Integrated Analysis of $C16orf54$ as a Potential Prognostic, Diagnostic, and Immune Marker across Pan-Cancer

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Chromosome 16 open reading frame 54 ($C16orf54$) is a protein coding gene, showing a biased expression in the bone marrow, lymph node, and 11 other tissues. Reports on the function of $C16orf54$ in the onset and development of tumours remain scarce. Clinical information and tumour expression profile data from The Cancer Genome Atlas (TCGA), Cancer Cell Line Encyclopedia (CCLE), and Genotype-Tissue Expression (GTEx) were utilized to determine the relationship between $C16orf54$ expression and prognosis, diagnosis, immune microenvironment, heterogeneity, and stemness across pan-cancer. The findings ascertained that $C16orf54$ was expressed at a low level in most cancers. Furthermore, $C16orf54$ could distinguish between cancer and normal tissues with high accuracy in most cancers, and the prognostic significance of low $C16orf54$ mRNA levels differed across cancers. $C16orf54$ expression was positively linked to the stromal, immune, and ESTIMATE scores. On the other hand, $C16orf54$ was reported to be negatively correlated with tumour purity in most cancers. Further, $C16orf54$ expression was positively correlated with immune cell infiltration and the expression of immune regulatory genes, including chemokines, receptors, major histocompatibility complexes, immune inhibitory, and immune stimulatory genes, in most cancers. Additionally, $C16orf54$ expression was significantly associated with tumour heterogeneity indicators, such as tumour mutation burden (TMB) and microsatellite instability (MSI), and was significantly correlated with DNAss and RNAss tumour stemness indicators. Moreover, Kyoto Encyclopaedia of Genes and Genomes (KEGG) analysis, as well as Gene Set Enrichment analysis (GSEA), revealed that $C16orf54$ expression was closely linked to the signalling pathways of immune cells and factors. The integrated analysis of $C16orf54$ indicates it as a potential prognostic, diagnostic, and immune marker, which could be adopted as a novel target for adjuvant immunotherapy across pan-cancer.

1. Introduction

The latest global cancer burden data issued by the International Agency for Research on Cancer (IARC) reported 19.29 million new malignancy individuals and nearly 10 million cancer-related mortalities globally in 2022 [1]. Reducing the cancer mortality rate remains a major hurdle as currently there is no absolute cure for cancer.

Recently, tumour immunotherapy, especially the advent of immune checkpoint inhibitors, has become another prominent and effective tumour treatment measure. Different from traditional chemotherapy and targeted therapy, immune checkpoint inhibition therapy does not directly act on tumour cells, but on the immune cells. It can recognize and kill tumour cells by restoring the previously "suppressed" immune system. The emergence of immunotherapy has greatly improved current cancer treatments. However, the interaction between tumour cells and the immune system is a continuous, dynamic, and evolving process. Furthermore, tumour malignancy largely depends on the development of the immune escape function of tumour cells [2], which hinders the efficacy of cancer immunotherapy. The main mechanism of tumour...
cell immune escape is the reduction of cytokine (CXCL9, CXCL10, and CCL3) secretion and killer immune cells, that is, T cells and natural killer (NK) cell infiltration levels. The increased secretion of IL-6, IL-33, and CXCL7 and the high expression of PD-L1 immune checkpoint ligand inhibit the function of T cells and reduce their infiltration. Various tumours have the potential to inhibit the effective recognition and killing of tumour cells by the immune system via various pathways, thereby producing immune tolerance and promoting tumour occurrence and development. The identification of biomarkers that can accurately predict the treatment response, aid in formulating individualised treatment regimens, and reduce the injury and burden caused by overtreatment and improper treatment is not only an urgent clinical need but also the biggest challenge faced by tumour immunotherapy [3–5].

Tumour multiomics databases, including The Cancer Genome Atlas (TCGA), offer endless possibilities for identifying novel diagnostic, therapeutic, and immunotherapy relevant markers. Chromosome 16 open reading frame 54 (C16orf54) is a protein coding gene which located at 16p11.2 and is expressed in 11 normal tissues, such as the bone marrow and lymph node. Moreover, the encoded protein is mainly located in the cell membrane. To the best of our knowledge, the role of C16orf54 in tumourigenesis, development, and immune remains unknown. In this study, multiple bioinformatics tools are used to integrate multiple multiomics high-throughput data, such as TCGA data, to analyse the differential expression of C16orf54 and its prognostic and diagnostic roles in pan-cancer. Furthermore, the link between C16orf54 expression and tumour immune microenvironment (TIME), tumour heterogeneity, and stemness are analysed. Additionally, the possible molecular mechanism of C16orf54 across pan-cancer is elucidated using Kyoto Encyclopaedia of Genes and Genomes (KEGG) analysis and Gene Set Enrichment analysis (GSEA).

