INTRODUCTION

Esophageal cancer (EC) was a kind of disease with vague symptoms, such as difficult to swallow some solid foods, pain with swallowing, or unexplained weight loss. It ranked 7th in incidence and 6th in mortality worldwide, while in China, the incidence rate and mortality of EC were 17.9/100,000 and 13.7/100,000, respectively, and its five-year survival rate was 19%, all of which caused great burden to patients, families, and society. Therefore, it is of great importance to early detection and find new biomarkers to diagnose EC for timely prevention and treatment. In clinical, it was divided into two main histological subtypes: esophageal adenocarcinoma (EAC) and esophageal squamous cell carcinoma (ESCC), while it is estimated that the people in central and southeastern Asia mostly occurred.
with ESCC subtypes, which was also common in China. Besides, the highly epidemiological characteristics were happened in Linxian of Henan Province and Cixian of Hebei Province.

Nowadays, with the development of medical medicine and high-tech preventive screening methods, such as microarray, next-generation sequencing (NGS) and even single strand Adaptor Library Preparation-sequencing (SALP-seq) in combination with machine learning, the genetic alternations were more convenient to find in cancer samples, which was also accelerated the research of small-molecule anti-cancer targeted drugs. Hence, in the study in cancer samples, which was also accelerated the research of learning, the genetic alternations were more convenient to find in cancer samples, which was also accelerated the research of small-molecule anti-cancer targeted drugs.

The First Affiliated Hospital of USTC, Division of life Sciences and Medicine, University of Science and Technology of China. 

2.2 | Immunohistochemistry

We collected 45 esophageal cancer tissues (15 poorly differentiated, 15 moderately differentiated, 15 well differentiated) and 15 para-carcinoma tissue (2-3 cm from the resection margin) from the patients who underwent esophagectomy at The First Affiliated Hospital of USTC, Division of life Sciences and Medicine, University of Science and Technology of China during 2017.5-2019.12. The tumor differentiation degree was determined by the pathological report of tissue samples, while the metastasis status was judged from the CT or PET-CT or ultrasonography method. Besides, all recruited patients were provided with written informed consent before surgical resection, and the study was approved by the Ethics Committee of The First Affiliated Hospital of USTC, Division of life Sciences and Medicine, University of Science and Technology of China.

2.3 | Western blot analysis

The liquid nitrogen stored esophageal cancer tissues and para-carcinoma tissue were washed and lysed with RIPA Lysis Buffer containing with PMSF (catalog: 329-98-6, Sigma); then, we analyzed the protein concentration in each sample with BCA Protein Assay Kit (catalog: PA115-01, TIANGEN). Afterward, the protein were de-natured and detected with the SDS-PAGE gel; besides, the incubated primary antibody were anti-TNK2 (#:ab185726, Abcam), anti-CDC42 (#:ab187643, Abcam), anti-Akt (#:ab8805, Abcam), anti-EGFR (#:ab52894, Abcam), and anti-GAPDH (#:ab8245, Abcam). And the secondary goat anti-rabbit antibody was IgG (#:ab172730, Abcam). Afterward, the blots were conjugated with ECL and were visualized with the bandscan; then, we analyzed the proteins’ gray intensity with Image J Software.

2.4 | Clinical implication of TNK2 expression in esophageal cancer

We assayed the survival curves of TNK2 protein expression with 184 esophageal cancer patients from TCGA database. Besides, with multiple linear regression analysis, we detected the correlations of TNK2 expression associated with tumor differentiation degree and metastasis status in esophageal cancer.

2.5 | Statistical analysis

Statistical analysis was analyzed with the SPSS 19.0 statistical software package (SPSS Inc., Chicago, USA). A one-way ANOVA analysis, a Kaplan-Meier plot, and a Spearman correlation coefficient analysis were used to evaluate statistical significance. Data were shown as the mean ± SEM. p < 0.05 was considered to be statistical significance. Besides, the graphs were depicted with GraphPad Prism 6.0 software.

