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Cancerous Inhibitor of Protein Phosphatase 2A as a Molecular Marker for Aggressiveness and Survival in Oral Squamous Cell Carcinoma

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Cancerous inhibitor of protein phosphatase 2A (CIP2A) has been identified as one of the most commonly altered proteins in human cancers. It blocks the tumor-suppressive action of protein phosphatase 2A (PP2A) complex and enhances malignancy. Thirty-five patients with squamous cell carcinoma of the oral cavity underwent surgical resection of the tumor. CIP2A was assessed by quantitative real-time PCR in the resected tumor tissues and in their adjacent normal tissues. CIP2A was found to be overexpressed in all oral squamous cell carcinoma (OSCC) specimens in comparison to their surrounding normal tissue. CIP2A overexpression was statistically correlated with poor prognostic feature of the tumor. Thus, a high expression level of CIP2A was associated with shorter survival. In conclusion, CIP2A is upregulated in OSCC, and its overexpression is correlated with aggressiveness of the tumor and poor outcome and survival. It may serve as a prognostic marker of OSCC.

Key Words Cancerous inhibitor of protein phosphatase 2A, Oral squamous cell carcinoma

INTRODUCTION

Oral squamous cell carcinoma (OSCC) represents about 1% of all malignancies [1]. OSCC is usually associated with high morbidity and mortality [2]. Modifications in the post-translational protein usually influence their function. One of the post-translational modifiers is the phosphorylation [3]. The protein phosphatase 2A (PP2A) substances have been found as tumor suppressors as they inhibit cellular transformation, proliferation, migration, and senescence [4-7]. In spite of the decreased PP2A activity in human cancers, DNA mutations are uncommon in their constituent genes [8]. This indicates that alternative mechanisms may exist that inhibit PP2A activity in human cancers. Many cellular proteins that inhibit PP2A activity in tumors have been recently identified. These include SET (SET nuclear oncogene), T-cell immunomodulatory protein, phosphatase methyl esterase-1 and cancerous inhibitor of protein phosphatase 2A (CIP2A) [5]. CIP2A has been identified as one of the most commonly altered proteins in human cancers [9,10]. As an intrinsic suppressor of PP2A, CIP2A blocks the tumor-suppressive action of PP2A complex and enhances malignancy [11]. More than 70% of specimens obtained from patients with solid tumors and hematological malignancies have CIP2A overexpression. This protein is of considerable interest as a promising therapeutic target or a potential diagnostic marker given the relatively low levels of CIP2A expression in normal tissues (apart from the testis) [12-14].

Surgery is the mainstay of treatment for localized and small OSCC. In advanced tumors, chemo-radiotherapy is added to surgery to obtain good locoregional control. Nevertheless, it is widely known that the high chemo-radioresistance of OSCC cells is one of the main obstacles to efficient treatment. Thus despite recent advancements in adjuvant treatments and imaging procedures, the overall prognosis of advanced OSCC of the head and neck (head and neck squamous cell carcinoma, HNSCC) has not been improved with the overall five-year survival around 50% [15]. A range of different strategies to identify the cause of OSCC aggressiveness and poor patient survival have been implemented recently.

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These include mutation analysis [16,17], examination of HNSCC cancer locoregional diversity [18], and investigation of radio/chemosensitivity mechanisms [19,20]. In spite of these practices, there is still a poor understanding of the mechanisms behind the poor survival and recurrence of HNSCC. Recognizing molecular factors involved in HNSCC resistance may provide for new therapeutic strategies.

The current study was conducted to explore the prognostic role of CIP2A in patients with OSCC and its relation to their survival.

MATERIALS AND METHODS

This study was carried out at Zagazig University Hospitals between May 2014 and June 2019 and included 35 patients with squamous cell carcinoma of the oral cavity. All patients have staged clinically and radiologically according to the American Joint Committee on Cancer 8th edition [21]. The study was approved by the Ethical Committee of Zagazig University. Informed consent was obtained from all patients who participated in the study.

