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ORIGINAL ARTICLE

Expression of PAR-4 and PHLDA1 is prognostic for overall and disease-free survival in oral squamous cell carcinomas

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Abstract PAR-4 is a tumor suppressor protein with a pro-apoptotic function and down-regulation of PAR-4 is seen in a variety of tumors. PHLDA1 gene overexpression has been shown to reduce cell proliferation and induce cell death in a variety of cell types. In this study, 229 cases of oral squamous cell carcinoma (OSCC), arranged in a tissue microarray, were analyzed by immunohistochemistry. PAR-4 expression was predominantly moderate to strong and expression of PHLDA1 was predominantly negative or weak. Cytoplasmic expression of PAR-4 was associated with advanced clinical stage. Expression of PHLDA1 was associated with advanced clinical stage of the tumour. Five-year overall and disease-free survival rates differed significantly between cases that did and cases that did not express PHLDA1, and by multivariate analysis, expression of PHLDA1 and PAR-4 were independent prognostic factors in OSCC patients. Expression of PAR-4 and PHLDA1 is altered in OSCC and might be a valuable prognostic indicator for this disease.

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Introduction

Squamous cell carcinoma (SCC) constitutes at least 90 % of all oral malignancies and is the eighth-most prevalent cancer worldwide [1, 2]. OSCC is also highly prevalent in Brazil, where 15,170 new cases of oral cancer were estimated to have developed in 2012 [3]. The prognosis of oral cancer patients has remained stable over the last 20 years. The identification of prognostic factors will help clinicians to establish a more appropriate therapeutic plan, according to the rate of recurrence.

The primary function of apoptosis is to eliminate senescent or altered cells that are useless or harmful to a multicellular organism. Altered expression levels of apoptosis-associated proteins have been reported in several cancers, including oral cancer [4–6]. The development of resistance to apoptosis is a hallmark of malignant cells, enabling them to survive, despite apoptosis-inducing environmental signals, and the loss of normal survival signals [7, 8].

PAWR [PKC apoptosis WT1 regulator, or PAR-4 (prostate apoptosis response-4)] was originally identified in androgen-independent prostate cancer cells that were undergoing apoptosis. *PAR-4* is located on chromosome 12q21 and encodes a 38-kDa protein that contains 2 putative nuclear localization sequences in its N-terminus, a SAC (selective apoptosis induction in cancer cells) domain in the central region (137–195 aa), and a leucine zipper in the C terminus that allows PAR-4 to bind and form complexes with various proteins, including WT1, PKC, DAXX, and p62 [9]. PAR-4 overexpression selectively induces apoptosis in cancer cells, and alterations in PAR-4 mRNA and protein levels occur in various types of tumors [9].

PAR-4 is downregulated in many cancers, such as renal cell carcinomas[10], neuroblastoma [11], acute lymphoblastic leukemia, chronic lymphocytic leukemia [12], breast cancer [13],

and endometrial cancer [14]. Recently, Lee et al. (2010) noted robust and extensive expression of PAR-4 in nasopharyngeal carcinoma, compared to other epithelial malignant neoplasms of the head and neck (hypopharyngeal carcinoma and oral cavity cancers) [15].

PHLDA1 (pleckstrin homology-like domain, family A, member 1; also called TDAG51) is located on chromosome 12q15 and encodes a 401-amino-acid protein that harbors a pleckstrin homolog (PHL) domain that spans residues 150 to 283, interrupted by a small proline/glutamine-rich sequence (QQ), and protein–protein interaction domains in its carboxy-terminal region, such as proline-glutamine (PQ)- and proline-histidine (PH)-rich tracts [16]. Overexpression of PHLDA1 impairs cell proliferation and induces cell death in many cell types, including T cells and neuronal, endothelial, melanoma, and cervical carcinoma cells [17–19]. Loss of PHLDA1 mRNA and protein correlates with the progression of breast adenocarcinoma and melanoma in clinical samples [20, 21].