3 | RESULTS

3.1 | TNK2 was a potential diagnostic marker in esophageal cancer via activating EGFR-AKT signaling

Immunohistochemistry (IHC) results revealed that TNK2 was highly expressed in the cytoplasm of esophageal cancer tissues (Figure 1A) compared with para-carcinoma tissues (Figure 1B, p < 0.05); besides,
IHC and Western blot analysis all proved that TNK2 was obviously up-regulated in poorly differentiated esophageal cancer tissues (Figures 1, 2); meanwhile, the proteins of CDC42, EGFR, and Akt were also up-regulated in different levels in esophageal cancer tissues compared with para-carcinoma tissues (Figure 2).

3.2 | Overall survival time and multiple linear regression analysis of TNK2 protein expression in esophageal cancer

With data from TCGA database of 184 esophageal cancer patients (n = 46, high expression, n = 138, low/medium expression), we found that there was no significant difference of the overall survival time associated with TNK2 protein expression (Figure 3, p = 0.37). However, in the included study samples of our study, there was a positive coefficient between TNK2 protein expression and differentiation degree in esophageal cancer with multiple linear regression analysis ($R = 0.928$, 95% confidence interval (0.085–0.12)) (Figure S1).

DISCUSSION

As cancer treatment had come into a new era of targeted therapy, hence, in this study, we studied the molecular target of esophageal cancer with clinical tumor samples. We found that TNK2 was highly expressed in the cytoplasm of esophageal cancer tissues, especially, in the poorly differentiated tissues; besides, in the esophageal cancer tissues, the proteins of CDC42, EGFR, and Akt were also up-regulated in different levels. As TNK2 was an intracellular tyrosine kinase, which was found to be implicated in regulating multiple aspects of tumor progression. Likely, in the study of George J N Tetley...
et al. revealed that Cdc42 bound to TNK2 CRIB region via hydrophobic interactions, while CDC42 was identified to play pivotal roles in controlling the actin cytoskeleton and it was engaged in oncogenic signaling networks. Besides, TNK2 (ACK1) was proved to regulate in controlling the actin cytoskeleton and it was engaged in oncogenic progression. Hence, the activated TNK2 was potential to bound and trigger AKT Tyr176 phosphorylation to promote prostate cancer progression.

Moreover, it is showed that the activated TNK2 (ACK1) could activate CDC42 to promote esophageal cancer progression through activating EGFR-AKT signaling. Even more, it is showed that the activated TNK2 (ACK1) could activate AKT Tyr176 phosphorylation to promote prostate cancer progression. Hence, the activated TNK2 was potential to bound and trigger CDC42 to promote esophageal cancer progression through activating EGFR-AKT signaling. Moreover, it may be the limitation of limited study samples that we found no significant difference of the overall survival time about TNK2 protein expression (p = 0.37), while there was positive coefficient between TNK2 protein expression and differentiation degree in esophageal cancer. This finding was similar to the study of Binhui Xie et al., in hepatocellular carcinoma study. Therefore, TNK2 was a potential diagnostic marker in esophageal cancer. However, due to the defects of limited study samples, there was necessity to conduct extensive and large cohort studies of esophageal cancer patients to further ensure the potential diagnostic marker, TNK2. Taken together, the oncogene TNK2 potentially promoted esophageal cancer progression through activating EGFR-AKT signaling.

5 | CONCLUSION

TNK2 was a potential diagnostic marker, and it possibly promoted esophageal cancer progression through activating EGFR-AKT signaling.

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CONFLICT OF INTEREST

The authors declare that they have no competing interests.

AUTHORS’ CONTRIBUTIONS

Each author has made an important scientific contribution to the study and has assisted with the drafting or revising of the manuscript.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The research protocol was reviewed and approved by the Ethical Committee and Institutional Review Board of The First Affiliated Hospital of USTC, Division of life Sciences and Medicine, University of Science and Technology of China.

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**SUPPORTING INFORMATION**

Additional supporting information may be found online in the Supporting Information section.

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