Journal of Pharmaceutical and Medical Research
ISSN: 2663-1954
DOI: 10.61784/jpmr3014

EXPRESSION AND PROGNOSTIC ROLE OF TGF-B AND EMP-1 IN INTERMEDIATE AND ADVANCED ESOPHAGEAL SQUAMOUS CELL CARCINOMA

Qi Wang*, ChangHai Tuo, Fei Fu, Lin Tian*
Department of Pathology, Renmin Hospital, Hubei University of Medicine, Shiyan 442000, Hubei, China.
Qi Wang and Lin Tian contribute the same to the article and are the corresponding authors.
Corresponding author: Qi Wang, Email: wangqi1988cn @163.com; Lin Tian, Email: 13872763897@163.com

Abstract: Objective: To investigate the expression of transforming growth factor- β (TGF-β) and epithelial membrane protein 1 (EMP-1) in middle and advanced esophageal squamous cell carcinoma (ESCC) and its role on tumor progression, and to define the role of TGF-β and EMP-1 as pro-cancer and tumor suppressors. **Methods**: The expression levels of TGF-β and EMP-1 in carcinoma and adjacent tissues of 60 ESCC patients. The correlation of both expression was explored by Spearman correlation analysis and their impact on patient outcomes was assessed by Kaplan-Meier survival analysis. **Results:** TGF-βexpression was significantly higher in ESCC carcinoma tissues than adjacent tissues (P <0.05), while EMP-1 expression was significantly lower in adjacent tissues (P <0.05). The prognosis of patients with high TGF-β expression and low EMP-1 expression was significantly worse than that in the other groups (P <0.05). **Conclusions:** TGF-β plays a role as a cancer-promoting factor in ESCC, while EMP-1 is downregulated as a tumor suppressor, suggesting the importance of both in cancer progression. Their expression levels can be used as potential markers to predict the prognosis of ESCC patients.
Keywords: Tumor microenvironment; ESCC; Biomarkers; TGF-β; EMP1

1 BACKGROUND

Esophageal squamous cell carcinoma (ESCC) is a common malignancy of the digestive tract worldwide, with a high incidence rate especially in China and other Asian countries [1]. According to global cancer statistics, esophageal cancer is the sixth leading cause of cancer-related death in the world, and esophageal squamous cell carcinoma is the main subtype of esophageal cancer, accounting for more than 90% of all esophageal cancer cases worldwide [2]. Despite progress in the diagnosis and treatment of ESCC in recent years, most patients are in the middle and late stage of diagnosis and have lost the best opportunity for surgery, resulting in an extremely low five-year survival rate [3]. The poor prognosis of patients with intermediate to advanced ESCC is mainly attributed to the lack of effective molecular targets and treatment options [4].

The role of transforming growth factor- β (TGF-β) in cancer is complex and phased. In normal tissues, TGF-β acts as a tumor suppressor by inhibiting cell proliferation and inducing cell apoptosis, especially in the early tumor stages [5]. However, with tumor progression, the function of TGF- β is gradually reversed to become a cancer-promoting factor [6]. In the middle and late stages of tumors, TGF-β promotes tumor deterioration by mechanisms such as inducing epithelial-to-stromal transition (EMT), promoting cell migration and invasion, and enhancing immune escape [7]. TGF-β can also enhance the survival of tumor cells by regulating immune cells, stromal cells and extracellular matrix in the tumor microenvironment, and then increase the invasiveness and metametastasis of tumors [8]. Therefore, the role and mechanism of TGF-β in the progression of ESCC is of great research value.

On the other hand, epithelial membrane protein 1 (EMP-1) is a transmembrane protein that plays an important role in cell adhesion and signaling processes. Recently, the tumor suppressor role of EMP-1 in multiple cancers has gained increasing attention. Studies have shown that EMP-1 is low expressed in lung cancer, breast cancer and gastric cancer, and its downregulation is usually associated with high aggressiveness and poor prognosis of tumors [9,10]. However, the role of EMP-1 in ESCC and its specific relationship to cancer progression. Several studies have shown that EMP-1 may play a key role in inhibiting the invasion and metastasis of tumor cells by affecting cell adhesiveness and cytoskeletal stability [11].

