those who died of other unrelated causes (30). The DFS was calculated as the time between the date of onset of treatment and the date of the first recurrence or last follow-up. OS was calculated as the date of onset of treatment to the date of death or last follow-up.

Immunohistochemistry

Immunostaining was performed on 4-μm-thick sections, serially cut of tumor representative areas from selected blocks. The mounted poly-L-lysine-coated glass slides were deparaffinized, rehydrated, immersed in 10 mmol/l citrate buffer, pH 6.0, and submitted to heat-induced epitope retrieval using a vapor lock for 45 min. The slides were briefly rinsed with phosphate-buffered saline (PBS) and subjected to immunostaining using monoclonal anti-p63 (4A4, Santa Cruz Biotechnology, Palo Alto, CA, USA, 1:100) and monoclonal anti-p53 (DO-7, Novocastra Laboratories, Newcastle upon Tyne, UK, 1:100) primary antibodies, according to the manufacturer's protocol (Novostain Super ABC Kit, Universal,

Table 1 Demographics and clinical features

	Primary site					
	Floor of mouth	*Tongue*	*Hard palate*	*Lower lip*	*Other*	*Total*
Cases	34 (32.1)	25 (23.6)	21 (19.8)	15 (14.1)	11 (10.4)	106 (100)
Age						
≤ 60 years	27 (79.4)	15 (60)	13 (61.9)	7 (46.7)	5 (45.5)	67 (63.2)
> 60 years	7 (20.6)	10 (40)	8 (38.1)	8 (53.3)	6 (54.5)	39 (36.8)
Gender						
Male	30 (88.2)	19 (76)	19 (90.6)	12 (80)	9 (81.8)	89 (84)
Female	4 (11.8)	6 (24)	2 (9.4)	3 (20)	2 (18.2)	17 (16)
Differentiation						
Well	19 (55.9)	11 (44)	5 (23.8)	8 (53.3)	5 (45.5)	48 (45.3)
Moderate	9 (26.5)	10 (40)	11 (52.4)	7 (46.7)	4 (36.4)	41 (38.7)
Poor	6 (17.6)	4 (16)	5 (23.8)	-	2 (18.1)	17 (16)
Recurrence						
No	15 (44.1)	13 (52)	10 (47.6)	5 (33.3)	4 (36.4)	47 (44.3)
Yes	19 (55.9)	12 (48)	11 (52.4)	10 (66.7)	7 (63.6)	59 (55.7)
Metastasis						
No	7 (20.6)	11 (44)	10 (47.6)	6 (40)	3 (27.3)	37 (34.9)
Yes	27 (79.4)	14 (56)	11 (52.4)	9 (60)	8 (72.7)	69 (65.1)

Novocastra Laboratories, Newcastle upon Tyne, UK). The labeling was developed with 3,3-diaminobenzidine (DAB) (D5638, Sigma Chemical Co., St Louis, MO). The slides were counterstained with Harris hematoxylin and mounted with Entellan (Merck, Darmstadt, Germany). The adjacent normal mucous basal cells were used as the positive control for p63. Negative controls were prepared by omission of the primary antibody. Only nuclear staining of tumoral cells was considered, at 100× magnification. The expression of p53 and p63 was evaluated and scored. The reactions were repeated once to evaluate the coherence with the first evaluation. For p53 analysis, only tumors with 10% or more positive nuclear staining in neoplastic cells were considered positive (10, 22, 24, 29). A semi-quantitative assessment of p63 expression was performed in tumoral cells and recorded as: 0 = no stained cells; 1 = 1–25% positive cells; 2 = 26–50% positive cells; 3 = 51–75% positive cells, and 4 = more than 75% positive cells (19, 25). The intensity of immunostaining was also evaluated as absence (0), weak (+), moderate (++), or strong (+++) (32, 33).

