Heliyon 10 (2024) e34461

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Research article

Pan-cancer analysis of the prognostic and immunological role of RPL4

Yan Liu [a], Wei Li [b], Shiyang Zhou [c], Min Cui [c,**,1], Lin Zhang [a,*,1]

[a] Department of Plastic Surgery and Burns, Zhuhai People's Hospital (Zhuhai Clinical Medical College of Jinan University), China
[b] Hepatobiliary and Pancreatic Surgery, Zhuhai People's Hospital (Zhuhai Clinical Medical College of Jinan University), China
[c] Thyroid&Galactophore Surgery, Zhuhai People's Hospital (Zhuhai Clinical Medical College of Jinan University), China

ARTICLE INFO

Keywords:
RPL4
Pan-cancer
Biomarker
Immunotherapy
Omics integrative analysis

ABSTRACT

Ribosomal proteins (RPs) play an important role in the overall stability, function, and integrity of ribosomes. Ribosomal protein L4 (RPL4), which is encoded by *RPL4*, is assumed to play different roles in different cancers due to the strong correlation between them. However, research based on the underlying mechanisms of this correlations is limited. Therefore, this study investigated the biological role of RPL4 in various cancers. The Cancer Genome Atlas (TCGA) and Genotype-Tissue Expression (GTEx) databases were used to compare the differential expression of RPL4 in tumor and normal tissues. The Sangerbox database and Kaplan–Meier method were employed to assess RPL4's impact on the prognosis of pan-cancer. Analyses using the cBioPortal tool, Shiny Methylation Analysis Resource Tool (SMART), and MethSurv provided insights into the methylation and epigenetic alterations of *RPL4*. Gene enrichment analysis revealed that *RPL4* is involved in ribosome biogenesis through multiple pathways, and its enrichment in signaling pathways directly or indirectly influence tumor development. Tumor Immune Single-cell Hub (TISCH) was used to analyze *RPL4* expression levels and cellular functions in the tumor microenvironment. Tumor Immune Estimation Resource Database 2.0 (TIMER2.0) and Tumor-Immune System Interactions Database (TISIDB) tools revealed that RPL4 affected the immune infiltration potential of tumors. Furthermore, the application of the ROC mapper and CellMiner databases indicated an association between RPL4 and sensitivity to multiple antitumor drugs. Additionally, RPL4 was found to remodel the tumor immune microenvironment, leading to the development of chemoresistance. In conclusion, the findings suggest that RPL4 can be used as a potential tumor biomarker and may serve as a target for immunotherapy in various cancers. Genetic testing of RPL4 provides a foundation for the diagnosis, prognosis, and treatment of clinical tumors.

1. Preface

Cancer is a significant global health concern, with escalating incidence and mortality rates worldwide [1]. Experts project that if the

* Corresponding author. Zhuhai People's Hospital (Zhuhai Clinical Medical College of Jinan University), No.79 Kangning Road, Zhuhai, Guangdong, 519000, China.
** Corresponding author. Zhuhai People's Hospital (Zhuhai Clinical Medical College of Jinan University), No.79 Kangning Road, Zhuhai, Guangdong, 519000, China.
E-mail addresses: cuimw@hotmail.com (M. Cui), zhangleen1983@163.com (L. Zhang).
[1] The corresponding authors have contributed equally to with work.

https://doi.org/10.1016/j.heliyon.2024.e34461
Received 8 May 2024; Received in revised form 29 June 2024; Accepted 9 July 2024
Available online 14 July 2024

current trends in the prevalence of major cancer types continue, the incidence of all cancers is expected to double by 2070 compared with 2020. This alarming trajectory raises the possibility that cancer will surpass cardiovascular disease as the leading or second-leading cause of premature death in many countries, posing a formidable challenge to increasing life expectancy [2,3]. In the United States, the overall cancer mortality rate reduced by 33 % since 1991, which is attributed to advanced treatment strategies. Despite this positive trend, the rising incidence of specific cancers, notably prostate, breast, and uterine cancers may still pose challenges to future societal progress [4].

The ribosome is an intracellular factory that facilitates protein synthesis and comprises of a small 40S and a large 60S subunit. These structurally distinct protein complexes consist of four rRNAs and approximately 80 small ribosomal protein (RP) subunits. In eukaryotic cells, rRNAs are transcribed in the nucleolus compartment, whereas RPs are synthesized in the cytoplasm and transported to the nucleolus for the assembly of ribosomal subunits [5]. *RPL4* is a member of the L4E family and encodes a protein that is a constituent of the ribosomal 60S subunit. Previous findings have implicated RPL4 in influencing tumor formation, either directly or indirectly, through its involvement in RP biogenesis. For instance, increased RPL4 expression has been observed in hepatocellular carcinoma (HC) tissues, where it interacts with c-Myb and activates pathways that promote HC development and metastasis [6,7]. Other studies have identified *RPL4* as a suitable reference gene for normalizing target gene expression in benign, junctional, and malignant ovarian tumors [8]. RPL4's role extends to the ribosomal pathway and plays a pivotal role in circulating tumor cells in colorectal cancer [9]. Furthermore, gene expression profiling has associated *RPL4* with poor prognosis in breast cancer, opening new avenues for research on triple-negative breast cancer [10].

However, information based on the members of the ribosomal family remains insufficient, and RPL4 is a contentious molecule implicated in a variety of cancers. Numerous studies have demonstrated that the RPL4 protein is inextricably linked to the expression, ubiquitination, and regulation of various proto-oncogenes, signalling pathways, protein translation, and apoptosis. A comprehensive pan-cancer analysis of RPL4 is lacking, and emerging evidence suggests the existence of a distinct class of ribosomes in cancer cells known as oncogenic ribosomes. These specialized ribosomes promote oncogenic translational programs, regulates cellular functions, and facilitates metabolic redistribution, thereby increasing the risk of malignancy. Recent studies have established links between mutations in RPs, aberrant ribosomes, and disease onset, all of which contribute to poor prognosis. This underscores the significance of ribosome-targeted therapies as a promising avenue for tumor treatment research [11]. Targeting ribosome biogenesis is a promising therapeutic strategy, and a deeper understanding of the constraints associated with RPL4 will enhance therapeutic approaches aimed at preventing cancer development and promoting regression. Therefore, this study aims to investigate the expression and prognostic role of RPL4, and the correlation between RPL4 and pan-cancer. Moreover, it aims to establish a new clinical foundation for diagnosis and treatment of different cancer types.

2. Materials and methods

2.1. Expression analysis of RPL4

Tumor Immune Estimation Resource 2.0 (TIMER2.0, http://timer.cistrome.org/) [12] was used to examine the differential expression of *RPL4* in tumor and normal tissues, and Sangerbox (http://sangerbox.com) was used to analyze *RPL4* expression in tumors for which TIMER2.0 did not have control data. Gene transcriptome data from the University of California Santa Cruz (UCSC) Xena (https://xenabrowser.net/datapages/) [13], was utilized and analyzed. Protein expression analysis of RPL4 was performed using the Clinical Protein Tumor Analysis Consortium dataset (CTPAC) from the University of Alabama at Birmingham Cancer Data Analysis Portal (UALCAN, https://ualcan.path.uab.edu/) [14]. The Human Protein Atlas (HPA, https://www.proteinatlas.org) provides expression profiles of normal human tissues, gene locations, and immunohistochemical images of tumor tissues [15].

2.2. Pan-cancer prognostic analysis of RPL4

The pan-cancer prognostic analysis used the Sangerbox database, and the pan-cancer dataset was obtained from the UCSC database in a unified and standardized format. The analyses included assessments of overall survival (OS), disease-specific survival (DSS), disease-free interval (DFI), and progression-free interval (PFI) for all types of cancer. A forest plot was generated using a one-way COX regression analysis.

2.3. Mutant personality analysis

The mutational profile of RPL4 in multiple cancers was assessed using the cBioPortal (http://www.cbioportal.org/) [16]. The distribution of methylation probes on the chromosomes was determined using the Shiny Methylation Analysis Resource Tool (SMART) App database (http://www. bioinfo-z. com/smartapp/) [17]. Multivariate survival analysis was conducted using MethSurv [18] (http://biit.cs.ut.ee/MethSurv/), an online tool. DNA methylation data was inserted into this tool.

2.4. Functional and enrichment analysis of RPL4

A protein-protein interaction (PPI) analysis was performed using the Search Tool for the Retrieval of Interacting Genes (STRING) database (http://cn.string-db.org/). To display the PPI network, Cytoscape version 3.10.1 was utilized. The "similar gene detection" characteristic of the Gene Expression Profiling Interactive Analysis database (GEPIA2, http://gepia2.cancer-pku.cn/) [19] was used to

