**To the Editor:** Esophageal squamous cell carcinoma (ESCC) is one of the most aggressive cancer types and places a heavy burden on human health. The early diagnosis and prognosis monitoring of ESCC is important for therapy. Despite recent progress in treatment regimens for ESCC, the prognosis of ESCC remains poor.[1]

Therefore, it is important to find new molecular therapeutic targets and prognostic monitoring biomarkers for ESCC patients. In this study, we aimed to explore new prognostic biomarkers for ESCC.

Based on the two public Illumina HiSeq platform RNA sequencing datasets downloaded from the Gene Expression Omnibus (GEO) database (https://www.ncbi.nlm.nih.gov/geo/), we collected the expression data of 33 ESCC tissues and 33 adjacent normal tissues from GSE130078 and GSE149609 to investigate the protein-coding differentially expressed genes (DEGs), using DESeq2 (http://www.bioconductor.org/packages/release/bioc/html/DESeq2.html) under the threshold of \[\log_{2} \text{Fold-Change} \geq 1\] and the adjusted \(P\) value of \(\leq 0.05\). As a result, a total of 3885 and 3477 DEGs were identified in dataset GSE130078 and GSE149609, respectively. Then, a total of 1914 overlapping DEGs in these two datasets were detected. To explore the interactive relationships among these DEGs, protein-protein interaction (PPI) network analysis was performed for the 1914 DEGs using STRING database (http://string-db.org/cgi/input.pl). Then, Cytoscape (version 3.5.1, https://cytoscape.org/) was used to construct the PPI network, and the Molecular Complex Detection (MCODE) app in Cytoscape was used to analyze the modules in the PPI network (degree cutoff = 2, max. depth = 100, k-core = 2, and node score cutoff = 0.200). The module criteria were a MCODE score \(>20\) and the number of nodes \(>10\). Consequently, the first module (MCODE score = 29.156), which included 46 hub genes, was chosen. Specifically, the node degree of Fc fragment of immunoglobulin G receptor Ila (FCGR2A) was 19.863, which indicated that FCGR2A might play an important role in maintaining the structure and function in the ESCC PPI network. In addition, Kyoto Encyclopedia of Genes and Genomes pathway results for the 46 hub genes indicated that they were mainly enriched in cytokine–cytokine receptor interactions, the interleukin (IL)-17 signaling pathway, and the chemokine signaling pathway. These pathways have been reported to be correlated with the cancer survival or prognosis, which suggested that these 46 hub genes might be linked to ESCC prognosis.

Afterward, the expression of the 46 hub genes was validated with 182 ESCC tissues and 286 adjacent tissues from The Cancer Genome Atlas (TCGA) and Genotype Tissue Expression (GTEx) databases. As a result, only 23 genes, including FCGR2A (Figure 1A), were found to be significantly and highly expressed in ESCC tissues compared with normal tissues [Supplementary Figure 1, http://links.lww.com/CM9/A768]. Then, we assessed the effect of the 23 hub genes on disease-free survival (DFS) in ESCC based on TCGA data using Gene Expression Profiling Interactive Analysis (GEPIA) (http://geopia.cancer-pku.cn/index.html). Cox proportional hazards regression models with estimations of hazard ratios (HR) and
95% confidence intervals (95% CI) were used to evaluate the impact of genes on DFS, and hub genes that were associated with significantly worse DFS (P < 0.05) were selected. The Kaplan–Meier curve with log-rank test results [Figure 1B] revealed that ESCC patients with higher mRNA expression levels of FCGR2A (HR: 1.700, 95% CI: 1.110–7.605, P = 0.032), fibronectin 1 (FN1) (HR: 2.000, 95% CI: 1.663–5.368, P = 0.007) and secreted phosphoprotein 1 (SPP1) (HR: 2.300, 95% CI: 2.150–3.796, P = 0.001) had significantly lower DFS [Supplementary Figure 2, http://links.lww.com/CM9/A768]. In addition, the expression of the three significant hub genes
in ESCC at different stages was analyzed based on one-way analysis of variance test. Consequently, compared with stage I, FCGR2A (F = 2.980, P = 0.033) [Figure 1C], FN1 (F = 4.840, P = 0.003) and SPP1 (F = 5.710, P < 0.001) [Supplementary Figure 2, http://links.lww.com/CM9/A768] were found to be more significantly expressed in the advanced clinical stages of ESCC (stage II, III, and IV). These results demonstrated that overexpression of the three genes was associated with the poor prognosis in ESCC.