Statistical analysis

GraphPad Prism v.4.0 (GraphPad Software Inc., San Diego, CA, USA) for Windows was used for all statistical analyses. Descriptive statistics were used to summarize study data. Statistical comparisons were performed using the Fisher's exact test and the non-parametric Kruskall–Wallis test. The DFS and OS were quantified. Survival curves were estimated using the Kaplan–Meier method (34) and the analysis was computed by comparing the categories of positive tumor cells. Survival data were censored for patients alive at the last follow-up. In addition, the survival data were censored for those without any recurrence of DFS. The long-rank test was used to compare survival outcomes. Statistical significance was defined as a two-tailed $P \leq 0.05$.

Results

The clinical data of the study population are summarized in Table 1. p63 immunoreactivity was found in 93 (87.8%) tumors (Fig. 1). The quantitative labeling index

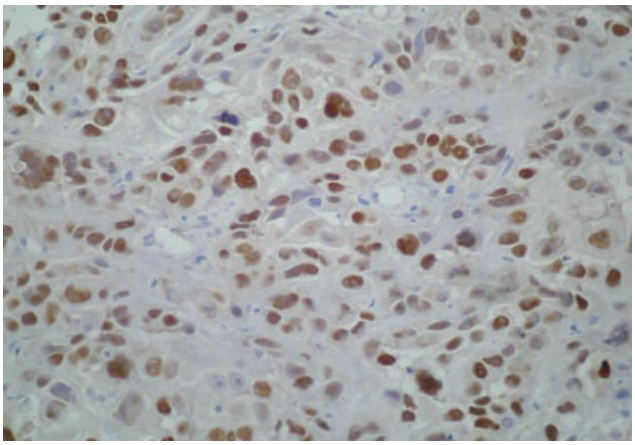

Figure 1 Neoplastic cells positive for p53 (dark nuclear staining, immunohistochemistry, original magnification ×400).

varied between 0% and 100%. The reactions were repeated once and their results showed coherence with the first evaluation. The distribution and intensity of p63 expression is specified in Tables 2 and 3 respectively. p63 expression was noted mainly in moderate and well-differentiated tumors; however, in general, it was absent in terminally differentiated cells and in the keratin-pearl areas. p53 immunoreactivity was found in 56 (52.8%) tumors (Fig. 2). The majority of negative tumors showed a well-differentiated pattern (Table 4).

There was no statistically significant correlation between p63 immunoexpression quantity status and both tumor recurrences and metastasis (Table 2, $P = 0.439$ and 0.687 respectively); however, there was a slight tendency toward lower p63 immunoexpression in less differentiated tumors ($P = 0.719$). The cases with lower p63 immunoexpression intensity showed a higher number of metastasis than those with strong immunoexpression (Table 3). There was no statistically significant correlation of p63 immunoexpression intensity status regarding tumor metastasis and recurrence ($P = 0.244$ and 0.172 respectively). In the same way, no significant result was reached in the p63 immunoexpression intensity according to differentiation ($P = 0.397$). No correlation was found between p53 staining and recurrence and histological differentiation

Table 2 p63 quantitative immunoexpression, differentiation, recurrences and metastasis analysis

	p63 quantitative immunoexpression					
	0	*1*	*2*	*3*	*4*	*Total*
Cases	13 (12.2)	26 (24.6)	23 (21.7)	17 (16)	27 (25.5)	106 (100)
Differentiation						
Well	2 (15.4)	9 (34.6)	9 (39.1)	11 (64.7)	17 (63)	48 (45.3)
Moderate	3 (23.1)	12 (46.2)	12 (52.2)	5 (29.4)	9 (33.3)	41 (38.7)
Poor	8 (61.5)	5 (19.2)	2 (8.7)	1 (5.9)	1 (3.7)	17 (16)
Recurrence						
No	8 (61.5)	8 (30.8)	7 (30.4)	11 (64.7)	13 (48.1)	47 (44.3)
Yes	5 (38.5)	18 (69.2)	16 (69.6)	6 (35.3)	14 (51.9)	59 (55.7)
Metastasis						
No	4 (30.8)	4 (15.4)	7 (30.4)	7 (41.2)	15 (55.6)	37 (34.9)
Yes	9 (69.2)	22 (84.6)	16 (69.6)	10 (58.8)	12 (44.4)	69 (65.1)

