High SIRPA Expression Predicts Poor Prognosis and Correlates with Immune Infiltrates in Patients with Esophageal Carcinoma

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Background. Signal regulatory protein alpha (SIRPA) is an inhibitory receptor expressed in macrophages and a potential therapeutic target in cancers. This study aims to investigate the functional role of SIRPA in esophageal carcinoma (ESCA).

Methods. Based on the Oncomine and The Cancer Genome Atlas (TCGA) database, SIRPA expression and clinical value were determined. Gene set enrichment analysis (GSEA) was performed to predict the mechanism underlying the oncogene role of SIRPA. Spearman’s correlation analysis was used to analyze the effects of SIRPA on the molecular relationship and immune landscape.

Results. SIRPA was highly expressed across Oncomine and TCGA databases and correlated with poor overall survival and disease-specific survival. There was an expression difference among clinical characteristics. Functional annotation showed that cancer-related biological function and pathways were enriched in the high SIRPA expression group. Besides, SIRPA strongly and extensively affected the immune infiltrates.

Conclusion. SIRPA might be an oncogene and a target of immunotherapy in ESCA.

1. Introduction

Esophageal carcinoma (ESCA) is the eighth most common malignant and the sixth most deadly tumor in the world [1]. It is also one of the common malignant tumors of the digestive tract in China, with the main histological types being squamous carcinoma and adenocarcinoma [2]. ESCA has a poor prognosis, with a 5-year survival rate of less than 30% [3]. Standardized surgery is the main treatment for ESCA, but for locally advanced patients, surgery alone often fails to achieve radical results and becomes a potential risk factor for local recurrence and distant metastases [4]. In recent years, research on radiotherapy and targeted therapy for ESCA has been carried out, and some patients have benefited from the integrated treatment [5]. However, about 70% of patients are already advanced at the time of consultation, and 40% still experience local recurrence after receiving concurrent radiotherapy, making treatment for these patients particularly challenging [6]. Active clinical research to identify effective targets and develop effective drugs to control tumor cell infiltration and metastasis is the key to improving the prognosis of ESCA patients.

Signal regulatory protein-α (SIRPa) is the first member of the SIRP family to be identified and is mainly expressed on the membrane surface of myeloid cells and neuronal cells but also, to a lesser extent, in smooth muscle cells and vascular endothelial cells [7]. The extracellular region of SIRPa contains three immunoglobulin superfamily domains and multiple glycosylation sites, while the cytoplasmic region is highly conserved and has immunosuppressive motifs rich in tyrosine residues [8]. SIRPa can be phosphorylated by protein kinases such as Src and binds to proteins containing the SH2 domain such as tyrosine phosphatases SHP1 and SHP2, thereby inhibiting protein tyrosine kinase (PTK) dependent cell activation and suppressing immune cell activation [9]. A study conducted by Kaur et al. showed that...
2. Methods

2.1. Data Collection. SIRPA expression in ESCA was determined in the Oncomine database (https://www.oncomine.org) [12] with the thresholds of the p value of 0.05, all fold change and all gene rank. RNAseq data in the transcripts per million reads (TPM) format processed with Toil software [13] were downloaded from UCSC Xena (https://xenabrowser.net/ datapages/) and log2 transformed. A total of 182 ESCA cases from TCGA dataset and 666 corresponding normal cases from the GTEx dataset were extracted. All data used were from Oncomine, TCGA, and GTEx, and therefore, this study did not require ethical approval or informed consent.

2.2. Clinical Significance of SIRPA in ESCA. In addition to level-3 HTSeq-FPKM data from the TCGA ESCA project, prognostic data from a study conducted by Liu et al. [14] were also used. Normal samples were removed, and samples with clinical information were retained. The diagnostic and prognostic value of SIRPA for ESCA was tested by the pROC package (version 1.17.0.1), survivor package (version 3.2-10), and survminer package (version 0.4.9).

2.3. Differential Expression Analysis. SIRPA median expression values were used to divide the ESCA samples into the SIRPAhigh group and SIRPALow group. The DESeq2 package (version 1.26.0) [15] was used to identify DEGs between the two groups. The expression of protein-coding genes was visualized by the volcano plot using the ggplot2 package (version 3.3.3), with genes meeting LogFC≥1 and p value <0.05 as upregulated genes and genes meeting LogFC≤−1 and p value <0.05 as downregulated genes.

