Prognostic values and clinical relationship of TYK2 in laryngeal squamous cell cancer

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Abstract
Laryngeal squamous cell cancer (LSCC) is the second most common head and neck cancer with the increasing mortality. The tyrosine kinase 2 (TYK2) has previously been reported to play an important role in various cancers excepting LSCC. We used available data from the cancer genome atlas program (TCGA), gene expression omnibus, and gene expression profiling interactive analysis (GEPIA) to evaluate the role of TYK2 in LSCC.

The difference of TYK2 expression level between normal and tumor samples was analyzed based on TCGA, gene expression omnibus, and GEPIA databases. The relationship between clinical features and TYK2 were analyzed using the Wilcoxon signed-rank test. We applied Cox regression and the Kaplan–Meier method to finding which clinical characteristics is associated with overall survival. Also, we used GEPIA database to validate the relationship between TYK2 and overall survival. At last, we performed gene set enrichment analysis based on TCGA data set.

The expression level of TYK2 in LSCC was significantly associated with gender, lymph node status and metastasis (P-values <.05). Kaplan–Meier survival analysis, as same as GEPIA validation, demonstrated that LSCC with TYK2-low had a worse prognosis than that with TYK2-high. The univariate analysis showed that TYK2-high correlated significantly with a better overall survival (hazard ratio: 0.351, 95% confidence interval: 0.194–0.637, P < .001). The multivariate analysis revealed that TYK2 remained independently associated with overall survival (hazard ratio: 0.36, 95% confidence interval: 0.185–0.699, P = .003). Gene set enrichment analysis shows that Janus kinases–STAT signaling pathway, p53 signalling pathway and natural killer cell mediated cytotoxicity, etc are enriched in TYK2 high expression phenotype.

Gene TYK2 may be a potential prognostic molecular marker for LSCC. Moreover, the Janus kinases–STAT signaling pathway and p53 signalling pathway are probably the key pathway associated with TYK2 in LC.

Abbreviations: CI = confidence interval, Cx43 = Connexin43, GEO = gene expression omnibus, GEPIA = gene expression profiling interactive analysis, GSEA = gene set enrichment analysis, HR = hazard ratio, JAK = Janus kinases, LSCC = laryngeal laryngeal squamous cell cancer, NK = natural killer, TCGA = the cancer genome atlas program, TYK2 = tyrosine kinase 2.

Keywords: bioinformatic analysis, gene set enrichment analysis, laryngeal cancer, the cancer genome atlas program, tyrosine kinase 2

1. Introduction
Laryngeal cancer, in recent years, is the second most common head and neck cancer, and over 95% of histological types are laryngeal squamous cell cancer (LSCC). According to the Global Cancer Observatory report, there were a total of 177,442 new cases of laryngeal cancer, and about 10,000 patients dying of this disease in 2018. In 2020, an estimated 12,370 new cases of laryngeal cancer will be diagnosed in America, and there will be approximately 3750 patients dying of it. Admittedly, both the social–economic loss and the medical burden are enormous. Tobacco and alcohol consumption are generally accepted as the most significant risk factors for LSCC, and the function of the human papillomavirus is also, to some extent, involved in tumorigenesis. Approximately half of the patients have been at stage III or IV disease when first diagnosed. Although, there are, currently, several effective treatment in the management of LSCC, including surgery, radiation therapy, and chemotherapy, LSCC, unfortunately, is 1 of a few tumors in which the 5-year survival rate has decreased over the past 40 years, from 66% to 63%. Therefore, in order to improve the therapeutic effect and the survival rate of patients with LC, it’s necessary to proceed further research finding novel biomarkers for tumor detection with prognostic value.
The tyrosine kinase 2 (TYK2), located on human chromosome 19p13.2, is one of the protein coding genes. TYK2 plays important roles in various biological processes, including cytokines activation, growth factors response, immune, or inflammatory reaction, to name a few. It’s reported that TYK2-deficient patients are vulnerable to viral, fungal or bacterial infections. With further research, some previous studies declared that TYK2 is an essential molecule involved in tumour immunosurveillance. Notably, there is a possibility that TYK2 is, to some extent, implicated in the pathogenesis of cancer. Recently, the function of TYK2 in various cancers, such as prostate cancer, ovarian, breast tumor, ovarian tumor, and so on, has been widely reported. 

