Association between tumor necrosis factor receptor 2 and progression and poor prognosis of tumor stage 2-3 esophageal squamous cell carcinoma and stratified analysis

ZIFENG LIU[1,2], MEI REN[2], SHASHA JIA[2], SEN QIAO[3] and DONG YANG[4]

[1]Department of Oncology, Shandong University of Traditional Chinese Medicine, Jinan, Shandong 250355, P.R. China;
[2]Department of Oncology, Jining No. 1 People's Hospital, Jining, Shandong 272029, P.R. China;
[3]Department of Hepatological Surgery, Jining No. 1 People's Hospital, Jining, Shandong 272029, P.R. China;
[4]Department of Oncology, Affiliated Hospital of Jining Medical University, Jining, Shandong 272000, P.R. China

Received April 10, 2024; Accepted July 31, 2024

DOI: 10.3892/ol.2024.14638

Abstract. Although tumor necrosis factor receptor 2 (TNFR2) may serve a protumor role in several types of tumors, the clinical significance of TNFR2, including the diagnostic and prognostic value in tumor (T) stage 2-3 esophageal squamous cell carcinoma (ESCC), remains unclear. Therefore, the present study aimed to explore the clinical significance of TNFR2 in stage T2-3 ESCC. The present study collected the mRNA expression data of TNFR2 from two databases and confirmed the high expression of TNFR2 in ESCC tissue. TNFR2 expression in stage T2-3 ESCC tissue (n=404) was detected using immunohistochemistry and a stratified analysis was performed. For all patients with stage T2-3 ESCC, TNFR2 expression was associated with clinical stage, invasion depth and metastatic lymph nodes. Stage T3 and low differentiation was associated with an increase in the risk of lymph node metastasis, but older age was associated with a decrease. TNFR2 expression was associated with poor overall survival (OS) of all patients with stage T2-3 ESCC and stratified patients with stage T3 ESCC. Moreover, TNFR2 expression and metastatic lymph nodes were independent prognostic factors for these patients. For stratified patients aged ≤60 years, TNFR2 expression was associated with clinical stage and metastatic lymph nodes. In addition, TNFR2 expression was associated with poor OS in stratified patients with stage T2 ESCC. The presence of metastatic lymph nodes was also an independent prognostic factor for these patients. For stratified patients aged >60 years, TNFR2 expression was associated with invasion depth. TNFR2 expression was also associated with poor OS in all patients with stage T2-3 ESCC and stratified patients with stage T3 ESCC. TNFR2 expression and metastatic lymph nodes were identified as independent prognostic factors for these patients. In conclusion, TNFR2 expression is associated with progression and poor prognosis in patients with stage T2-3 ESCC as an independent prognostic factor, except in the subgroup of patients with stage T2-3 ESCC aged ≤60 years.

Correspondence to: Dr Dong Yang, Department of Oncology, Affiliated Hospital of Jining Medical University, 89 Guhuai Road, Jining, Shandong 272000, P.R. China
E-mail: yangdongatjining@163.com

Dr Sen Qiao, Department of Hepatological Surgery, Jining No. 1 People's Hospital, 6 Jiankang Road, Jining, Shandong 272029, P.R. China
E-mail: qsenqiao@sina.com

Key words: tumor necrosis factor receptor 2, esophageal squamous cell carcinoma, invasion depth, immunohistochemistry, overall survival

Introduction

Esophageal cancer (ESCA) is a type of common malignant tumor of the digestive tract, with the incidence ranking seventh and the mortality ranking sixth globally among all cancer types (1). Esophageal squamous cell carcinoma (ESCC) is the main pathological type of ESCA, accounting for ~90% of cases (2). Despite the emergence of immunotherapy and the combination of several treatment methods such as surgery, radiotherapy and chemotherapy, the prognosis of advanced ESCC is not favorable with a 5-year survival rate of <20%. This is largely attributed to the characteristics of ESCC, including the insidious early symptoms and the lack of specific markers for diagnosis and evaluating prognosis (3-5). Therefore, it is essential to identify more efficient markers which can be used for the diagnosis, prediction of prognosis and treatment of patients with ESCC.

According to the invasion depth described in Tumor (T)-Node (N)-Metastasis (M) staging system (6), ESCC can be divided into T1-4 stages. Moreover, a previous study reported that the proportions of 1,033 postoperative patients with ESCC at stages T1-4 were 19.2, 23, 55.7 and 2.1%, with 5-year survival rates of 74.6, 47.3, 32.8 and 15.6%, respectively (7). These data suggest that, in postoperative ESCC, stage T2-3 ESCC accounts for the vast majority (~80%) of cases and the prognosis markedly deteriorates from stage T2 onwards. Therefore, it is of great clinical significance to perform comprehensive

research on stage T2-3 ESCC to assess the potential prognostic markers and therapeutic targets.

Tumor necrosis factor (TNF) receptor (TNFR)2, also known as TNF receptor superfamily member 1b, is a member of the TNFR family and includes membrane-binding TNFR2 and soluble (s)TNFR2 (8). The role of TNFR2 in cancer has attracted increasing attention. Babic *et al* (9) reported that high sTNFR2 in the blood was associated with a poor prognosis in patients with colorectal cancer. Furthermore, Torrey *et al* (10) reported that targeting TNFR2 with antagonistic antibodies inhibited the proliferation of ovarian cancer cells and tumor-associated regulatory T cells. However, in the current era of advocating precision therapy, the roles of TNFR2 in different subgroups of tumors need more detailed research as the clinical significance of TNFR2 in patients with stage T2-3 ESCC remains unclear.

The current study retrieved the mRNA expression data of TNFR2 from online databases and detected the expression of TNFR2 in esophageal tissues from 404 patients with stages T2-3 ESCC and 40 healthy patients using immunohistochemistry (IHC) staining. The association between TNFR2 with the clinical parameters and overall survival (OS) of patients with stage T2-3 ESCC was then assessed. Further stratified analysis based on age and invasion depth was also performed to analyze the clinical significance of TNFR2 more deeply. The results of the present study will help clinicians to have a more accurate understanding of the role of TNFR2 in different subgroups of patients with ESCC, providing a basis for a more precise use of TNFR2 as a prognostic marker and therapeutic target.

Materials and methods

Database analysis of the expression of TNFR2 mRNA in human cancers. The Tumor IMmune Estimation Resource (TIMER; http://timer.cistrome.org/) and The Cancer Genome Atlas (TCGA; https://cancergenome.nih.gov/) databases were used to compare the expression of TNFR2 mRNA in tumor tissues of patients with ESCA and adjacent normal tissues from some of the ESCA cases. $P<0.05$ was considered to indicate a statistically significant difference.

Collection of tissue samples. The present study was performed in accordance with the principles of The Declaration of Helsinki and approved by the Ethics Committee of the Affiliated Hospital of Jining Medical University (Jining, China; approval no. 2017-Research-01). Tumor tissues from a total of 404 patients with stage T2-3 ESCC between January 2008 and December 2014 diagnosed by pathologists were used in the present study for IHC staining. The inclusion criteria were as follows: i) Radical resection of ESCC, diagnosed by pathologists; ii) stage T2 or 3 confirmed according to the TNM staging of esophageal cancer of the 7th edition of the American Joint Commission on Cancer (T2, tumor invading intrinsic muscularis; and T3, tumor exceeding muscularis and invading the esophageal fibrous membrane) (6,11); and iii) no administration of chemotherapy, radiotherapy or immunotherapy before surgery. Normal esophageal tissues from 40 healthy outpatients obtained by gastroscopy were used as controls.