2. Methods

2.1. Data Processing and Pan-Cancer Analysis of C16orf54 Expression. Normalised expression profile data and clinical data from TCGA and Genotype-Tissue Expression (GTEx) were retrieved from the website known as UCSC XENA (https://xenabrowser.net/datapages/) [6]. The Cancer Cell Line Encyclopedia (CCLE) data were downloaded from the DepMap portal (https://depmap.org/portal/download/). Besides, C16orf54 expression profile data were log2 transformed for comparison between groups. The Simple Nucleotide Variation dataset of level 4 of every TCGA sample was processed using the MuTect2 software and such data were downloaded from GDC (https://portal.gdc.cancer.gov/). The tmr function of the R software package “maftools” was used to calculate each tumour mutation burden (TMB). Based on the previous study [7], we obtained the microsatellite instability (MSI) score and the purity data for every tumour. Similarly, tumour stemness scores of DNA and RNA for each tumour were obtained from a previous study [8].

The R software (version 3.6.4) was used to compute C16orf54 expression across pan-cancer, and “ggplot2” was used for visualisation. The immunohistochemical results of C16orf54 protein were queried and downloaded from the Human Protein Atlas (HPA) website (https://www.proteinatlas.org/). p < 0.05 indicated statistical significance for the difference between tumour and normal tissues.

2.2. Prognostic and Diagnostic Significance Analysis. R software package “survival” for Cox regression analysis was utilized to study the relationship between C16orf54 expression and the prognosis of tumour patients, such as overall survival (OS), disease-specific survival (DSS), disease-free interval (DFI), and progression-free interval (PFI). Additionally, the prognostic differences between the two groups were analysed using “survminer” and “survival” R packages.

The potential value of C16orf54 expression in each tumour diagnosis was analysed using the R software package “pROC” and visualised using the “ggplot2” package.

2.3. Tumour Microenvironment (TME) Analysis. The stromal, immune, and ESTIMATE scores of each patient were calculated using the R software package “ESTIMATE.” The purity data of each tumour were obtained from a previous study [9], and the corr.test function of the R software package “psych” was used to calculate the Pearson’s correlation coefficient of C16orf54 expression in each tumour along with the immune infiltration score and tumour purity.

2.4. Analysis of Immune Cell Infiltration. The link between C16orf54 expression and each immune cell infiltration (p < 0.05) was analysed using the TIMER2.0 (http://timer.cistrome.org/) platform [10].

2.5. Analysis of Tumour Immunoregulatory Genes. Pearson’s correlation coefficient of C16orf54 and immunoregulatory gene expression in each tumour [11] was analysed using the corr.test function of the R software package “psych.”

2.6. Analysis of Tumour Heterogeneity and Stemness. Pearson's correlation coefficient between C16orf54 expression and TMB, MSI, DNA, and RNA in each tumour was computed using the corr.test function found in the R software package “psych.”

2.7. C16orf54 Functional Enrichment Analysis. The biological significance of C16orf54 expression in tumours was elucidated using the KEGG signal pathway analysis [12] and GSEA [13].

The R software package “stat” was utilized to compute the link between protein-coding genes and C16orf54 expression in each tumour, and the top 300 genes that positively linked to C16orf54 expression were obtained. The “clusterProfiler” and “org.Hs.eg.db” packages were used for KEGG enrichment analysis, using the “ggplot2” package for visualisation.

GSEA was performed using the “clusterProfiler” package, wherein the samples were categorized into two groups based on the C16orf54 expression levels, and the h.all.v7.4.symbols.gmt subset was retrieved from the Molecular Signatures Database to ascertain the correlation between pathways and molecular mechanisms [14], p.adjust < 0.05, and false discovery rate (FDR) < 0.25 were statistically significant.
2.8. Statistical Analyses. The difference in C16orf54 expression between normal tissues and different tumour cell lines was analysed with the aid of Kruskal–Wallis test. Pan-cancer analysis was performed utilizing unpaired Wilcoxon Rank Sum as well as Signed Rank Tests for significant difference analysis. Cox regression and log-rank tests were conducted to analyse the differences in survival time distribution in different groups. The link between C16orf54 expression and immune indexes was analysed by the Pearson’s test (no significant ns); \( p \geq 0.05; \quad * p < 0.05; \quad ** p < 0.01; \quad ***p < 0.001. \) The findings demonstrated that the area under the curve (AUC) value in the receiver operating characteristic (ROC) curve was closer to 1, indicating better diagnostic effects. AUC has low, certain, and high accuracy when it falls within 0.5–0.7, 0.7–0.9, and above 0.9, respectively.