Table 3 p63 intensity immunoexpression, differentiation, recurrences and metastasis analysis

	p63 intensity immunoexpression				
	0	*+*	*++*	*+++*	*Total*
Cases	13 (12.2)	20 (18.9)	53 (50)	20 (18.9)	106 (100)
Differentiation					
Well	2 (15.4)	8 (40)	26 (49.1)	12 (60)	48 (45.3)
Moderate	3 (23.1)	8 (40)	23 (43.4)	7 (35)	41 (38.7)
Poor	8 (61.5)	4 (20)	4 (7.5)	1 (5)	17 (16)
Recurrence					
No	8 (61.5)	5 (25)	24 (45.3)	10 (50)	47 (44.3)
Yes	5 (38.5)	15 (75)	29 (54.7)	10 (50)	59 (55.7)
Metastasis					
No	4 (30.8)	2 (10)	19 (35.8)	12 (60)	37 (34.9)
Yes	9 (69.2)	18 (90)	34 (64.2)	8 (40)	69 (65.1)

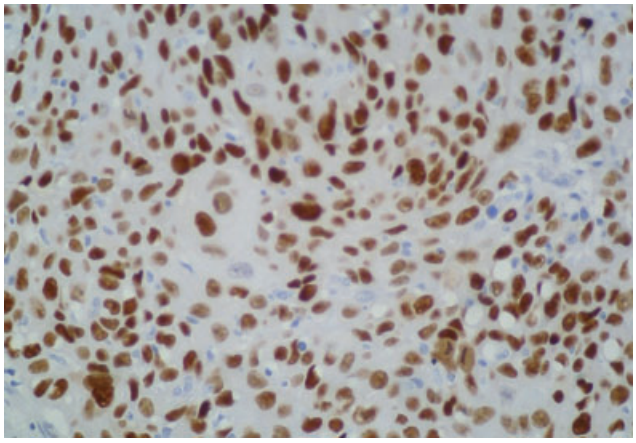

Figure 2 Neoplastic cells positive for p63 (dark nuclear strong staining, immunohistochemistry, original magnification ×400).

Table 4 p53 immunoexpression, differentiation, recurrences and metastasis analysis

	p53 immunoexpression		
	Positive	*Negative*	*Total*
Cases	56 (52.8)	50 (47.2)	106 (100)
Differentiation			
Well	21 (37.5)	27 (54)	48 (45.3)
Moderate	22 (39.3)	19 (38)	41 (38.7)
Poor	13 (23.2)	4 (8)	17 (16)
Recurrence			
No	24 (42.9)	23 (46)	47 (44.3)
Yes	32 (57.1)	27 (54)	59 (55.7)
Metastasis			
No	12 (21.4)	25 (50)	37 (34.9)
Yes	44 (78.6)	25 (50)	69 (65.1)

(Table 4, $P = 0.845$ and 0.156 respectively). Nevertheless, correlation was found between p53 expression and metastasis ($P = 0.002$), with the p53-positive cases showing a higher metastasis rate (78.6%).

No statistical significance was reached between p53 and p63 staining quantity ($P = 0.438$). In the p63 staining intensity analysis, the moderate and strong cases had the majority of the p53 negative cases (78%), but also without statistical significance between the p53 and p63 staining intensities ($P = 0.212$, Table 5).