IHC staining and scoring. The tissue specimens from patients with ESCC were fixed in 10% formalin for 6-72 h at room temperature, followed by dehydration and paraffin embedding. ESCC tissue was cut into 4-μm thick paraffin sections, then deparaffinized in xylene and rehydrated in graded ethanol. Antigen retrieval was performed by microwaving in 10 mmol/l citrate buffer (pH 6.0) for 20 min. After treatment with 0.3% Triton X-100 for 30 min at room temperature, sections were immersed in 3% H_2O_2 for 10 min to block endogenous peroxidase and in goat serum (ready-to-use; AR0009; Wuhan Boster Biological Technology, Ltd.) for 15 min at room temperature to block nonspecific antigens. After incubation at room temperature for 2 h with the primary antibody of TNFR2 (1:400; 28746-1-AP; Proteintech Group, Inc.), sections were washed with phosphate-buffered saline and incubated in horseradish peroxidase goat antirabbit/mouse IgG polymer (ready-to-use; KIT5010; Fuzhou Maixin Biotechnology Development Co., Ltd.) at room temperature for 30 min. Finally, sections were stained with 3,3'-diaminobenzidine for 30 sec at room temperature and counterstained with hematoxylin for 3 sec at room temperature. The proportion score (0, 1, 2 or 3) represented the estimated fraction of positive staining tumor cells (0, 0-25%; 1, 26-50%; 2, 51-75%; and 3, >75% cell staining). The intensity score (0, 1, 2 and 3) represented the estimated average staining intensity of positive tumor cells (0, negative; 1, weak; 2, moderate; and 3, strong). The expression of TNFR2 was evaluated using the product of the proportion score and the intensity score in five random fields at x400 magnification under a light microscope (DM2500; Leica Microsystems GmbH), and the mean value was obtained (≤4, low expression; and >4, high expression).

Statistical analysis. The association between TNFR2 and clinical parameters was analyzed using the χ^2 test. TNFR2 expression detected by IHC and the numbers of metastatic lymph nodes were compared between two groups using the Mann-Whitney U test. Factors which were associated with lymph node metastasis were identified using logistic regression analysis. Factors which were associated with OS were identified using Cox regression analysis. The aforementioned statistical analyses were performed using SPSS 29.0 software (IBM Corp.). TNFR2 expression was compared between tumor tissue and normal tissue in TIMER and TCGA databases using the Wilcoxon rank sum test in R 4.2.1 software (The R Foundation). Survival analysis was performed using a log-rank test in R 4.2.1 software. The correlation between two factors was analyzed using Spearman's correlation analysis in R 4.2.1 software. $P<0.05$ was considered to indicate a statistically significant difference.

Results

TNFR2 is associated with clinical stage, invasion depth, metastatic lymph node and poor OS in patients with stage T2-3 ESCC. Compared with those in normal tissues, the TIMER database revealed that the TNFR2 mRNA levels were significantly higher in ESCA, glioblastoma multiforme (GBM), head and neck squamous cell carcinoma (HNSC), kidney renal clear cell carcinoma (KIRC), stomach

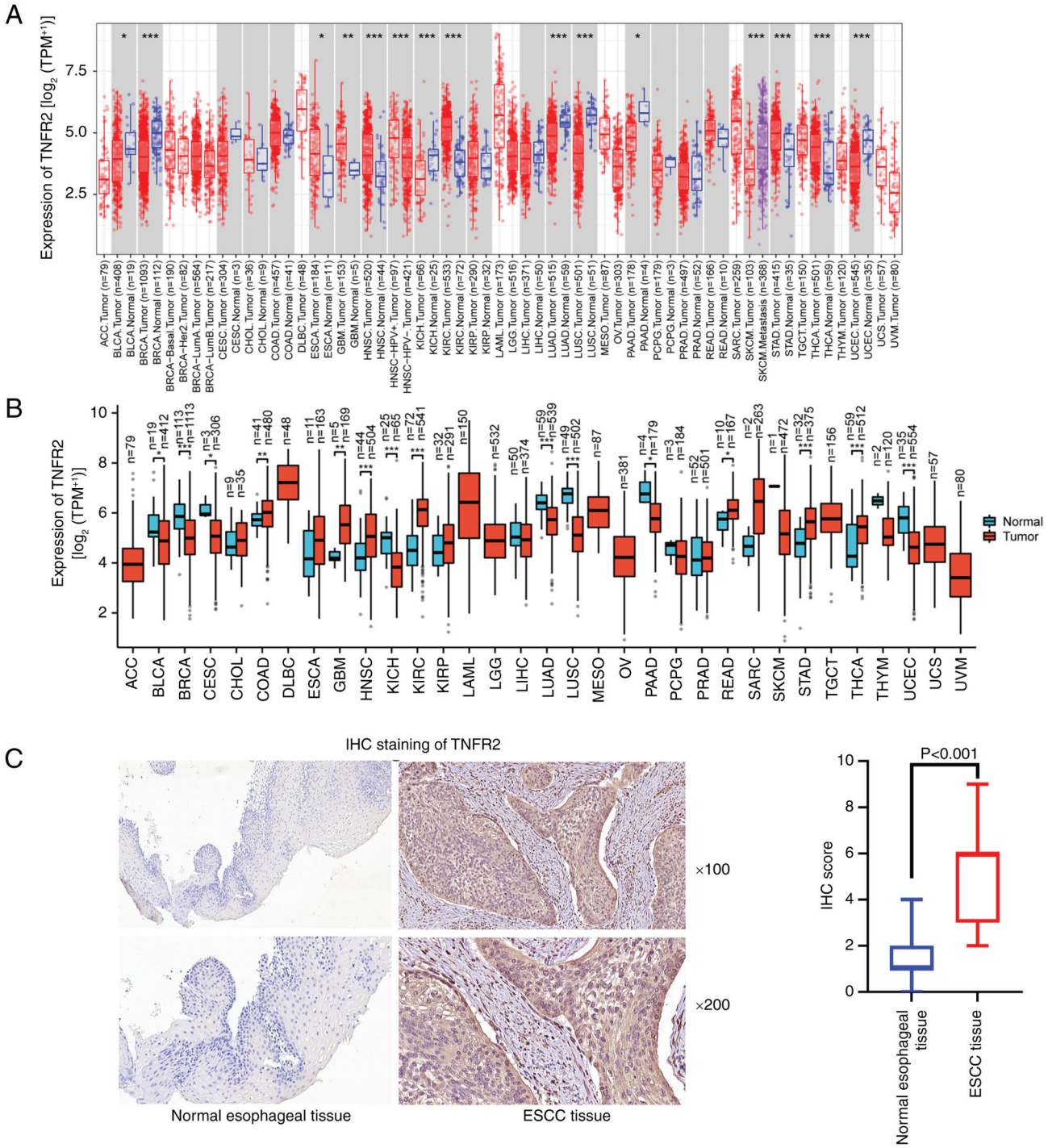

Figure 1. Expression of TNFR2 in tumor and normal tissues. mRNA expression of TNFR2 in different types of tumors and normal tissues in the (A) Tumor IMmune Estimation Resource and (B) The Cancer Genome Atlas databases. (C) Expression of TNFR2 in ESCC and normal esophageal tissues using IHC staining. *P<0.05, **P<0.01, ***P<0.001. TNFR2, tumor necrosis factor receptor 2; IHC, immunohistochemistry; ESCC, esophageal squamous cell carcinoma; TPM, transcripts per million.

