RESEARCH ARTICLE

Astrocyte Elevated Gene 1 (AEG-1): A Promising Candidate for Molecular Targeted Therapy in Oral Squamous Cell Carcinomas

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Abstract

Background: Astrocyte elevated gene 1 (AEG-1), also known as metadherin, is an oncogene which is overexpressed in various types of cancer, playing important roles in invasion, metastasis, angiogenesis and chemotherapy resistance. Hence it might be used as a therapeutic target. The aim of this study was to evaluate the expression of AEG-1 as a novel molecular marker in oral squamous cell carcinomas and establish correlations with clinicopathologic factors. **Materials and Methods: T**hirty formalin fixed paraffin-embedded blocks of OSCC cases and 30 samples of normal oral mucosa with minimal inflammation were selected and stained immunohistochemically for AEG-1. Staining intensity and percentage of stained cells were scored according to nuclear and cytoplasmic staining of epithelial cells. Relationship between immunoreactivity and clinicopathologic factors were examined by T-test and Mann-Whitney. **Results:** AEG-1 expression in OSCCs was greater than in normal oral mucosa ($P<0.05$). However, nuclear and cytoplasmic expression of AEG-1 was not associated with any of the clinicopathologic factors, age and gender of patients, tumor location, smoking history, tumor staging and grading, metastasis to lymph nodes and distant metastasis ($P>0.05$). **Conclusion:** The current results support some role of AEG-1 in genesis of oral squamous cell carcinomas.

Keywords: Oral squamous cell carcinoma- metastasis- prognosis- AEG − 1- immunohistochemical staining

Asian Pac J Cancer Prev, **18 (12)**, 3301-3305

Introduction

Squamous cell carcinoma is the most common oral cancer in the world that threatens public health. Since most of the oral squamous cell carcinoma (OSCC) usually has no visible changes in early stage, so early detection is crucial to improve survival rate of patient (Yu et al., 2009). Despite the progressing in diagnostic techniques, SCC has a high incidence in different parts of the world. Recent studies have demonstrated an increased rate in the incidence of OSCC in Iranian young patients. (Seyedmajidi et al., 2014)

Several histopathologic and clinical criteria have been proposed to evaluate the prognosis of oral squamous cell carcinoma, yet, the most important prognostic factor is TNM staging. Recently, there is much interest in novel molecular markers for predicting of tumor prognosis and estimating of overall survival rate of patients. (Shakib et al., 2014)

Astrocyte Elevated Gene 1 (AEG-1) which is known as metadherin (MTDH), 3D3 or Lysin-Rich CEACAM 1 (LYRIC) is a transmembrane protein that has no functional area and overexpressed in different types of human cancers (Liu et al., 2013). This proteins increases immortal cells by help of deformed phenotype oncogene Ha-Ras and plays an important role in tumor progression and metastasis (Liao et al., 2011). Many studies showed AEG-1 overexpression in esophageal and laryngeal squamous cell carcinoma rather than normal tissue (Yu et al., 2009; Zun-fu et al., 2013). Also, the relationship between the expression of AEG-1 with lymph node metastasis, grading of tumor, clinical stage and risk of recurrence is observed in the head and neck, salivary glands and tongue squamous cell carcinoma (Wang et al., 2013; Liao et al., 2011; Zun-fu et al., 2013). Previous studies concluded that AEG-1 overexpression decreased survival time in esophageal, salivary glands, laryngeal and tongue squamous cell carcinoma (Yu et al., 2009, Liao et al., 2011; Liu et al., 2013). Also, overexpression of AEG-1 also can increase non-cancerous growth of melanocytes and astrocytes and the likelihood of breast cancer metastasis to the lungs (Liu et al., 2014).

There are a few studies on the use of AEG-1 as a target therapeutic molecule. Furthermore, there are few studies

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that evaluate the AEG-1 expression in OSCC and there is no comprehensive study of the relationship between the expression of AEG-1 and the clinicopathologic factors; this study is aimed to investigate the expression of AEG-1 in oral squamous cell carcinoma and its correlation with clinicopathologic factors such as age and gender of patients, location of tumor, smoking, tumor staging, grading of tumor, metastasis to lymph nodes and distant metastasis.

Materials and Methods

After approval from the ethics in research committee of Babol university of medical sciences (Code:1360), 30 formalin fixed and paraffin-embedded blocks of oral squamous cell carcinoma (the same samples used in the previous study) (Seyedmajidi et al.,In press) and 30 formalin fixed and paraffin-embedded blocks of normal oral mucosa with minimal inflammation in clinical and histopathological feature obtained from crown lengthening surgery in patients who had referred, were included.

