Int J Clin Exp Pathol 2016;9(3):3448-3453
www.ijcep.com /ISSN:1936-2625/IJCEP0021751

Original Article

Correlation of expression of ADAR1 in oral squamous cell carcinoma with clinicopathologic parameters

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Received December 13, 2015; Accepted February 18, 2016; Epub March 1, 2016; Published March 15, 2016

Abstract: The aim of the study is to determine the expression Double-stranded RNA-specific adenosine deaminase-1 (ADAR1) in subjects with and without oral squamous cell carcinoma, and to explore the correlation between expression of ADAR1 and clinicopathologic parameters, so as to confirm the role and significance of ADAR1 expression in development of oral squamous cell carcinoma. Immunohistochemical staining was performed to detect the expression of ADAR1 in oral mucosa of 23 case normal tissues and 124 cases with oral squamous cell carcinoma. We found that ADAR1 positive expressed in all subjects with normal oral mucosa, high expressed in 13 out of 23 subjects. In subjects with oral squamous cell carcinoma, ADAR1 is positive expression in 118 out of 124 subjects, high expression in 79 out 129 subjects. No significance differences was found between positive of ADAR1 and age, gender, tumor location and lymph node metastasis, except for tumor diameter, lclinical stage , histopathologic grade and relapse (P<0.05). Our results suggested that RNA editing of ADAR1 may be not linked to oral cancer occur but to progress of oral cancer, which indicated that the regulation of ADAR1 protein existed in the development of oral cancer. Thus, the detection of ADAR1 expression may have certain reference to evaluate prognosis of patients with oral cancer.

Keywords: Oral squamous cell carcinoma, immunohistochemistry, RNA-editing enzyme, ADAR1

Introduction

Head and neck squamous cell carcinoma (HNSCC) ranks sixth among the most common cancers worldwide with an incidence of over 500,000 new cases each year [1]. Oral squamous cell carcinoma (OSCC) is the most prevalent malignancy in oral cavity [2], which is a disease found particularly in low income communities and mainly a problem of older men, 90% being in >45 year age group who are exposed to the known risk factors of tobacco and/or alcohol [3].

DNA mutations play an important role in the occurrence and development of tumor. In recent years, many scholars have found that the imbalance of RNA editing is an important factor to accelerate tumor progression. RNA editing biology diversity and complexity have important role to guarantee, the most common RNA editing is adenine into hypoxanthine I, which carry genetic information and its coding protein structure and function change. In recent years, the study found that many diseases occurrence and RNA editing is closely related to the abnormal, in the process of occurrence and development of tumor, RNA editing disorders may lead to anti oncogene inactivation of living or oncogene activation and promote tumor progression. ADAR1 is widespread in human tissues and its role in the substrate are glutamate receptors and serotonin receptor mRNA, when in the presence of specific substrates, ADAR1 will from the nucleus into plasma cells play a catalytic role [4], thus, the function of ADAR1 is closely related with intracellular localization. It has been found that there are a variety of ADAR1 genes regulating the intracellular localization of the related sequences, which affect the intracellular localization of ADAR1, resulting in a different role in the outcome of different substrate. Study found that RNA editing function of ADAR1 in antiviral and anti-inflammatory

response plays an important role [5]. Meanwhile, abnormal of RNA editing in many malignant tumors also have influence on tumor occurrence and progress [6].

RNA editing is a common phenomenon that exists in organism, which can make genetic information change, and RNA editing enzyme plays an important role in the process. ADAR1 is a double chain RNA editing enzyme, one of the members of the ADAR family; so far the family has found three members: ADAR1, ADAR2 and ADAR3. ADAR1 is located on human chromosome 1q21.1-q21.2, with a total length of about 30 KB, with 15 exons [7]. After selective cutting, multiple transcripts can be generated, which contain P150 and P110, P150 are distributed in the cytoplasm and the nucleus, P110 only concentrated in the nucleus [8]. Increasing number of study show that the disorder of RNA editing may be closely related to the occurrence and progression of tumor. Oral cancer is a squamous cell carcinoma (cell carcinoma squamous) which occurs in the oral mucosa, accounting for 90% of the oral malignant tumors. At present, the five year survival rate is only maintained at 50%-60%, and most of the patients eventually died due to tumor metastasis and recurrence. Currently about ADAR1 and cancer research focus in brain tumor, liver cancer, laryngeal cancer, blood disease and gastric cancer, colon cancer and other tumors of the digestive tract. Little is known about the expression and function of ADAR1 in oral cancer worldwide. Thus, the purpose of present study is to detect the expression of ADAR1 in normal oral mucosa and oral cancer by immunohistochemical staining method, to analysis the relationship between the expression degree of ADAR1 and clinical pathological character. Moreover, exploring the role of ADAR1 in the development of oral cancer, which will offer certain reference for the mechanism of metastasis and recurrence and prognosis of oral cancer in future research.

