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RAPID COMMUNICATION

OStme: Online consensus survival analysis web server for tumor microenvironment components of pan-cancers

Tumor microenvironment (TME) is the environment around the tumor cells, consisting of extracellular matrix, nonmalignant stromal cells, and various types of immune cells (like macrophages, B and T lymphocytes).[1] TME has been recurrently reported to play important roles in the progression of cancers.[1] The mechanism study of TME may offer opportunities for targeted/immune therapies. However, how TME components impact the prognosis of cancer patients remains to be determined. In this study, we constructed a web server named OStme (Online consensus Survival analysis web server for Tumor MicroEnvironment components of pan-cancers) to analyze the association of cancer prognosis and various types of tumor-infiltrating cells (TICs), and to investigate the fraction variations of TICs across different stages/grades or variations along with metastasis/recurrence or variations regarding therapies. OStme is freely available at http://bioinfo.henu.edu.cn/Immune/Immune.html. OStme provides 12 types of survival terms (such as OS, DSS, and RFS), offers four analysis modules for cancer patient survival analysis, differential analysis per clinical characteristics (such as different stage, tumor versus normal), comprehensive TME fraction estimation, and the correlation between gene expression and TIC abundance of pan-cancers. We believe OStme will serve as a valuable resource and tool to systematically evaluate the impact of TICs on the prognosis, metastasis, recurrence, progression, and therapy of pan-cancers.

To gain more comprehensive and robust results of TIC estimation in a large scale of cancer tissue samples in pan-cancers, we first collected 141 gene expression datasets including 23,917 unique cancer cases from 31 distinct malignancies from The Gene Expression Omnibus (GEO), The Cancer Genome Atlas (TCGA), CGGA (Chinese Glioma Genome Atlas), and literature (Table S1). The detailed process of how to obtain these datasets was described in the supplementary file. Then through four state-of-the-art algorithms[2] including CIBERSORTx, xCell, EPIC, and ESTIMATE, we estimated the fractions of TICs in cancer samples. Finally, we built a freely available web server OStme using HTML5, JSP, and JavaScript. The workflow and typical output schema were shown in Figure 1A. OStme provides four analysis modules: survival analysis, differential analysis, infiltration estimation, and infiltration association (Fig. 1B). To demonstrate the performance and reliability of OStme, case studies were added (supplementary file), and the detailed instructions for each analysis module are also available on the online OStme Help page.

Tumor-infiltrating lymphocytes (TILs) are associated with cancer outcomes. To quickly assess the association of various TIL/TIC abundance and patient survival across 31 cancer types, we built the "survival analysis" module (Fig. 1B). Briefly, OStme uses a log-rank test for hypothesis evaluation and adopts a Cox proportional hazard model to evaluate the effects of TICs on outcomes. To apply survival analysis for TICs in OStme, a user needs to select the TIC estimation tool and the enquired cancer type to be analyzed, survival event, stratification cutoff, and confounding factors including tumor stage and gender. After that, the user can click on the "Plot" button to examine how much the TICs (high versus low) influence the patient's survival by Kaplan–Meier (KM) curves. The Cox proportional hazard ratio (HR) with a 95% confidence interval is also shown on the survival plot.

Chemotherapy, radiotherapy, and targeted molecular therapy have been shown to have great benefits for cancer patients although some patients may respond to these treatments distinctly.[3] Recently, TME has been recurrently reported to be associated with the sensitivity and resistance of chemotherapy and/or radiotherapy, and immune infiltrating cells (such as macrophages and neutrophils) are mainly found in poorly differentiated or undifferentiated