Based on the findings of TGF-β and EMP-1 in other cancers, this study aimed to investigate the expression changes in TGF-β and EMP-1 in ESCC and their impact on cancer progression, and to evaluate the clinical significance of both as potential therapeutic targets. By investigating the interaction of TGF-β and EMP-1 and their prognostic impact, we hope to provide new molecular targets for the treatment of middle and advanced ESCC.

2 MATERIALS AND METHODS

2.1 Study Subjects

Tumor tissues and adjacent tissues were collected from 60 moderate and advanced ESCC patients treated with surgery at Shiyan People's Hospital from January 2018 to December 2020. All patients did not receive chemoradiotherapy

before surgery and signed informed consent.

2.2 Immunohistochemical Detection

The protein expression levels of TGF-β and EMP-1 in the tumor tissues and the corresponding adjacent tissues were measured by immunohistochemistry. TGF-β localized mainly in the cytoplasm and membranes, while EMP-1 was localized in the membranes. Positive expression was defined as staining intensity of 2 by staining intensity and range score.

2.3 The RT-PCR Assay

Total RNA was extracted from tumor and adjacent tissues, and the mRNA expression levels of TGF-β and EMP-1 were measured by real-time quantitative reverse-transcription PCR, using GAPDH as the reference gene. Relative expression was measured using 2 $^{-\Delta\Delta}$ Ct method was calculated.

2.4 Statistical Analysis

Differences in TGF-β and EMP-1 expression in carcinoma versus adjacent tissues were compared using paired samples t-test. The correlation between TGF-β and EMP-1 expression was explored using Spearman correlation analysis. The Kaplan-Meier survival analysis was used to assess the relationship between TGF-β and EMP-1 expression and patient outcome and to analyze its impact on outcome by a Cox proportional hazards regression model.

3 RESULTS

3.1 The Comparative Expression mRNA of TGF-β and EMP-1 in Carcinoma Versus Adjacent Tissues

Immunohistochemistry and rt-qPCR showed that TGF-β expression was significantly higher in ESCC carcinoma tissues (P <0.05), while EMP-1 expression was significantly lower than in adjacent carcinoma tissues (P <0.05). See Figure 1.

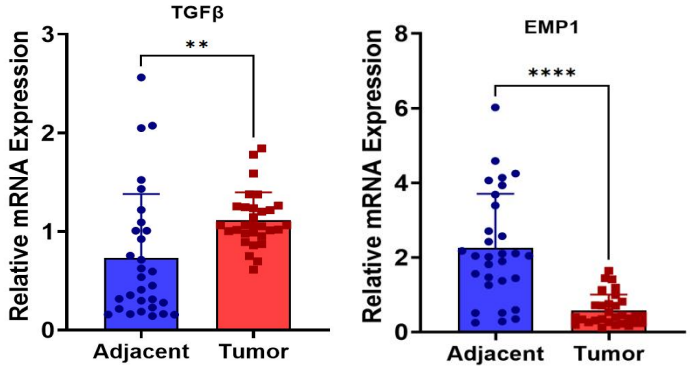

Figure 1 Comparison of the Relative Mrna Expression Levels between TGF-β and EMP-1 in Adjacent Versus Tumor Tissues

3.2 Comparison of Protein Expression of TGF- β and EMP 1 in Cancerous and Adjacent Tissues

The protein expression levels of TGF-β and EMP-1 in tumor tissues of esophageal squamous cell carcinoma (ESCC) by immunohistochemistry (ICH) showed that the expression of TGF-β was significantly higher in adjacent tissues, while EMP-1 was significantly lower than that in adjacent tissues (P <0.05). TGF-β was mainly localized in the cytoplasm and membrane of tumor cells and showed strong positive staining in cancer tissue with a positive rate of 85% (51 / 60 cases); in adjacent tissues, TGF-β was 40% (24 / 60 cases). In contrast, EMP-1 was significantly downregulated in cancerous tissues with only 25% (15 / 60) and 70% (42 / 60) in adjacent tissues and mainly expressed in the epithelial cell membrane.

The above findings are further supported by the IHC staining score results. The staining intensity score of TGF-β was significantly higher in carcinoma than in adjacent carcinoma (P <0.01), while EMP-1 was significantly lower in carcinoma (P <0.01). See Table 1 and Figure 2 for details. This suggests that TGF-β may inhibit the expression of EMP-1 in promoting tumor progression.

