p27^{Kip1} expression as a prognostic marker for squamous cell carcinoma of the head and neck (Review)

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Abstract. Regulation of the cell cycle is essential for carcinogenesis. The cell cycle is controlled by cyclin-dependent kinases (CDKs), which are upregulated by cyclins and downregulated by CDK inhibitors (CDKIs). Decreased p27^{Kip1} expression has been associated with survival rate, tumor size, histological differentiation and the presence of lymph node metastasis in patients with various types of cancer. The aim of the current study is to provide a literature review on the association between p27^{Kip1} expression and the clinical and pathological aspects of head and neck squamous cell carcinoma (HNSCC), and the expression of other CDKIs of the Cip/Kip family and cyclins. Throughout the literature, different methodologies were used to determine the immuno-histochemical expression of p27^{Kip1}; thus, results concerning p27^{Kip1} expression in HNSCC vary widely. However, it has now been confirmed that p27^{Kip1} is underexpressed in SCC cells. p27 may be a promising marker for determining the prognosis of HNSCC, despite the marked variability of the results obtained. An association between p27 expression and survival rate, time to recurrence and tumor stage has been observed. Based on the information currently available, it is premature to recommend the analysis of p27^{Kip1} expression in guiding HNSCC treatment planning. However, although relatively unstudied, the correlation between p27^{Kip1} expression and other tumor suppressor genes may turn out to be important in determining the prognosis of HNSCC. Further prospective studies utilizing standardized laboratory methodologies and statistics that facilitate meta-analyses are required to confirm this proposal.

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Contents

1. Introduction
2. p27 expression in HNSCC
3. Progression of p27 expression
4. Correlation between expression of p27 and genes from the Cip/Kip family
5. Correlation between p27 expression and cyclins
6. Conclusions

1. Introduction

Head and neck cancer is growing in prevalence in many regions of the world. Oral and oropharyngeal cancer (excluding naso-pharyngeal cancer) together represent the sixth most common malignant neoplasm, with an estimated annual incidence of 275,000 cases for oral cancer and 130,300 for oropharyngeal cancer, two thirds of which occur in developing countries. Approximately 90% of head and neck cancers are squamous cell carcinomas (1,2).

The carcinogenetic process includes various phases that are necessary for the development and evolution of the neoplasm. Cancer is the result of a series of genetic and epigenetic alterations that occur in multiple steps in a repeated and inter-connected manner influenced by the genetic predisposition of the individual and by exogenous environmental factors (1,3). Collectively, these factors result in a series of molecular altera-tions, including the inactivation of tumor suppressor genes and the activation of oncogenes through deletions, specific muta-tions, promoted methylation and gene amplification (3,4).

Tumor suppressor genes are implicated in various cell divi-sion processes, including gene expression regulation, cell cycle control, apoptosis programming and genome stability (5). The loss of activity of these genes results in an inability to respond to the control mechanisms that regulate cell division, resulting in uncontrolled cell proliferation, the development of neoplasms and their evolution towards more aggressive processes, from mild or moderate dysplasia to *in situ* or invasive carcinoma (5).

Regulation of the cell cycle is an important factor in carci-nogenesis (6). The cell cycle is controlled by cyclin-dependent kinases (CDKs), the activity of which is upregulated by

cyclins and downregulated by CDK inhibitors (CDKIs). CDKs receive signals promoting or inhibiting cell division, thereby coordinating the progress of the cell cycle (6). For example, the transition from G1-S phase is regulated by the activity of cyclin G1/CDK complexes composed of CDK4 and CDK6, which are activated upon association with cyclin D, and CDK2, which is activated upon binding with cyclin E (6). This activity is essential for transition of the restriction point at the late stage of G1 (6,7).