Although changes in PAR-4 expression occur in head and neck neoplasms, expression of PAR-4 has been evaluated by immunohistochemistry in only a small set of OSCC samples [15], and no study has examined the expression of PHLDA1 in OSCC. The aim of this study was to characterize the expression of PAR-4 and PHLDA1 by immunohistochemistry on a tissue microarray (TMA), containing 229 cases of OSCC, and determine whether their expression is associated with the clinicopathological features and clinical outcome of patients with OSSC.

Materials and methods

Tissue samples

Paraffin-embedded tissue samples from 229 primary site oral squamous cell carcinoma cases were obtained from the files of the Department of Pathology, AC Camargo Cancer Center, São Paulo, Brazil. All retrieved cases had been untreated and underwent surgery as the initial treatment at the hospital between 1970 and 1992 and had been followed-up for at least 5 years. All cases were primary tumors; no tissues from cases of recurrences or metastases were examined. The clinical and histological details of these cases are shown in Table 1. The Institutional Ethics Committee approved this study (Protocol number 985/07).

Tissue microarray (TMA)

To construct the tissue microarray, H&E sections were analyzed, and a representative area of the deepest tumor sheet was marked on the slide. The tissues that corresponded to the selected areas were sampled from the donor block using a tissue micro-arrayer (Beecher Instruments, Silver Springs, USA). Each sample was arrayed twice with a 1.0-mm-diameter core, spaced 0.2 mm apart. After the array was constructed, tissue microarray blocks were sectioned at thicknesses of 4 μm.

Immunohistochemistry

The expression of PAR-4 and PHLDA1 was examined in OSCC tissue samples on a tissue microarray. Immunohistochemical staining was performed on duplicate tissue slides; duplicate sections were separated by 40 μm. The following antibodies were purchased from Santa Cruz Biotechnology: PAR-4, clone A-10, working titer 1:100; and PHLDA1, clone M-20, working titer 1:100.

The slides were deparaffinized, rehydrated, and subjected to antigen retrieval using citrate buffer, pH 6.0. The sections were incubated in 3 % aqueous hydrogen peroxide for 15 min to quench endogenous peroxidase activity and Protein Block Serum-Free (Dako, Carpinteria, CA, USA) for 20 min at room temperature to suppress nonspecific binding of subsequent reagents. Next, we incubated the sections with primary antibody for 2 h at room temperature.

The antigen–antibody complexes were visualized using a streptavidin-biotin peroxidase LSAB kit (Dako, Carpinteria, CA, USA) and incubated with 3′3 diaminobenzidine tetrachloride (DAB; Dako, Carpinteria, CA, USA) for 5 min. The sections were counterstained with Mayer's hematoxylin, dehydrated, and mounted with a glass coverslip and xylene-based mounting media. Positive controls were used as per the manufacturer's recommendations.

Semiquantitative analysis of the results was performed using a conventional optical microscope, considering the following scores, based on the number of cells that were stained and the staining intensity: 0, negative/weak staining (up to 10 % of cells stained and only visible at ×40); 1, moderate staining (10 % to 50 % of cells stained and visible at ×20); and 2, strong staining (more than 50 % of cells stained and easily detectable at ×10). All cores had been previously evaluated using a ×20 objective lens, and the results were only considered when there tumor was present in over 50 % of the core represented. For statistical analysis, we grouped the cases into two categories: negative (negative/weak expression) and positive (moderate/strong expression).

Statistical analysis

Associations between protein levels and demographic and clinicopathological characteristics of the patients were analyzed by chi-square test. We analyzed differences in expression between the following categories: T stage (T1/T2 and T3/T4a), clinical stage (I/II and III/IV), tumor site (oral tongue, floor of mouth, or other sites), lymph node metastasis (yes or no), vascular invasion (yes or no), perineural infiltration (yes or no), and

Table 1 Summary of clinicopathological characteristics of oral squamous cell carcinoma (OSCC) patients

