Cancer
Research

TIMER: A Web Server for Comprehensive Analysis of Tumor-Infiltrating Immune Cells

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Abstract

Recent clinical successes of cancer immunotherapy necessitate the investigation of the interaction between malignant cells and the host immune system. However, elucidation of complex tumor–immune interactions presents major computational and experimental challenges. Here, we present Tumor Immune Estimation Resource (TIMER; cistrome.shinyapps.io/timer) to comprehensively investigate molecular characterization of tumor–immune interactions. Levels of six tumor-infiltrating immune subsets are precalculated for 10,897 tumors from 32 cancer types. TIMER provides 6 major analytic modules that allow users to interactively explore the associations between immune infiltrates and a wide spectrum of factors, including gene expression, clinical outcomes, somatic mutations, and somatic copy number alterations. TIMER provides a user-friendly web interface for dynamic analysis and visualization of these associations, which will be of broad utilities to cancer researchers. *Cancer Res; 77(21); e108–10. ©2017 AACR.*

Introduction

Recent advances in immunotherapy, especially checkpoint blockade, have resulted in clinical success in treating late-stage cancers (1). Immune infiltrates obtained from different patients with the same tumor type are heterogeneous and may impact clinical outcome (2). Because of the complexity of tumor genomes and the plasticity of the host immune system, it remains challenging to characterize the interaction between cancer cells and immune infiltrates (3). Recently, a variety of computational methods to infer immune infiltrates have been developed to investigate tumor immunology and develop new effective immunotherapies (4–7). One seminal study measures effector cell cytolytic activity using the transcript levels of two genes, *GZMA* and *PRF1*, to elucidate possible mechanisms of immune evasion (8). Immune signature genes have also been used to characterize immune infiltrates and predict clinical outcome (4). One influential deconvolution method, CIBERSORT, utilizes microarray data with a predefined immune signature matrix to estimate the fraction of 22 tumor-infiltrating immune cells (TIIC) within a given sample (5). Its results have been presented as online resources, such as PRECOG (6)

and TCIA (7), to analyze the impact of TIICs and individual gene expression levels on clinical outcome. However, CIBERSORT estimations are potentially affected by statistical multicollinearity due to the inclusion of highly correlated immune cell types, leading to high estimations of uncertainty (Supplementary Material). In addition, PRECOG and TCIA have limited analysis and visualization functions.

Despite these efforts, a comprehensive computational tool for cancer researchers to conveniently explore and visualize tumor immunologic and genomics data is still lacking. Therefore, we developed an interactive web application, Tumor Immune Estimation Resource (TIMER; cistrome.shinyapps.io/timer), as a public resource to enable cancer biologists to analyze and visualize the abundance of TIICs in a comprehensive and flexible manner. TIMER applies a deconvolution method we previously published (9) to infer the abundance of TIICs from gene expression profiles. In this work, we reanalyze gene expression data, which includes 10,897 samples across 32 cancer types from The Cancer Genome Atlas (TCGA) to estimate the abundance of six TIIC subsets (B cells, CD4 T cells, CD8 T cells, macrophages, neutrophils, and dendritic cells). The workflow is based on our previous work of statistical deconvolution of immune infiltrates (9). In brief, we select informative genes, which are negatively correlated with tumor purity (percentage of malignant cells in a tumor tissue) for each cancer type (10) and apply constrained least squares fitting on the expression of selected genes to predict the abundance of six TIIC subsets.

Results

To dynamically explore and visualize the interactions between TIICs and important tumoral genomic changes, we introduce TIMER, a user-friendly web interface (cistrome. shinyapps.io/timer). TIMER consists of six functional modules, including the association of TIIC abundance with gene expression (gene), overall survival (survival), somatic mutations (mutation), and DNA somatic copy number alterations (SCNA),
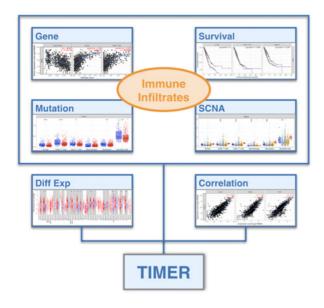
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Figure 1.
Overview of TIMER modules on the website. TIMER comprises six modules. Four modules in the top box explore the associations of TIICs with gene expression (gene), overall survival (survival), somatic mutation (mutation), and somatic copy number alteration (SCNA), as well as analysis of differential gene expression (DiffExp) and correlation between two groups of genes (correlation). Examples of visualization for each module are displayed in the corresponding text boxes.

as well as analysis of differential gene expression (DiffExp) and gene–gene correlations (correlation; Fig. 1). Each module allows users to input function-specific parameters, with resulting figures dynamically generated and displayed at the bottom of the TIMER webpage. Detailed descriptions of each module are described below.

Gene module

Malignant cells and TIICs interact through multiple gene products and pathways during tumor development and progression. The "gene" module allows users to input a gene symbol, cancer types, and immune cell types of interest. A scatterplot displaying gene expression levels against tumor purity is first displayed. This is because the tumor microenvironment is a complex milieu of noncancerous cells mainly consisting of immune cells around tumor cells, and genes highly expressed in cells in the microenvironment are expected to have negative associations with tumor purity, while the opposite is expected for genes highly expressed in the tumor cells. Therefore, this panel is helpful to identify the cancer type and specific TIIC subsets that are associated with the expression of a certain gene of interest. For example, if users input the granzyme A gene (*GZMA*), breast cancer (BRCA), and all six TIIC subsets, a negative correlation between *GZMA* expression and tumor purity is observed because granzymes are produced in the tumor microenvironment. Six panels, showing the purity-corrected partial Spearman correlation and statistical significance between *GZMA* expression and each TIIC subset, would also be displayed. To facilitate easy comparisons across different cancer types, TIMER allows users to choose multiple cancer types and simultaneously displays all correlations.

