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Original Article

Prognostic value of elevated KDM5B expression in patients with laryngeal squamous cell carcinoma

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Abstract: Previous studies confirmed that KDM5B expression is dysregulated in most human tumors. However, KDM5B expression in human laryngeal squamous cell carcinoma (HLSCC) has not been reported. In this paper, the relationship between KDM5B expression and clinical features of HLSCC is clarified, and its prognostic value in HLSCC patients is evaluated. In our study, KDM5B expression was examined by immunohistochemical analysis in 63 HLSCC clinical tissue samples and 20 adjacent normal tissue samples. Subsequently, the relationship between KDM5B expression and clinicopathologic factors in 63 HLSCC patients was clarified, and its prognostic value was evaluated according to Cox model analysis. Our results showed that KDM5B was over-expressed in HLSCC cells and over-expression of KDM5B was related to the histologic type, clinical stages, lymph node metastasis, and recurrence of tumor. Furthermore, over-expression of KDM5B had poor five-year overall survival in HLSCC patients. The result of a multivariate analysis indicated that over-expression of KDM5B was an independent risk factor for poor prognosis. These results indicated that over-expression of KDM5B was closely correlated with tumorigenesis, metastasis, and poor overall survival in HLSCC patients. Furthermore, KDM5B might serve as a specific and novel prognostic biomarker in HLSCC patients.

Keywords: KDM5B, laryngeal squamous cell carcinoma, prognosis

Introduction

Laryngeal carcinoma is one of the most common cancers in the head and neck. According to GLOBOCAN 2012, there is an incidence of 2.1/100,000 and mortality of 1.1/100,000 worldwide [1]. In China, the incidence and the mortality of laryngeal carcinoma are 1.23/100,000 and 0.63/100,000, respectively, and these are higher in urban areas thanin rural areas [2]. Males are more commonly affected, and most patients are aged over 40 years. In the field of the etiology, several important factors have been proposed (e.g. active cigarette smoking, unlimited alcohol use, air pollution and human papillomavirus infection). Several treatment protocols have been proven effective for laryngeal carcinoma. Surgical options have been developed for decades, and they are crucial for the treatment of laryngeal carcinoma. Besides total laryngectomy, organ preservation surgical techniques (e.g. open partial laryngectomy and transoral laser microsurgery) have been accepted by a growing number of surgeons and patients. One study recruiting 1115 patients showed that the five-year survival rate of partial laryngectomy reached 85%, higher than that of total laryngectomy (68%) [3]. Chemotherapy and radiotherapy have been employed in the treatment of laryngeal carcinoma. It is necessary to have combined therapy options for patients according to their body conditions, pathologic types, and clinical stages of carcinoma, but the overall survival rate of advanced laryngeal carcinoma remains poor. Accordingly, molecular-targeted treatment against oncogenes may be a potential method as it has good outcomes in some malignant tumors.

Lysine-specific demethylase 5B (KDM5B), namely JARID1B and PLU-1, a crucial member of the KDM5 family, contains five conserved

domains, including the catalytic JmjC domain, N-terminal JmjN domain, ARID domain, PHD finger domain, as well as the C5CH2 domain [4]. To be specific, KDM5B removes methyl residues from tri-, di-, and monomethylated lysine 4 on histone H3 (H3K4) residues, facilitating the access of RNA polymerase II to DNA. Though KDM5B is found to be expressed primarily in the normal testis, expression of KDM5B has been shown to be significantly upregulated in most human solid tumors, e.g. breast cancer [5], gastric cancer [6], lung cancer [7], and hepatocellular carcinoma [8]. KDM5B silencing is observed to inhibit the migration and the invasion of oral squamous cell carcinoma [9]. Immunohistochemical results showed that KDM5B is over-expressed in esophageal squamous cell carcinoma [10]. KDM5B has also been reported to be vital for leukemia [11]. The above findings demonstrated that KDM5B could contribute to the proliferation, apoptosis and invasion of malignant tumors. Hence, KDM5B is considered an oncogene in all these tumors, and it can be a potential anticancer drug target [12]. However, whether KDM5B is over-expressed in human laryngeal squamous cell carcinoma (HLSCC) has been unclear. In this study, the over-expression of KDM5B in HLSCC patients and the relationship between KDM5B expression and the clinical features of HLSCC patients are revealed. We determined whether over-expressed KDM5B can serve as a potential prognostic biomarker.

