Expression of FAM135B and Lysine Acetyltransferase in Uyghur Esophageal Squamous Cell Carcinoma Post-print

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Abstract

Objective: To investigate the expression patterns of Family with Sequence Similarity 135 Member B (FAM135B) and Lysine Acetyltransferase 5 (KAT5) in esophageal squamous cell carcinoma (ESCC) in the Uyghur population. Methods: The expression of FAM135B and KAT5 was detected using a Roche automated immunohistochemistry system in 40 pairs of ESCC and adjacent noncancerous tissues from Uyghur patients, and the correlation between the two proteins and their associations with clinicopathological features were analyzed. Results: In Uyghur ESCC specimens, FAM135B and KAT5 were expressed in 92.50% (37/40) and 15.00% (6/40) of cases, respectively; the proportion of strong positive FAM135B expression was higher in cancer tissues than in adjacent tissues [45.00% (18/40) vs. 22.50% (9/40), ^{2} = 4.528, P = 0.033]; the proportion of KAT5 negative expression in cancer tissues showed no statistically significant difference compared with adjacent tissues [85.00% (34/40) vs. 87.50% (35/40), ^{2} = 0.105, P = 0.745]; there was a good positive correlation in strong positive FAM135B expression between ESCC tissues and their paired adjacent tissues (Kendall' s correlation coefficient = 0.707, P < 0.001); strong positive FAM135B expression in cancer tissues showed a significant negative correlation with KAT5 expression (Kendall' s correlation coefficient = -0.946, P < 0.001); the expression of FAM135B and KAT5 showed no significant correlation with gender, age, tumor location, differentiation grade, depth of invasion, lymph node metastasis, or clinical stage of ESCC patients (P > 0.05). Conclusion: Strong positive expression of FAM135B may be an important molecular basis for the development of ESCC in the Uyghur population, and this molecule may exert its effect through the negative expression of KAT5.

Full Text

Abstract

Objective To investigate the expression patterns of family with sequence similarity 135 member B (FAM135B) and lysine acetyltransferase 5 (KAT5) in esophageal squamous cell carcinoma (ESCC) among Uygur patients. **Methods** The expression of FAM135B and KAT5 was detected in 40 pairs of ESCC tissues and adjacent non-cancerous tissues from Uygur patients using the Roche Benchmark XT automated immunohistochemistry platform. Correlations between the two proteins and their associations with clinicopathological characteristics were analyzed. **Results** Positive expression of FAM135B and KAT5 was observed in 92.50% (37/40) and 15.00% (6/40) of ESCC specimens, respectively. The proportion of strong FAM135B expression was significantly higher in cancer tissues than in adjacent tissues [45.00% (18/40) vs. 22.50% (9/40); ^{2} = 4.528, P = 0.033]. However, the rate of negative KAT5 expression did not differ significantly between cancer and adjacent tissues [85.00% (34/40) vs. 87.50% (35/40); ^{2} = 0.105, P = 0.745]. Strong FAM135B expression in ESCC showed a strong positive correlation with that in paired adjacent tissues (Kendall' s coefficient = 0.707, P < 0.001). In ESCC tissues, strong FAM135B expression demonstrated a significant negative correlation with KAT5 expression (Kendall' s coefficient = -0.946, P < 0.001). Neither FAM135B nor KAT5 expression correlated significantly with patient gender, age, tumor location, differentiation grade, invasion depth, lymph node metastasis, or clinical stage (all P > 0.05). **Conclusion** Strong FAM135B expression may represent an important molecular basis for ESCC development in Uygur patients, potentially exerting its effects through negative regulation of KAT5 expression.

Keywords: FAM135B; KAT5; Uygur; esophageal squamous cell carcinoma

Introduction

Esophageal cancer exhibits high incidence in Asian countries, with approximately 70% of cases occurring in China, where over 90% are esophageal squamous cell carcinoma (ESCC). Due to the lack of effective treatment options for advanced disease, the five-year survival rate for middle-to-late stage patients remains below 20%. Identifying key molecular drivers of ESCC development is therefore crucial for elucidating pathogenic mechanisms and developing targeted therapies. While most ESCC research has focused on Han Chinese populations, the Kashgar region in southwestern Xinjiang presents a distinct epidemiological context, with Uygurs comprising 92.16% of the local population and ESCC representing a major health burden. Investigating molecular markers in this ethnic group is thus essential.