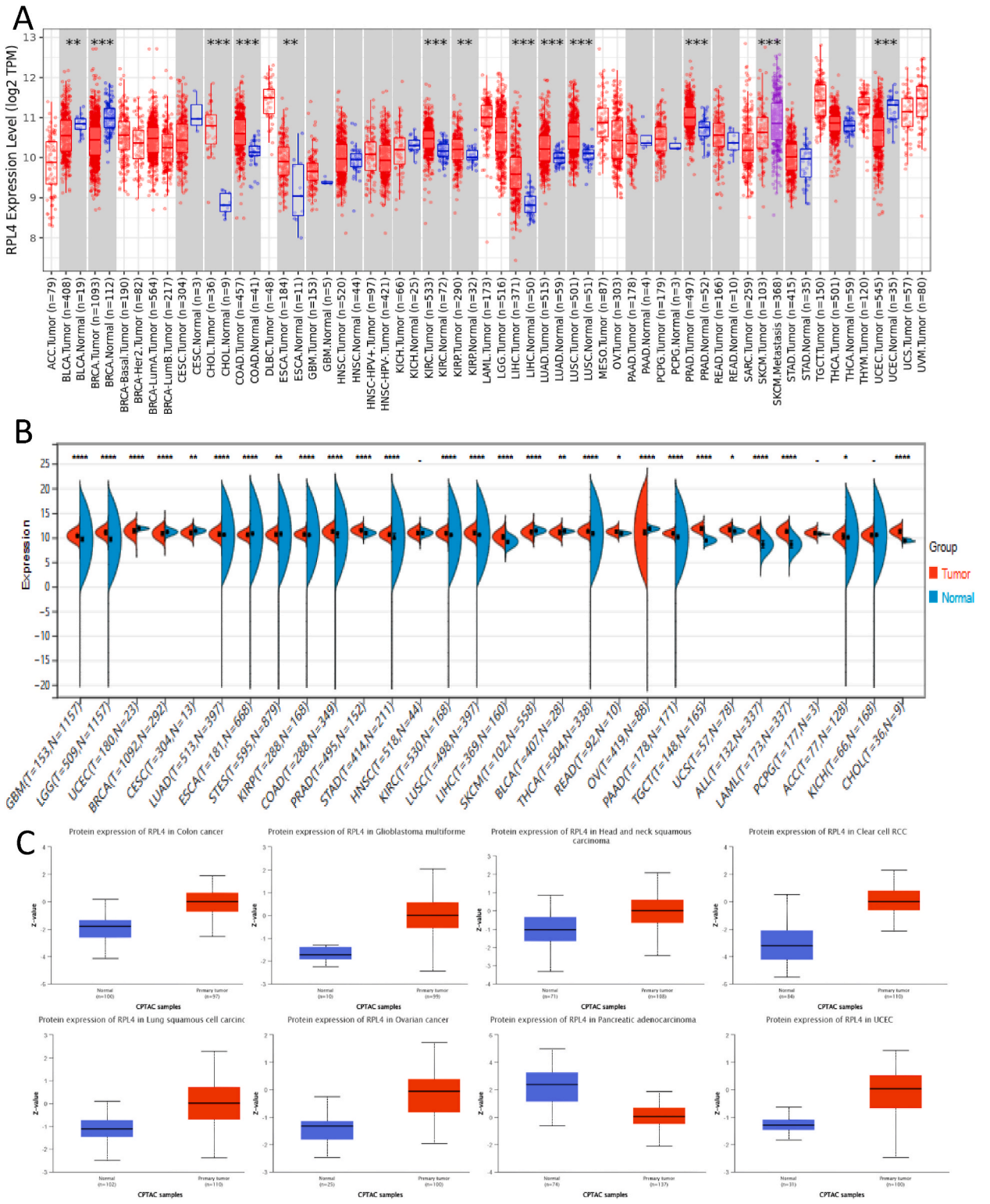

Fig. 1. RPL4 gene and protein expression in normal tissues and pan-cancer tissues. (A) Expression of RPL4 throughout pan-cancer tissues. (Blue represents normal tissues, red represents tumor tissues, and purple represents metastatic tumors. *P < 0.5; **P < 0.1; ***P < 0.01) (B) RPL4 mRNA expression levels were acquired by matching the TCGA and GTEx datasets. (C) The CPTAC demonstrates RPL4's overall protein expression level in both normal and malignant tissues. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

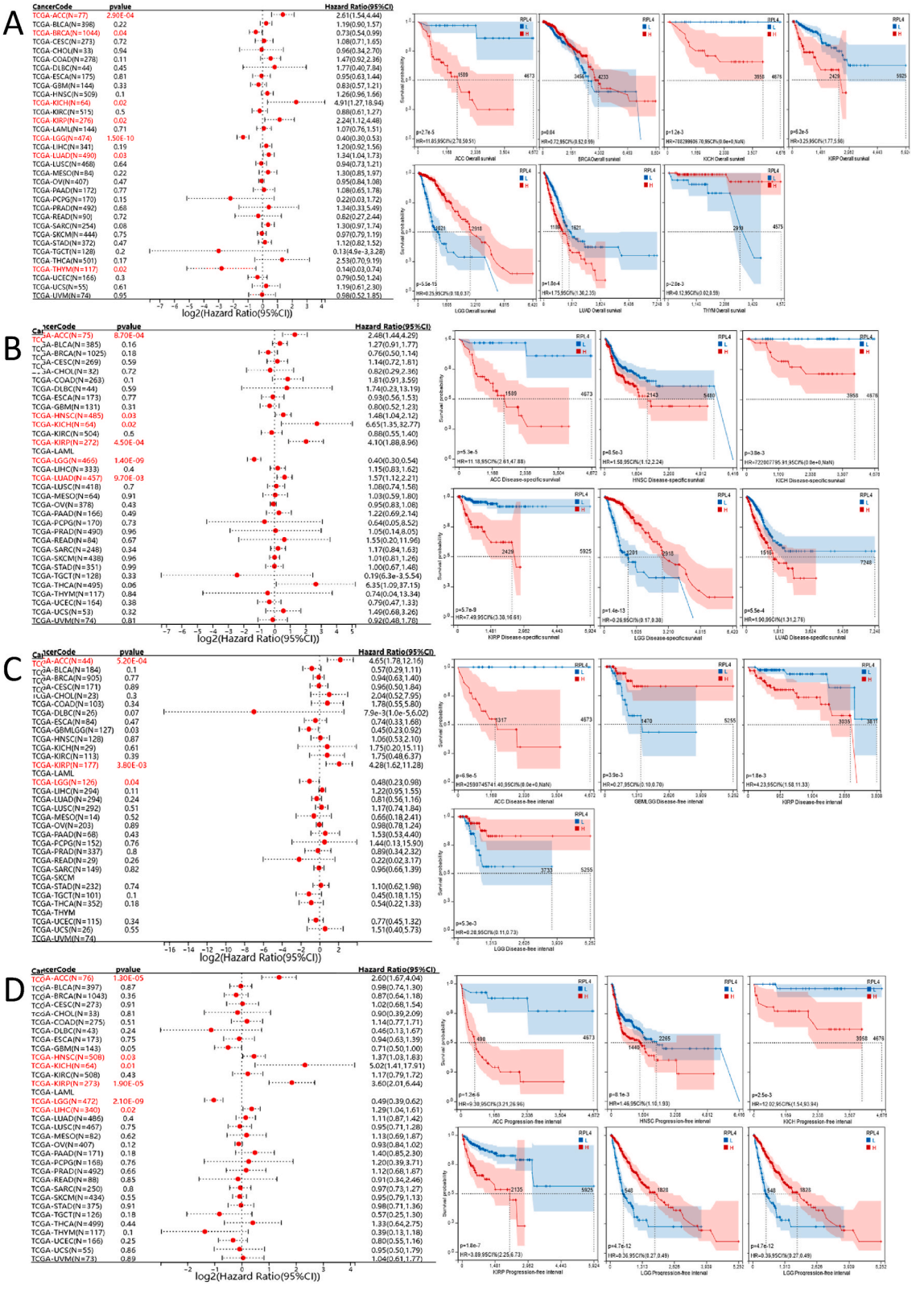

Fig. 2. Forest plot of survival analysis of RPL4 expression in 33 tumors based on the SangerBox database. (A) RPL4 expression and OS correlation. (B) RPL4 expression and DSS correlation. (C) RPL4 expression and DFI correlation. (D) RPL4 expression and PFI correlation.

detect 100 genes that have sequences similar to that RPL4. Heatmaps of the prognostic results of the co-members are available in the Survival Analysis section of the GEPIA 2 online tool. Using the Database for Annotation, Visualization, and Integrated Discovery (DAVID, http://david.ncifcrf.gov), co-expressed gene-based gene ontology (GO), and the Kyoto Encyclopedia of Genes and Genomes (KEGG) [20] were used. Functional enrichment study of the KEGG pathway encyclopedia was conducted. A range of enrichment analysis methodologies were supported by the WEB-based Gene Collection AnaLysis Toolkit (WEBGestalt, https://www.webgestalt.org/) [21]. From this, KEGG-enriched gene collection, which was then visualized using the Microbiotics online tool, was acquired.

2.5. Single-cell functional analysis

Tumor Immunosingle Cell Center (TISCH, http://tisch1.comp-genomics.org) [22] is an scRNA-seq database that focuses on the tumor microenvironment. Relevant single-cell studies have been conducted using this web-based program. Heatmaps and scatter plots were used to quantify and visualize the RPL4 expression levels in each cell type.

2.6. Immunoassay of RPL4

Tumor-Immune System Interactions Database (TISIDB, http://cis.hku.hk/TISIDB/index.php) [23] is an online resource tool that emphasizes the portal between tumors and the immune system. It was used to assess the probable relationship between RPL4 expression and various immunological subtypes of tumors. Additionally, it provides an analysis of immune cell infiltration and immunomodulation. In pan-cancerous tissues, the TIMER2.0 tool was used to examine the relationship between *RPL4* expression and 21 immune cell subpopulations. The SangerBox website addresses the association between the expression of RPL4 and tumor mutation load (TMB), microsatellite instability (MSI), and homologous recombination deficiencies (HRD).

2.7. Drug sensitivity analysis of RPL4

The ROC mapper tool (http://rocplot.org) [24] was utilized to assess the prognostic significance of RPL4 expression in predicting tumor therapy response. The CellMiner database (http://discover.nci.nih.gov/cellminer/) [25] provides web-based tools for accessing genomic and pharmacological data pertaining to the NCI-60 cell line. Using this database, we obtained RNA-seq expression data and NCI-60 chemoactivity data, and specifically selected Food and Drug Administration (FDA)-approved antitumor agents to investigate the correlation between RPL4 and drug sensitivity.

3. Results

3.1. Differential expression analysis of RPL4

The expression of *RPL4* was higher in cholangiocarcinoma (CHOL), colon adenocarcinoma (COAD), esophageal carcinoma (ESCA), glioblastoma multiforme (GBM), kidney renal clear cell carcinoma (KIRC), kidney renal papillary cell carcinoma (KIRP), liver hepatocellular carcinoma (LIHC), lung adenocarcinoma (LUAD), lung squamous cell carcinoma (LUSC), pheochromocytoma and paraganglioma (PCPG), prostate adenocarcinoma (PRAD), rectal adenocarcinoma (READ), and thyroid carcinoma (THCA) compared to that of normal tissue. However, the expression of *RPL4* was lower in bladder urothelial carcinoma (BLCA), breast invasive carcinoma (BRCA), cervical squamous cell carcinoma and endocervical adenocarcinoma (CESC), kidney chromophobe (KICH), pancreatic adenocarcinoma (PAAD), and uterine corpus endometrial carcinoma (UCEC) than in normal tissues (Fig. 1A). The Cancer Genome Atlas (TCGA) and Genotype-Tissue Expression (GTEx) databases were combined to assess the expression of RPL4 across various types of cancer. As shown in Figs. 1B and 27 widespread tumors had distinct expression characteristics of RPL4.