Materials and methods

Patients and tissue samples

The data of 69 patients with laryngeal carcinoma admitted to our department between 2009 and 2013 were collected. All patients met the following criteria: (1) laryngeal squamous cell carcinoma according to pathological results; (2) no history of chemotherapy and radiotherapy treatment before surgery. Thus, 63 HLSCC patients were recruited in this study as approved by the hospital ethics committee. The information of 63 patients was reviewed, including their ages, genders, smoking or not, alcohol consumption, five-year survival states. Other information (e.g. the histological type, clinical stages, position, lymph node metastasis and recurrence of tumor) was reviewed. A total of 63 HLSCC tissue samples and 20 adjacent normal tissue samples were used to detect KDM5B expression.

Immunohistochemistry

All tissue samples were fixed with formaldehyde. Conventional paraffin embedding was performed. For immunohistochemical staining, rehydrated sections were subjected to antigen retrieval and their endogenous peroxidase activity blocked in 1% H_2O_2/PBS solution. After tissue sections were blocked for 1 h and subsequently incubated with rabbit anti-KDM5B antibody (1:500, ab198884, Abcam, England) at 4°C overnight, horseradish peroxidase-conjugated secondary antibody was added and then incubated for 30 min at 37°C. PBS was used as the negative control instead of primary antibody. The sections were developed according to the instructions of a diaminobenzidine tetrahydrochloride (DAB) immunohistochemistry color development kit and subsequently counterstained with hematoxylin for 1 min. After dehydration and sealing steps were finished, ten visual fields were randomly selected under the microscope.

Evaluation of immunostaining

Brown particle staining was considered positive for KDM5B expression. The intensity of the immunostaining was classified as follows: 0, no brown particle staining; 1, light brown particles; 2, moderate brown particles; 3, dark brown particles. The extent of immunostaining was quantified according to the percentage of positive cells and split into four groups: 0, below 10% positive cells; 1, 10~25% positive cells; 2, 26~50% positive cells; and 3, 51~75% positive cells; 4, above 76% positive cells. By multiplying the score for the extent and the score for intensity, a final immunostaining score was calculated as follows: 0, negative (-); 1~4, weakly positive (+); 5~9, moderately positive (++); 10~12, strongly positive (+++). Tumors were categorized into two groups according to final score: high expression (scored as 5~12) and low expression (scored as 0~4). To avoid the interindividual bias of immunohistochemical staining differentiations, all slides were evaluated by two experienced pathologists.

Follow-up

Follow-up of 63 patients after surgery was performed. Four patients were lost to follow-up. Recurrence and metastasis were determined according to clinical and pathological examination. The follow-up period was continued for 60

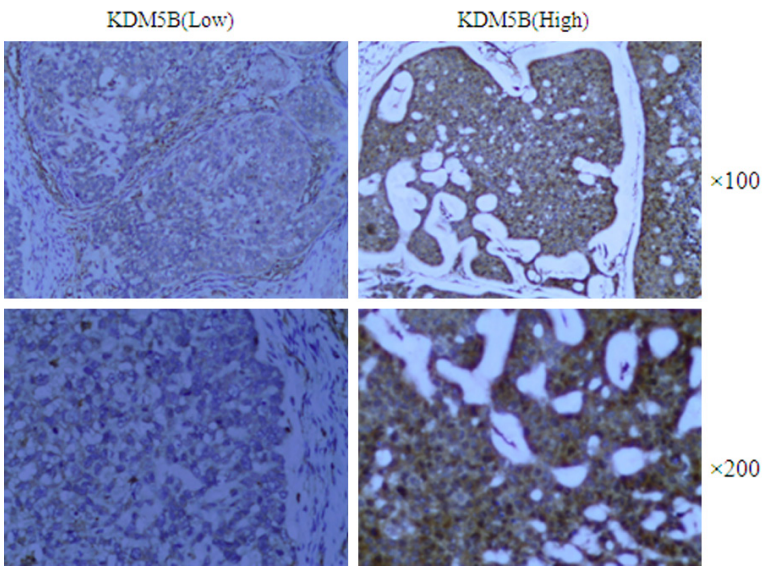

Figure 1. KDM5B protein expression in HLSCC tissues, as determined by immunohistochemistry. Original magnification: 100× (upper) and 200× (lower).