Required information such as age and gender of patients, location of tumor, smoking, clinical staging, grading of tumor, metastasis to lymph nodes and distant metastasis achieved from pathologic reports or by contact with patients. Two sections of paraffin-embedded blocks were prepared. The first section with 4 microns thickness was stained by hematoxylin-eosin to confirm the initial diagnosis and select the convenient blocks with enough tissue.

Polyclonal Anti-Rabbit AEG-1 antibody as primary antibody (ART NO: ab124789, Abcam CO, USA) with 1: 100 dilution was used for immunohistochemical staining. These sections were washed with Tris Buffered Saline (pH = 7.4). In next step post primary block solution was used to enhance the penetration of the subsequent polymer reagent. Then the surfaces of the tissue were covered with secondary antibody (Goat Anti-Rabbit IgG H and L (HRP) ART NO: ab97051-1 mg Abcam CO, USA) for 30 minutes. Diaminobenzidine or DAB chromogen (Dako, Substrate Buffer- 00046018, Germany) was used as chromogen. The sections were counterstained with Harris hematoxylin. Finally slides were dehydrated in graded alcohol and then cleaned with xylene and covered with a coverslip.

Positive control was breast cancer and negative control was obtained by omission the primary antibody. Immunohistochemical expression of AEG-1 was evaluated using an optical microscope (Olympus BX41, Olympus, Tokyo, Japan) by two independent pathologists who were not aware of the clinical specifications of the samples. For evaluation of AEG-1 expression, five randomly selected microscopic fields were evaluated and percentage of stained cells from cytoplasm and nuclear aspects with 400X magnification was considered.. For cells staining as 0% to 5% , we have score of 0, 6% to 25%, score of 1, 26% to 50%, score of 2, 51% to 75%, score of 3 and 76% to 100%, score of 4. Cytoplasmic and nuclear staining intensity of epithelial tumor cells stained by AEG-1 was evaluated on a scale from 0 to 4 (without staining was score 0; low, score 1; intermediate, score 2; and intense, score 3). Immunoreactivity or Total score was shown by sum of two score (the percentage of stained cells and the intensity of staining) that considered as 0 up to 2 for negative and from 3 to 7 for positive. (Wang et al., 2013; Liu et al., 2013).

Data were analyzed using statistical software SPSS 20, Mann-Whitney test and T test and Pearson correlation. Statistical significance was set at $P<0.05$. To examine the relationship between gender and age of the patient, location of tumor, smoking, clinical staging, grading of tumor, metastasis to lymph node and distant metastasis with the expression of AEG-1, the Mann-Whitney test was used.

Results

Sixty samples consisted of 30 paraffin-embedded blocks of oral squamous cell carcinoma from 11 male patients (36.7%) and 19 female patients (63.3%) in the age range 32 to 94 years and 30 samples of normal oral mucosa obtained from lengthening surgery crown from patients who were referred for this purpose, with at least inflammation from the clinical and histopathologic aspects related to 30 patients in the age range 35 to 73 years.

Oral squamous cell carcinoma specimens examined in this study, include 15 patients (50%) with Grade I, 12 patients (40%) with Grade II and 3 patients (10%) with Grade III.

Nuclear and cytoplasmic staining of AEG-1 in squamous cells was evaluated. AEG-1 expression in the cytoplasm of tumor cells shows diffuse distribution in neoplastic islands of oral squamous cell carcinoma. (Figure 1).

The frequency distribution of the cells in terms of nuclear and cytoplasmic staining was shown in Tables 1 and 2 respectively.

Final Nuclear expression showed that none of the normal mucosal cells with AEG-1 has staining, but in terms of the cytoplasmic staining, (30%) 9 cases were positive. In the evaluation of tumor cells, it was observed that in 22 cases (73.3%) of AEG-1 cells has not staining,

Table 1. Final Nuclear Score in OSCC and Normal Oral Mucosa

Expression	Group	Score	Number	Percent
Final Nuclear score	Oral squamous cell carcinoma	0	22	73.30%
		1	0	0
		2	0	0
		3	2	6.70%
		4	2	6.70%
		5	3	10%
		6	1	3.30%
	Normal oral mucosa	0	30	100%
		1	0	0
		2	0	0
		3	0	0
		4	0	0
		5	0	0
		6	0	0

