Association of High Expression of Mitochondrial Fission Regulator 2 with Poor Survival of Patients with Esophageal Squamous Cell Carcinoma

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Abstract

Background: Mitochondrial fission regulator 2 (MTFR2) is associated with mitochondrial fission, while few studies has assessed the associations between MTFR2 expression and clinical characteristics and prognosis of esophageal squamous cell carcinoma (ESCC).

Methods: We compared the expression of MTFR2 in 6 ESCC tumors and relative normal tissues by immunochemistry (IHC). To assess the effect of MTFR2 expression on clinicopathologic characteristics and survival, totally 115 paraffin embedded ESCC tissue samples were assessed by IHC staining. Furthermore, the associations between clinicopathological properties and MTFR2 expression in patients with ESCC was examined. The survival analysis was performed using the Cox regression models.

Results: We found that MTFR2 expression was significantly increased in ESCC tumors compared with normal esophageal epithelial cells (NEECs). IHC analysis of 115 paraffin embedded ESCC tumor specimens of the patients showed that the expression of MTFR2 was significantly associated with clinical stage (P <0.001), tumor classification (P <0.001), histological grade (P<0.001), and other clinicopathological characteristics. Both univariate and multivariate analyses showed that MTFR2 expression was significantly associated with the survival of ESCC patients.

Conclusion: The expression of MTFR2 is significantly associated with clinicopathologic characteristics and prognosis of ESCC. Thus, *MTFR2* expression could serve as a potentially important prognostic biomarker and clinical target for patients with ESCC.

Introduction

Esophageal cancer is one of the most common malignant tumors in the upper digestive tract of the chest(1, 2). Esophageal squamous cell carcinoma (ESCC) is one of most common types of esophageal cancer(3, 4). Approximately 300,000 people die of esophageal cancer annually worldwide, with more than half in China[5]. Moreover, the mortality of esophageal cancer is ranked fourth in China(5). Recurrence and metastasis of ESCC are still the main causes for death and treatment failure, and have crucial impact on the prognosis of ESCC(6, 7). Although considerable progress has been made in diagnosis and treatment, the relevant predictors for early diagnosis and prognosis remain lacking. Therefore, identification of molecular biomarkers will promote the development of new diagnostic and prognostic predictors for early diagnosis and personalized treatment of patients with ESCC.

Mitochondrial fission regulator 2 (MTFR2), also known as similarity 54 family member A (FAM54A), belongs to the MTFR1/FAM54 family(8, 9). It has two subtypes and is produced by selective shearing. MTFR2 plays important roles in mitochondrial division and aerobic respiration(10), while few studies have assessed the effects of MTFR2 expression on clinicopathological characteristics and prognosis of ESCC. Currently, some studies have shown that MTFR2 is overexpressed in glioblastoma (GBM)(9), while the role of MTFR2 expression in ESCC remains unclear.

In this study, we analyzed the expression of MTFR2 in six paired ESCC and normal specimens and 115 paraffin embedded ESCC tumor samples by immunohistochemistry (IHC). We evaluated the associations of MTFR2 expression with clinicopathological characteristics of ESCC patients, and further explored the effect of expression of *MTFR2* in ESCC on clinical outcome.

Materials And Methods

Study patients and tissue specimens

The study was approved by the Yantai Yuhuangding Hospital ethics committee and obtained consent from each patient before study. Besides the 6-pairs of ESCC patients, additional 115 paraffin-embedded tumor specimens from patients, who were pathologically diagnosed with ESCC from 2005 to 2010, were enrolled for the study at Yantai Yuhuangding Hospital. Detailed clinical information of the patients is shown in Table 1. Written informed consents were given to all patients and this study was approved by the IRB committee of Yantai Yuhuangding Hospital.

Table 1
Clinicopathological characteristics of patients and MTFR2 expression in ESCC

Characteristics	Number of cases (%)
Sex	
Female	6 (5.22)
Male	109 (94.78)
Age(years)	
>60	56 (48.70)
≤60	59 (51.30)
Clinical stage	
I/II	79 (68.70)
III/IV	36 (31.30)
T stage	
1	20 (17.39)
2	36 (31.30)
3	40 (34.78)
4	19 (16.52)
N stage	
0	76 (66.09)
1	33 (28.70)
2	6 (5.22)
M stage	
0	110 (95.65)
1	5 (4.35)
Vital status	
Alive	53 (46.09)
Dead	62 (53.91)
Histological differentiation	
High	22(19.13)

Characteristics	Number of cases (%)
Moderate	67 (58.26)
Low	26 (22.61)
Expression of MTFR2	
Low	77 (67.00)
High	38 (33.00)