The activity of these CDK enzymes is restricted by the inhibitory action of two major groups of CDKIs: The INK4 family, which comprises the inhibitors p15[INK4B], p16[INK4A], p18[INK4C] and p19[WAF1]; and the Cip/Kip family, which comprises p21[Cip1] (8), p27[Kip1] and p57[Kip2]. The Cip/Kip family inhibits cyclin/CDK complexes, including cyclin D/CDK4, cyclin D/CDK2, cyclin E/CDK2 and cyclin A/CDK2 (Fig. 1) (9,10).

First discovered in 1993, p27[Kip1] exhibits a unique responsiveness pattern to a wide range of mitogenic and antimitogenic signals, making it notably different from the other two members of the CDKI Cip/Kip family. For example, p27 can inhibit directly without mediators cyclin D and CDK4/6 (11). Although p27 mutations are rare in human tumors, decreased p27 expression has been associated with survival rate, tumor size, histological differentiation and the presence of lymph node metastasis in patients with various types of cancer (12-15). The mechanism by which p27 is silenced remains unclear: Whilst its expression is regulated transcriptionally and translationally, its levels are predominantly regulated by ubiquitin-dependent proteolysis mechanisms (16).

The aim of the current study is to provide a literature review on the association between p27 expression and the clinical and pathological aspects of HNSCC, the expression of CDKIs of the Cip/Kip family and cyclins.

2. p27 expression in HNSCC

The Cochrane database, MEDLINE and EMBASE were searched on January 27, 2014, using the following keywords: 'p27 oral squamous cell carcinoma', 'p27 head and neck squamous cell carcinoma' and 'p27 mouth neoplasms'. From this search, 29 studies of p27 expression in HNSCC were identified, the results of which varied widely (17-45). Different methodologies were used to determine the immunohistochemical expression of p27: Certain studies used a quantitative analysis of the percentage of stained cells, whereas the vast majority used a semi-quantitative analysis, although with different degrees of cell and sample staining. The various antibodies used were also applied at different concentrations. In order to compare the results, Table I shows the percentage of tumors with positive expression as reported by various studies; different definitions of positivity have been used, depending on the methodology used in each case. A wide variability was observed, with a range of 3-89%. By contrast, the difference in positivity rate between studies that employed a quantitative analysis was markedly lower, with the p27 score varying from 10±10 to 56.4±16.2% (Table II).

Thus, differences in expression between the different working groups may be due to differences in immunohistochemistry, in addition to population characteristics and specimen variation. Similarly, it is essential to standardize semi-quantitative analysis methods to allow methodological unification of the different studies, and thus enable performance of systematic reviews and meta-analyses.

3. Progression of p27 expression

Numerous studies have investigated p27 expression throughout various phases of HNSCC progression, comparing normal mucosa with dysplastic lesions, carcinoma *in situ*, verrucous carcinoma and SCC (Table III). In certain of these studies, the comparison was performed qualitatively on the basis of positive or negative expression. Thus, Kudo *et al* (25) observed that the percentage of tumors with positive expression was significantly lower in SCC and in lesions with severe dysplasia compared with that in normal mucosa (P<0.05). Queiroz *et al* (39) also found that p27 expression is lower in oral squamous cell carcinoma (OSCC) and in benign lesions compared with in normal tissue (P<0.05). By contrast, Schoelch *et al* (22) and Shintani *et al* (33) found no statistically significant differences. In other studies, a quantitative analysis of the percentage of stained cells (p27 score) was conducted. Thus, Jordan *et al* (19) reported that p27 expression in lesions with moderate dysplasia, severe dysplasia and SCC is lower than that in normal mucosa (P<0.05), whilst Saito *et al* (23) observed lower p27 expression in verrucous carcinoma compared with in SCC (P<0.001). Similarly, Kuo *et al* (29) obtained significantly reduced p27 expression values in SCC compared with that in dysplastic lesions, and in the latter compared with in normal mucosa.

With regard to the age of the patient at the onset of the neoplasm, no statistically significant differences were detected. In certain studies, the mean age of patients with tumors positive and negative for p27 expression was calculated, and no differences were observed between the two (18,21,28). Similarly, other studies compared the percentage of tumors positively expressing p27 in younger and older patients (below and above the mean cohort age, respectively) and also identified no differences (29,32,41,46).