The expression profile of *RPL4* in normal human tissues was analyzed using the HPA database. The findings from the Consensus DataSet, which integrated data from the HPA and GTEx, revealed that the expression pattern of *RPL4* were not significantly restricted to any tissue. However, elevated levels of RPL4 mRNA were observed in the ovaries, tonsils, thorax, and bone marrow (Supplementary Fig. 1B) of patients with different cancer types. Supplementary Fig. 1 displays the expression of RPL4 in the HPA (A), GTEx (C), and FANTOM5 (D) datasets. The disparities in RPL4 protein expression levels among different cancer types were analyzed using the CPTAC dataset. The results showed an increase in total protein expression of RPL4 in tissues affected by COAD, GBM, head and neck squamous cell carcinoma (HNSC), KIRC, LUSC, ovarian serous cystadenocarcinoma (OV), and UCEC. However, in the case of PAAD, the level of RPL4 total protein exhibited was reduced compared to that in healthy tissues (Fig. 1C).

3.2. Prognostic analysis of RPL4 in pan-cancer

Having established the overexpression of RPL4 in most cancers, the potential correlation between RPL4 expression and pan-cancer prognosis was investigated. RPL4 was significantly expressed on the OS of patients with various types of cancers (Fig. 2A), including adrenocortical carcinoma (ACC, $p < 0.01$), BRCA ($p = 0.04$), KICH ($p < 0.05$), KIRP ($p < 0.05$), brain lower-grade glioma (LGG, $p < 0.01$), LUAD ($p < 0.05$), and thymoma (THYM, $p < 0.05$). A higher RPL4 expression was associated with a shorter OS rate in ACC, KICH, KIRP, and LUAD. Conversely, a shorter OS was associated with a lower RPL4 expression in BRCA, LGG, and THYM. After considering potential non-tumor death factors during the follow-up period, we examined tThe correlation between RPL4 expression and DSS was examined after considering potential non-tumor death factors during the follow-up period. As shown inIn Fig. 2B, it was

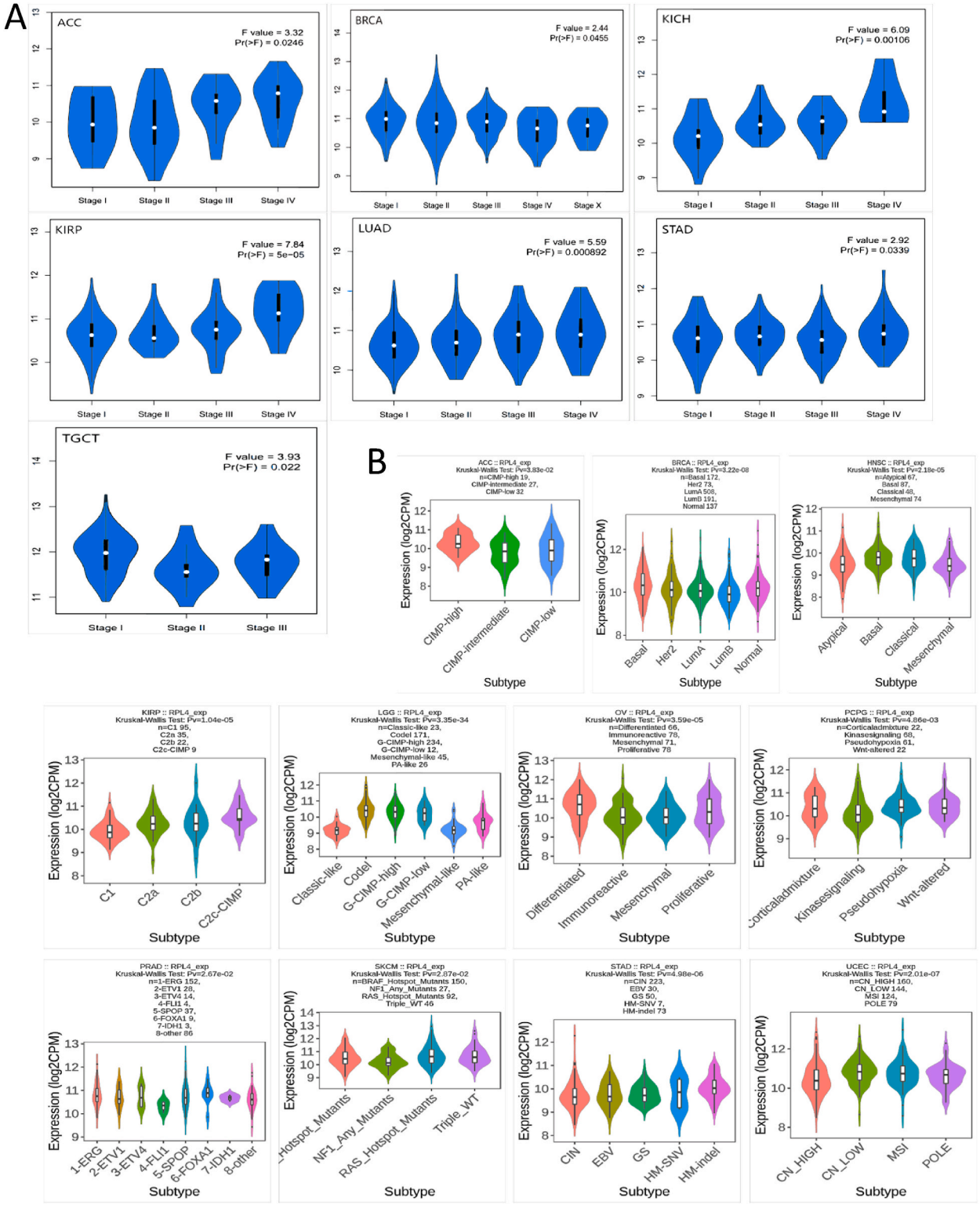

Fig. 3. Association of RPL4 gene expression with tumor stage and molecular subtype. (A) Correlation of RPL4 gene expression with tumor staging. (B) Correlation of RPL4 expression with molecular subtypes in tumors.

observed that a high we acquired that high expression of RPL4 was significantly associated with a higher risk of DSS shortening in ACC ($p < 0.01 = 8.7e-4$), HNSC ($p =< 0.053$), KICH ($p < 0.05 = 0.02$), KIRP ($p < 0.01 = 4.5e-4$), and LUAD ($p < 0.01 = 9.7e-3$). Conversely, a high RPL4 expression prolonged DSS in patients with LGG ($p < 0.01$). Regarding the relationship between RPL4 expression and DFI, it was found that a high expression of RPL4 was associated with longer a DFI in glioma (GBMLGG, $p < 0.05$) and LGG ($p < 0.05$), but a shorter DFI in ACC ($p < 0.01$) and KIRP ($p < 0.01$) (Fig. 2C). There was a strong positive correlation between a high RPL4 expression and a shorter PFI in ACC ($p < 0.01$), HNSC ($p < 0.05$), KICH ($p = 0.01$), KIRP ($p < 0.01$), and LIHC ($p < 0.05$). Additionally, there was a significant negative correlation between a high RPL4 expression and a shorter PFI (Fig. 2D).

3.3. Analysis of clinical stage and tumor molecular subtypes of RPL4 in pan-cancer

As shown in Fig. 3A, substantial differences in the expression of RPL4 between the clinical phases of ACC, BRCA, KICH, KIRP, LUAD, stomach adenocarcinoma (STAD), and testicular germ cell tumors (TGCT) were observed. The TISIDB database was used to investigate the relationship between RPL4 expression and tumor molecular subtypes (Fig. 3B). RPL4 exhibited distinct expression patterns in the molecular subtypes of 11 different malignancies: ACC, BRCA, HNSC, KIRP, LGG, OV, PCPG, PRAD, skin cutaneous melanoma (SKCM), STAD, and UCEC.

3.4. Methylation and epigenetic analysis of RPL4 in pan-cancer

DNA methylation was one of the first forms of epigenetic modification of genes to be identified. Abnormal DNA methylation is considered one of the hallmarks of cancer. If epigenetic changes in genes are not detected in time and continue to evolve, they will cause genetic changes such as mutations. As shown in Fig. 4A, the prevalence of *RPL4* mutations in pleural mesothelioma was reported to be 3.45 %, with most mutations occurring in the form of "Amplification." However, most types of *RPL4* gene mutations were found in breast cancer, including "Mutation," "Structural Variant," "Amplification" and "Multiple Alterations." Therefore early detection of abnormal methylation sites and expression profiles of tumour-associated genes enables early prevention and treatment. Because DNA methylation is mainly targeted at CpG islands present in regulatory regions, such as gene promoters or enhancers [26]. We leveraging the SMART App and MethSurv databases, we did research into the methylation level analysis of the RPL4 gene's CpG islands in patients with pan-cancer. A total of 23 methylation probes were discovered for RPL4, as shown in Fig. 4B, all of which are located on human chromosome 15.

Additionally, in BLCA, COAD, KIRC, LIHC, LUAD, LUSC, PRAD, READ, and UCEC, the methylation level of *RPL4* probes were considerably lower than that in normal tissues, whereas it was greater in BRCA and KIRP (Supplementary Fig. 2A). Based on the dataset in the database (Supplementary Fig. 2B), the DNA methylation status of *RPL4* in patients with LIHC were examined. For the probes of the CpG island of *RPL4*, the methylation levels of CG13048962, CG20792206, and CG04866357 were positively correlated with *RPL4* expression, while CG17352276, CG00090787, CG18091264, CG12660445, CG18864581, and CG16035638 had a negative correlation with methylated gene expression.

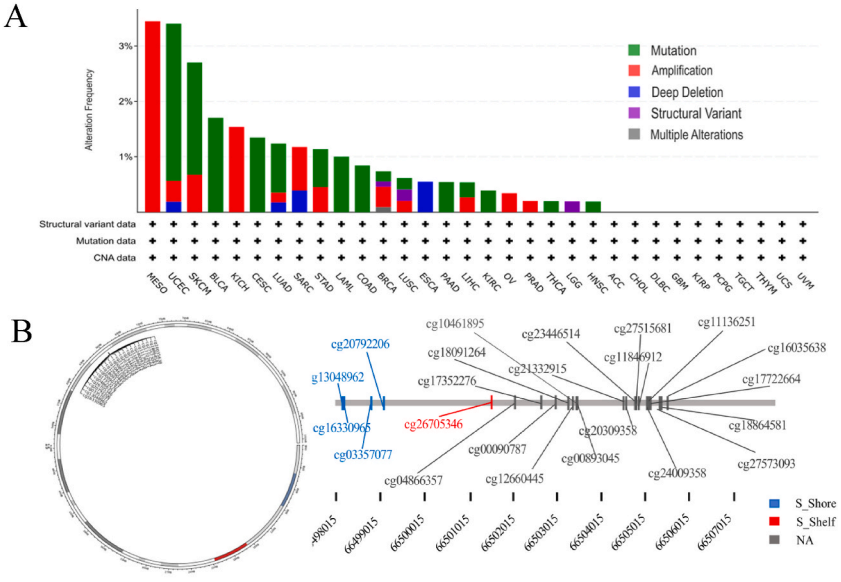

Fig. 4. Gene mutation types and RPL4 probe distribution in pan-cancer. (A) Analysis of RPL4 gene mutation types within different tumor tissues. (B) Chromosomal distribution of RPL4-related methylation probes.

