

Comprehensive Pan-cancer Analysis of 33 Human Cancers Reveals the Immunotherapy Value of *FOXP3*

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Abstract

Background: Previous studies reported that *FOXP3* is involved in the regulation of tumor microenvironment and the regulation of tumor local immunity. However, its mechanism has not been fully studied. This study aims to explore the potential relationship between *FOXP3* and cancer immunotherapy in 33 human cancers.

Methods: The gene expression data and clinical characteristics of 33 cancers were retrieved from the Cancer Genome Atlas database. The immunotherapy cohort includes GSE157893, GSE67501, and IMvigor210, which come from a comprehensive gene expression database and are included in previously published studies. Analyse clinical parameters, including patient age, gender, and tumor stage to assess the prognostic value of *FOXP3*. At the same time, we conducted survival analysis and correlation analysis of tumor microenvironment. The correlation between *FOXP3* and immunosuppressive agents and stimulants, as well as major histocompatibility complexes was also analysed. Potential pathways related to *FOXP3* signalling in cancer have also been explored. In addition, the correlation between *FOXP3* and two immunotherapy biomarkers (tumor mutation burden and microsatellite instability) was studied. Finally, the immunotherapy response relationship between *FOXP3* and the immunotherapy cohort was explored.

Results: Among 33 cancer types, *FOXP3* expression is different in different clinical groups (gender, age, and tumor stage) in certain cancers, and it also shows potential prognostic value in predicting patient survival. *FOXP3* is correlated with immune cell infiltration, immunomodulatory and immunotherapy markers. In addition, low *FOXP3* expression is significantly associated with certain pathways. However, no significant correlation was observed between *FOXP3* and immunotherapy response.

Conclusion: This study explored the immunotherapy value of *FOXP3* expression in 33 human cancers, and provided evidence and insights for the application of *FOXP3* expression in tumor immunotherapy. However, considering the bioinformatics methods used in this study, the findings of the study are only preliminary, and more relevant experimental verifications are needed.

Key words: Bio-informatics; Pan cancer; *FOXP3*; Immunity

Abbreviations: ACC: Adrenocortical Carcinoma; BLCA: Bladder Urothelial Carcinoma; BRCA: Breast Invasive Carcinoma; CESC: Cervical Squamous Cell Carcinoma and Endocervical Adenocarcinoma; CHOL: Cholangiocarcinoma; COAD: Colon Adenocarcinoma; DLBC: Lymphoid Neoplasm Diffuse Large B-cell; ESCA: Esophageal Carcinoma; GBM: Glioblastoma Multiforme; HNSC: Head and Neck Squamous Cell Carcinoma; KICH: Kidney Chromophobe; KIRC: Kidney Renal Papillary Cell Carcinoma; KIRP: Kidney Renal Papillary Cell Carcinoma; LAML: Acute Myeloid Leukaemia; LGG: Brain Lower Grade Gliomas; LIHC: Liver Hepatocellular Carcinoma; LUAD: Lung Adenocarcinoma; LUSC: Lung Squamous Cell Carcinoma; MESO: Mesothelioma; OV: Ovarian Serous Cyst Adenocarcinoma; PAAD: Pancreatic Adenocarcinoma; PCPG: Pheochromocytoma and Paraganglioma; PRAD: Prostate Adenocarcinoma; PEAD: Rectum Adenocarcinoma; SARC: Sarcoma; SKCM: Skin Cutaneous Melanoma; STAD: Stomach Adenocarcinoma; TGCT: Testicular Germ Cell Tumours; THCA: Thyroid Carcinoma; THYM: Thymoma; UCEC: Uterine Corpus Endometrial Carcinoma; UCS: Uterine Carcinosarcoma; UVM: Uveal Melanoma.

Introduction

Humoral immunity and cellular immunity are the two main effect mechanisms of human anti-tumor immunity. Of the two, cellular immunity plays a more important role in anti-tumor. Among them, T lymphocytes are the main participating cells. Regulatory T cells are the main cell population for anti-tumor immunosuppression. There is a

fixed ratio of immune cells in the normal body. If this ratio is changed, it will lead to the body's immune tolerance or weakened immune function, which will allow tumor cells to escape the host immune system. Identification and attack [1,2]. Studies have found that Treg plays an important role in tumor immunity. A high proportion of regulatory T cells have been found in various tumors such as non-small cell lung cancer and gastric cancer, and they are positively correlated with tumor metastasis and invasion [3-5]. Fork head box protein 3 (FOXP3) is an important member of the fork head/wing-shaped spiral transcription factor family and is mainly expressed in Treg. It is a landmark molecule of Treg. It regulates regulatory T cells (regulatory T cells). Cells, Treg and immune suppression [6,7]. At the same time, *FOXP3* can exert an immunosuppressive function by regulating the content of anti-

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inflammatory factors such as IL-10 and TGF β , and the expression of thousands of proteins in the body is also regulated by it [8]. TGF- β 1 is a classic inhibitory cytokine that inhibits the immune function of DCs in the tumor microenvironment. Down-regulating the expression of *FOXP3* can significantly reduce the secretion of TGF- β 1. It can be seen that *FOXP3* participates in the regulation of tumor microenvironment and the regulation of tumor local immunity [9]. Studies have reported that the high expression of *FOXP3* in tumor tissues due to the impaired tumor immune function leads to poor anti-tumor response. In breast cancer, cervical cancer, liver cancer, lung cancer and other tumor tissues, the increased expression of *FOXP3* is associated with poor anti-tumor response, but the high expression of *FOXP3* has been reported to be associated with good clinical results in patients with colorectal cancer [10-12]. However, few previous studies have paid attention to the immunotherapy value of *FOXP3* in human cancer.

In this study, the expression of *FOXP3* in 33 different cancers was demonstrated, and the potential impact of *FOXP3* on the tumor immune microenvironment was studied. At the same time, in this article, we studied the relationship between *FOXP3* expression and Tumor Mutational Burden (TMB), Microsatellite Instability (MSI) and other immunomodulatory and dynamic immune biomarkers. The relationship between *FOXP3* expression and three immunotherapy cohorts was analysed. In general, the research in this article provides a basis for the immunotherapy of *FOXP3* in cancer, which may provide directions for further experiments.