Table 5 p53 × p63 immunoexpression relation

	p53 immunoexpression		
	Positive	*Negative*	*Total*
Cases	56 (52.8)	50 (47.2)	106 (100)
p63 immunoexpression			
Staining absence	9 (16.1)	4 (8)	13 (12.2)
p63 quantitative			
1 (1–25%)	8 (14.3)	18 (36)	26 (24.6)
2 (26–50%)	12 (21.4)	11 (22)	23 (21.7)
3 (51–75%)	9 (16.1)	8 (16)	17 (16)
4 (76–100%)	18 (32.1)	9 (18)	27 (25.5)
p63 intensity			
+ (weak)	13 (23.2)	7 (14)	20 (18.9)
++ (moderate)	28 (50)	25 (50)	53 (50)
+++ (strong)	6 (10.7)	14 (28)	20 (18.9)

Overall survival rate was 32% at 5 years (SE 7.3%). The patients had a median survival of 35 months. On the other hand, the DFS rate was only 10% at 5 years (SE 3.4%) and a 19 months median DFS. The histological grade did not affect the survival curves (DFS: $P = 0.381$ and OS: $P = 0.113$). In the p63 quantitative immunoexpression, patients with a high index of p63 immunoexpression (4 = 75–100%) had higher DFS and OS; however, no statistical significance was found by comparison between the curves ($P = 0.271$ and 0.184 respectively).

The p63-positive tumors with strong staining had a higher DFS and the tumors with no staining had the smallest DFS, yet no statistical difference was observed ($P = 0.406$). Concerning the OS, a significant association between the intensity of p63 staining and increased odds of OS ($P = 0.008$) was found (Fig. 3).

In the p53 immunoexpression survival curves, no significant differences were found concerning DFS between p53 positive (≥10%) and negative (<10%) patients ($P = 0.334$). In relation to OS, patients with a negative p53 expression had a statistically significant better prognosis compared with those with p53 positive staining ($P = 0.046$, Fig. 4).

Discussion

To avoid the influence of the diverse treatment strategies in the prognostic evaluation, it is important to study

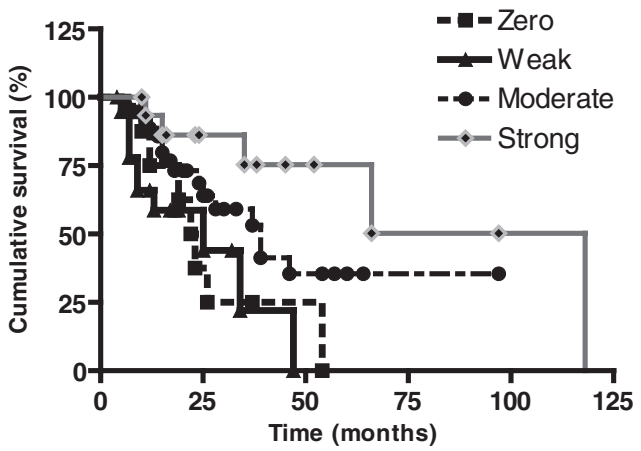

Figure 3 p63 intensity immunoexpression relationship and overall survival.

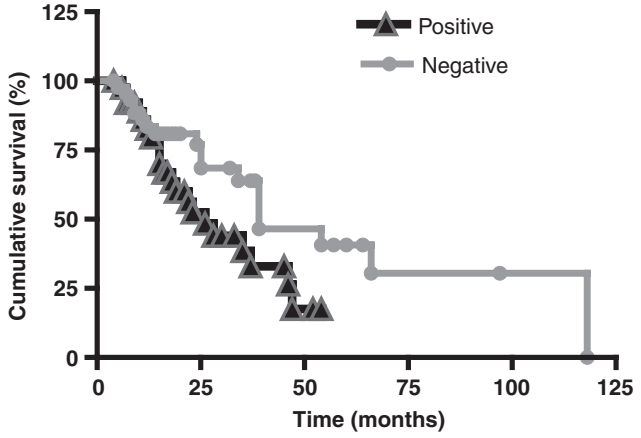

Figure 4 p53 immunoexpression relationship and overall survival.

primary tumors without multicentric lesions and nodal involvement. Moreover, it is also necessary to have an adequate selection of primary site, focusing only on the oral lesions. According to Ha and Califano, there are several prognostic studies that include oropharyngeal lesions in prognostic prediction of OSCC (35). However, this primary site can hold different radiotherapy sensitivity, besides specific biological behavior, as well as clinical and prognostic characteristics that differ for OSCCs (29, 35). In OSCCs, it is still not clear why some patients do better than others with the same stage and site of disease (31).