Regarding gender, no differences in p27 expression were observed between males and females (21,28,32,41).

The results concerning survival are controversial (17,18, 21,24-26,28,29,32,38,41,43,45,46). In the majority of solid tumors, p27 expression silencing is associated with a decrease in the five-year survival rate. As can be seen in Table IV, in the case of HNSCC, the majority of studies have also reported similar findings, some with statistically significant differences (21,24,27-29,33,38,41). However, in studies where improved survival rates were observed for tumors with negative p27 expression, the differences were not statistically significant (18,32,42,45).

Relapse rate has been analyzed to a lesser extent. Studies by Perisanidis *et al* (43) and Monteiro *et al* (42) analyzed the risk of relapse and observed no significant differences between tumors with different p27 expression values. Canzonieri *et al* (44) observed a lower p27 expression positivity in tumors with relapse, however, the differences were not statistically significant. With regard to the time at which relapse occurred, Venkatesan *et al* (24) reported that the mean was 324 days in patients with tumors exhibiting low p27 expression levels, significantly lower than for patients with high expression levels.

Table I. Semi-quantitative analysis of immunohistochemical p27^{Kip1} expression in HNSCC.

Author, year (reference)	HNSCC cases, n	Location	Definition of positivity, %	Positive cases, %	Antibody dilution
Kudo *et al*, 1998 (17)	70	Oral	30	13	Transduction 1:100
Fujieda *et al*, 1999 (18)	60	Oral/oropharynx	40	30	Oncogene
Ito *et al*, 1999 (20)	43	Oral	30	14	Transduction 1:500
Mineta *et al*, 1999 (21)	94	Tongue	50	26	Transduction 1:100
Schoelch *et al*, 1999 (22)	20	Oral/oropharynx	33	75	Novocastra 1:25
Venkatesan *et al*, 1999 (24)	35	Oral/oropharynx	11	77	Transduction 1:1,000
Kudo *et al*, 2000 (25)	17	Oral	30	59	Transduction 1:100
Kapranos *et al*, 2001 (26)	31	Oral	10	65	Santa Cruz 1:50
Kudo *et al*, 2001 (27)	37	Oral	5	49	Transduction 1:100
Harada *et al*, 2002 (28)	81	Oral	50	63	Novocastra 1:100
Kuo *et al*, 2002 (29)	63	Oral	11	25	Transduction 1:500
Shintani *et al*, 2002 (30)	117	Oral	5	64.1	Dakopatts 1:1,000
Choi *et al*, 2003 (32)	30	Oral[a]	5	36.6	Neo Markers 1:100
Shintani *et al*, 2003 (33)	75	Oral	25	26.6	Transduction 1:100
Kitajima *et al*, 2004 (35)	63	Oral	30	51	Transduction 1:100
Rodolico *et al*, 2004 (36)	95	Lower lip	19.7	70	Transduction 1:1,200
Rodolico *et al*, 2005 (37)	97	Lower lip	20	78	Transduction 1:1,200
Filies *et al*, 2007 (38)	189	Oral	1	20	Pharmingen 1:1,000
Queiroz *et al*, 2010 (39)	34	Oral	50	3	Santa Cruz 1:100
Martín-Ezquera *et al*, 2011 (40)	49	Oral	5	38	BD Biosciences 1:100
Gao *et al*, 2012 (41)	206	Oral	10	60.2	Zhongshan 1:30
Monteiro *et al*, 2012 (42)	51	Oral	25	89	Novocastra 1:20
Perisanidis *et al*, 2012 (43)	111	Oral/oropharynx	50	69	Neo Markers
Canzonieri *et al*, 2012 (44)	25	Oropharynx/hypopharynx/larynx	5	25	Transduction 1:100

[a]All but two tumors (in the larynx and parotid) were oral. HNSCC, head and neck squamous cell carcinoma.