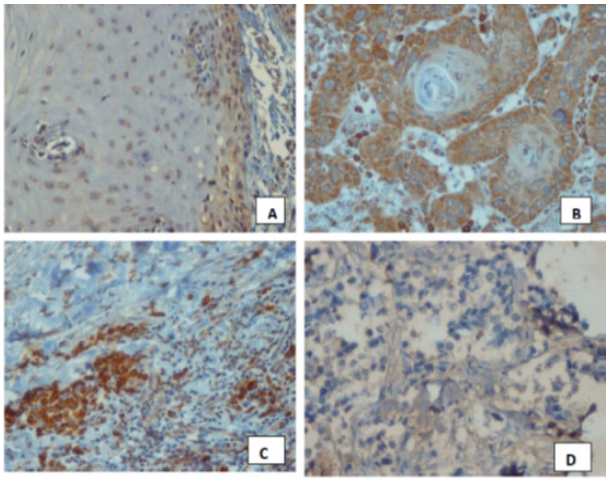

Figure 1. Immunohistochemical Staining of AEG-1 in A, Normal oral mucosa; B,Well differentiated squamous cell carcinoma; C, Moderately differentiated squamous cell carcinoma; D, Poorly differentiated squamous cell carcinoma (400X magnification)

but all were positive in terms of cytoplasmic view.

Immunoreactivity of the nucleus and cytoplasm of tumor cells and normal oral mucosa is shown in Table 3. According to Table 3, AEG-1 in the nuclear and cytoplasm of tumor cells, was expressed significantly (P=0.002 and P<0.001 respectively).

AEG-1 expression in terms of nuclear and cytoplasmic has no significant association between different grade of squamous cell carcinoma and location of the tumor. (Respectively P=0.282 and P=0.816).

To compare the mean expression of AEG-1 between normal oral mucosa and oral squamous cell carcinoma, Mann-Whitney test was used. The AEG-1 expression in OSCC was more than normal oral mucosa.

To investigate the relationship between age of patient and expression of AEG-1, patients were divided into two groups: less than or equal to 70 years and over 70 years. From 13 patients with oral SCC with age less than or equal

Table 2. Final Cytoplasmic Score in OSCC and Normal Oral Mucosa

Expression	Group	Score	Number	Percent
Final cytoplasmic score	Oral squamous cell carcinoma	0	0	0
		1	0	0
		2	0	0
		3	0	0
		4	6	20%
		5	13	43.30%
		6	11	36.70%
	Normal oral mucosa	0	9	30%
		1	4	13.30%
		2	8	26.70%
		3	4	13.30%
		4	5	16.70%
		5	0	0
		6	0	0

Table 3. Nuclear and Cytoplasmic Immunoreactivity in OSCC and Normal Oral Mucosa

Expression	group	Immunoreactivity	Number	P value
Nuclear Immunoreactivity	OSCC	Negative	22 (73.3%)	
		Positive	8 (26.7%)	
	Normal mocusa	Negative	30 (100%)	0.002
		Positive	0	
Cytoplasmic Immunoreactivity	OSCC	Negative	0	
		Positive	30 (100%)	<0.0001
	Normal mocusa	Negative	21 (70%)	
		Positive	9 (30%)	

Table 4. The Comparison Between AEG-1 Expression in OSCC and Normal Oral Mucosa

Group	Normal oral mucosa Mean Rank	OSCC Mean Rank	P value
Expression			
percentage of stained nuclei	27.5	33.5	0.011
Nuclear staining intensity	26	35	0.001
Final nuclear score	26.5	34.5	0.003
Percentage of stained cytoplasms	15.95	45.05	<0.0001
Cytoplasmic staining intensity	21/93	39.07	<0.0001
Final cytoplasmic score	16	45	<0.0001

to 70 years, 4 patients (8.30%) and 17 patients above 70 years (23.5%) was showed as positive nuclear expression of AEG-1, but this relationship was not significant (P =0.698). AEG-1 was expressed in the cytoplasm of all of cells in oral squamous cell carcinoma.

To examine the relationship between gender and age of patients, location of tumor, smoking, clinical staging, grading of tumor, metastasis to lymph nodes and distant metastasis with the expression of AEG-1, the Mann-Whitney test was used. In evaluation of the relationship between AEG-1 positive with clinicopathologic factors, a significant relationship was not observed in any case (P>0.05).

Discussion

The present results suggest that the higher expression of AEG-1 in oral squamous cell carcinoma compared to normal oral mucosa is a confirmation for its role in carcinogenesis and progression of OSCC. Other studies have shown that tumor cells in tongue carcinoma, metastatic cancers and oral squamous cell carcinoma exhibit increased expression of AEG-1 (Zun-fu et al., 2013; Liu et al., 2014; Xia et al., 2014), which is consistent with the results of present study.