Previous efforts to correlate p53 status with clinical outcome for patients with OSCCs have been inconclusive. The ambiguity of these results is attributed to factors, such as small and heterogeneous patient samples, type of tissue analyzed (frozen and paraffin-embedded) and its pretreatment, antibodies applied, and arbitrary threshold of the percentage of stained cells used to characterize sections as positive (31). Following other studies (9, 10, 24, 26, 31), we have chosen a 10% stained nuclei as a threshold level for p53 immunoexpression prognostic analysis.

The proportion of p53 positive tumors found in our study (52.8%) is in agreement with that previously reported by different authors who found a range of p53 positive staining between 34% and 81% (36, 37).

Despite the inconsistency of previous studies concerning the p53 prognostic value in OSCCs, in our series a prognostic importance has been demonstrated, and the patients with negative p53 expression had a better prognosis and a significantly reduced number of metastases. Similar to our study, an assay investigating only OSCCs without initial neck node metastases found that p53 expression was a significant prognostic factor associated with longer survival in patients with p53 negative tumors (31). In relation to metastasis, this work is in agreement with Khademi *et al*., which also found a significant association (38). Studying only tongue cancers, Unal *et al*. (1999) reported that p53 alterations might occur before metastasis and promote the metastatic potential of neoplastic cells (39). Although no significant differences were found in the DFS curves, accordingly with Siegelmann-Danieli *et al*., a trend of shorter DFS curve was seen with p53-positive tumors (40).

In agreement with Massion *et al*. and Bortoluzzi *et al*., our results showed no correlation between p63 and p53 immunostaining (33, 41). These results can suggest independent roles for p53 and p63 during OSCCs tumorigenesis.

In this study, our 106 cases represent one of the most extensive series for p63 IHC investigation. p63 detection by IHC was always evident in OSCCs, and the elevated expression found (87.8%) is in agreement with previous studies (1, 19, 24, 42), supporting the idea that p63 is involved in OSCC tumorigenesis. However, there are few publications about the immunohistochemical prognostic significance of p63 immunoexpression in OSCCs.

There are many variations in IHC evaluation of p63 protein. There are some investigations showing that the TAp63 isoform expression has been undetectable by IHC using the commonly employed 4A4 monoclonal antibody to p63 (16, 43). However, it has been suggested that it can be showed at the low levels of TAp63 expressed, and most of the investigations demonstrate the use of 4A4 antibody in the detection of both isoforms (19, 20, 32, 44–46).

The studies also show a high variability in the IHC score pattern. The majority of investigations only evaluated the quantity of cells stained for p63, without taking into account its intensity (19, 23–26). In this study, both staining intensity pattern and quantity of stained cells could not be used to distinguish significantly the degree of differentiation of the lesions studied, and the same results were also found by Reis-Filho *et al*. and Bortoluzzi *et al*. (18, 41).

In the p63 intensity immunoexpression assessment, despite no correlation being found in recurrence and metastasis, a significant relationship was found between strong intensity staining tumors with better survival curves of OS. The strong staining intensity for p63 correlated with a 5-year OS of 75.4%, compared with 0% in weak and absent cases respectively. This might

suggest that, in OSCCs, p63 can play a role toward a less aggressive behavior. The only study concerning the relationship between the intensity of p63 immunoexpression and prognosis found in the literature was performed by Massion *et al.*, which also reported better survival in p63 strong intensity staining lung squamous carcinomas (33).

In relation to the quantity of p63 positive cells, there are several studies demonstrating correlation of p63 overexpression with a better prognosis. In a recent study with esophageal squamous cell carcinoma performed by Takahashi *et al.*, at a 50% established cut-off value for p63 expression, the 5-year OS was significantly longer in p63-positive (46.4%) than in p63-negative patients (11.1%, $P = 0.05$) (47). In the same way, some authors found that a lower p63 expression was significantly associated with a poor prognosis in carcinomas of the bladder (48, 49). In our quantitative evaluation for p63 immunoreactivity with clinical behavior, in agreement with Choi *et al.* (24), we found no significant association between the quantity of p63 protein expression and survival curves, and similar to the Reis-Filho *et al.* study in cutaneous carcinomas (18), no significant association was seen between recurrence and metastasis. On the other hand, Lo Muzio *et al.* recently reported that high p63 expression is associated with a more aggressive phenotype and poor prognosis in a study with 94 OSCCs cases (19).