2.4. Gene Set Enrichment Analysis (GSEA). GSEA [16] was performed using the clusterProfiler package (version 3.14.3) [17] to annotate differential genes between the SIRPAhigh group and SIRPALow group, providing us another option to screen out significant differential biological functions derived after bariatric surgery. The gene set arrangement was performed 1000 times per analysis. Gene sets were considered to be significantly enriched with an alpha or p value <5% and a false discovery rate (FDR) <25%. Datasets with false discovery rate (FDR) <0.25 and p. adjust <0.05 were considered significantly enriched.

2.5. SIRPA-Correlated Gene Screening. SIRPA-correlated lncRNA and protein-coding genes were analyzed using the stat package (version 3.6.3) and visualized as heatmaps using the ggplot2 (version 3.3.3) package. Under Spearman’s analysis, genes with correlation coefficient ≥0.5 and p value <0.05 were screened as positively correlated genes, and genes with correlation coefficient ≤−0.5 and p value <0.05 were screened as negatively correlated genes. Then, the SIRPA-correlated genes were imported into Cytoscape software (version 3.8.0) [18] to construct coexpression networks, with negatively correlated genes in green, positively correlated genes in red, lncRNAs in diamond, and protein-coding genes in ellipse.

2.6. Immune Infiltration Analysis. The tumor purity, immune score, and stromal score were calculated for all samples using the estimate package (version 1.0.13) [19]. In addition, the infiltration of 24 common immune cells was assessed using the built-in algorithm ssGSEA in the R package GSVA [20]. Spearman’s correlation analysis was used to investigate the correlation between SIRPA and immune cell infiltration and to compare the level of immune cell infiltration between the SIRPAhigh group and SIRPALow group by Wilcoxon rank-sum test. Furthermore, the correlation between SIRPA and macrophage markers [21] was calculated by the Spearman method to assess macrophage polarization. In addition, correlations between SIRPA and immunomodulators, chemokines, chemokinereceptors, and immune checkpoints were also analyzed.

2.7. Statistical Analyses. All statistical analyses and plots were performed using R (version 3.6.3), except for Oncomine analysis and Cytoscape. Both chi-square tests and Fisher’s exact test were used for the analysis of clinical information. Statistical significance was defined as ns, * p <0.05, ** p <0.01, and *** p <0.001.

3. Results

3.1. Elevated Expression of SIRPA in ESCA. From the Oncomine database, we found that SIRPA was highly expressed in ESCA (Figure 1(a) and Table 1). Through integrating TCGA and GTEx database, we observed the overexpression of SIRPA again (Figure 1(b)). Moreover, ROC curve showed that there was some accuracy in the diagnostic value of SIRPA in predicting tumor and normal outcomes. Besides, high SIRPA expression correlated with poor overall survival (Figure 1(d)) and disease-specific survival (Figure 1(e)). It was indicated that SIRPA was an oncogene in ESCA.

3.2. SIRPA Expression Associated with Clinicopathologic Variables. To further investigate whether SIRPA remains prognostically relevant in different ESCA patients, we stratified the ESCA patients according to different clinical information. As shown in Figure 2, SIRPA was also an important prognostic gene. Based on the case classification, we analyzed SIRPA expression (Figure 3). The results...
showed that SIRPA expression was distinct among ESCA patients with different clinicopathologic characteristics. It suggested that higher SIRPA expression in ESCA patients correlated with poor survival.

3.3. The Impact of SIRPA in ESCA. To clarify the role of SIRPA in ESCA, we divided the ESCA samples into high and low SIRPA groups (Figure 4(a)). Then, we performed the GSEA and obtained 120 enriched biological functions as well as 354 enriched pathways. It depicted that SIRPA might inhibit macrophage activation, promote epithelial cell proliferation (Figure 5(a)), and promote known procancer pathways, such as PI3K/AKT and Wnt signaling pathways (Figure 5(b)). In terms of genes affected by SIRPA, we analyzed the SIRPA-correlated genes and presented the top 10 correlated genes in Figure 4(b). Then, the correlated genes were imported into Cytoscape for visualization (Figure 4(c)).

