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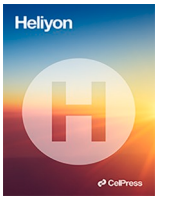

Research article

Pan-cancer analysis revealed prognosis value and immunological relevance of RAMPs

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ABSTRACT

Whether receptor activity-modifying proteins (RAMPs) play a key role in human cancer prognosis and immunity remains unknown. We used data from the public databases, The Cancer Genome Atlas, Therapeutically Applicable Research to Generate Effective Treatments, and the Genotype-Tissue Expression project. We utilized bioinformatics methods, R software, and a variety of online databases to analyze RAMPs. In general, RAMPs were significantly and differentially expressed in multiple tumors, and RAMP expression was closely associated with prognosis, immune checkpoints, RNA-editing genes, tumor mutational burden, microsatellite instability, ploidy, and stemness indices. In addition, the expression of RAMPs is strongly correlated with tumor-infiltrating lymphocytes in human cancers. Moreover, the RAMP co-expression network is largely involved in many immune-related biological processes. Quantitative reverse transcription polymerase chain reaction and Western blot proved that RAMP3 was highly expressed in glioma, and RAMP3 promoted tumor proliferation and migration. RAMPs exhibit potential as prognostic and immune-related biomarkers in human cancers. Moreover, RAMPs can be potentially developed as therapeutic targets or used to enhance the efficacy of immunotherapy.

1. Introduction

At present, three members of the receptor activity-modifying protein (RAMP) family have been identified, namely, RAMP1, RAMP2, and RAMP3 [1], which belong to a single family of transmembrane proteins. They were initially discovered during the cloning of the human calcitonin gene-related peptide (CGRP) receptor [2]. Since their initial discovery, RAMPs have been revealed to interact with various G protein-coupled receptors (GPCRs), including parathyroid hormone receptors, glucagon-like peptide-1 receptors, vasoactive peptide receptors, calcium-sensing receptors, calcitonin receptors, calcitonin receptor-like receptors, and corticotropin-releasing factor receptor 1 [2–6]. This interaction modulates the trafficking, pharmacological properties, and signaling

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functions of these receptors. Although extensive studies have been conducted on the cellular and biochemical characteristics of these GPCRs and their ligands, the functional significance and role of RAMPs in human diseases are not yet fully elucidated.

New emerging evidence strongly suggests the involvement of CGRP and adrenomedulin (AM) in various aspects of cancer biology. The neuropeptide CGRP interacts with immune cells (macrophages , dendritic cells, and T cells) through the RAMP1 signaling pathway, promoting lymphangiogenesis and exerting anti-inflammatory and pro-tumorigenic effects. Furthermore, CGRP in the nervous system promotes pathological angiogenesis, including tumor angiogenesis. Unexpectedly, recent research results indicate that CGRP produced during injury sensing can directly impair the function of cytotoxic $CD8^+$ T cells, limiting their ability to eliminate melanoma [7]. Meanwhile, AM has been identified as a potential therapeutic target against pathological angiogenesis [8]. It is expressed in various cancers, including pancreatic, breast, and lung cancers [9–12]. In particular, the AM–RAMP2 system plays a crucial role in tumor angiogenesis and metastasis [13]. The depletion of RAMP2 in endothelial cells reduces tumor angiogenesis and inhibits locally transplanted tumor growth [14]. In addition, RAMP2 deficiency induces endothelial-to-mesenchymal transition (EndMT)-like changes and enhances carcinoma-associated fibroblast (CAF) production, promoting tumor progression and metastasis through the secretion of tumor growth factors [13,15,16]. EndMT-induced cellular changes disrupt cell adhesion and enhance migration, further facilitating cancer progression and metastasis [17,18]. Targeting RAMP2 can effectively inhibit tumor metastasis by controlling vascular integrity. RAMP3, with high expression in tumor periphery, plays a pro-tumor role in DI-E-RAMP2−/−mice [19]. RAMP3-deficient tumors exhibit significantly reduced metastasis and a corresponding decrease in the number of CAF-expressing podoplanin, leading to suppressed tumor proliferation and metastasis [19]. Moreover, RAMP3 is involved in epithelial-to-mesenchymal transition. Overall, the RAMP system represents a promising therapeutic target for regulating tumor microenvironment and controlling tumor progression and metastasis.

However, the underlying mechanisms of altered RAMP expression in these tumors and the functional significance of RAMPs in tumorigenesis and tumor development are not yet fully understood. Therefore, exploring the role of RAMPs in pan-cancer from new and multiple perspectives is important in tumor research. Our pan-cancer analysis was performed using multiple public databases to illustrate the role of RAMPs in the prognosis and immunotherapy of cancer. The correlations of RAMPs with immune checkpoint genes (ICGs), RNA-editing genes, tumor mutational burden (TMB), microsatellite instability (MSI), neoantigens, and clinical stage and immune-infiltrating cells were investigated. In summary, we hypothesized that RAMPs may regulate immune activity and tumor metastasis in the majority of pan-cancers, suggesting that RAMPs have prognostic value and may be potential therapeutic targets.

2. Materials and methods

2.1. RAMP expression in pan-cancer analysis

The TIMER database and the SangerBox website were used to screen the difference in the RAMP expression of cancers and paired normal tissues in humans [20,21].

2.2. Promoter DNA methylation and protein expression of RAMPs in human cancers

The database of the University of Alabama at Birmingham Cancer Data Analysis Portal (UALCAN) was used to assess the differences between cancer tissues and normal tissues in the promoter DNA methylation of RAMPs, which was obtained from The Cancer Genome Atlas (TCGA), and the differences in the protein expression of RAMPs, which was obtained from the mass spectrometry-based proteomics of the Clinical Proteomic Tumor Analysis Consortium (CPTAC) [22].

2.3. Prognostic value of RAMPs in pan-cancer

A Cox proportional hazards regression model was established using R package survival to explore the relationship between RAMP expression and cancer patient prognosis. This model involves overall survival (OS), disease-specific survival (DSS), progression-free interval (PFI), and disease-free interval (DFI) [23]. Prognostic significance was obtained via the log-rank test ($P < 0.05$). Moreover, the Kaplan–Meier (KM) plotter was used to conduct KM survival analysis by dividing patients into low- and high-expression groups in accordance with the best cutoff expression value of RAMPs with regard to the aspects of OS and relapse-free survival (RFS). Moreover, we analyzed the prognostic value of RAMPs in immunotherapy cohorts with regard to the aspects of OS or progression-free survival (PFS). In addition, the PrognoScan database [24] was used to verify the relationship between RAMP expression and clinical outcomes. Furthermore, the varying expression levels of RAMP genes were evaluated in each tumor at different clinical stages.

2.4. Relation of RAMP expression to ICGs immunological regulators, and RNA-editing genes

The standardized pan-cancer datasets were obtained from the University of California Santa Cruz database, which was composed of TCGA, Therapeutically Applicable Research to Generate Effective Treatments (TARGET), and the Genotype-Tissue Expression (GTEx) cohorts [25]. We obtained 44 RNA modification genes (10 m1A, 13 m5C, and 21 m6A) [25–30], 150 marker genes for 5 immune-related pathways [18 for receptor, 21 for major histocompatibility complex, 24 for immunoinhibitor, 41 for chemokine, and 46 for immunostimulator], and 60 ICGs (24 inhibitory and 36 stimulatory) from previous studies. Then, the mRNA matrix of these genes and RAMP genes in each sample was obtained. Normal samples and those with an expression level of 0 were removed.

2.5. Relation of RAMP expression to immune infiltration cells

The expression profiles from each tumor were individually extracted, and the infiltration of 22 immune cells per patient in each tumor were assessed using the deconvo_CIBERSOR method of R package IOBR (version 0.99.9) [31]. In addition, the deconvo_epic, deconvo_ips, deconvo_mcpcounter, deconvo_quantiseq, TIMER, and deconvo_xCell methods were used to assess the infiltration scores of immune cells per patient in each tumor.

2.6. Relation of RAMP expression to TMB, MSI, ploidy, and ESTIMATE

The level 4 Simple Nucleotide Variation dataset for all the samples in the TCGA cohorts were processed using MuTect2 software [32], then maftools of R package (version 2.8.05) was used to calculate TMB for each sample [33]. In addition, we obtained the MSI scores, ploidy data, and NEO data of all the samples from previous research. The MSI, ploidy, TMB, and RAMP expression of each sample were integrated. In addition, samples with an expression level of zero for RAMPs and cancer species with less than three samples in a single cancer species were excluded. Finally, the full expression data were obtained. Moreover, we used ESTIMATE in R package (version 1.0.13) to calculate stromal, immune, and ESTIMATE scores for each patient in each tumor [34].

2.7. Relation of RAMP expression to tumor-infiltrating lymphocytes (TILs), molecular subtypes, and immune subtypes in human cancers

We obtained the distribution of RAMP expression across immune and molecular subtypes from the TISIDB database, which integrates a rich human cancer dataset from the TCGA database and immunology data from seven public databases [35]. TISIDB enables the analyses of associations of RAMPs with TILs, molecular subtypes, and/or immune subtypes for various cancer types.

2.8. Relation of RAMP expression to stemness indices in human cancers

We obtained the stemness indices of each tumor in TCGA cohorts from previous studies. The stemness indices were proven related to tumor pathology, drug sensitivity, oncobiology, and same clinical data. RNA expression-based stemness (RNAss) and epigenetically regulated RNA expression-based stemness (EREG.EXPss) were the RNA-based stemness indices used in this study, while DNA expression-based stemness (DNAss) was based on DNA methylation [36]. The stemness indices and RAMP expression data were integrated for each sample.

2.9. Co-expression networks of RAMPs

The LinkedOmics database, including multi-omics data from TCGA and CPTAC cohorts [37], was used for statistically exploring the co-expression genes of RAMPs by using Pearson's correlation coefficient. Then, the biological processes in the Gene Ontology (GO) and pathways in the Kyoto Encyclopedia of Genes and Genomes (KEGG) of the co-expression genes of RAMPs and itself were investigated via gene set enrichment analysis (GSEA) [38].

2.10. Data preparation

Four glioblastoma (GBM) tissues and the corresponding para-tumor tissues were obtained from the Guizhou Provincial People's Hospital for quantitative real-time polymerase chain reaction (qPCR) and Western blot analysis. The procedure was approved by the Ethics Committee of Guizhou Provincial People's Hospital.

2.11. qPCR

Total RNA extraction was performed using RNAiso Plus (TaKaRa, Beijing, China). Complementary DNA (cDNA) was synthesized from the purified RNA specimens by using the Primescript RT reagent kit (TaKaRa) after measuring the concentrations of these RNA samples by using a spectrophotometer. The primer sequence and product size were as follows: GAPDH forward 5′-TTCCAGCCTTCC TTCCTGGG-3′ and reverse 5′-TTGCGCTCAGGAGGAGCAAT-3′ and RAMP3 forward 5′-T CGGTGAAGAACTATGAGACAGC-3′ and reverse 5′- AAGCCCAGGTCAAACAACTC-3′. The temperature was 95 °C for 30 s, followed by 40 cycles at 95 °C for 15 s, and 60 °C for 45 s, which was set under amplification conditions. The $2-\Delta\Delta CT$ method was used to calculate relative fold changes of mRNA levels.

2.12. Antibodies

RAMP3 antibodies were purchased from Abcam (UK). Horseradish peroxidase HRP-conjugated secondary antibodies were purchased from Cell Signaling Technology (USA).

2.13. Cell culture and transfections

Four GBM cell lines, namely, U87, U251, U118, and T98, were purchased from the Chinese Academy of Sciences Cell Bank (Shanghai, China), and T98 was procured from Procell Life Science and Technology (Wuhan, China). All the cells were maintained in

