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Prognostic values of STAT3 and HIF-1α in esophageal squamous cell carcinoma

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Abstract. – OBJECTIVE: To investigate the expressions of signal transduction and activator of transcription 3 (STAT3) and hypoxia-inducible factor 1-alpha (HIF-1α) in esophageal squamous cell carcinoma (ESCC), and their potential roles in the pathology of ESCC.

PATIENTS AND METHODS: Tumor tissues and clinical data of 202 ESCC patients treated in our hospital from January 2011 to June 2013 were collected. Expressions of STAT3 and HIF-1α in tumor tissues and normal esophageal tissues were detected by immunohistochemical S-P method. Correlation of STAT3 and HIF-1α with clinicopathological parameters and prognosis of ESCC were analyzed.

RESULTS: STAT3 was positively expressed in 82/202 ESCC tissues, with a positive expression rate of 40.59%, and HIF-1α was positively expressed in 142/202 ESCC tissues, with a positive expression rate of 70.30%. Both STAT3 and HIF-1α were highly expressed in ESCC tissues than those normal esophageal tissues, showing statistically significant differences ($p<0.05$). The expression of STAT3 was positively correlated with that of HIF-1α in ESCC tissues (r=0.401, $p<0.05$). Overall survival (OS) and disease-free survival (DFS) of ESCC patients with positive STAT3 and HIF-1α expression were markedly worse than those with negative expression ($p<0.05$). STAT3 and HIF-1α were related to the infiltration depth (T stage) of ESCC ($p<0.05$). Univariate and multivariate analyses revealed that the expression of STAT3 was associated with OS and DFS ($p<0.05$) and was an independent prognostic factor for ESCC.

CONCLUSIONS: High expressions of STAT3 and HIF-1α are closely related to ESCC. STAT3 is an independent prognostic risk factor for ESCC, and HIF-1α may be a poor prognostic survival factor for ESCC, both of which can be used as indicators to predict the prognosis of ESCC patients.

Key Words
Esophageal squamous cell carcinoma, STAT3, HIF-1α, Clinicopathology, Prognosis.

Introduction

Esophageal carcinoma is one of the top ten malignant tumors in the world, with high morbidity and mortality rates[1]. The incidence of esophageal carcinoma remains high in China, accounting for about 50% of the global morbidity and mortality cases. Unlike Western countries, squamous cell carcinoma is the major pathological subtype of esophageal carcinoma in China[2]. At present, the treatment for esophageal carcinoma mainly includes surgery, chemotherapy and radiotherapy, but most of the patients used to be already in the advanced stage when diagnosed, with a poor prognosis. With the rapid development of precision medicine, the molecular targeted therapy has become a new direction of cancer treatment[3,4]. However, therapeutic targets in esophageal carcinoma are still lacked, and search for new markers and treatment methods have become a research focus in this field. Signal transduction and transcription activator 3 (STAT3) exists in the cytoplasm and can be transferred into the nucleus to bind to deoxyribonucleic acids (DNAs) after activation, which has dual functions of signal transduction and transcription regulation[5,6]. Hypoxia-inducible factor 1-alpha (HIF-1α) is a nuclear transcription factor that plays an active role in hypoxia[7]. Studies have shown that HIF-1α expression is closely related to the tumor growth, lymphocyte proliferation, angiogenesis and metastasis. However, the relationship between STAT3 and HIF-1α in esophageal squamous cell carcinoma (ESCC) is not very clear. Therefore, in this paper, immunohistochemical method was used to detect the expressions of STAT3 and HIF-1α in ESCC tissues. We further analyzed their correlations with clinicopathological factors and prognosis of ESCC, so as to seek for new prevention and treatment targets for ESCC patients.

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Patients and Methods

Patients

Clinical data of ESCC patients in the First Affiliated Hospital of Xiamen University from January 2011 to June 2013 were collected. Inclusion criteria were: 1) patients with complete clinical data undergoing radical resection of esophageal carcinoma; 2) those who gained 0 or 1 point in the Eastern Cooperative Oncology Group scoring and did not receive preoperative adjuvant treatment, such as radiotherapy and chemotherapy; 3) those who were definitely diagnosed with ESCC by two experienced pathologists in our hospital. A total of 202 cases were included in this study, including 158 males (78.22%) and 44 females (21.78%) aged 36-82 years old, with average age of (58.72±8.69) years old. According to the tumor-node-metastasis (TNM) staging standard for esophageal carcinoma[8], there were 20 cases in stage I, 110 cases in stage II, 63 cases in stage III and 9 cases in stage IV (no distant metastasis was found in preoperative examination, and the pathology was M1 after operation). This study was approved by the Ethics Committee of The First Affiliated Hospital of Xiamen University. Signed written informed consent was obtained from all participants before the study.