Materials and Methods

Data collection

The expression data, mutation data, survival data and pan-cancer clinical data of 33 cancers are from UCSC Xena: (<http://xena.ucsc.edu/>). For the treatment cohort, a systematic search was conducted to determine the immune checkpoint lockout cohort, which can be retrieved and reported complete clinical information publicly. This study finally adopted three immunotherapy cohorts: Engineered IL-15 cytokine mutein fused with anti-PD1 antibody can improve T cell function and anti-tumor immunity in tumors (GSE157893 cohort downloaded from GEO); use of Niwudan Treatment-resistant renal cell carcinoma (GSE67501 cohort downloaded from GEO); advanced urothelial carcinoma and atezolizumab intervention (IMvigor210 cohort downloaded from a previously published study) [13].

The clinical correlation between *FOXP3* expression and various cancers

Use the limma package in the R studio software to extract the expression level of the target gene *FOXP3* and analyse the difference of the target gene *FOXP3* to observe whether there is a difference between normal tissues and tumor tissues. We also investigated whether *FOXP3* expression is different between different clinical groups (gender, age, and tumor stage). At the same time, use the limma package, GSVA package and GSEA Base package in the R studio software to score gene activity.

According to the expression level of the target gene, it is divided into two groups of high and low expression. The survival package in R is used to perform Kaplan-Meier survival analysis to study the time-dependent value of *FOXP3* in 33 cancers. The contents of the study include overall survival (OS: The time from the start of treatment to death), disease-free survival (DFS: The period from the start of treatment to the recurrence of the disease), and disease-specific survival (DSS: The outcome index

changes to specific Death caused by disease), progression-free survival (PFS: the period from the start of treatment to the progression of the disease). p value<0.05 is considered to be a difference in survival between the high and low expression groups.

Analyse the potential relationship between *FOXP3* expression and immune-related factors

First, use the ESTIMATE package in the R studio software to calculate the matrix score and immune score of each case; ESTIMATE is a tool used to predict tumor purity and the presence of infiltrating matrix and immune cells in tumor problems [14]. The ESTIMATE algorithm is based on a single-sample GSEA and generates four final scores: Matrix score (the higher the score, the more stromal cells in the tumor tissue), the immune score (the higher the score, the more immune cells in the tumor tissue), and the comprehensive score (The higher the score, the higher the content of stromal cells and immune cells in the tumor tissue), and the purity of the tumor (the higher the overall score, the lower the purity of the tumor in the sample). Subsequently, use the ggplot2 package, ggpubr package and gg Extra package in the R studio software to analyse the correlation of the tumor microenvironment; use the e1071 package, pre-process Core package and Linna package in the R studio software to perform the immune cell infiltration analysis to obtain the immunity in each sample Cell content, followed by correlation analysis of immune cells to get the correlation between *FOXP3* expression in tumor tissues and immune cell content; then correlation analysis of immune genes, in addition, through the TISIDB website (<http://cis.hku.hk/TISIDB/index.php>) explored the potential relationship between *FOXP3* expression and immunosuppressive genes, immune stimulatory genes and MHC-related genes. Then highlight the two most relevant results and show them in the graph. Finally, in order to further study related signalling pathways, GSEA analysis was performed to determine the differential pathways between the low *FOXP3* expression group and the high *FOXP3* expression group. These pathways were from the Kyoto Encyclopaedia of Genes and Genome database. If the relevant signal pathway meets a specific standard (p <0.05), save the relevant signal pathway in the output graph. If the relevant signal pathway in the enrichment result is greater than 5, the top 5 most significant enrichment will be displayed. If the result of the collection pathway is less than 5, it shows all the enrichment pathways.

Immunotherapy response analysis

This study included and analysed three related independent immunotherapy cohorts. Under normal circumstances, immunotherapy produces four outcomes: Complete Remission (CR), Partial Remission (PR), Disease Progression (PD), and Stable Disease (SD). Perform immunotherapy analysis on three data sets to study whether the expression of target gene *FOXP3* affects the effect of patients receiving immunotherapy. In this study, those who achieved complete remission and partial remission were classified as the response group, and those who achieved disease progression and stable disease were classified as the non-response group, and then the two groups were compared.

Results

FOXP3 expression in 33 cancers

In order to better show the method and content of this research, we show the details in Figure 1. The 33 cancers involved in this research are shown in abbreviations. In Figure 2A, the expression of *FOXP3* is different in 33 types of tumors (CESC, CHOL, COAD, HNSC, KIRC,

LUAD, PRAD, SKCM, UCEC). In Figure 2B, *FOXP3* is highly expressed in elderly patients in the ESCA, LAML, LGG, and LUAD groups, but is low in elderly patients in the BRCA and SKCM groups. Figure 2C shows

that *FOXP3* expression is different in different stages of certain tumors, including ACC, COAD, ESCA, KICH, KIRC, KIRP, SKCM, STAD, TGCT, and THCA. Figure 2D shows the difference in the expression of