adenocarcinoma (STAD) and thyroid carcinoma (THCA) (Fig. 1A). TCGA revealed that TNFR2 mRNA levels were significantly higher in colon adenocarcinoma, GBM, HNSC, KIRC, rectum adenocarcinoma, STAD and THCA tumor tissues compared with normal tissues (Fig. 1B). In addition, TCGA revealed that TNFR2 mRNA levels in ESCA tissues were also higher than those in normal tissue, although the difference was not statistically significant (Fig. 1B). Furthermore, strong IHC staining of TNFR2 was detected in the cytoplasm and membrane of ESCC tissues, which was significantly higher than the weak staining observed in the normal esophageal tissues (P<0.001; Fig. 1C). All 404 specimens from patients with stage T2-3 ESCC were divided into two groups according to the expression level of TNFR2 stained by IHC (Table I). Out of 223 cases with high expression of TNFR2, 180 were at stage III-IV, compared with 106/181 cases for the group with low expression of TNFR2 (P<0.001). A total of 165/223 cases in the high TNFR2

Table I. Association of tumor necrosis factor receptor 2 expression with the clinical parameters of patients with tumor stage 2-3 esophageal squamous cell carcinoma.

	TNFR2 expression		
Clinical parameter	Low (n=181)	High (n=223)	P-value
Sex			0.588
Male	146 (80.66)	175 (78.48)	
Female	35 (19.34)	48 (21.52)	
Age			0.537
≤60 years	90 (49.72)	104 (46.63)	
>60 years	91 (50.28)	119 (53.37)	
Clinical stage			<0.001[a]
I-II	75 (41.44)	43 (19.28)	
III-IV	106 (58.56)	180 (80.72)	
Invasion depth			<0.001[a]
T2	76 (41.98)	58 (26.01)	
T3	105 (58.02)	165 (73.99)	
Metastatic lymph node			0.008[a]
No	74 (40.88)	63 (28.25)	
Yes	107 (59.12)	163 (71.75)	
Differentiation			0.368
Low	86 (47.51)	116 (52.02)	
Moderate/high	95 (52.49)	107 (47.98)	
Tumor diameter			0.328
≤4 cm	107 (59.12)	121 (54.26)	
>4 cm	74 (40.88)	102 (45.74)	

Data are presented as n (%). [a]P<0.05 was considered statistically significant. TNFR2, tumor necrosis factor receptor 2; T, tumor.

expression group had a stage T3 invasion depth, compared with 105/181 cases in the low TNFR2 expression group (P<0.001). Moreover, 163/223 cases in the high TNFR2 expression group had metastatic lymph nodes, compared with 107/181 cases in the group with low expression of TNFR2 (P=0.008). There was no significant difference in sex, age, differentiation and tumor diameter between the two groups.

To evaluate the role of TNFR2 in predicting prognosis, survival curves were drawn and compared using the log-rank test. For patients with stage T2-3 ESCC, the OS rate in the group with high TNFR2 expression was much significantly worse than that in the group with low TNFR2 expression, beginning from ~12 months after surgery [hazard ratio (HR), 1.769; 95% confidence interval (CI), 1.284-2.436; P<0.001; Fig. 2A]. For patients with stage T2 ESCC only, the difference in OS between the two groups was not statistically significant; however, the OS rate of the group with high TNFR2 expression was notably improved compared with the low expression group within 25 months after surgery (HR, 1.297; 95% CI, 0.686-2.455; P=0.417; Fig. 2B). For patients with stage T3 ESCC only, the OS rate in the high TNFR2 expression group was significantly worse than that in the group with low expression of TNFR2 (HR, 1.852; 95% CI, 1.278-2.684; P=0.002; Fig. 2C), and the difference began earlier than in patients with stage T2-3 ESCC.

Analysis of factors associated with lymph node metastasis in patients with stage T2-3 ESCC and survival analysis. To further assess the factors affecting lymph node metastasis, logistic regression analysis was performed. Univariate logistic regression analysis revealed that an age of >60 years, low differentiation, an invasion depth of T3 and high expression of TNFR2 were significantly associated with lymph node metastasis (P=0.004, P<0.001, P<0.001 and P=0.008, respectively; Fig. 3A). Further multivariate logistic regression analysis demonstrated that low differentiation and an invasion depth of T3 significantly increased the risk of lymph node metastasis by 3.019-fold and 11.929-fold, respectively (P<0.001 and P<0.001, respectively; Fig. 3B); however, an age of >60 years significantly reduced the risk of lymph node metastasis by 46.5% (P=0.019; Fig. 3B). Moreover, TNFR2 expression was not significantly associated with lymph node metastasis (P=0.318; Fig. 3B). To further assess the relationship between age, differentiation and invasion depth with lymph node metastasis, the association of these three factors with the number of metastatic lymph nodes was evaluated. The results revealed that the median number of metastatic lymph nodes in the group with T3 invasion depth was 2 (range, 1-3), which was significantly higher than in the group with T2 invasion depth (median, 0; range, 0-1; P<0.001; Fig. 3C). Moreover, the median number of metastatic lymph nodes in the group with

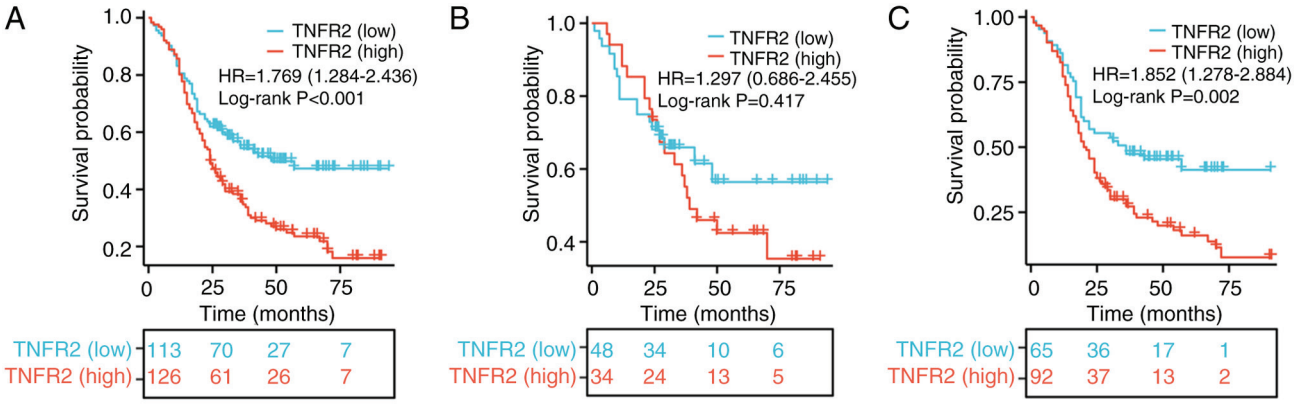

Figure 2. Survival analysis of patients with stage T2-3 ESCC. (A) Comparison of the survival probability between the high and low TNFR2 expression groups of patients with stage T2-3 ESCC. Stratified comparison of survival probability between the high and low TNFR2 expression groups of patients with stage (B) T2 and (C) T3 ESCC. TNFR2, tumor necrosis factor receptor 2; ESCC, esophageal squamous cell carcinoma; HR, hazard ratio; T, tumor.