months. Once we lost contact with the patients due to the changes of their telephone number or address, the follow-up ended. Deaths from other causes were treated as censored cases.

Statistical analysis

Measurement data were denoted as mean ± standard deviation ($\bar{X}$ ± S). Kaplan-Meier test was used for survival analysis and survival curves between two groups were compared using the log-rank test. Cox's proportional hazards model was applied in univariate and multivariate analyses to identify the factors that acted as independent indicators of prognosis. The X^2 test was used forcomparison in the expression of KDM5B and clinical features. SPSS 19.0 software was applied in the statistical analyses. $P < 0.05$ was considered significant.

Results

KDM5B expression in HLSCC tissues and adjacent normal tissues

To clarify the expression of KDM5B protein, immunohistochemistry was performed. **Figure 1** suggests that KDM5B immunoreactivity was primarily distributed in the cytoplasm of HLSCC. The positive rate of KDM5B expression in HLSCC tissue samples reached 81.0% (51/63). High expression accounted for a large proportion (39/51). However, there was low KDM5B expression in adjacent normal tissue in 2 cases (2/20). There was a significant difference ($P < 0.001$).

Relationship between over-expressed KDM5B and clinical features of HLSCC patients

Table 1 reveals the relationship between over-expressed KDM5B and clinical features of HLSCC patients. Our study showed high KDM5B expression was related to the histological type, clinical stage, and lymph node metastasis ($P = 0.016$, 0.043 and 0.032, respectively). There was no significant differences between high KDM5B expression and age ($P = 0.580$), gender ($P = 1.000$), smoking ($P = 0.850$), alcohol consumption ($P = 0.502$), tumor position ($P = 0.777$) and recurrence ($P = 0.153$). **Figure 2** draws a comparison of the survival curve between high and low KDM5B expression in HLSCC patients. The five-year overall survival rate of HLSCC patients with low KDM5B expression was 83.3%, much more than that with high KDM5B expression (46.2%). The results of the log-rank test suggested that there was a significant difference ($P = 0.002$).

Univariate analysis for five-year overall survival of HLSCC patients

As **Table 2** shows, over-expressed KDM5B was negatively related to five-year overall survival of HLSCC patients ($P = 0.006$). The following factors were also related to prognosis of HLSCC patients: clinical stage, lymph node metastasis, and recurrence (all $P < 0.05$). However, factors including histological type, tumor position, age, gender, smoking, and alcohol consumption were not related to the prognosis of HLSCC patients (All $P > 0.05$).

Multivariate Cox model analysis for five-year overall survival of HLSCC patients

To find out whether high KDM5B expression is considered as an independent prognostic factor for post-surgical outcome of HLSCC patients, multivariate Cox model analysis was used (**Table 3**). The results showed that high KDM5B

Table 1. Correlation between KDM5B expression and clinicopathological features

Factors	N	KDM5B expression		χ^2	P
		Low (n = 24)	High (n = 39)		
Age					
≥ 50 y	52	19	33	0.306	0.580
< 50 y	11	5	6		
Gender					
Male	60	23	37	0.000	1.000
Female	3	1	2		
Smoking					
Yes	57	21	36	0.036	0.850
No	6	3	3		
Alcohol consumption					
Yes	50	18	32	0.451	0.502
No	13	6	7		
Histologic type					
Highly-moderately differentiated	38	19	19	5.755	0.016
Poorly differentiated	25	5	20		
Clinical stage					
0+I+II	40	19	21	4.109	0.043
III+IV	23	5	18		
Tumor position					
Supraglottic	12	5	7	0.080	0.777
Glottic	51	19	32		
Lymph node metastasis					
N0	47	22	25	4.592	0.032
N+	16	2	14		
Recurrence					
Yes	9	1	8	2.044	0.153
No	54	23	31		