A hypothesis for the different results in p63 expression patterns in the OSCC prognostic results could be related to the differential regulation of p63 isoforms in oncogenesis. According to Thurfjell *et al.*, p63 expression in HNSCC might influence the tumor cell differentiation (15). As the p63 isoform population changes throughout tumor formation and progression, distinct functions of p63 may appear and recede. Designation of p63 as an oncogene or a tumor suppressor could be problematic as the many isoforms may perform opposing functions. Thus, what may be important is not the presence or absence of a given isoform, but rather the p63 predominant isoform regulation.

Conclusion

p53 overexpression, as well as the decreased intensity of p63 immunostaining, is associated with metastases and correlates with a poor outcome. However, despite its high immunoexpression in OSCCs, p63 protein status provides little information about the prognosis of these primary tumors.

References

1. Nylander K, Dabelsteen E, Hall PA. The p53 molecule and its prognostic role in squamous cell carcinomas of the head and neck. *J Oral Pathol Med* 2000; **29**: 413–25.
2. Garcia-Montesinos-Perea B, Val-Bernal JF, Saiz-Bustillo R. Epidermoid carcinoma of the lip: an immunohistochemical study. *Med Oral Patol Oral Cir Bucal* 2005; **10**: 454–61.
3. Varley JM, Brammar WJ, Lane DP, Swallow JE, Dolan C, Walker RA. Loss of chromosome 17p13 sequences and mutation of p53 in human breast carcinomas. *Oncogene* 1991; **6**: 413–21.
4. Macgeoch C, Barnes DM, Newton JA et al. p53 protein detected by immunohistochemical staining is not always mutant. *Dis Markers* 1993; **11**: 239–50.
5. Xu L, Chen YT, Huvos AG et al. Overexpression of p53 protein in squamous cell carcinomas of head and neck without apparent gene mutations. *Diagn Mol Pathol* 1994; **3**: 83–92.
6. Sauter ER, Ridge JA, Gordon J, Eisenberg BL. p53 overexpression correlates with increased survival in patients with squamous carcinoma of the tongue base. *Am J Surg* 1992; **164**: 651–53.
7. Gapany M, Pavelic ZP, Gapany SR et al. Relationship between immunohistochemically detectable p53 protein and prognostic factors in head and neck tumors. *Cancer Detect Prev* 1993; **17**: 379–86.
8. Raybaud-Diogène H, Tétu B, Morency R, Fortin A, Monteil RA. p53 overexpression in head and neck squamous cell carcinoma: review of the literature. *Eur J Cancer B Oral Oncol* 1996; **32B**: 143–49.
9. Sittel C, Ruiz S, Volling P, Kvasnicka HM, Jungehulsing M, Eckel HE. Prognostic significance of Ki-67 (MIB1), PCNA and p53 in cancer of the oropharynx and oral cavity. *Oral Oncol* 1999; **35**: 583–89.
10. Grabenbauer GG, Muhlfriedel C, Rodel F et al. Squamous cell carcinoma of the oropharynx: Ki-67 and p53 can identify patients at high risk for local recurrence after surgery and postoperative radiotherapy. *Int J Radiat Oncol Biol Phys* 2000; **48**: 1041–50.
11. Gonzalez-Moles MA, Galindo P, Gutierrez-Fernandez J et al. P53 protein expression in oral squamous cell carcinoma survival analysis. *Anticancer Res* 2001; **21**(4B): 2889–94.
12. Narayana A, Vaughan AT, Gunaratne S, Kathuria S, Walter SA, Reddy SP. Is p53 an independent prognostic factor in patients with laryngeal carcinoma? *Cancer* 1998; **82**: 286–91.
13. Vielba R, Bilbao J, Ispizua A et al. p53 and cyclin D1 as prognostic factors in squamous cell carcinoma of the larynx. *Laryngoscope* 2003; **113**: 167–72.
14. Sauter ER, Ridge JA, Litwin S, Langer CJ. Pretreatment p53 protein expression correlates with decreased survival in patients with end-stage head and neck cancer. *Clin Cancer Res* 1995; **1**: 1407–12.
15. Thurfjell N, Coates PJ, Boldrup L et al. Function and importance of p63 in normal oral mucosa and squamous cell carcinoma of the head and neck. *Adv Otorhinolaryngol* 2005; **62**: 49–57.
16. Yang A, Kaghad M, Wang Y et al. p63, a p53 homolog at 3q27-29, encodes multiple products with transactivating, death-inducing, and dominant-negative activities. *Mol Cell* 1998; **2**: 305–16.
17. Ghioni P, Bolognese F, Duijf PH, Van Bokhoven H, Mantovani R, Guerrini L. Complex transcriptional effects of p63 isoforms: identification of novel activation and repression domains. *Mol Cell Biol* 2002; **22**: 8659–68.
18. Reis-Filho JS, Torio B, Albergaria A, Schmitt FC. p63 expression in normal skin and usual cutaneous carcinomas. *J Cutan Pathol* 2002; **29**: 517–23.
19. Lo Muzio L, Santarelli A, Caltabiano R et al. p63 overexpression associates with poor prognosis in head and neck squamous cell carcinoma. *Hum Pathol* 2005; **36**: 187–94.