Variables	Categories	Number of patients (%)
Age (years)[a]	≤56	117 (51.1)
	>56	112 (48.9)
Gender	Male	194 (84.7)
	Female	35 (15.3)
Tobacco smoking	No	19 (8.3)
	Yes	182 (79.5)
	n/a[b]	28 (12.2)
Alcohol consumption	No	45 (19.7)
	Yes	153 (66.8)
	n/a	31 (13.5)
T stage	T1/T2	131(57.2)
	T3/T4a	98 (42.8)
Clinical Stage	I/II	77 (33.6)
	III/IV	152 (66.4)
Tumor site	Oral tongue	122 (53.3)
	Floor of mouth	55 (24.0)
	Other	52 (22.7)
Lymph node metastasis (pN)	No	92 (40.2)
	Yes	112 (48.9)
	n/a	25 (10.9)
Perineural infiltration	No	129 (56.3)
	Yes	86 (37.6)
	n/a	14 (6.1)
Vascular invasion	No	72 (31.4)
	Yes	141 (61.6)
	n/a	16 (7.0)
Histological grade	Well differentiated	177 (77.3)
	Moderately/poorly differentiated	46 (20.1)
	n/a	6 (2.6)
Treatment	Surgery	118 (51.5)
	Surgery+radiotherapy	111 (48.5)

[a] Median value of all patients' age was adopted as a cut-off

[b] n/a information not available

histological grade (well differentiated or moderatelly/poorly differentiated).

Overall and disease-free survival probabilities were calculated by Kaplan–Meier method, using log-rank test to determine statistical significance. Relative risk was evaluated by the multivariate Cox proportional hazards model. The multivariate model was adjusted by T stage, clinical stage, and lymph node metastasis. The significance level was set to 5 % for all statistical tests. Statistical analyses were performed using R, version 2.13 (R Development Core Team (2010), Vienna, Austria, www.R-project.org).

Results

PAR-4 and PHLDA1 were expressed in the primary oral squamous cell carcinoma (OSCC) samples that we studied at varying levels: PAR-4 showed cytoplasmic/nuclear staining and PHLDA1 was cytoplasmic in neoplastic and control cells. Twenty-three cases (11.6 %) showed negative/weak nuclear staining of PAR-4, 126 cases (63.3 %) showed moderate staining, and 50 cases (25.1 %) showed strong staining. Seventy-two cases (36.2 %) showed negative/weak cytoplasmic staining for PAR-4. These cases were mostly moderately differentiated OSCC and presented large areas of basaloid neoplastic cells. In 106 cases (53.3 %), PAR-expression was moderate and cytoplasmic. In 21 cases (10.5 %), strong cytoplasmic expression of PAR-4 was noted. These cases were well-differentiated OSCC, and PAR-4 immunoreactivity was concentrated in the cytoplasm of keratinizing neoplastic cells surrounding keratin pearls. The majority of the cases (124,62.3 %) showed (moderate or strong) both cytoplasmic and nuclear staining for PAR-4. For PHLDA1, 122 cases (60.7 %) showed negative/weak staining; 75 cases (37.3 %) showed moderate staining, and 4 cases (2.0 %) showed strong staining. This pattern was present regardless the differentiation status of the neoplasm. PAR-4 was expressed at moderate intensity in the middle and lower layers of normal oral squamous mucosa, whereas PHLDA1 was absent (Fig. 1). This pattern was similar in dysplastic areas of the oral epithelium.

For statistical analysis, we grouped the cases into 2 categories: negative (negative/weak expression) and positive (moderate/strong expression). Loss of cytoplasmic expression of PAR-4 was associated with early T stage of the disease (p=0.03; Table 2). Cytoplasmic expression of PAR-4 correlated with advanced clinical and pathological stages of the disease (p=0.01 and p=0.03, respectively; Table 2). Expression of PHLDA1 was associated with advanced clinical stage of the tumor (p=0.02). Loss of PHLDA1 expression was observed more frequently in with well-differentiated tumors (p<0.001; Table 2).