Table II. Quantitative analysis of immunohistochemical p27^{Kip1} expression in HNSCC.

Author, year (reference)	HNSCC cases, n	Location	p27 score, %	Antibody dilution
Jordan *et al*, 1998 (19)	8	Oral	28.7±5.1	Transduction 1:500
Fujieda *et al*, 1999 (18)	60	Oral/oropharynx	31.1±30	Oncogene
Saito *et al*, 1999 (23)	44	Oral	24±6	Transduction 1:5,000
Kapranos *et al*, 2001 (26)	31	Oral	56.4±16.2	Santa Cruz 1:50
Kuo *et al*, 2002 (29)	63	Oral	10±10	Transduction 1:500
Rodolico *et al*, 2004 (36)	95	Lower lip	19.7	Transduction 1:1,200
Rodolico *et al*, 2005 (37)	97	Lower lip	26.4	Transduction 1:1,200
Zhang *et al*, 2013 (45)	110	Oral	55.46	Zhongshan

HNSCC, head and neck squamous cell carcinoma.

The presence of lymph node metastasis is a clinicopathological factor known to be associated with poorer prognosis. As such, it is important to determine whether any association exists between p27 silencing and the appearance of lymph node metastasis. The majority of published studies report that patients with a positive lymph node metastasis status have reduced p27 expression levels compared with those without lymph node metastasis (21,27,33,36); in studies that reported contrasting results, the differences were not statistically significant (20,29).

Advanced tumor stages are also associated with poorer prognosis in HNSCC. Thus, in theory, lower p27 expression levels should be observed in tumors of more advanced stages. Indeed, all studies identified in the literature that

Table III. Progression of p27Kip1 expression during development of HNSCC as assessed by semi-quantitative or quantitative analysis.

A, Semi-quantitative analysis

Author (reference)	Definition of positivity	p27+, % [n]								P-value
		NM	OBL	MiD	MoD	SD	ISC	VC	HNSCC	
Schoelch et al (22)	>30%	100 [8]	100 [4]	100 [9]	93.3 [15]	57 [7]	0 [3]	ND	75 [20]	ND
Kudo et al (25)	>30%	ND	ND	100 [4]	100 [7]	69 [6]a	ND	ND	18 [17]a	P<0.05a
Shintani et al (30)	>5%	100 [20]	ND	100 [12]	100 [12]	92.9 [14]	ND	ND	64.1 [117]	ND
Queiroz et al (39)	>25%	81.3 [32]	66.7 [30]a	ND	ND	ND	ND	ND	2.9 [34]a	P<0.05a

B, Quantitative analysis

Author (reference)	p27+, % [n]								P-value
	NM	OBL	MiD	MoD	SD	ISC	VC	HNSCC	
Jordan et al (19)	49.6±5.8 [10]	ND	46.2±7.4 [8]	33.0±6.5 [11]a	23.4±2.7 [17]a	ND	ND	28.7±5.1 [8]a	P<0.05a
Saito et al (23)	53.0±4.28 [10]	ND	40.7±7.2 [23]	32.1±7.19 [23]	28.4±13.5 [11]	ND	52.6±15.7 [15]	24.0±6.0 [44]	P<0.001b
Kuo et al (29)	65±10[10]a	ND	ND	32±11 [19]a*	ND	ND	ND	10±10[63]a	P<0.05a

aP<0.05 vs. normal mucosa. bP<0.001 vs. normal mucosa. *Includes all patients with mild to severe dysplasia. NM, normal mucosa; OBL, oral benign lesion; MiD, mild dysplasia; MoD, moderate dysplasia; SD, severe dysplasia; ISC, in situ carcinoma; VC, verrucous carcinoma; HNSCC, head and neck squamous cell carcinoma; ND, not determined.

Table IV. Analysis of the P-value obtained when comparing survival rates for tumors with high and low p27^{Kip1} expression.