3. Results

3.1. Differential Expression of C16orf54 across Pan-Cancer. C16orf54 expression was analysed across pan-cancer and normal tissues. Different tissues from GTEx showed significantly different C16orf54 expression (Figure 1(a), \( p < 0.001 \)), with high expression in the blood, spleen, small intestine, and other tissues. C16orf54 expression in different cell line from CCLE was also significantly different (\( p < 0.001 \)), with higher expression in haematopoietic and lymphatic cells (Figure 1(b), \( p < 0.001 \)). TCGA data analysis revealed that C16orf54 expression was substantially upregulated in glioblastoma multiforme (GBM), head and neck squamous cell carcinoma (HNSC), and kidney renal clear cell carcinoma (KIRC), whereas it was downregulated in bladder urothelial carcinoma (BLCA), breast invasive carcinoma (BRCA), colon adenocarcinoma (COAD), lung squamous cell carcinoma (LUSC), rectum adenocarcinoma (READ), liver adenocarcinoma (LUAD), uterine corpus endometrial carcinoma (UCEC), and thyroid carcinoma (THCA) compared to the corresponding samples from normal tissues (Figure 1(c)). TCGA integrated GTEx data showed that the C16orf54 gene was significantly upregulated in BRCA, cervical squamous cell carcinoma and endocervical adenocarcinoma (CESC), GBM, KIRC, ovarian serous cystadenocarcinoma (OV), HNSC, kidney renal papillary cell carcinoma (KIRC), brain lower grade glioma (LGG), liver hepatocellular carcinoma (LIHC), acute myeloid leukaemia (LAML), pancreatic adenocarcinoma (PAAD), stomach adenocarcinoma (STAD), testicular germ cell tumours (TGCT), skin cutaneous melanoma (SKCM), esophageal carcinoma (ESCA), and THCA but downregulated in BLCA, adenocortical carcinoma (ACC), lymphoid neoplasm diffuse large B-cell lymphoma (DLBC), LUSC, and thymoma (THYM) compared to their respective samples from normal tissues (Figure 1(d)). The analysis of paired samples revealed that C16orf54 was substantially upregulated in KIRC but significantly downregulated in BRCA, BLCA, COAD, LUSC, LUAD, UCEC, and THCA in (Figure 1(e)). Furthermore, immunohistochemical results showed that protein levels of C16orf54 in endometrial cancer, thyroid cancer, liver cancer, and urothelial cancer were decreased compared with those in normal tissues (Figures 1(f)–1(i)). Therefore, C16orf54 expression in various tissues and tumours is heterogeneous.

3.2. The Prognostic and Diagnostic Value of C16orf54. To explore the clinical significance of C16orf54 expression in tumours, the correlation between C16orf54 expression and the prognosis of patients, including OS, DSS, DFI, and PFI, and diagnosis in each tumour was analysed.

Cox proportional hazards regression model analysis confirmed that high C16orf54 expression was related to poor OS in LGG, LAML, pan-kidney cohort (KIPAN), and uveal melanoma (UVM), whereas low expression was associated with a poor prognosis in SKCM, SKCM-M, sarcoma (SARC), CESC, LUAD, LIHC, and OV (Figure 2(a)). Kaplan–Meier analysis revealed that low C16orf54 expression was related to poor OS in SKCM, LUAD, CESC, SKCM-M, and SARC (Figures 2(b)–2(f)), whereas high expression was associated with poor OS in UVM, LGG, LAML, and KIPAN (Figures 2(g)–2(j)). DSS analysis confirmed that the high expression of C16orf54 was linked to poor prognosis in LGG and UVM, but low expression was associated with a poor prognosis in SKCM, SKCM-M, CESC, SARC, LUAD, LIHC, THCA, and OV (Figure 2(k)). Forest plots of PFI revealed that high C16orf54 mRNA levels were associated with a poor prognosis in LGG and UVM while low levels were associated with a poor prognosis in LIHC, CESC, SKCM, SKCM-M, BRCA, LUAD, cholangiocarcinoma (CHOL), ACC, and SARC (Figure 2(l)). DFI analysis further revealed that low C16orf54 expression was associated with a poor prognosis in LIHC and OV (Figure 2(m)). Additionally, Kaplan–Meier analyses demonstrate the correlation between C16orf54 expression and DSS, DFI, and PFI. DSS analysis revealed that low C16orf54 expression was linked to a lower DSS in OV, SKCM, SKCM-M, SARC, CESC, and LUAD and a higher DSS in LIHC, UVM, and LGG (Supplementary Figure 1). DFI and PFI analyses showed that the low expression of C16orf54 was associated with a lower PFI in CESC, SKCM, SKCM-M, SARC, LUAD, LIHC, BRCA, CESC, LGG, and UVM and a lower DFI in LIHC and OV (Supplementary Figure 2).

Additionally, the ROC curve analysis revealed that C16orf54 had a high accuracy in anticipating tumour and normal outcomes in LAML (AUC = 0.98), LUSC (AUC = 0.98), PAAD (AUC = 0.98), GBM (AUC = 0.98), READ (AUC = 0.91), COAD (AUC = 0.90), had a certain accuracy in SKCM (AUC = 0.98), LIHC (AUC = 0.98), LGG (AUC = 0.98), CESC (AUC = 0.88), OV (AUC = 0.86), LUAD (AUC = 0.84), BLCA (AUC = 0.75), and DLBC (AUC = 0.73), but lower accuracy in other tumours (Figure 3).