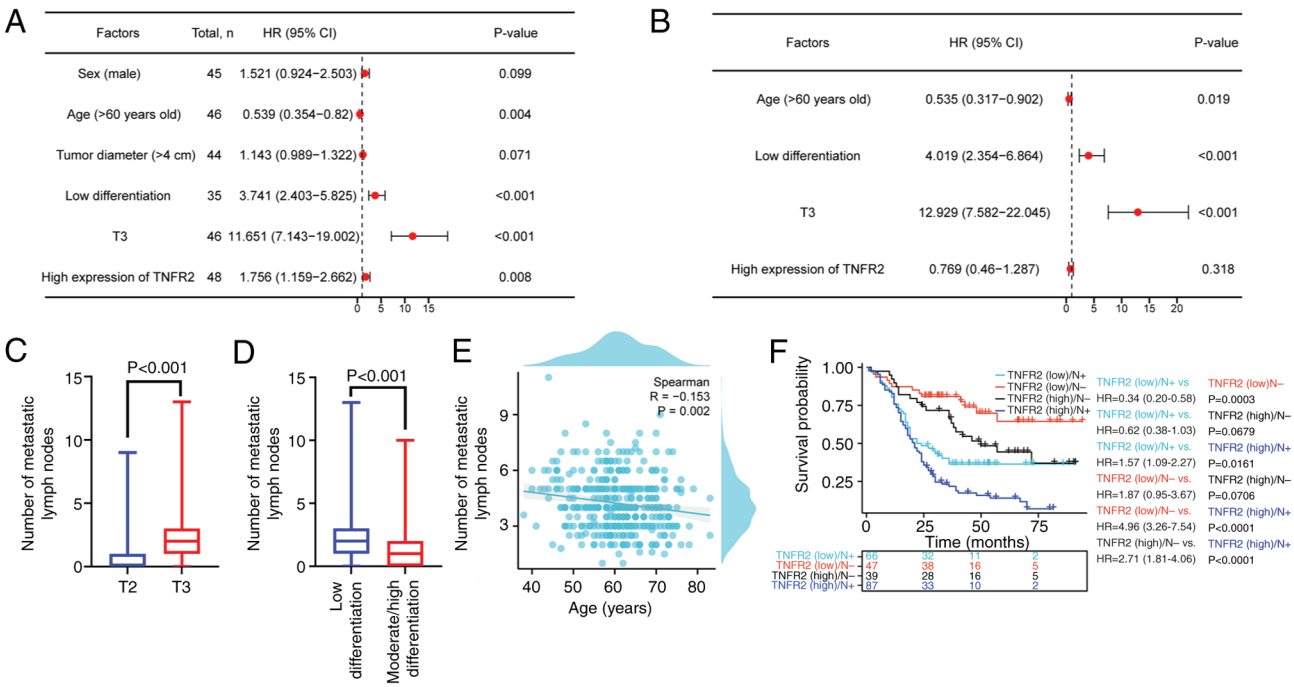

Figure 3. Analysis of factors associated with lymph node metastasis in patients with stage T2-3 esophageal squamous cell carcinoma and survival analysis. (A) Univariate and (B) multivariate logistic regression analysis of factors associated with lymph node metastasis. Comparison of the number of metastatic lymph nodes between the (C) stage T2 and T3 patient groups and the (D) low differentiation and moderate/high differentiation groups. (E) Spearman's analysis of the correlation between age and the number of metastatic lymph nodes. (F) Comparison of survival probability between groups with different expression levels of TNFR2 and lymph node metastasis. TNFR2, tumor necrosis factor receptor 2; HR, hazard ratio; CI, confidence interval; T, tumor; N, node.

low differentiation was 2 (range, 1-3), which was significantly higher than in the group with moderate/high differentiation (median, 1; range, 0-2; P<0.001; Fig. 3D). Furthermore, the Spearman's analysis demonstrated that age was significantly negatively correlated with the number of metastatic lymph nodes (R=-0.153; P=0.002; Fig. 3E).

Survival analysis of TNFR2 and lymph node metastasis was performed and the results revealed that the group with low expression of TNFR2 and lymph node metastasis had a markedly worse OS compared with that in the group with high expression of TNFR2 and no lymph node metastasis, although the difference was not statistically significant (HR, 0.62; 95% CI, 0.38-1.03; P=0.0679; Fig. 3F). With the exception of the comparison between the aforementioned two groups, groups with a high expression of TNFR2 demonstrated worse OS than the groups with low expression of TNFR2: The low TNFR2 expression/lymph node metastasis group vs. the high TNFR2 expression/lymph node metastasis group (HR, 1.57; 95% CI, 1.09-2.27; P=0.0161), the low TNFR2 expression/no lymph node metastasis group vs. the high TNFR2 expression/no lymph node metastasis group (HR, 1.87; 95% CI, 0.95-3.67; P=0.0706) and the low TNFR2 expression/no lymph node metastasis group vs. the high TNFR2 expression/lymph node metastasis group (HR, 4.96; 95% CI, 3.26-7.54; P<0.0001; Fig. 3F). This revealed the association between TNFR2 expression combined with lymph node metastasis and prognosis of patients with ESCC.

Table II. Association of tumor necrosis factor receptor 2 expression with the clinical parameters of patients with tumor stage 2-3 esophageal squamous cell carcinoma aged ≤60 years.

	TNFR2 expression		
Clinical parameter	Low (n=90)	High (n=104)	P-value
Sex			0.791
Male	74 (82.22)	87 (83.65)	
Female	16 (17.78)	17 (16.35)	
Clinical stage			0.002[a]
I-II	34 (37.78)	19 (18.27)	
III-IV	56 (62.22)	85 (81.73)	
Invasion depth			0.055
T2	34 (37.78)	26 (25.00)	
T3	56 (62.22)	78 (75.00)	
Metastatic lymph node			0.004[a]
No	33 (36.67)	19 (18.27)	
Yes	57 (63.33)	85 (81.73)	
Differentiation			0.596
Low	57 (63.33)	62 (59.62)	
Moderate/high	33 (36.67)	42 (40.38)	
Tumor diameter			0.773
≤4 cm	46 (51.11)	51 (49.04)	
>4 cm	44 (48.89)	53 (50.96)	

Data are presented as n (%). [a]P<0.05 was considered statistically significant. TNFR2, tumor necrosis factor receptor 2; T, tumor.

TNFR2 is associated with clinical stage, lymph node metastasis and poor OS in patients with stage T2-3 ESCC aged ≤60 years. A total of 194 patients with stage T2-3 ESCC aged ≤60 years were divided into a low TNFR2 expression group (n=90) and a high TNFR2 expression group (n=104) using IHC staining. In the group with high TNFR2 expression, 85/104 cases had a clinical stage of III-IV, which was significantly greater than the 56/90 cases in the group with low TNFR2 expression (P=0.002). Moreover, 85/104 cases in the high TNFR2 expression group had metastatic lymph nodes, which was significantly higher than the 57/90 cases in the group with low expression of TNFR2 (P=0.004). However, there were no significant differences demonstrated for sex, invasion depth, differentiation and tumor diameter between the two groups (Table II).