Author (reference)	Cases, n	Minimum follow-up, years	Definition of p27 positivity	Survival, %		P-value	Test used
				Low p27 expression	High p27 expression		
Kudo et al (17)	70	5	5%	50	80	<0.05	Kaplan-Meier curve (Mantel-Cox Test)
Fujieda et al (18)	60	5	40%	57.8	50.7	0.69	Kaplan-Meier curve (Log-Rank Test)
Mineta et al (21)	94	5	50%	44	68	0.04	Kaplan-Meier curve (Log-Rank Test)
Venkatesan et al (24)	35	2	35%	~20	~65	0.0001	Kaplan-Meier curve (Log-Rank Test)
Kudo et al (25)	37	5	5%	~50	~80	ND	Kaplan-Meier curve (Mantel-Cox Test)
Kapranos et al (26)	31	5	10%	19.2	21	0.11	Kaplan-Meier curve (Log-Rank Test)
Harada et al (28)	81	5	50%	56.7	80.4	0.009	Kaplan-Meier curve (Log-Rank Test)
Kuo et al (29)	63	1	10%	~65	~92	0.01587	Kaplan-Meier curve (Log-Rank Test)
Choi et al (32)	30	3	5%	63.2[a]	36.4[b]	ND	None
Shintani et al (33)	75	2	25%	~50	~75	0.027	Kaplan-Meier curve (Log-Rank Test)
Fillies et al (38)	189	5	1%	~25	~60	0.03	Kaplan-Meier curve (Log-Rank Test)
Gao et al (41)	206	5	10%	~40	~70	0.001	Kaplan-Meier curve
Monteiro et al (42)	51	3	25%	66.7	51.3	0.233	Cox proportional hazards model
Perisanidids et al (43)	111	5	50%	ND	ND	0.41	Univariate Cox regression
Zhang et al (45)	110	5	2.8[c]	73	64	0.27	Cox proportional hazards model

[a]12/19 cases. [b]4/11 cases. [c]Mean protein expression, used as cut-off value for high/low expression. Values that appear as approximate are those estimated upon analysis of the Kaplan-Meier curves. ND, not determined.
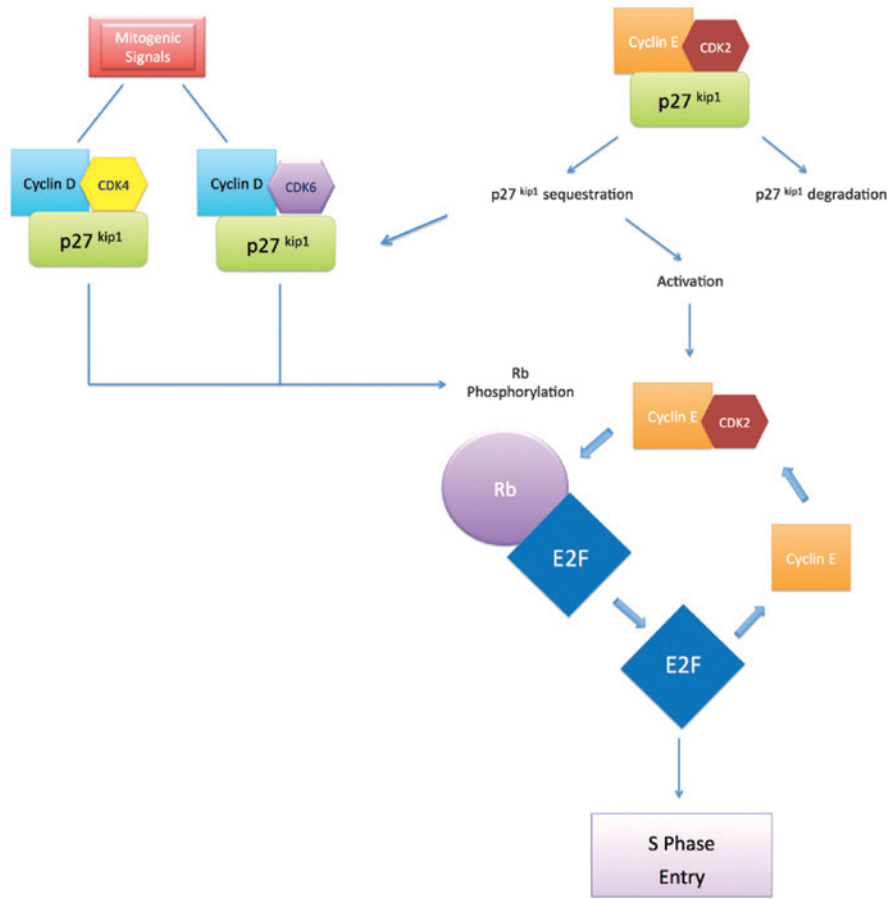
Figure 1. p27[Kip1] activity in regulation of the G1/S phase transition. Mitogenic signals promote the formation of cyclin D/CDK4 and cyclin D/CDK6 complexes that are inhibited by p27[Kip1]. Sequestration of p27[Kip1] lowers the inhibitory activity and facilitates the activation of cyclin E/CDK2 complex. The formation of cyclin D and cyclin E/CDK complexes contribute to Rb phosphorylation, activating E2F family members required for progression of the cell cycle to S phase. Cyclin E/CDK2 antagonizes p27[Kip1] by phosphorylation, which promotes its degradation. CDK, cyclin-dependent kinase; Rb, retinoblastoma protein.

