Overexpression of Keratin17 is Associated With Prognosis of Oral Cancer in the Chinese Population

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Abstract

Background: Oral cancer represents an common cancer among head and neck malignancies. The early metastasis contributes the poor prognosis of this disease. Keratin (KRT) family has ever been found to be involved in some crucial biological processes in normal cells and human cancer cells. As a member of this family, keratin 17 (*KRT17*) has been studied in some different cancers. In the present study, we aimed at assess the prognostic value of *KRT17* in patients with oral cancer.

Methods: Expression of *KRT17* in 135 paired oral cancer tissues and noncancerous tissues was estimated by using quantitative Real-Time RT-PCR (qRT-PCR). The prognostic value of *KRT17* was assessed with Kaplan-Meier survival analysis and multivariate Cox regression analysis.

Results: Upregulated expression of *KRT17* was detected in oral cancer tissues compared with the matched normal tissues ($P < 0.001$). The expression of *KRT17* was found associated with lymph node metastasis ($P = 0.025$) and clinical stage ($P = 0.005$). The Kaplan-Meier survival curves showed that the patients with high *KRT17* expression possessed worse overall survival than those with low level cases (log-rank $P = 0.000$). From the data of Cox analysis, we considered the *KRT17* expression was an independent prognostic biomarker in oral cancer patients with HR of 2.489, 95% CI of 1.333-4.648 and P of 0.004.

Conclusion: In summary, the expression of *KRT17* was found upregulated in oral cancer tissue specimens and it was proved to serve as an independent prognostic biomarker in patients with oral cancer.

Background

Oral cancer is a common malignancy worldwide, which mainly originates from the head and neck region [1]. The rate of incidence has been found increased in the last few years for patients with oral cancer [2]. The etiologic factors of oral cancer are well-established, including genetic polymorphisms and some environmental factors (cigarette smoking, betel quid chewing and alcohol consumption), which contribute the high incidence of this devastating disease [3, 4]. Among all the oral cancer types, the squamous cell carcinoma represents the most common one, accounting for more than 90% of all oral cancers [5]. Evidences have shown that oral cancer is characterized by high recurrence rate, high incidence of metastasis and poor prognosis [6], leading the increased mortality and poor quality of life in patients with oral cancer. Despite a lot of advances in therapy against this malignant disease, such as chemotherapy and radiotherapy or their combinations, the long-term overall survival remains dismal, especially in the cancer cases with advanced lesions [7]. Given the poor outcomes of oral cancer patients, the prognosis of this disease urgently needs to be improved.

In the recent studies, molecular biomarkers have directed the way of malignancy researches [8, 9]. The prognosis of human cancers has been vastly improved thanks to the application of prognostic biomarkers [10–12]. Thus, it is important to find novel prognostic factors for developing the treatment of cancers. Keratin (*KRT*) represents the largest gene family in humans and possesses 54 functional genes,

which can be divided into two types: type I and type II [13]. Studies found that the expression of *KRTs* is dysregulated in different cells, contributing their functional roles in differentiation [14, 15]. Besides, they have also been reported to be correlated with regulation of metabolic processes, epithelium growth and intracellular signaling pathway [16]. Keratin 17 (*KRT17*), one of this family, has ever been described to act as an crucial role in human cancers, such as colorectal carcinoma [17]. In oral cancer, Rumana and his colleagues demonstrated that *KRT17* could promote the growth of oral cancer cells [18]. However, whether the *KRT17* has clinicopathological significance in prognosis of oral cancer has been rarely studied.

In the current study, we aimed at to estimate the expression levels of *KRT17* in tissues of oral cancer patients, and to explore its prognostic value for patients with this disease.

Methods

Patients and tissue specimens collection

In this study, we enrolled 135 oral cancer patients. All of the patients were pathologically diagnosed with oral cancer in Chinese PLA General Hospital. Oral cancer tissues and adjacent noncancerous tissues were collected from the 135 patients, who underwent surgery as the first and only treatment, and immediately frozen in liquid nitrogen and then stored at -80 °C until further analyses. Moreover, we recorded the clinicopathological characteristics of these cancer patients for the analysis of their influences on the expression of *KRT17*. Table 1 summarized all the clinical parameters, including age, gender, smoking history, drinking, differentiation, lymph node metastasis and clinical stage. All the experiment procedures of this study were approved by the Ethics Committee of Chinese PLA General Hospital. The informed consent was provided for each of the participator.