20. Parsa R, Yang A, Mckeon F, Green H. Association of p63 with proliferative potential in normal and neoplastic human keratinocytes. *J Invest Dermatol* 1999; **113**: 1099–105.
21. Hibi K, Trink B, Patturajan M et al. Ais is an oncogene amplified in squamous cell carcinoma. *Proc Natl Acad Sci USA* 2000; **97**: 5462–67.
22. Di Como CJ, Urist MJ, Babayan I et al. p63 expression profiles in human normal and tumor tissues. *Clin Cancer Res* 2002; **8**: 494–501.
23. Wang TY, Chen BF, Yang YC et al. Histologic and immunophenotypic classification of cervical carcinomas by expression of the p53 homologue p63: a study of 250 cases. *Hum Pathol* 2001; **32**: 479–86.
24. Choi HR, Batsakis JG, Zhan F, Sturgis E, Luna MA, EL-Naggar AK. Differential expression of p53 gene family members p63 and p73 in head and neck squamous tumorigenesis. *Hum Pathol* 2002; **33**: 158–64.
25. Weber A, Bellmann U, Bootz F, Wittekind C, Tannapfel A. Expression of p53 and its homologues in primary and recurrent squamous cell carcinomas of the head and neck. *Int J Cancer* 2002; **99**: 22–8.
26. Zigeuner R, Tsybrovskyy O, Ratschek M, Rehak P, Lipsky K, Langner C. Prognostic impact of p63 and p53 expression in upper urinary tract transitional cell carcinoma. *Urology* 2004; **63**: 1079–1083.
27. Association of Directors of Anatomic and surgical Pathology. Recommendations for the reporting of specimens containing oral cavity and oropharynx neoplasms. *Virchows Arch* 2000; **437**: 345–47.
28. Wahi PN, Cohen B, Luthra UK et al. Histological consideration. In: Wahi PN, Cohen B, Luthra UK et al., eds. *Histological typing of oral and oropharyngeal tumors*. Geneva, Switzerland: World Health Organization, 1977; 15–19.
29. Ord RA. Discussion. Primary oral squamous cell carcinoma: a review of 92 cases. *J Oral Maxillofac Surg* 1996; **54**: 955.
30. Sobin LH, Wittekind CH. *TNM classification of malignant tumors*. New York: Wiley-Liss, 1997.
31. De Vicente JC, Junqueira LM, Zapatero AH, Forcelledo MF, Hernández-Vallejo G, Arranz JS. Prognostic significance of p53 expression in oral squamous cell carcinoma without neck node metastases. *Head Neck* 2004; **26**: 22–30.
32. Ramalho LN, Maggiori MS, Ribeiro-Silva A, Peres LC. P63 expression in hydropic abortion and gestational trophoblastic diseases. *Placenta* 2006; **27**: 740–43.
33. Massion PP, Taflan PM, Jamshedur Rahman SM et al. Significance of p63 amplification and overexpression in lung cancer development and prognosis. *Cancer Res* 2003; **63**: 7113–21.
34. Kaplan EL, Meier P. Nonparametric estimation from incomplete observations. *J Am Stat Assoc* 1958; **53**: 457–81.
35. Ha PK, Califano JA. The role of human papillomavirus in oral carcinogenesis. *Crit Rev Oral Biol Med* 2004; **15**: 188–96.