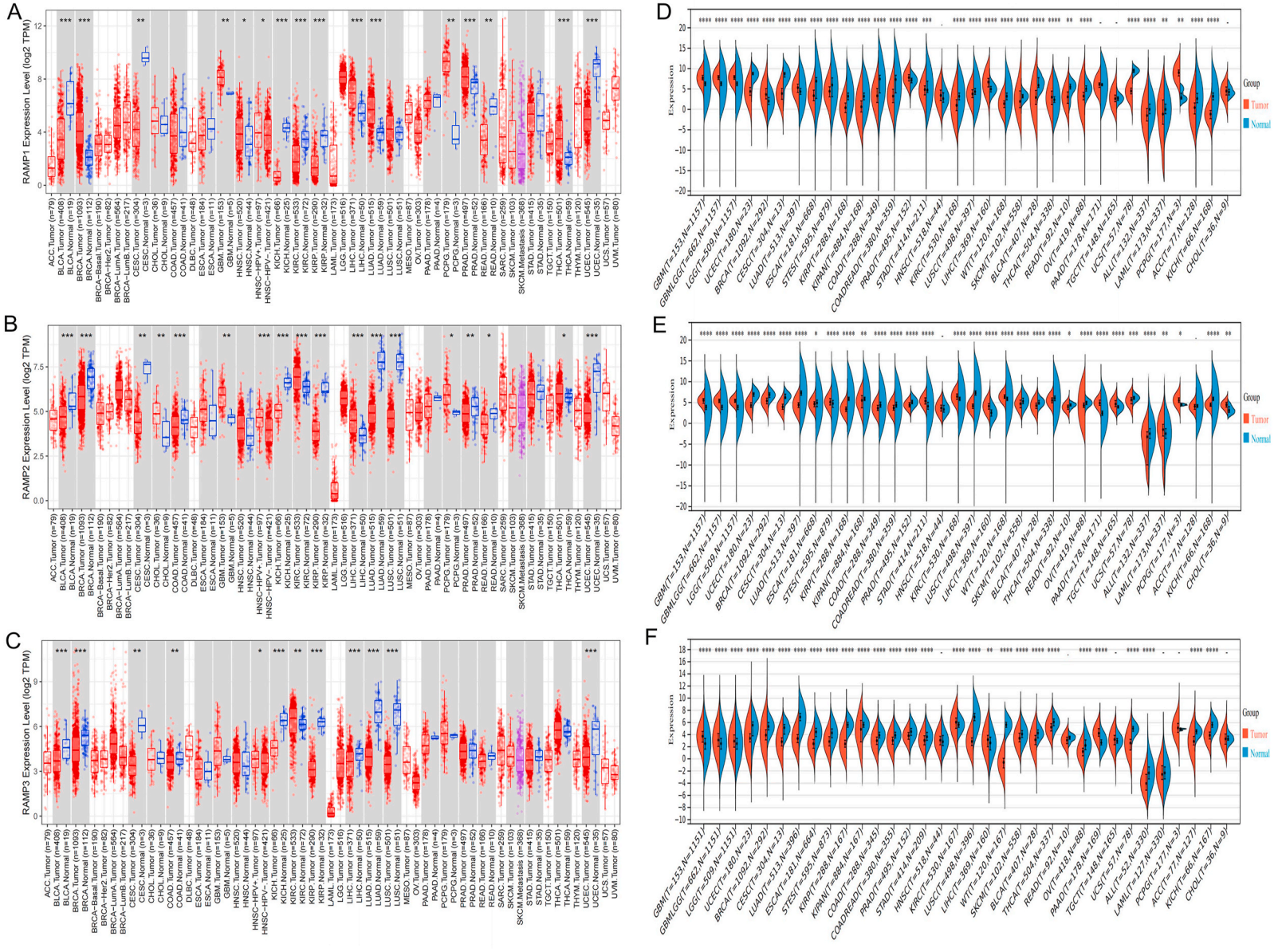

Fig. 1. RAMPs expression levels in human cancers. RAMP1 (A), RAMP2 (B), and RAMP3 (C) expression levels in different cancer types from the TCGA database analyzed by the TIMER database. RAMP1 (D), RAMP2 (E), and RAMP3 (F) expression levels in different cancer and normal tissue from the TCGA, TARGET, and GTEx database analyzed by the Sangerbox database. (*P < 0.05, **P < 0.01, ***P < 0.001).

Dulbecco's Modified Eagle's Medium (DMEM) (Gibco, USA) supplemented with 10 % fetal bovine serum (FBS) (Gibco, USA) and 1 % penicillin and streptomycin purchased from Beyotime Biotechnology (Shanghai, China) The cells were cultured at 37 °C in 5 % CO_2. For transient knockdown studies, U87 cell lines were maintained at 60 % confluence in six-well plates. Viral solutions were added to a cell culture medium that contained 8 μg/mL of polybrene (Solarbio, China) and then selected using neomycin (Beyotime, China). Expression plasmids for RAMP3 were generated via PCR and cloning into LentiORF pLEX-MCS vector (Invitrogen, USA). The short hairpin RNA (shRNA) system (pLKO-neo from Addgene) was used to knock down endogenous RAMP3. The target sequence was GGAAGGCTTTCGCAGACATGA.

2.14. Cell proliferation analysis (CCK-8 assay)

CCK-8 assay (Beyotime, China) was performed to measure cell proliferation ability. Cells, including U87 cells transfected with p-RAMP3 or p-cDNA3.1 overexpressed plasmid and U87 cells transfected with shRNA or scrRNA lentiviruses, were seeded into 96-well plates at a density of 5000 cells/well. The U87 cells were incubated for 1 h at 37 °C after adding 10 μL of CCK-8 working solution to each well. Absorbance at 450 nm was confirmed at 0, 24, 48, 72, and 96 h with an enzyme-linked instrument.

2.15. Transwell migration and invasion assay

For migration assay, glioma cell suspensions were placed in a 24-well upper chamber (Corning, USA), while 1 % penicillin/streptomycin (Beyotime, China) and 10 % FBS (Gibco, USA) were supplemented in the lower chambers. After incubation for 24 h at 37 °C, the glioma cells were fixed with 4 % paraformaldehyde and then stained with crystal violet (0.1 %; Beyotime, China). Migrated glioma cells were counted under a microscope. For the invasion assay, Matrigel (BD, USA) was applied to the upper chamber and then initially maintained at 37 °C for half an hour.

2.16. Western Blot

Total proteins were extracted in a lysis buffer with a protease inhibitor (Solarbio, China) to protect the proteins from degradation. Nuclear and cytoplasmic proteins were extracted from homogenized cells by using nuclear and cytoplasmic protein extraction kits (Solarbio, China), respectively. After denaturation at 100 °C for 10 min, equal amounts of protein per sample were subjected to sodium dodecyl sulfate–polyacrylamide gel electrophoresis and then the proteins were transferred to a polyvinylidene fluoride transfer membrane (Millipore, USA). Subsequently, 5 % bovine serum albumin (Beyotime, China) blocked the membranes at room temperature for 1 h. The membranes were rinsed three times for 10 min each with 0.5 % PBST (0.5 mL of Tween-20 dissolved in 1 L of 0.01 M phosphate-buffered saline). This process was followed by overnight incubation with the indicated primary antibody at 4 °C and three washes with 0.5 % PBST for 10 min each, followed by the addition of HRP-labeled goat anti-mouse or anti-rabbit immunoglobulin G for 1 h, and three washes with 0.5 % PBST for 10 min each. Finally, enhanced chemiluminescence (ECL) was performed using an ECL kit (Millipore, United States).

2.17. Statistical analysis

Pearson's correlation analysis was used. Prognostic significance was determined by performing the log-rank test. Unpaired Wilcoxon rank sum and signed rank tests were adopted for difference significance analysis between pairs, and Kruskal–Wallis test was used for multiple groups. R software (version 3.6.4) was used to conduct all the statistical analysis. A P value < 0.05 indicates statistical significance.

3. Results

3.1. RAMP expression in human cancers and normal tissues

The TIMER database showed that RAMP1 expression was significantly higher in BRCA, GBM, LIHC, LUAD, THCA, PRAD, and PCPG than in adjacent normal tissues. Meanwhile, RAMP2 expression was significantly higher in LIHC, CHOL, ESCA, GBM, KIRC, PCPG, and THCA. Moreover, RAMP3 expression was significantly higher in KIRC (Fig. 1A–C). In accordance with the results from the SangerBox website, displayed as supplementary results of cancers with paired normal tissues, we observed a significantly higher expression of RAMP1 in 9 types of tumors (GBMLGG, LGG, GBM, BRCA, LUAD, PRAD, PCPG, THCA, and LIHC) and significantly lower expression of RAMP1 in 20 types of tumors (e.g., UCEC, KIRC ESCA, STES, KIPAN, COAD, COAD, KIRP, READ, STAD, and CESC). In addition, RAMP2 expression was significantly higher in 12 types of tumors (GBM, GBMLGG, LGG, STES, STAD, KIRC, LIHC, WT, PAAD, TGCT, PCPG, and CHOL) and significantly lower in 20 types of tumors. Meanwhile, RAMP3 expression was significantly higher in 6 types of tumors (e.g., GBMLGG, LGG, GBM, KIRC, and PAAD) and significantly lower in 22 types of tumors (Fig. 1D–F).

The UALCAN database showed that the promoter methylation level of PAMA1 was significantly lower in BLCA, BRCA, LIHC, LUSC, PRAD, TCGT, and THCA, but significantly higher in CESC, ESCA, KIRP, KIRC, and UCEC. The promoter methylation level of PAMA2 was significantly lower in TGCT and significantly higher in UCEC, KIRP, HNSC, READ, PRAD, LUSC, LUAD, LIHC, KIRC, ESCA, CESC, CHOL, COAD, BRCA, and BLCA. Moreover, the promoter methylation level of PAMA3 was significantly lower in MLCA, HNSC, KIRC, LIHC, LUSC, PAAD, READ, and TGCT, but significantly higher in CHOL, KIRP, LUAD, PRAD, and UCEC. Furthermore, the protein

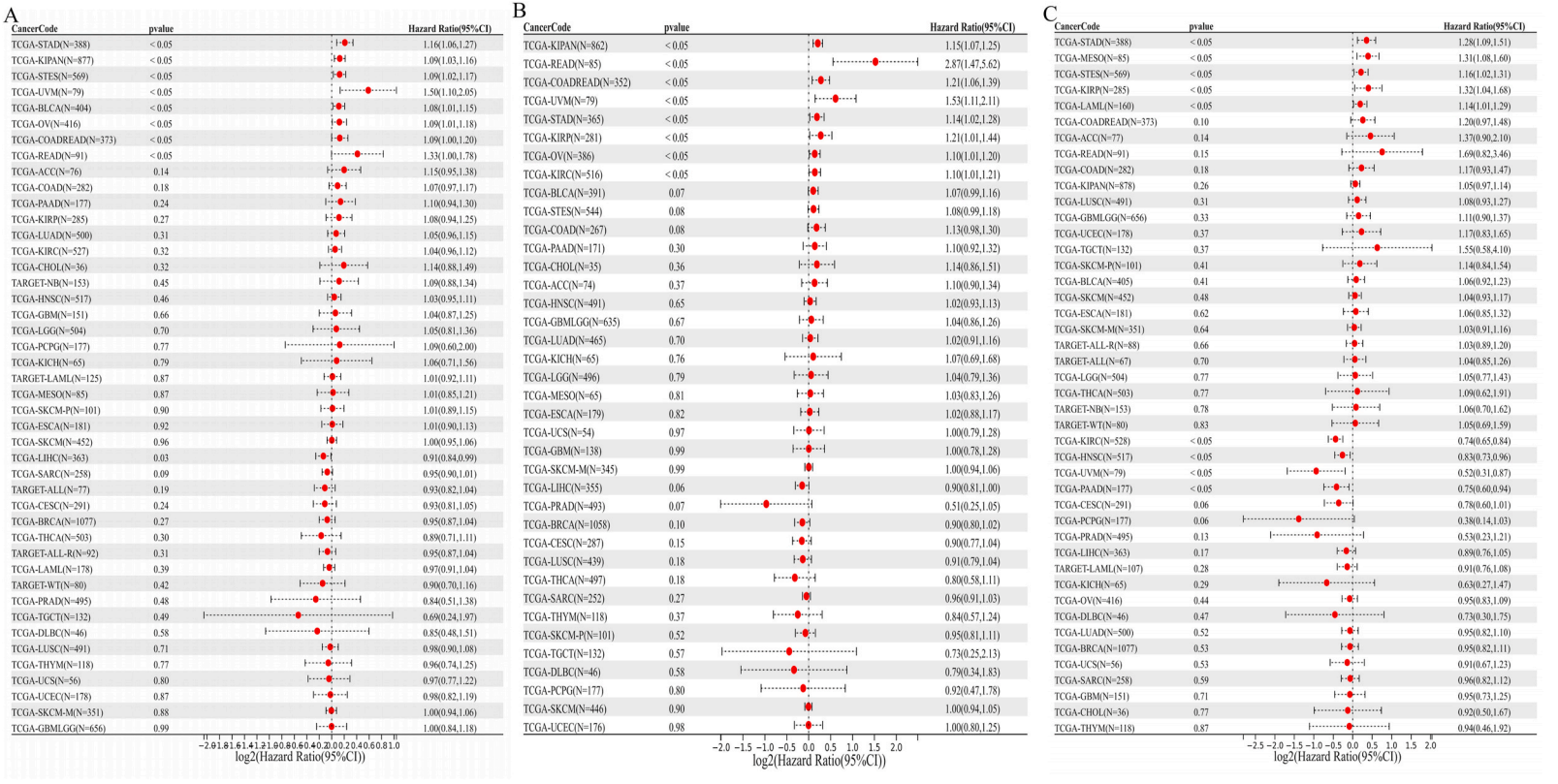

Fig. 2. Prognostic effect of RAMPs in the pan-cancer dataset of the UCSC database. (A–C) Forest plots showing the results of the univariate Cox regression analysis between overall survival (OS) and RAMP1 (A), RAMP2 (B), and RAMP3 (C) expression in human cancers. (D–F) Forest plots showing the results of the univariate Cox regression analysis between disease-specific survival (DSS) and RAMP1 (D), RAMP2 (E), and RAMP3 (F) expression in human cancers.