3.3. Correlation between the TME and C16orf54 Expression. To explore the possible roles of C16orf54 in TME, the link between C16orf54 expression and TME index and tumour purity were analysed [15, 16]. C16orf54 expression was found to be substantially positively related to immune indicators, including stromal, immune, and ESTIMATE scores (Figure 4(a)). Additionally, it was negatively linked to tumour purity in the majority of the tumours (Figure 4(b)).
Gene expression of \( \text{C16orf54} \ \log_2 (\text{TPM+1}) \)

- Adipose tissue
- Adrenal gland
- Blood vessel
- Blood
- Bone marrow
- Brain
- Breast
- Cervix uteri
- Colon
- Esophagus
- Fallopian tube
- Heart
- Kidney
- Liver
- Lung
- Muscle
- Ovary
- Pancreas
- Pituitary
- Prostate
- Salivary gland
- Skin
- Small intestine
- Spleen
- Stomach
- Testis
- Thyroid
- Uterus
- Vagina

**GTeX**

- Kruskal–Wallis test \( p < 0.001 \)

**CCLE**

- Kruskal–Wallis test \( p < 0.001 \)

**Figure 1:** Continued.
The expression of C16orf54 log2 (TPM+1) was compared between normal and tumor tissues across different cancer types. The expression levels are displayed using box plots, with statistical significance indicated by asterisks; multiple asterisks denote higher significance levels.

**Figure 1: Continued.**
Figure 1: Continued.
3.4. Relationship between Immune Cell Infiltration and C16orf54 Expression. The relationship between C16orf54 expression and immune cell infiltration levels in different tumour types was evaluated utilizing the TIMER website tool. This tool serves as a comprehensive resource for immune infiltrate-related systematic analysis across different cancer types [10]. Although the results of different algorithms may vary slightly, the overall trend remains the same. This study shows that C16orf54 expression in the majority of tumour types was significantly positively linked to immune cell infiltration, including T cell CD4+, T cell CD8+, T cell regulatory (Tregs), B-cell, neutrophil, NK cell, monocyte, endothelial cell, macrophage, T cell follicular helper, and myeloid dendritic cell, and significantly negatively correlated with common lymphoid progenitor and eosinophil and myeloid-derived suppressor cells infiltration. Additionally, the correlation between C16orf54 expression and the immune cell infiltration of cancer-associated fibroblast, common myeloid progenitor, granulocyte-monocyte progenitor, T cell gamma delta, hematopoietic stem cell, T cell NK and mast cell across pan-cancer was not significant (Figure 5).

3.5. Relationship between Immunoregulatory Genes and C16orf54 Expression. To further investigate the role of C16orf54 in TIME, the link between C16orf54 expression and immunoregulatory genes in various tumour types was analysed. The Pearson’s correlation coefficient between C16orf54 expression and 60 immune checkpoint genes, including 24 inhibitory genes and 36 stimulatory genes (Figure 6), was calculated, which showed that almost all immune checkpoint genes, except IFNA1 and IFNA2, were significantly positively coexpressed with C16orf54 in most tumours. Furthermore, the Pearson’s correlation analysis of C16orf54 and immunoregulatory genes (Figure 7), including 41 chemokine, 18 receptor, and 21 major histocompatibility complex (MHC), showed that C16orf54 was significantly positively coexpressed with most immunomodulatory genes across pan-cancer.

3.6. Analysis of the Tumour Heterogeneity, Stemness, and C16orf54 Expression. Tumour heterogeneity can modulate immunotherapy effects. Therefore, the Pearson’s correlation coefficients between C16orf54 and tumour heterogeneity indicators, TMB and MSI, were analysed. C16orf54 expression was discovered to be significantly negatively related to TMB in CHOL, ACC, LUAD, STAD, and BLCA; significantly positively associated with TMB in CRC, COAD, OV, and UCEC (Figure 8(a)); and significantly negatively related to MSI in DLBC, TGCT, ACC, KIPAN, KIRP, GBM, LGG, LUSC, STAD, HNSC, and OV (Figure 8(b)).

