RESEARCH ARTICLE

Decreased Expression of FADS1 Predicts a Poor Prognosis in Patients with Esophageal Squamous Cell Carcinoma

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Abstract

FADS1 (fatty acid desaturase 1) plays a crucial role in fatty acid metabolism, and it was recently reported to be involved in tumorigenesis. However, the role of FADS1 expression in esophageal squamous cell carcinoma (ESCC) remains unknown. In the current study, we investigated the expression and clinical pathologic and prognostic significance of FADS1 in ESCC. Immunohistochemical analyses revealed that 58.2% (146/251) of the ESCC tissues had low levels of FADS1 expression, whereas 41.8% (105/251) exhibited high levels of FADS1 expression. In positive cases, FADS1 expression was detected in the cytoplasm of cells. Correlation analyses demonstrated that FADS1 expression was significantly correlated with tumor location (p=0.025) but not with age, gender, histological grade, tumor status, nodal status or TNM staging. Furthermore, patients with tumors expressing high levels of FADS1 had a longer disease-free survival time (p<0.001) and overall survival time (p <0.001). Univariate and multivariate analyses revealed that, along with nodal status, FADS1 expression was an independent and significant predictive factor (p<0.001). In conclusion, our study suggested that FADS1 might be a valuable biomarker and potential therapeutic target for ESCC.

Keywords: FADS1 - Esophageal squamous cell carcinoma - prognosis - survival

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Introduction

Esophageal squamous cell carcinoma (ESCC) is one of the most common malignant neoplasms in the digestive tract, with the highest rates occurring in southern and eastern Africa and eastern Asia, where it accounts for more than 90% of esophageal malignant tumors (Gholipour et al., 2008; Jemal et al., 2011). The incidence rate of ESCC has demonstrated an increasing trend in recent years, and the mechanism underlying its occurrence may be associated with a high-temperature diet, smoking and alcohol consumption (Engel et al., 2003; Lee et al., 2005; Wu et al., 2006). Currently, there are less effective clinical approaches, including surgery, chemotherapy, radiotherapy and their combinations, for the treatment of ESCC, resulting in a limited five-year survival rate (Chen et al., 2013; Song et al., 2014). Therefore, there is an urgent need to identify new markers for tumor staging and new strategies for the treatment of ESCC.

Recently, an increasing number of studies have revealed a relationship between fatty acid metabolism and tumor progression (Cairns et al., 2011; Carracedo et al., 2013; Currie et al., 2013). FADS1 (fatty acid desaturase 1) is a member of the fatty acid desaturase (FADS) gene family. FADS1 participates in fatty acid metabolism by regulating the instauration of fatty acids through the introduction of double bonds into defined carbons of the fatty acyl chain (Wang et al., 2007; Glaser et al., 2011; Wang et al., 2014). FADS1 has 2 human homologs, namely FADS2 and FADS3 (Marquardt et al., 2000; Blanchard et al., 2011), both of which consist of an N-terminal cytochrome b5-like domain and a C-terminal multiple transmembrane desaturase domain (Marquardt et al., 2000). Some reports have described an association between epigenetic modifications or changes in transcript levels of FADS families and cancer (Yamashita et al., 2006; Liu et al., 2007; Fan et al., 2012; Zhang et al., 2014). However, the correlation between FADS protein levels and tumor prognosis remains unknown. The role of FADS1 in ESCC has never been investigated in relatively large samples. Therefore, in the present study, we used immunohistochemistry (IHC) to investigate the expression of FADS1 in ESCC and its impact on patient survival.

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Materials and Methods

Clinical samples

Patients enrolled in the current study were diagnosed with ESCC from October 2000 to April 2007 at the Sun Yat-sen University Cancer Center (SYSUCC) and underwent curative resection. A total of 251 ESCC tissues and adjacent paracancerous tissues were sectioned and subjected to IHC staining. The histological grade and clinical stage were diagnosed according to the 7th edition of the TNM classification of the International Union Against Cancer (2009). The clinical data for the patients were obtained from the hospital records after surgery. This study was approved by the research ethics committee of SYSUCC.