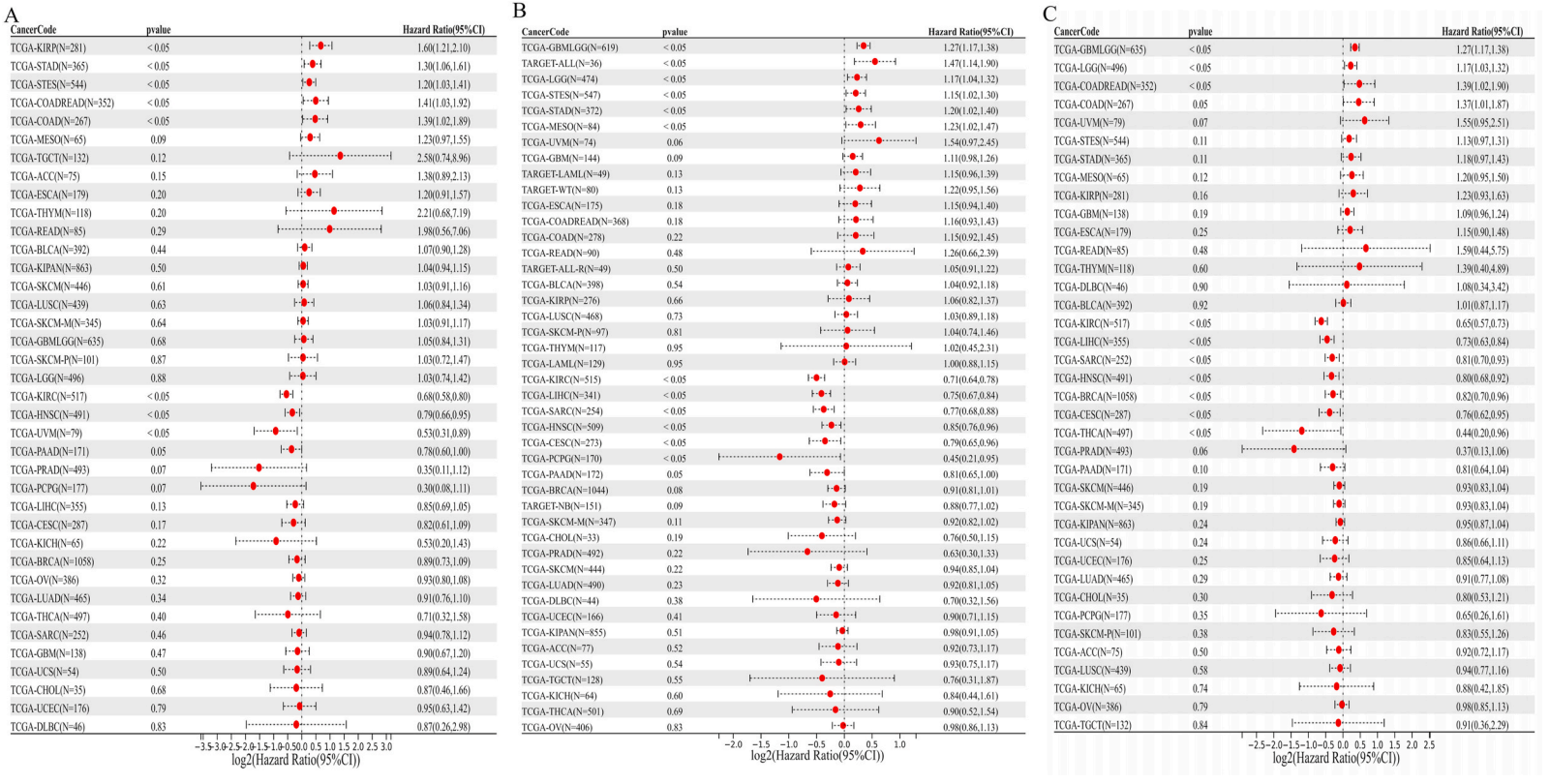

Fig. 3. The association heatmaps between PARPs expression and other important gene levels. (A–C) The association heatmaps between RNA modification genes and PARP1 (A), RAMP2 (B), and RAMP3 (C) expression. (D–F) The association heatmaps between five Immune-Related Pathways genes and PARP1 (D), RAMP2 (E), and RAMP3 (F) expression. (G–I) The association heatmaps between immune checkpoint genes and PARP1 (G), RAMP2 (H), and RAMP3 (I) expression.

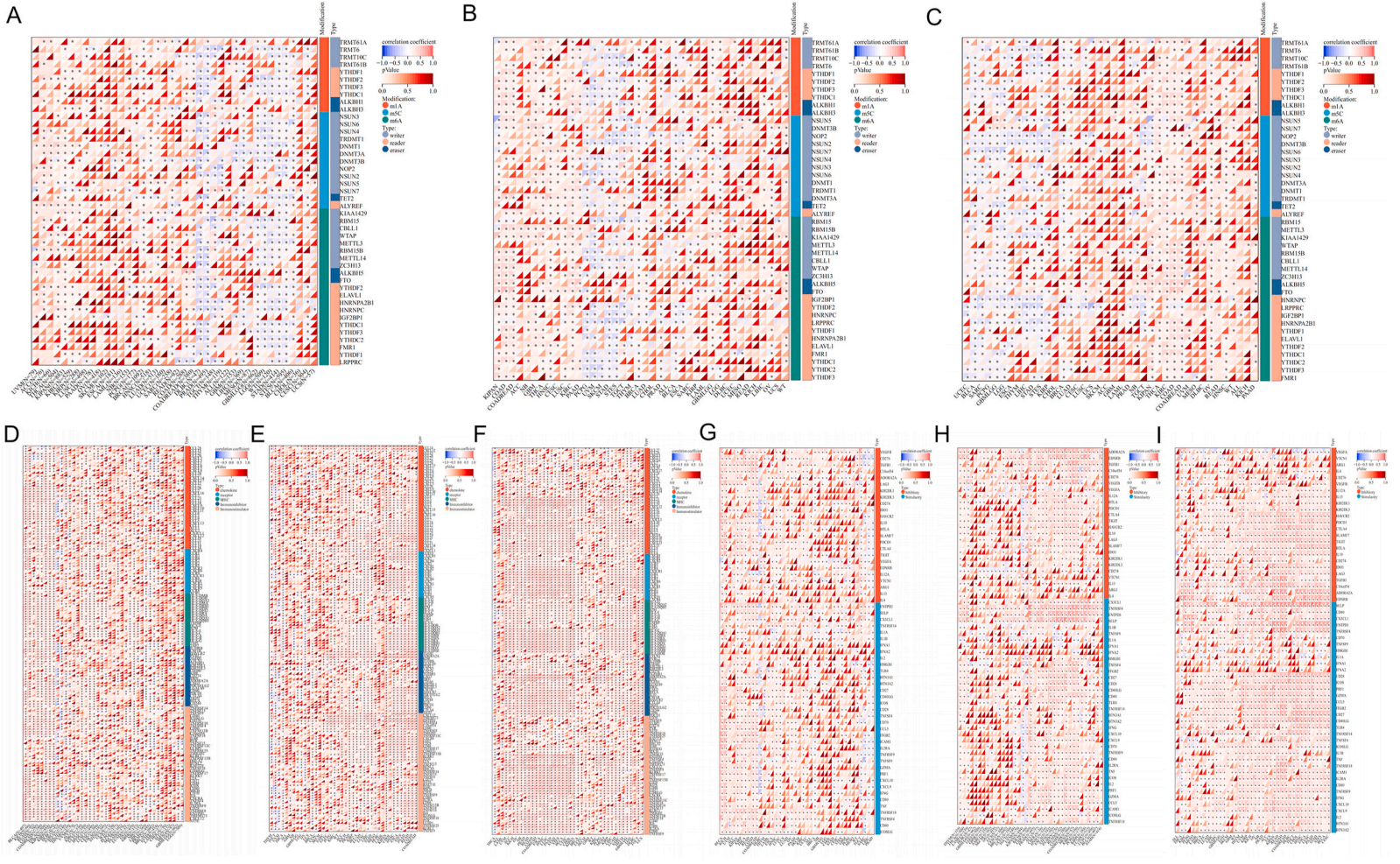

Fig. 4. The relationship between RAMPs expression and pan-cancer immune subtypes or molecular subtypes. (A, B) The relationship between RAMP1 expression and pan-cancer immune subtypes (A) or molecular subtypes (B). (C) The relationship between RAMP1 expression and immune subtypes in BLCA. (D) The relationship between RAMP1 expression and molecular subtypes in BRCA. (E, F) The relationship between RAMP2 expression and pan-cancer immune subtypes (E) or molecular subtypes (F). (G) The relationship between RAMP2 expression and immune subtypes in BRCA. (H) The relationship between RAMP2 expression and molecular subtypes in BRCA. (I, J) The relationship between RAMP3 expression and pan-cancer immune subtypes (I) or molecular subtypes (J). (K) The relationship between RAMP3 expression and immune subtypes in KIRC. (L) The relationship between RAMP3 expression and molecular subtypes in BRCA.

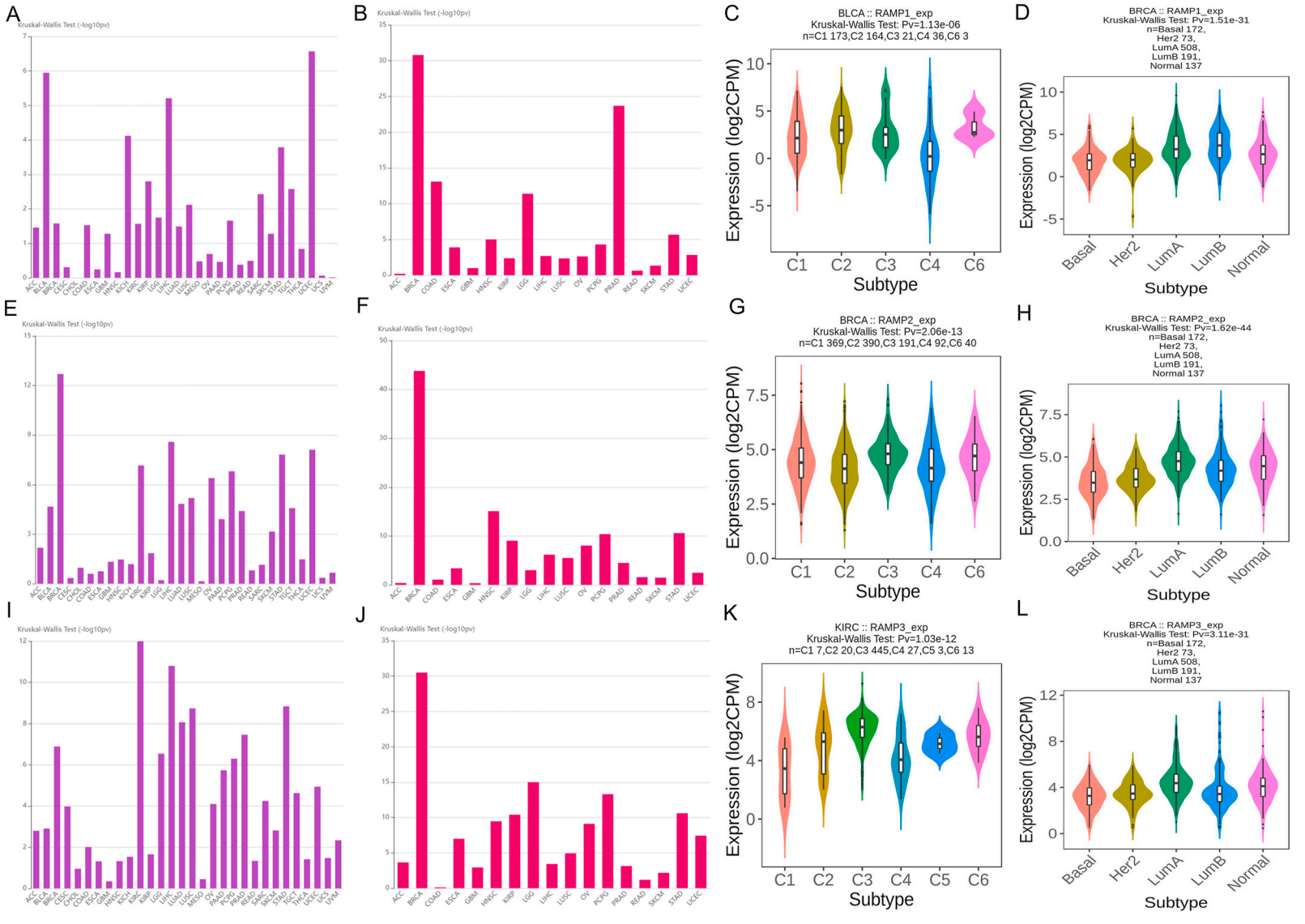

Fig. 5. The association between immune score and PARP1 (A), RAMP2 (B), and RAMP3 (C) expression in human tumors.

expression of RAMP1 was significantly higher in GBM and significantly lower in hepatocellular carcinoma. The protein expression of RAMP2 was higher in hepatocellular carcinoma and RCC, but significantly lower in HNSC, LUAD, and UCEC. Meanwhile, the protein expression of RAMP3 was lower in LUAD (Supplementary Fig. 1).

3.2. Prognostic and predictive values of RAMPs

A Cox proportional hazards regression model was established to explore the relationship between RAMP gene expression and prognosis. Higher RAMP1 expression was associated with poorer OS in six tumor types (STES, KIPAN, STAD, BLCA, OV, and UVM), while lower RAMP1 expression was associated with poorer OS in LIHC (Fig. 2A). Higher RAMP1 expression was related to poorer DSS in eight tumor types (KIRP, KIPAN, COADREAD, STAD, KIRC, READ, OV, and UVM), as shown in Fig. 2B. Higher RAMP1 expression was related to poorer DFI in eight tumor types (Supplementary Fig. 2A). In addition, higher RAMP1 expression might indicate poorer PFI in six tumor types (Supplementary Fig. 2B). Moreover, higher RAMP2 expression might mean poorer OS in five tumor types (STES, KIRP, STAD, MESO, and LAML), and patients with lower RAMP2 expression has poorer OS in four tumor types (HNSC, KIRC, UVM, and PAAD) (Fig. 2C). Patients with higher RAMP2 expression has poorer DSS in five tumor types (STES, KIRP, COAD, COADREAD, and STAD), and lower RAMP2 expression was associated with poorer DSS in four tumor types (HNSC, KIRC, UVM, and PAAD) (Fig. 3A). Higher RAMP2 expression was associated with poorer DFI in three tumor types (Supplementary Fig. 2C). Furthermore, the higher the RAMP2 expression, the poorer the PFI in three tumor types (Supplementary Fig. 2D). In addition, the higher the RAMP3 expression, the poorer the OS in six tumor types (GBMLGG, LGG, STES, MESO, STAD, and ALL); the lower the RAMP3 expression, the poorer the OS in seven tumor types (CESC, SARC, HNSC, KIRC, LIHC, PAA, and PCPG) (Fig. 3B). Moreover, higher RAMP3 expression was relevant to poorer DSS in four tumor types (GBMLGG, LGG, COAD, and COADREAD) (Fig. 3C). Higher RAMP3 expression was relevant to poorer PFI in GBMLGG (Supplementary Fig. 2E).