Tumour stemness is not only associated with metastasis and heterogeneity, but also with immune checkpoint gene expression and cell infiltration of the immune system [8]. The analysis of C16orf54 expression and tumour stemness indicators, DNAss and RNAss, suggested that C16orf54 expression was substantially negatively correlated with DNAss in 15 tumour types (Figure 8(c)), including THYM, GBM, and LUSC; significantly positively correlated with DNAss in 7 tumour types, including LGG, KIRP, and THCA; significantly negatively correlated with RNAss in 30 tumour types (Figure 8(d)), including GBM, LGG, CESC, and LUAD; and significantly positively correlated with RNAss in THYM.
| Cancer         | HR (95%CI)     | p value |
|----------------|----------------|---------|
| SKCM (N = 444) | 0.85 (0.80–0.90) | <0.001  |
| LGG (N = 474)  | 1.40 (1.20–1.64) | <0.001  |
| SKCM–M (N = 347) | 0.86 (0.81–0.92) | <0.001  |
| LAML (N = 209)  | 1.36 (1.16–1.61) | <0.001  |
| SARC (N = 254)  | 0.85 (0.77–0.93) | <0.001  |
| UVM (N = 74)   | 1.30 (1.09–1.55) | 0.003   |
| CESC (N = 273) | 0.83 (0.72–0.95) | 0.007   |
| LUAD (N = 490)  | 0.86 (0.77–0.96) | 0.008   |
| LIHC (N = 341) | 0.87 (0.77–0.98) | 0.02    |
| OV (N = 407)   | 0.93 (0.87–0.99) | 0.03    |
| KIPAN (N = 855) | 1.10 (1.00–1.20) | 0.04    |
| BRCA (N = 1044) | 0.91 (0.82–1.01) | 0.06    |
| SKCM–P (N = 97) | 0.83 (0.68–1.01) | 0.06    |
| TGCT (N = 128)  | 2.52 (0.76–8.37) | 0.12    |
| CHOL (N = 33)  | 0.83 (0.64–1.07) | 0.15    |
| LUSC (N = 468) | 1.07 (0.97–1.19) | 0.18    |
| DLBC (N = 44)  | 1.36 (0.87–2.13) | 0.18    |
| UCS (N = 55)   | 1.12 (0.92–1.35) | 0.25    |
| GBM (N = 144)  | 1.10 (0.90–1.34) | 0.34    |
| HNSC (N = 509) | 0.97 (0.90–1.04) | 0.41    |
| ACC (N = 77)   | 0.94 (0.81–1.09) | 0.42    |
| PAAAD (N = 172) | 0.96 (0.86–1.07) | 0.43    |
| STAD (N = 372) | 1.04 (0.94–1.14) | 0.44    |
| THCA (N = 501) | 0.88 (0.64–1.22) | 0.45    |
| STES (N = 547) | 1.03 (0.96–1.10) | 0.47    |
| KIRC (N = 515) | 0.96 (0.85–1.08) | 0.47    |
| KICH (N = 64)  | 1.14 (0.78–1.67) | 0.5     |
| THYM (N = 117) | 0.86 (0.54–1.35) | 0.5     |
| MESO (N = 84)  | 0.94 (0.79–1.13) | 0.51    |
| ESCA (N = 175) | 1.04 (0.93–1.16) | 0.52    |
| BLCA (N = 398) | 0.98 (0.91–1.05) | 0.52    |
| UCEC (N = 166) | 0.97 (0.81–1.16) | 0.74    |
| PCPG (N = 170) | 0.92 (0.50–1.72) | 0.8     |
| COAD (N = 278) | 0.99 (0.86–1.14) | 0.87    |
| READ (N = 90)  | 0.98 (0.72–1.32) | 0.89    |
| PRAD (N = 492) | 1.01 (0.63–1.62) | 0.96    |
| KIRP (N = 270) | 1.00 (0.80–1.25) | 0.99    |

Figure 2: Continued.
Figure 2: Continued.
| Cancer   | HR (95%CI) | p value |
|----------|------------|---------|
| SKCM (N = 438) | 0.85 (0.79–0.91) | <0.001 |
| LGG (N = 466)   | 1.45 (1.23–1.70) | <0.001 |
| SKCM-M (N = 341) | 0.86 (0.80–0.92) | <0.001 |
| CESC (N = 269)   | 0.76 (0.65–0.88) | <0.001 |
| SARC (N = 248)   | 0.84 (0.75–0.93) | <0.001 |
| LUAD (N = 457)   | 0.81 (0.71–0.93) | 0.004 |
| UVM (N = 74)   | 1.28 (1.07–1.54) | 0.007 |
| LIHC (N = 333)   | 0.84 (0.72–0.97) | 0.02  |
| THCA (N = 495)   | 0.59 (0.38–0.93) | 0.03  |
| OY (N = 378)   | 0.92 (0.86–0.99) | 0.03  |
| SKCM-P (N = 97) | 0.79 (0.63–1.00) | 0.05  |
| TGCT (N = 128)   | 2.74 (0.75–10.06) | 0.12  |
| BRCA (N = 1025)  | 0.90 (0.79–1.03) | 0.13  |
| CHOL (N = 32)   | 0.84 (0.65–1.10) | 0.2   |
| STAD (N = 351)   | 1.08 (0.95–1.22) | 0.23  |
| STES (N = 524)   | 1.05 (0.96–1.15) | 0.26  |
| COAD (N = 263)   | 1.12 (0.92–1.37) | 0.27  |
| KIPAN (N = 840)  | 1.06 (0.95–1.19) | 0.29  |
| ACC (N = 75)   | 0.92 (0.79–1.07) | 0.29  |
| GBM (N = 171)   | 1.12 (0.89–1.39) | 0.33  |
| READ (N = 84)   | 1.27 (0.77–2.11) | 0.36  |
| HNSC (N = 485)   | 0.96 (0.87–1.06) | 0.38  |
| KIRC (N = 504)   | 0.93 (0.80–1.09) | 0.39  |
| ESCA (N = 173)   | 1.06 (0.93–1.21) | 0.4   |
| MESO (N = 64)   | 0.92 (0.74–1.15) | 0.48  |
| THYM (N = 117)   | 0.84 (0.64–1.54) | 0.57  |
| PRAD (N = 490)   | 0.84 (0.43–1.64) | 0.61  |
| PCPG (N = 170)   | 1.20 (0.55–2.63) | 0.65  |
| BLCA (N = 385)   | 0.98 (0.90–1.07) | 0.66  |
| DLBC (N = 44)   | 1.14 (0.63–2.05) | 0.67  |
| KIRP (N = 273)   | 0.94 (0.72–1.25) | 0.68  |
| UCEC (N = 164)   | 1.05 (0.82–1.34) | 0.69  |
| LUSC (N = 418)   | 0.98 (0.84–1.13) | 0.74  |
| UCS (N = 53)   | 1.03 (0.85–1.24) | 0.76  |
| PAAD (N = 166)   | 0.98 (0.87–1.11) | 0.77  |
| KICH (N = 64)   | 1.01 (0.65–1.57) | 0.96  |