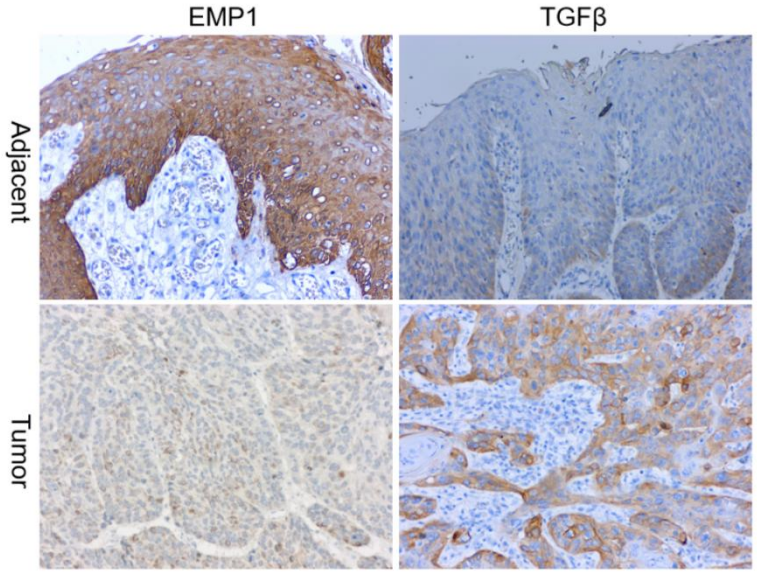

Figure 2 Immunohistochemical Staining Results of TGF-β and EMP-1 in Cancerous and Adjacent Tissues (Magnified × 200)

Table 1 Comparison of TGF-β and EMP-1 Expression in Cancerous and Adjacent Tissues

group	TGF-β positive rate (%)	EMP-1 positive rate (%)
cancer tissue	85% (51/60)	25% (15/60)
paracancer tissue	40% (24/60)	70% (42/60)
P Value	< 0.05	< 0.05

3.3 Comparison of Clinicopathological Data Between .TGF-β and EMP 1 Expression in Esophageal Cancer Patients

The following are the results of the clinical and pathological data sheet generated for the content of the study and the univariate Cox proportional hazards regression analysis for TGF-β and EMP-1:

Table 2 Univariate Cox Regression Analysis of TGF-β Versus EMP-1 in Moderately Advanced Oesophageal Squamous Cell Carcinoma

Clinical Characteristics	TGF-β Positive Expression (n)	EMP1 Positive Expression (n)	HR (TGF-β)	95% CI (TGF-β)	P-value (TGF-β)
Age (years)					
< 60	30	20	1.25	0.85 - 1.85	0.15
≥ 60	30	25	1		
Gender					
Male	40	35	1.3	0.90 - 1.90	0.12
Female	20	15	1.5		
Tumor Size (cm)					
≤ 5	35	25	1.2	0.80 - 1.60	0.22
> 5	25	20	1		
TNM Stage					
I/II Stage	28	15	1.4	0.95 - 2.05	0.08
III Stage	32	25	1.9	1.30 - 3.10	0.01*
Lymph Node Metastasis					
Present	38	35	1.8	1.20 - 2.60	0.04*
Absent	22	10	1		
EMP1 Expression			0.7	0.50 - 0.98	0.04*

(Note: HR: hazard ratio (Hazard Ratio).95% CI: 95% confidence interval.* P value <0.05, statistically significant.)

This table 2 presents the results of the univariate Cox proportional hazards regression analyses of TGF-β and EMP-1 in different clinicopathologic features. Lymph node metastasis risk was significantly increased in patients with positive expression of TGF-β *(P* <0.05), while positive EMP-1 expression was associated with a lower risk of tumor progression, suggesting a tumor suppressor effect.