The results of the KM plotter showed that higher RAMP1 expression was relevant to poorer OS in urothelial carcinoma, bladder carcinoma, PRCC, pheochromocytoma, paraganglioma, ovarian cancer, stomach adenocarcinoma, and rectum adenocarcinoma, with poorer RFS in PRCC and breast cancer and poorer PFS in melanoma. Moreover, higher RAMP1 expression was relevant to poorer PFS in melanoma treated with anti-PD1. However, lower RAMP1 expression was relevant to poorer OS and PFS in glioblastoma treated with anti-PD1. Higher RAMP1 expression was associated with poorer OS in urothelial carcinoma treated with anti-PDL1 (Supplementary Fig. 3). Higher RAMP2 expression was associated with poorer OS in urothelial carcinoma, PRCC, rectum adenocarcinoma, stomach adenocarcinoma, and testicular germ cell tumor, with poorer RFS in esophageal adenocarcinoma, stomach adenocarcinoma, and PRCC, and poorer PFS in glioblastoma and melanoma. Moreover, higher RAMP2 expression was associated with poorer PFS in esophageal adenocarcinoma, glioblastoma, and melanoma treated with anti-PD1, and poorer OS in melanoma treated with anti-PD1. Higher RAMP2 expression was also relevant to poorer OS and PFS in melanoma treated with anti-CTLA4 (Supplementary Fig. 4). Moreover, higher RAMP3 expression was associated with poorer OS in esophageal adenocarcinoma, PRCC, and stomach adenocarcinoma, with poorer RFS in esophageal adenocarcinoma and HNSC, and poorer PFS in glioblastoma without treatment or treated with anti-PD1. Additional details are provided in Supplementary Fig. 5. The PrognoScan results are presented as supplementary results (Supplementary Tables 1–3). All the aforementioned pieces of evidence proved that the expression of RAMPs is strongly correlated with the prognosis of a variety of tumors. Moreover, the expression levels of RAMP1, RAMP2, and RAMP3 were significantly different in various T stages of 9, 10, and 10 tumor types, respectively (Supplementary Fig. 6, Supplementary Tables 4–6).

3.3. RAMP expression is related to RNA modification, immune-related, and immune checkpoint genes

Our findings revealed that RAMP gene expression was notably associated with RNA modification genes across human cancers and positively associated with multiple cancers (Fig. 4A–C). In addition, RAMP gene expression was positively associated with marker genes for five immune-related pathways in multiple cancers, indicating that RAMPs exert an immune effect and help identify the types of cancers that may benefit from RAMP-targeted immunotherapy (Fig. 4D–I).

3.4. RAMP expression is related to molecular and immune subtypes in cancer

Subsequently, we investigated the differences in the expression of RAMPs in human cancer immune and molecular subtypes. Immune subtypes were classified into six: C1 for wound healing, C2 for interferon gamma dominant, C3 for inflammatory, C4 for lymphocyte depleted, C5 for immunologically quiet, and C6 for transforming growth factor beta dominant. RAMP1 expression was related to different immune subtypes in 16 cancer types (Fig. 5A). In addition, RAMP1 expression varied in different molecular subtypes of 14 cancer types (Fig. 5B). Presenting BLCA as an example, RAMP1 was highly expressed in C2 and C6 types and lowly expressed in C4 types (Fig. 5C). For each BRCA molecular subtype, RAMP1 presented high expression in LumA and LumB types, while low RAMP1 expression was found in basal and Her2 types (Fig. 5D). RAMP2 expression varied in different immune subtypes of 19 cancer types and molecular subtypes of 14 cancer types (Fig. 5E and F). Presenting BRCA as an example, high RAMP2 expression was observed in C3 and LumA types, whereas low RAMP2 expression was observed in basal and Her2 types (Fig. 5G and H). RAMP3 expression varied in different immune subtypes of 27 cancer types and molecular subtypes of 15 cancer types (Fig. 5I and J). Presenting KIRC as an example, RAMP3 exhibited high expression in C3 (Fig. 5K). For BRCA, RAMP3 demonstrated high expression in LumA (Fig. 5L).

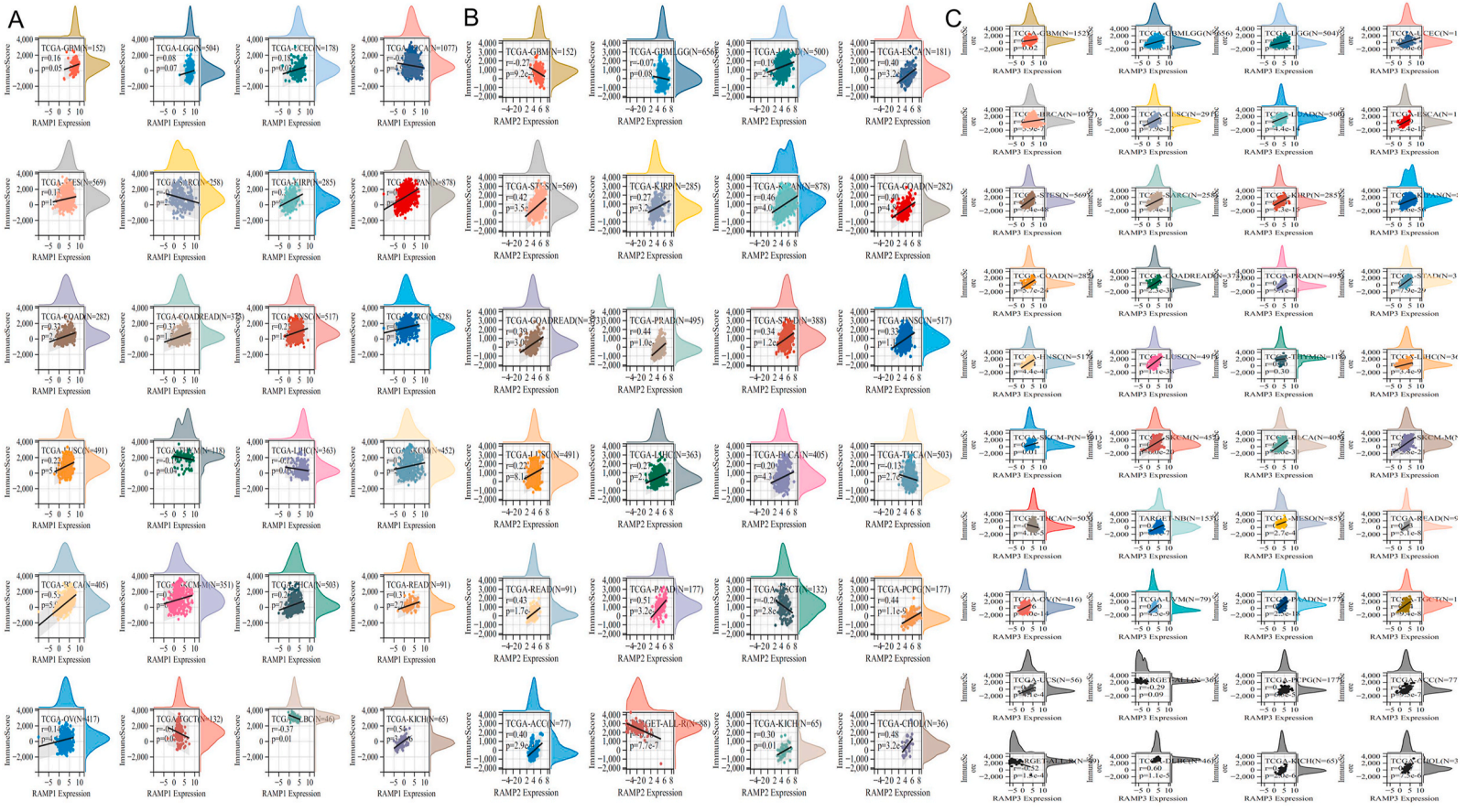

Fig. 6. The association between tumor-infiltrating lymphocytes and RAMP1. (A), RAMP2 (B), and RAMP3 (C) expression in human tumors. (D) The correlation between TMB and RAMPs expression in human tumors. (E) The correlation between MSI and RAMPs expression in human tumors. (F) The correlation between ploidy and RAMPs expression in human tumors.

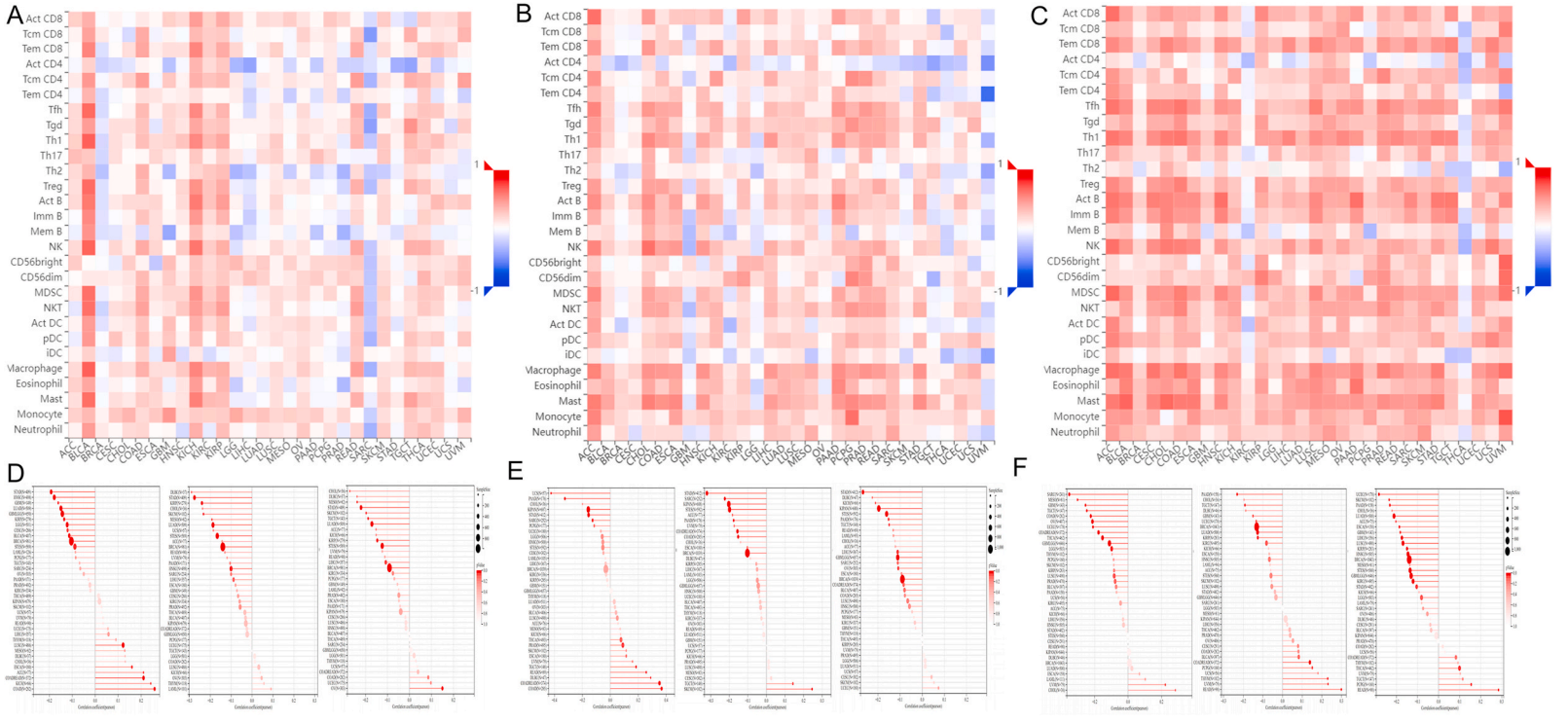

Fig. 7. (A, B) The association between RAMP1 expression and RNAss stemness indices (A) or DNAss stemness indices (B) in human tumors. (C, D) The association between RAMP2 expression and RNAss stemness indices (C) or DNAss stemness indices (D) in human tumors. (E, F) The association between RAMP3 expression and RNAss stemness indices (E) or DNAss stemness indices (F) in human tumors.