Methods

Sources of material

A total of 124 OSCC specimens were taken from department of pathology affiliated stomatology hospital of nanjing medical university (Jiangsu province dental hospital) from August, 2008 to April, 2012, which consist of 72 male and 52 female. The age of the study patients was from 34 to 88 years old at the time of diagnosis. Of these 142 patients, 99 case over 50 years old and 25 case under 50 years old, 52 cases of tongue cancer, 31 patients with buccal carcinoma, 23 cases with gingival carcinoma, 18 cases with other type of oral cancer (mouth floor carcinoma, palate carcinoma, lip and chin carcinoma).

According to the tumor diameter, 21 cases in T1 phase, in 68 cases in T2 stage , 25 cases and 10 cases in T3 and T4,respectively. 52 cases with lymph node metastases, and72 cases without lymph node metastasis. Only 1 out of 124 patients with distant metastasis. According to the degree of clinical stage, 12 cases, 42 cases, 34 cases and 36 cases in stage I, II, III and IV. Depending on the degree of tumor differentiation, 75, 41 and 8 cases in high, medium and low differentiated of squamous cell carcinoma (SCC), respectively. Followed-up of all patients in the March, 2015 found, 2 out of 124 patients lost to follow-up. Among the remaining 122 cases, 41 cases with tumor recurrence (including 32 cases of death because of recurrence, 9 cases of recurrence but is still alive).

Another 23 cases of normal oral mucosa tissues as control, all the normal oral mucosa tissues were derived from oral mucosa which was more than 2 cm from the tumor edge, and confirmed by pathological examination of oral mucosal negative. Two investigators assessed the slides without knowledge of the clinicopathological features and were blinded to each other's evaluation. They were in agreement on all the slides examined. The diagnosis, differential diagnosis and pathological classification of oral squamous cell carcinoma are based on the seventh edition "oral histopathology and pathology" [9].

Antibodies

Goat anti human ADAR1 polyclonal antibody was purchased from Santa Cruz Ltd; hypersensitivity two-step immuno group of detection kit (PV-9003) was purchased from Beijing Zhongshan Jinqiao Biological Technology Co., Ltd.; PBS buffer (ph7.2-7.4) and citrate buffer PH6.0 purchased from Wuhan boster Biological Engineering Co., Ltd.; DAB kit, hematoxylin, immune group of water soluble mounting

reagent were purchased from Nanjing Jiancheng Bioengineering Institute.

Experimental methods

Super sensitive two step (Polink-2 Plus) immunohistochemical staining method was used in our research. Sliced the specimen under 65°C oven, bake for 30 min. Then, taking slice into solution of dimethyl benzene and alcohol for regular dewaxing and gradient dehydration. In citrate buffer salt (PH 6.0), after the microwave antigen repair kit in 3% H_2O_2 deionized water incubation for 10 min, to block endogenous peroxidase; Add a resistance to 1:30 0 (concentration) in 4°C refrigerator overnight. The next day, in turn, add kit of reagent 1 (polymer auxiliary agent), reagent 2 (horseradish enzyme mark goat IgG resistant polymer), the incubation 10-20 min; After the DAB chromogenic redyeing, dehydration, transparent sealing piece. Every step between 2 min is washed with PBS buffer was used washing for three times ,every time last 2 min. repeat 3 times. Using known hippocampus slices as positive control, with PBS instead of one as a negative control.