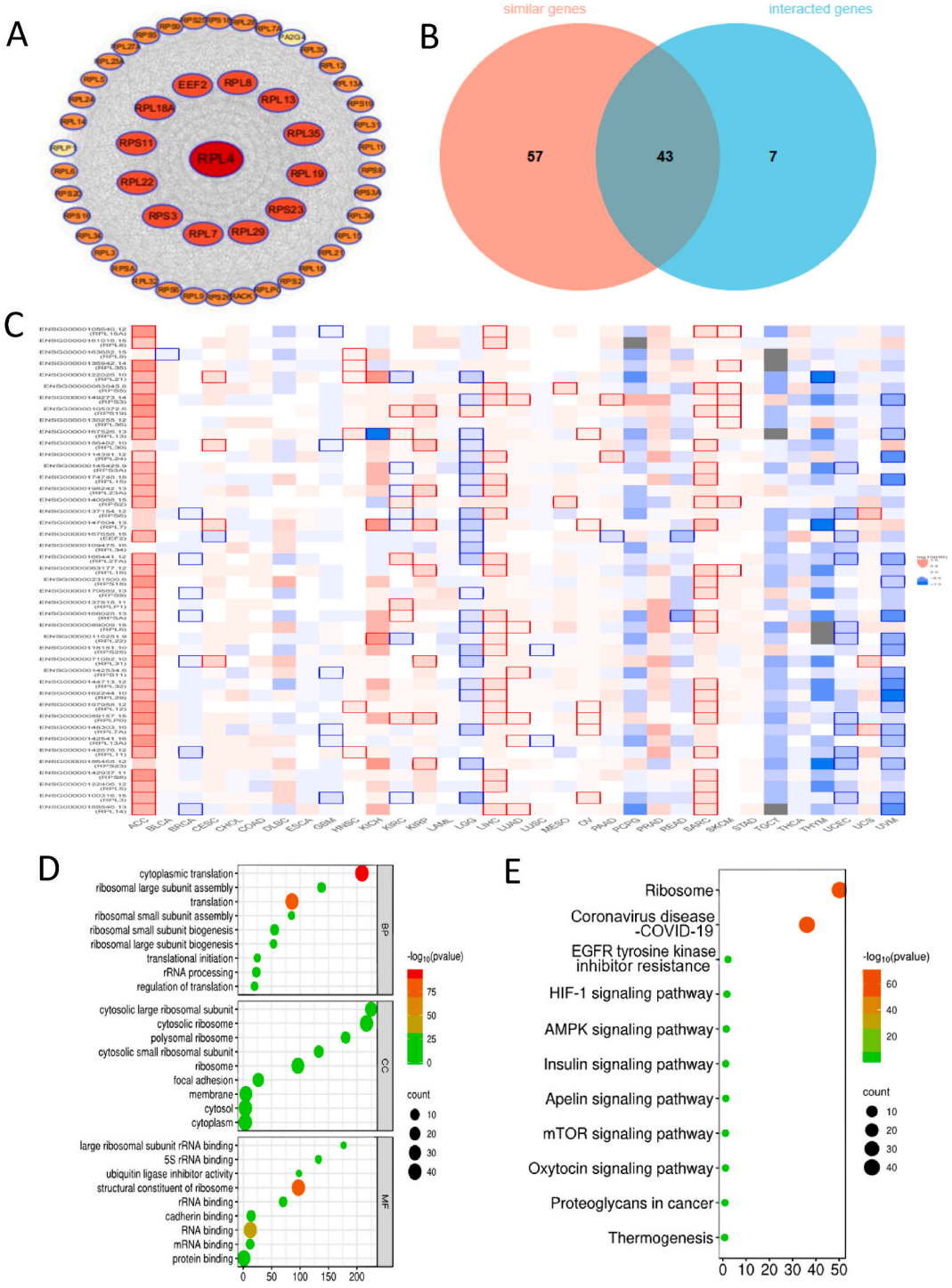

Fig. 5. The functional enrichment analysis and PPI network of 43 RPL4 common members. (A) PPI network, including 50 proteins that interact with RPL4. (B) Venn diagram of 50 interacting proteins with 100 related genes. (C) A heatmap showing the pan-cancer prognosis for 43 co-members. (D) GO enrichment analysis (including BP, CC, and MF) based on 43 genes associated with RPL4. (E) Analysis of the KEGG pathway based on 43 genes associated with RPL4.

3.5. RPL4-related gene-protein interaction and functional enrichment analysis

To gain an in-depth understanding of the biological functions and molecular mechanisms of RPL4 in human cancers, 50 RPL4-interacting proteins from the STRING database (Fig. 5A) were retrieved. Correlation analysis conducted in GEPIA2 identified 100 genes that exhibited a high degree of similarity. The 50 proteins interacting with RPL4 had 43 common members (Fig. 5B). Heat maps depicting the predictive outcomes of these genes in various tumor types were generated (Fig. 5C). Next, 43 co-members were incorporated for functional condensation analysis. GO analysis (Fig. 5D) indicated that their primary biological processes (BPs) consisted of cytoplasmic translation, biogenesis of ribosomal small and large subunits, rRNA processing, ribosomal small subunit assembly, regulation, and initiation of translation. The primary processes by which cellular components (CC) are formed include the

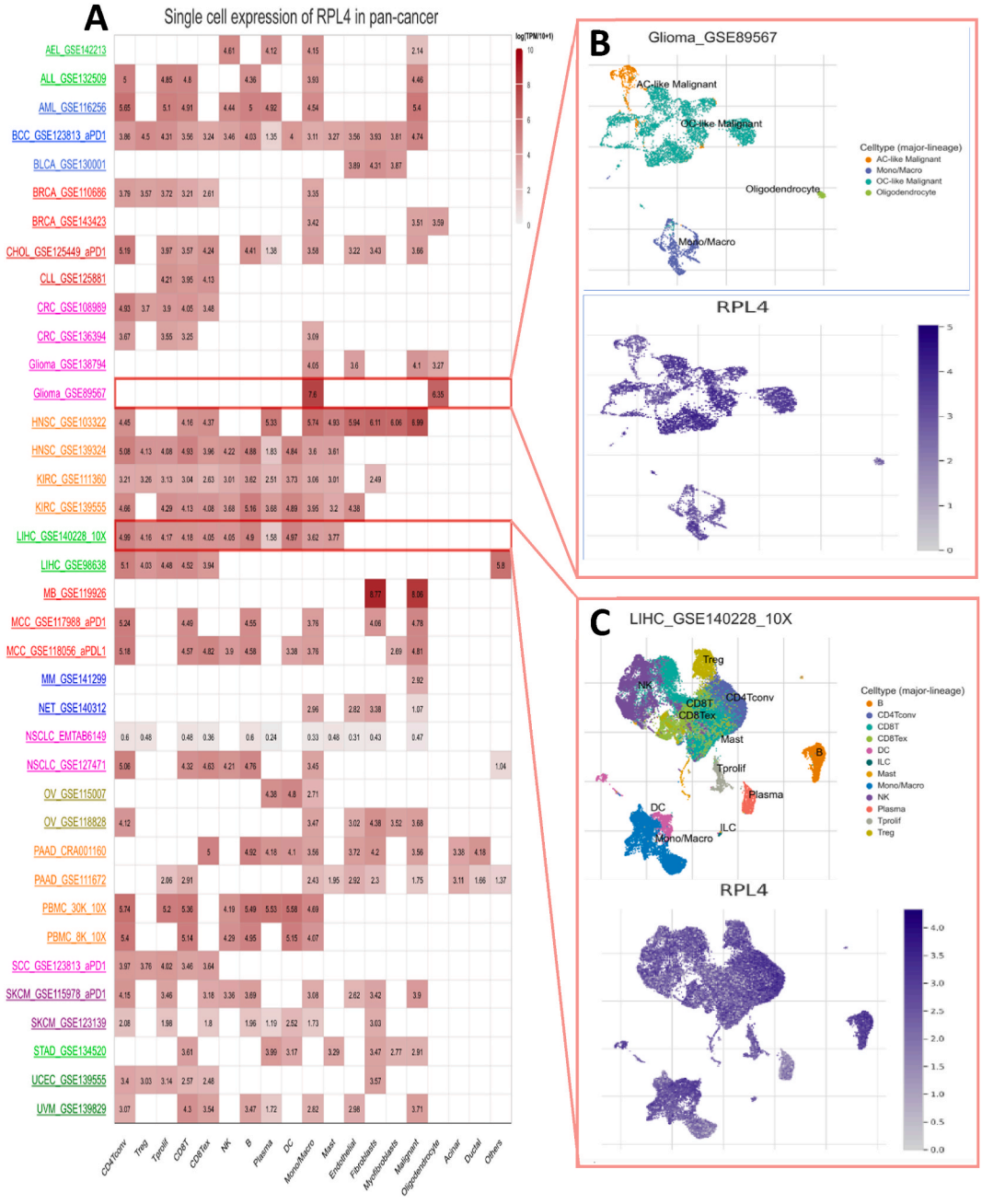

Fig. 6. RPL4 single-cell analysis. (A) Expression of RPL4 in several cell types. (B) Scatter plot displaying the distribution of 4 different cell types in the GSE89567 dataset and RPL4 expression levels in the dataset cells. (C) Scatter plot displaying the distribution of 12 different cell types in the GSE140228 dataset and RPL4 expression levels in the dataset cells.

formation of cytoplasmic ribosomes and polyribosomes. Small and large cytoplasmic ribosomal subunits, the cytoplasm, and cell membranes. Molecular functions (MF) mainly focused on ribosomal structural components, certain RNAs, protein binding, stem cell binding, large ribosomal subunit rRNA binding, and ubiquitin ligase inhibitor activity. Combining the WEBGestalt and DAVID databases, KEGG enrichment analysis (Fig. 5E) revealed that *RPL4* was involved in 11 signaling pathways, including the ribosome, coronavirus disease-COVID-19, epidermal growth factor receptor (EGFR) tyrosine kinase inhibitor resistance, hypoxia-inducible factors (HIF)-1 signaling pathway, 5′ AMP-activated protein kinase (AMPK) signaling pathway, insulin signaling pathway, apelin signaling pathway, mammalian target of rapamycin signaling pathway, oxytocin signaling pathway, proteoglycan, and thermogenesis in cancer, but was enriched in ribosomes and coronavirus disease-COVID-19.