relate p27 expression with tumor stage report that expression is reduced in the advanced stages compared with in earlier stages (18,21,27-29,32,33,41). Furthermore, the majority of studies reported statistically significant differences (18,21,27,41), thereby, to some extent, confirming that p27 silencing is important role in the development of HNSCC. Thus, if p27 expression is associated with tumor stage, tumor size should also be related. However, in the few studies that have investigated the correlation between tumor size and p27 expression, the results obtained were not consistent with this hypothesis. Mineta *et al* (21), for example, reported statistically significant differences for tumor stage, but no such differences for tumor size. These findings were in accordance with those of Harada *et al* (28) and Kuo *et al* (29), who observed a trend in which p27 was overexpressed in smaller tumors.

With regard to tumor differentiation, although reduced p27 expression may be expected in poorly differentiated tumors, studies into the correlation between p27 expression and tumor differentiation indicate a tendency for this gene to be silenced in poorly differentiated tumors; however, the vast majority of studies report no statistically significant differences (20,21,27,29,32,33,41). Harada *et al* (28) is the only study to have identified a significant correlation between histological grade and decreased p27 expression.

Concerning other clinicopathological factors, such as smoking habit, alcohol consumption, tumor invasion and tumor depth, no statistically significant differences indicating any association with these factors and p27 expression have been reported in any studies, to the best of our knowledge (17-45).

Thus, regarding the multistep progression of oral cancer, almost all studies confirm that p27 expression is reduced as the degree of dysplasia or the differentiation of the tumor increases.

4. Correlation between expression of p27 and genes from the Cip/Kip family

In a similar manner to p27, the other members of the Cip/Kip family, p21[Cip1] and p57[Kip2], also inhibit cyclin/CDK complexes by blocking the cell cycle at the G1-S phase checkpoint. However, although Fan *et al* (47) considered p57 expression to be a good prognostic marker for OSCC, it has received relatively little attention. Therefore, the current review will focus more on the correlation between p21 and p27. As is the case for p27, a loss of p21 expression is observed in HNSCC. However, according to Shintani *et al* (30), this occurs during the initial stages of carcinogenesis and, probably as a result, the correlation between p21 expression and the clinicopathological parameters is highly variable, although the appearance of lymph node metastases is often related to p21 silencing. With regard to the association between p21 and p27 expression, the results in the literature are somewhat unclear. Whilst

Choi *et al* (32) reported an inverse correlation (P=0.08) between the two, this was not related to clinicopathological parameters. Similarly, a study by Zhang *et al* (45) revealed a statistically significant correlation between the expression of the two genes by way of a Pearson analysis, whereas Fillies *et al* (38) obtained different results, although a different statistical test (Cox regression model) was used and thus, the results are not comparable. Upon analyzing tumors of the oral cavity and larynx together, Kapranos *et al* (26) also failed to find a correlation between the expression of these genes, or any differences in survival rate between the four possible expression combinations.