3.4. Correlation between SIRPA Expression and Immune Infiltration. Considering that SIRPA was reportedly involved in a macrophage checkpoint pathway [22], we analyzed the association of SIRPA and immune infiltration. As
Figure 2: Kaplan–Meier survival curves comparing the high and low expression of SIRPA in ESCA patients with clinicopathologic characteristics.
Figure 3: Association of SIRPA expression with clinicopathologic characteristics. (a) T stage. (b) Radiation therapy. (c) Race. (d) Weight. (e) BMI. (f) Reflux history. (g) Columnar mucosa dysplasia. (h) Columnar metaplasia. (i) Barrett’s esophagus. (j) Pathologic stage. (k) Histological stage. (l) Tumor central location. * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$. 
Figure 4: SIRPA-coexpressed genes’ screening and network construction. (a) Expression change between SIRPA\textsuperscript{high} and SIRPA\textsuperscript{low} groups shown in the volcano plot. (b) Expression profile of top 10 correlated genes shown in the heatmap. (c) Coexpression network of SIRPA-coexpressed lncRNAs and protein-coding genes, with negatively correlated genes in green, positively correlated genes in red, lncRNAs in diamond, and protein-coding genes in ellipse. *** $p < 0.001$. 

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\caption{SIRPA-coexpressed genes’ screening and network construction. (a) Expression change between SIRPA\textsuperscript{high} and SIRPA\textsuperscript{low} groups shown in the volcano plot. (b) Expression profile of top 10 correlated genes shown in the heatmap. (c) Coexpression network of SIRPA-coexpressed lncRNAs and protein-coding genes, with negatively correlated genes in green, positively correlated genes in red, lncRNAs in diamond, and protein-coding genes in ellipse. *** $p < 0.001$.} 
\end{figure}
Figure 5: Enrichment plots from GSEA showing enriched biological process and pathway. (a) Enriched biological function. (b) Enriched pathways.
Figure 6: Continued.
shown in Figures 6(a)–6(c), SIRPA was positively correlated with StromalScore, ImmuneScore, and ESTIMATEScore, respectively. And between the SIRPA<sup>high</sup> group and SIRPA<sup>low</sup> group, StromalScore and ESTIMATEScore were distinct (Figure 6(d)). Besides, 24 immune cell infiltration levels were assessed in ESCA samples (Figures 6(e) and 6(f)). Interestingly, SIRPA was positively correlated with M2 macrophage markers, including CD163, VSIG4, and MS4A4A, which suggested that SIRPA could be correlated with M2 macrophage polarization (Figure 6(g)). Furthermore, the role of SIRPA in immune landscape was explored. Spearman’s correlation analysis showed that SIRPA was correlated with many immune checkpoints (Figure 7(a)), immunostimulators (Figure 7(b)), immunoinhibitors (Figure 7(c)), MHC (Figure 7(d)), chemokines (Figure 8(a)), and chemokine receptors (Figure 8(b)).

### 4. Discussion

In this study, SIRPA was found to be highly expressed in ESCA tissues compared to corresponding normal esophagus tissues. Clinically, SIRPA possessed certain diagnostic and prognostic value, analyzed by the ROC curve and survival curve. Besides, among different patients of various clinical characteristics, SIRPA was also prognostically relevant and expressed distinctly. Functional annotation by GSEA demonstrated that SIRPA might affect tumor growth and the immune cell. This assumption was further supported by Spearman’s correlation analysis on SIRPA expression and immune score, 24 immune cell infiltration levels, and immune-related markers. Moreover, SIRPA was also correlated with three reported M2 macrophage markers. These results implied that SIRPA should be considered as a potential therapeutic target for ESCA. SIRPα engagement by CD47-Fc prevents the phenotypic and functional maturation of immature DC and still inhibits cytokine production by mature DC. Therefore, we have also found high correlation with iDC and Th1 cells.

In the stratified analysis of prognosis, SIRPA expression could distinguish disease-specific survival across patients with different clinical characteristics, exhibiting a strong prognostic predictive capability. In expression analysis, we noticed that the expression of SIRPA increased as the T stage or pathologic stage rose, indicating that SIRPA might contribute to ESCA progression, which was consistent with reports that blocking the CD47-SIRPα pathway had an antitumor effect against solid tumors [23–25]. Interestingly, patients who received radiation therapy had higher SIRPA expression. This phenomenon provided a hypothesis that radiation therapy might induce SIRPA expression, and tumor with high SIRPA expression might gain the ability to immune escape. In terms of race, Asians had higher SIRPA expression than Whites. Besides, SIRPA expression also correlated with weight, body mass index (BMI), reflux history, columnar mucosa dysplasia, columnar metaplasia, Barrett’s esophagus, histological type, and tumor central location.