Main Reagents and Methods

STAT3 and HIF-1α anti-mouse monoclonal antibodies were purchased from Cell Signaling (Danvers, MA, USA), and S-P kits and diaminobenzidine (DAB) chromogenic solution were purchased from Fuzhou Maixin Biotechnologies Co., Ltd. (Fuzhou, China). ESCC tissues and normal tissues of the esophageal mucosa > 5 cm from the edge of the tumor were routinely and serially cut into 4 μm-thick slices for immunohistochemical staining.

Result Assessment

The appearance of pale brown granules in the cytoplasm represented positive expression of STAT3, while the appearance of yellow to medium brown granules in the cell membrane or cytoplasm indicated positive expression of HIF-1α. According to the scores of the positive rate of $STAT3^+$ cells or HIF-$1\alpha^+$ cells, 0 point represented for 0-10%, 1 point for 11-25%, 2 points for 26-50%, 3 points for 51-75%, and 4 points for 76-100%. According to the color intensity score, 1 point stood for light yellow, 2 points for brown yellow, and 3 points for medium brown. The two scores multiplied, and the median, 6 points, was taken as the cut-off value, with lower than or equal to 6 points being negative and higher than 6 points being positive. All the above results were independently judged by two pathologists *via* the blind method.

Statistical Analysis

All statistics were performed using Statistical Product and Service Solutions (SPSS) 20.0 software (IBM, Armonk, NY, USA). The paired sample *t*-test, x^2-test, and Spearman correlation analysis were performed to evaluate the relationship between STAT3 and HIF-1α. The Kaplan-Meier curve, univariate logistic regression analysis and multivariate logistic regression factor analysis were used to evaluate the prognostic factors of ESCC. $p<0.05$ suggested that the difference was statistically significant.

Results

Expressions of STAT3 and HIF-1α in ESCC

The positive expression rate of STAT3 was 40.59% (82/202) in ESCC tissues and 3.47% (7/202) in normal esophageal tissues. The positive expression rate of HIF-1α was 70.30% (142/202) in ESCC tissues and 4.95% (10/202) in normal esophageal tissues. The positive expression rates of STAT3 and HIF-1α in ESCC tissues were both significantly higher than those in normal esophageal tissues, and the differences were statistically significant ($p<0.05$, Table I). Both STAT3 and HIF-1α were positively expressed in 74 cases (36.63%). Spearman correlation analysis manifested that the expression of STAT3 was positively correlated with that of HIF-1α ($r=0.401$, $p<0.05$).

Table I. Correlation between expression of STAT3 and HIF-1α [no. (%)].

HIF-1α	STAT3		Sum
	–	+	
–	52 (25.74)	8 (3.96)	60 (29.70)
+	68 (33.66)	74 (36.63)	142 (70.30)
Sum	120 (59.41)	82 (40.59)	202 (100)

Table II. Relationships between STAT3 expression and clinical characteristics of ESCC.

Characteristics	No.	STAT3 expression (%)	p
Age (y)			0.425
<60	98	36 (36.73)	
≥60	104	46 (44.23)	
Gender			0.716
Male	158	65 (41.14)	
Female	44	17 (38.64)	
Differentiation degree			0.089
Undifferentiated	2	0 (0.00)	
Poorly differentiated	60	16 (26.67)	
Moderately differentiated	126	56 (44.44)	
Highly differentiated	14	10 (71.43)	
T Stage			0.027*
T_1/T_2	73	25 (34.25)	
T_3/T_4	129	57 (44.19)	
N Stage			0.652
N_0	120	47 (39.17)	
N_{1-3}	82	35 (42.68)	
TNM Stage			0.078
I	20	11 (55.00)	
II	110	36 (32.73)	
III	63	33 (52.38)	
IV	9	2 (22.22)	

Note: *, $p<0.05$ indicated significant difference.