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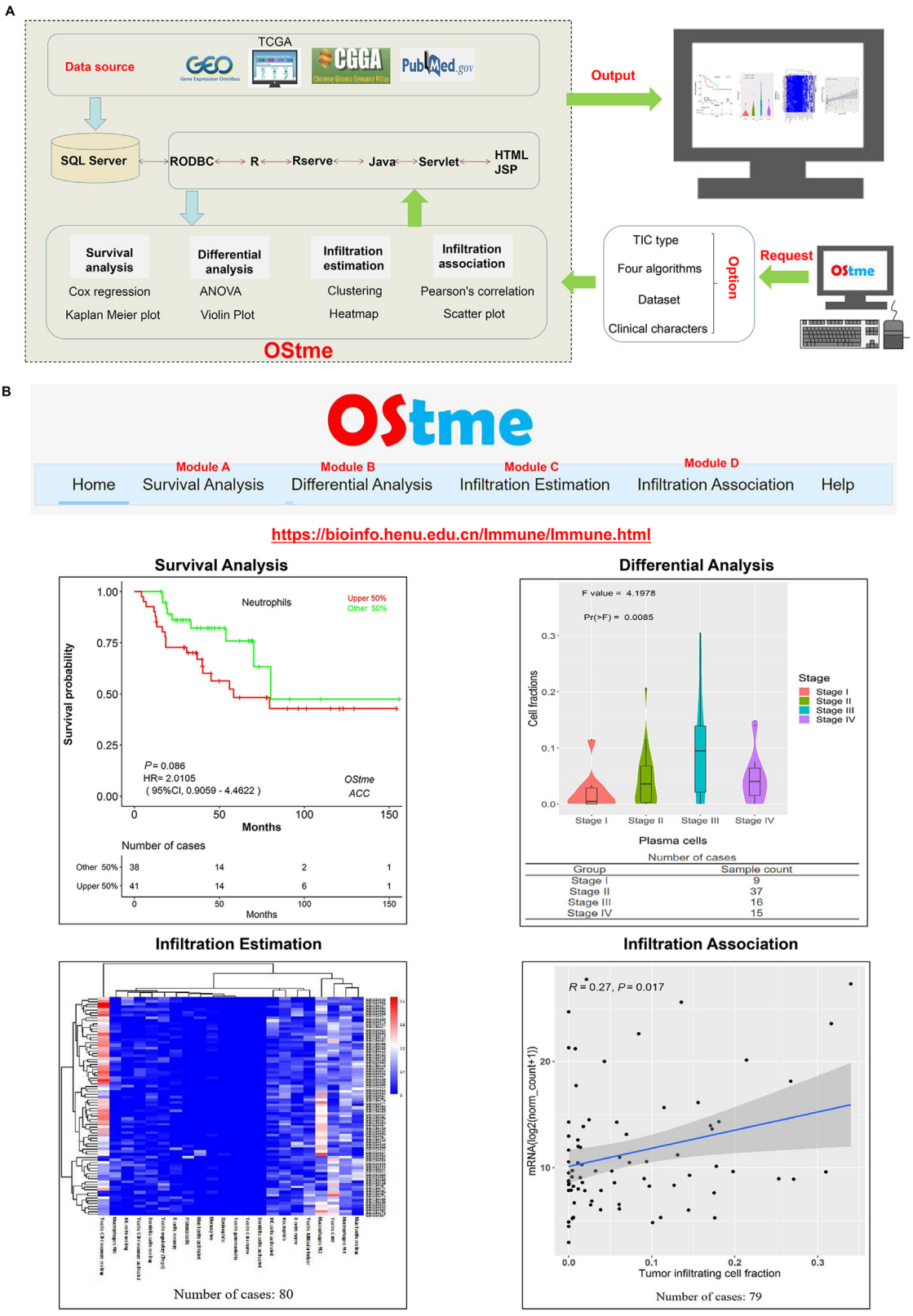

Figure 1 The construction process and functional modules of OStme. **(A)** The flowchart of the web server architecture **(B)** Snapshot of OStme usage and functions. OStme contains four analysis modules: survival analysis (Module A), differential analysis (Module B), infiltration estimation (Module C), and infiltration association (Module D). Kaplan—Meier plot shows the association of neutrophil level and ACC outcomes; Violin plot shows the variation of plasma cells in different ACC stages in the TCGA dataset; Heatmap shows the clustered 22 types of TIC levels of LIHC cases in the GSE20140 dataset; Scatter plot shows the association of *CD47* mRNA expression and infiltrated fractions in TCGA-ACC. The expression of CD47 mRNA was positively related to the dendritic

late-TNM stage cancers. In addition, the TME and immunoscore are found to be critical determinants of tumor cell dissemination to distant metastasis.[4] To examine the relationship between TICs and the clinicopathological features, we established the "differential analysis" module (Fig. 1B), which allows users to analyze the fraction variation of TIC for 9 clinicopathological characters (including stage, grade, tumor versus normal, neoadjuvant treatment, radiation therapy, targeted molecular therapy, therapy outcome, metastasis, and recurrence). For the TIC fraction comparison between tumor stages, we used the pathological stage (stages I, II, III, IV) as a subgroup variable to calculate the difference of TIC fractions across different stages. For comparing TIC fractions between different tumor grades, we used histological grade (G1, G2, G3, G4, and GX) as a subgroup variable to measure the TIC fraction variations across different tumor grades. Users can also use OStme to investigate the TIC fraction changes by neoadjuvant chemotherapy in various cancers. The TME estimation may guide the selection of proper immunotherapy for cancer patients and may be important in determining the success or failure of radiotherapy as well.[5] Therefore, users can explore the differences in TICs between cancer patients with radiotherapy and without radiotherapy by OStme. For therapy outcome comparison, users can apply outcomes (complete remission/response, partial remission/response, progressive disease, stable disease) as subgroup variables to determine the variations of TIC fractions between the above four subgroups. Tumor metastasis is responsible for nearly 90% of all cancer-related deaths. TME is one of the main actors in the metastasis process. For comparing the TIC variation along with tumor metastasis, users can utilize metastasis as a subgroup variable to calculate the differences of TIC fractions alongside metastasis. In addition, the differences in TICs between normal and tumor tissues can also be analyzed. After selecting the clinicopathological character for TIC comparison, users then need to select the intended immune estimation tool, the interested TIC type, cancer type, and tendentious cohort, and click on the analysis button "Plot". After that, the analysis results will be presented as a violin plot.