Five-year cancer-specific and disease-free survival rates differed significantly between patients with and without expression of PHLDA1 (p=0.02 and p=0.01, respectively). Median 5-year cancer-specific survival was 62.0 % for patients who did not express PHLDA1 and 46.0 % for those who did express PHLDA1. Median 5-year disease-free survival was 61.0 % for patients who did not express PHLDA1 and 42.0 % for patients who did express PHLDA1 (Tables 3, 4, and 5).

For PAR-4 expression, 5-year cancer-specific and disease-free survival rates did not differed between patients with negative and positive expression, even when both subcellular localizations where considered (Tables 3, 4, and 5).

Considering the expression of both PAR-4 and PHLDA1, 5-year cancer-specific and disease-free survival rates

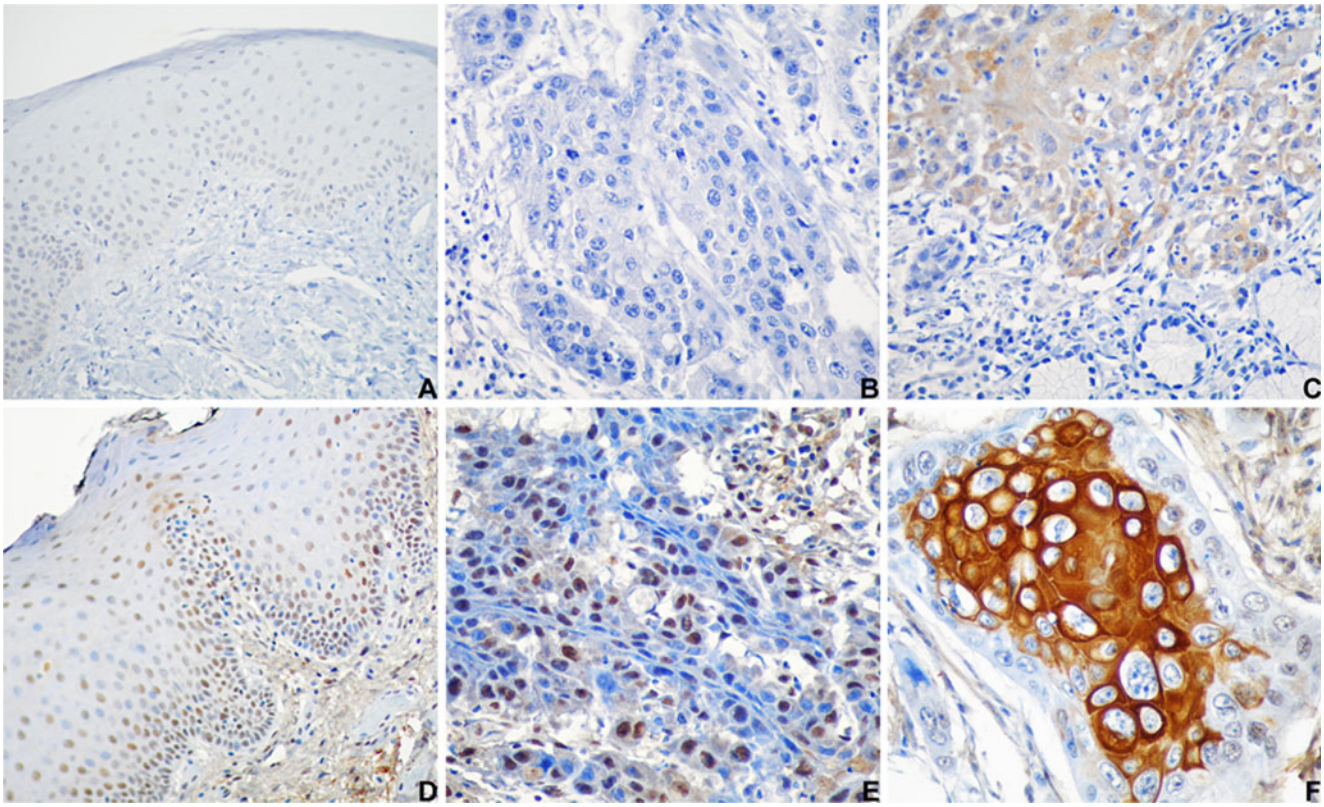

Fig. 1 Expression of PAR-4 and PHLDA1 in oral squamous cell carcinoma and normal oral mucosa. **a**, PHLDA1 expression in normal oral mucosa; **b**, negative PHLDA1 expression in oral squamous cell carcinoma; **c**, positive PHLDA1 expression in oral squamous cell carcinoma; **d**, PAR-4 expression in normal oral mucosa; **e**, PAR-4 nuclear expression in oral squamous cell carcinoma; **f**, PAR-4 cytoplasmic expression in oral squamous cell carcinoma