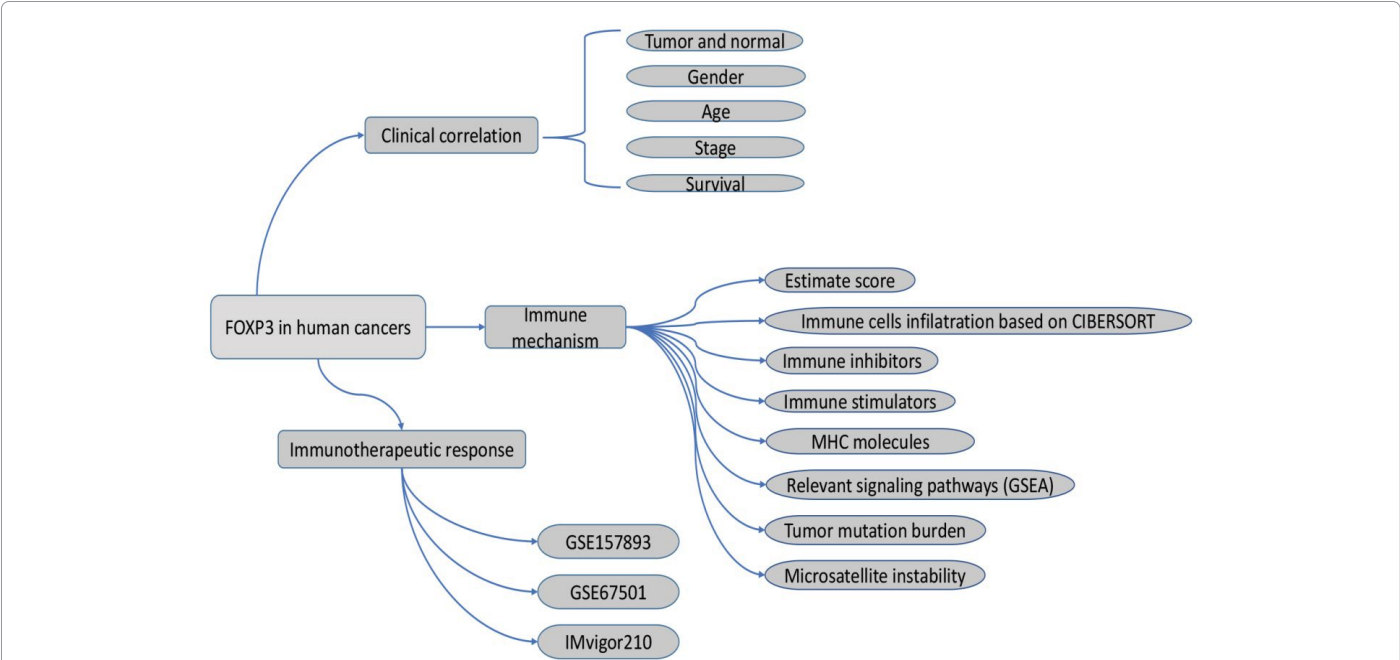


Figure 1: Shows the analysis and indicators used in this study. In the clinically relevant part, differential *FOXP3* expression analysis was performed between different tissues (tumor and normal), age (≤ 65 and >65), gender (male and female), and stage (I, II, III, IV). Kaplan-Meier was used to perform correlation analysis of survival analysis. In the part of immune mechanism, GSEA explored related signal pathways based on the expression of *FOXP3*. In the immunotherapy response part, Wilcoxon test was performed according to the expression of *FOXP3* in the response group and the non-reaction group.

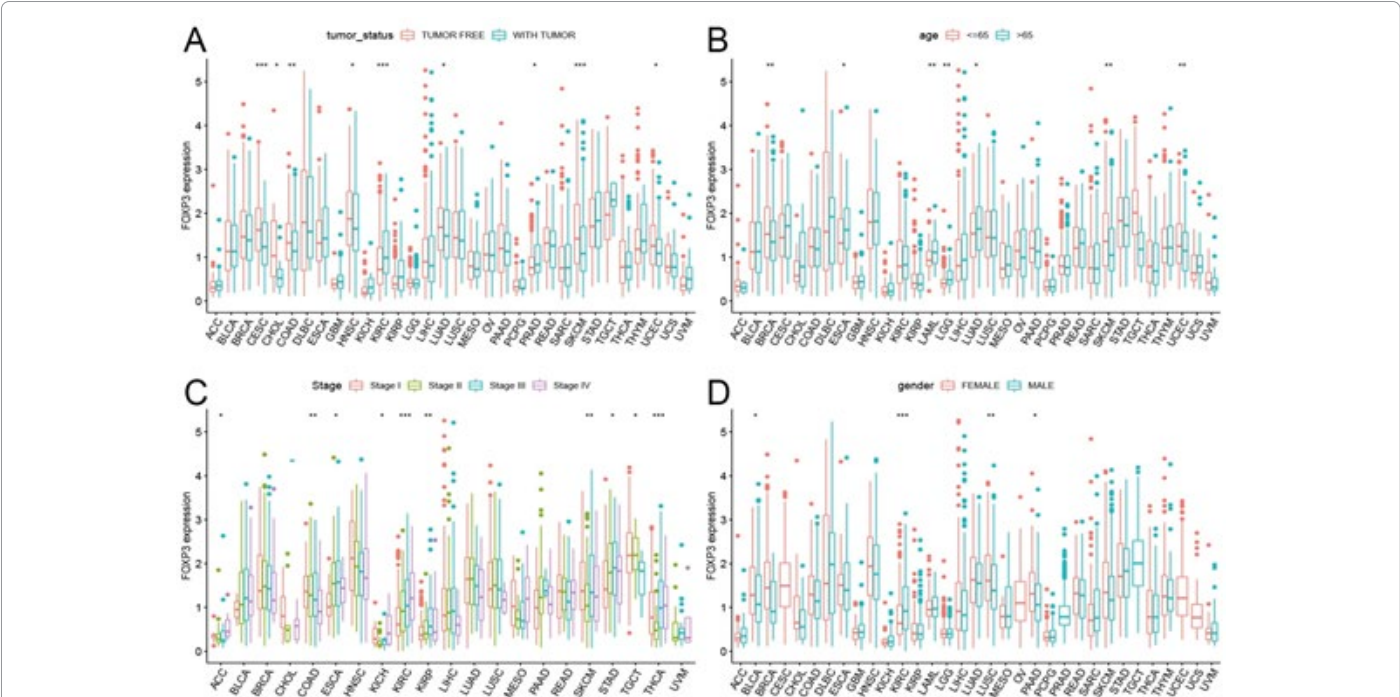
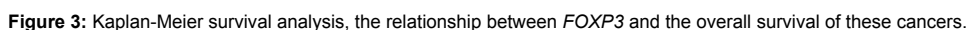


Figure 2: Clinical correlation analysis of *FOXP3*. (A) Shows the differential expression analysis of *FOXP3* tumor group and normal group in 33 cancers; (B) represents the correlation between age and *FOXP3*; (C) shows the correlation between tumor stage and *FOXP3*; (D) Shows the correlation between gender and *FOXP3*.
Note: *means $p < 0.05$, **means $p < 0.01$, and ***means $p < 0.001$.