However, the correlation between TYK2 and the prognosis of LSCC has yet been reported. In this study, we performed bioinformatic analyses to identify the TYK2 expression between normal and tumor samples using high throughput RNA-sequencing data from gene expression omnibus (GEO) and the cancer genome atlas program (TCGA) databases. Also, the survival analysis were performed based on the TCGA profile. Therefore, the main aim of the present study was to evaluate the potential prognostic value and clinical correlation of TYK2 expression in LC. What’s more, gene set enrichment analysis (GSEA) was performed to gain further insight into the biological pathways involved in LSCC pathogenesis related TYK2-regulatory network.

2. Methods

2.1. Data acquisition and bioinformatic analysis

The data of microarray datasets GSE59102 from the GEO database were chosen for analysis (https://www.ncbi.nlm.nih.gov/geo/). There were 13 normal samples and 29 tumor samples in dataset GSE59102 (Last update date is Jan 23, 2019; Platform: GPL6480 Agilent-014850 Whole Human Genome Microarray 4x44K G4112F). In total, 12 normal samples and 111 LSCC samples were obtained from TCGA database. Notably, the data category was transcriptome profiling, while the data type was gene expression quantification. What’s more, experimental flow type was HTSeq - Counts. Also, clinical characteristic data including gender, age, tumor stage, etc were downloaded at the same time. We used R software (v.4.0.3) to observe whether the statistical difference (P-value <0.05) of the expression of TYK2 existed between the normal and tumor samples.

2.2. GEPIA validation and statistical analysis

Gene expression profiling interactive analysis (GEPIA) database (http://gepia.cancer-pku.cn/), a newly opening interactive web server for cancer and normal gene expression profiling and interactive analyses, included 9736 tumors and 8587 normal samples from TCGA and the GTEx projects. We further estimated the differences of TYK2 expression between normal and tumor tissue based on GEPIA database. What’s more, according to the median expression values of TYK2, we divided tumor samples into 2 groups (high expression of TYK2 and low expression of TYK2). The survival analysis of TYK2 was performed by Kaplan–Meier method and log-rank test. Also, the outcome of survival analysis would be verified in GEPIA database. The Wilcoxon signed-rank test were used to evaluate statistical differences between clinical pathologic features and TYK2. Univariate Cox regression analysis was applied to identify single factor of clinical characteristics that were strongly correlated with survival. Besides, we also implemented multivariate Cox regression analysis in order to oversee the impact of both TYK2 expression and other clinical characteristics on survival. All statistical analyses were performed based on R software (v.4.0.3). Furthermore, P-value <.05 was regarded as significance in all statistical analysis.

2.3. Gene set enrichment analysis (GSEA)

GSEA is a computational tool and method. We can use GSEA to investigate whether a priori defined set of genes shows statistically significant, accordant differences between 2 biological states. We applied GSEA software (version 3.0) to the analysis of functional enrichment. Firstly, genes are ranked in GSEA based on the correlation between their expression and the expression of TYK2. Subsequently, GSEA was carried out to identify significant signaling pathways between low and high TYK2 expression data sets. The annotated gene set files (c2.cp. kegg.v7.0.symbols.gmt and h.all.c2.v7.2.symbols.gmt) were as references. Gene set permutations were performed 1000 times for each analysis. The phenotype label was expression level of TYK2. What’s more, if the nominal P-value <.05 and the false discovery rate q-value <0.25, signaling pathway would be considered as statistically significant.

3. Results

3.1. TYK2 expression comparison and patient clinical characteristics

In order to explore whether the difference of the expression level of TYK2 existed between LSCC tissue and normal tissue, we employed GEO, TCGA, and GEPIA databases to analyze the expression level of it. As shown in Figure 1A, the expression of TYK2 in tumor tissue samples (n=13) was all significantly higher than normal tissue samples (n=29) in GSE59102 (P=1.683e–04). Significantly higher expression of TYK2 in TCGA dataset was observed in 111 LSCC patients than in 12 normal samples (P=1.365e–04, Fig. 1B). Also, we observed the same trend in GEPIA database (P < .01, Fig. 1C) between tumor samples (n=111) and normal samples (n=12). What’s more, clinical characteristics of 111 LSCC patients from TCGA were exhibited in Table 1.