Correlations of the Expressions of STAT3 and HIF-1α in Cancer Tissues with Clinicopathology

The positive expression of STAT3 was related to the T stage, displaying a statistically significant difference ($p<0.05$, Table II). However, it was not correlated with age, gender, differentiation degree, lymph node metastasis and clinical stage of ESCC patients, showing no statistically significant differences ($p>0.05$, Table II). The expression of HIF-1α was associated with the T stage of ESCC patients, and the difference was statistically significant ($p<0.05$, Table III). However, it was not correlated with the differentiation degree, lymph node metastasis and clinical stage, and the differences were not statistically significant ($p>0.05$, Table III). The

Table III. Relationships between HIF-1α expression and clinical characteristics of ESCC.

Clinical characteristics	No.	HIF-1α expression (%)	p
Differentiation degree			0.736
Undifferentiated	2	2 (100.00)	
Poorly differentiated	60	44 (73.33)	
Moderately differentiated	126	86 (68.25)	
Highly differentiated	14	10 (71.43)	
T Stage			0.003*
T_1/T_2	73	49 (67.12)	
T_3/T_4	129	93 (72.09)	
N Stage			0.695
N_0	120	82 (68.33)	
N_{1-3}	82	60 (73.17)	
TNM Stage			0.082
I	20	11 (55.00)	
II	110	74 (67.27)	
III	63	50 (79.37)	
IV	9	7 (77.78)	

Note: *, $p<0.05$ indicated significant difference.

Table IV. Relationships between co-expression of STAT3 and HIF-1α and clinical characteristics of ESCC.

Clinical characteristics	No.	Co-expression of STAT3 and HIF-1α (%)	*p*
Differentiation degree			0.318
Undifferentiated	2	0 (0.00)	
Poorly differentiated	60	16 (26.67)	
Moderately differentiated	126	48 (38.10)	
Highly differentiated	14	10 (71.43)	
T Stage			0.016*
T1/T2	73	18 (24.66)	
T3/T4	129	56 (43.41)	
N Stage			0.764
N0	120	43 (35.83)	
N1-3	82	31 (37.80)	
TNM Stage			0.215
I	20	10 (50.00)	
II	110	32 (29.09)	
III	63	30 (47.62)	
IV	9	2 (22.22)	

Note: *, $p<0.05$ indicated significant difference.

positive expressions of both STAT3 and HIF-1α were associated with the T stage of ESCC patients, and the differences were statistically significant ($p<0.05$, Table IV). We did not find correlation between their expressions with the differentiation degree, lymph node metastasis and clinical stage, and the differences were not statistically significant ($p>0.05$, Table IV).

Correlations of the Expressions of STAT 3 and HIF-1α in Cancer Tissues with Prognosis

The Kaplan-Meier survival curve showed that the median overall survival (OS) and disease-free survival (DFS) in ESCC patients with negative STAT3 expression were 36 and 24 months, respectively, while those presenting positive STAT3 expression were 24 and 16 months, respectively, showing statistically significant differences ($p<0.05$, Figure 1). Similarly, the median OS and DFS in ESCC patients with negative HIF-1α expression were 42 and 26 months, respectively, while those with positive HIF-1α expression were 27 and 18 months, respectively, displaying statistically significant differences ($p<0.05$, Figure 2). According to different expression patterns of STAT3 and HIF-1α, ESCC patients were grouped into $STAT3^+/HIF\text{-}1\alpha^+$ (S^+/H^+) group, $STAT3^-/HIF\text{-}1\alpha^-$ (S^-/H^-) group, $STAT3^+/HIF\text{-}1\alpha^-$ (S^+/H^-) group and $STAT3^-/HIF\text{-}1\alpha^+$($S^-/H^+$) group. Among them, the mean OS [(47.05±22.93) months] and mean DFS [(31.42±20.87) months] in S^-/H^- group were