Figure 5 shows the relationship between the expression level of *FOXP3* and the content of stromal cells and immune cells. There is a positive correlation in BLCA, CESC, CHOL, COAD, ESCA, HNSC, KICH, LUAD, LUSC, PAAD, READ, SKCM, THCA and UVM (Figure 6). In terms of immune cell infiltration, *FOXP3* expression is positively correlated with the content of Plasma cells in ACC and UVM; *FOXP3* expression is positively correlated with the content of T cells follicular helper in ACC; *FOXP3* expression is negatively correlated with NK cells activated in KICH and CHOL Correlation:



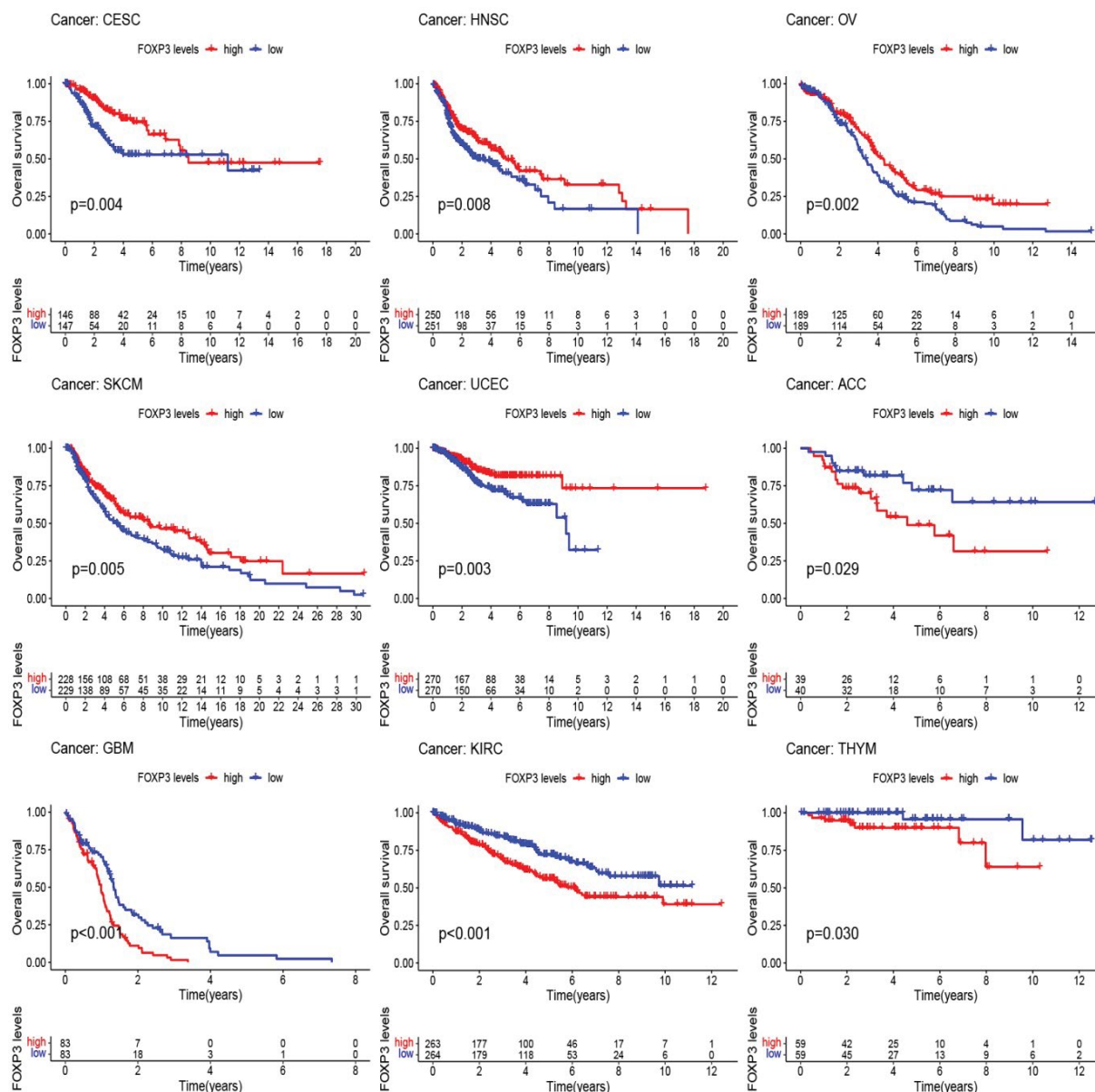


Figure 4: Kaplan-Meier survival analysis, the relationship between *FOXP3* and these cancers in disease-free survival, disease-specific survival, and progression-free survival.

FOXP3 expression is positively correlated with T cells CD8 in KICH, SARC, UVM. At the same time, the correlation between *FOXP3* expression and immunomodulatory was also studied. In the analysis of immunosuppressive agents, Figure 7 shows that *FOXP3* expression is positively correlated with BRCA TIGIT, and negatively correlated with THCA KDR. Figure 8 shows that in the analysis of immune stimulants, the expression of *FOXP3* is positively correlated with the ICOS of ESCA and negatively correlated with the CD276 of TGCT. Figure 9 shows that *FOXP3* expression is positively correlated with HLA-DRA of THCA, and negatively correlated with HLA-DMB of LGG. In Figure 10, considering the strong correlation between *FOXP3* and THCA, BRCA, TGCT, ESCA, and LGG, we conducted a GSEA analysis to study the relationship between *FOXP3* signalling pathways and their relationships. The pathway Cell adhesion molecule cam was enriched in the *FOXP3* high expression group in ESCA and THCA. In Figure

11, *FOXP3* expression is positively correlated with tumor mutation burden in THYM, UCEC, BRCA, and LGG, and negatively correlated with DLBC, HNSC, LUAD, LUSC, PAAD, and TGCT. In the correlation analysis of microsatellite instability, *FOXP3* is positively correlated with UCEC and THCA, but negatively correlated with DLBC, ESCA, HNSC, KIRC, LIHC, LUSC, OV, SKCM, STAD, TGCT.

***FOXP3* immunotherapy response**

In Figure 11, in the three immunotherapy cohorts involved in this study, there was no significant difference in the expression of *FOXP3* between the response group and the non-responder group. The observed trends indicate that it seems that patients with low *FOXP3* expression are more effective for immunotherapy.