FAM135B was first identified as an ESCC-associated gene in Han Chinese populations, with studies demonstrating that FAM135B mutations significantly promote proliferation, migration, and invasion of ESCC cells and correlate posi-

tively with poor prognosis. At the protein level, FAM135B has been shown to interact with lysine acetyltransferase 5 (KAT5), a well-established tumor suppressor. However, the expression patterns and clinical significance of FAM135B and KAT5 in Uygur ESCC patients remain unexplored. This study addresses this knowledge gap by examining the expression and correlation of these two proteins in Uygur ESCC.

Methods

1.1 Clinical Specimens

We retrieved archived paraffin-embedded specimens of ESCC and paired adjacent non-cancerous tissues from 40 Uygur patients who underwent surgical resection at the First People' s Hospital of Kashgar, Xinjiang in 2015. All patients had complete clinical records and had not received preoperative radiotherapy or chemotherapy. The cohort included 28 males and 12 females with a mean age of 62.3 ± 7.5 years. Tumor differentiation was high in 8 cases, moderate in 5 cases, and low in 27 cases. Tumor locations were upper thoracic (n = 6), middle thoracic (n = 19), and lower thoracic (n = 15). Invasion depth was T2 in 9 cases, T3 in 29 cases, and T4 in 2 cases. Lymph node metastasis was present in 23 cases and absent in 17 cases. Clinical stage distribution was stage II in 19 cases and stage III in 19 cases.

1.2 Equipment and Reagents

Key equipment included a Leica RM2245 rotary microtome, Motic BA400 microscope, and Roche Benchmark XT automated immunohistochemistry system. Primary antibodies consisted of rabbit anti-human FAM135B polyclonal antibody (Sigma) and rabbit anti-human KAT5 polyclonal antibody (Abcam).

1.3 Immunohistochemical Staining

Immunohistochemical staining was performed on the Roche Benchmark XT platform according to standardized protocols. The procedure included baking slides, washing with reaction buffer, deparaffinization with EZprep, incubation with 3% H O , antigen retrieval using CCI solution with independent temperature-controlled slide heating, application of LCS solution to prevent evaporation, primary antibody incubation (100 L), secondary antibody incubation, DAB chromogenic development, hematoxylin counterstaining with bluing, gradient alcohol dehydration, and final xylene clearing and mounting.

1.4 Evaluation of Immunohistochemical Results

Staining was evaluated by first identifying the most densely stained area under 40× magnification, followed by cell counting under 100× magnification. The final score was calculated as the product of the proportion of stained cells (0% = 0; 1–24% = 1; 25–49% = 2; 50–74% = 3; 75–100% = 4) and staining intensity

(negative = 0; light yellow = 1; orange-yellow = 2; brown = 3). Expression levels were classified as: negative (-) for 0 points, weakly positive (+) for 1–4 points, moderately positive (++) for 5–8 points, and strongly positive (+++) for 9–12 points.

1.5 Statistical Analysis

Data analysis was performed using SPSS 20.0 software. Comparisons between groups were conducted using ^{2} tests, and bivariate correlations were analyzed using Spearman correlation analysis. Statistical significance was defined as $P < 0.05$.

Results

2.1 Expression of FAM135B and KAT5 in Uygur ESCC and Adjacent Tissues

FAM135B expression was detected in 92.50% (37/40) of ESCC specimens and 87.50% (35/40) of adjacent tissues. The proportion of strong FAM135B expression was significantly higher in cancer tissues than in adjacent tissues [45.00% (18/40) vs. 22.50% (9/40); $^2 = 4.528$, $P = 0.033$]. Among the 18 ESCC cases with strong FAM135B expression, 6 showed strong expression in paired adjacent tissues, demonstrating a strong positive correlation between ESCC and paired adjacent tissues (Kendall' s coefficient = 0.707, $P < 0.001$).