Table 2 Association between protein expression and clinicopathological characteristics of oral squamous cell carcinoma (OSCC) patients

Characteristic	Category	PHLDA1 expression		*p* value[a]	PAR-4 cytoplasmic expression		*p* value[a]	PAR-4 nuclear expression		*p* value[a]
		Negative *n* (%)	Positive *n* (%)		Negative *n* (%)	Positive *n* (%)		Negative *n* (%)	Positive *n* (%)	
Tumor site	Tongue	65 (53.3)	41 (51.9)	0.64	43 (59.7)	62 (48.8)	0.19	14 (60.9)	91 (51.7)	0.67
	Floor of mouth	26 (21.3)	21 (26.6)		17 (23.6)	30 (23.6)		4 (17.4)	43 (24.4)	
	Other sites	31 (25.4)	17 (21.5)		12 (16.7)	35 (27.6)		5 (21.7)	42 (23.9)	
T stage	T1/T2	71 (58.2)	36 (45.6)	0.08	48 (66.7)	64 (50.4)	**0.03**	17 (73.9)	95 (54.0)	0.07
	T3/T4a	51 (41.8)	43 (54.4)		24 (33.3)	63 (49.6)		6 (26.1)	81 (46.0)	
Clinical stage	I/II	45 (36.9)	17 (21.5)	**0.02**	32 (44.4)	34 (26.8)	**0.01**	10 (43.5)	56 (31.8)	0.26
	III/IV	77 (63.1)	62 (78.5)		40 (55.6)	93 (73.2)		13 (56.5)	120 (68.2)	
Histological grade	Well-differentiated	104 (88.1)	51 (66.2)	<**0.01**	57 (80.3)	97 (78.9)	0.81	21 (91.3)	133 (77.8)	0.22
	Moderately/poorly differentiated	14 (11.9)	26 (33.8)		14 (19.7)	26 (21.1)		2 (8.7)	38 (22.2)	
Vascular invasion	No	35 (31.8)	28 (36.8)	0.48	29 (42.6)	35 (29.2)	0.06	8 (40.0)	56 (33.3)	0.55
	Yes	75 (68.2)	48 (63.2)		39 (57.4)	85 (70.8)		12 (60.0)	112 (66.7)	
Perineural infiltration	No	65 (58.6)	46 (59.7)	0.87	44 (64.7)	67 (55.4)	0.21	11 (52.4)	100 (59.5)	0.53
	Yes	46 (41.4)	31 (40.3)		24 (35.3)	54 (44.6)		10 (47.6)	68 (40.5)	
Lymph node metastasis	No	48 (44.4)	30 (40.0)	0.55	32 (54.2)	47 (39.2)	0.06	11 (57.9)	68 (42.5)	0.20
	Yes	60 (55.6)	45 (60.0)		27 (45.8)	73 (60.8)		8 (42.1)	92 (57.5)	

[a] *p* value obtained by chi-square test

Table 3 Cancer-specific and disease-free survival rates for oral squamous cell carcinoma (OSCC) patients