IHC

MTFR2 expression was performed in 115 paraffin embedded ESCC specimens and 6 pairs of ESCC and normal tissue samples by IHC, as previously described(11). In IHC, we used rabbit monoclonal antibody as target anti-MTFR2 antibody (1:200). Samples without any antibody were used as the corresponding blank control(11). In qualitative analysis, we selected 6 samples, which contained cancer and adjacent normal tissues, for comparison(12). Two independent pathologists who did not know the relevant clinical information evaluated the results of IHC staining of tumor Sect. (13). The positive staining score of cancer cells was divided into the following five grades: 0 (0), 1 (< 10%), 2 (11–50%), 3 (51–75%), and 4 (> 75%). The staining intensity was graded as follows: 0(negative = no staining), 1 (weak = light yellow), 2 (moderate = yellow brown), and 3 (strong = brown). The staining index (SI) was defined as the staining intensity score × the proportion of positive staining tumor cells (ranging from 0 to 12). Simultaneously, we defined SI ≥ 6 as high expression and SI < 6 as low expression.

Statistical analysis

The relationships between MTFR2 expression level and sex, age, TNM stage and clinical stage were analyzed using the Spearman correlation method. The effect of MTFR2 expression on clinicopathological characteristics was assessed using the test or Fisher's exact test. For survival analysis, the effect of clinicopathological characteristics and MTFR2 expression on prognosis of ESCC were examined by the Kaplan-Meier analysis and log rank test, and further evaluated by univariate and multivariate Cox regression analyses. All statistical analyses were performed using SPSS software, version 20 (SPSS Inc., Chicago, IL, USA). Statistical significance was set at $P < 0.05$.

Results

MTFR2 is highly expressed in ESCC tissues

After IHC in 115 specimens, we found that MTFR2 was highly expressed in ESCC tissues. To validate the expression of MTFR2 in ESCC, we further analyzed the expression of MTFR2 in 6 pairs of ESCC tumor and adjacent normal tissues. Compared to that in normal tissues, the expression of MTFR2 in ESCC tissues was significantly overregulated as shown in Fig. 1. Therefore, these results indicated that MTFR2

was overexpressed in ESCC tumors. In addition, to evaluate the association between MTFR2 expression and clinicopathological characteristics of patients with ESCC, we analyzed such associations in 115 ESCC patients. These patients included 25 patients with stage I, 53 patients with stage II, 32 patients with stage III, and 5 patients with stage IV, respectively. Out IHC staining showed that 38 cases (33.00%) had high MTFR2 protein expression and 77 cases (67.00%) had low or no expression (Table 1).

Association between MTFR2 expression clinicopathologic characteristics of ESCC

As shown in Table 2, we found that there was no significant difference in MTFR2 expression for age and sex ($P > 0.05$), while the significant differences in MTFR2 expression were found for clinical stage ($P < 0.001$), pathological grade ($P = 0.029$), T/N stage ($P < 0.001$) and M classification ($P = 0.040$) (Table 2). These results suggested that MTFR2 expression was significantly associated with advanced tumors. Additionally, we also analyzed the correlations between MTFR2 expression and clinicopathologic characteristics. As shown in Table 3, there was statistically significant correlations between MTFR2 expression and clinical stage (r = 0.460, P 0.001) (Fig. 2), T stage (r = 0.318, $P = 0.001$), N stage (r = 0.373, P 0.001), M stage (r = 0.213, $P = 0.040$), and histological differentiation (r = 0.192, $P = 0.038$). Therefore, MTFR2 was significantly related to overall clinical stage, TNM stage, and poor pathological differentiation of ESCC, supporting a role of MTFR2 in cancer progression of ESCC.

Table 2
The association of MTFR2 expression with clinicopathological characteristics of ESCC patients

Characteristics	No. of cases	MTFR2 expression		test *P*	Fisher's exact test
		Low (%)	High (%)		*P*
Sex				0.351	0.267
Female	6	5 (83.33)	1 (16.77)		
Male	109	72 (66.06)	37 (33.94)		
Age(years)				0.213	0.097
≤60	56	40 (71.43)	16 (28.57)		
>60	59	37 (62.71)	22 (37.29)		
Clinical stage				□0.001	□0.001
I/II	79	65 (82.29)	14 (17.71)		
III/IV	36	12 (33.33)	24 (66.67)		
T stage				□0.001	□0.001
1	20	17 (85.00)	3 (15.00)		
2	36	27 (75.00)	9 (25.00)		
3	40	27 (67.50)	13 (32.50)		
4	19	6 (31.58)	13 (68.42)		
N stage				□0.001	□0.001
0	76	60 (78.95)	16 (21.05)		
1	33	16 (48.48)	17 (51.52)		
2	6	1 (16.67)	5 (83.33)		
M stage				0.040	0.037
0	110	76 (69.09)	34 (30.91)		
1	5	1 (20.00)	4 (80.00)		
Histological differentiation				0.029	0.015
High	22	17 (77.27)	5 (22.73)		
Moderate	67	47 (70.15)	20 (29.85)		
Low	26	13 (50.00)	13 (50.00)		