Discussion

A comprehensive study of the differential expression of *FOXP3* in

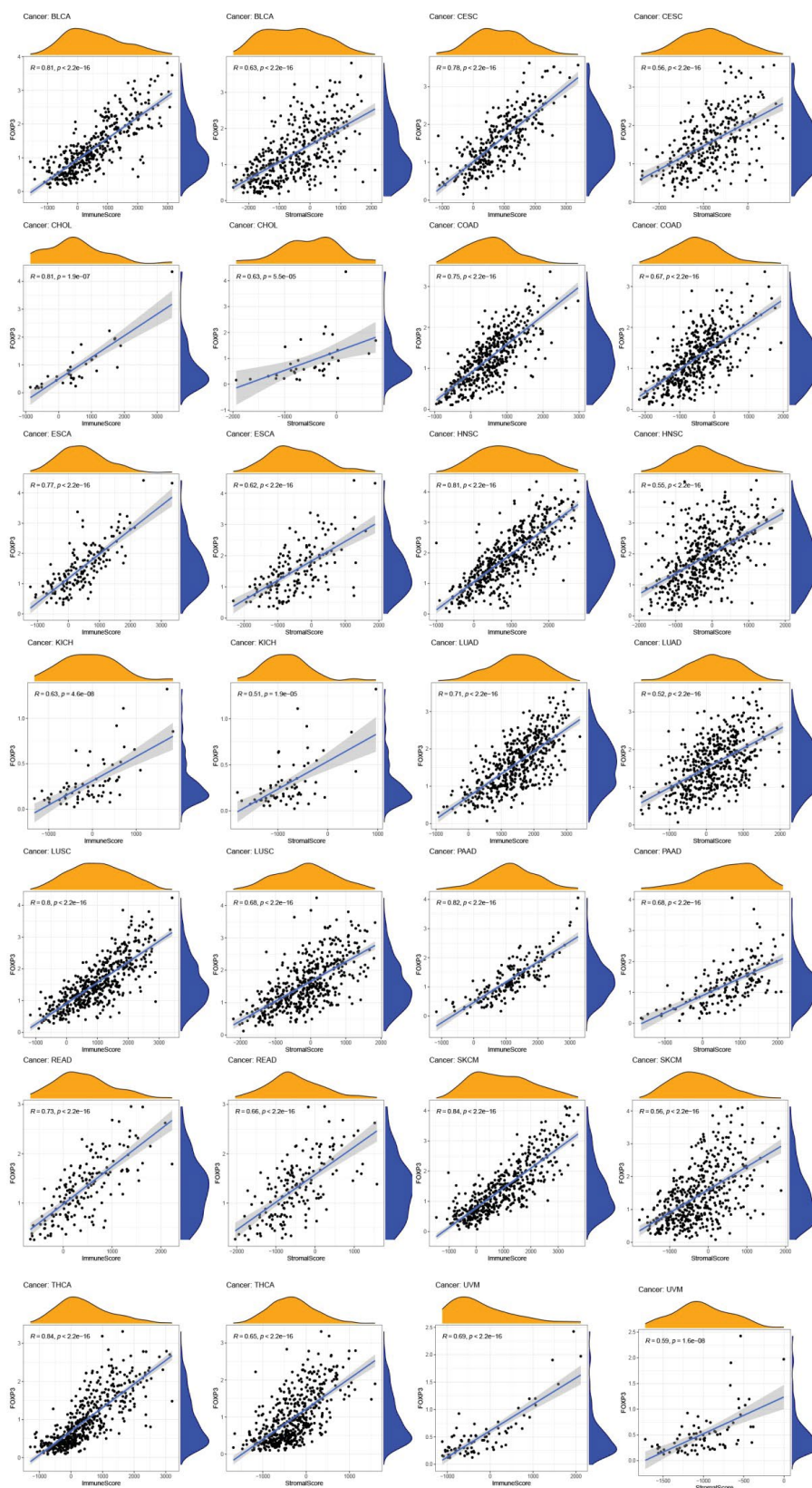


Figure 5: *FOXP3* expression and ESTIMATE score. ESTIMATE score includes matrix score (indicating whether there are stromal cells in tumor tissue) and immune score (indicating the infiltration of immune cells in tumor tissue).

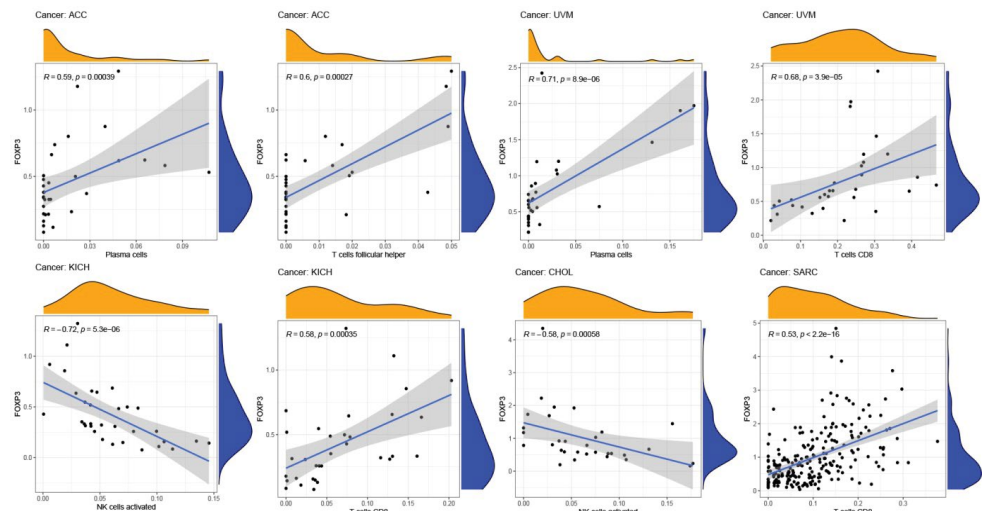


Figure 6: Correlation between *FOXP3* expression and immune cell infiltration. The CIBERSORT algorithm was used to calculate the immune cell infiltration, and the results of $R>0.5$ and $p<0.05$ were retained.