3.2. Survival outcomes and Cox analysis

As exhibited in Figure 2A, the result of Kaplan–Meier survival analysis indicated that tumor tissue with low expression of TYK2 was considerably associated with a worse overall survival (P < .0001). Similarly, the survival analysis in GEPIA database also shown that low expression of TYK2 in tumor tissues had a worse overall survival than TYK2-high expression (P=.00027, Fig. 2B). In the univariate analysis, 5 factors including TYK2 expression level (hazard ratio [HR]: 0.331, 95% confidence interval [CI]: 0.194–0.637, P < .001), gender (HR: 0.297 95% CI: 0.151–0.586, P < .001), tumor stage (HR: 1.451, 95% CI: 1.363–1.656, P =.044), lymph node status (HR: 1.894, 95% CI: 1.060–3.383, P=.038) and metastasis (HR: 4.86, 95% CI: 1.670–14.146, P=.004) exerted significant influence over
survival. In the multivariate analysis, TYK2 expression (HR: 0.36, 95% CI: 0.185–0.699, \(P = .003\)) remained associated with overall survival, along with gender (HR: 0.508, 95% CI: 0.279–0.714, \(P = .009\)), lymph node status (HR: 1.996, 95% CI: 1.013–3.935, \(P = .046\)) and metastasis (HR: 2.147, 95% CI: 1.632–7.292, \(P = .021\)). The detailed information of Cox analysis was shown in Table 2.

### 3.3. Association with TYK2 expression and clinicopathologic features in LSCC patients

On the one hand, as shown in Figures S1–S6, Supplemental Digital Content, http://links.lww.com/MD2/A356, http://links.lww.com/MD2/A357, http://links.lww.com/MD2/A358, http://links.lww.com/MD2/A359, http://links.lww.com/MD2/A360, http://links.lww.com/MD2/A361, age, AJCC stage, tumor stage, race, alcohol history, and smoking history were not significantly different in TYK2 expression (\(P > .05\)). On the other hand, the expression level of TYK2 was significantly associated with gender (Fig. 3A), lymph node status (Fig. 3B) and metastasis (Fig. 3C). Increased expression of TYK2 significantly correlated with low lymph node status (\(P = .036\)), no-metastasis (\(P = .031\)) and male (\(P = .026\)).

### 3.4. TYK2-related potential signaling pathways based on GSEA

GSEA was conducted to evaluate the potential biological mechanism related to TYK2 expression. The most significantly enriched signaling pathways were identified according to their normalized enrichment score (NES). As delineated in Figure 4, the GSEA indicated that expression of TYK2 was related to “Janus kinases (JAK)–STAT signaling pathway” (Fig. 4A), “p53 signalling pathway” (Fig. 4B), “natural killer (NK) cell mediated cytotoxicity” (Fig. 4C), “pyrimidine metabolism” (Fig. 4D), “DNA replication” (Fig. 4E) and “homologous recombination” (Fig. 4F). The detailed information of signaling pathways were shown in Table 3.

### 4. Discussion

LSCC is the second most common head and neck cancer with the increasing mortality in the United States.\(^7\) As a result, it’s
Table 2

Univariate and multivariable Cox regression analysis on overall survival.