Immunohistochemistry (IHC)

Two hundred and fifty-one ESCC specimens were subjected to IHC staining to visualize the expression of FADS1. First, the specimens were deparaffinized and rehydrated. After three washes in PBS, the slides were boiled for 15 minutes in citric acid in a microwave oven to retrieve antigen, followed by treatment with 3% hydrogen peroxide buffered in methanol to quench the endogenous peroxidase activity. Next, 1% BSA was used to block non-specific binding. After blocking, the sections were incubated with rabbit anti-human monoclonal antibody against FADS1 (1:400 dilution; ab126706; Abcam) buffered in blocking buffer at 4°C overnight in a moist chamber. Blocking buffer without primary antibody was used as a negative control. The sections were then incubated with horseradish peroxidase for 30 minutes at 37°C, followed by an incubation with 3,30-diaminobenzidine solution for visualization.

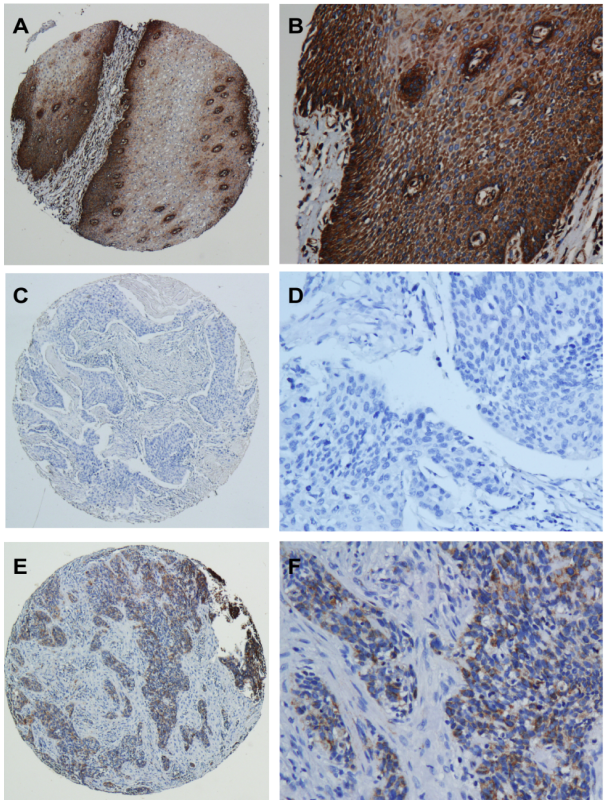

Figure 1. FADS1 Expression by Immunohistochemical Staining. a,b Normal esophageal mucosa demonstrating a high expression level of FADS1 protein in the cytoplasm of cells (magnification: a x40, b x200). c, d Low expression level of FADS1 in ESCC (magnification: c x40, d x 200). e, f High expression level of FADS1 in ESCC (magnification: e x40, f x200)

The staining results were scored based on two criteria: (1) the proportion of positive tumor cells in the tumor tissue: 0 (0%), 1 (1% to 10%), 2 (11% to 50%), 3 (51% to 75%), and 4 (76% to 100%); and (2) the intensity of staining: 0, absent; 1, weak; 2, moderate and 3, strong. ESCC patients were divided into two groups according to the median score of anti-FADS1 IHC staining, and a score >8 was considered to indicate a high level of expression.

Statistical analysis

The statistical analysis was performed using the SPSS 17.0 software statistical software package. The ROC curve was used to define the FADS1 IRS cutoff value. The ROC curve was analyzed by using the Med-Calc statistical software package 11.0.1 (MedCalc Software bvba, Ostend, Belgium). The correlation between FADS1 expression and clinicopathological variables was evaluated using Pearson's χ^2 test. Disease-free survival (DFS) and overall survival (OS) curves were generated using the Kaplan-Meier method and compared with the log-rank test. A Cox proportional hazard model was used for the multivariate survival analysis. A two-sided $p<0.05$ was considered statistically significant.

Results

FADS1 expression in ESCC tissues and normal esophageal mucosal tissues

To determine the expression and localization of FADS1 in ESCC tissues, immunohistochemistry analysis was conducted in 251 paraffin-embedded specimens. The study included 180 males and 71 females. The clinicopathological characteristics of the 251 patients are listed in Table 1. FADS1 expression was significantly downregulated in ESCC tissues compared with the adjacent normal tissues (Figure 1). A high level of FADS1 protein expression was detected in 41.8% (105/251) of the ESCC tissues, whereas a low expression level of FADS1 was observed in 58.2% (146/251) of the ESCC tissues. IHC staining showed FADS1 localization to the cytoplasm both in ESCC and normal tissues (Figure 1).