Result determination

ADAR1 staining was mainly localized in the cell nuclear and cell cytoplasm, the positive expression of yellow or brown/brown coloring. Immunohistochemical staining results were determined by semi quantitative scoring method, based on the expression of intensity integration: no coloring is 0; the yellow is 1; brown is 2; Black-brown is 3. At low magnification, selected specimens positive cells and uniform distribution area, the mean percentage of random counting 5 unique, non overlapping high power field of positive cells, according to the percentage of positive cell integral: 0-5% to 0; 6-25% to 1; 26-50% to 2; 51-75% to 3, more than 75% to 4. Finally the two integral multiplications, the integral is greater than or equal to 1 divided into positive. 0 score means "-"; 1-4 score means "+"; 5-8 score means "++"; 6-12 score means "+++"; "-" or "+" means low expression, "++" or "+++" means - ~; low high expression.

Statistical analysis

Statistical analysis was performed by R program language, percent of gender, age, tumor location, lymph node metastasis, differential degree, tissue location and positive expression of ADAR1 between two groups was compared by chi-square test. A *P*-value of less than 0.05 was considered to be statistically significant.

Results

Expression of ADAR1 in the normal oral mucosa

ADAR1 positive expression in epithelial tissue of normal oral mucosa of 23 cases, the positive expression rate was 100% (23/23), the high expression rate was 56.5% (13/23). Positive staining located in the nuclei and cytoplasm, basal epithelial cell layer negative expression; nuclear staining positive cells scattered in distribution in the epithelium, which coloring number of stratum spinosum cell nuclei above the basal layer was, brownish yellow or brown; near surface epithelial prickle cells in the cytoplasm of mainly was yellow brown or yellow.

Expression of ADAR1 in oral cancer

The positive expression rate of ADAR1 in 124 cases of oral cancer was 95.2% (118/124), the high expression rate was 63.7% (79/124). The expression of positive expression rate and expression intensity of ADAR1 in normal oral mucosa was not statistically significant. Positive staining also positioning in the nuclei and cytoplasm, scattered uniformly in the cancer tissues, yellow, brown or yellow brown, some cancer nests of peripheral cells was negative (**Figures 1**-**3**).

ADAR1 expression in oral squamous cell carcinoma and its relation to the clinic pathological characteristics

Analysis results revealed that no correlation existed between ADAR1 expression level and patients age, sex, site of lesion and lymph node metastasis (P>0.05), but tumor size, clinical staging, pathological grading and recurrence. The high expression rate of ADAR1 in T1 and T2 stage and in T3 and T4 stage of oral cancer was 58.4% (52/89) and 77.1% (27/35), respectively.

The high expression of ADAR1 in stage I and II and in stage III and IV of oral cancer rate is

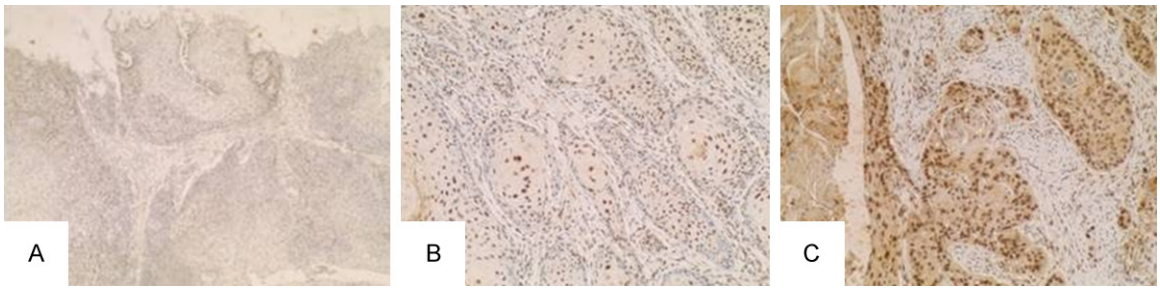

Figure 1. The expression of ADAR1 in highly differentiated oral cancer; positive staining was localized in the nucleus and cytoplasm. A: ADAR1 negative expression (A×200); B: ADAR1 weak positive expression (B×200), cancer nest surrounding cells was negative expression; C: ADAR1 strong positive expression, positive staining of nuclear staining (C×200).