Although the results are not entirely convincing, it appears that the activity of p27 is independent of other cell cycle regulators of the Cip/Kip family.

5. Correlation between p27 expression and cyclins

The present study focussed on reviewing the association between p27 expression and cyclin expression. In contrast to p27 expression, a number of studies have reported that cyclins A, D, and E are overexpressed in SCC compared with in normal mucosa, thereby confirming the cyclin-inhibitory role of p27. Various studies have also reported that overexpression of cyclins is associated with tumor stage, histological differentiation of the tumor and the presence of lymph node metastases, thereby affecting the prognosis of patients in terms of survival rate and time to recurrence. Pignataro *et al* (48) investigated the correlation between p27 and cyclin D1 expression in HNSCC, including tumors of the larynx, and observed an inverse correlation whereby the majority of tumors that exhibit positive p27 expression also present negative cyclin D1 expression, and those with positive cyclin D1 expression exhibit negative p27 expression. The same study also reported that patients with cyclin D1+/p27- tumors have a poorer prognosis, whilst those with cyclin D1-/p27+ tumors have a better prognosis in terms of survival rate (P=0.0015) and time to recurrence (P=0.0001). Rodolico *et al* (37) found that tumors overexpressing cyclin D1 and underexpressing p27 are associated with the appearance of lymph node metastases in patients with SCCs of the lower lip. Kuropkat *et al* (31) believe that the results obtained in their study, in which no relationship was identified between cyclin D1 expression and time to recurrence when p27 expression was also considered, are unreasonable, as both overexpression or very low expression of cyclin D1 were associated with shorter time-to-recurrence. Fujieda *et al* (18) obtained statistically significant results that contradict those found in the literature and current theories of cell cycle regulation (12,48,37,31): The results revealed that tumors with positive cyclin D1 expression exhibit a higher percentage of cells stained by the p27 antibody compared with that of tumors with negative cyclin D1 expression (42.7±31.9 vs. 25.2±29.3%; P=0.001), whereas the majority of previous studies have demonstrated an inverse correlation between between cyclin D1 and p27 expression.

Ito *et al* (20) reported a strongly significant inverse correlation between cyclin E and p27 expression (cyclin E+/p27-, 33/42; cyclin E+/p27+, 3/42; cyclin E-/p27-, 2/42; and cyclin E-/p27+, 4/42; P<0.001) but did not investigate this association with regard to clinicopathological

factors. Additionally, Jordan *et al* (19) identified an association between increased cyclin A expression and reduced p27 expression in SCC.

Thus, although further confirmation is required, the literature to date suggests that the expression of CDKIs is inversely correlated with the expression of cyclins A, D, and E (12).

6. Conclusions

A wide variety of tumor behaviors have been reported at the different sites in the head and neck (21). Thus, in future, more objective analyses and immunohistochemical expression studies must be performed at specific anatomical locations, as already undertaken by a number of research groups (21,36,37).

Various studies of p27 expression in HNSCC have been performed over the last two decades, and there is now no doubt that p27 is underexpressed in SCC cells (12,13). p27 may be a promising marker for determining the prognosis of HNSCC, despite the marked variability of the results obtained. An association between p27 expression and survival rate, time to recurrence and tumor stage has been observed (24,48). Based on the information currently available, it is too early to recommend the analysis of p27 expression for guiding HNSCC treatment planning. Although relatively unstudied, the correlation between p27 expression and other tumor suppressor genes may turn out to be important for determining the prognosis of HNSCC. However, further prospective studies and studies involving standardized laboratory methodologies and statistics that allow meta-analyses to be performed are required to confirm this proposal.

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