3.6. Single-cell analysis of RPL4 expression

Seventy-nine cancer samples to were used to identify the primary cellular categories in which RPL4 is present in tumors and microenvironments at the single-cell level. The expression levels of RPL4 in 33 different cell types (immune, stromal, malignant, and functional cells) among the 79 datasets obtained using the TISCH web tool is shown in Fig. 6A. These findings demonstrated that RPL4 is mainly expressed in immunological and malignant cells. More specifically, RPL4 was widely expressed in malignant and immune cells mono/macro in the GBMLGG microenvironment in the GSE89567 database, which comprises 6341 cells from 10 patients with GBMLGG who did not receive any therapy (Fig. 6B). *RPL4* was substantially expressed in immune cells in the HC microenvironment, notably dendritic cells and $CD4^{+}$ cells, according to our analysis of 62,530 cells from five patients with HC in the GSE140228 HC dataset (Fig. 6C).

Fig. 7. RPL4 expression and functional status of 19 malignancies are correlated.

Next, *RPL4*'s possible single-cell roles in tumor cells (Fig. 7) were investigated. These findings demonstrate that *RPL4* expression has a major impact on the biological processes of tumor cells, including DNA damage, DNA repair, inflammation, cell cycle, stemness, angiogenesis, metastasis, hypoxia, apoptosis, proliferation, EMT, and invasion.

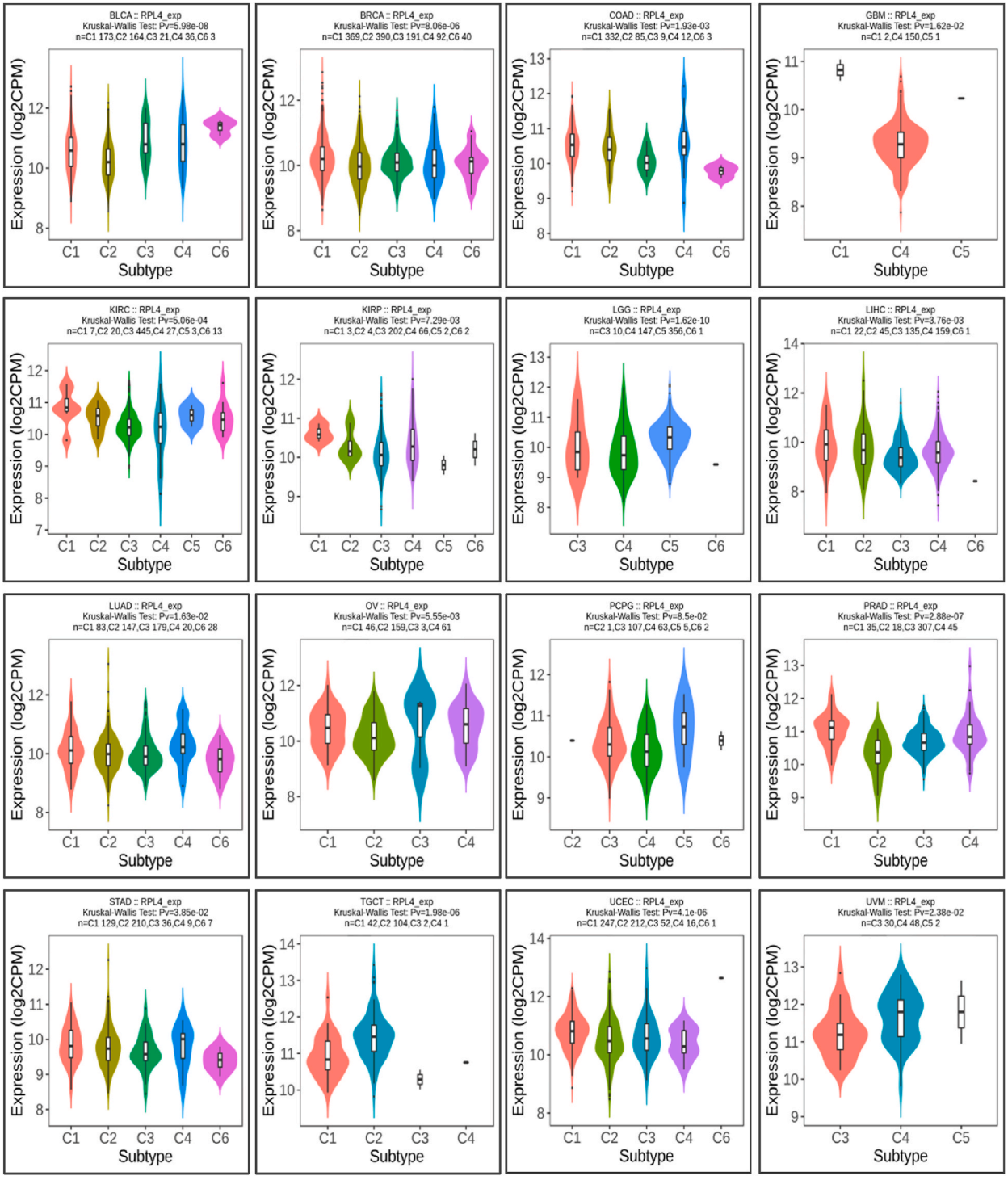

Fig. 8. Correlation between RPL4 expression with tumor immune subtypes based on the TISIDB database. Among the immunological subtypes is C1.: wound healing,C2: IFN-gamma dominant, C3: inflammatory, C4: lymphocyte depleted, C5: immunologically quiet,C6: TGF-b dominant.

3.7. RPL4 expression is associated with immune infiltration

Ongoing interactions between tumor cells, microenvironment, and immune cells play a critical role in tumor initiation, progression, and therapeutic response. Understanding the relationship between immune cells and cancer can help predict the effects of immunotherapy and identify new targets for immunotherapy [27,28]. Using immunoassays, it was determined that the immunological subtypes of 16 cancers, including BLCA and BRCA, were substantially correlated with the expression of *RPL4* (Fig. 8). According to a subsequent study of immune regulation, RPL4 expression has been linked to five immunological pathways, including immunosuppressants, immunostimulatory factors, MHC, chemokines, and receptors (Fig. 9). We investigated whether *RPL4* expression influences carcinogenesis, prognosis, and therapy by affecting the immune invasion process of malignancies. It was possible to identify the exact location of *RPL4* expression in immune infiltration of tumors using TIMER2.0. These findings indicated a correlation between the expression of *RPL4* and various immune cells in various tumors, suggesting that RPL4 might mediate the infiltration of immune cells into the tumor microenvironment (Fig. 10, Supplementary Fig. 3).

3.8. Correlation of RPL4 expression with TMB, MSI and HRD

TMB is a novel target for predicting the effectiveness of tumor immunotherapy because it induces the creation of highly immunogenic and tumor-specific antibodies. Mismatch repair of damaged microsatellites results in MSI, which affects the prognosis of tumors by causing gene replication abnormalities and tumor proliferation. HRD status is a crucial determinant of therapy selection and prognosis. It is also strongly associated with sensitivity to PARP inhibitors and platinum-based chemotherapeutic drugs.

In this study a positive correlation was observed between RPL4 expression and TMB in stomach and esophageal carcinomas (STES, $p < 0.001$), STAD ($p < 0.001$), PRAD ($p < 0.05$), HNSC ($p < 0.05$), READ ($p < 0.05$), ACC ($p < 0.01$), and KICH ($p < 0.05$). Conversely, there was a negative correlation between *RPL4* expression and TMB in the LUAD ($p < 0.05$) and CHOL ($p < 0.01$) groups (Fig. 11A). The expression of *RPL4* showed a positive correlation with TMB in BRCA ($p < 0.05$), STES ($p < 0.001$), KIRP ($p < 0.01$), STAD ($p < 0.001$), UCEC ($p < 0.01$), lymphoid neoplasm diffuse large B-cell lymphoma (DLBC; $p < 0.01$), and HNSC ($p < 0.01$), while in LGG ($p < 0.05$) and TGCT ($p < 0.01$) showed a negative correlation with MSI (Fig. 11B). *RPL4* expression positively correlates with HRD in GBM ($p < 0.001$), LGG ($p < 0.05$), KIRP ($p < 0.01$), LIHC ($p < 0.05$), MESO ($p < 0.01$), and ACC ($p < 0.001$). In contrast, in LUAD ($p < 0.001$), COAD ($p < 0.05$), acute myeloid leukemia (LAML; $p < 0.05$), STES ($P < 0.001$), sarcoma (SARC; $p < 0.05$), STAD ($p < 0.001$), PRAD ($p < 0.001$), UCEC ($p < 0.001$), and BLCA ($p < 0.001$), the expression of *RPL4* had a negative relationship with HRD (Fig. 11C).

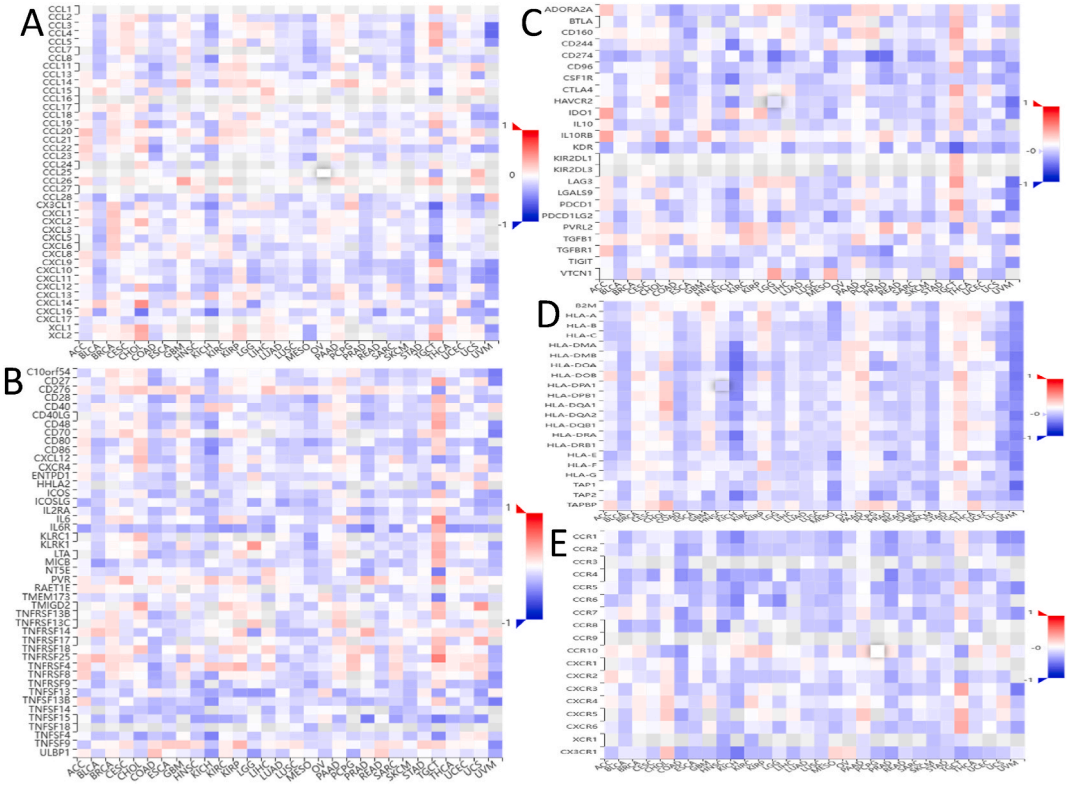

Fig. 9. RPL4 expression and tumor immune pathways in relation to each other. RPL4 expression in human malignancies is correlated with (A) immunosuppressive drugs; (B) immunostimulatory factors; (C) chemokines; (D) MHC; and (E) receptors.