Characteristic	Category	*n*	Cancer-specific survival (5 years; %)	*n*	Disease-free survival (5 years; %)
PAR-4 cytoplasmic expression	Negative	72	62.0	72	58.0
	Positive	127	54.0	127	53.0
PAR-4 nuclear expression	Negative	176	64.0	176	60.0
	Positive	23	56.0	23	54.0
PAR-4: cytoplasmic/nuclear expression	Negative/negative	20	65.0	20	61.0
	Negative/positive	52	61.0	52	57.0
	Positive/positive	124	54.0	124	53.0
	Positive/negative	3	50.0	3	50.0
PHLDA1 expression	Negative	122	62.0	122	61.0
	Positive	79	46.0	79	42.0
PAR-4 cytoplasmic/PHLDA1 expression	Negative/negative	35	69.0	35	66.0
	Positive/negative	76	60.0	76	60.0
	Negative/positive	27	49.0	27	42.0
	Positive/positive	51	46.0	51	43.0
PAR-4 nuclear/PHLDA1 expression	Negative/negative	12	56.0	12	56.0
	Positive/negative	99	64.0	99	63.0
	Positive/positive	75	47.0	75	44.0

differed between patients without and those with nuclear expression of PAR-4 and PHLDA1. Median 5-year cancer-specific survival was 64.0 % for patients who were positive for PAR-4 and negative for PHLDA1 and 56.0 % and 47.0 % for patients who were negative for both proteins or positive for both proteins, respectively (p=0.05). Only three cases were negative for PAR-4 and positive for PHLDA1, so this category was not included in the analysis. Median 5-year disease-free survival was 63.0 % for patients who were positive for nuclear PAR-4 and negative for PHLDA1 and 56.0 % and 44.0 % for patients who were negative for both proteins or positive for both proteins, respectively (p=0.04). No association was found between cytoplasmic expression of PAR-4 and PHLDA1 expression (p=0.13 and p=0.08; Tables 3, 4, and 5).

In further multivariate analyses based on a Cox proportional hazard model, we found that clinical stage, lymph node metastasis, PHLDA1 expression, and PAR-4 expression remained

Table 4 Univariate cancer-specific survival analysis of oral squamous cell carcinoma (OSCC) patients and relative risk with estimated 95 % confidence intervals by Cox regression

Characteristic	Category	*N*	RR	CI (95 %)		*p* value[a]
PAR-4 cytoplasmic expression	Negative	72	1	–	–	0.51
	Positive	127	1.17	0.73	1.88	
PAR-4 nuclear expression	Negative	176	1	–	–	0.82
	Positive	23	1.27	0.46	3.48	
PAR-4: cytoplasmic/nuclear expression	Negative/negative	20	1	–	–	0.89
	Negative/positive	52	1.06	0.43	2.65	
	Positive/positive	124	1.22	0.52	2.85	
	Positive/negative	3	1.86	0.22	15.51	
PHLDA1 expression	Negative	122	1	–	–	**0.02**
	Positive	79	1.65	1.07	2.55	
PAR-4 cytoplasmic/PHLDA1 expression	Negative/negative	35	1	–	–	0.13
	Positive/negative	76	1.15	0.57	2.33	
	Negative/positive	27	1.84	0.82	4.10	
	Positive/positive	51	1.91	0.94	3.86	
PAR-4 nuclear/PHLDA1 expression	Negative/negative	12	1	–	–	**0.05**
	Positive/negative	99	0.63	0.25	1.63	
	Positive/positive	75	1.12	0.44	2.85	

[a] *p* value obtained by log-rank test

Table 5 Univariate disease-free survival analysis of oral squamous cell carcinomas (OSCC) patients and relative risk with estimated 95 % confidence intervals by Cox regression

Characteristic	Category	*N*	RR	CI (95 %)		*p* value*
PAR-4 cytoplasmic expression	Negative	72	1	–	–	0.98
	Positive	127	0.99	0.66	1.51	
PAR-4 nuclear expression	Negative	176	1	–	–	0.77
	Positive	23	1.48	0.54	4.05	
PAR-4: cytoplasmic/nuclear expression	Negative/negative	20	1	–	–	0.97
	Negative/positive	52	0.93	0.44	1.99	
	Positive/positive	124	0.94	0.46	1.89	
	Positive/negative	3	1.44	0.18	11.41	
PHLDA1 expression	Negative	122	1	–	–	**0.01**
	Positive	79	1.69	1.13	2.51	
PAR-4 cytoplasmic/PHLDA1 expression	Negative/negative	35	1	0	0	0.08
	Positive/negative	76	0.99	0.54	1.84	
	Negative/positive	27	1.84	0.92	3.68	
	Positive/positive	51	1.61	0.86	3.00	
PAR-4 nuclear/PHLDA1 expression	Negative/negative	12	1	–	–	**0.04**
	Positive/negative	99	0.65	0.27	1.53	
	Positive/positive	75	1.11	0.47	2.60	