To evaluate the role of TNFR2 in predicting the prognosis of patients with stage T2-3 ESCC aged ≤60 years, a survival analysis was performed. The results revealed that the OS of the group with high TNFR2 expression was markedly worse than that of the group with low TNFR2 expression, beginning ~20 months after surgery; however, the difference was not statistically significant (HR, 1.593; 95% CI, 0.991-2.56; P=0.05; Fig. 4A). For patients aged ≤60 years with stage T2 ESCC only, the OS rate of the high TNFR2 expression group was significantly worse than that of the group with low TNFR2 expression (HR, 3.121; 95% CI, 1.166-8.355; P=0.017; Fig. 4B). However, for patients aged ≤60 years with stage T3 ESCC only, the OS rate in the high TNFR2 expression group was notably poorer than that in the group with low TNFR2 expression, although the difference was not statistically significant (HR, 1.153; 95% CI, 0.67-1.986; P=0.602; Fig. 4C).

TNFR2 is associated with clinical stage, lymph node metastasis and poor OS in patients with stage T2-3 ESCC aged >60 years. A total of 210 patients with stage T2-3 ESCC aged >60 years old were divided into a low TNFR2 expression group (n=91) and a high TNFR2 expression group (n=119) using IHC staining. In the group with high expression of TNFR2, 87/119 cases had a T3 invasion depth, which was significantly higher than the 49/91 cases in the low TNFR2 expression group (P=0.004). However, there was no significant difference in sex, clinical stage, metastatic lymph node, differentiation and tumor diameter between the two groups (Table III).

To evaluate the role of TNFR2 in predicting the prognosis of patients with stage T2-3 ESCC aged >60 years, a survival analysis was performed. The results demonstrated that the OS rate of the high TNFR2 expression group was significantly worse than that of the group with low expression of TNFR2, beginning ~10 months after surgery (HR, 1.92; 95% CI, 1.239-2.975; P=0.006; Fig. 5A). However, for patients with stage T2 ESCC aged >60 years, the log-rank test revealed no significant difference in OS between the

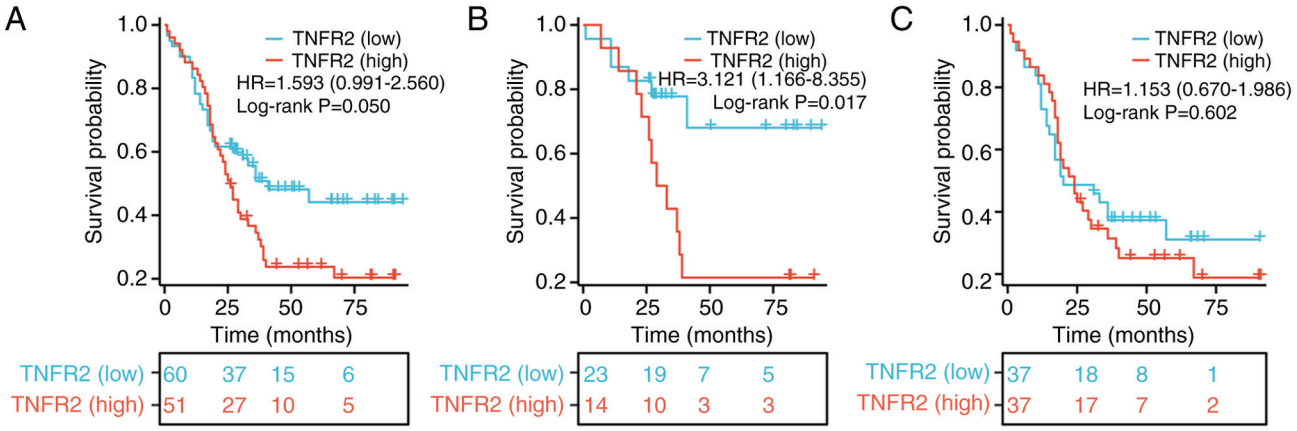

Figure 4. Survival analysis of patients with stage T2-3 ESCC aged ≤60 years. (A) Comparison of survival probability between the high and low TNFR2 expression groups of patients with stage T2-3 ESCC aged ≤60 years. Stratified comparison of survival probability between the high and low TNFR2 expression groups of patients with stage (B) T2 and (C) T3 ESCC aged ≤60 years. TNFR2, tumor necrosis factor receptor 2; ESCC, esophageal squamous cell carcinoma; HR, hazard ratio; T, tumor.

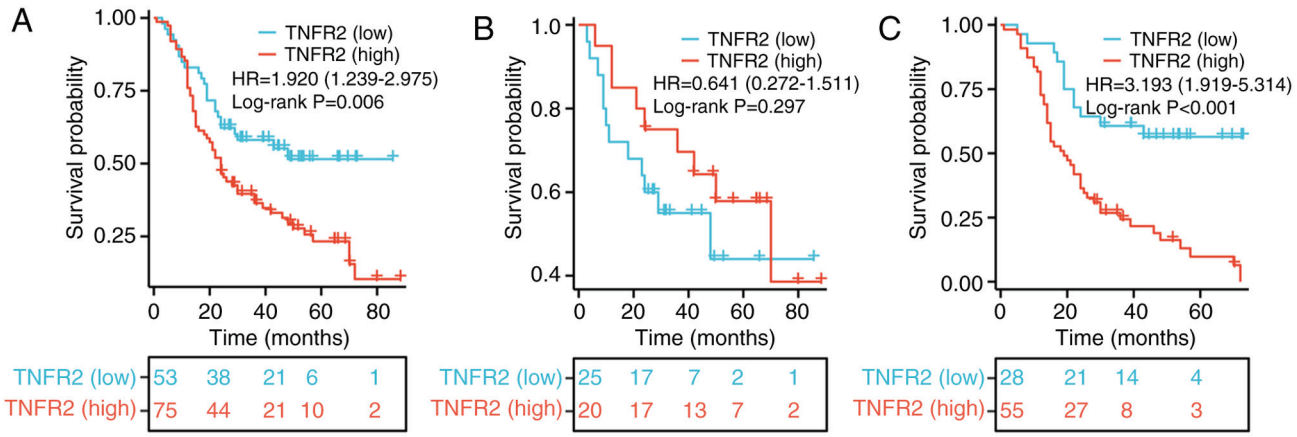

Figure 5. Survival analysis of patients with stage T2-3 ESCC aged >60 years. (A) Comparison of survival probability between the high and low TNFR2 expression groups of patients with stage T2-3 ESCC aged >60 years. Stratified comparison of survival probability between the high and low TNFR2 expression groups of patients with (B) T2 and (C) T3 ESCC aged >60 years. TNFR2, tumor necrosis factor receptor 2; ESCC, esophageal squamous cell carcinoma; HR, hazard ratio; T, tumor.

group with high expression of TNFR2 and the group with low expression of TNFR2 (HR, 0.641; CI, 0.272-1.511; P=0.297; Fig. 5B). Moreover, for patients aged >60 years with stage T3 ESCC only, the OS rate of the group with high expression of TNFR2 was significantly worse than that in the group with low expression of TNFR2, beginning ~3 months after surgery (HR, 3.193; 95% CI, 1.919-5.314; P<0.001; Fig. 5C).