Selection of the FADS1 immunoreactivity score cutoff value

The ROC curve was used to define the FADS1 IRS cutoff value. The point with both maximum sensitivity and specificity was selected as the cutoff value. The cutoff value for FADS1 expression was 8 (Figure 2). As a consequence, high and low levels of FADS1 expression were defined as an IRS greater than or less than 8, respectively.

Relationship between FADS1 expression and clinicopathological variables

The correlation between FADS1 expression and

patient clinicopathological variables was examined. As summarized in Table 1, FADS1 expression was significantly correlated with the tumor location (p=0.025) but was not statistically correlated with age, gender, histological grade, tumor status, nodal status or TNM staging (p=0.28, 0.932, 0.197, 0.401, 0.787 and 0.948, respectively).

FADS1 expression and survival

We analyzed the correlation between FADS1 expression and the survival of patients with ESCC using Kaplan-Meier methodology (log-rank test). The median observation period was 55 months (range, 4 to 150 months), and 143 patients were deceased and 108 were alive at the end of the follow-up. The 5-year OS rate and DFS rate for the entire cohort was 47.0% and 44.2%, respectively. Patients with a high level of FADS1

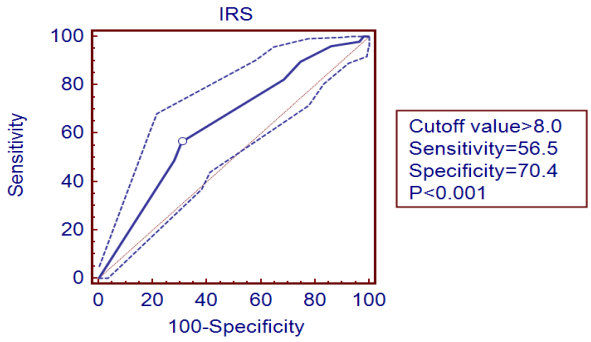

Figure 2. Receiver Operating Curve for the FADS1 Expression Cutoff Value Plotted By Survival Status

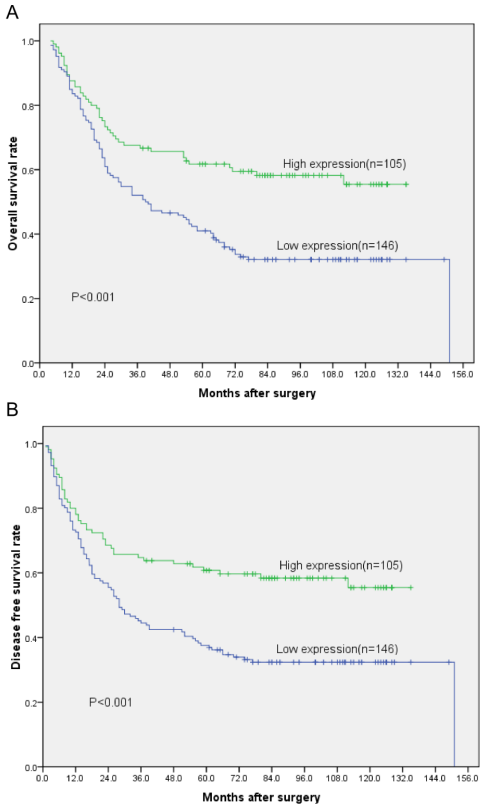

Figure 3. Overall Survival (OS) and Disease-Free Survival (DFS) Curves for ESCC Patients According to their FADS1 Expression Status. a OS curves: patients with low and high expression levels of FADS1. b DFS curves: patients with low and high expression levels of FADS1

Table 1. Correlation between FADS1 Expression and Clinicopathological Variables of ESCC Cases