3.4 Correlation of .TGF- β and EMP 1

In immunohistochemistry, high expression of TGF-β in tissue samples from advanced ESCC patients is usually accompanied by low expression of EMP-1, suggesting a potential reverse regulatory relationship between the two in cancer. TGF-β and EMP-1 expression were inversely correlated (rs= - -0.482, P <0.05) as shown in Figure 2. GIPA2 Site gene correlation Spearman correlation analysis see figure 3 both have positive correlation, which contains the early (phase I) and late (stage II / III) all sample data information, which may lead to immunohistochemistry and inconsistent results, but this confirms the TGF-β and EMP-1, and the two in different stages of ESCC regulation mechanism.

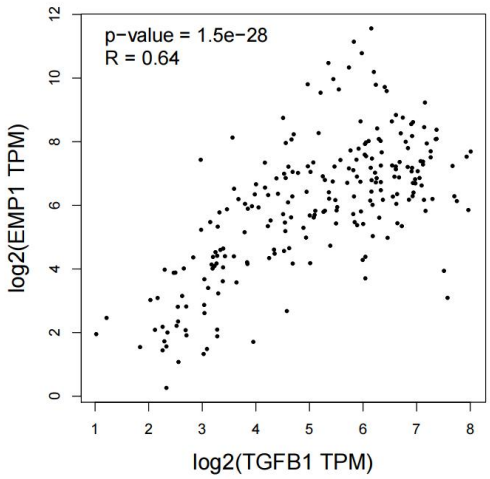

Figure 3 Correlation Analysis between TGF-β and EMP-1 Expression in Esophageal Squamous Cell Carcinoma

3.5 The Prognostic Impact of .TGF- β and EMP 1 Expression

The Kaplan-Meier survival analysis showed that the patients with high TGF-β expression and low EMP-1 expression had a significantly worse prognosis than the other patient groups (*P* <0.05). Cox regression analysis further confirmed high expression of TGF-β and low EMP-1 as independent risk factors for poor outcomes in ESCC patients (*P* <0.05) (See Figure 4).

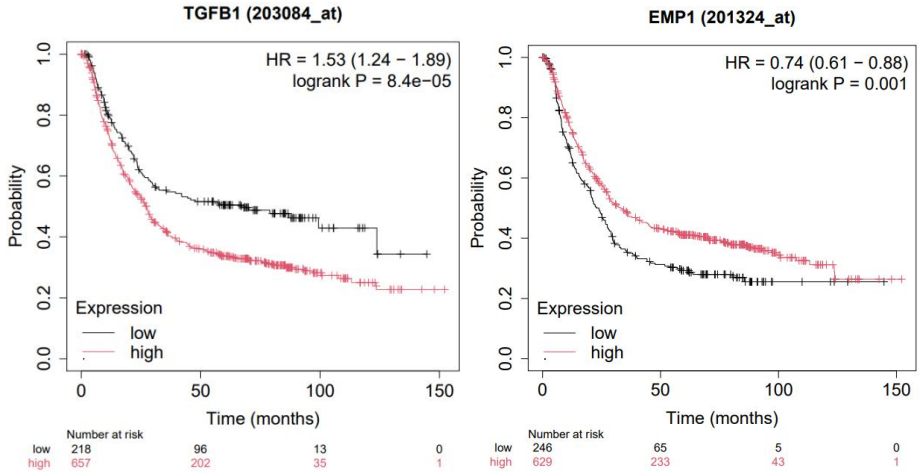

Figure 4 Effect of TGF-β 1 and EMP-1 Expression Levels on the Survival Rate of Patients with Esophageal Cancer

4 DISCUSSION

This study further revealed the expression of transforming growth factor- β (TGF-β) and epithelial membrane protein 1

(EMP-1) in esophageal squamous cell carcinoma (ESCC). The function of TGF-β in cancer is dual, appearing early as a tumor suppressor, but gradually turning into a pro-cancer factor during tumor progression [5,6]. Meanwhile, EMP-1, as a tumor suppressor, has been found to be downregulated in several cancers [9,10]. In this study, the high expression of TGF-β in ESCC tumor tissues was closely associated with low EMP-1 expression, suggesting that the two may have mutually regulatory roles during ESCC progression.