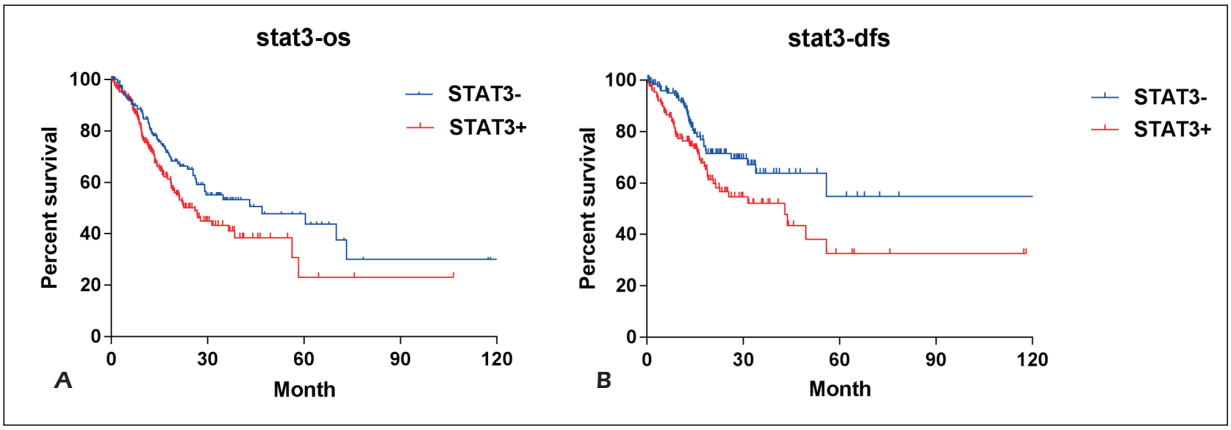

Figure 1. Kaplan-Meier survival analysis in negative and positive STAT3 group. **A**, The median overall survival (OS); **B**, Disease-free survival (DFS).

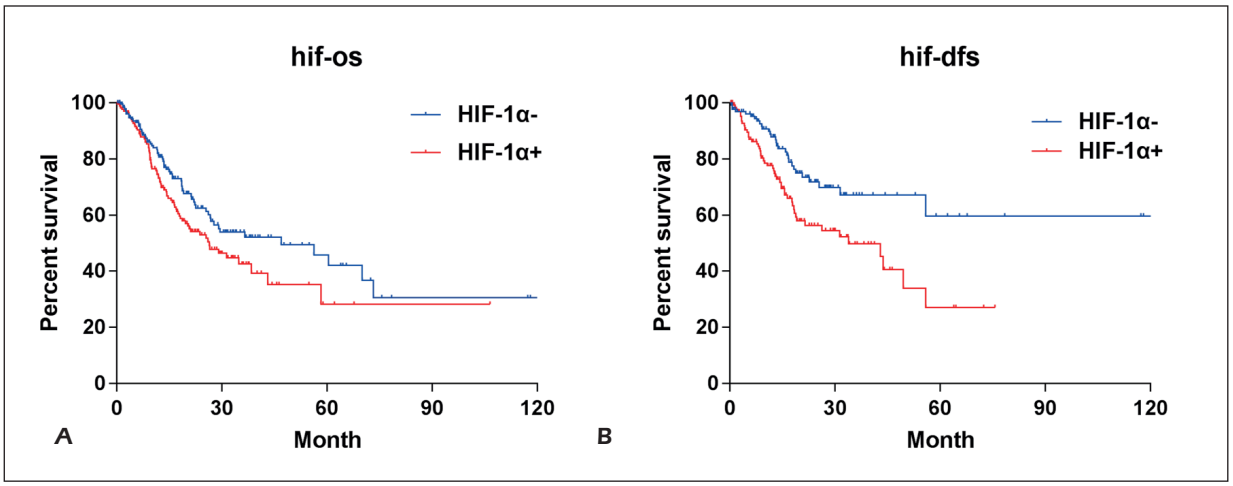

Figure 2. Kaplan-Meier survival analysis in negative and positive HIF-1α group. **A**, The median overall survival (OS); **B**, Disease-free survival (DFS).

longer than those in S^+/H^+ group, and the differences were statistically significant ($p<0.05$, Figure 3). The median OS in S^-/H^- group was 62 months, and the median DFS was 28 months, both of which were significantly longer than those in the other three groups ($p<0.05$, Figure 3). There were no statistically significant differences on OS and DFS among the other three groups ($p>0.05$).

Univariate and Multivariate Analyses

The univariate analysis revealed that both STAT3 and HIF-1α were poor prognostic factors for ESCC ($p<0.05$, Table V). Through multivariate analysis, only STAT3 was found to be an independent poor prognostic factor for esophageal carcinoma ($p<0.05$, Table VI).

