Fc fragment of immunoglobulin G receptor IIa (*FCGR2A*) as a new potential prognostic biomarker of esophageal squamous cell carcinoma

Sifen Lu[1], Nan Li[2], Zhiqiang Peng[3], Zuhong Lu[4], Xingyue Tu[5], Wengeng Zhang[1], Yulin Kang[5]

[1]Precision Medicine Key Laboratory of Sichuan Province and Precision Medicine Center, West China Hospital, Sichuan University, Chengdu, Sichuan 610041, China;
[2]Department of Information Center and National Clinical Research Center for Geriatrics, West China Hospital, Sichuan University, Chengdu, Sichuan 610041, China;
[3]State Key Laboratory of Proteomics, National Center for Protein Sciences (Beijing), Beijing Institute of Lifeomics, Beijing 100850, China;
[4]State Key Lab of Bioelectronics, National Demonstration Center for Experimental Biomedical Engineering Education, School of Biological Science and Medical Engineering, Southeast University, Nanjing, Jiangsu 210096, China;
[5]Institute of Environmental Information, Chinese Research Academy of Environmental Sciences, Beijing 100012, China.

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 ≤ 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 IIa (*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://gepia.cancer-pku.cn/index.html). Cox proportional hazards regression models with estimations of hazard ratios (HR) and

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Correspondence to: Dr. Yulin Kang, Institute of Environmental Information, Chinese Research Academy of Environmental Sciences, Beijing 100012, China
E-Mail: kangyulin@craes.org.cn
Dr. Wengeng Zhang, Precision Medicine Key Laboratory of Sichuan Province and Precision Medicine Center, West China Hospital, Sichuan University, Chengdu, Sichuan 610041, China
E-Mail: wengeng.zhang@wchscu.cn

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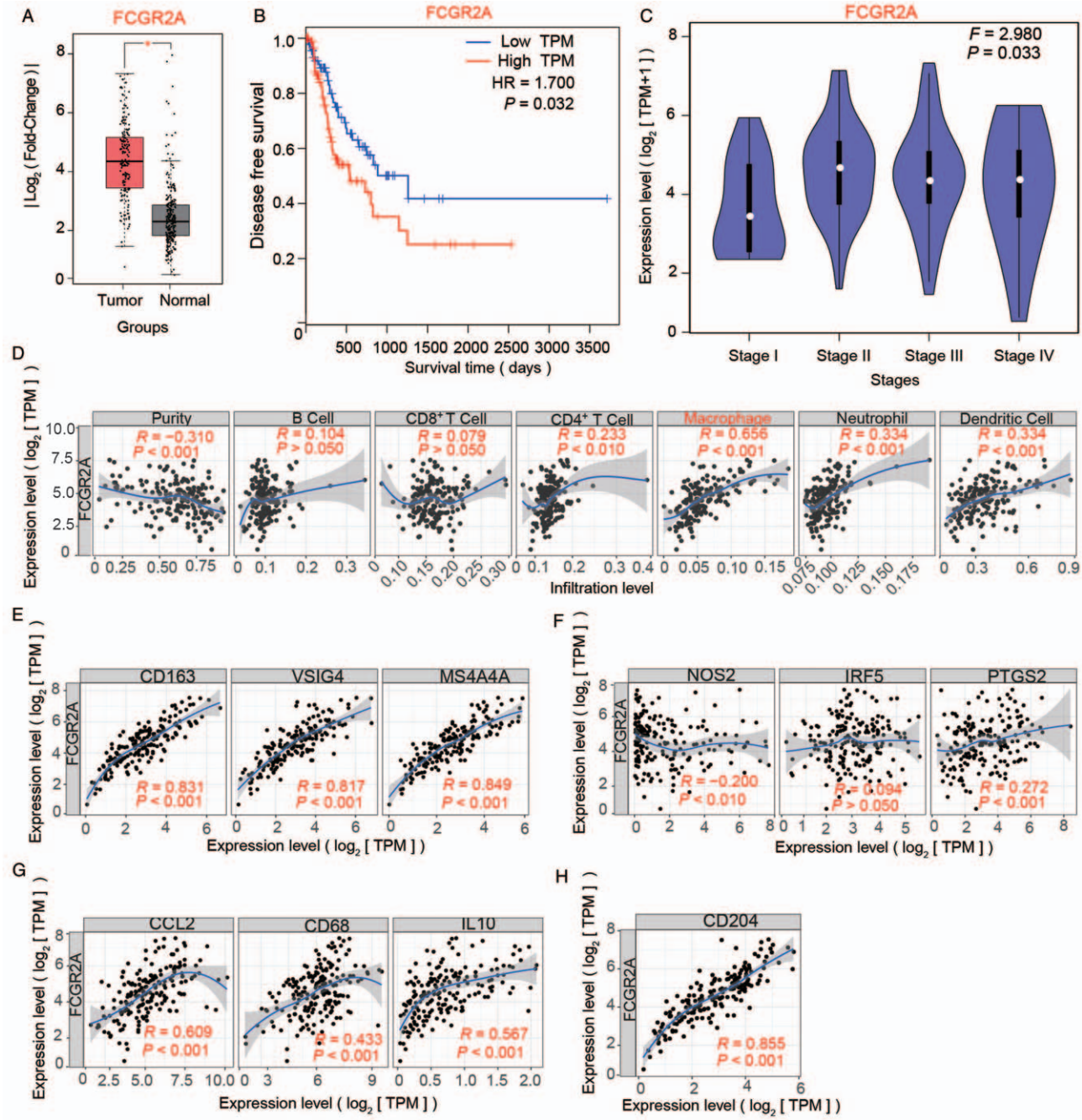

Figure 1: *FCGR2A* as a potential prognostic biomarker of ESCC. (A) The expression level of *FCGR2A* in tumor tissues ($n = 182$) and normal tissues ($n = 286$) in ESCC from TCGA and GTEx databases. ESCC tissues and normal tissues are shown in red and black, respectively. $^{*}P < 0.05$. (B) An increased expression level of *FCGR2A* was associated with a poor DFS in ESCC. The samples with gene highly expressed or gene lowly expressed are shown in red and blue, respectively. The *P* value was verified by the log-rank test. (C) *FCGR2A* expression varied significantly in different clinical stages of ESCC ($P < 0.05$). The *F* value and *P* value were calculated by one-way ANOVA test. (D) Spearman correlations between *FCGR2A* expression and infiltration level of TIICs. (E–G) Correlations between the expression of *FCGR2A* and the gene markers in each subtype of macrophages, including (E) M2 macrophages, (F) M1 macrophages, and (G) TAMs. (H) Correlation between the expression of *FCGR2A* and TAMs associated marker CD204. ANOVA: Analysis of variance; CCL2: C-C motif chemokine ligand 2; CD: Cluster of differentiation; DFS: Disease-free survival; ESCC: Esophageal squamous cell carcinoma; *FCGR2A*: Fc fragment of immunoglobulin G receptor IIa; GTEx: Genotype Tissue Expression; HR: Hazard ratio; IL10: Interleukin 10; IRF5: Interferon regulatory factor 5; MS4A4A: Membrane spanning 4-domains A4A; NOS2: Nitric oxide synthase 2; PTGS2: Prostaglandin-endoperoxide synthase 2; TAMs: Tumor-associated macrophages; TCGA: The Cancer Genome Atlas; TIIC: Tumor-infiltrating immune cells; TPM: Transcripts per million; VSIG4: V-set and immunoglobulin domain-containing 4.

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.[2,3] 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.[4] 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 be correlated with the poor prognosis in ESCC.[3] 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,[2,3] 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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