Treatment

The principal treatment was surgical removal of the primary tumor with radical neck dissection and postoperative irradiation with selective patients treated with 5-fluorouracil and cisplatin as induction therapy concurrently with radiotherapy.

Tissue specimens

The diagnosis of OSCC was established by pathological examination of the specimens. Tissue specimens were obtained by endoscopic biopsies or during surgery. Tumor specimens and the corresponding non-cancerous adjacent tissues were collected and stored in liquid nitrogen until use.

RNA extraction, reverse transcription PCR and real-time quantitative PCR

Total RNA was extracted from all tissue specimens by using the miRNeasy Mini Kit (Qiagen, Hilden, Germany) according to the instruction of the manufacturer. Quantitative real-time PCR (qRT-PCR) was performed using SYBR Green PCR Master Mix (Perfect Real Time; TaKaRa Biotechnology, Shiga, Japan) according to company instructions based on the Bio-Rad CFX96 sequence detection system (Bio-Rad Laboratories Inc., Hercules, CA, USA).

Two μg of total RNA and ReverTra Ace qPCR RT kit (Toyobo Co., Ltd., Osaka, Japan) was used for qRT-PCR of both CIP2A, Oct4 and the housekeeping gene glyceraldehyde 3-phosphate dehydrogenase (GAPDH). The primers used for CIP2A: 5′-CCATATGC TCACTCAGATGATGT-3′ (forward) and 5′-GTGTATCATCTCCA CAGAGAGTT-3′ (reverse); for Oct4: 5′-AGGGCTTCTCCTTCTGGGTCT-3′ (forward) and 5′-TGAGAAAGGAGACCCAGCAG-3′ (reverse); GAPDH: 5′-TGACTTCAACAGCGACACCCA-3′ (forward) and 5′-CACCCTGTTGCTGTAGCCAAA-3′ (reverse).

Reactions were done in triplicate and the relative expression of CIP2A and Oct4 was normalized to the expression level of GAPDH. The expression was calculated by the $2^{-\Delta\Delta Cq}$ method [22].

Statistical analysis

Results were expressed as the means ± SD of at least three independent experiments. Statistical analysis was performed using ver. 9.4 of the SAS software package (SAS Institute, Inc., Cary, NC, USA). $P < 0.05$ was considered significant.

RESULTS

Patient characteristics

The clinical and pathological characteristics of patients are presented in Table 1. Males constitute the majority of patients included in the study (91.4%) with 62.9% less than sixty years of age. American Joint Committee on Cancer (AJCC) clinical stage III and IV constitute 57.1% of all patients. The majority of patients (74%) have N0 cervical lymph node status.

CIP2A expression in squamous cell carcinoma and adjacent normal tissues

The expression level of CIP2A was high in OSCC in comparison to their adjacent normal tissues. The mean level

Table 1. Patients characteristics

Characteristic	Number (%)
Sex	
Male	32 (91.4)
Female	3 (8.6)
Age (yr)	
< 60	22 (62.9)
> 60	13 (37.1)
T (tumor size)	
I + II	22 (62.9)
III + IV	13 (37.1)
N (lymph node)	
N0	25 (71.4)
N1 + N2	10 (28.6)
AJCC stage	
I + II	15 (42.9)
III + IV	20 (57.1)
Tumor grade	
Well	28 (80.0)
Moderate + poor	7 (20.0)
Treatment	
Surgery	15 (42.9)
Radiotherapy	17 (48.6)
Chemotherapy	10 (8.5)
Chemoradiotherapy	5 (14.3)

AJCC, American Joint Committee on Cancer.

of expression of CIP2A in OSCC was 8.144 while in their adjacent normal tissues it was 0.651. This difference was statistically significant (Student's *t*-test = 16.597, $P < 0.0001$) (Fig. 1).

CIP2A expression and clinicopathological characteristics

The relation between CIP2A expression and clinicopathological characteristics of patients are presented in Table 2. A higher level of expression with a statistically significant difference was observed with the sex and age of patients. The mean level of CIP2A expression was elevated and correlated significantly with the bad clinical and pathological features as large tumor size ($P < 0.0001$), cervical lymph involvement ($P < 0.0001$), advanced clinical stage (AJCC clinical stage) ($P < 0.0001$), and poor tumor histopathological differentiation ($P < 0.0001$).