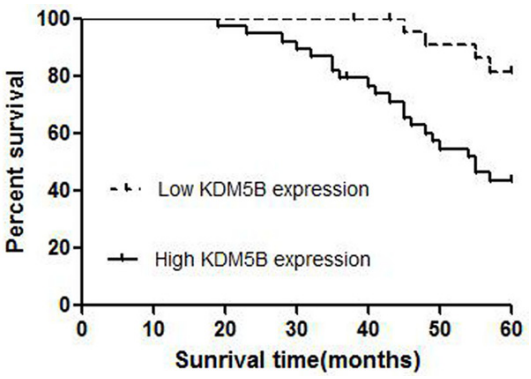

Figure 2. Overexpressed KDM5B was correlated with poor clinical outcome in HLSCC patients. Kaplan-Meier test was used for a survival curve in HLSCC patients with differential expression of KDM5B.

expression can be an independent prognostic factor (P = 0.004). Also, clinical stage, lymph node metastasis and recurrence can serve as independent prognostic factors for predicting poor prognosis of HLSCC patients (P = 0.048, 0.000 and 0.003, respectively). Among these factors, lymph node metastasis was the most important independent prognostic factor.

Discussion

Post-translational modifications on histones are critical determinants of transcriptional activity. Similar to other modifications of chromatin including acetylation, phosphorylation and ubiquitination, methylation of H3-K4 also plays a key role in histone modification. A previous study revealed that methylation modification of H3K4 is dynamically regulated. Two families of enzymes can set the balance of methylation of H3K4. Histone methyltransferase can improve the level of H3K4me3 by targeting H3K4me1 and H3K4me2. However, H3K4 demethylation is accomplished by the jumonji AT-rich interactive domain 1 (JARID1/KDM5) family. KDM5B belongs to the lysine demethylase family, which specifically removes the methyl group of histone H3K4. KDM5B can be vital to regulate H3K4 methylation markers near promoters, gene bodies, and enhancers in embryonic stem cells and during differentiation. However, the relationship between the up-regulated KDM5B expression and cancer has aroused much attention from researchers.

Numerous previous studies demonstrated that KDM5B expression was dysregulated in most human tumors. KDM5B was first identified as a gene up-regulated by the tyrosine kinase HER2 signaling in breast cancer. Subsequent studies

Table 2. Univariate analysis for five-year overall survival in HLSCC patients

Factors	B	*P* value	Exp (B)	95.0% CI for Exp (B)	
				lower	upper
KDM5B expression	1.498	0.006	4.471	1.531	13.053
Age	0.631	0.306	0.532	0.159	1.779
Gender	3.103	0.393	0.045	0.000	55.302
Smoking	0.476	0.519	1.069	0.379	6.837
Alcohol consumption	0.172	0.732	1.187	0.445	3.166
Histological type	0.147	0.719	1.158	0.520	2.580
Clinical stages	1.411	0.001	4.098	1.802	9.323
Tumor position	0.431	0.358	0.650	0.259	1.628
Lymph node metastasis	2.021	0.000	7.544	3.341	17.034
Recurrence	1.693	0.000	5.438	2.308	12.816

Table 3. Multivariate Cox model analysis of five-year overall survival in HLSCC patients