![Graph](image-url)
3.7. KEGG and GSEA Analysis. To determine the biological significance of C16orf54 in different tumour tissues, KEGG and GSEA analyses, were performed for 6 selected tumours. KEGG analysis revealed that C16orf54 and its coexpressed top 300 genes mainly participated in the immune-related pathways of the cytokine-cytokine receptor interaction, Th1, Th2, and Th17 cell differentiation, T cell receptor signalling pathway, and hematopoietic cell lineage in KIRC, LUAD, lung squamous cell carcinoma (LUSC), COAD, BLCA, and READ (Figures 9(a)–9(f)). Moreover, the pathways of cell adhesion molecules, chemokine signalling pathway, autoimmune thyroid disease, type 1 diabetes mellitus, primary immunodeficiency, and graft versus host diseases were enriched in KIRC and LUAD (Figures 9(a) and 9(b)), and the signalling pathway of asthma, allograft rejection, viral myocarditis, the internal immune network for IgA production, and Staphylococcus aureus infection were enriched in LUSC and READ (Figures 9(c) and 9(f)). Furthermore, GSEA revealed that C16orf54 expression was significantly related to various immune-related pathways, such as PI3K/Akt/mTOR and WNT/BETA CATENIN signalling pathways in BLCA (Figure 10(a)), IL2/STAT5, inflammatory response, IL6/JAK-STAT3, TNFA/NFKB, and interferon-gamma response signalling pathways in COAD, KIRC, LUAD, LUSC, and READ (Figures 10(b)–10(f)). Interestingly, apoptotic signalling pathways were enriched in various tumours (Figures 10(a)–10(f)). Hence, C16orf54 is closely related to various immune signalling pathways.

4. Discussion

Tumour malignancy is a significant threat to human health and life. In the past, clinical treatments mainly focused on surgery, radiotherapy, and chemotherapy, which included various limitations, such as prominent side effects, drug resistance, easy recurrence, and metastasis. Different from conventional treatment, tumour immunotherapy uses the human immune system to resist the attack and growth of tumour cells. It is currently one of the most promising therapies. Tumour immunotherapy usually includes cellular immunotherapy and immune checkpoint inhibitor therapy [4, 17]. Cellular immunotherapy directly recognises tumour
| Cancer  | AUC  | CI            |
|---------|------|---------------|
| LAML    | 1.000| 1.000–1.000   |
| TGCT    | 0.986| 0.974–0.999   |
| LUSC    | 0.968| 0.955–0.982   |
| PAAD    | 0.968| 0.948–0.987   |
| GBM     | 0.967| 0.954–0.979   |
| READ    | 0.917| 0.873–0.962   |
| COAD    | 0.902| 0.862–0.942   |
| SKCM    | 0.898| 0.881–0.916   |
| LGG     | 0.895| 0.879–0.911   |
| KIRC    | 0.883| 0.843–0.924   |
| OV      | 0.865| 0.825–0.905   |
| LUAD    | 0.84 | 0.801–0.880   |
| BLCA    | 0.759| 0.682–0.836   |
| DLBC    | 0.734| 0.661–0.807   |
| THYM    | 0.691| 0.645–0.737   |
| CESC    | 0.69 | 0.521–0.859   |
| BRCA    | 0.664| 0.623–0.705   |
| THCA    | 0.657| 0.581–0.733   |
| ACC     | 0.651| 0.565–0.737   |
| HNSC    | 0.636| 0.558–0.714   |
| OSCC    | 0.62 | 0.524–0.715   |
| UUCEC   | 0.615| 0.540–0.689   |
| KIRP    | 0.586| 0.479–0.694   |
| UCS     | 0.585| 0.483–0.688   |
| LIHC    | 0.578| 0.503–0.653   |
| CHOL    | 0.565| 0.385–0.744   |
| KICH    | 0.563| 0.441–0.684   |
| STAD    | 0.56 | 0.445–0.676   |
| ESCA    | 0.522| 0.365–0.680   |
| PRAD    | 0.521| 0.433–0.609   |