[a] *p* value obtained by log-rank test

as independent prognostic factors with regard to disease-free survival and cancer-specific survival (Tables 6 and 7).

Discussion

In this study, we evaluated the expression of PAR-4 and PHLDA1 in 229 cases of oral squamous cell carcinoma (OSCC) by tissue microarray (TMA). Although the patterns of PAR-4 and PHLDA1 expression have been examined in several types of cancer, their expression in OSCC has not been extensively addressed. Only Lee et al. (2010) have studied PAR-4 expression in nasopharyngeal/hypopharynx/oral cancer; PHLDA1 expression in OSCC has not been reported [15].

Apoptosis has a significant function in OSCC. We recently demonstrated that decreased expression of caspases is associated with classical prognostic factors, as downregulation of caspase-3 correlated with lymph node metastasis and decreased caspase-7 levels were associated with disease-free survival in oral cancer [6]. We also examined the expression of 12 Bcl-2 family proteins by immunohistochemistry and observed that high expression of Bim-Long was linked to overall survival and that elevated PUMA levels were associated with disease-free survival in oral cancer patients [5].

In this study, we noted widespread nuclear and cytoplasmic expression of PAR-4 in OSCC cases. Cytoplasmic expression of PAR-4 correlated with advanced clinical stage and tended to be associated with the presence of lymph node metastasis and vascular invasion. Disease-free survival rates were better in patients with nuclear expression of PAR-4 when compared to patients without expression. Although these results were not statistically significant, our multivariate analysis suggested that nuclear expression of PAR-4 is an independent prognostic factor in OSCC patients. The association of cytoplasmic expression with poor prognosis might reflect that PAR-4 function depends on its subcellular localization [9, 22, 23].

PAR-4 is a proapoptotic tumor suppressor. It contains a leucine zipper and was first identified in prostate cancer cells that were undergoing apoptosis in response to an exogenous insult. PAR-4 is ubiquitously expressed in normal tissues and cell types, primarily in the cytoplasm, and does not induce apoptosis unless a second apoptotic insult occurs. In contrast, PAR-4 is coexpressed in the cytoplasm and nucleus in many but not all cancer cells and clinical specimens, and the

Table 6 Multivariate analysis for cancer-specific survival of oral squamous cell carcinoma (OSCC) patients

Variables	Categories	*p* value	HR (hazard ratio) multivariate (95 %CI)
Lymph node metastasis	No	<0.001	1.0 (ref)
	Yes		3.00 (1.65–5.45)
PHLDA1 expression	Negative	0.023	1.0 (ref)
	Positive		1.86 (1.09–3.16)

Table 7 Multivariate analysis for disease-free survival of oral squamous cell carcinomas (OSCC) patients

Variables	Categories	*p* value	HR (hazard ratio) multivariate (95 % CI)
Stage	I/II	0.038	1.0 (ref)
	III/IV		1.74 (1.03–2.95)
Lymph node metastasis	No	0.020	1.0 (ref)
	Yes		1.90 (1.11–3.25)
Nuclear PAR-4 expression	Positive	0.011	1.0 (ref)
	Negative		3.19 (1.31–7.77)
PHLDA1 expression	Negative	0.003	1.0 (ref)
	Positive		2.21 (1.30–3.74)

ability of PAR-4 to induce apoptosis directly is associated with its nuclear translocation [9, 22, 23].

