Identification of Potential Therapeutic Targets Inducible Co-Stimulator (ICOS) in Cancer Immunotherapy Using Bioinformatics Analysis

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Abstract: Clinical trials testing Inducible Co-Stimulator (ICOS) agonists in cancers are under way. However, Co-expression and Interaction of ICOS at the Gene and Protein Levels, the correlations of ICOS to prognosis and tumor-infiltrating lymphocytes in different cancers remain unclear. ICOS expression was analyzed via the Oncomine database and Tumor Immune Estimation Resource (TIMER) site. Analysis of the expression difference of ICOS shows that the expression of ICOS is significantly increased in BRCA, ESCA, HNSC, KIRC, KIRP, LIHC, STAD and UCEC. We evaluated the influence of ICOS on clinical prognosis using Kaplan-Meier plotter and Gene Expression Profiling Interactive Analysis (GEPIA). This analysis confirmed that low ICOS expression was significantly correlated with poor overall survival (OS) and progression-free survival (PFS) in ovarian cancer. The correlations between ICOS and cancer immune infiltrates were investigated via TIMER. Metascape and Protein-protein interaction (PPI) analysis suggest that ICOS plays an important role in the process of immune activation. ICOS is a potential target for the development of antibody drugs.

Keywords: ICOS; Cancer; Lymphocytes; Immunotherapy; Prognosis

1. Introduction

In the past decade, it has been widely recognized that the immune system can control the occurrence and development of tumors. ICOS is one of the members of the CD28 family. It has attracted interest as a T-cell enhanced costimulatory receptor. Hutloff et al. first described ICOS as a T cell-specific costimulatory molecule that enhances T cell responses to foreign antigens. ICOS is a complex central immune response and homeostasis center. Whether ICOS can be used as a drug target needs to be further determined.

Here, we evaluated the expression level of ICOS mRNA in different types of cancer and evaluated the association with the prognosis of various types of cancer in a common database (e.g., Oncome, Kaplan-Meier plotter, GEPIA). We also use the Metascape and String database to reveal the important role of ICOS in the biological process, and provide the current situation of anti ICOS antibody agonists.

2. Material and methods

2.1 Oncomine database analysis

ICOS mRNA expression levels in different types of human cancers were identified in the Oncomine database (https://www.oncomine.org/resource/login.html) [1]. The threshold was a P-value of 0.001, a fold change of 1.5, a top 10% gene ranking, and the data had to be from mRNA.

2.2 TIMER database analysis

We analyzed ICOS expression and the correlation of ICOS expression with the abundance of 6 types of infiltrating immune cells (B cells, CD4+ T cells, CD8+ T cells, neutrophils, macrophages, and dendritic cells) in in different types of human cancers via The Tumor IMmune Estimation Resource (TIMER) algorithm database (https://cistrome. shinyapps. io/timer/) [2].

2.3 Co-expression and Interaction Analysis

COXPRESdb database was used to predict the co expression genes of ICOS. The Metascape database (http://metascape.org) is used to analyze the functional enrichment relationship of ICOS [3]. String database (https://string-db.org/cgi/input.pl) was used to predict the biological process and function of ICOS protein, and analyze protein-protein interaction [4].

3. Results

3.1 The mRNA Expression Levels of ICOS in Different Types of Human Cancers

In order to determine the difference of ICOS expression between tumor and normal tissues, the Oncomine database were used to analyze the level of ICOS mRNA in different tumor and normal tissues of multiple cancer types (Figure 1A). The Oncomine database analysis revealed that ICOS mRNA expression was lower in sarcoma. Higher expression was observed in breast cancer, kidney cancer, Melanoma.