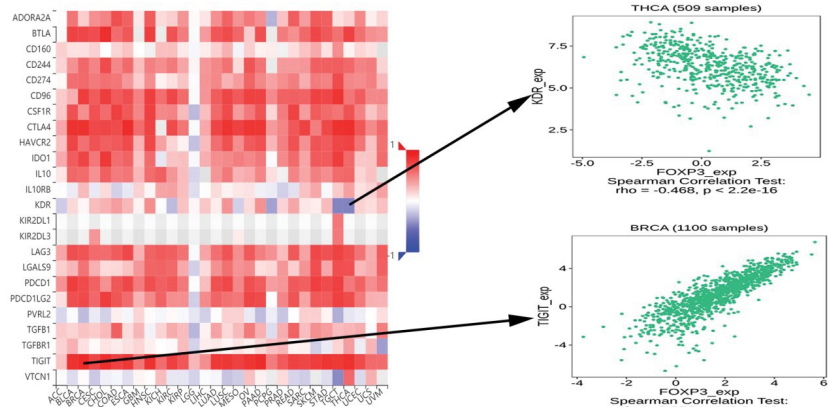


Figure 7: The correlation between *FOXP3* and the expression of immunosuppressive agents. Red indicates positive correlation, and blue indicates negative correlation. Take the top 2 strongest associations and display them through dot diagrams.

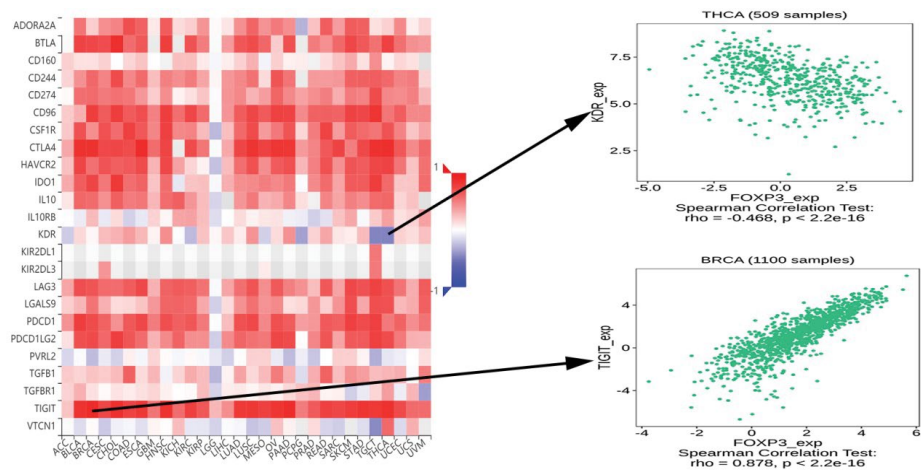


Figure 8: The correlation between *FOXP3* and the expression of immunostimulants. Red indicates positive correlation, and blue indicates negative correlation. Take the top 2 strongest associations and display them through dot diagrams

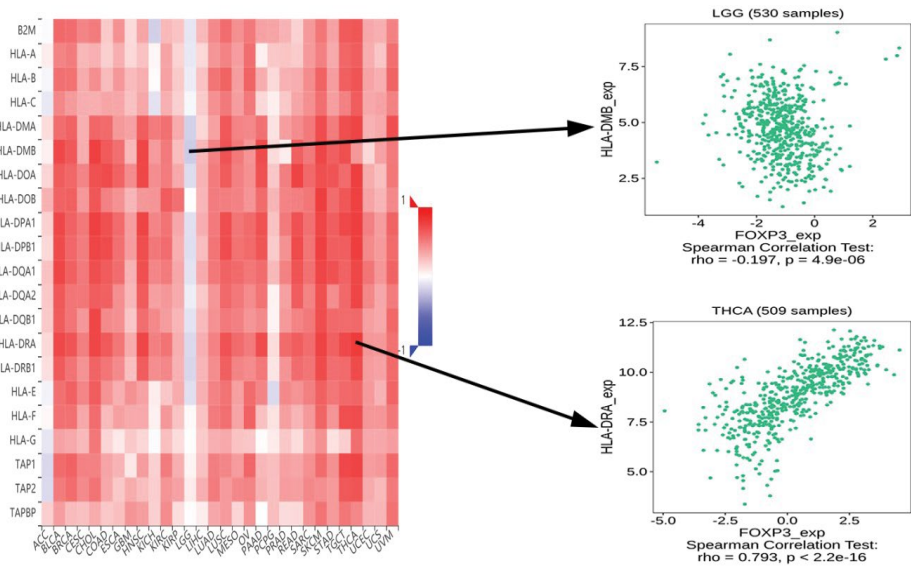


Figure 9: The correlation between *FOXP3* and MHC molecule expression. Red indicates positive correlation, and blue indicates negative correlation. Take the top 2 strongest associations and display them through dot diagrams.