To evaluate the abundance of up to 64 types of TICs in 31 cancer types, we established the "infiltration estimation" module (Fig. 1B). After selecting the TIC estimation tool, specified tumor type, and gene expression cohort, OStme could generate a heatmap of clustered TIC fractions in the specified cancer cohort. To get a high-resolution heatmap for further investigation and publication, users can click on the heatmap, and then a heatmap with a high-resolution PDF version will be output. A total of 8, 22, and 64 kinds of TICs estimated by EPIC, CIBERSORT, and xCell will be shown in the heatmap. Immune scores and stromal scores calculated by ESTIMATE will be also shown in the heatmap.

To further explore the relationship between gene expression and the abundance of various TICs, we established the "infiltration association" module (Fig. 1B). This module allows users to identify such relationships in a fast and systematic way. When a user selects one gene of interest, a TIC type, and a cancer type, OStme will generate a scatter plot of the expression of the input gene and the abundance of TICs with a Pearson correlation coefficient. This display allows users to view whether the expression of their input gene is associated with a TIC type.

OStme is an interactive web server for TIC prognosis and differential analysis in pan-cancers, it allows users to (i) measure the effect of TICs on patient survival, (ii) analyze the TIC variations across different tumor stages, grades, and other clinicopathological subgroups, (iii) estimate the abundance of all TIC types in pan-cancers, and (iv) evaluate the correlation between gene expression and the abundance of TICs in pan-cancers. To our knowledge, OStme is the first web server that allows users to perform integrative analysis of TME component variations along with tumor progression, metastasis, recurrence, and prognosis beyond TCGA data. OStme will greatly help cancer researchers, biologists, and clinicians without many computational skills to quickly explore the TICs and their clinical associations, and to identify novel prognostic TME biomarkers and potential therapeutic TME targets in pan-cancers. In the future, we will add a new function for uploading and analyzing a specific dataset by user to increase the potential user community.

Author contributions

LX, QW, and XG contributed to the conceptualization; XM, GZ, ZY, and HL collected the datasets; QW, LX, and XG built the web server; QW, LX, WZ, ZZ, and XG wrote, edited, and reviewed the paper. All authors read and approved the final manuscript.

Conflict of interests

The authors have declared no conflict of interests.

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cell activated fraction ($R = 0.27$, $P = 0.017$). The strength can be evaluated by these general guidelines (which may vary by discipline): $0.1 < |R| < 0.3$ represents small/weak correlation; $0.3 < |R| < 0.5$ represents medium/moderate correlation; and $|R| > 0.5$ represents large/strong correlation.

Data availability

The datasets generated during and/or analyzed during the current study are available from the corresponding authors upon reasonable request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.gendis.2023.02.043.

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Longxiang Xie [a,1], Qiang Wang [a,1], Xiaoyu Ma [a], Guo Zhao [a], Zhongyi Yan [a], Huimin Li [a], Wan Zhu [b], Zhanjie Zuo [c], Xiangqian Guo [a,*]

[a] *Department of Preventive Medicine, Institute of Biomedical Informatics, Bioinformatics Center, Henan Provincial Engineering Center for Tumor Molecular Medicine, Academy for Advanced Interdisciplinary Studies, School of Software, School of Basic Medical Sciences, Henan University, Kaifeng, Henan 475004, China*
[b] *Department of Anesthesia, Stanford University, Stanford, CA 94305, United States*
[c] *Thoracic Cancer Treatment Center, Armed Police Beijing Corps Hospital, Beijing 100027, China*

*Corresponding author.
E-mail address: xqguo@henu.edu.cn (X. Guo)

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[1] These authors contributed equally to this work.