<table>
<thead>
<tr>
<th>Variables</th>
<th>HR</th>
<th>95% CI</th>
<th>P-value</th>
<th>HR</th>
<th>95% CI</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>TYK2 (high vs low)</td>
<td>0.351</td>
<td>0.194 to 0.637</td>
<td>&lt;.001</td>
<td>0.36</td>
<td>0.185 to 0.699</td>
<td>.003</td>
</tr>
<tr>
<td>Age (&gt;60 vs ≤60)</td>
<td>0.806</td>
<td>0.454 to 1.429</td>
<td>.46</td>
<td>0.774</td>
<td>0.416 to 1.441</td>
<td>.419</td>
</tr>
<tr>
<td>Gender (male vs female)</td>
<td>0.297</td>
<td>0.151 to 0.586</td>
<td>&lt;.001</td>
<td>0.508</td>
<td>0.279 to 0.714</td>
<td>.009</td>
</tr>
<tr>
<td>AJCC stage (III–IV vs I–II)</td>
<td>0.782</td>
<td>0.366 to 1.672</td>
<td>.057</td>
<td>0.823</td>
<td>0.192 to 3.524</td>
<td>.793</td>
</tr>
<tr>
<td>Tumor stage (T3–4 vs T1–2)</td>
<td>1.451</td>
<td>1.363 to 1.656</td>
<td>.044</td>
<td>1.017</td>
<td>0.292 to 3.541</td>
<td>.978</td>
</tr>
<tr>
<td>Lymph node status (N2–3 vs N0–1)</td>
<td>1.894</td>
<td>1.060 to 3.383</td>
<td>.038</td>
<td>1.996</td>
<td>1.013 to 3.935</td>
<td>.046</td>
</tr>
<tr>
<td>Metastasis (M1 vs M0)</td>
<td>4.86</td>
<td>1.670 to 14.146</td>
<td>.004</td>
<td>2.147</td>
<td>1.632 to 7.922</td>
<td>.021</td>
</tr>
<tr>
<td>Race (White vs no White)</td>
<td>1.421</td>
<td>0.748 to 2.699</td>
<td>.284</td>
<td>0.587</td>
<td>0.286 to 1.203</td>
<td>.146</td>
</tr>
<tr>
<td>Alcohol history (no vs yes)</td>
<td>1.497</td>
<td>0.846 to 2.650</td>
<td>.166</td>
<td>1.273</td>
<td>0.670 to 2.421</td>
<td>.461</td>
</tr>
<tr>
<td>Smoking history (no vs yes)</td>
<td>1.061</td>
<td>0.588 to 1.912</td>
<td>.166</td>
<td>1.134</td>
<td>0.604 to 2.129</td>
<td>.695</td>
</tr>
</tbody>
</table>

CI = confidence interval, HR = hazard ratio, TYK2 = tyrosine kinase 2.

Figure 2. (A) Impact of TYK2 expression on overall survival in LSCC patients in TCGA cohort. (B) Impact of TYK2 expression on overall survival in GEPIA database. GEPIA = gene expression profiling interactive analysis, LSCC = laryngeal laryngeal squamous cell cancer, TCGA = the cancer genome atlas program, TYK2 = tyrosine kinase 2.

Figure 3. Association with TYK2 expression and clinical characteristics. (A) Gender. (B) Lymph node status. (C) Metastasis. TYK2 = tyrosine kinase 2.
Figure 4. Enrichment plots from GSEA. (A) JAK-STAT signaling pathway. (B) P53 signaling pathway. (C) Natural killer cell mediated cytotoxicity. (D) Pyrimidine metabolism. (E) DNA replication. (F) Homologous recombination. GSEA = gene set enrichment analysis, JAK = Janus kinases.
necessary to find new biomarkers benefiting the diagnosis, treatment and prognosis assessment. To our knowledge, the function of TYK2 and its potential prognostic impact on LSCC has not yet been reported. The gene TYK2 is located on chromosome 19p13.2. The protein TYK2 belongs to the family of JAK, which structurally has 4 functional domains—FERM-domain, SH2-like domain, JH1 domain, and JH2 domain. N-terminal FERM and SH2 domains, the important mediators in peptide interactions, are able to facilitate JAK for connecting with cytokine receptors. JH1 domain is a tyrosine kinase and JH2 is regarded as a kinase-like or pseudokinase which could inhibit the kinase activity of JH1. Notably, TYK2 is closely related to a variety of cytokines and immune cells. In the studies of TYK2 knockout mice, TYK2-deficient mice become more susceptible to infection, which can be explained by impaired Th1 and Th17 development. On the contrary, low level TYK2 led to enhanced lung inflammation because of enhanced Th2 response and highest IL-4 level. What’s more, TYK2 plays critical role not only in effector Th cell signaling but also in NK cells and dendritic cells. The presence of TYK2 is necessary for dendritic cells to prime CD8+ T cells generating IFN-γ. And NK cells would abrogate Th1 differentiation and IFN-c generation in the absence of TYK2. Besides, previous research have established that TYK2 inhibitors have shown exciting preclinical efficacy in various autoimmune diseases, such as psoriasis, lupus, and inflammatory bowel disease.