Table 3
Spearman correlation analysis between MTFR2 expression and clinicopathologic characteristics

Variables	MTFR2 expression level Spearman's correlation	*P*
Clinical stage	0.460	<0.001
T stage	0.318	0.001
N stage	0.373	<0.001
M stage	0.213	0.040
Vital status	0.427	<0.001
Histological differentiation	0.192	0.038

MTFR2 expression is associated with survival of ESCC

In the patients with low MTFR2 expression, the rate of cumulative 5-year survival was 61.0% (95% CI, 60.6%, 75.9%), while that was only 15.8% (95% CI, 15.2%, 28.1%) in patients with high MTFR2 expression, indicating that the 5-year survival rate in the low MTFR2 expression group was significantly higher than in high MTFR2 group

Furthermore, we found that the survival time of ESCC patients was negatively correlated with MTFR2 expression (r = -0.580, *P*<0.001), and the patients with high MTFR2 expression had worse survival than those with low expression (log-rank: *P*<0.001, Fig. 3A). Our stratified analysis also showed the survival differences in several subgroups. There were significant differences in survival between MTFR2 expression in patients with early disease stage (stage I /II) (log-rank, *P* < 0.001, Fig. 3B) and those with advanced disease (stage III/IV) (*P*<0.001, Fig. 3C). Similarly, there were significant survival differences between the MTFR2 expression in patients with T1-2, T3-4, positive lymph nodes, negative lymph nodes, non-distant metastasis and distant metastasis groups (All *P*<0.001, Fig. 4), respectively. These findings in the subgroups further indicated that high expression of MTFR2 had shorter survival than low expression of MTFR2, supporting the role of MTFR2 expression could be potential prognostic biomarkers for ESCC.

Finally, we performed both univariate and multivariate Cox regression analyses to assess the associations between MTFR2 expression and survival of patients with ESCC. Our results showed that the expression of MTFR2 was a significantly independent prognostic predictor for ESCC (aHR, 3.04, 95%CI, 1.75–5.30; *P*<0.001; Table 4).

Table 4
Univariate and multivariable analysis on association between MTFR2 expression and survival in patients with ESCC

Variables	Univariate analysis				Multivariate analysis		
	No. of patients	*P*	cHR†	95% CI	*P*	aHR*	95% CI
MTFR2 expression		0.001			0.001		
Low	77		1.00			1.00	
High	38		3.12	1.66–5.27		3.04	1.75–5.30

†cHR: crude hazard ratio

*aHR: adjusted hazard ratio, HR was adjusted by age, sex, clinical stage, T/N/M stage, and Histological differentiation

Discussion

In this study, *MTFR2* expression were relatively higher in tumor lesions than that in normal tissues. *MTFR2* was highly expressed in ESCC tumor specimens, and it was significantly correlated with clinical stage, T, N, M classification, histological differentiation, and metastasis of ESCC. The expression of *MTFR2* increased with the progression of ESCC, indicating that high expression of *MTFR2* could be related to the progression of ESCC. Our current findings demonstrated that *MTFR2* was an independent prognostic factor for prognosis of patients with ESCC, and the patients with high *MTFR2* expression had worse survival than those with corresponding low expression. Therefore, it is likely that *MTFR2* expression may have significant clinical values as a new prognostic predictor and one of potential novel targets for future targeted treatment of ESCC.

As a mitochondrion fission factor, MTFR2 is related to mitochondrion fission, participates in aerobic respiration and has antioxidant effects(8, 14). While MTFR2 may play a key role in the induction of intrinsic apoptosis(15, 16). Compared with normal cells, mitochondrion induces tumor-related programming and accelerates growth of tumor cells and blood vessels(17, 18). Some studies have shown that MTFR2 may transcriptionally regulate dual specificity protein kinase TTK(9). MTFR2 is one of the key genes related to TTK, which can be regulated by activating the promoter of TTK(19, 20). Furthermore, it is involved in regulation of proliferation of glioma stem cells (GSCs) and thus may affect the prognosis of glioblastoma(9, 17). Most recently, MTFR2 has been reported to promote the proliferation, migration, and invasion of oral squamous carcinoma(21), and MTFR2-dependent regulation of TTK was involved in maintaining GSCs in glioblastoma and might serve as a potential novel druggable target for glioblastoma. It might regulate the expression of TTK for participation in up-regulation and expression of GSCs in glioblastoma. In addition to cell proliferation, TTK was also involved in centrosome

duplication, DNA damage response, and organ development(22). Another study showed that reduced TTK levels could result in abnormal mitoses, activate apoptosis and reduce survival of breast cancer(23).