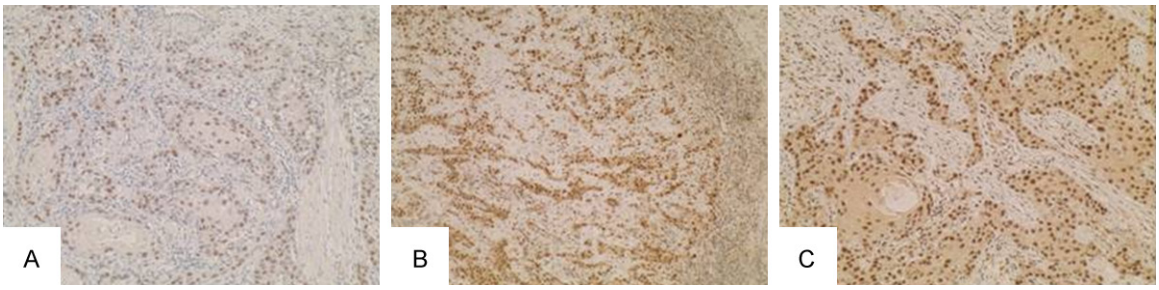

Figure 2. Expression of ADAR1 in moderately differentiated oral cancer, positive staining was localized in the nucleus and cytoplasm. A: The weak positive expression of ADAR1 (A×200); B: Strong positive expression of ADAR1 (B×100); C: Strong positive expression of ADAR1 (C×200).

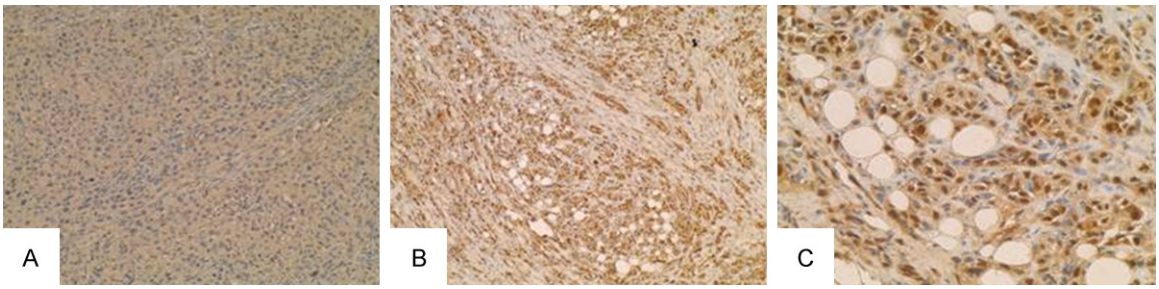

Figure 3. The expression of ADAR1 in poorly differentiated oral cancer was localized in the nucleus and cytoplasm. A: The weak positive expression of ADAR1 (A×200), the cell cytoplasm was weak positive expression, cell nuclear expression was negative; B: ADAR1 strong positive expression (B×100); C: ADAR1 strong positive expression (C×400).

53.7% (29/54) and 71.4% (50/70). Chi-square test results showed that ADAR1 expression level increased with increasing of clinical stages (chi-square=4.142, $P<0.05$). ADAR1 high expression rate in high, middle and low differentiation of oral cancer was 56.0% (42/75), 73.2% a (30/41) and 87.5% (7/8). Chi square test results show that ADAR1 expression rate increases with decreasing the degree of differentiation of oral cancer (chi-square =5.415, P<0.05). Because of recurrent cancer of the oral cavity lead to death rate of the high expression of ADAR1 in died cases with recurrent was 75% (24/32), which is significantly higher than in survival case with recurrent 33.3% (3/9) (chi-square=5.423, P<0.05) and that poor clinical prognosis of patients with ADAR1 expression was enhanced (**Table 1**).