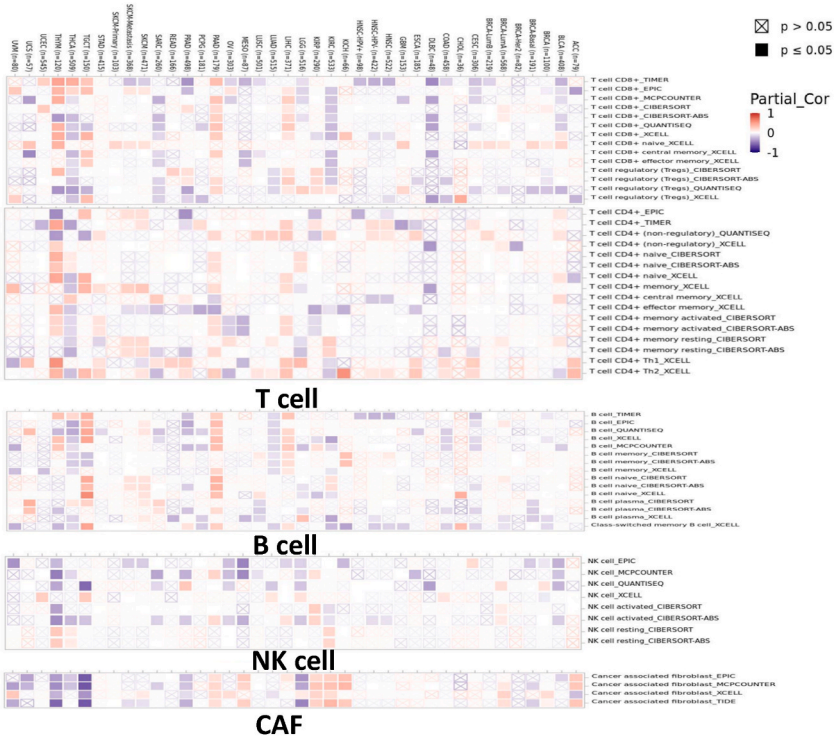

Fig. 10. Correlation between RPL4 expression and immune infiltration in cancer tissues (data from TIMER2). TC $CD8^{+}$, TC $CD4^{+}$, B cells, NK cells, and cancer-associated fibroblasts.

3.9. Sensitivity analysis of RPL4 expression and antitumor drugs

Using transcriptome data, the relationship between gene expression and the prediction of treatment response in glioblastoma, colorectal, breast, and ovarian malignancies were analyzed. In contrast to individuals with high *RPL4* expression, the data indicated that patients with low *RPL4* expression in ovarian and breast malignancies were more likely to be chemoresistant. Conversely, there was no discernible variation in the treatment response between colorectal cancer and glioblastoma with respect to *RPL4* expression (Fig. 12A).

Sensitivity to antitumor drugs, including methotrexate, hydroxyurea, lomustine, camptothecin, ifosfamide, aclubicin, valrubicin, and oxaliplatin, as well as cytarabine, timazid, vorinostat, belinostat, nellarabine, and palbociclib, was positively correlated with the expression level of *RPL4*. *RPL4* expression level and the sensitivity of anticancer medicines including mithramycin, irofulven, everolimus, pazopanib, actinomycin D, idelalisib, dasatinib, duvelisib (IPI-145), rositinib (CO-1686), erdatinib (JNJ-42756493), and copanlisib were negatively correlated. This implies that *RPL4* differential expression promotes carcinogenesis and cancer development, but also indirectly affects tumor treatment effectiveness and lapse (Fig. 12B).

4. Discussion

Ribosomal biogenesis is the rate-limiting step in cell growth and proliferation. RPs are essential components of ribosomes that are involved in the recruitment and localization of ribosomal subunits and are necessary for the integrity and function of ribosomes. RPL4 is involved in ribosome biogenesis, and its protein structure and properties affect translation speed and accuracy. In addition, PRL4 interacts with other proteins to regulate the activity and selectivity of ribosomes, thus affecting protein synthesis and cell growth. Although a strong correlation between RPL4 and cancer has been demonstrated, our understanding of the underlying mechanisms of this correlation remains limited. RPL4 may play different roles in different cancers, owing to the heterogeneity and complexity of tumors. To gain a more systematic and comprehensive understanding of the role of RPL4 in cancer, we explored RPL4 from a pan-cancer perspective.

Differential expression analysis showed that RPL4 is widely distributed throughout the human body and has low tissue specificity, as PRL4 expression is significantly higher in tumor tissues than in normal tissues. This affects the prognosis of tumors and shows a significant correlation with the clinical stage of each tumor and the tumor immune subtypes. Prognostic analyses have indicated that RPL4 has the potential to be a prognostic biomarker for many types of cancer.

Recent research has shown that immune cells, including CD4 + T cells, CAF, MDSC, neutrophils, and macrophages play critical roles in cancer immunotherapy [29], highlighting the importance of immune cells in cancer treatment. To further investigate the value

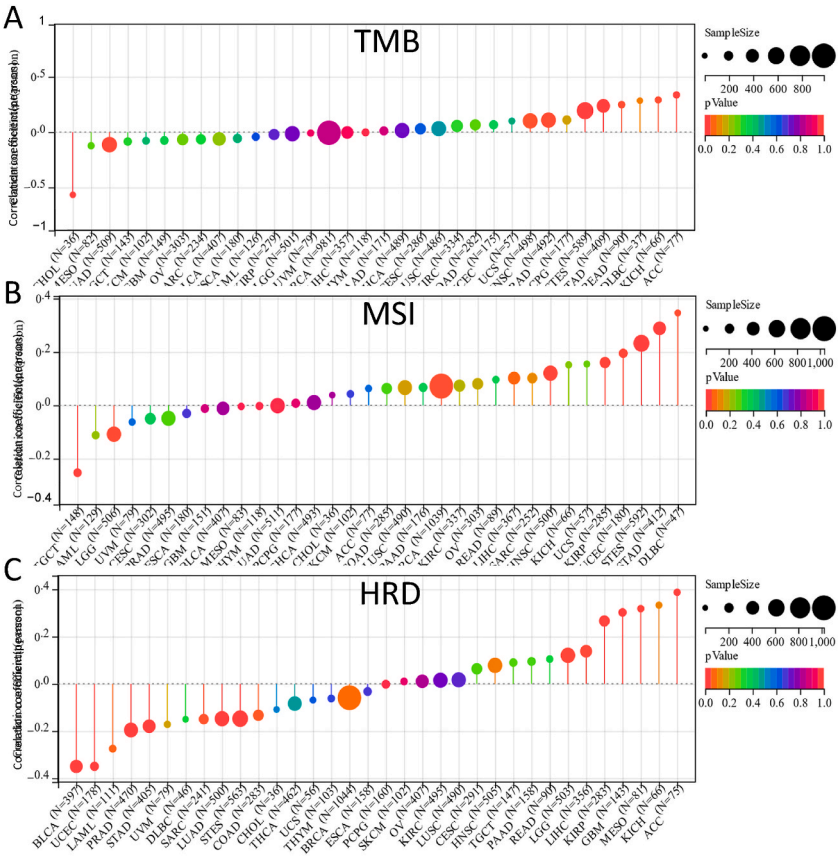

Fig. 11. RPL4 expression and immunological markers in relation to each other. (A) Relationship between RPL4 expression and TMB. (B) Relationship between RPL4 expression and MSI. (C) Relationship between RPL4 expression and HRD.

and importance of RPL4 in the tumor microenvironment, single-cell analyses were performed, which revealed that RPL4 was mainly expressed in immune and malignant cells, which are associated with multiple cellular functions. This suggests that immune cell function depends on RPL4 expression. This analysis also revealed that immune cell infiltration into the tumor microenvironment was affected by RPL4 expression. Natural killer/T-cell lymphoma (T/NK) cells were initially designed to recognize and kill cancer cells, and tumor tissues can shape the immune tumor microenvironment to facilitate immune evasion. Particularly, tumors can disrupt T/NK cell infiltration, cell function, and antigen presentation via soluble and cell surface mediators (such as PD-L1) and immunosuppressive cells. Thus, elucidating tumor-immune cell interactions will aid in the prediction of immunotherapeutic responses and develop novel targets for immunotherapy. Simultaneously, the expression of RPL4 determines immune cell infiltration, which may be applied to the development of new targeted drugs for certain cancer immunotherapies and may benefit many patients with cancer.

In addition, enrichment analyses suggest that *RPL4* is involved in the initiation and regulation of ribosome composition and biogenesis, as well as translation. This pathway was mainly enriched in ribosomes and coronavirus disease-19 (COVID-19). Activation of ribosome biogenesis, over-translation of proteins, or changes in ribosome mass caused by an increase in protein production and a decrease in translation fidelity may lead to tumorigenesis [30,31]. This may be one of the pathways through which RPL4 expression affects tumor formation. *RPL4* is involved in cell adhesion, tumor progression, and metastasis through related pathways such as proteoglycan, TOR, and AMPK signaling pathways in cancer. Combined with drug sensitivity analysis, *RPL4* expression was negatively correlated with the drug sensitivity to dasatinib, rositinib, and erdatinib, which may be related to the enrichment of RPL4 for drug resistance to EGFR tyrosine kinase inhibitors.