Discussion

The STAT family is a kind of DNA binding protein that can be activated by various ligands, which is an important substrate of the JAK/STAT pathway showing a vital role in the signal transduction of cytokines[9,10]. Activated STAT3 promotes angiogenesis, interferes with cell cycle and inhibits cell apoptosis by regulating downstream genes such as VEGF, Cyclin D1, Bcl-2, Bcl-XL, C-myc, MMP-2, and MMP-9. Researches[5,6] have shown that STAT3 is abnormally expressed in various tumor tissues and cell lines such as liver cancer, lung cancer, breast cancer and gallbladder cancer. However, the potential role of STAT3 in clinicopathological of esophageal carcinoma tissues is controversial [11-13].

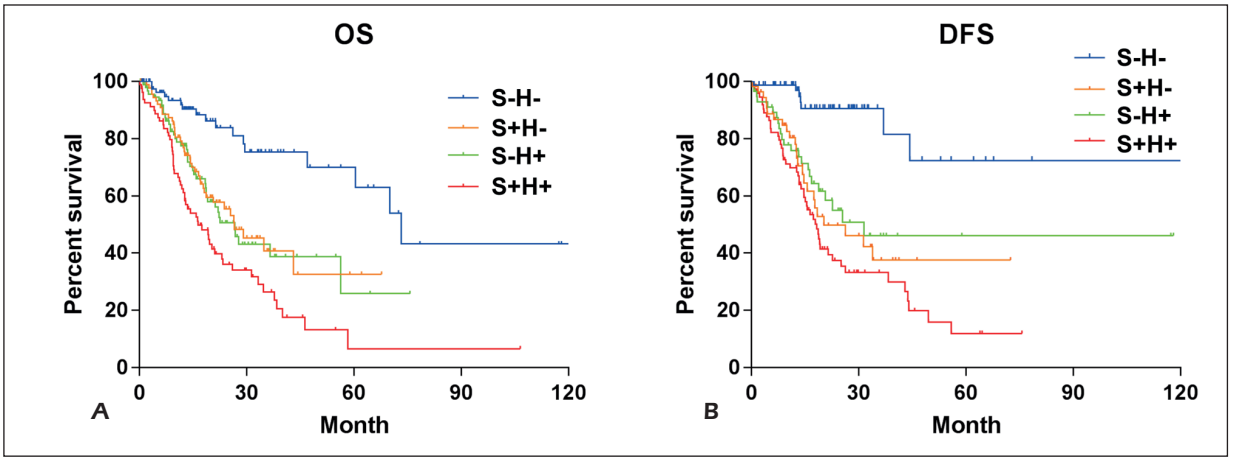

Figure 3. Kaplan-Meier survival analysis in STAT3+/HIF-1α+ (S+/H+) group, STAT3-/HIF-1α- (S-/H-) group, STAT3+/HIF-1α- (S+/H-) group and STAT3-/HIF-1α+(S-/H+) group. **A**, The median overall survival (OS); **B**, Disease-free survival (DFS).

Table V. Univariate and multivariate analysis of ESCC for OS.

	Univariate analysis			Multivariate analysis		
	β	*p*	HR (95%CI)	β	*p*	HR (95%CI)
Age	-0.016	0.437	0.98 (0.957-1.014)	-0.008	0.627	0.99 (0.926-1.017)
Gender	0.032	0.824	1.02 (0.632-1.595)	-0.015	0.286	0.99 (0.724-1.379)
Differentiation	-0.038	0.792	0.97 (0.698-1.338)	0.022	0.528	1.05 (0.786-1.262)
T Stage	0.074	0.510	1.06 (0.879-1.289)	-0.147	0.615	0.92 (0.679-1.328)
N Stage	0.315	0.296	1.29 (0.906-1.632)	-0.026	0.459	0.97 (0.537-1.474)
TNM Stage	0.263	0.145	1.18 (0.928-1.426)	0.381	0.364	1.31 (0.708-2.515)
STAT3	0.737	0.001*	2.02 (1.295-2.847)	0.479	0.012*	1.76 (1.023-2.891)
HIF-1α	0.486	0.004*	1.94 (1.251-2.643)	0.403	0.223	1.52 (0.862-2.383)

Note: *, $p<0.05$ indicated significant difference.