CIP2A was expressed in all OSCC specimens; to evaluate the survival of patients we used the mean value of CIP2A expression, with 8.144 as a cutoff value. Nineteen patients had expression levels more than the mean value and sixteen patients had expression levels below the mean value. The mean values of low and high expression levels were 5.62 and 10.27, respectively.

Analysis of survival by Kaplan–Meier estimate revealed that patients with low expression of CIP2A have a higher survival rate than that with high expression level ($P = 0.021$) (Fig. 2).

Multivariate analysis of clinical and pathological features and CIP2A expression

Multivariate analysis of clinical and pathological characteristics of the patients revealed that CIP2A upregulation was correlated with the clinical stage (AJCC stage) and the histopathological grade of the tumor with *P*-values was 0.0006 and 0.0301, respectively Table 3. These results indicate that CIP2A expression is an independent molecular marker for squamous cell carcinoma (SCC) of the oral cavity.

DISCUSSION

Local recurrence and metastasis occur frequently in OSCC in spite of the significant advancement in diagnosis and treatment approach, leading to unsatisfactory survival and poor prognosis [23]. Thus, new treatment modalities and sensitive molecular biomarkers are required that can lower OSCC mortality. HNSCC is a tumor with multiple diverse, and sophisticated genetic aberrations.

Reversible protein phosphorylation is one of the most

Table 2. CIP2A expression and clinicopathological characteristics

Characteristic	Mean	T[a]	*P*-value
Sex			
Male	7.85	2.29	0.0285
Female	11.00		
Age (yr)			
< 60	6.74	5.767	< 0.0001
> 60	10.52		
T (tumor size)			
I + II	6.74	5.767	< 0.0001
III + IV	10.52		
N (lymph node)			
N0	7.11	4.746	< 0.0001
N1 + N2	10.74		
AJCC stage			
I + II	5.45	12.232	< 0.0001
III + IV	10.17		
Tumor grade			
Well	7.36	4.381	0.0001
Moderate + poor	11.27		

CIP2A, cancerous inhibitor of protein phosphatase 2A; AJCC, American Joint Committee on Cancer. [a]Student's *t*-test.

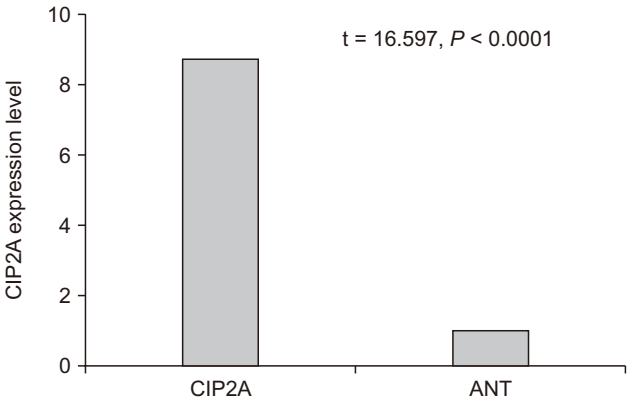

Figure 1. The mean level of expression of cancerous inhibitor of protein phosphatase 2A (CIP2A) in oral squamous cell carcinoma and their adjacent normal tissues. The mRNA expression of CIP2A was measured as described in Materials and Methods. ANT,adjacent normal tissues.