Table 1
Relationship between *KRT17* expression and clinicopathological data of oral cancer patients

Features	Total No. n = 135	<i>KRT17</i> expression		<i>P</i> values
		Low (n = 64)	High (n = 71)	
Age (years)				0.848
≤ 50	56	26	30	
> 50	79	38	41	
Gender				0.965
Female	53	25	28	
Male	82	39	43	
Smoking history				0.391
No	56	29	27	
Yes	79	35	44	
Drinking				0.633
No	52	26	26	
Yes	83	38	45	
Differentiation				0.733
Negative	57	28	29	
Positive	78	36	42	
Lymph node metastasis				0.025
Negative	52	31	21	
Positive	83	33	50	
Clinical stage				0.005
I-II	55	34	21	
III-IV	80	30	50	

RNA extraction and quantitative Real-Time RT-PCR (qRT-PCR)

The extraction of total RNA was performed with the TRIzol reagent (Invitrogen, CA, USA) from the fresh tissue samples. To estimate the purity and concentration of RNA, ultraviolet spectrophotometer was adopted to measure the absorbance at 260 nm and 280 nm. Purity of RNA was determined by calculating the ratio of A OD260/OD280, and the RNA was subsequently used in the further analyses when the ratio

value was close to 2.0. RNA was then used to synthesize the single stranded cDNA by using the PrimeScript reverse transcriptase (RT) reagent kit (TaKaRa, Shiga, Japan). Total of the experiments above were carried out following the manufacturer's guidelines.

QRT-PCR was used to assess the expression of *KRT17* in oral cancer tissues and normal specimens using the SYBR Green PCR master mix (Applied Biosystems, USA) on the 7300 Real-Time PCR System (Applied Biosystems, USA). In addition, *GAPDH* gene was selected to be the endogenous control gene to normalize the expression of *KRT17*. The final relative expression of *KRT17* was computed with the method of $2^{-\Delta\Delta C_t}$. Each sample was examined in triplicate.

Statistical analysis

In the present study, SPSS version 18.0 statistical software (SPSS Inc., Chicago, IL, USA) was used to perform all the statistical analyses. Total of the data was presented in the form of mean $\pm$ SD. The differences between two groups were analyzed by using Student's t-test. Chi-square test was performed to explore the relationship between *KRT17* expression and clinicopathological features of oral cancer patients. To examine the correlation of *KRT17* expression with the overall survival of cancer patients, the survival analysis was conducted with the Kaplan-Meier method and compared with log-rank test. The Cox regression was adopted to further confirm the prognostic value of *KRT17* in patients with oral cancer. The differences were considered as statistically significant when the $P < 0.05$.

Results

Upregulated expression of *KRT17* in oral cancer patients

To estimate the expression of *KRT17* in both oral cancer tissues and corresponding noncancerous tissue samples, qRT-PCR was conducted in this study. The expression of *KRT17* was significantly upregulated in oral cancer tissue specimens compared with the adjacent normal tissue samples with a P value of < 0.001 (Fig. 1).

Association of *KRT17* expression with clinicopathological data of oral cancer patients

Given the abnormal expression patterns of *KRT17* in oral cancer tissues, we wondered if there is a relationship between *KRT17* and progression of oral cancer. Thus, Chi-square test was carried out to assess the correlation between *KRT17* expression and oral cancer patients' clinical characteristics. To facilitate this analysis, expression of *KRT17* was classified into low expression and high expression with the case numbers of 64 and 71, respectively, according to the mean expression value. Total of the parameters were listed in Table 1. The results revealed that the expression of *KRT17* was correlated with lymph node metastasis ($P = 0.025$) and clinical stage ($P = 0.005$). However, *KRT17* expression was not obviously associated with age, gender, smoking and drinking history and differentiation (all $P > 0.05$).

Prognostic value of *KRT17* in patients with oral cancer

In the present study, survival analysis was carried out to investigate the relationship between *KRT17* expression and overall survival of oral cancer patients according to the Kaplan-Meier methods and log-rank test. The survival curves showed in Fig. 2 suggested that the patients with high levels of *KRT17* had short survival time than those with low *KRT17* levels (log-rank $P=0.000$). Multivariate Cox regression analysis was used to verify whether the *KRT17* was an independent risk factor for outcomes of oral cancer patients. The results in Table 2 showed that the elevated expression of *KRT17* was an independent prognostic biomarker in patients with oral cancer with a $P=0.004$. The HR value was 2.489 and 95% CI was 1.333–4.648.

Table 2
Multivariate Cox regression analysis for *KRT17* in oral cancer patients

Variables	Multivariate analysis		
	HR	95%CI	P value
<i>KRT17</i>	2.489	1.333–4.648	0.004
Lymph node metastasis	2.411	1.209–4.809	0.012
HR: hazard ratio; CI: confidence interval.			