For further evaluate ICOS expression in human cancers, we examined ICOS expression using TCGA RNA-sequencing data by TIMER. The differential expression of tumor and adjacent normal tissue for ICOS in all TCGA tumors is shown in Figure 1B. ICOS expression was significantly lower in LUAD (lung adenocarcinoma), LUSC (Lung squamous cell carcinoma), THCA (thyroid carcinoma) compared with adjacent normal tissues. However, LAYN expression was significantly higher in BRCA (breast invasive carcinoma), ESCA (Esophageal carcinoma), HNSC (head and neck cancer), KIRC (kidney renal clear cell carcinoma), KIRP (Kidney renal papillary cell carcinoma), LIHC (liver hepatocellular carcinoma), STAD (stomach adenocarcinoma) and UCEC (uterine corpus endometrial carcinoma) compared with adjacent normal tissues.

3.2 Co-expression and Interaction Analyses of ICOS at the Gene and Protein Levels

We used the COXPRESdb database to analyze the genes co-expressed with ICOS. The results showed that the gene treasure co-expressed with ICOS included CTLA4, ITK, CD28, TRAT1, CD2, SH2D1A, CD3D, CD3G, UBASH3A, SIRPG, IFNG, THEMIS and TIGIT. The functions of ICOS and genes co-expressed were predicted by analyzing GO and KEGG in Metascape. Metascape analysis found that the following processes were affected by ICOS gene alterations: Lymphocyte activation; T cell receptor signaling pathway; Hematopoietic cell lineage; Cytokine-cytokine receptor interaction; Cell adhesion molecules (CAMs); negative T cell selection; regulation of lymphocyte differentiation (Figure 2A and B). These results indicate that ICOS plays an important role in immune regulation.

For further investigate the molecular function and biological process of ICOS, String database is used to analyze the

Protein-protein interaction (PPI) of ICOS. String predicted functional partners included ICOL, RC3H1, CD40LG, B7RP1, PIK3R1, PIK3CB, PIK3CD, PIK3R3, PIK3R2 and PIK3CA. As shown in Figure 4C, these functional molecules form a PPI network in String database. The biological process analysis of ICOS shows that ICOS plays an important role in positive regulation of immune system process, phosphatidylinositol phosphorylation, T cell costimulation, phosphatidylinositol-3-phosphate biosynthetic process (Figure 2D). The molecular function analysis of ICOS shows that phosphatidylinositol 3-kinase activity, 1-phosphatidylinositol-3-kinase activity, phosphatidylinositol-4, 5-bisphosphate 3-kinase activity, 1-phosphatidylinositol-3-kinase regulator activity, 1-phosphatidylinositol-4-phosphate 3-kinase activity, phosphotransferase activity, alcohol group as acceptor are important molecular functions of ICOS (Figure 2E). These results suggest that ICOS plays an important role in the process of immune activation.

4. Discussion

Nowadays, immunotherapy has become an important means of cancer treatment. The clinical manifestations of anti-CTLA-4 and anti-PD-1 antibodies also provide a basis for immunotherapy. Combined immunotherapy is an effective method to improve the effect of immunotherapy monotherapy. Therefore, we provide three development strategies: 1) develop bispecific antibody related to ICOS; 2) develop fusion protein with agonist function based on ICOSL gene sequence; 3) combine anti-ICOS antibody agonist with other drugs for tumor treatment

In addition, it is worth noting that ICOS can activate both killer T cells and regulatory T cells. Autoimmune disease is a kind of chronic disease with multiple organs and systems damage caused by the disorder of the body's autoimmune system, such as systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), Sjogren's syndrome (SS), polymyositis and dermatomyositis (DM). The development of ICOS antagonists is expected to be a therapeutic drug for autoimmune diseases.

Conflict of interest

The authors declare that there are no conflicts of interest.

Fig. 1. ICOS expression levels in different types of human cancers. (A) ICOS in data sets of different cancers in the Oncomine database. (B) Human ICOS expression levels in different tumor types from TCGA database were determined by TIMER (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$).