One of the essential apoptotic functions of PAR-4 is its inhibition of the NF-κB pathway. PAR-4 inhibits the Ras- or Raf-induced transcriptional activity of NF-κB in the nucleus and activates the extrinsic death pathway by enabling Fas and Fas ligand (FasL) to be trafficked to the plasma membrane. PAR-4 binds to the zinc finger domain of WT-1 through its leucine zipper and downregulates the anti-apoptotic gene Bcl-2 at the transcriptional level [9]. Qiu et al. (1999) reported mutually exclusive expression patterns of Bcl-2 and PAR-4 in human prostate tumors [24]. We also investigated the expression of Bcl-2 in these samples [5] but were unable to note any association between Bcl-2 and PAR-4 expression, because Bcl-2 was negative in most samples, independent of PAR-4 status (data not shown).

Recently, Burikhanov et al. (2009) demonstrated that PAR-4 is spontaneously secreted by normal and cancer cells in culture independently of caspase activation or apoptosis [25]. Notably, extracellular PAR-4 induces apoptosis by binding to the stress response protein glucose-regulated protein-78 (GRP78), which is expressed on the surface of cancer cells. The interaction of extracellular PAR-4 and cell surface GRP78 effects apoptosis through ER stress and activation of the FADD/caspase-8/caspase-3 pathway. This pathway is essential for apoptosis by TRAIL, which, like PAR-4, induces cancer-specific apoptosis.

Sharma et al. (2011) also observed that wild-type tumor growth was impaired due to the secretion of PAR-4 from distant tumors; the identification of an extracellular function of PAR-4 significantly expands its therapeutic potential for primary and metastatic tumors [26]. Another recent study provided novel evidence that PAR-4 is a physiological substrate of caspase-3 and is cleaved during apoptosis and that PAR-4 degradation facilitates the induction of apoptosis [27].

There are limited data concerning the function of PHLDA1 in cancer, and no study has examined the expression patterns of PHLDA1 in oral cancer. In this study, PHLDA1 was not expressed in 60.7 % of samples, notably in well-differentiated tumors; PHLDA1 expression was associated with advanced clinical stages of the disease, suggesting that PHLDA1 has a function in oral tumorigenesis. Further, overall and disease-free survival rates were significantly better in patients who were negative for PHLDA1, and our multivariate analysis suggested that PHLDA1 is an independent prognostic factor in OSCC patients.

PHLDA1 is a cell death mediator that sensitizes cells to apoptosis or has antiproliferative activity [20, 21, 28]. PHLDA1 is induced by external stresses, such as heat shock, and can be modulated by the IGF-I (insulin-like growth factor I) and ERK (extracellular-regulated kinase) pathways [19, 28, 29]. Toyoshima et al. (2004) demonstrated that in IGFR NIH3T3 cells, PHLDA1 is a crucial mediator of the antiapoptotic effects of IGF1 [29]. Similarly, PHLDA1 is highly expressed in pancreatic tumors that are resistant to apoptosis and chemotherapeutic agents [30].

Recently, Johnson et al. (2011) demonstrated that PHLDA1 levels are regulated by a post-translational modification: Aurora A negatively regulates PHLDA1 levels through phosphorylation in breast cancer cells, and PHLDA1 negatively affects Aurora A levels in a feedback loop [31]. Another recent study concluded that PHLDA1 is a stem cell marker in human small and large intestine, contributing to migration and proliferation in colon cancer cells [32]. It also has been suggested that PHLDA1 is a marker of hair follicle stem cells that can be used to differentiate between various dermatological tumors [33–35].

In summary, our study provides evidence of alterations in PAR-4 and PHLDA1 expression in OSCC: PAR-4 is prominently expressed and PHLDA1 is occasionally expressed in OSCC samples. Subcellular localization of PAR-4 appears to be a significant feature of oral tumorigenesis and nuclear expression of PAR-4 might be an independent prognostic factor in OSCC patients. Loss of PHLDA1 expression is associated with better prognosis, and expression of PHLDA1 might be an independent prognostic factor in OSCC patients. Further in vitro and in vivo studies are necessary to better understand the mechanisms by which PAR-4 and PHLDA1 are regulated and their association with proliferation indices, invasion, and metastatic potential in OSCC.

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