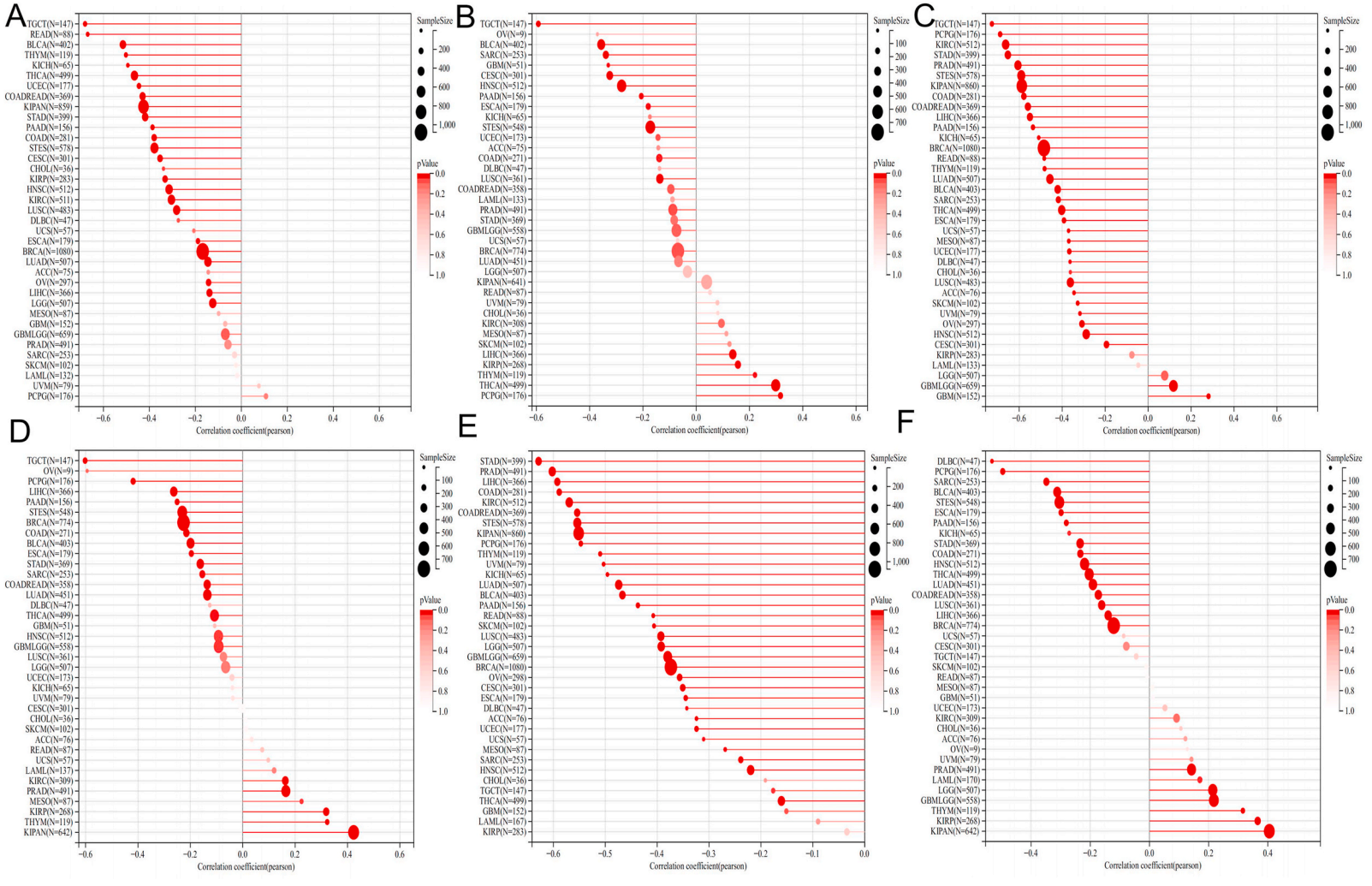

Fig. 8. RAMPs coexpression genes in cancer analyzed by the LinkedOmics database. (A) Highly correlated genes of RAMP1 tested by Pearson test in BLCA cohort. (B, C) Top 50 negative coexpression genes (B) and positive coexpression genes (C) of PAMP1 in heat map in BLCA. (D) GO analysis (biological process) of RAMP1 in BLCA cohort. (E) KEGG pathways of RAMP1 in BLCA cohort. (F) Highly correlated genes of RAMP2 tested by Pearson test in KIRP cohort. (G, H) Top 50 negative coexpression genes (G) and positive coexpression genes (H) of PAMP2 in heat map in KIRP. (I) GO analysis (biological process) of RAMP2 in KIRP cohort. (J) KEGG pathways of RAMP2 in KIRP cohort. (K) Highly correlated genes of RAMP3 tested by Pearson test in GBMLGG cohort. (L, M) Top 50 negative coexpression genes (L) and positive coexpression genes (M) of PAMP3 in heat map in GBMLGG. (N) GO analysis (biological process) of RAMP3 in GBMLGG cohort. (O) KEGG pathways of RAMP3 in GBMLGG cohort.

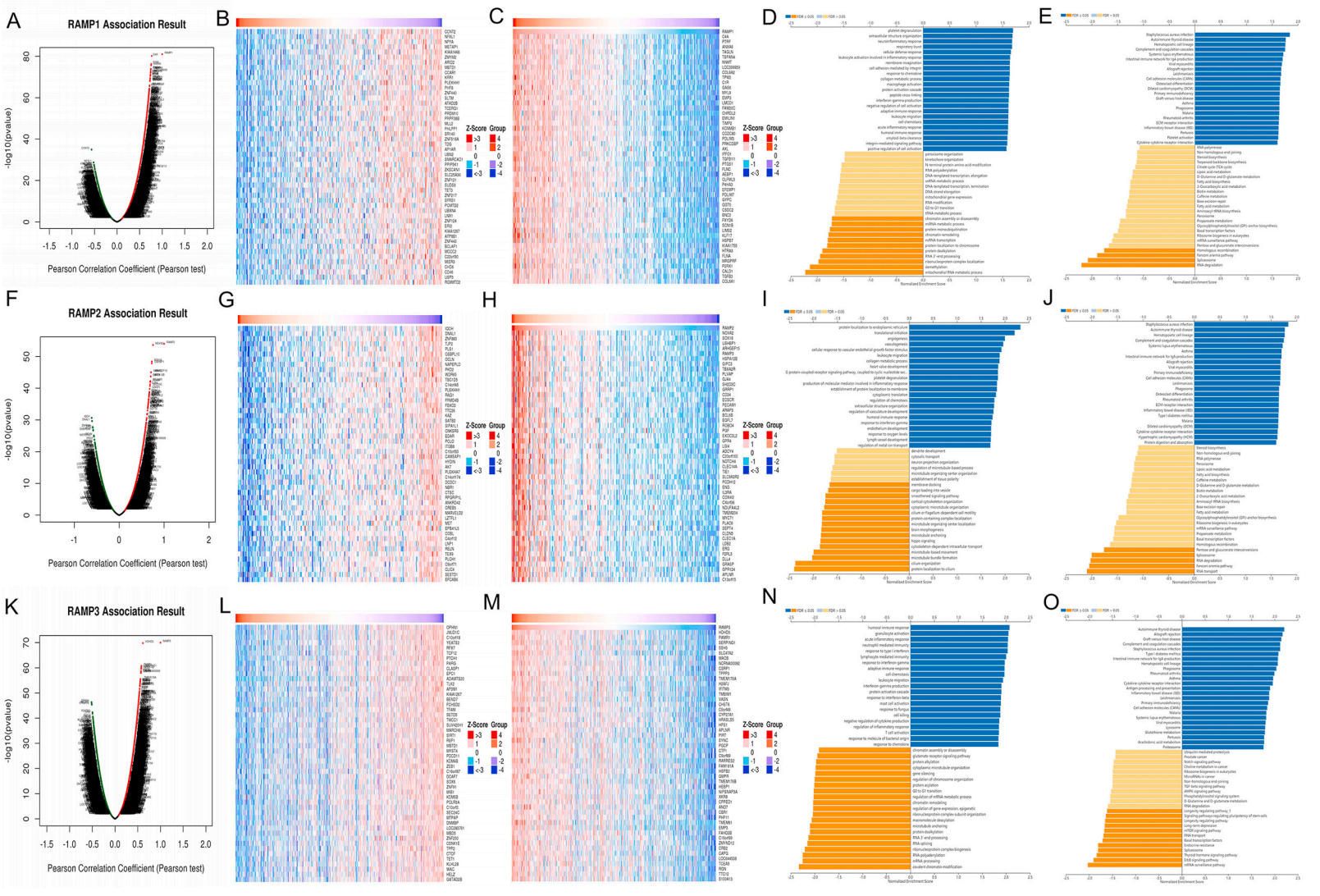

Fig. 9. RAMP3 mRNA and protein expression levels in glioma. (A) qRT-PCR result of RAMP3 expression was higher in GBM tissues (GBM) compared to corresponding para-tumor tissues (Normal). (B, C) Western Blot result of RAMP3 expression was higher in GBM tissues (T/GBM) compared to the corresponding para-tumor tissues (N/Normal). (D, E) Western Blot result of RAMP3 expression was higher in 4 glioma cell lines (U118, U87, U251, T98) compared to Normal human astrocytes (NHA). (*P < 0.05, **P < 0.01, ***P < 0.001).

3.5. RAMP expression is related to ESTIMATE, immune cells, TMB, MSI, and ploidy

The correlations of RAMPs with immune, stromal, and ESTIMATE scores were measured. Then, the significant results are illustrated in Fig. 6 and Supplementary Fig. 7. RAMP1 expression was significantly related to immune score in 21 cancer types. Among which, 16 were positively correlated and 5 were negatively correlated. RAMP1 expression was significantly related to stromal score in 25 cancer types. Among which, 23 were positively correlated and 2 were negatively correlated. This gene expression was significantly related to ESTIMATE score in 20 cancer types. Among which, 18 were positively correlated and 2 were negatively correlated. In addition, a significant correlation was observed between RAMP2 expression and immune score in 38 cancer types, with 37 positive and 1 negative correlations. RAMP2 expression was significantly correlated with stromal score in 23 cancer types. Of which, 19 were positively and 4 were negatively correlated. RAMP2 expression was significantly correlated with ESTIMATE score in 33 cancer types. Of which, 31 were positively and 2 were negatively correlated. Moreover, RAMP3 expression was significantly correlated with immune score in 40 cancer species, with 39 positively and 1 negatively correlated. RAMP3 expression was significantly correlated with stromal score in 38 cancer species. Of which, 36 were positively and 2 were negatively correlated. RAMP3 expression was significantly related to ESTIMATE score in 40 cancer species, with 38 positively and 2 negatively correlations.

RAMPs were significantly correlated with TILs in many cancer types (Fig. 7A–C). Therefore, 7 algorithms were used to estimate immune cell infiltration scores in 38 cancer types. The results confirmed that RAMPs may play an important role in multiple immune infiltrating cells in a variety of cancer types. Supplementary Figs. 8–10 present the detailed results. As depicted in Fig. 7D, RAMP1, RAMP2, an RAMP3 expression levels were significantly associated with TMB in 15, 7, and 10 cancer types, respectively ($p < 0.05$). The coefficients indicated that the expression of three genes was associated with MSI in 11, 9, and 7 cancer types, respectively (Fig. 7E; $p < 0.05$). In addition, the correlation between RAMPs and ploidy is shown in Fig. 7F. The expression of those genes was associated with ploidy in 12, 9, and 17 cancer types, respectively ($p < 0.05$).

3.6. RAMP expression is related to stemness indices

RAMP1 was associated with RNAss stemness indices in 25 tumors, and all associations were negative (Fig. 8A, $p < 0.05$). RAMP1 was associated with DNAss stemness indices in 16 tumors, including positive associations in 5 tumors and negative associations in 11 tumors (Fig. 8B, $p < 0.05$). RAMP2 was correlated with RNAss stemness indices in 34 tumors, with positive correlations in 2 tumors and negative correlation in 32 tumors (Fig. 8C, $p < 0.05$). RAMP2 was associated with DNAss stemness indices in 22 tumors, including positive correlations in 6 tumors and negative correlations in 16 tumors (Fig. 8D, $p < 0.05$). For RAMP3, all negative associations were found with RNAss stemness in 33 tumors (Fig. 8E, $p < 0.05$). RAMP3 was associated with DNAss stemness in 24 tumors, 7 tumors were positively associated and 17 tumors were negatively (Fig. 8F).

3.7. Co-expression networks of RAMPs in specific cancer types

To further investigate the specific biological function of RAMPs in tumors, we explored RAMP1 co-expression networks in BLCA to illustrate the potential function. In BLCA, 5469 genes (dark green dots) were negatively correlated with RAMP1, while 6507 genes (dark red dots) were positively correlated (FDR <0.01) (Fig. 9A, Supplementary Table 7). The top 50 negatively and top 50 positively correlated genes with RAMP1 are depicted in Fig. 9B and C. Subsequently, the major GO terms of RAMP1 co-expression genes were determined via GSEA (Fig. 9D–Supplementary Table 8). RAMP1 and its co-expression genes primarily participated in neuroinflammatory response, cellular defense response, cell adhesion mediated by integrin, response to chemokine, and leukocyte activation involved in inflammatory response. The KEGG pathway analysis showed that co-expressed genes were enriched during extracellular matrix (ECM)–receptor interaction, intestinal immune network for IgA production, primary immunodeficiency, and complement and coagulation cascades (Fig. 9E–Supplementary Table 9). Moreover, RAMP2 co-expression networks in KIRP were analyzed. A total of 5014 genes (dark green dots) were significantly negatively correlated with RAMP2, and 5912 genes (dark red dots) were positively correlated (FDR <0.01) (Fig. 9F–Supplementary Table 10). Fig. 9G and H shows the top 50 negatively and positively correlated genes with RAMP2. Subsequently, the major GO terms of RAMP1 co-expression genes were enriched in vasculogenesis, cellular response to vascular endothelial growth factor stimulus, leukocyte migration, production of a molecular mediator involved in inflammatory response, and G protein-coupled receptor signaling pathway (Fig. 9I–Supplementary Table 11). The KEGG pathway enrichment analyses of RAMP2 and its co-expression genes were mostly enriched in complement and coagulation cascades, leukocyte transendothelial migration, the cGMP-PKG signaling pathway, ECM–receptor interaction, and the calcium signaling pathway (Fig. 9J–Supplementary Table 12). In addition, we analyzed RAMP3 co-expression networks in GBMLGG. The results showed that 5801 genes (dark green dots) were significantly negatively related to RAMP1, while 8178 genes (dark red dots) were positively related (FDR <0.01) (Fig. 9K–Supplementary Table 13). Fig. 9L and M presents the top 50 negatively and top 50 positively correlated genes with RAMP3. The GO enrichment analysis showed that humoral immune response, granulocyte activation, acute inflammatory response, neutrophil-mediated immunity, response to Type I interferon/interferon gamma, lymphocyte-mediated immunity, and adaptive immune response were mostly enriched. Meanwhile, the KEGG enrichment analysis showed that complement and coagulation cascades, primary immunodeficiency, antigen processing and presentation, phagosome, cytokine–cytokine receptor interaction, and intestinal immune network for IgA production were mostly enriched (Fig. 9N and O, Supplementary Tables 14 and 15).