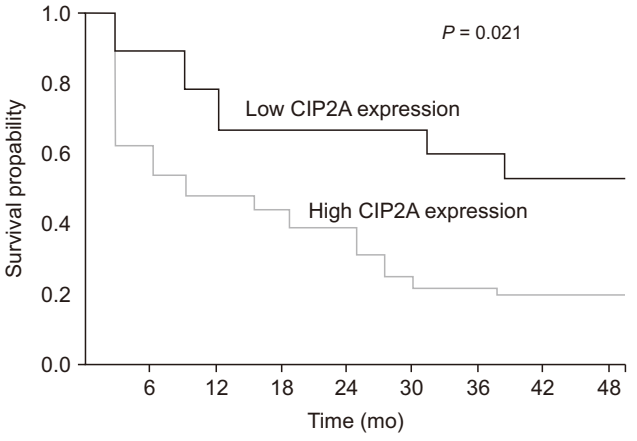

Figure 2. Kaplan–Meier survival according to cancerous inhibitor of protein phosphatase 2A (CIP2A) expression.

Table 3. Multivariate analysis of clinical and pathological features and CIP2A expression

	Coefficients	SE	t Stat	*P*-value	95% CI
Intercept	2.3662	0.5251	4.5064	0.0001	1.2923
AJCC stage	1.4165	0.3680	3.8491	0.0006	0.6638
Histopathological grade	1.1508	0.5045	2.2812	0.0301	0.1190
T	0.1815	0.4453	0.4076	0.6865	–0.7291
N	–0.8990	0.5893	–1.5254	0.1380	–2.1043

CIP2A, cancerous inhibitor of protein phosphatase 2A; t Stat, t statistics; AJCC, American Joint Committee on Cancer.

critical molecular mechanisms for signal transduction. It is strictly regulated by protein kinases and phosphatases to preserve the balance of the phosphorylation state of the protein and to regulate its biological functions [24]. There is, however, considerable evidence that the disturbance of this harmony, including the activation of protein kinases and phosphatase inhibition, contributes to the source and development of several diseases, including cancer [25]. One of the significant forms of serine/threonine phosphatase is PP2A, which is inhibited in human cancers and hence considered as a tumor suppressor [24]. In several types of cancer, downregulation of CIP2A promotes the catalytic phosphatase function of the PP2A complex [26].

In the current study, upregulation of CIP2A was observed in OSCC specimens whereas all their corresponding adjacent normal tissues showed downregulation.

In a study conducted with Taiwanese OSCC patients, Velmurugan et al. [27] observed a significant increase of CIP2A expression in the tumor tissues, compared with their corresponding non-cancerous tissues. In another study, CIP2A was upregulated in nasopharyngeal carcinoma (NPC) [28]. Moreover, CIP2A is overexpressed in many other types of cancers at high frequencies [29-33].

Analysis of the clinical and pathological characteristics revealed that, upregulation of CIP2A was correlated with the sex and age of patients. In addition, upregulation was significantly associated with features of poor prognosis, such as the large tumor size, cervical lymph node involvement, undifferentiated tumors, and advanced clinical stage. Our observations were verified in OSCC [27], NPC [28], breast [31], non-small cell lung cancer (NSCLC) [34], and many other malignancies [35].

In our study, low expression of CIP2A was associated with a high survival rate. Our results were supported by Liu et al. [28] in NPC, Dong et al. [34] in NSCLC, Birkman et al. [20] in rectal cancer, Li et al. [36] in esophagogastric junction adenocarcinoma, Ji et al. [37] in gastric carcinoma, and Kim et al. [38] in HNSCC.

Multivariate analysis of clinical and pathological characteristics of the patients revealed that CIP2A upregulation was correlated with the clinical stage (AJCC stage) and the histopathological grade of the tumor with *P*-values 0.0006 and 0.0301, respectively (Table 3). These results indicate that CIP2A expression is an independent molecular marker for OSCC, which is in agreement with observation by Velmurugan et al. [27].

Multivariate analysis of the clinical and pathological characteristics revealed a significant correlation between tumor differentiation and the clinical stage of patients.

Based on our current study and previous ones by others, the therapeutic targeting of CIP2A could promote a new therapeutic strategy in the management of OSCC and other malignancies [39].

In conclusion, we found that CIP2A is upregulated in OSCC. Its overexpression is related to aggressiveness of the tumor and poor outcome and survival. It may serve as a prognostic molecular marker for OSCC.

CONFLICTS OF INTEREST

No potential conflicts of interest were disclosed.

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