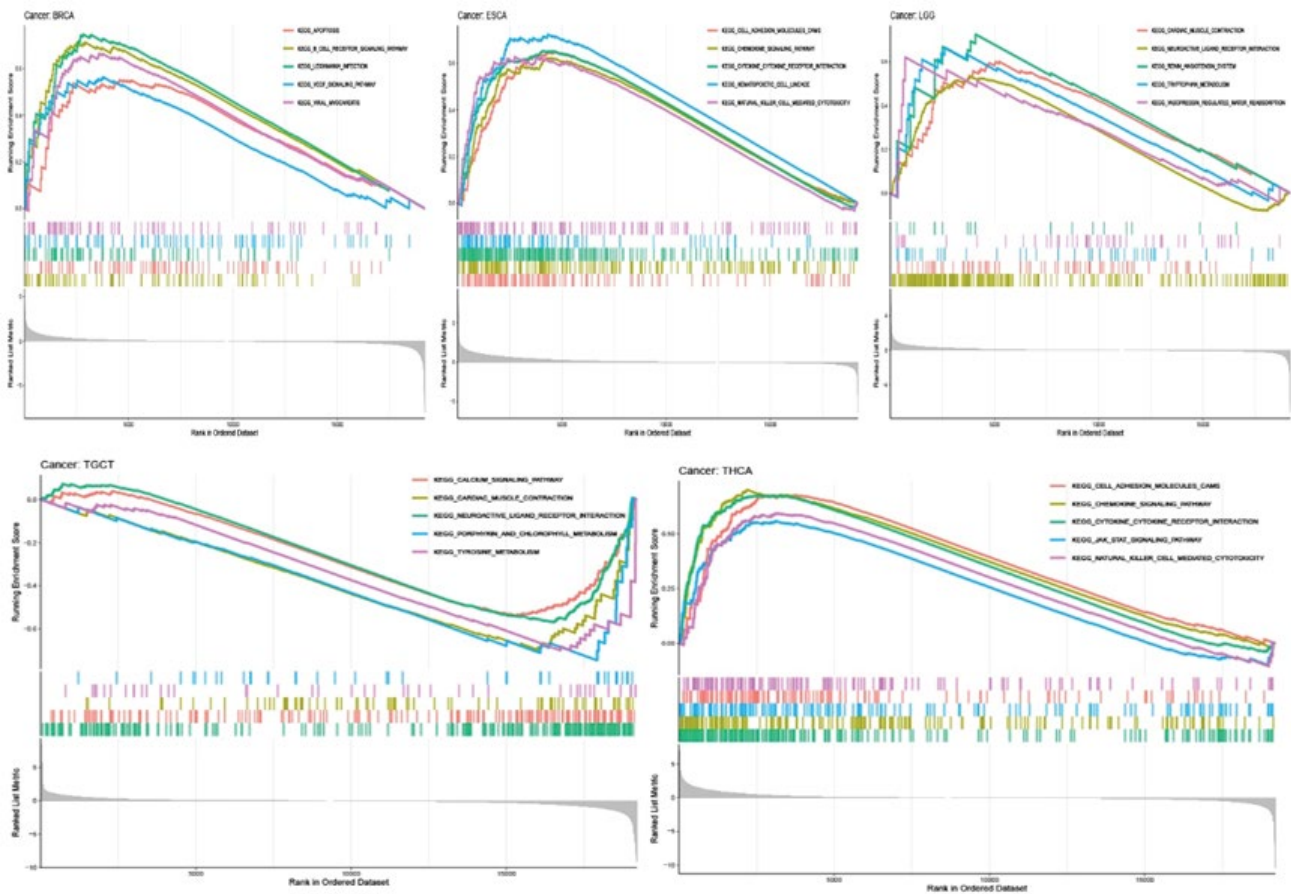
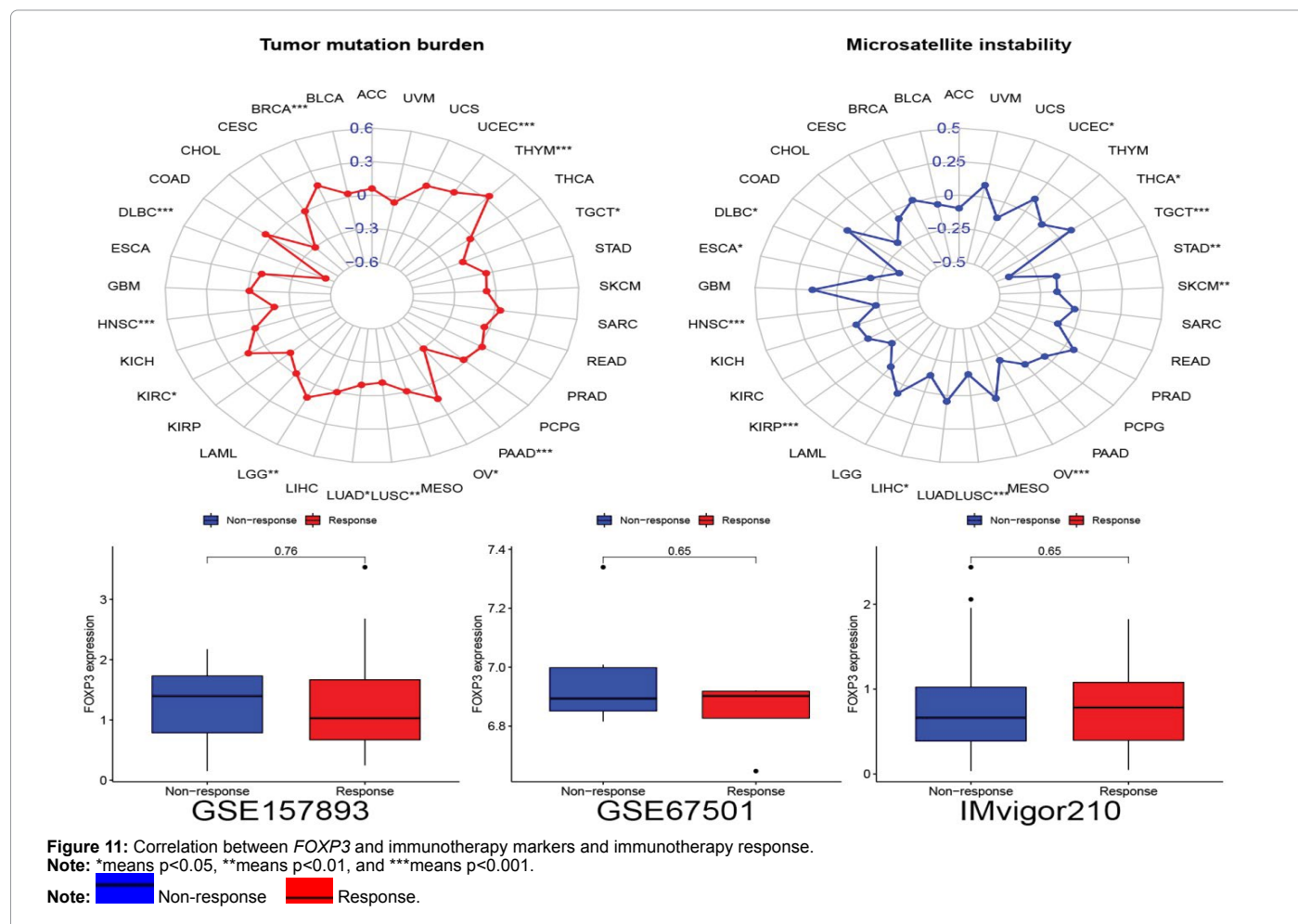


Figure 10: GSEA results based on KEGG database. In each graph, the curve on the left indicates that the pathway is enriched in the high *FOXP3* expression group, while the curve on the right indicates that the pathway is enriched in the low *FOXP3* expression group.