3.8. mRNA and protein expression levels of RAMP3 in glioma

The high expression of RAMP3 in glioma tissues was verified in mRNA and protein levels compared with the corresponding para-tumor tissues. This result is consistent with our in-silico bioinformatics findings described above (Fig. 10A–C). In addition, the protein level of RAMP3 in glioma cell lines was explored through Western blot analyses. As shown in Fig. 10D and E, RAMP3 was more highly expressed in glioma cell lines (U118, U251, U87, and T98) compared with normal human astrocytes (NHAs).

3.9. RAMP3 promotes glioma cell proliferation, migration, and invasion

Previous studies have indicated that RAMP3 is associated with metastasis in other cancer types. To explore the function of RAMP3 in glioma further, overexpression plasmids and the shRNA system were designed to construct U87 cells that were highly expressed in RAMP3 genes and inhibited RAMP3 endogenous expression, respectively. In addition, the RAMP3 expression of U87 cells was appreciably upregulated after the transfection of overexpressed plasmids (Fig. 11A and B, $p < 0.01$), and RAMP3 expression of U87 cells was appreciably downregulated after the transfection of sh-RAMP3 (Fig. 12A and B, $p < 0.01$). The CCK-8 assays indicated that RAMP3 overexpression significantly enhanced cell proliferation at 48, 72, and 96 h post-transfection (Fig. 11C). Meanwhile the proliferation of U87-Sh-RAMP3 cells was significantly inhibited at 48, 72, and 96 h post-transfection (Fig. 12C). In addition, transwell migration and invasion experiments indicated that the overexpression of RAMP3 upregulated the migration and invasion abilities of U87 cells compared with those of the controls (Fig. 11D and E). Meanwhile, the knockdown of RAMP3 weakened the migration and invasion abilities of U118 cells compared with those of the controls (Fig. 12D and E).

4. Discussion

In a recent study, the researchers used a series of genes and drug treatments in cell and animal experiments to determine whether melanoma cells can "sensitize" nociceptor nerve cells, which, in turn, promote the release of a neuropeptide called CGRP [7]. This neuropeptide can bind to the RAMP-1 receptors of cytotoxic $CD8^+$ T cells to promote their exhaustion. RAMP-1 is a subtype of RAMPs, which comprise a new family of single-transmembrane proteins that determine the ligand specificity of CLR. To date, the regulatory roles of RAMPs and their ligands in various cancers or other diseases have been extensively studied [39–42]. However, the molecular functions and clear mechanisms of RAMPs in most tumors remain unexplored. On the basis of evidence obtained from previous studies, an inference can be made that RAMPs may play a critical role in tumorigenesis as regulatory proteins of G-protein-coupled receptors.

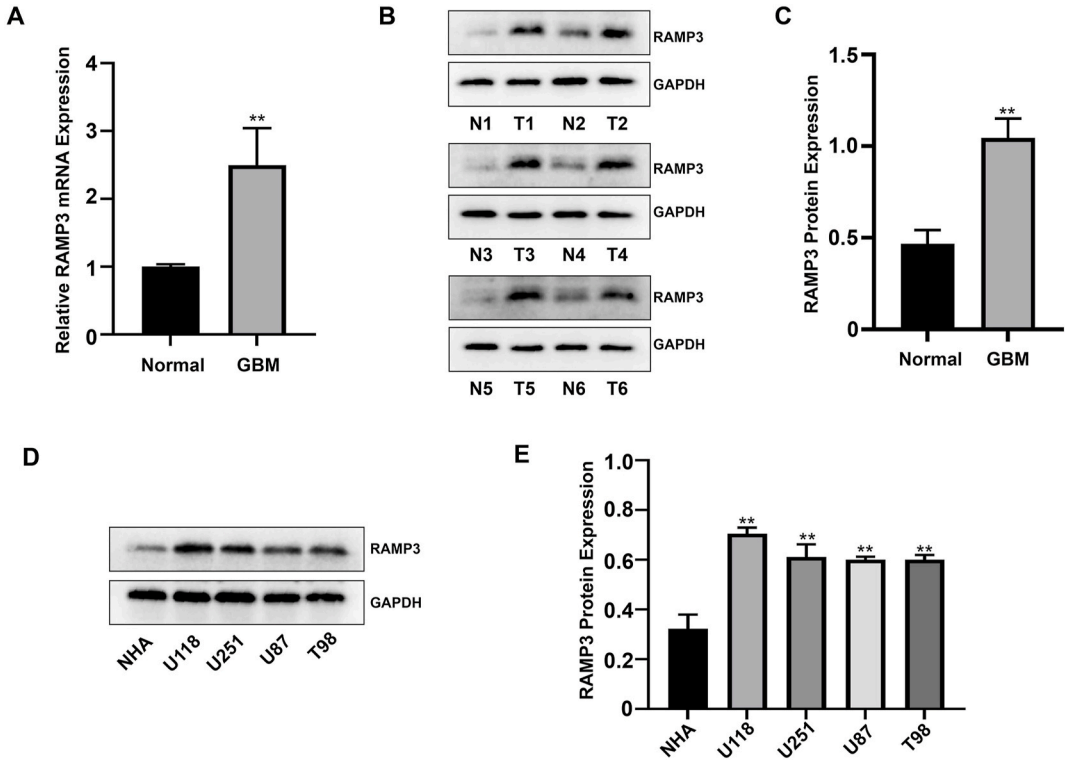

Fig. 10. Overexpression of RAMP3 promotes glioma cells proliferation, migration, and invasion in vitro. (A, B) Western blot analysis of the RAMP3 expression in U87-Ctrl cells and U87-over-RAMP3 cells. (C) CCK8 assay of overexpression of RAMP3 promotes glioma cell proliferation. (D, E) Transwell migration and invasion assay of overexpression of RAMP3 promotes glioma cell migration and invasion (**$P < 0.01$, ***$P < 0.001$).

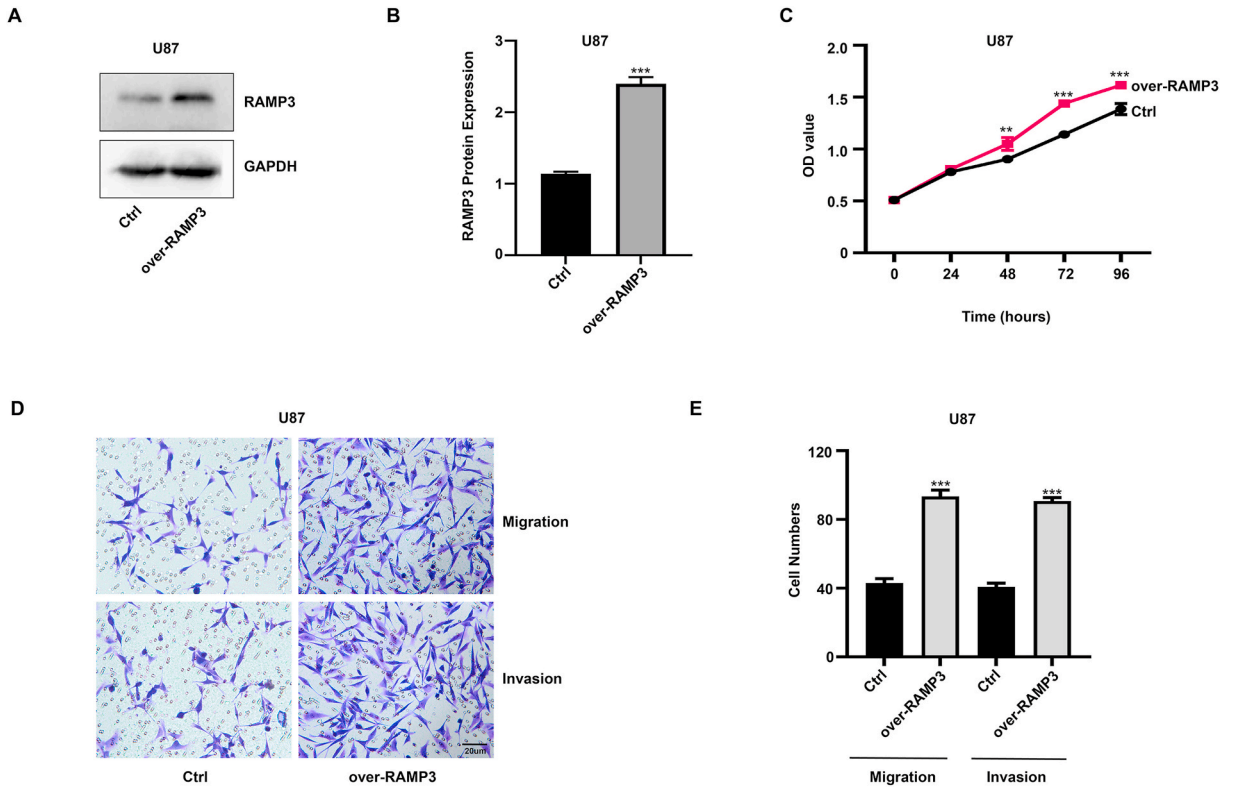

Fig. 11. Overexpression of RAMP3 promotes glioma cells proliferation, migration, and invasion in vitro. (A, B) Western blot analysis of the RAMP3 expression in U87-Ctrl cells and U87-over-RAMP3 cells. (C) CCK8 assay of overexpression of RAMP3 promotes glioma cell proliferation. (D, E) Transwell migration and invasion assay of overexpression of RAMP3 promotes glioma cell migration and invasion (**P < 0.01, ***P < 0.001).

These proteins are involved in the modulation of various signaling pathways, including those related to tumor growth, invasion, and metastasis. Therefore, RAMPs can potentially influence the characteristics and behavior of tumor cells during cancer development, leading to changes associated with tumor initiation and the tumor immune microenvironment. Moreover, RAMPs may regulate the function and abundance of immune cells within the tumor immune microenvironment. Immune cells play a pivotal role in antitumor immune response; they are capable of attacking and eliminating tumor cells. RAMPs may modulate the interaction between tumor cells and immune cells, affecting the infiltration and activity of immune cells, and thus, shaping the characteristics of the tumor immune microenvironment. In addition, RAMPs may influence diagnostic outcomes through their effects on tumor cell metabolism and proliferation. Studies have revealed the involvement of RAMPs in the regulation of tumor cell metabolism, potentially leading to alterations in the sensitivity of tumor cells to specific therapeutic approaches, ultimately influencing diagnostic outcomes.

In the current study, RAMP expression was compared between normal and tumor tissues in pan-cancer. The reliability of the data was verified through multiple databases, and RAMPs were significantly differentially expressed in various tumors. In addition, the promoter methylation levels of PAMAs were different between multiple tumors and normal tissues. The prognostic value of RAMPs has also been reflected in the current study. In accordance with our results, we can conclude that RAMPs are closely related to OS, DSS, DFI, PFI, PFS, and other survival indicators.

Abundant previous studies have also confirmed that clinical stages, molecular subtypes, and immune subtypes can indicate the prognostic value of tumors [43–46]. Therefore, we analyzed the expression in different tumor subgroups of RAMPs. In accordance with the TISIDB database analysis, we confirmed that RAMPs can predict clinical stage, immune subtype, and molecular subtype in a variety of tumors to a certain extent, indicating that its expression level exhibits a relationship with the development and occurrence of tumors. Overall, RAMPs may be important biomarkers associated with prognosis in different tumors.