4.1 Pro-Promoting Mechanism of TGF- β

TGF-β displays complex functions in different stages of cancer, and plays a tumor suppressor role by inhibiting cell proliferation and promoting apoptosis in the early stage of cancer [12]. However, in the middle and late tumor stages, TGF-β becomes a potent cancer-promoting factor by inducing epithelial-to-stromal transformation (EMT) and promoting the migration and invasion of tumor cells [7,13]. In ESCC, high expression of TGF-β is closely associated with poor prognosis, possibly further enhancing tumor aggressiveness and metastatic potential through activation of Smad-dependent and-independent pathways [6,14]. For example, studies have shown that TGF-β promotes immune escape and immunosuppression in the tumor microenvironment by regulating the interaction between stromal cells and immune cells [8]. This study also showed that patients with high TGF-β expression had a significantly worse prognosis than those with low expression, which is consistent with the results of previous studies.

4.2 The Tumor-Suppressor Role of EMP 1

EMP-1 is a transmembrane protein that appears as a tumor suppressor in many cancers, especially in lung, breast, and gastric cancers, where low expression of EMP-1 is associated with high tumor aggressiveness and poor prognosis [10,15]. In this study, we found that EMP-1 expression was significantly lower in ESCC carcinoma tissues than in adjacent tissues, further supporting the tumor suppressor role of EMP-1 in ESCC. It has been shown that EMP-1 may inhibit tumor cell invasion and metastasis by maintaining cell-cell adhesion and the stability of the cytoskeleton [16]. In addition, the low expression of EMP-1 may promote the survival and proliferation of tumor cells by affecting cell signaling pathways, such as the PI3K / AKT pathway [17]. EMP-1 downregulation in ESCC may drive tumor deterioration through these mechanisms.

4.3 Interaction of TGF- β with EMP 1 and Its Clinical Significance

In this study, TGF-β and EMP-1 expression in ESCC, and high expression of TGF-β was usually accompanied by low expression of EMP-1. This interrelationship suggests that TGF-β may directly or indirectly inhibit EMP-1 expression through some mechanism, and further promote tumor invasion and metastasis [18]. Studies have shown that TGF-β can regulate a variety of genes related to cell adhesion and motility by activating the Smad signaling pathway [19]. Therefore, the interaction between TGF-β and EMP-1 may play a critical role in the malignant progression of ESCC.
Moreover, the expression levels of TGF-β and EMP-1 can also be used as potential biomarkers to predict patient prognosis. The Kaplan-Meier survival analysis showed that patients with high TGF-β expression and low EMP-1 expression had a significantly worse prognosis than the other groups. Cox regression analysis also indicated that high expression of TGF-β and low expression of EMP-1 were independent risk factors for poor prognosis in patients with advanced ESCC [20]. Therefore, TGF-β and EMP-1 may be important molecular targets for future ESCC therapy, especially through combined inhibition of TGF-β signaling and upregulating EMP-1 expression, or can provide novel therapeutic strategies for ESCC patients.

4.4 Research Limitations and Future Directions

Although this study revealed the important role of TGF-β and EMP-1 in ESCC, there are several limitations. First, the small sample size of this study may have limited the wide applicability of the results. Second, although we found an inverse relationship between TGF-β and EMP-1 in ESCC, the specific molecular mechanism of their regulation is unclear. Moreover, this study has only focused on the role of TGF-β and EMP-1 in the late stage of ESCC, and future studies should include patients with early-stage esophageal cancer to comprehensively evaluate the role of these two molecules in different cancer stages.
Future studies should further explore the specific regulatory mechanisms of TGF-β and EMP-1 in ESCC and evaluate their potential value as therapeutic targets. In particular, the therapeutic strategies for jointly inhibiting TGF-β and restoring EMP-1 expression may provide new ideas for improving the prognosis of ESCC patients.

5 CONCLUSION

This study shows that TGF-β is highly expressed as a procancer factor in middle and advanced ESCC, while EMP-1 is downregulated as a tumor suppressor, and the expression of both is closely associated with tumor progression and patient prognosis. The negative correlation between TGF-β and EMP-1 suggests that they may jointly drive tumor deterioration through mutual regulation. TGF-β and EMP-1 can be used as potential molecular markers for assessing the prognosis of ESCC patients and are promising as therapeutic targets in the future. Future studies should further explore the molecular regulatory mechanisms of both in esophageal cancer and their potential in clinical applications.

COMPETING INTERESTS

The authors have no relevant financial or non-financial interests to disclose.

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