Survival module

Many studies have reported a prominent impact of TIICs on clinical outcome of cancer patients (11). The "survival" module allows users to explore the clinical relevance of one or more TIIC subsets, with the flexibility to correct for multiple covariates in a multivariable Cox proportional hazards model. Clinical covariates include patient age, gender, ethnicity, and tumor stages, where T, N, and M delineate primary, lymph node spreading, and metastatic tumors, respectively. Users are also allowed to include a list of gene expression values into the Cox model to investigate the clinical relevance of genes as well as TIICs. TIMER outputs the Cox regression results, including HRs and statistical significance. Users can easily explore the clinical impact of a gene of interest and correct for potential confounding factors. For example, applying this module, we could find that CD40 ligand (*CD40LG*) independently predicts better outcome (HR = 0.79, P = 0.003) in lung adenocarcinoma corrected for patient age, stage, and TIICs. This result is consistent with the established role of CD40/CD40LG in regulating immune cell function (12). For each survival analysis, TIMER outputs Kaplan–Meier plots for TIICs and genes to visualize the survival differences between the upper and lower x percentile of patients, where x can be adjusted using a slider. A log-rank P value is also calculated and displayed for each Kaplan–Meier plot.

Genetic aberration modules

Tumor development and progression are associated with multiple genomic aberrations, which in turn may influence TIICs. The "mutation" and "SCNV" modules are designed to facilitate the investigation of such associations. In the "mutation" module, users are able to compare the abundance of TIICs with or without the presence of a given somatic mutation. To identify potential neoantigens or genes that are related to immune infiltration, we only include nonsynonymous mutations that can cause amino acid changes in the protein sequence. Therefore, we chose the top 50 genes with the most frequent nonsynonymous mutations as options in the "mutated gene" field for each cancer type. Box plots are generated for each TIIC subset, to compare the distribution of the abundance of TIICs with different gene mutation status, with statistical significance estimated using two-sided Wilcoxon rank sum test. For example, when head and neck cancer and TP53 mutation are selected, the returned box plots show significantly lower infiltration for most immune cells in tumors with TP53 mutations. This result is potentially related to the important role that p53 plays in regulating the innate immune response (13). The "SCNA" module provides the comparison of the abundance of TIICs among tumors with different somatic copy number aberrations for a given gene. SCNAs are defined by GISTIC 2.0 (14), including deep deletion, shallow deletion, diploid/normal, low-level gain, and high amplification. Box plots are presented to show the distributions of each TIIC subset for each copy number status in selected cancer types using the same statistical tests as in the "mutation" module.

Gene comparison modules

TIMER provides two additional modules, "DiffExp" and "correlation", for researchers to explore interesting genes expressed in tumor and adjacent normal tissue or gene–gene associations that may be related to cancer immunity. The "DiffExp" module allows users to study the differential

expression between tumor and adjacent normal tissues for any gene of interest across all TCGA tumors. Distributions of gene expression levels are displayed using box plots, with statistical significance of differential expression evaluated using Wilcoxon test. Users can identify genes that are up- or downregulated in the tumors compared with normal tissues for each cancer type, as displayed in gray columns when normal data are available. The "correlation" module plots the expression scatterplots between a pair of user-defined genes in a given cancer type, together with the Spearman correlation and estimated statistical significance, which could be adjusted by tumor purity or age optionally. A brief video tutorial is provided in the Supplementary Material (See Supplementary Video S1) and the main page of TIMER website.

Discussion

The increasing number and complexity of cancer immunotherapy studies in recent years pose new challenges to analyze and visualize the rapidly accumulating clinical and cancer genomics data. Characterization of the immune infiltration landscape is necessary for the investigation of tumor–immune interactions, which requires innovative computational methods to integrate and deconvolve multidimensional datasets. Our study provides a comprehensive analytic web tool for cancer researchers to conveniently access the tumor immunologic, clinical, and genomic features. Intuitive inputs and outputs of the six functional modules in TIMER simplify the analysis of the interactions between genes of interest and immune cells in multiple cancer types. To the best of our knowledge, TIMER is the first method that allows users to perform integrative analysis of tumor immunologic, clinical, and genomics data. The development and maintenance of the TIMER web server will be carried out by our laboratory for a minimum of 5 years. With the increasing numbers of studies of cancer immunology, we anticipate TIMER to attract broad interest from the cancer research community and aid the discovery of novel therapeutic targets and new immune evasion mechanisms.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

Conception and design: T. Li, X.S. Liu
Development of methodology: T. Li, J.S. Liu
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): T. Li, J. Fan, B. Li
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): T. Li, J. Fan, B. Wang
Writing, review, and/or revision of the manuscript: T. Li, Q. Chen, J.S. Liu, B. Li, X.S. Liu
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): T. Li, N. Traugh, Q. Chen
Study supervision: Q. Chen, J.S. Liu, B. Li, X.S. Liu

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