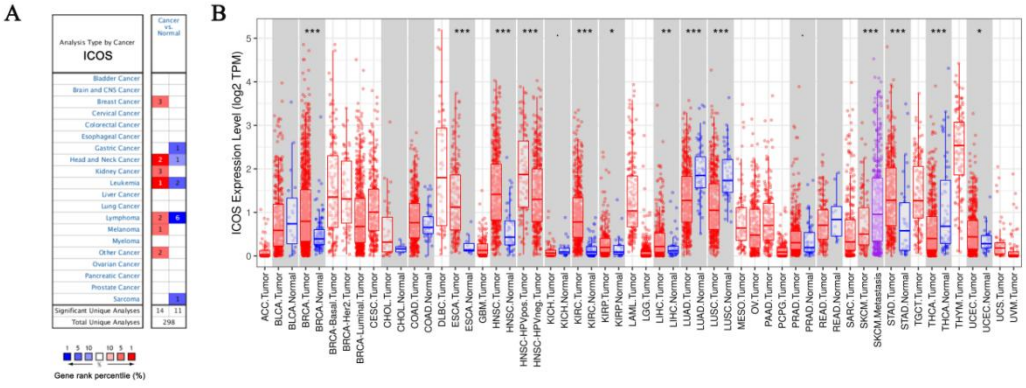
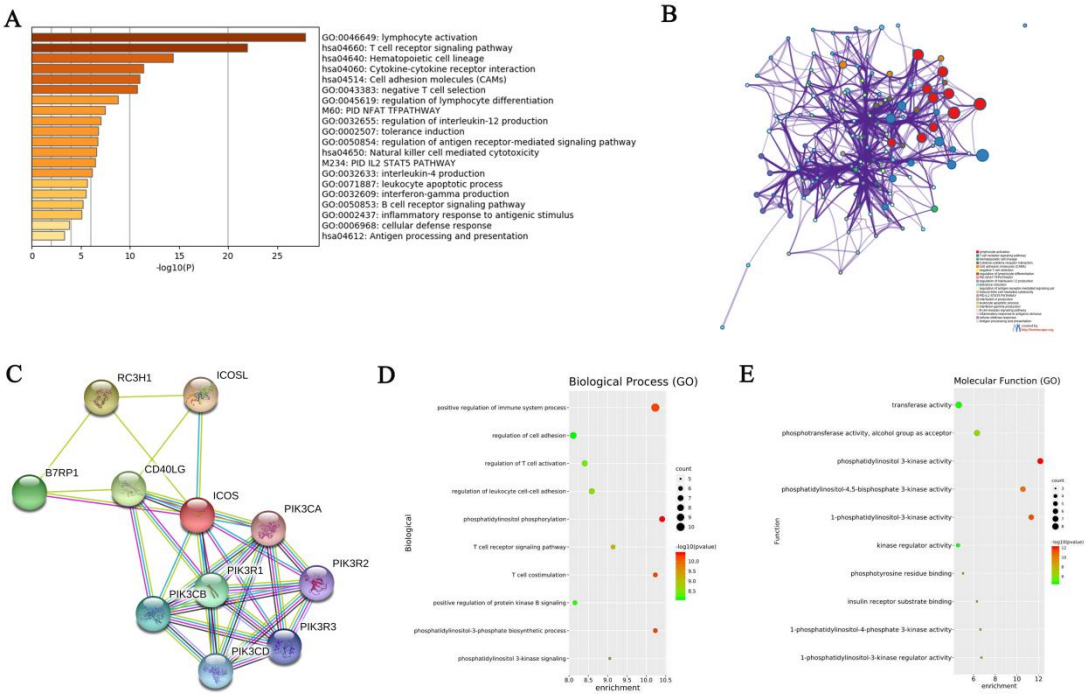


Fig. 2. Co-expression and Interaction Analyses of ICOS at the Gene and Protein Levels. (A) Metascape bar graph and (B) Metascape enrichment network visualization showing the functions of ICOS and genes co-expressed by analyzing GO and KEGG. (C) Protein–protein interaction network of ICOS in the String database. (D) biological process and (E) molecular function of ICOS enrichment analyses. The x-axis represents the −log10 (p value), and the y-axis represents the GO term. The GO terms were measured by the rich factor, p value and number of genes enriched. The greater the Rich factor is, the greater the degree of enrichment and the greater the p value [0, 1]. The brighter the color of red is, the more significant



the term.

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