As we know, SIRPA inhibits the phagocytosis of tumor cells by macrophages by interacting with its ligand CD47 [26]. Our GSEA showed that the negative regulation of cytokine production involved in immune response and negative regulation of macrophage activation were enriched in high SIRPA tissues. Besides, Spearman’s correlation analysis showed that SIRPA affected immune landscape, especially macrophage polarization. Previous studies have shown that tumor-associated macrophages are mainly composed of M2 macrophages, suppressing antitumor immune responses and promoting tumor progression, including ESCA [27–29]. Our results implied that SIRPA might promote ESCA progression through inducing M2 macrophage polarization.

This study has some limitations. For example, we analyzed the SIRPA role only in public microarray data based on the Oncomine database or sequencing data based on TCGA database, so the results lacked corroboration by evidence from experiments. Because of this, hypothesis based on SIRPA expression was cast in the air. Besides, although we found that the expression level of SIRPA was closely related to the development of ESCA, the role of SIRPA in ESCA still needs further in-depth study and exploration.

In conclusion, this study examined the expression of SIRPA in ESCA and found it correlated with a short survival and poor prognosis and affected tumor-associated pathways.
Figure 7: Coexpression of SIRPA and immune-related genes. (a) Immune checkpoints. (b) Immunostimulator. (c) Immunoinhibitor. (d) MHC. *p < 0.05, **p < 0.01, and ***p < 0.001.
and immune landscape. SIRPA could be used as a biomarker to assess ESCA patient prognosis and an attractive target for the treatment.

Data Availability

The data used to support this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

References

[1] D. J. Uhlenhopp, E. O. Then, T. Sunkara, and V. Gaduputi, "Epidemiology of esophageal cancer: update in global trends, etiology and risk factors," Clinical Journal of Gastroenterology, vol. 13, no. 6, pp. 1010–1021, 2020.
[2] Y. Lin, M. Totsuka, B. Shan et al., "Esophageal cancer in high-risk areas of China: research progress and challenges," Annals of Epidemiology, vol. 27, no. 3, pp. 215–221, 2017.
[3] S. Pasquale, G. Yim, R. S. Vohra et al., "Survival after n and adjuvant treatments compared to surgery alone for resectional carcinoma," Annals of Surgery, vol. 265, no. 3, pp. 481–491, 2017.
[4] S. Mönig, M. Chevallay, N. Niclauss et al., "Early esophageal cancer: the significance of surgery, endoscopy, and chemoradiation," Annals of the New York Academy of Sciences, vol. 1434, no. 1, pp. 115–123, 2018.
[5] M. Watanabe, R. Otake, R. Kozuki et al., "Recent progress in multidisciplinary treatment for patients with esophageal cancer," Surgery Today, vol. 50, no. 1, pp. 12–20, 2020.
[6] B. A. Vellayappan, Y. Y. Soon, G. Y. Ku, C. N. Leong, J. J. Lu, and C. Tey, "Chemoradiotherapy versus chemoradiotherapy plus surgery for ESCA," Cochrane Database of Systematic Reviews, vol. 8, p. CD010511, 2017.
[7] M. E. W. Logtenberg, F. A. Scheeren, and T. N. Schumacher, "The CD47-sirpα checkpoint," Immunity, vol. 52, no. 5, pp. 742–752, 2020.
[8] J. Zhang, S. Jin, X. Guo, and W. Qian, "Targeting the CD47-sirpα signaling axis: current studies on B-cell lymphoma immunotherapy," Journal of International Medical Research, vol. 46, no. 11, pp. 4418–4426, 2018.
[9] K. Oshima, A. R. Ruhul Amin, A. Suzuki, M. Hamaguchi, and S. Matsuda, "SHPS-1, a multifunctional transmembrane glycoprotein," FEBS Letters, vol. 519, no. 1-3, pp. 1–7, 2002.
[10] S. Kaur, A. G. Elkahloun, S. P. Singh et al., "A function-blocking CD47 antibody suppresses stem cell and EGF signaling in triple-negative breast cancer," Oncotarget, vol. 7, no. 9, pp. 10133–10152, 2016.
[11] N. Koga, A. R. Ruhul Amin, A. Suzuki, M. Hamaguchi, and S. Matsuda, "SHPS-1, a multifunctional transmembrane glycoprotein," FEBS Letters, vol. 52, pp. 101–105, 2002.
[12] D. R. Rhodes, S. Kalyana-Sundaram, V. Mahavisno et al., "Oncomine 3.0: genes, pathways, and networks in a collection of 18,000 cancer gene expression profiles," Neoplasia, vol. 9, no. 2, pp. 166–180, 2007.
[13] J. Vivian, A. A. Rao, F. A. Nothaft et al., "Toil enables reproducible, open source, big biomedical data analyses," Nature Biotechnology, vol. 35, no. 4, pp. 314–316, 2017.
[14] J. Liu, T Lichtenberg, K. A Hoadley et al., “An integrated TCGA pan-cancer clinical data resource to drive high-quality survival outcome analytics,” Cell, vol. 173, no. 2, pp. 400–e11, 2018.