Cox regression analysis of potential factors affecting the OS of patients with stage T2-3 ESCC. To assess the potential factors which may affect the OS of patients with stage T2-3 ESCC, univariate and multivariate Cox regression analyses were performed. For patients with stage T2-3 ESCC, univariate Cox regression analysis revealed that metastatic lymph node, low differentiation, clinical stage III-IV, invasion depth of T3 and high expression of TNFR2 were significantly associated with a poor OS (P<0.001, P=0.018, P<0.001, P<0.001 and P<0.001, respectively; Fig. 6A). Moreover, multivariate Cox regression analysis demonstrated that metastatic lymph node and high expression of TNFR2 significantly increased the risk of death by 1.866- and 0.661-fold, respectively (P<0.001 and P=0.003 respectively; Fig. 6B). For patients with stage T2-3 ESCC aged ≤60 years, univariate Cox regression analysis revealed that metastatic lymph node, low differentiation, clinical stage III-IV and invasion depth of T3 were significantly associated with a poor OS (P<0.001, P=0.003, P<0.001 and P=0.019, respectively; Fig. 6C). Furthermore, multivariate Cox regression analysis demonstrated that only metastatic lymph node significantly increased the risk of death by 2.479-fold (P<0.001; Fig. 6D). For patients with stage T2-3 ESCC aged >60 years, univariate Cox regression analysis revealed that metastatic lymph node, clinical stage III-IV, invasion depth of T3 and high expression of TNFR2 were significantly associated with a poor OS (P<0.001, P<0.001, P=0.014 and P=0.007, respectively; Fig. 6E). Moreover, multivariate Cox regression analysis demonstrated that metastatic lymph node and high expression of TNFR2 significantly increased the risk of death by 1.636- and 0.914-fold, respectively (P<0.001 and P=0.008, respectively; Fig. 6F).

Table III. Association of tumor necrosis factor receptor 2 expression with the clinical parameters of patients with tumor stage 2-3 esophageal squamous cell carcinoma aged >60 years.

	TNFR2 expression		
Clinical parameter	Low (n=91)	High (n=119)	P-value
Sex			0.383
Male	72 (79.12)	88 (73.95)	
Female	19 (20.88)	31 (26.05)	
Clinical stage			0.237
I-II	41 (45.05)	44 (36.97)	
III-IV	50 (54.95)	75 (63.03)	
Invasion depth			0.004[a]
T2	42 (46.15)	32 (26.89)	
T3	49 (53.85)	87 (73.11)	
Metastatic lymph node			0.237
No	41 (45.05)	44 (36.97)	
Yes	50 (54.95)	75 (63.03)	
Differentiation			0.494
Low	37 (40.66)	54 (45.38)	
Moderate/High	54 (59.34)	65 (54.62)	
Tumor diameter			0.224
≤4 cm	61 (67.03)	70 (58.82)	
>4 cm	30 (32.97)	49 (41.18)	

Data are presented as n (%). [a]P<0.05 was considered statistically significant. TNFR2, tumor necrosis factor receptor 2; T, tumor.

Discussion

TNFR2 is a promising factor in terms of predicting prognosis and finding therapeutic targets of ESCC, as it is highly expressed in several types of tumor cells and normal cells such as interstitial fibroblasts, endothelial cells, immune cells and hematopoietic cells (12-14). In recent years, several studies reported the role of TNFR2 in tumor occurrence and development in different manners: Gao *et al* (15) reported that TNFR2 can promote the proliferation, migration and invasion of pancreatic cancer cells via the NF-κB signaling pathway; Wang *et al* (16) reported that TNFR2 can promote the switch from fibroblasts to cancer-associated fibroblasts in the microenvironment of colorectal cancer, which facilitates cancer metastasis; and Qu *et al* (17) reported that activation of the TNF-α/TNFR2 pathway promotes the immunosuppressive phenotype and function of Tregs in gastric cancer, resulting in cancer progression. Tumor heterogeneity is an important reason for poor treatment outcomes, which may exist among different types of tumors, different patients with the same type of tumor or even different parts of the same tumor (18). Although TNFR2 exhibits significant protumor effects, it is unclear whether its role in different subgroup patients is also the same. Therefore, the present study focused on assessing the role of TNFR2 in stage T2-3 ESCC and further stratified subgroup patients to provide more accurate data on the role of TNFR2 in ESCC.

The present study demonstrated a high expression of TNFR2 both at the mRNA and protein level and revealed that high expression of TNFR2 was positively associated with advanced clinical stage, invasion depth and lymph node metastasis, which is in line with the role of TNFR2 in tumors reported by the aforementioned studies (15-17). Moreover, age is an important factor in the occurrence and development of malignant tumors. Previous research has reported that the incidence of malignant tumors increases with age but, compared with young patients, the tumors of older patients tend to exhibit inert phenotypes and different patterns of management (19). Patel *et al* (19) reported that older patients with colon cancer had a decreased rate of distant metastasis and lymph node metastasis compared with younger patients. Recently, Lin *et al* (20) reported that 60 years was the optimal cut-off age for differences in OS and progression-free survival (PFS) in a study including 568 patients with ESCC. Moreover, several studies on ESCC used 60 years as the cutoff for age grouping and reported marked differences in variables, such as gene expression, biological behavior and prognosis (21-25). Therefore, the present study performed a subgroup analysis based on the age of 60 years to further evaluate the clinical significance of TNFR2 in patients with stage T2-3 ESCC. Notably, different to patients aged ≤60 years old, for patients aged >60 years old, high expression of TNFR2 was only associated with invasion depth, but not advanced clinical stage or lymph node metastasis. This demonstrates the heterogeneity among subgroups and may be explained by the inert