In addition to its typical ribosomal functions, RPL4 has extra-ribosomal functions, including the activation of p53-dependent or -independent pathways in response to stress. Enrichment analyses revealed that *RPL4* is involved in the regulation of ubiquitin ligase activity, leading to cell cycle arrest and apoptosis. After activation of the RP response, RPL4 competitively binds to MDM2 and p53, leading to reduced p53 ubiquitination and degradation. RPL4 can bind to PRDX2, a member of the peroxiredoxin family, which regulates the expression of p53 through the RPL-MDM2-p53 pathway, thereby promoting p53 ubiquitination and protein degradation. External stimuli, such as chemotherapy and radiotherapy, can activate the ribosomal response and increase p53-dependent cell cycle arrest and apoptosis, thereby influencing the therapeutic effects of radiotherapy. Previously, it has been demonstrated that the balance between RPL4 expression is essential for normal p53 levels and cellular homeostasis. Conversely, overexpression of RPL4 impairs ribosomal biogenesis, moreover, RPL4 knockdown triggers ribosomal stress, significantly inducing p53 activation and cell cycle arrest

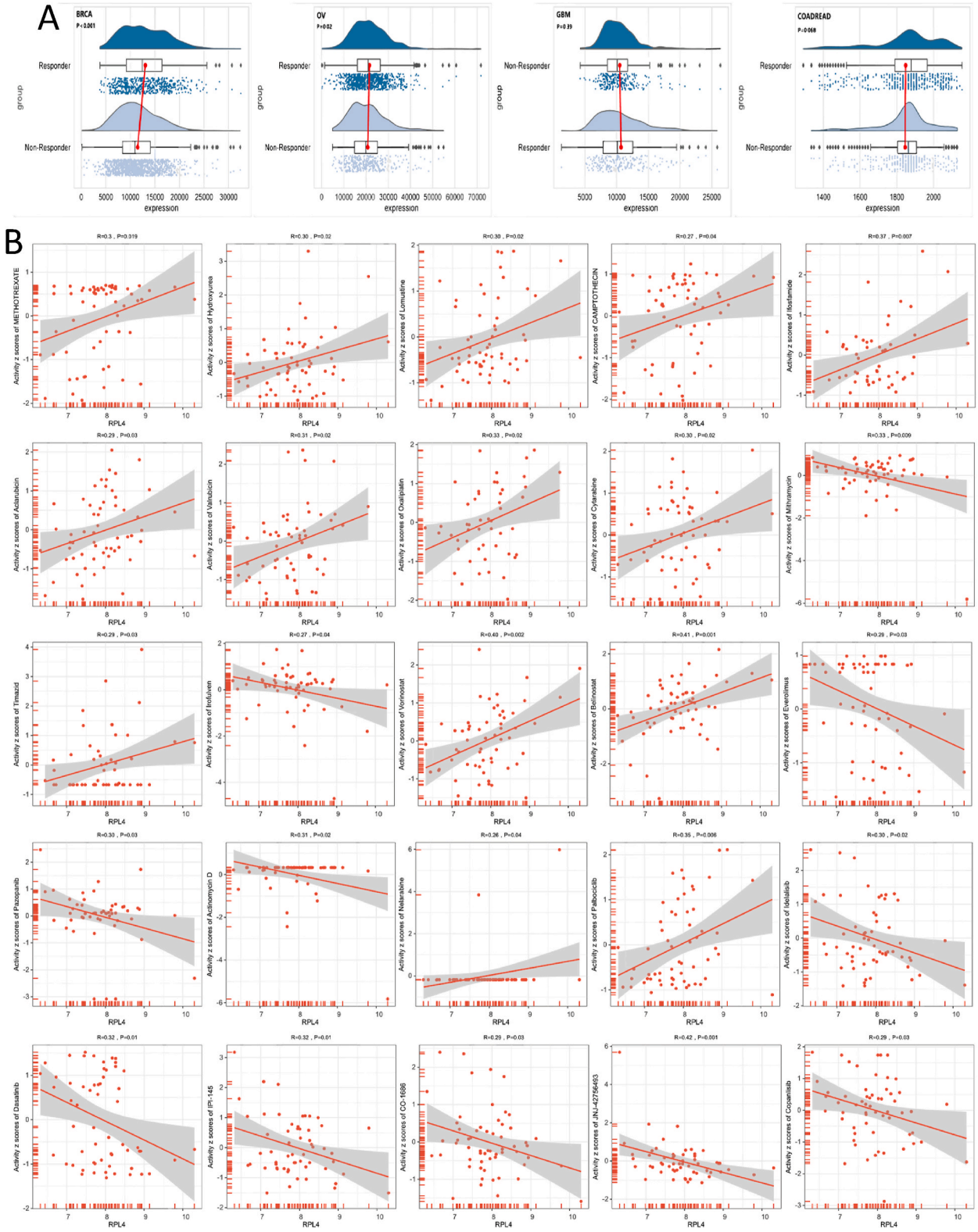

Fig. 12. RPL4 response to chemotherapy and drug sensitivity analysis. (A) Relationship between RPL4 expression level and response to chemotherapy in BRCA (5-year recurrence-free survival as response), OV (recurrence-free survival at 12 months as response), GBM (response referenced to overall survival at 16 months), and COADREAD (response based on RECIST criteria). (B) Analysis of the correlation between RPL4 expression and antitumor drug sensitivity.

[30,32]. Proteins are the true drivers or mediators of cellular functions. Therefore, targeted proteins are the first choice for targeted cancer therapy. Targeting RPL4 may help improve the effects of chemotherapy and reverse drug resistance in tumor cells [33].

The implications of RPL4 in disease genesis are much greater than its known functions. According to a previous study, *RPL4* was identified as a novel disease gene related to diamond Blackfan anemia (DBA). However, mutations in RPL4 have not been shown to be associated with human disease. This was assumed to be related to somatic cell reversal events [34]. In 2022, Pillet et al. revealed that RPL4 levels could be controlled by yeast. However, the occurrence in humans or other mammalian cells are not well understood. These findings may contribute to our understanding of diseases caused by defects in RPs, such as DBA, as well as neurodegenerative disorders caused by clumps of proteins formed in cells [35]. Overexpression of mRPL4 leads to a significant dose-dependent reduction in viral particle production [36], but Shen et al. determined that the RPL4 protein affects latent Epstein–Barr virus (EBV) infection [37].

Since most RP gene promoter regions are conserved, it is believe that their regulatory and synthetic roles in the cell cycle promote ribosome biogenesis [5]. Therefore, ribosome biogenesis must be tightly regulated to match normal cell proliferation. The ribosomal pathway plays a crucial role in protein synthesis, and increased ribosome biosynthesis is a common feature of active proliferation in organisms. The more active the ribosomal pathway in tumor cells, the higher the proliferation rate. It is noteworthy that numerous studies have demonstrated that the oncogenic pathway of RPL4 includes not only the ribosomal pathway but also the interaction between RPL4 and the proto-oncogene product c-Myb in response to growth factors and nutrient signaling pathways. This interaction plays an important role in the expression of the oncogene c-Myc [38] and protects unassembled RPL4 from Tom1-mediated cellular degradation [39]. As a transcriptional regulator, RPL4 regulates the expression of CD40, a member of the tumor necrosis factor (TNF) receptor superfamily. This indirectly suggests that RPs perform ribosome-independent functions in tumorigenesis, immune signaling, and development [40]. Moreover, for a large family of RPs, aberrant expression of various proteins can result in different cellular outcomes [41]. For instance, dysregulation of RPL5 affects HDM2/p53-mediated ribosomal biogenesis, contributing to T-cell-causing acute lymphoblastic leukemia and melanoma. Upregulation of RPL15 is associated with intestinal cancer, whereas silencing RPL19 inhibits prostate cancer tumor growth in vivo. RPL19 overexpression predicts poor prognosis in patients with HC, and high levels of phosphorylated RPS6 correlate with shorter metastasis-free survival in lung cancer [11].

The findings of this study may contribute to unraveling the pathophysiological mechanisms of RPL4, raising new hope for more aggressive research on RPL4 in future guidelines, including experimental studies and longitudinal clinical trials. This can better characterize the true magnitude of RPL4's impact on cancer formation risk. Identification of novel biological pathways related to RPL4 is important for the development of new preventive and therapeutic strategies.

5. Conclusion

In this study, bioinformatics analyses revealed that the pan-carcinogenic gene RPL4 is differentially expressed in tumor and normal tissues and is involved in immune cell infiltration in the tumor microenvironment. RPL4 has potential as a pan-cancer biomarker and immunotherapeutic target. The identification of new biomarkers is expected to improve patient outcomes. However, this study has some limitations. Most of these findings are based on data analysis, and further experiments are required to validate and explore the potential mechanisms.

Ethics

This study complied with the guidelines published by TCGA and UCSC, and therefore ethical approval and informed consent of the patients was waived.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

DATA availability statement

The data associated with my study has been deposited into a publicly available repository. This publicly available datasets can be found in the TCGA database, the UCSC database, GETx, and the GEO database. The data will be made available on request.

CRediT authorship contribution statement

Yan Liu: Writing – original draft. **Wei Li:** Methodology, Formal analysis, Conceptualization. **Shiyang Zhou:** Visualization. **Min Cui:** Supervision, Project administration, Conceptualization. **Lin Zhang:** Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e34461.

References

[1] H. Sung, J. Ferlay, R.L. Siegel, et al., Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries, CA Cancer J Clin 71 (3) (2021) 209–249, https://doi.org/10.3322/caac.21660.

[2] I. Soerjomataram, F. Bray, Planning for tomorrow: global cancer incidence and the role of prevention 2020-2070, Nat. Rev. Clin. Oncol. 18 (10) (2021) 663–672, https://doi.org/10.1038/s41571-021-00514-z.

[3] F. Bray, M. Laversanne, E. Weiderpass, I. Soerjomataram, The ever-increasing importance of cancer as a leading cause of premature death worldwide, Cancer 127 (16) (2021) 3029–3030, https://doi.org/10.1002/cncr.33587.

[4] R.L. Siegel, K.D. Miller, N.S. Wagle, A. Jemal, Cancer statistics, 2023, CA Cancer J Clin 73 (1) (2023) 17–48, https://doi.org/10.3322/caac.21763.

[5] N. Nosrati, N.R. Kapoor, V. Kumar, Combinatorial action of transcription factors orchestrates cell cycle-dependent expression of the ribosomal protein genes and ribosome biogenesis, FEBS J. 281 (10) (2014) 2339–2352, https://doi.org/10.1111/febs.12786.