HIF-1α is the main regulator of oxygen homeostasis[7]. Its activity exerts a crucial effect on maintaining energy metabolism, promoting angiogenesis and stimulating apoptosis of tumor cells. HIF-1α could be affected by the regulation of various oncogenes and tumor-suppressor genes. Previous researches have reported the potential immune correlation between STAT3 and HIF-1α. Investigations have confirmed that STAT3 can regulate the Akt expression and then up-regulate the HIF-1α expression induced by growth factors. In this study, the research methods and sample numbers were further improved. We collected prognostic data of ESCC patients for analyzing the prognostic values of STAT3 and HIF-1α in ESCC. In this investigation, STAT3 and HIF-1α were found to be highly expressed in ESCC tissues, and they were positively correlated with each other. In addition, expressions of STAT3 and HIF-1α were closely related to the tumor T stage and had nothing to do with other pathological factors of ESCC patients. It is suggested that STAT3 and HIF-1α may promote the proliferation and invasion of ESCC cells, thus participating in the development of ESCC. Meanwhile, the multivariate analysis suggested that STAT3 was an independent prognostic factor for OS and DFS in ESCC, while HIF-1α was not an independent prognostic factor, which may affect the prognosis by influencing the expression level of STAT3. STAT3 is a poor prognostic factor for digestive tract tumors, especially esophageal cancer. In this study, OS and DFS in ESCC patients presenting positive STAT3 and HIF-1α expressions were significantly worse than those with negative expressions. However, scholars believed that the STAT3 expression level was not correlated with disease prognosis. We speculated that the main reasons for the different conclusions are as follows: First, the two studies adopted different evaluation criteria. Secondly, the criteria for patients entering the group were different. Some of patients underwent preoperative neoadjuvant treatment, and others underwent non-R0 resection in the study. These factors led to different results. In this study, the difference in the T stage between S^-/H^- group and S^+/H^+ group was statistically significant. Moreover, OS and DFS in S^-/H^- group were markedly better than those in $S^+/$

Table VI. Univariate and multivariate analysis of ESCC for DFS.

	Univariate analysis			Multivariate analysis		
	β	*p*	HR (95%CI)	β	*p*	HR (95%CI)
Age	-0.025	0.072	0.97 (0.942-1.004)	-0.020	0.216	0.98 (0.951-1.014)
Gender	0.062	0.654	1.09 (0.725-1.517)	0.003	0.827	0.99 (0.652-1.436)
Differentiation	0.053	0.726	1.04 (0.694-1.502)	0.271	0.634	1.15 (0.804-1.605)
T Stage	0.047	0.685	1.06 (0.708-1.328)	-0.136	0.225	0.87 (0.589-1.128)
N Stage	0.468	0.092	1.35 (0.986-1.932)	0.284	0.592	1.26 (0.647-1.925)
TNM Stage	0.219	0.186	1.23 (0.917-1.685)	0.159	0.873	1.09 (0.568-1.802)
STAT3	0.704	0.004*	1.81 (1.125-2.096)	0.527	0.039*	1.63 (1.130-2.283)
HIF-1α	0.691	0.008*	1.92 (1.204-2.379)	0.458	0.621	1.58 (0.903-2.256)

Note: *, $p<0.05$ indicated significant difference.

H^+ group, indicating that the prognosis of patients can be better judged by jointly detecting the expressions of STAT3 and HIF-1α. Previous authors have pointed out that although the expression of STAT3 decreases, it still remains a relatively high level after the inhibition on HIF-1α, suggesting that STAT3 may have other signal pathways besides the HIF-1α pathway[14,15]. A relative study showed that STAT3 and HIF-1α inhibitors have achieved certain curative effects in tumor treatment, with the advantages of good tolerance, small side effects and effective treatment. However, their application in ESCC is rarely reported. With the rapid development of molecular biology, the development prospects of STAT3 and HIF-1α inhibitors are exciting. This work provided a theoretical basis and a strategy for targeted treatment of ESCC, and the research on STAT3 and HIF-1α dual-target treatment needs to be further carried out. To sum up, expressions of STAT3 and HIF-1α in ESCC are higher than those in normal tissues, and the two are closely related to each other. Expression levels of STAT3 and HIF-α are significantly related to the T stage, OS and DFS of ESCC patients, and they are adverse prognostic factors for ESCC. The above results suggested that the STAT3/HIF-1α signaling pathway plays an important role in esophageal carcinoma and provide a preliminary experimental basis for the targeted therapy. A large number of cases and related basic research are still needed for further investigation in the future.

Conclusions

We revealed that STAT3 and HIF-1α are highly expressed in ESCC and are closely related to each other, suggesting that they can be used as prognostic indicators for ESCC patients.

Conflict of Interest

The authors declare that they have no conflict of interest.

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