33 types of cancers, normal tissues and tumor tissues, has found the potential immunotherapy value of *FOXP3*. *FOXP3* not only participates in the regulation of tumor microenvironment, but also participates in the regulation of tumor local immunity. Therefore, we have conducted related research including tumor microenvironment, immune cells, and immunomodulatory and immunotherapy response. In this study, our goal is to gain a deeper understanding of the potential immunological association between *FOXP3* and 33 human cancers. First, we studied the correlation between *FOXP3* and clinical parameters and found that only a small number of cancers have significant differences in *FOXP3* expression with gender, age, and tumor stage, including CESC, CHOL, COAD, HNSC, KIRC, LUAD, PRAD, SKCM, UCEC. Amaral MGD found that the age difference between *FOXP3* expression and oral tongue squamous cell carcinoma was not significant [15]. Another study showed that in colorectal cancer, the expression of *FOXP3* is related to gender and Dukes staging [16], which is consistent with the results of this study. *FOXP3* has a certain prognostic value in certain cancers, and related studies have also shown that the high density of *FOXP3* in tumor tissues is a powerful independent prognostic marker related to mortality [17]. Many previous studies have considered *FOXP3* as an independent factor affecting the poor prognosis of various cancers. For example, in patients with tongue squamous cell carcinoma and Oropharyngeal squamous cell carcinoma, high expression of *FOXP3* is

associated with low overall survival rate [18,19]. In breast cancer, the expression of *FOXP3* in breast cancer tissue is significantly higher than that in normal breast tissue, and the increase in *FOXP3* expression in tumor tissue is related to poor prognosis [20,21]. The above content illustrates the usefulness of *FOXP3* in the prognosis of cancer. Therefore, we hypothesize that the regulation of *FOXP3* expression in various types of cancer may be clinically beneficial.

In order to further study the potential value of *FOXP3*, the correlation between *FOXP3* and immune cell infiltration was discussed. In terms of immune cell infiltration, *FOXP3* expression is positively correlated with plasma cell content in ACC and UVM. *FOXP3* expression is positively correlated with the content of T cells follicular helper in ACC; *FOXP3* expression is positively correlated with T cells CD8 in KICH, SARC and UVM. Previous studies have shown that CD8 and TGF- β 1 are the two main factors in the tumor immune microenvironment. CD8⁺ T cells are the most important effector executive cells in the tumor immune microenvironment. Inhibiting their activity will affect the body's immune defence function. *FOXP3* can help tumor cells escape by inhibiting or killing CD8⁺ T cells [22]. In KICH and CHOL, we observed that *FOXP3* expression is negatively correlated with NK cells activated. In the analysis of various immunosuppressive agents, we found that the expression of *FOXP3* was correlated with the TIGIT of TGCT, and negatively correlated with the KDR of TGCT. TIGIT is

an immunosuppressive receptor of T cell immunoglobulin, which is mainly expressed in T cells and natural killer cells, and can inhibit the function of immune cells through a variety of mechanisms to affect the prognosis of cancer patients [23,24]. Based on these data, we propose that there may be potential mechanisms for *FOXP3*, *TIGIT* and T cells, but this requires further experimental verification. In the analysis of immune stimulants and MHC molecules, the expression of *FOXP3* was positively correlated with ICOS and HLA-DRA of TGCT, but negatively correlated with CD276. This discovery may lead to CD276 becoming a new type of immunomodulatory. Considering the relationship between *FOXP3* and THCA, BRCA, TGCT, ESCA, LGG, we conducted a GSEA analysis to study the relationship between *FOXP3* signalling pathways and these cancers. The results showed that the pathway Cell adhesion molecules was enriched in the *FOXP3* high expression group in ESCA and THCA. There have been few reports on *FOXP3* and this pathway. The discovery of this pathway may provide some ideas for immunotherapy research. In addition, in this study, we found that tumor mutation burden and microsatellite instability are shown to be related to *FOXP3* in some cancers. Tumor mutation load can be used to predict the efficacy of immune checkpoint blockade and the evaluation of tumor neoantigen load, and it has become a biomarker in certain cancer types [25]. Microsatellite instability is a hallmark feature of DNA mismatch repair defects, and it is an important diagnostic and prognostic cancer biomarker [26]. *FOXP3* is positively correlated with TMB and MSI in UCEC, and negatively correlated with two immunotherapy biomarkers in DLBC, HNSC, and LUSC. This indicates that *FOXP3* has an indirect effect on the immunotherapy of UCEC, DLBC, HNSC, and LUSC. Finally, we studied the correlation between *FOXP3* and the three immunotherapy cohorts. However, no significant difference was found in the three immunotherapy cohorts. We hypothesized that *FOXP3* may be used to image immunotherapy response through *TIGIT*, ICOS, HLA-DRA, etc. Our research only involves three immunotherapy cohorts, and we cannot fully clarify the actual immunotherapy value of *FOXP3*. In the future, more immunotherapy cohorts should be studied.

This article studies the value of *FOXP3* in 33 cancer types, provides valuable insights into the role of *FOXP3* in cancer immunotherapy, and explores *FOXP3* and important immunological indicators (immune cell infiltration, immune modulators, and immune biology Markers). These findings may help to understand the potential link mechanism between *FOXP3* and immunotherapy. Not all of the 33 human cancers in this study showed an association with *FOXP3* in the tumor immune microenvironment, indicating that *FOXP3* only plays an immunological role in specific cancer types. These findings will help immune targeted therapy Research. However, considering that bioinformatics analysis was used in this study, the findings of the study are only preliminary. We also need to follow up with more relevant experimental verification.

Conclusion

This study is the first to explore the immunotherapy value of *FOXP3* in 33 human cancers, investigate the value of *FOXP3* in 33 cancer types, provide valuable insights into the role of *FOXP3* in cancer immunotherapy, and explore the role of *FOXP3* and *FOXP3* in cancer immunotherapy. We believe these findings will help provide new insights into tumor immunotherapy research.

Data Availability Statement

The datasets presented in this study can be found in online repositories.

The names of the repository/repositories and accession number(s) can be found in the article/Supplementary Material.

Author Contributions

Yiyang Chen and Xi Ou conceived of the presented idea. Data mining and gene analysis were carried out by Yiyang Chen, and Yiju Gong. Yiyang Chen and Jikui Liu wrote the manuscript. Yiyang Chen, Yiju Gong contributed to the manuscript revision. All authors contributed to the article and approved the submitted version.

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Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Declarations

Ethics approval and consent to participate

This study is approved by the Ethics Committee of Peking University Shenzhen Hospital.

Consent for publication

Not applicable

Competing interests

The authors declare that they have no competing interests.

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