TTK depletion might seriously damage the viability and ability to form colonies of TNBC cell lines(24). Thus, *TTK* might be an independent predictor of prognosis, and it appears biologically plausible that *MTFR2* might play important roles in activation of regulation, promotion of expression of *TTK* in ESCC tumors, and occurrence and development of ESCC. However, the exact mechanisms underlying such associations require further exploration.

Hassan et al.(10) showed that the expression of MTFR2 was related to poor prognosis, suggesting that MTFR2 might be involved in the progression and invasion of several cancers(25, 26). Such findings may highlight the potential role of MTFR2 in ESCC as future new targets for treatment.

Although our study found that MTFR2 was overexpressed in ESCC, and was significantly associated with advanced disease and prognosis, which was consistent with the research on glioma[9], our research has some limitations. First, we only analyzed the expression levels of cell protein, while we did not specifically analyze other aspects of genetic alterations. Second, our sample size is relatively small, and all study patients are from a single hospital, and our findings could be due to by chance. Moreover, the selection bias may also exist. Nevertheless, the current preliminary findings from this small study might serve as a basis for future hypothesis generation and testing as well as validation in large well-designed prospective studies such consortia, multi-center study. Finally, more studies are needed to explore the exact molecular mechanisms underlying the associations.

Conclusions

In conclusion, in the current study, we found that high expression of *MTFR2* is overexpressed in and correlated with ESCC progression. In addition, *MTFR2* expression was significantly associated with survival of patients with ESCC, particularly in aggressive tumors. Thus, our current findings might support that *MTFR2* could be an independent biomarker for prognosis and serve as a potential new therapeutic target for ESCC.

Abbreviations

MTFR2, Mitochondrial fission regulator 2; ESCC, esophageal squamous cell carcinoma; IHC, immunochemistry; NEECs, normal esophageal epithelial cells, GSCs, glioma stem cells.

Declarations

Ethics approval and consent to participate

All procedures performed in studies involving human participants were in accordance with the ethical standards of the Ethics Committee of the Yantai Yuhuangding Hospital Affiliated Hospital of Qingdao

University and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Consent for publication

Not applicable.

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

HL, XZ, WZ, WL, and JM carried out the majority of the experiment, data analysis, and wrote the manuscript. They were all helped by CL, YZ, RZ, and YS. All authors reviewed and approved the final manuscript.