Figure 3: Continued.
cell surface proteins and induces cancer cell death by stimulating autoimmune cells. However, owing to the heterogeneity of tumour antigens and the failure of tumour-infiltrating T lymphocytes, the effect of cellular immunotherapy in treating solid tumours was unsatisfactory [18]. With the in-depth study of tumour immunotherapy and pathogenesis, immunomodulatory genes have become potential targets of immunotherapy, with immune checkpoint inhibitors widely used as tumour immunotherapy drugs. The immune checkpoint is a significant factor in tumour immune tolerance. The intervention of immune checkpoints can reactivate T cells, thereby inducing tumour cell death [19–21]. CTLA-4 inhibitor and PD-1 inhibitor (PD-1/PD-L1 inhibitor) have strong antitumour activities in different tumours including

Figure 3: The ROC curve of C16orf54. (a) The ROC results of C16orf54 across pan-cancer. (b–m) ROC curves of C16orf54 in various tumours.
C16orf54 is a protein-coding gene with heterogeneous expression in different normal and tumour tissues. Expression profile and immunohistochemical results analysis showed that C16orf54 expression in BLCA, BRCA, COAD, LUAD, LUSC, and other tumour tissues decreased significantly, suggesting that C16orf54 could play a role in the occurrence and development of different tumours. Furthermore, evaluating the association between C16orf54 expression and OS, DSS, DFI, and PFI showed that C16orf54 expression was significantly positively associated with the three microenvironment scores of most tumours and negatively correlated with tumour purity, suggesting that C16orf54 affects the TME. Interestingly, recent research has revealed that C16orf54 can affect the morphology of lipid droplets (LDs) that exist in a variety of cells and can be used to generate metabolic energy and cell membrane [28]. This suggests that C16orf54 may regulate homeostasis of cell energy supply and cell membrane and thereby affect the crosstalk between various cells in the TME of various tumours.

The tumour microenvironment (TME) significantly affects the survival, proliferation, immune escape, diffusion, metastasis, and clinical prognosis of tumour [25, 26]. As the main component of the microenvironment, stromal, immune, and estimate scores in the microenvironment change with the interaction between tumour cells and the microenvironment, which has a significant impact on the immune metabolism of tumour cells [27]. The correlation analysis between C16orf54 expression and TME confirmed that C16orf54 expression was significantly positively associated with the three microenvironment scores of most tumours and negatively correlated with tumour purity, suggesting that C16orf54 affects the TME. Interestingly, recent research has revealed that C16orf54 can affect the morphology of lipid droplets (LDs) that exist in a variety of cells and can be used to generate metabolic energy and cell membrane [28]. This suggests that C16orf54 may regulate homeostasis of cell energy supply and cell membrane and thereby affect the crosstalk between various cells in the TME of various tumours.

Furthermore, the function of C16orf54 in the modulation of the tumour immune microenvironment (TIME) was investigated. In addition to tumour cells, TIME also includes nontumour cells, such as fibroblasts, endothelial cells, stromal cells, and immune cells, that interact with tumour cells [29–31]. Immune cells in TIME, such as macrophages, lymphocytes, and NK cells, have a critical impact on tumour immune escape and tumour immunotherapy resistance. Previous studies have reported that the antitumour immune efficacy can be improved by modifying the TIME, such as reconstructing the immune

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**Figure 4:** The correlation between C16orf54 expression and TME. (a) Correlation between C16orf54 and stromal, immune and ESTIMATE scores. (b) The correlation between C16orf54 expression and tumour purity. *p < 0.05; **p < 0.01; ***p < 0.001.
microenvironment by inducing immune cell death in cancer cells, inhibiting glucose metabolism, and repolarising tumour-related macrophages [32]. Therefore, TIME is of great significance in tumour initiation, development, metastasis, and treatment. Immunotherapy shows obvious therapeutic differences among patients, which is largely attributed to the heterogeneity of TIME. Clinical studies have shown that the degree of immune cell infiltration has a critical impact on the prognosis of patients with tumours. Wang et al. report that the tumour-infiltrating IL17+ cells can activate the antitumour response of the TME [33], and M2 macrophages contribute to metastasis and invasiveness.
of lung and liver tumours [34, 35]. Therefore, immune cell infiltration could be used as an indicator of the prognosis of disease and response to treatment. In the present research, C16orf54 expression was remarkably positively associated with the infiltration level of different immune cells in most tumour types, especially UVM, UCEC, THYM, and SKCM.

Figure 6: C16orf54 expression and immune checkpoint genes. *p < 0.05.
suggesting that C16orf54 can participate in tumour progression and immunotherapy by regulating immune cell infiltration.