Our study also demonstrated the correlation of RAMP expression with TMB, MSI, and tumor neoantigens in many types of tumors. TMB is closely related to the efficacy of immunotherapy. MSI is also a clinically important tumor marker that results from defective DNA mismatch repair [47–49]. Neoantigens are tumor-specific antigens derived from non-synonymous mutations; they have attracted considerable attention in tumor immunotherapy [50,51]. Tumor neoantigens are widely found in tumor cells, and they exhibit significant tumor heterogeneity and immunogenicity. In the clinical trials of a variety of solid tumors, vaccines developed by neoantigens have been used for tumor immunotherapy [52]. ICGs are important targets for antitumor immunotherapy approaches in a variety of tumors [53,54]. In the present study, RAMP expression has been shown to be significantly positively correlated with most of the 47 ICGs in most cancers, such as CD40, CTLA-4, CD86, and CD276. Similarly, RAMP expression is highly relevant to 150 genetic markers of 5 classes of the immune pathways in most tumors. Unexpectedly, RAMP expression also significantly affected patient outcomes in

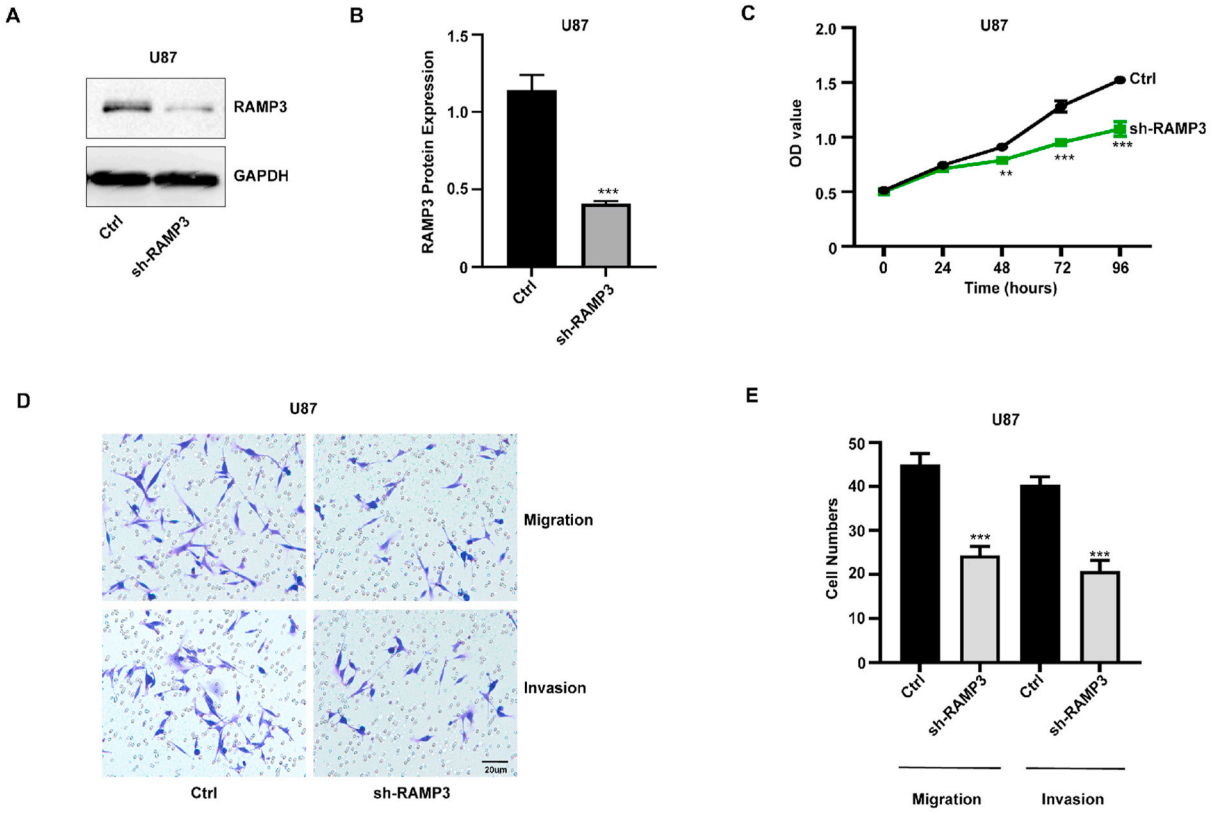

Fig. 12. Knockdown of RAMP3 suppresses glioma cells proliferation, migration, and invasion in vitro. (A, B) Western blot analysis of the RAMP3 expression in U87-Ctrl cells and U87-Sh-RAMP3 cells. (C) CCK8 assay of knockdown of RAMP3 suppresses glioma cell proliferation. (D, E) Transwell migration and invasion assay of knockdown of RAMP3 suppresses glioma cell migration and invasion (**P < 0.01, ***P < 0.001).

the immunotherapy cohort. For example, higher RAMP1 expression might mean poorer PFS in melanoma treated with anti-PD1. Moreover, higher RAMP2 expression might indicate poorer PFS in esophageal adenocarcinoma, GBM, and melanoma treated with anti-PD1 and poorer OS in melanoma treated with anti-PD1. Higher RAMP2 expression might also indicate poorer OS and PFS in melanoma treated with anti-CTLA4. Moreover, higher RAMP3 expression might mean poorer PFS in GBM treated with anti-PD1. Therefore, RAMPs are extremely important immunotherapy and prognostic targets that may enhance the therapeutic effects of current immune checkpoint inhibitors. Subsequently, we analyzed the relationship between RAMP and immune cells in TME. TILs have been reported to predict prognosis and immunotherapy efficacy independently in cancer patients [55,56]. The results from the TISDB database suggested a strong correlation between RAMPs and TILs in most tumors. In addition, immune cells are closely related to the occurrence, development, and metastasis of tumors, and they are also related to the development of drugs for immunotherapy. Our study found that RAMP expression was negatively correlated with the content of immune cells in the tumor microenvironment in a variety of tumors. It also determined that RAMPs were strongly correlated with the immune, stromal, and ESTIMATE scores in most cancer types, negatively in some tumors and positively in others, suggesting that RAMPs play different immunomodulations in various cancers. However, the relationship between RAMPs and immunity in various tumors and the mechanism of action still require further analyses.

The gradual reduction of cell differentiation capacity and the acquisition of stem cell-like features are the major factors that drive tumors [57,58]. The stemness index is used to evaluate the degree of stem cell-like characteristics of tumor cells, and it is largely related to active biological processes and a high degree of differentiation [59]. We calculated the stemness index in individual tumors by using a variety of algorithms, and the significant correlation between RAMPs and stemness index was confirmed in our study. m1A, m5C, and m6A RNA methylation have been reported to play an important role in cancer [60], and it is expected to become a therapeutic target. RAMPs were significantly correlated with RNA methylation genes, further indicating the importance of RAMPs in tumors.

To comprehensively analyze the possible biological functions of RAMPs in tumors, co-expression and GSEA analyses were performed in specific tumors, and the results showed that RAMPs were significantly related to a variety of oncogenic pathways and immune functions. However, our results suggest that the mechanisms of RAMPs vary in different tumors, and further studies are necessary for each tumor to understand the effects of RAMPs on the immune function of specific tumors and mechanisms.

At present, the study of RAMPs in pan-cancer remains lacking, and their immunological effect has been rarely reported. In the current study, we not only analyzed the prognostic value of RAMPs and the predictive value of immunotherapy in pan-cancer, but also

examined the relationship between RAMPs and immunology from several perspectives. In addition, our results were cross-validated in different databases, strengthening the credibility of our results. The specific mechanism of RAMPs in pan-cancer deserves more attention, and the current study also provided a reference for future in-depth research. We emphasized that we used quantitative reverse transcription PCR (qRT-PCR) and Western blot analysis to confirm that RAMP3 was expressed significantly higher in glioma tissues than in paracancer tissues. Moreover, RAMP3 was higher in glioma cell lines than normal cell lines. The overexpression of RAMP3 upregulated the migration and invasion abilities of U118 cells compared with those of the controls, while the knockdown of RAMP3 weakened the migration and invasion abilities of U118 cells compared with those of the controls.

Our study has limitations. First, although we used a large number of bioinformatics methods to analyze the relationship of RAMPs with prognosis and tumor immune microenvironment, in vivo/in vitro experiments are still required to prove our findings. In addition, clinical cohort studies help further evaluate the potential of RAMPs in many cancers. Second, although we have reasonably demonstrated that RAMPs are closely related to TIME and prognosis, the mechanism by which RAMPs participate in immune regulation remains unclear, and thus, more in-depth studies are necessary. In addition, no anti-RAMP treatment has been developed and clinically tested. Therefore, further molecular and clinical validations are necessary for the development of novel antitumor immunotherapy drugs. Finally, the results of data from multiple databases or analysis algorithms are not always consistent. Therefore, further analysis is required to verify our results.

5. Conclusion

In conclusion, our study shows that RAMPs are differentially expressed in multiple tumors and associated with patient outcomes. Meanwhile, RAMPs are significantly correlated with immune scores, ICGs, and immune-infiltrating cells. In addition, RAMPs are significantly associated with RNA-editing genes, TMB, MSI, tumor neoantigens, and stemness index. Furthermore, RAMP3 promotes glioma cell proliferation and migration. We speculate that RAMPs may affect the progression of multiple tumors through multiple signaling pathways, particularly immune-related pathways. Our study reveals the potential of RAMPs as a possible biomarker and therapeutic target. Further study of RAMPs may help improve immunotherapy outcomes and prognosis in patients.

Availability of data and materials

The datasets generated and/or analyzed during this study are publicly available in the TIMER database (https://cistrome.shinyapps. io/timer/), Sangerbox website (http://sangerbox.com), UALCAN database (UALCAN (uab.edu)), Kaplan–Meier plotter (https://kmplot.com/analysis/), PrognoScan (http://dna00.bio.kyutech.ac.jp/PrognoScan/index.html), UCSC database (https://xenabrowser.net/), TCGA GDC database (https://portal.gdc.cancer.gov/), TISIDB database (http://cis.hku.hk/TISIDB), and LinkedOmics database (http://www.linkedomics.org).

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Ethics approval and consent to participate

The patients' consent and approval from the Institutional Research Ethics Committee of Guizhou Provincial People's Hospital were obtained for research purposes [(2019) 122].

Consent for publication

Not applicable.

Data availability statement

Data included in article/supplementary material in article.The datasets generated and/or analyzed during this study are publicly available in the online databases.

CRediT authorship contribution statement

Sha Yang: Writing – original draft, Visualization, Validation, Software, Project administration, Methodology, Formal analysis, Data curation, Conceptualization. **Renzheng Huan:** Formal analysis, Data curation, Conceptualization. **Mei Deng:** Methodology, Formal analysis. **Tao Luo:** Writing – original draft, Software. **Shuo Peng:** Writing – review & editing, Software. **Yunbiao Xiong:** Writing – review & editing, Software. **Guoqiang Han:** Writing – review & editing, Methodology, Data curation. **Jian Liu:** Writing – review & editing, Methodology, Funding acquisition, Data curation, Conceptualization. **Jiqin Zhang:** Writing – review & editing, Writing – original draft, Software, Funding acquisition. **Ying Tan:** Writing – review & editing, Validation, Project administration,

Funding acquisition, Data curation, Conceptualization.

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.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e24849.