Factors	B	*P* value	Exp (B)	95.0% CI for Exp (B)	
				lower	upper
KDM5B expression	1.433	0.026	4.192	1.191	14.753
Clinical stages	0.944	0.048	2.570	1.007	6.559
Lymph node metastasis	1.744	0.000	5.722	2.234	14.658
Recurrence	1.464	0.003	4.323	1.670	11.190

showed that KDM5B could inhibit breast cancer cell proliferation by down-regulating tumor suppressor genes (e.g. BRCA1, CAV1 and HOXA5) [13], leading to an improved level of H3K4me3 at the chromatin region of these target genes. This suggested that the level of KDM5B could significantly regulate cancer cell proliferation. Quantitative RT-PCR analysis confirmed that KDM5B expression was significantly higher in human bladder cancer tissues than that in normal bladder tissues. One further study showed that over-expressed KDM5B was involved in the proliferation of cancer cells by the E2F/RB pathway [14]. High KDM5B expression has been described in other cancers. Dai et al. demonstrated that KDM5B could promote glioma proliferation partly by down-regulating the expression of p21 [15]. Over-expressed KDM5B in lung cancer cells exhibited greatly decreased p53 expression, whereas silencing of KDM5B expression dramatically increased p53 expression at both the mRNA and protein levels [16]. Thus, the results suggest over-expressed KDM5B plays roles in proliferation and invasion in lung cancer cells partly through regulating the p53 expression. These results imply involvement of KDM5B in tumorigenesis. Another study confirmed a crucial role of KDM5B in stimulating metastatic behaviors of hepatocellular carcinoma cells [17]. Further study found KDM5B exerted its function through modulation of H3K4me3 at the PTEN gene promoter, which is related to inactivation of PTEN transcription.

HSLCC is a common cancer in the head and neck. The main pathologic type is highly differentiated squamous cell carcinoma. In recent years it seems that the incidence of HSLCC increased gradually. However, there is still a research gap for the relationship between KDM5B expression and proliferation and invasion of HSLCC. Our results showed there was over-expression of KDM5B in HLSCC tissues. However, only low KDM5B expression was found in a few adjacent normal tissues. The results suggest that over-expression of KDM5B may be related to the occurrence of HSLCC. Our study demonstrated that over-expression of KDM5B can elevate the risk of lymph node metastasis of HSLCC. According to comparison of different clinical stages, KDM5B expression in the advanced carcinoma (III+IV) was much more than that in early carcinoma (0+I+II). Accordingly, over-expression of KDM5B is related to development and metastasis of HLSCC. Though the number of patients with high KDM5B expression was greater than that with low KDM5B expression, our results showed that there was not significant relationship between over-expression of KDM5B and recurrence of HLSCC (P = 0.153). The possible cause is that only 9 HSLCC patients relapsed in our study. If there had been more samples, a significant difference between over-expression of KDM5B and recurrence of HLSCC may have occurred. In brief, we conclude that high KDM5B expression may be related to invasiveness and poor prognosis in HSLCC patients.

The study of Kuo et al. revealed that KDM5B can promote tumor aggressiveness through

multiple biological paths which are related to activation of c-Met signaling. Based on the results, it was concluded that KDM5B can be a novel prognostic biomarker of non-small cell lung cancer [18]. One study of correlation between KDM5B expression and prognosis revealed the patients with KDM5B positive in hepatocellular carcinoma had much poorer prognosis than those who were KDM5B negative, especially in hepatocellular carcinoma derived from persistent infection of hepatitis B virus (HBV) or hepatitis C virus (HCV) [19]. The results imply that over-expression of KDM5B results in poor prognosis in hepatocellular carcinoma, especially due to HBV/HCV. Thus, we also want to clarify the relationship between over-expression of KDM5B and prognosis of HSLCC patients. Based on univariate analysis, our study proved high KDM5B expression was related to poor prognosis in HLSCC patients. Other factors, including clinical stage, lymph node metastasis, and recurrence were identified as the main predictors of poor prognosis in HLSCC patients. To exclude interference from other factors on KDM5B, a multivariate Cox model analysis was used. The result showed over-expression of KDM5B is an independent prognostic factor. Accordingly, over-expression of KDM5B has good value in predicting poor prognosis of HLSCC. Furthermore, clinical stages, lymph node metastasis, and recurrence of tumor are independent factors for prognosis. Lymph node metastasis is still the best among the above-mentioned factors for poor prognosis in HLSCC patients.

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Disclosure of conflict of interest

None.

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