Variables	Cases (n=251)	FADS1 expression		*P* value
		Low	High	
Age (years)				0.28
<60	143	79(31.5)	64(25.5)	
≥60	108	67(26.7)	41(16.3)	
Gender				0.932
Male	180	105(41.8)	75(29.9)	
Female	71	41(16.3)	30(12.0)	
Tumor location				0.025*
Upper	14	13(5.2)	1(0.4)	
Middle	173	98(39.0)	75(29.9)	
Lower	64	39(15.5)	25(10.0)	
Histological grade				0.197
Well differentiated (G1)	56	36(14.2)	20(8.0)	
Moderately differentiated (G2)	161	87(34.7)	74(29.5)	
Poorly differentiated (G3)	34	23(9.2)	11(4.4)	
Tumor status (T)				0.401
T1	6	3(1.2)	3(1.2)	
T2	61	41(16.3)	20(8.0)	
T3	181	100(39.8)	81(32.3)	
T4	3	2(0.8)	1(0.4)	
Nodal status (N)				0.787
N0	134	79(31.5)	55(21.9)	
N>0	117	67(26.7)	50(19.9)	
TNM Staging				0.948
I	9	5(2.0)	4(1.6)	
II	146	84(33.5)	62(24.7)	
III	96	57(22.7)	39(15.5)	

*p<0.05, statistically significant

Table 2. FADS1 Expression in ESCC Patients by Kaplan-Meier Survival Analysis (Log-Rank Test)

Variable	Case	DFS (months)			OS (months)		
		Mean	Median	P-value	Mean	Median	*P*-value
Total							
Low expression	146	63.7	28	<0.001	68.2	39	<0.001
High expression	105	86	NR		88.8	NR	
T categories							
T1-2							
Low expression	44	69.6	36	0.002	74	54	0.002
High expression	23	111.4	NR		112.8	NR	
T3-4							
Low expression	102	60.5	27	0.011	65.1	38	0.01
High expression	82	75.6	NR		82.3	NR	
N categories							
N=0							
Low expression	79	91.4	151	0.018	94.8	151	0.018
High expression	55	106.8	NR		108.3	NR	
N=1/2/3							
Low expression	67	27.8	17	0.001	33.9	23	0.001
High expression	50	59.4	26		63.5	37	
Histologic grade							
G1							
Low expression	36	79	62	0.264	81.9	68	0.252
High expression	20	84.3	NR		88.6	NR	
G2-3							
Low expression	110	58.1	26	<0.001	63.1	34	<0.001
High expression	85	85.4	NR		87.9	NR	

*ESCC: esophageal squamous cell carcinoma; DFS: disease free survival; OS: overall survival; NR: not reached

Table 3. Univariate and Multivariate Analyses of Overall Survival of ESCC Patients

Variables	Univariate analyses			Multivariate analyses		
	HR	(95%CI)	p value	HR	(95%CI)	*p* value
Age (years),(<60 *vs*. ≥60)	1.065	0.766-1.482	0.707			
Gender (male vs. female)	1.227	0.844-1.785	0.284			
Tumor location(upper/middle/lower)	0.938	0.676-1.301	0.701			
Histological grade, (G3/G2/G1)	1.331	1.004-1.763	0.047*	1.23	0.933-1.620	0.142
Tumor status (T4/T3/T2/T1)	1.5	1.046-2.152	0.028*	1.411	0.980-2.032	0.064
Nodal status (N>0/N0)	3.185	2.251-4.506	<0.001*	3.228	2.272-4.588	<0.001*
FADS1 expression, (High/Low)	0.529	0.371-0.756	<0.001*	0.481	0.336-0.689	<0.001*

expression demonstrated a significantly better OSand DFS compared with those with a low level of FADS1 expression (Figure 3). The mean OS was 68.2 and 88.8 months in the low and high FADS1 expression groups, respectively (Table 2). The mean DFS was 63.7 and 86.0 moths in the low and high FADS1 expression groups, respectively (Table 2).

We performed univariate and multivariate survival analyses using a Cox proportional hazard model to determine the effect of each independent factor on OS. As presented in Table 3, the univariate survival analysis revealed histological grade (HR=1.331, p=0.047), tumor status (HR=1.500, p=0.028), nodal status (HR=3.185, p<0.001) and FADS1 expression level (HR=0.529, p<0.001) as independent predictors of OS, whereas the multivariate survival analysis suggested that only nodal status (HR=3.228, p<0.001) and FADS1 expression level (HR=0.481, p<0.001) were independent predictors of OS. These data demonstrated that the expression level of FADS1 had a significant association with the clinicopathological characteristics of the patients. Altogether, FADS1 appears to be a good predictive factor in ESCC, supporting its potential use as a prognostic marker of ESCC.