Discussion

Oral cancer is reported to be one of the most frequent cancers among men and women around the world, and most of the cases are present in male [19–21]. From the results of epidemiologic study, a wide of environmental carcinogens have been found correlate with the tumorigenesis of oral cancer, and the most prevalent members including abuse of alcohol, betel quid and tobacco [22, 23]. The overall survival of patients with early stage oral cancer is better than the other malignancies, but it is rather poor in the patients at advanced lesion [24]. Thus, it is necessary to develop new prognostic tools and effective therapeutics that may be beneficial for improving the clinical management of oral cancer. In the recently studies, application of prognostic biomarkers has directed the research in the tumor studies [25, 26]. For example, Xu et al. have found that fibulin-5 could serve as an independent prognostic factor and promote the proliferation and invasion in glioma [27]. Similarly, there are some biomarkers have been found to be associated with prognosis of oral cancer. Kumar et al. have demonstrated that nuclear heterogeneous nuclear ribonucleoprotein D was correlated with cellular proliferation and overall survival, and acted as a potential prognostic factor for oral cancer patients [28]. It is helpful to improve the prognosis and treatment of oral cancer by finding more efficient prognostic biomarkers.

KRT family represents a series of intermediate filament proteins in abundant epithelial cells [15]. In cancer, keratins have traditionally been used as diagnostic tools, but accumulating evidence points to their importance as prognostic markers and active regulators of epithelial tumorigenesis [29]. *KRT17*, a member of KRT family, has been originally described as a major KRT in the basal cell skin cancers [30].

The studies in the last decade have demonstrated that *KRT17* could be used to distinguish the cancer cases from healthy controls for diverse human carcinomas, such as lung cancer, prostate carcinoma and pancreaticobiliary adenocarcinomas [31–33]. All these data suggest its potential functional role in human cancers. In oral cancer, *KRT17* has also been investigated and proved to facilitate the growth of oral cancer cells and promote the tumorigenesis [18].

In the present study, we found the deregulated expression of *KRT17* in oral cancer tissues compared with the paired normal tissues by using qRT-PCR method. Then we wonder if *KRT17* was involved in the progression of oral cancer. Thus, the relationship between *KRT17* expression and oral cancer patients' clinicopathological characteristics was assessed with Chi-square test. By this analysis, the remarkable relationship between *KRT17* expression and lymph node metastasis and clinical stage was found. These results indicated that expression of *KRT17* was involved in the development of oral cancer.

As the *KRT17* expression was found dysregulated and correlated with progression of oral cancer, we further assessed its clinical significance in prognosis of this malignant disease. In the current study, we first examined the relationship between *KRT17* expression with overall survival of oral cancer patients, and found that the high levels of *KRT17* was associated with poor overall survival in the oral cancer patients. Then, we adopted the multivariate Cox regression to perform the influences of *KRT17* on the overall survival of oral cancer patients. From the results of Cox analysis, we considered that the increased expression of *KRT17* was an independent prognostic biomarker in patients with oral cancer.

Conclusions

In conclusion, expression of *KRT17* was found upregulated in oral cancer tissues. Besides, *KRT17* expression was considered as an independent prognostic biomarker for patients with oral cancer. Although data in our study provided evidences for *KRT17* to serve as a prognostic factor of oral cancer, the further studies are still urgently needed to uncover the potential molecular mechanisms of *KRT17* acting in oral cancer cells.

List Of Abbreviations

Keratin (KRT)

Keratin 17 (KRT17)

Quantitative Real-Time RT-PCR (qRT-PCR)

Reverse transcriptase (RT)

Declarations

Ethics approval and consent to participate

This study was supported by the Ethics Committee of Chinese PLA General Hospital and also has been carried out in accordance with the World Medical Association Declaration of Helsinki.

The subjects had been informed the objective. Certainly, written consents were signed by every subject in this study.

Consent for publication

We obtaining permission from participants to publish their data.

Availability of data and materialsAll data generated or analysed during this study are included in this published article.**Competing interests**The authors declare that they have no competing interests.**Authors' contributions** L.W. design of the work; H.S. the acquisition, analysis, S.Y. interpretation of data; L.W. the creation of new software used in the work; H.S. have drafted the work or substantively revised it. All authors read and approved the final manuscript.**Acknowledgements** Not applicable.

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Figures

Figure 1

Expression levels of KRT17 in 135 paired oral cancer tissues and adjacent noncancerous tissues. The KRT17 expression was upregulated in oral cancer samples compared with the matched noncancerous samples ($P < 0.001$).

Figure 2

Kaplan-Meier survival curves based on different KRT17 expression levels in oral cancer patients. The high expression levels of KRT17 was associated with the poor overall survival of oral cancer patients (log-rank test, $P=0.000$).