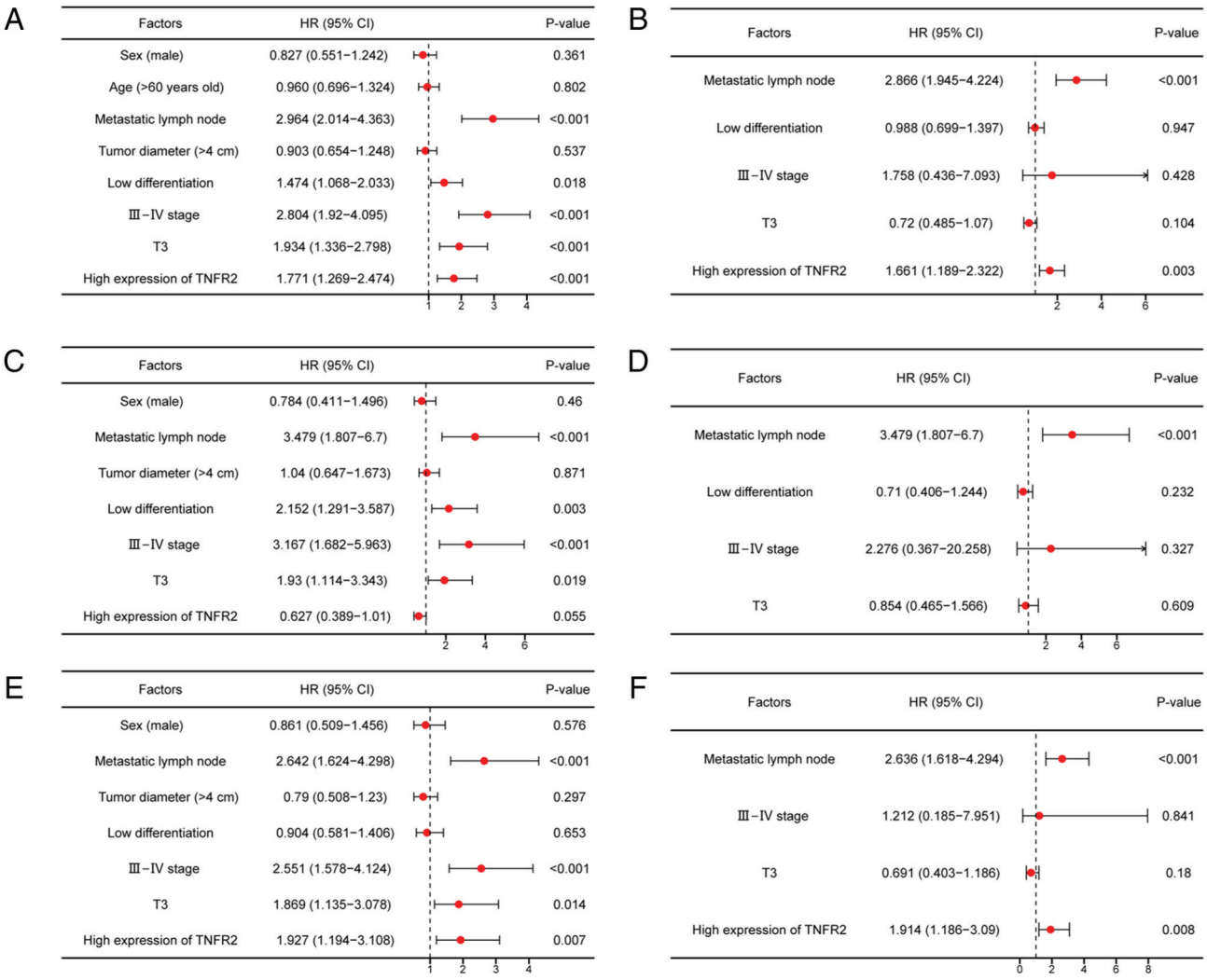

Figure 6. Cox regression analysis of factors affecting OS. (A) Univariate and (B) multivariate Cox regression analysis of factors affecting OS of patients with stage T2-3 ESCC. (C) Univariate and (D) multivariate Cox regression analysis of factors affecting OS of patients with stage T2-3 ESCC aged ≤60 years. (E) Univariate and (F) multivariate Cox regression analysis of factors affecting OS of patients with stage T2-3 ESCC aged >60 years old. OS, overall survival; TNFR2, tumor necrosis factor receptor 2; ESCC, esophageal squamous cell carcinoma; T, tumor.

characteristics of malignant tumors in elderly patients reported previously (19). In addition, no association between TNFR2 and tumor size was demonstrated in total T2-3 cases or stratified subgroups split by age (60 years old). This result is not consistent with the promoting effect of TNFR2 in pancreatic cancer reported by Gao *et al* (15), and the difference may be explained by the heterogeneity derived from different tumor types. Further detailed cell experiments are required for validation. Meanwhile, the present study confirmed that an age of >60 years reduced the risk of lymph node metastasis, and age was associated with the number of metastatic lymph nodes. This again reflects the inert characteristics of ESCC in elderly patients, in line with the report by Patel *et al* (19).

The association between TNFR2 with prognosis has been reported in several types of tumors but at present it remains controversial. In 2021, Silva Raju *et al* (26) reported that patients in Malaysia with a high level of TNFR2 expression in ovarian cancer tissue exhibited no significant difference in PFS interval compared with patients with a low level of TNFR2. In 2019, Zhang *et al* (27) reported that TNFR2 was expressed in non-small cell lung cancer tissues and was related to the poor prognosis of patients in China. The present study demonstrated that a high expression of TNFR2 is associated with the poor prognosis of patients with stage T2-3 ESCC, but this was not associated with the presence of lymph nodes metastasis. This finding is in line with the report of Zhang *et al* (27) but inconsistent with the results of Silva Raju *et al* (26), which may be explained by differences in tumor origin or ethnicity. Further stratified analysis revealed there was no significant effect of TNFR2 on OS of patients with stage T2 ESCC, patients with stage T2-3 ESCC aged ≤60 years old, patients with stage T3 ESCC aged ≤60 years old, or patients with stage T2 ESCC aged >60 years old. The aforementioned results indicate differences in the role of TNFR2 in different subgroups of patients with stage T2-3 ESCC, suggesting that TNFR2 may not be suitable as a potential prognostic marker for these four stratified subgroup patients.

The occurrence and development of tumors is a complex process and prognosis is influenced by a combination of different factors. Therefore, the independent prognostic factors among different subgroups may be different. For all patients

with stage T2-3 ESCC, metastatic lymph nodes and a high expression of TNFR2 were independent prognostic factors. This was also demonstrated for patients with stage T2-3 ESCC age >60 years. However, for patients with stage T2-3 ESCC aged ≤60 years, only the presence of metastatic lymph nodes was an independent prognostic factor, whilst TNFR2 expression did not exhibit a significant effect on prognosis even in univariate Cox regression analysis. These results further confirm the unsuitability of TNFR2 as a potential prognostic marker for patients with T2-3 ESCC aged ≤60 years. Moreover, these different results may be related to age-based biological differences of tumors; however, the impact of a limited number of cases on the result is also unavoidable, especially the limited number of cases in subgroups.

In addition to the limited number of cases considered, there are other limitations in the present study: Nearly all cases were from one area, so the data were regional and the universality of the results is limited. A larger number of cases from multiple centers will provide more reliable and universal results. In addition, the present study was retrospective and the results could have easily been influenced by bias and confounding effects; therefore, further validation in prospective studies is needed.

In conclusion, the results of the present study demonstrated the association of TNFR2 expression with progression and poor prognosis in patients with stage T2-3 ESCC and different stratified subgroups. These findings will enrich the current knowledge of the roles of TNFR2 in tumors. Moreover, they will help clinicians have a more accurate understanding of the clinical significance of TNFR2 in different subgroups of patients with ESCC, providing a basis for more precise utilization of TNFR2 as a prognostic marker and therapeutic target.

Acknowledgements

Not applicable.

Funding

The present study was supported by the Postdoctoral Program of the Affiliated Hospital of Jining Medical University (grant no. JYFY321205), the Postdoctoral Program of Jining No.1 People's Hospital (grant no. 2023-BSH-003) and the Jining Research and Development Program (grant no. 2023YXNS046).

Availability of data and materials

The data generated in the present study may be requested from the corresponding author.