[6] X. Yang, L. Sun, L. Wang, B. Yao, H. Mo, W. Yang, LncRNA SNHG7 accelerates the proliferation, migration and invasion of hepatocellular carcinoma cells via regulating miR-122-5p and RPL4, Biomed. Pharmacother. 118 (2019) 109386, https://doi.org/10.1016/j.biopha.2019.109386.

[7] T. Lan, K. Yuan, X. Yan, et al., LncRNA SNHG10 facilitates hepatocarcinogenesis and metastasis by modulating its homolog SCARNA13 via a positive feedback loop, Cancer Res. 79 (13) (2019) 3220–3234, https://doi.org/10.1158/0008-5472.CAN-18-4044.

[8] Z. Kolkova, A. Arakelyan, B. Casslén, S. Hansson, E. Kriegova, Normalizing to GADPH jeopardises correct quantification of gene expression in ovarian tumours - IPO8 and RPL4 are reliable reference genes, J. Ovarian Res. 6 (1) (2013) 60, https://doi.org/10.1186/1757-2215-6-60. Published 2013 Aug 30.

[9] R. Pan, C. Yu, Y. Shao, et al., Identification of key genes and pathways involved in circulating tumor cells in colorectal cancer, Anal. Cell Pathol. 2022 (2022) 9943571, https://doi.org/10.1155/2022/9943571. Published 2022 Jan 27.

[10] J. Chen, C. Liu, J. Cen, et al., KEGG-expressed genes and pathways in triple negative breast cancer: protocol for a systematic review and data mining, Medicine (Baltim.) 99 (18) (2020) e19986, https://doi.org/10.1097/MD.0000000000019986.

[11] A.R. Elhamamsy, B.J. Metge, H.A. Alsheikh, et al., Ribosome biogenesis: a central player in cancer metastasis and therapeutic resistance, Cancer Res. 82 (13) (2022) 2344–2353, https://doi.org/10.1158/0008-5472.CAN-21-4087.

[12] T. Li, J. Fu, Z. Zeng, et al., TIMER2.0 for analysis of tumor-infiltrating immune cells, Nucleic Acids Res. 48 (W1) (2020) W509–W514, https://doi.org/10.1093/nar/gkaa407.

[13] Shen, et al., Sangerbox: a comprehensive, interaction-friendly clinical bioinformatics analysis platform, iMeta 1 (3) (2022) e36, https://doi.org/10.1002/imt2.36.

[14] D.S. Chandrashekar, S.K. Karthikeyan, P.K. Korla, et al., UALCAN: an update to the integrated cancer data analysis platform, Neoplasia 25 (2022) 18–27, https://doi.org/10.1016/j.neo.2022.01.001.

[15] M. Uhlén, L. Fagerberg, B.M. Hallström, et al., Proteomics. Tissue-based map of the human proteome, Science 347 (6220) (2015) 1260419, https://doi.org/10.1126/science.1260419.

[16] I. de Bruijn, R. Kundra, B. Mastrogiacomo, et al., Analysis and visualization of longitudinal genomic and clinical data from the AACR project GENIE biopharma collaborative in cBioPortal, Cancer Res. 83 (23) (2023) 3861–3867, https://doi.org/10.1158/0008-5472.CAN-23-0816.

[17] Y. Li, D. Ge, C. Lu, The SMART App: an interactive web application for comprehensive DNA methylation analysis and visualization, Epigenet. Chromatin 12 (1) (2019) 71, https://doi.org/10.1186/s13072-019-0316-3. Published 2019 Dec 5.

[18] V. Modhukur, T. Iljasenko, T. Metsalu, K. Lokk, T. Laisk-Podar, J. Vilo, MethSurv: a web tool to perform multivariable survival analysis using DNA methylation data, Epigenomics 10 (3) (2018) 277–288, https://doi.org/10.2217/epi-2017-0118.

[19] Z. Tang, B. Kang, C. Li, T. Chen, Z. Zhang, GEPIA2: an enhanced web server for large-scale expression profiling and interactive analysis, Nucleic Acids Res. 47 (W1) (2019) W556–W560, https://doi.org/10.1093/nar/gkz430.

[20] B.T. Sherman, M. Hao, J. Qiu, et al., DAVID: a web server for functional enrichment analysis and functional annotation of gene lists (2021 update), Nucleic Acids Res. 50 (W1) (2022) W216–W221, https://doi.org/10.1093/nar/gkac194.

[21] Y. Liao, J. Wang, E.J. Jaehnig, Z. Shi, B. Zhang, WebGestalt 2019: gene set analysis toolkit with revamped UIs and APIs, Nucleic Acids Res. 47 (W1) (2019) W199–W205, https://doi.org/10.1093/nar/gkz401.

[22] D. Sun, J. Wang, Y. Han, et al., TISCH: a comprehensive web resource enabling interactive single-cell transcriptome visualization of tumor microenvironment, Nucleic Acids Res. 49 (D1) (2021) D1420–D1430, https://doi.org/10.1093/nar/gkaa1020.

[23] B. Ru, C.N. Wong, Y. Tong, et al., TISIDB: an integrated repository portal for tumor-immune system interactions, Bioinformatics 35 (20) (2019) 4200–4202, https://doi.org/10.1093/bioinformatics/btz210.

[24] J.T. Fekete, B. Győrffy, ROCplot.org: validating predictive biomarkers of chemotherapy/hormonal therapy/anti-HER2 therapy using transcriptomic data of 3,104 breast cancer patients, Int. J. Cancer 145 (11) (2019) 3140–3151, https://doi.org/10.1002/ijc.32369.

[25] W.C. Reinhold, M. Sunshine, H. Liu, et al., CellMiner: a web-based suite of genomic and pharmacologic tools to explore transcript and drug patterns in the NCI-60 cell line set, Cancer Res. 72 (14) (2012) 3499–3511, https://doi.org/10.1158/0008-5472.CAN-12-1370.

[26] A. Nishiyama, M. Nakanishi, Navigating the DNA methylation landscape of cancer, Trends Genet. 37 (11) (2021) 1012–1027, https://doi.org/10.1016/j.tig.2021.05.002.

[27] Y. Xiao, D. Yu, Tumor microenvironment as a therapeutic target in cancer, Pharmacol. Ther. 221 (2021) 107753, https://doi.org/10.1016/j.pharmthera.2020.107753.

[28] B. Lv, Y. Wang, D. Ma, et al., Immunotherapy: reshape the tumor immune microenvironment, Front. Immunol. 13 (2022) 844142, https://doi.org/10.3389/fimmu.2022.844142. Published 2022 Jul 6.

[29] Z. Tu, Q. Ouyang, X. Long, et al., Protein disulfide-isomerase A3 is a robust prognostic biomarker for cancers and predicts the immunotherapy response effectively, Front. Immunol. 13 (2022) 837512, https://doi.org/10.3389/fimmu.2022.837512. Published 2022 Mar 25.

[30] J. Pelletier, G. Thomas, S. Volarević, Ribosome biogenesis in cancer: new players and therapeutic avenues, Nat. Rev. Cancer 18 (1) (2018) 51–63, https://doi.org/10.1038/nrc.2017.104 [published correction appears in Nat Rev Cancer. 2018 Jan 25;18(2):134].

[31] J. Kang, N. Brajanovski, K.T. Chan, J. Xuan, R.B. Pearson, E. Sanij, Ribosomal proteins and human diseases: molecular mechanisms and targeted therapy, Signal Transduct. Targeted Ther. 6 (1) (2021) 323, https://doi.org/10.1038/s41392-021-00728-8. Published 2021 Aug 30.

[32] X. He, Y. Li, M.S. Dai, X.X. Sun, Ribosomal protein L4 is a novel regulator of the MDM2-p53 loop, Oncotarget 7 (13) (2016) 16217–16226, https://doi.org/10.18632/oncotarget.7479.

[33] W. Wang, J. Wei, H. Zhang, et al., PRDX2 promotes the proliferation of colorectal cancer cells by increasing the ubiquitinated degradation of p53, Cell Death Dis. 12 (6) (2021) 605, https://doi.org/10.1038/s41419-021-03888-1. Published 2021 Jun 11.

[34] M.C.J. Jongmans, I.J. Diets, P. Quarello, E. Garelli, R.P. Kuiper, R. Pfundt, Somatic reversion events point towards RPL4 as a novel disease gene in a condition resembling Diamond-Blackfan anemia, Haematologica 103 (12) (2018) e607–e609, https://doi.org/10.3324/haematol.2018.200683.

[35] B. Pillet, A. Méndez-Godoy, G. Murat, et al., Dedicated chaperones coordinate co-translational regulation of ribosomal protein production with ribosome assembly to preserve proteostasis, Elife 11 (2022) e74255, https://doi.org/10.7554/eLife.74255. Published 2022 Mar 31.

[36] L. Green, B. Houck-Loomis, A. Yueh, S.P. Goff, Large ribosomal protein 4 increases efficiency of viral recoding sequences, J. Virol. 86 (17) (2012) 8949–8958, https://doi.org/10.1128/JVI.01053-12.
[37] C.L. Shen, C.D. Liu, R.I. You, et al., Ribosome protein L4 is essential for epstein-barr virus nuclear antigen 1 function, Proc Natl Acad Sci U S A 113 (8) (2016) 2229–2234, https://doi.org/10.1073/pnas.1525444113.
[38] A. Egoh, S. Nosuke Kanesashi, C. Kanei-Ishii, T. Nomura, S. Ishii, Ribosomal protein L4 positively regulates activity of a c-myb proto-oncogene product, Gene Cell. 15 (8) (2010) 829–841, https://doi.org/10.1111/j.1365-2443.2010.01421.x.
[39] F.M. Huber, A. Hoelz, Molecular basis for protection of ribosomal protein L4 from cellular degradation, Nat. Commun. 8 (2017) 14354, https://doi.org/10.1038/ncomms14354. Published 2017 Feb 2.
[40] M. Zou, X. Zhang, D. Jiang, et al., Transcriptional regulation of CD40 expression by 4 ribosomal proteins via a functional SNP on a disease- associated CD40 locus, Genes 11 (12) (2020) 1526, https://doi.org/10.3390/genes11121526. Published 2020 Dec 21.
[41] I. Takemasa, N. Kittaka, T. Hitora, et al., Potential biological insights revealed by an integrated assessment of proteomic and transcriptomic data in human colorectal cancer, Int. J. Oncol. 40 (2) (2012) 551–559, https://doi.org/10.3892/ijo.2011.1244.