Discussion

FADS1 was first identified in the 11q12-13.1 cluster in the human genome in 2000 (Marquardt et al., 2000). This genetic locus is associated with a variety of immune-associated diseases, such as asthma, atopy and osteoarthritis (Wang et al., 2007; He et al., 2012). The protein product of FADS1, △5 desaturase, regulates the instauration of fatty acids through the introduction of double bonds into defined carbons of the fatty acyl chain (Wang et al., 2007). Abnormal lipid metabolism is observed in tumor cells (Cairns et al., 2011), and FADS1 is one of the disrupted genes in several cancers. For example, the FADS1 gene is associated with the risk of colorectal cancer (Zhang et al., 2014), is dysregulated

in hepatocellular carcinoma (Liu et al., 2007) and is methylated in gastric carcinoma (Yamashita et al., 2006). The limitation of these studies is the detection of only the genetic state or transcript level of FADS1 in the corresponding cancer; no studies have investigated whether the expression of FADS1 is associated with tumor prognosis. The present study is the first to demonstrate a correlation between FADS1 expression and ESCC.

Our data revealed reduced levels of FADS1 in ESCC tissues compared with adjacent nonmalignant esophageal mucosal tissues. Among these ESCC tissues, 58.2% (146/251) showed low FADS1 expression, whereas 41.8% (105/251) exhibited high FADS1 expression. Further analysis revealed that the expression of FADS1 was significantly related to the tumor location (p=0.025) but was not statistically correlated with age, gender, histological grade, tumor status, or nodal status. At present, surgical resection continues to be the main therapy for ESCC patients. However, even patients with the same TNM stage experience different therapy outcomes. Therefore, additional predictive factors of prognosis are needed to evaluate the outcomes of therapy and provide suggestions for individualized treatment. The Cox multivariate analysis showed that decreased FADS1 expression correlated with a poor prognosis in ESCC patients. Patients with higher FADS1 expression demonstrated a longer DFS and OS. Thus, patients with a low level of FADS1 expression could be recommended to undergo aggressive radiotherapy and chemotherapy. Collectively, our data indicate that FADS1 is a potentially independent and predictive factor in the survival of ESCC patients.

Previous reports have shown that FADS1 expression is closely related to inflammatory diseases and that the inhibition of FADS1 can promote significant anti-inflammatory effects (Calder, 2008; Fan et al., 2012; Wang et al., 2014). FADS1 could influence inflammation through the metabolites of its substrates: long-chain polyunsaturated fatty acids (PUFAs) (Eschwege et al., 2001; Sala-Vila et al., 2008). These metabolites include arachidonic acid (AA), and eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), all of which play an important role in inflammatory reactions and cancer progression. AA is pro-inflammatory (Kuehl and Egan, 1980; Mullen et al., 2010) and promotes tumor progression through its metabolite, prostaglandin(Yang et al., 2014), whereas EPA and DHA are less inflammatory, or anti-inflammatory (Mullen et al., 2010), and they suppress cell proliferation and diminish the migration and invasion of cancer cells in various carcinomas (Rahman et al., 2013; Li et al., 2014; Liu and Ma, 2014). The association between inflammation and cancer progression has been well characterized (Karin and Greten, 2005). FADS1 has been suggested to regulate inflammation by modifying the metabolite profiles of fatty acids and thus may influencing the progression of cancer. Therefore, decreased expression of FADS1 may disrupt the balance of AA with EPA and DHA, which benefits the growth and development of cancer cells. However, further studies are required to reveal the mechanism underlying the activity of FADS1 in ESCC. In the present study, we show that a high expression level of FADS1 is a protective factor in ESCC.

In conclusion, we demonstrated that FADS1 levels were decreased in ESCC. Decreased expression of FADS1 in ESCC patients was positively associated with the tumor location and poor prognosis. Thus, the detection of FADS1 expression by IHC could serve as an independent predictor of patient survival in ESCC patients.

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Conflict of Interest: The authors declare that they have no conflicts of interest.

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