Authors' contributions

DY and SQ were responsible for conception, design, definition of intellectual content and final approval of the version to be published. MR and SJ were responsible for immunohistochemical staining of esophageal squamous cell carcinoma tissue and data analysis. ZL was responsible for immunohistochemical staining of esophageal squamous cell carcinoma tissue, data analysis, and drafting the article and revising it critically for important intellectual content. DY and ZL confirmed the authenticity of all the raw data. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

The present study was performed in accordance with the principles of the Declaration of Helsinki and approved by the Ethics Committee of the Affiliated Hospital of Jining Medical University (Jining, China; approval no. 2017-Research-01). Written informed consent was provided by the patients or their guardians.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Morgan E, Soerjomataram I, Rumgay H, Coleman HG, Thrift AP, Vignat J, Ferlay J and Arnold M: The global landscape of esophageal squamous cell carcinoma and esophageal adenocarcinoma incidence and mortality in 2020 and projections to 2040: new estimates from GLOBOCAN 2020. Gastroenterology 163: 649-658.e2, 2022.
2. Codipilly DC and Wang KK: Squamous cell carcinoma of the esophagus. Gastroenterol Clin North Am 51: 457-484, 2022.
3. Voron T, Julio C and Pardo E: Esophageal carcinoma: Novelties and challenges in surgery. Bull Cancer 110: 533-539, 2023.
4. Xu J, Kato K, Raymond E, Hubner RA, Shu Y, Pan Y, Park RS, Ping L, Jiang Y, Zhang J, *et al*: Tislelizumab plus chemotherapy versus placebo plus chemotherapy as first-line treatment for advanced or metastatic oesophageal squamous cell carcinoma (RATIONALE-306): A global, randomised, placebo-controlled, phase 3 study. Lancet Oncol 24: 483-495, 2023.
5. Waters JK and Reznik SI: Update on management of squamous cell esophageal cancer. Curr Oncol Rep 24: 375-385, 2022.
6. Edge SB and Compton CC: The American joint committee on cancer: The 7th edition of the AJCC cancer staging manual and the future of TNM. Ann Surg Oncol 17: 1471-1474, 2010.
7. Wang J, Wu N, Zheng QF, Yan S, Lv C, Li SL and Yang Y: Evaluation of the 7th edition of the TNM classification in patients with resected esophageal squamous cell carcinoma. World J Gastroenterol 20: 18397-18403, 2014.
8. Mensink M, Tran TNM, Zaal EA, Schrama E, Berkers CR, Borst J and Kivit SD: TNFR2 costimulation differentially impacts regulatory and conventional CD4(+) T-Cell Metabolism. Front Immunol 13: 881166, 2022.
9. Babic A, Shah SM, Song M, Wu K, Meyerhardt JA, Ogino S, Yuan C, Giovannucci EL, Chan AT, Stampfer MJ, *et al*: Soluble tumour necrosis factor receptor type II and survival in colorectal cancer. Br J Cancer 114: 995-1002, 2016.
10. Torrey H, Butterworth J, Mera T, Okubo Y, Wang L, Baum D, Defusco A, Plager S, Warden S, Huang D, *et al*: Targeting TNFR2 with antagonistic antibodies inhibits proliferation of ovarian cancer cells and tumor-associated Tregs. Sci Signal 10, 2017.
11. Wei MX, Song X, Zhao XK, Han WL, Bao Q, Han XN, Xu RH, Li XM, Fan ZM, Wang R, *et al*: Clinicopathological characteristics and postoperative prognosis of patients with nuclear pedigree of esophageal squamous cell carcinoma. Front Oncol 13: 1190457, 2023.
12. Zhang H and Xiao W: TNFR1 and TNFR2 differentially mediate TNF-α-induced inflammatory responses in rheumatoid arthritis fibroblast-like synoviocytes. Cell Biol Int 41: 415-422, 2017.
13. Bai J, Ding B and Li H: Targeting TNFR2 in Cancer: All roads lead to rome. Front Immunol 13: 844931, 2022.

14. He T, Zhao Y, Zhao P, Zhao L, Zakaria J and Wang K: Signaling pathway(s) of TNFR2 required for the immunoregulatory effect of CD4(+)Foxp3(+) regulatory T cells. Int Immunopharmacol 108: 108823, 2022.
15. Gao Z, Zhang Q, Chen H, Chen J, Kang J, Yu H, Song Y and Zhang X: TNFR2 promotes pancreatic cancer proliferation, migration, and invasion via the NF-κB signaling pathway. Aging 15: 8013-8025, 2023.
16. Wang L, Yang D, Tian J, Gao A, Shen Y, Ren X, Li X, Jiang G and Dong T: Tumor necrosis factor receptor 2/AKT and ERK signaling pathways contribute to the switch from fibroblasts to CAFs by progranulin in microenvironment of colorectal cancer. Oncotarget 8: 26323-26333, 2017.
17. Qu Y, Wang X, Bai S, Niu L, Zhao G, Yao Y, Li B and Li H: The effects of TNF-α/TNFR2 in regulatory T cells on the microenvironment and progression of gastric cancer. Int J Cancer 150: 1373-1391, 2022.
18. Kashyap A, Rapsomaniki MA, Barros V, Fomitcheva-Khartchenko A, Martinelli AL, Rodriguez AF, Gabrani M, Rosen-Zvi M and Kaigala G: Quantification of tumor heterogeneity: From data acquisition to metric generation. Trends Biotechnol 40: 647-676, 2022.
19. Patel SS, Nelson R, Sanchez J, Lee W, Uyeno L, Garcia-Aguilar J, Hurria A and Kim J: Elderly patients with colon cancer have unique tumor characteristics and poor survival. Cancer 119: 739-7347, 2013.
20. Lin Y, Ye Y, Huang Q, Zheng B, Yang Y, Chen Y, Li W, Ke H, Lin C, Zhang Y, *et al*: Influence of age as a continuous variable on survival outcomes and treatment options in patients with upper thoracic esophageal carcinoma. J Cancer 14: 1039-1048, 2023.
21. Zhang P, Wang S, Wu JZ and Song Q: Clinical and prognostic significance of perioperative change in red cell distribution width in patients with esophageal squamous cell carcinoma. BMC Cancer 23: 319, 2023.
22. Feng RB, Zhou QZ, Cheng R, Li P, Zhu ST, Min L and Zhang ST: Expression and significance of N-myc downstream regulated gene 2 in the process of esophageal squamous cell carcinogenesis. Bioengineered 13: 3275-3283, 2022.
23. Luo QY, Di T, Qiu MZ, Xia ZF, Du Y, Lin RD, Yang LQ, Sun YT, Yang DJ, Sun J and Zhang L: High AKAP8L expression predicts poor prognosis in esophageal squamous cell carcinoma. Cancer Cell Int 22: 90, 2022.
24. Xie C, Chen Z, Xu J, Meng Z, Huang Z and Lin J: Influence of lymphangio vascular (V) and perineural (N) invasion on survival of patients with resected esophageal squamous cell carcinoma (ESCC): A single-center retrospective study. Peer J 10: e12974, 2022.
25. Wang N, Li Y, Zhou RM, Wang GY, Wang CM, Chen ZF and Liu W: Hsa-miR-196a2 functional SNP is associated with the risk of ESCC in individuals under 60 years old. Biomarkers 19: 43-48, 2014.
26. Silva Raju J, Abd Aziz NH, Atallah GA, Teik CK, Shafiee MN, Mohd Saleh MF, Jeganathan R, Md Zin RR and Kampan NC: Prognostic value of TNFR2 and STAT3 among high-grade serous ovarian cancer survivors according to platinum sensitivity. Diagnostics (Basel) 11: 526, 2021.
27. Zhang YW, Chen QQ, Cao J, Xu LQ, Tang X, Wang J, Zhang J and Dong LX: Expression of tumor necrosis factor receptor 2 in human non-small cell lung cancer and its role as a potential prognostic biomarker. Thoracic Cancer 10: 437-444, 2019.