KAT5 expression was observed in 15.00% (6/40) of ESCC specimens and 12.50% (5/40) of adjacent tissues. The rate of negative KAT5 expression did not differ significantly between cancer and adjacent tissues [85.00% (34/40) vs. 87.50% (35/40); $^2 = 0.105$, $P = 0.745$]. Notably, among the 18 ESCC cases with strong FAM135B expression, 17 showed negative KAT5 expression, revealing a significant negative correlation between strong FAM135B expression and KAT5 expression in ESCC (Kendall' s coefficient = -0.946, $P < 0.001$) , [Figure 1: see original paper].

2.2 Correlation of FAM135B and KAT5 Expression with Clinicopathological Features

Analysis of the relationship between FAM135B/KAT5 expression and seven clinicopathological features in Uygur ESCC patients is summarized in . Strong FAM135B expression showed no significant correlation with gender, age, tumor location, differentiation grade, invasion depth, lymph node metastasis, or clinical stage (all $P > 0.05$). Similarly, negative KAT5 expression demonstrated no significant association with these seven parameters (all $P > 0.05$).

Discussion

FAM135B is located on chromosome 8q24.23. Previous studies have linked mutations in this gene to susceptibility to extrapulmonary tuberculosis and autism,

while its role in tumorigenesis remains poorly understood. Song et al. first implicated FAM135B as a driver of ESCC development in Han Chinese populations, though other research suggests its mutations primarily function during late-stage ESCC progression. Our study examined protein-level expression in Uygur patients, revealing high FAM135B expression rates in both ESCC (92.50%) and adjacent tissues (87.50%), with significantly stronger expression in cancer tissues. Moreover, strong FAM135B expression showed a robust positive correlation between cancer and paired adjacent tissues (Kendall' s coefficient = 0.707, $P < 0.001$), yet demonstrated no significant association with clinicopathological features.

Regarding KAT5 expression, previous studies reported KAT5 mRNA expression in 97.5% of tumor tissues from Kazakh and Han Chinese ESCC patients, significantly higher than in normal tissues, though protein-level differences were not statistically significant. Our findings show predominantly negative KAT5 protein expression in both ESCC and adjacent tissues among Uygur patients, with no significant difference between groups. Additionally, negative KAT5 expression did not correlate with any clinicopathological parameters. However, a striking negative correlation emerged between strong FAM135B expression and KAT5 expression in ESCC tissues (Kendall' s coefficient = -0.946, $P < 0.001$).

These findings suggest that strong FAM135B expression may constitute an important molecular basis for ESCC development in Uygur patients, potentially functioning through negative regulation of KAT5. Combined with previous Han Chinese studies, our results indicate shared pathogenic mechanisms between Uygur and Han ESCC. Indeed, Liu et al. reported similar mechanisms of esophageal squamous cell carcinogenesis induced by human papillomavirus infection in both ethnic groups. Nevertheless, other studies have identified differences in ESCC biomarkers between Han and Uygur patients. For instance, serum levels of vascular endothelial growth factor and endostatin, which correlate with ESCC stage and prognosis, are significantly higher in Han patients. Additionally, the xeroderma pigmentosum group D gene, associated with ESCC susceptibility, exhibits opposite predictive effects in Han versus Uygur populations. These ethnic-specific differences underscore the necessity of molecular mechanism studies in Uygur ESCC.

Our study has several limitations. First, most patients had middle-to-advanced stage ESCC, precluding investigation of FAM135B and KAT5 expression in early-stage disease or esophageal intraepithelial neoplasia. Since ESCC and its precancerous lesions share similar genetic mutations, identifying common markers across both stages could facilitate risk stratification. Second, our sample size was relatively small, necessitating validation in larger cohorts.

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Note: Figure translations are in progress. See original paper for figures.