[15] M. I. Love, W. Huber, and S. Anders, “Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2,” Genome Biology, vol. 15, no. 12, p. 550, 2014.

[16] A. Subramanian, P. Tamayo, V. K. Mootha et al., “Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles,” Proceedings of the National Academy of Sciences, vol. 102, no. 43, pp. 15545–15550, 2005.

[17] G. Yu, L.-G. Wang, Y. Han, and Q.-Y. He, “clusterProfiler: an R package for comparing biological themes among gene clusters,” OMICS: A Journal of Integrative Biology, vol. 16, no. 5, pp. 284–287, 2012.

[18] S. Killcoyne, G. W. Carter, J. Smith, and J. Boyle, “Cytoscape: a community-based framework for network modeling,” Methods in Molecular Biology, vol. 563, pp. 219–239, 2009.

[19] K. Yoshishara, M. Shahmoradgoli, E. Martinez et al., “Inferring tumour purity and stromal and immune cell admixture from expression data,” Nature Communications, vol. 4, no. 1, p. 2612, 2013.

[20] S. Hänzelmann, R. Castelo, and J. Guinney, “GSVA: gene set variation analysis for microarray and RNA-seq data,” BMC Bioinformatics, vol. 14, no. 1, p. 7, 2013.

[21] J.-h. Pan, H. Zhou, L. Cooper et al., “LAYN is a prognostic biomarker and correlated with immune infiltrates in gastric and colon cancers,” Frontiers in Immunology, vol. 10, p. 6, 2019.

[22] M. Feng, W. Jiang, B. Y. S. Kim, C. C. Zhang, Y.-X. Fu, and I. L. Weissman, “Phagocytosis checkpoints as new targets for cancer immunotherapy,” Nature Reviews Cancer, vol. 19, no. 10, pp. 568–586, 2019.

[23] X. W. Zhao, E. M. van Beek, K. Schornagel et al., “CD47-signal regulatory protein- (SIRP ) interactions form a barrier for antibody-mediated tumor cell destruction,” Proceedings of the National Academy of Sciences, vol. 108, no. 45, pp. 18342–18347, 2011.

[24] X. Zhang, J. Fan, S. Wang et al., “Targeting CD47 and autophagy elicited enhanced antitumor effects in non-small cell lung cancer,” Cancer Immunology Research, vol. 5, no. 5, pp. 363–375, 2017.

[25] T. Yanagita, Y. Murata, D. Tanaka et al., “Anti-SIRPalpha antibodies as a potential new tool for cancer immunotherapy,” JCI Insight, vol. 2, no. 1, p. e89140, 2017.

[26] S. B. Willingham, J. P Volkmer, A. J Gentles et al., “The CD47-signal regulatory protein alpha (SIRPa) interaction is a therapeutic target for human solid tumors,” Proceedings of the National Academy of Sciences of the United States of America, vol. 109, no. 17, pp. 6662–6667, 2012.

[27] A. Sica, T. Schioppa, A. Mantovani, and P. Allavena, “Tumour-associated macrophages are a distinct M2 polarised population promoting tumour progression: potential targets of anti-cancer therapy,” European Journal of Cancer, vol. 42, no. 6, pp. 717–727, 2006.

[28] Q.-w. Zhang, L. Liu, C.-y. Gong et al., “Prognostic significance of tumor-associated macrophages in solid tumor: a meta-analysis of the literature,” PLoS One, vol. 7, no. 12, p. e50946, 2012.

[29] X. Yuan, Y. Li, A. Z. Zhang et al., “Tumor-associated macrophage polarization promotes the progression of esophageal carcinoma,” Aging, vol. 13, no. 2, pp. 2049–2072, 2020.