Discussion

Our research results show that no statistically difference was found in ADAR1 expression

Table 1. The expression of ADAR1 in oral squamous cell carcinoma

Variable	ADAR1			
	Low	High	χ^2/Z	P
Gender				
Male (72)	27	45	0.109	0.742
Female (52)	18	34		
Age (year)				
≤50 (25)	10	15	0.186	0.666
>50 (99)	35	64		
Tumor location				
Tongue (52)	22	30	2.387	0.496
Buccal (31)	11	20		
Gingival (23)	8	15		
Other (18)	4	14		
Tumor diameter				
T1+T2 (89)	37	52	3.806	0.051
T3+T4 (35)	8	27		
Lymph node metastasis				
Yes (52)	17	35	0.501	0.479
No (72)	28	44		
Clinical stage				
1+2	25	29	4.142	0.042
3+4	20	50		
Differentiation				
High (75)	33	42	5.415[a]	0.020
Middle (41)	11	30		
Low (8)	1	7		
Relapse				
Death	8	24	5.423	0.019
Alive	6	3		

Note: a for Z value of rank-sum test.

between oral squamous cell carcinoma and normal oral mucosa and oral cancer occurrence may be not associated with catalysis of aberrant RNA editing. Some scholars have shown that the study of bladder cancer, cancer tissue and normal bladder tissue encoding and non encoding District RNA editing are not significantly different, but also shows that the occurrence of bladder cancer and RNA is not related to the abnormal editing [10]. Although no correlation was found between ADAR1 expression level and tumor size in present research (p=0.051), properly link maybe exist when we enlarge the sample size in future studies.

This study also found that ADAR1 expression is associated with tumor size, clinical stage, degree of differentiation and prognosis of oral squamous cell carcinoma. High expression of ADAR1 is positive associated with the tumor diameter, tumor TNM stage and tumor low differentiation degree. ADAR1 expression intensity is significantly higher in recurrence patients (already died) than in recurrence patients (surviving).

All of the above results suggest that the high expression of ADAR1 is closely related to the progression and prognosis of oral cancer. Thus, we hypothesis that abnormal phenomenon of RNA editing maybe occur in oral cancer. Tumor occurrence, development and recurrence and metastasis is a very complex process, in the gene expression process, there are a large number of transcription, reverse transcription and translation.RNA editing disorders may affect tumor progression. for example, pleomorphic malignant glioma in glur-b Q/R site of low RNA editing, will change the nature of the ion channels between cells, resulting in the imbalance of intracellular and extracellular ion concentration, leading to tumor growth and produced a series of clinical symptoms of reason [11]; Levanon *et al.* found in neuroblastoma in the existence of a large number of new RNA editing phenomenon [12]; nearly a quarter of multiple neurofibromatosis patients with abnormal RNA editing [13]. Meanwhile, the balance of RNA editing could increase the cell apoptosis and inhibit its proliferation, and reverse the growth and migration of malignant cells [14].

The present study showed that the expression of ADAR1 in different tumors showed a double side effect. ADAR1 showed low expression in tumors, such as glioma of the ADAR family of three molecular mRNA levels decreased [15]; Gu Yong *et al*. Research results showed that the expression of ADAR1 in digestive tract malignant tumor of gastric cancer, liver cancer and colon cancer than in normal tissues and cancer adjacent tissues [13], suggesting that ADAR1 may play an inhibitory role in cancer gene. On the other hand, ADAR1 expression enhancements will lead to a large number of apoptotic genes such as bcl2 and bcl10 reduced, enhanced the anti apoptotic effect of the body, leading to cell proliferation caused by cancer [16], ADAR1 seemed plays the role of oncogene.

Conclusion

From the point of our results, ADAR1 expression is not associated with occur of oral cancer but prognosis of oral cancer, which suggested that high expression of ADAR1 plays a role in prognosis of oral cancer. We should confirm that whether RNA editing disorder of ADAR1 catalyze activate of ontogenesis and tumor growth or inactivate of tumor suppressor genes and the progression of cancer in further research. In addition, ADAR1 high expression is not associated with lymph node metastasis in oral cancer but with tumor grade staging. In particular, the high expression of ADAR1 in patients with recurrent death is worthy of further study. It is expected to provide a new way to evaluate the prognosis of patients with oral cancer.

Acknowledgements

This work was supported by Provincial Natural Science Research Project of Anhui Colleges (No. KJ2014A269).

Disclosure of conflict of interest

None.

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