More importantly, the Gene Ontology analysis of FCGR2A was analyzed using KEGG Orthology Based Annotation System (KOBAS) (http://kobas.cbi.pku.edu.cn/kobas3) with a corrected P value of <0.05. Results indicated that FCGR2A may be associated with the immune response in ESCC. To explore its potential correlation with the immune environment in ESCC, the relationships between FCGR2A and tumor-infiltrating immune cells (TIICs) were determined using the Tumor Immune Estimation Resource (https://cistrome.shinyapps.io/timer/) database because some TIICs have been reported to predict poor prognosis in ESCC patients. Our results [Figure 1D] revealed that the expression of FCGR2A was most positively correlated with the infiltration level of macrophages in ESCC (R = 0.656, P < 0.001), while it had lower correlations with the level of tumor purity and infiltrating B cells, CD8+ T cells, CD4+ T cells, neutrophils, and dendritic cells (R < 0.500). Specifically, we focused on the correlation between FCGR2A and macrophages. Macrophages have two different phenotypes, the M1 phenotype, which is tumor suppressive, and the M2 phenotype, which is tumor supportive. Furthermore, tumor-associated macrophages (TAMs) differentiate into the M2 phenotype and contribute to progression of the disease. An increase in the number of infiltrating CD204+ TAMs has been reported to correlate with the poor prognosis in ESCC. To detect the relationship between FCGR2A and diverse immune infiltrating cells, we also analyzed correlations between FCGR2A and the gene markers of various immune cells in ESCC. Regarding the correlation between FCGR2A and macrophages, the expression of FCGR2A was significantly and positively correlated with the expression of CD163 molecule (CD163) (R = 0.831, P < 0.001), V-set and immunoglobulin domain-containing 4 (R = 0.817, P < 0.001) and membrane spanning 4-domains A4A (R = 0.849, P < 0.001), which are markers of M2 macrophages [Figure 1E]. However, FCGR2A was negligibly associated with nitric oxide synthase 2, interferon regulatory factor 5, and prostaglandin-endoperoxide synthase 2 in M1 macrophages [Figure 1F]. In addition, the expression of FCGR2A was significantly and positively correlated with the expression of C-C motif chemokine ligand 2 (R = 0.609, P < 0.001), CD68 molecule (R = 0.433, P < 0.001), and IL-10 (R = 0.567, P < 0.001), which are markers of TAMs [Figure 1G]. The expression of FCGR2A was also significantly and positively correlated with the expression of CD204 molecule (R = 0.855, P < 0.001), which is a TAMs associated marker [Figure 1H]. These results showed that FCGR2A was correlated with immune infiltrates in ESCC and significantly positively correlated with M2 macrophages and TAMs.

In the present study, we identified 1914 DEGs and 46 hub genes in ESCC using ESCC cohorts from GEO and we validated the 46 hub genes with ESCC cohorts from TCGA and GTEx databases. Among them, a higher expression level of FCGR2A had a significantly lower DFS. Moreover, the expression of FCGR2A was significantly and positively correlated with the expression of both CD163 and CD204. Strikingly, the elevated number of infiltrating CD204+ TAMs and CD163-positive M2 macrophages were reported to be correlated with the poor prognosis in ESCC, which indicated that overexpression of FCGR2A might be associated with a poor prognosis and correlated with immune infiltrates in ESCC. To the best of our knowledge, the present study is the first to explore the association between FCGR2A and TIICs and the prognostic value of FCGR2A in ESCC via bioinformatics methods. However, our bioinformatics results should be confirmed by biological experiments in the future. Despite its limitations, our findings provide direct evidence for research on prognostic biomarkers of ESCC. In conclusion, FCGR2A may be a novel potential prognostic biomarker of ESCC.

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Conflicts of interest

None.

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