The immunomodulatory factor is the medium of communication between infiltrating immune cells and tumour cells. It recruits or expels immune cells into the TME and

Figure 7: C16orf54 expression and immunomodulatory genes. *p < 0.05.
Figure 8: Analysis of tumour heterogeneity and stemness. (a) The relationship between C16orf54 and mutational burden (TMB), (b) microsatellite instability (MSI), (c) DNAss, and (d) RNAss.
Graft–versus–host disease
Cytokine–cytokine receptor interaction
Cell adhesion molecules
Chemokine signaling pathway
Natural killer cell mediated cytotoxicity
Th17 cell differentiation
T cell receptor signaling pathway
Th1 and Th2 cell differentiation
Hematopoietic cell lineage
Antigen processing and presentation
Viral myocarditis
Autoimmune thyroid disease
Type I diabetes mellitus
Primary immunodeficiency
Allograft rejection
Graft–versus–host disease

Counts

Counts

Figure 9: Continued.
Asthma
Cell adhesion molecules
Cytokine–cytokine receptor interaction
Hematopoietic cell lineage
Th17 cell differentiation
Chemokine signaling pathway
Th1 and Th2 cell differentiation
Staphylococcus aureus infection
Intestinal immune network for IgA production
B cell receptor signaling pathway
Leishmaniasis
Inflammatory bowel disease
Viral myocarditis
Primary immunodeficiency
Allograft rejection

Counts
○ 10
○ 20
○ 29

Figure 9: Continued.
mediates immune cells to kill or protect tumour cells. The interaction between tumour cells and the immune system is regulated by various immune regulatory factors. Tumour cells inhibit the antitumour immune response and induce immune escape by upregulating immunosuppressive factors or downregulating immune activators [36–38]. Certain positive regulatory factors, such as CD27, CD28, CD30, ICOS, and negative regulatory factors, such as CTLA4, PD-1,
Figure 10: Continued.
BTLA, TIM3, and LAG3, at immune checkpoints affect the recognition and apoptotic ability of the immune system against tumour cells [39–41]. C16orf54 gene is coexpressed with various immune inhibitors, immune-stimulatory factors, chemicals and chemokine receptors, MHC genes, and other immunomodulatory genes across pan-cancer, suggesting that C16orf54 can regulate the expression of immunomodulatory genes or it is itself an immunomodulatory gene.

During tumourigenesis and development, the clone type of tumour cells changes constantly due to differences in gene mutation, expression, or methylation [8]. These highly heterogeneous tumour cells give rise to varying responses to various tumour treatments. TMB and MSI are closely related to the prognosis of many tumour types after immunotherapy and can be used as biomarkers to predict immunotherapy efficacy [42]. The tumour remission results and survival benefits after nivolumab treatment were significantly better than those after chemotherapy for patients with high TMB levels [43]. MSI detection is also widely used in the clinical treatment of patients with COAD [44]. This study found that C16orf54 expression was significantly negatively linked to TMB and MSI in five and ten tumour types, respectively, further suggesting that C16orf54 could potentially predict patient response to immunotherapy. The relationship between C16orf54 and tumour stemness was also analysed. Cancer stem cells have the ability to renew themselves and can produce heterogeneous tumour cells. They play an essential function in tumour survival, proliferation, metastasis, and recurrence [45–47]. DNAss is the dryness index derived based on methylation data while RNAss is the stemness indices calculated based on expression data. When the stemness index is closer to 1, the degree of cell differentiation tends to be lower, whereas the stem cells’ characteristics become stronger. This study observed that C16orf54 expression was negatively correlated with DNAss in most tumours, such as THYM, GBM, and LUSC; similarly, it was significantly negatively correlated with RNAss in all tumours except THYM. This indicates low C16orf54 expression corresponds to strong tumour cell stemness, thus promoting tumour proliferation and metastasis. Furthermore, this correlation can be used as a predictor of the efficacy of immune checkpoint inhibition therapy.

In the end, the molecular mechanisms by which C16orf54 functions were analysed by KEGG and GSEA. KEGG analysis showed that C16orf54 could be involved in cytokine receptor interaction, Th1, Th2, and Th17 cell...
The correlation between C16orf54 and the PFI and DFI of LUAD, LIHC, UVM, and LGG. Supplementary Figure 2: The DSS of C16orf54 and DSS in OV, SKCM, SKCM-M, SARC, CESC, Meier analysis. (A-I) The relationship of C16orf54 to verify the conclusions of this study.

Materials) to carry out molecular, cellular, and animal experimentation for providing learning materials and integrating data analysis tools. The authors thank SangerBox and Xiantaoxueshu APP for providing learning materials and integrating data analysis tools.

Data Availability
All datasets generated for this study are included in the article/Supplementary Material.

Conflicts of Interest
The authors declare no conflict of interest.

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Supplementary Materials
Supplementary Figure 1: the DSS of C16orf54 by Kaplan–Meier analysis. (A-I) The relationship of C16orf54 expression and DSS in OV, SKCM, SKCM-M, SARC, CESC, LUAD, LIHC, UVM, and LGG. Supplementary Figure 2: the PFI and DFI of C16orf54 by Kaplan–Meier analysis. (A-J) The correlation between C16orf54 expression and PFI and (K-L) DFI in various tumours. (Supplementary Materials)

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