Occurrence of oral squamous cell carcinoma with genetic changes is due to lack of mechanisms of controlling in cell growth and cell differentiation. Tumor developing is related to genetic incidents due to lack of control on cell division with activation of oncogenes and

inactivation of tumor suppressor genes (Neville et al., 2016).

Molecular markers can play an indispensable role in predicting tumor aggressiveness. By identifying the biological markers, ability of clinical staging system can be increased and predicting prognosis and progression of oral squamous cell carcinoma can be done in a better way Polanska et al., (2014) by biological markerssuch as AEG-1.

Astrocyte Elevated Gene-1, or AEG-1 as metadherin (MTDH) or 3D3 or Lysin-Rich CEACAM 1 (LYRIC) is oncogenic which is increased in different human cancers (Wang et al., 2013; Xia et al., 2014; Yu et al., 2014; Shi and Wang 2014; Hu et al., 2009). This gene produces a trans-membrane protein existing in the cell membrane, cytoplasm, nuclear, the nucleolus and endoplasmic reticulum (Wang et al., 2013; Yu et al., 2014, Sutherland et al., 2004) and participate in the tight junction complex (Britt, 2004). This proteins increases immortal cells by help of deformed phenotype oncogene Ha-Ras and plays an important role in tumor progression and metastasis (Emdad et al., 2007).

In this study, the expression of AEG-1 was not found in the nuclear and cytoplasm of tumor cells, while Zun-fu et al., (2013) and Huang et al.,(2014) concluded that AEG-1 in colorectal cancer and squamous cell carcinoma has cytoplasmic expression. In the study of Song H et al., (2010), AEG-1 was expressed in the cytoplasm of tumor cells and its nuclear expression was associated with colorectal carcinoma in higher clinical stages.

In this study, the expression of AEG-1 was not associated by age and gender of patients; smoking, clinical staging that consistent with finding of the study of Liao et al about AEG-1 expression in carcinoma of the salivary glands (Liao et al., 2011). In the study of Zun-fu et al., (2013) AEG-1 expression in squamous cell carcinoma of the tongue did not related to age and gender of patients and smoking. Yu et al., (2009) in their study on esophageal squamous cell carcinoma did not find a significant relationship between AEG-1 expression and age of patients.

In this study, we did not observe the relationship between AEG-1 expression with metastasis to lymph nodes. Yu et al., (2009) showed that the expression of AEG-1 in esophageal squamous cell carcinoma is related with metastasis to lymph nodes. Wang et al., (2013) found increased AEG-1 expression in head and neck squamous cell carcinoma associated with metastasis and also Liu et al., (2013) obtained the same result in laryngeal squamous cell carcinoma. Participation of AEG-1 in metastasis has been reported in tongue carcinoma (Zun-fu et al., 2013), colorectal carcinoma (Song et al., 2010), cervical squamous cell carcinoma (Song et al., 2015) and gastric carcinoma (Dong et al., 2015).

In the present study, the percentage of nuclear staining of tumor cells was not related to clinical staging and this result is not consistent with results of studies of Dong et al.,(2015), Zun-fu et al., (2013) and Yu et al., (2009) which AEG-1 expression and tumor clinical stage was related.

According to the results of this study, a relationship between the patients survival with AEG-1 expression was not observed. AEG-1 expression as a prognostic indicator for survival in patients with carcinoma was considered in many studies. (Liu et al., 2013; Zun-fu, 2013; Xia et al., 2014).

Zun-fu et al., (2013), did not find any relationship between AEG-1 expression and smoking. Also according to results of present study smoking not related to survival of patients with AEG-1 positive oral squamous cell carcinoma.

AEG-1 expression in oral squamous cell carcinoma indicated its role in carcinogenesis, also AEG-1 may be considered as a target therapeutic molecule in treatment approach for patients with oral squamous cell carcinoma.

In conclusion, according to the results of the present study, AEG-1 expression in oral squamous cell carcinoma was more than normal oral mucosa that indicates its role in carcinogenesis. Thus, considering the results and the further studies in this field, AEG-1 may be used as a therapeutic target molecule in oral squamous cell carcinoma.

Acknowledgments

Thereby, we acknowledge the efforts of hospital staff in Shahid Rajai of Babolsar to collect information of patients. This study (No. 9338715) was funded by the Vice Chancellor for Research and Technology in Babol University of Medical Sciences.

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