36. Brachman DG, Graves D, Vokes E et al. Occurrence of p53 gene deletions and human papilloma virus infection in human head and neck cancer. *Cancer Res* 1992; **52**: 4832–36.
37. Ahomadegbe JC, Barrois M, Fogel S et al. High incidence of p53 alterations (mutation, deletion, overexpression) in head and neck primary tumors 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.
38. Khademi B, Shirazi FM, Vasei M et al. The expression of p53, c-erbB-1 and c-erbB-2 molecules and their correlation with prognostic markers in patients with head and neck tumors. *Cancer Lett* 2002; **184**: 223–30.
39. Unal OF, Ayhan A, Hosal AS. Prognostic value of p53 expression and histopathological parameters in squamous cell carcinoma of oral tongue. *J Laryngol Otol* 1999; **113**: 446–50.
40. Siegelmann-Danieli N, Ben-Izhack O, Hanlon A et al. P53 alteration in oral tongue cancer is not significantly associated with age at diagnosis or tobacco exposure. *Tumori* 2005; **91**: 346–50.
41. Bortoluzzi MC, Yurgel LS, Dekker NP, Jordan RC, Regezi JA. Assessment of p63 expression in oral squamous cell carcinomas and dysplasias. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2004; **98**: 698–704.
42. Hagiwara K, Mcmenamin MG, Miura K, Harris CC. Mutational analysis of the p63/p73L/p51/p40/CUSP/KET gene in human cancer cell lines using intronic primers. *Cancer Res* 1999; **59**: 4165–69.
43. Nylander K, Vojtesek B, Nenutil R et al. Differential expression of p63 isoforms in normal tissues and neoplastic cells. *J Pathol* 2002; **198**: 417–27.
44. Crook T, Nicholls JM, Brooks L, O'nions J, Allday MJ. High level expression of ΔN-p63: a mechanism for the inactivation of p53 in undifferentiated carcinoma. *Oncogene* 2000; **19**: 3439–44.
45. Pellegrini G, Dellambra E, Golisano O et al. p63 identifies keratinocyte stem cells. *Proc Natl Acad Sci USA* 2001; **98**: 3156–61.
46. Foschini MP, Gaiba A, Cocchi R et al. Pattern of p63 expression in squamous cell carcinoma of the oral cavity. *Virchows Arch* 2004; **444**: 332–9.
47. Takahashi Y, Noguchi T, Takeno S, Kimura Y, Okubo M, Kawahara K. Reduced expression of p63 has prognostic implications for patients with esophageal squamous cell carcinoma. *Oncol Rep* 2006; **15**: 323–28.
48. Urist MJ, Di Como CJ, LU ML et al. Loss of p63 expression is associated with tumor progression in bladder cancer. *Am J Pathol* 2002; **161**: 1199–206.
49. Koga F, Kawakami S, Fujii Y et al. Impaired p63 expression associates with poor prognosis and uroplakin III expression in invasive urothelial carcinoma of the bladder. *Clin Cancer Res* 2003; **9**: 5501–7.

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