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Figures

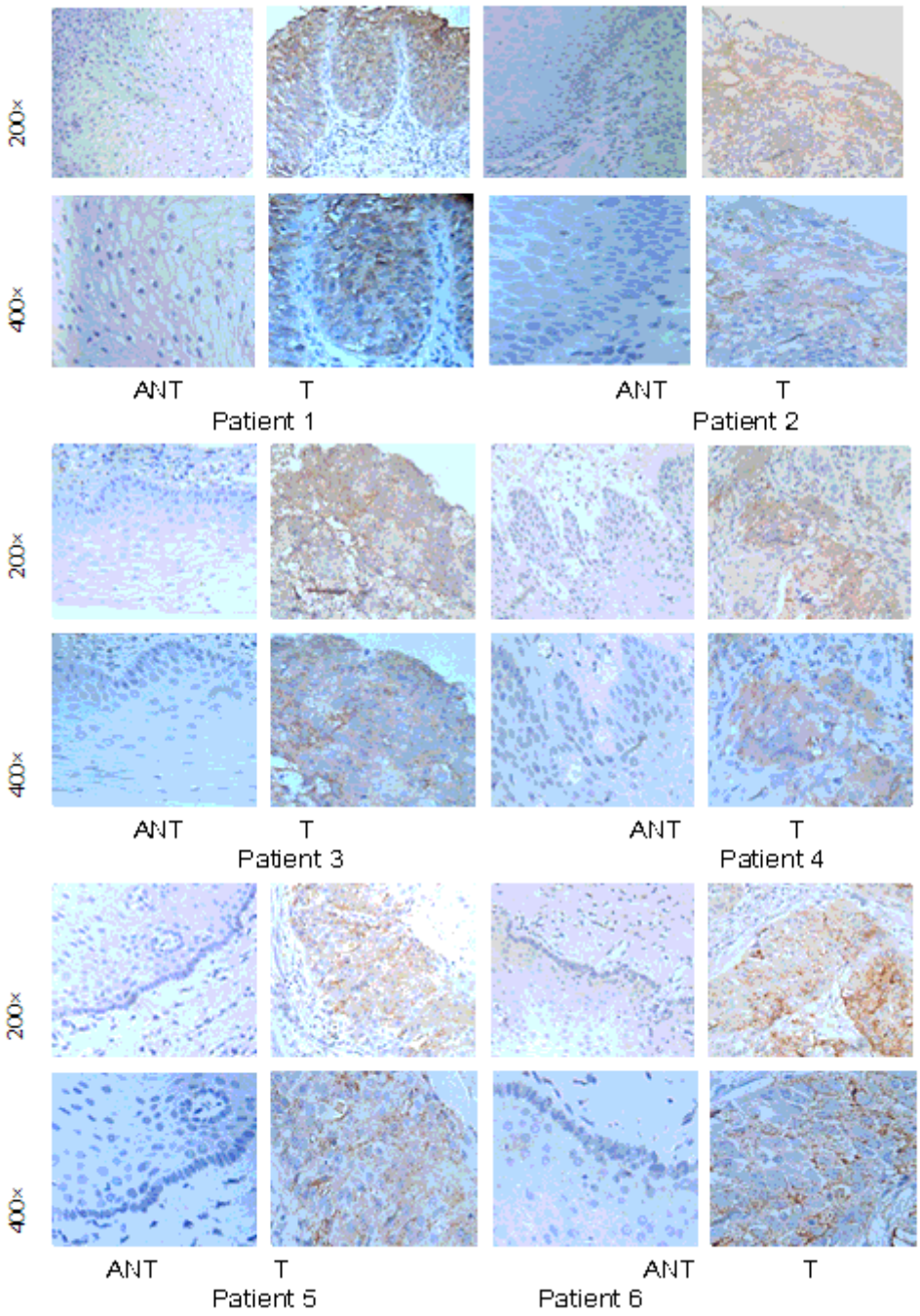

Figure 1

Upregulation of MTFR2 in ESCC tumor tissues.

Figure 2

MTFR2 expression in normal esophagus tissues and ESCC tumor specimens at different clinical stages.

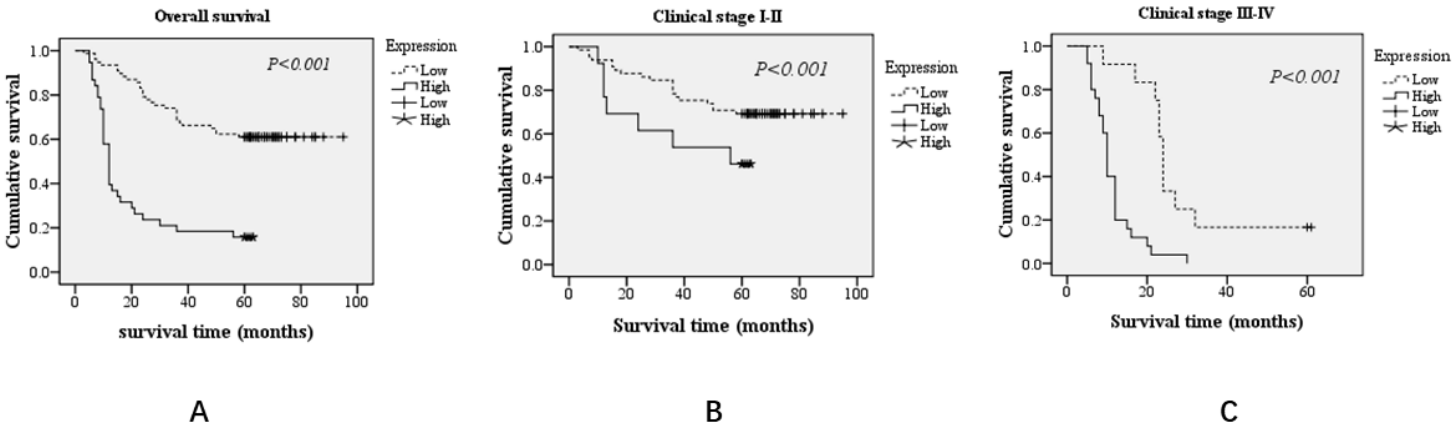

Figure 3

Effect of MTFR2 expression on survival in all patients, early-stage patients, and late-stage patients.

Figure 4

Effect of MTFR2 expression on survival in ESCC patients stratified by T classification, lymph node status, and metastasis status.