References

[1] D.L. Hay, A.A. Pioszak, Receptor activity-modifying proteins (RAMPs): new insights and roles, Annu. Rev. Pharmacol. Toxicol. 56 (2016) 469–487, https://doi.org/10.1146/annurev-pharmtox-010715-103120.
[2] A. Febres, O. Vanegas, M. Giammarresi, C. Gomes, E. Díaz, A. Ponte-Sucre, Is the activity of CGRP and adrenomedullin regulated by RAMP (-2) and (-3) in trypanosomatidae? An in-silico approach [published correction appears in infect genet evol. 2021 nov;95:105002], Infect. Genet. Evol. 61 (2018) 197–206, https://doi.org/10.1016/j.meegid.2018.04.003.
[3] D. Scuteri, M.T. Corasaniti, P. Tonin, P. Nicotera, G. Bagetta, Role of CGRP pathway polymorphisms in migraine: a systematic review and impact on CGRP mAbs migraine therapy, J. Headache Pain 22 (1) (2021) 87, https://doi.org/10.1186/s10194-021-01295-7. Published 2021 Jul 30.
[4] A.A. Pioszak, D.L. Hay, RAMPs as allosteric modulators of the calcitonin and calcitonin-like class B G protein-coupled receptors, Adv. Pharmacol. 88 (2020) 115–141, https://doi.org/10.1016/bs.apha.2020.01.001.
[5] D. Yang, A. Jacobson, K.A. Meerschaert, et al., Nociceptor neurons direct goblet cells via a CGRP-RAMP1 axis to drive mucus production and gut barrier protection, Cell 185 (22) (2022) 4190–4205, https://doi.org/10.1016/j.cell.2022.09.024, e25.
[6] A.F. Russo, D.L. Hay, CGRP physiology, pharmacology, and therapeutic targets: migraine and beyond [published online ahead of print, 2022 Dec 1], Physiol. Rev. (2022), https://doi.org/10.1152/physrev.00059.2021.
[7] W. Wu, B. Feng, J. Liu, et al., The CGRP/macrophage axis signal facilitates inflammation recovery in the intestine, Clin. Immunol. 245 (2022) 109154, https://doi.org/10.1016/j.clim.2022.109154.
[8] D. Stanić, E. Kuteeva, I. Nylander, T. Hökfelt, Characterization of CGRP protein expression in "satellite-like" cells and dendritic arbours of the mouse olfactory bulb, J. Comp. Neurol. 518 (6) (2010) 770–784, https://doi.org/10.1002/cne.22226.
[9] M. Levite, R. Safadi, Y. Milgrom, M. Massarwa, E. Galun, Neurotransmitters and Neuropeptides decrease PD-1 in T cells of healthy subjects and patients with hepatocellular carcinoma (HCC), and increase their proliferation and eradication of HCC cells, Neuropeptides 89 (2021) 102159, https://doi.org/10.1016/j.npep.2021.102159.
[10] S. Tsuru, Y. Ito, H. Matsuda, et al., RAMP1 signaling in immune cells regulates inflammation-associated lymphangiogenesis, Lab. Invest. 100 (5) (2020) 738–750, https://doi.org/10.1038/s41374-019-0364-0.
[11] L. Yang, T. Sakurai, A. Kamiyoshi, et al., Endogenous CGRP protects against neointimal hyperplasia following wire-induced vascular injury, J. Mol. Cell. Cardiol. 59 (2013) 55–66, https://doi.org/10.1016/j.yjmcc.2013.02.002.
[12] M. Balood, M. Ahmadi, T. Eichwald, et al., Nociceptor neurons affect cancer immunosurveillance, Nature 611 (7935) (2022) 405–412, https://doi.org/10.1038/s41586-022-05374-w.
[13] J.L. Deville, S. Salas, D. Figarella-Branger, L. Ouafik, L. Daniel, Adrenomedullin as a therapeutic target in angiogenesis, Expert Opin. Ther. Targets 14 (10) (2010) 1059–1072, https://doi.org/10.1517/14728222.2010.522328.
[14] V. Ramachandran, T. Arumugam, R.F. Hwang, J.K. Greenson, D.M. Simeone, C.D. Logsdon, Adrenomedullin is expressed in pancreatic cancer and stimulates cell proliferation and invasion in an autocrine manner via the adrenomedullin receptor, ADMR. Cancer Res. 67 (6) (2007) 2666–2675, https://doi.org/10.1158/0008-5472.CAN-06-3362.
[15] V. Ramachandran, T. Arumugam, R. Langley, et al., The ADMR receptor mediates the effects of adrenomedullin on pancreatic cancer cells and on cells of the tumor microenvironment, PLoS One 4 (10) (2009) e7502, https://doi.org/10.1371/journal.pone.0007502. Published 2009 Oct 22.
[16] V.A. Siclari, K.S. Mohammad, D.R. Tompkins, et al., Tumor-expressed adrenomedullin accelerates breast cancer bone metastasis, Breast Cancer Res. 16 (6) (2014) 458, https://doi.org/10.1186/s13058-014-0458-y. Published 2014 Dec 2.
[17] E. Gezelius, P.O. Bendahl, W. Gallo, et al., Circulating levels of the cardiovascular biomarkers ST2 and adrenomedullin predict outcome within a randomized phase III lung cancer trial (RASTEN), Cancers 14 (5) (2022) 1307, https://doi.org/10.3390/cancers14051307. Published 2022 Mar 3.
[18] M. Tanaka, T. Koyama, T. Sakurai, et al., The endothelial adrenomedullin-RAMP2 system regulates vascular integrity and suppresses tumour metastasis, Cardiovasc. Res. 111 (4) (2016) 398–409, https://doi.org/10.1093/cvr/cvw166.
[19] I. Kaafarani, S. Fernandez-Sauze, C. Berenguer, et al., Targeting adrenomedullin receptors with systemic delivery of neutralizing antibodies inhibits tumor angiogenesis and suppresses growth of human tumor xenografts in mice, Faseb. J. 23 (10) (2009) 3424–3435, https://doi.org/10.1096/fj.08-127852.
[20] J. Ren, L. Ding, D. Zhang, et al., Carcinoma-associated fibroblasts promote the stemness and chemoresistance of colorectal cancer by transferring exosomal lncRNA H19, Theranostics 8 (14) (2018) 3932–3948, https://doi.org/10.7150/thno.25541. Published 2018 Jun 24.
[21] J. Kong, H. Tian, F. Zhang, et al., Extracellular vesicles of carcinoma-associated fibroblasts creates a pre-metastatic niche in the lung through activating fibroblasts, Mol. Cancer 18 (1) (2019) 175, https://doi.org/10.1186/s12943-019-1101-4. Published 2019 Dec 3. d.
[22] G. Babaei, S.G. Aziz, N.Z.Z. Jaghi, EMT, cancer stem cells and autophagy; the three main axes of metastasis, Biomed. Pharmacother. 133 (2021) 110909, https://doi.org/10.1016/j.biopha.2020.110909.
[23] M. Saitoh, Involvement of partial EMT in cancer progression, J. Biochem. 164 (4) (2018) 257–264, https://doi.org/10.1093/jb/mvy047.
[24] K. Dai, M. Tanaka, A. Kamiyoshi, et al., Deficiency of the adrenomedullin-RAMP3 system suppresses metastasis through the modification of cancer-associated fibroblasts, Oncogene 39 (9) (2020) 1914–1930, https://doi.org/10.1038/s41388-019-1112-z.
[25] T. Li, J. Fan, B. Wang, et al., TIMER: a web server for comprehensive analysis of tumor-infiltrating immune cells, Cancer Res. 77 (21) (2017) e108–e110, https://doi.org/10.1158/0008-5472.CAN-17-0307.

[26] D. Wang, Y. Wang, X. Zou, et al., FOXO1 inhibition prevents renal ischemia-reperfusion injury via cAMP-response element binding protein/PPAR-γ coactivator-1α-mediated mitochondrial biogenesis, Br. J. Pharmacol. 177 (2) (2020) 432–448, https://doi.org/10.1111/bph.14878.
[27] D.S. Chandrashekar, B. Bashel, S.A.H. Balasubramanya, et al., UALCAN: a portal for facilitating tumor subgroup gene expression and survival analyses, Neoplasia 19 (8) (2017) 649–658, https://doi.org/10.1016/j.neo.2017.05.002.
[28] P.K. Andersen, R.D. Gill, Cox's regression model for counting processes: a large sample study, Ann. Stat. 10 (4) (1982) 1100–1120.
[29] H. Mizuno, K. Kitada, K. Nakai, A. Sarai, PrognoScan: a new database for meta-analysis of the prognostic value of genes, BMC Med. Genom. 2 (18) (2009), https://doi.org/10.1186/1755-8794-2-18. Published 2009 Apr 24.
[30] J. Navarro Gonzalez, A.S. Zweig, M.L. Speir, et al., The UCSC Genome Browser database: 2021 update, Nucleic Acids Res. 49 (D1) (2021) D1046–D1057, https://doi.org/10.1093/nar/gkaa1070.
[31] J. Wei, D.L. Fang, W. Zhou, Y.F. He, N6-methyladenosine (m6A) regulatory gene divides hepatocellular carcinoma into three subtypes, J. Gastrointest. Oncol. 12 (4) (2021) 1860–1872, https://doi.org/10.21037/jgo-21-378.
[32] E. Wang, Y. Li, R. Ming, et al., The prognostic value and immune landscapes of a m6A/m5C/m1A-Related LncRNAs signature in Head and neck squamous cell carcinoma, Front. Cell Dev. Biol. 9 (2021) 718974, https://doi.org/10.3389/fcell.2021.718974. Published 2021 Nov 30.
[33] P.C. Teng, Y. Liang, A.A. Yarmishyn, et al., RNA modifications and epigenetics in modulation of lung cancer and pulmonary diseases, Int. J. Mol. Sci. 22 (19) (2021) 10592, https://doi.org/10.3390/ijms221910592. Published 2021 Sep. 30.
[34] J. Li, Z. Zuo, S. Lai, et al., Differential analysis of RNA methylation regulators in gastric cancer based on TCGA data set and construction of a prognostic model, J. Gastrointest. Oncol. 12 (4) (2021) 1384–1397, https://doi.org/10.21037/jgo-21-325.
[35] Z. Ma, Q. Li, P. Liu, W. Dong, Y. Zuo, METTL3 regulates m6A in endometrioid epithelial ovarian cancer independently of METTl14 and WTAP, Cell Biol. Int. 44 (12) (2020) 2524–2531, https://doi.org/10.1002/cbin.11459.
[36] D. Zeng, Z. Ye, R. Shen, et al., IOBR: multi-omics immuno-oncology biological research to decode tumor microenvironment and signatures, Front. Immunol. 12 (2021) 687975, https://doi.org/10.3389/fimmu.2021.687975. Published 2021 Jul 2.
[37] R. Beroukhim, C.H. Mermel, D. Porter, et al., The landscape of somatic copy-number alteration across human cancers, Nature 463 (7283) (2010) 899–905, https://doi.org/10.1038/nature08822.
[38] A. Mayakonda, D.C. Lin, Y. Assenov, C. Plass, H.P. Koeffler, Maftools: efficient and comprehensive analysis of somatic variants in cancer, Genome Res. 28 (11) (2018) 1747–1756, https://doi.org/10.1101/gr.239244.118.
[39] K. Yoshihara, H. Kim, R.G. Verhaak, Estimate: estimate of stromal and immune cells in malignant tumor tissues from expression data, R package version 1 (13) (2016) r21.
[40] 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.
[41] T.M. Malta, A. Sokolov, A.J. Gentles, et al., Machine learning identifies stemness features associated with oncogenic dedifferentiation, Cell 173 (2) (2018) 338–354.e15, https://doi.org/10.1016/j.cell.2018.03.034.
[42] S.V. Vasaikar, P. Straub, J. Wang, B. Zhang, LinkedOmics: analyzing multi-omics data within and across 32 cancer types, Nucleic Acids Res. 46 (D1) (2018) D956–D963, https://doi.org/10.1093/nar/gkx1090.
[43] A. Subramanian, P. Tamayo, V.K. Mootha, et al., Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles, Proc. Natl. Acad. Sci. U. S. A. 102 (43) (2005) 15545–15550, https://doi.org/10.1073/pnas.0506580102.
[44] A. Jacob, R. Wu, P. Wang, Regulation of RAMP expression in diseases, Adv. Exp. Med. Biol. 744 (2012) 87–103, https://doi.org/10.1007/978-1-4614-2364-5_8.
[45] A.J. Desai, D.J. Roberts, G.O. Richards, T.M. Skerry, Role of receptor activity modifying protein 1 in function of the calcium sensing receptor in the human TT thyroid carcinoma cell line, PLoS One 9 (1) (2014) e85237, https://doi.org/10.1371/journal.pone.0085237. Published 2014 Jan 13.
[46] C. Weston, J. Lu, N. Li, et al., Modulation of glucagon receptor pharmacology by receptor activity-modifying protein-2 (RAMP2), J. Biol. Chem. 290 (38) (2015) 23009–23022, https://doi.org/10.1074/jbc.M114.624601.
[47] D.L. Hay, C.S. Walker, J.J. Gingell, G. Ladds, C.A. Reynolds, D.R. Poyner, Receptor activity-modifying proteins; multifunctional G protein-coupled receptor accessory proteins, Biochem. Soc. Trans. 44 (2) (2016) 568–573, https://doi.org/10.1042/BST20150237.
[48] J. Bliton, M. Parides, P. Muscarella, J.C. McAuliffe, K. Papalezova, H. Clinical stage of cancer affects perioperative mortality for gastrointestinal cancer surgeries, J. Surg. Res. 260 (2021) 1–9, https://doi.org/10.1016/j.jss.2020.11.023.
[49] G.N. Hortobagyi, S.B. Edge, A. Giuliano, New and important changes in the TNM staging system for breast cancer, Am. Soc. Clin. Oncol. Educ. Book 38 (2018) 457–467, https://doi.org/10.1200/EDBK_201313.
[50] E. Picard, C.P. Verschoor, G.W. Ma, G. Pawelec, Relationships between immune landscapes, genetic subtypes and responses to immunotherapy in colorectal cancer, Front. Immunol. 11 (2020) 369, https://doi.org/10.3389/fimmu.2020.00369. Published 2020 Mar 6.
[51] D.J. McConkey, W. Choi, Molecular subtypes of bladder cancer, Curr. Oncol. Rep. 20 (10) (2018) 77, https://doi.org/10.1007/s11912-018-0727-5. Published 2018 Aug 20.
[52] D.M. Merino, L.M. McShane, D. Fabrizio, et al., Establishing guidelines to harmonize tumor mutational burden (TMB): in silico assessment of variation in TMB quantification across diagnostic platforms: phase I of the Friends of Cancer Research TMB Harmonization Project, J. Immunother Cancer 8 (1) (2020) e000147, https://doi.org/10.1136/jitc-2019-000147.
[53] T.M. Pawlik, C.P. Raut, M.A. Rodriguez-Bigas, Colorectal carcinogenesis: MSI-H versus MSI-L, Dis. Markers 20 (4–5) (2004) 199–206, https://doi.org/10.1155/2004/368680.
[54] T.M. Pawlik, C.P. Raut, M.A. Rodriguez-Bigas, Colorectal carcinogenesis: MSI-H versus MSI-L, Dis. Markers 20 (4–5) (2004) 199–206, https://doi.org/10.1155/2004/368680.
[55] T.N. Schumacher, R.D. Schreiber, Neoantigens in cancer immunotherapy, Science 348 (6230) (2015) 69–74, https://doi.org/10.1126/science.aaa4971.
[56] X. Zhao, X. Pan, Y. Wang, Y. Zhang, Targeting neoantigens for cancer immunotherapy, Biomark. Res. 9 (1) (2021) 61, https://doi.org/10.1186/s40364-021-00315-7. Published 2021 Jul 28.
[57] M. Peng, Y. Mo, Y. Wang, et al., Neoantigen vaccine: an emerging tumor immunotherapy, Mol. Cancer 18 (1) (2019) 128, https://doi.org/10.1186/s12943-019-1055-6. Published 2019 Aug 23.
[58] S. Van Coillie, B. Wiernicki, J. Xu, Molecular and cellular functions of CTLA-4, Adv. Exp. Med. Biol. 1248 (2020) 7–32, https://doi.org/10.1007/978-981-15-3266-5_2.
[59] Y. Han, D. Liu, L. Li, PD-1/PD-L1 pathway: current researches in cancer, Am. J. Cancer Res. 10 (3) (2020) 727–742. Published 2020 Mar 1.
[60] S.T. Paijens, A. Vledder, M. de Bruyn, H.W. Nijman, Tumor-infiltrating lymphocytes in the immunotherapy era, Cell. Mol. Immunol. 18 (4) (2021) 842–859, https://doi.org/10.1038/s41423-020-00565-9.