To date, the expression and functions of TYK2 associated with or causative for tumorigenesis, including prostate, ovarian, cervical, and breast cancer, to name a few, have attracted more and more attention and is actively investigated. Accounts by many researchers of the ability of TYK2 to regulate invasiveness and metastasis of cancer cells have been widely reported. High levels of TYK2 showed a positive correlation with the invasion and metastasis of prostate cancer via gastrin-releasing peptide receptor. Also, TYK2 is involved in the urokinase-type plasminogen activator receptor system, which is central for tumor cell migration and metastasis. Strangely, it’s relatively lower TYK2 level in tumor samples that is regarded as an undesirable prognostic marker. It has been previously shown that normal or higher expression level of TYK2 is significantly associated with longer survival according to a meta-analysis about hepatocellular cancer patients. Consistent with this, our result also demonstrate that tumor tissue with low expression of TYK2 was considerably associated with a worse overall survival. The underlying mechanisms for these conflicting reports is still equivocal. One possible explanation for this observation is that TYK2 serves the function of inhibiting or activating BCL-2 family members which is able to prevent tumor from cell death. What’s more, the study led by Li et al demonstrated that there was a dual role of TYK2 in regulation of Connexin43 (Cx43) with both pro- and antitumorigenic function: on the 1 hand, TYK2 is capable of decreasing Cx43 stability via phosphorylating; on the other hand, TYK2 can increase Cx43 levels in a STAT3-dependent manner.

Admittedly, the above findings provide constructive references for further research of the mechanism of TYK2 in LSCC.

Our study pointed to the fact that the expression of TYK2 in LSCC not only probably correlated with tumorigenesis, but also, to some extent, could predict prognosis. Consequently, we can boldly speculate that TYK2 can contribute to the pathogenesis and metastasis in LSCC. To further investigate the functions of TYK2 in LSCC, GSEA was implemented via using TCGA data, GSEA showed that JAK–STAT signaling pathway, and P53 signaling pathway are differentially enriched in TYK2 high expression phenotype.

Both JAK–STAT signaling pathway and P53 signaling pathway are famous pathways associated with tumor. Originally, Leonard et al suggested that constitutive activation of JAKs and STATs was highly associated with malignancy. Subsequently, the JAK–STAT pathway is gradually regarded as one of the most important cancer pathways and directly contributes to tumorigenesis, progression, invasion, and metastasis. Various cancers, such as lung cancer, oral cancer, and pancreatic cancer, are closely related to JAK–STAT signaling pathway. Furthermore, P53 signaling pathway also has notable effect in tumorigenesis. Previous studies have demonstrated that p53 overexpression was obviously associated with LSCC as well as head and neck squamous cell carcinoma in immunohistochemical analysis. Recent study additionally reported that Lupeol can serve the function of regulating neoplastic growth and apoptosis in laryngeal cancer based on the antitumor effect mediated by p53. The aforementioned discoveries does shed a light on further study of mechanism of TYK2 in LSCC.

Additionally, this study has compelling limitation. Inevitably, a limitation of this analysis is that the sample size was relatively inadequate. No experimental study was conducted by us to explore the potential carcinogenic mechanism of TYK2 in the development of LSCC. Admittedly, additional studies are needed in order to explore the accurate functional mechanisms of TYK2 in LSCC. What’s more, the information acquired from all databases was limited, therefore improvement of the databases will lead to varied and credible outcomes.

5. Conclusion

In conclusion, this work postulated that TYK2 is probably a good prognostic factor in LSCC patients. The expression level of TYK2 decreased with the progression of tumor. Besides, JAK–STAT
signaling pathway and p53 signaling pathway might be the key pathway associated with TYK2 in LSCC. Admittedly, it’s necessary to perform further experimental validation including the molecular mechanism and deeper genomic research to prove the biological impact of TYK2.

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Author contributions

Data curation: Lucheng Fang, Qinjuan Chen.
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Project administration: Lucheng Fang, Licai Shi, Xingwang Rao.
Resources: Xingwang Rao.
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Visualization: Qinjuan Chen.
Writing – original draft: Wen Wang, Licai Shi.
Writing – review & editing: Lucheng Fang, Licai Shi, Qinjuan Chen, Xingwang Rao.

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