ORIGINAL RESEARCH

Prognostic Implications of RAB35 and its Relationship With Immune Microenvironment in Pan-cancer

Dongdong Pan, BM; Xueting Chen, MM; Yujie Zong, MM; Deru Lei, MM

ABSTRACT

Context • Ras-associated binding 35 (RAB35) is an oncogenic, guanosine triphosphate (GTP)ase that plays a role in cancer invasion, metastasis, and immune evasion. However, systematic and comprehensive research to identify the importance of RAB35 in various cancer types is still absent.

Objective • The study intended to explore the potential value of RAB35 as a molecular biomarker.

Design • The research team performed a genetic evaluation of RAB35.

Setting • The study took place at the Second Affiliated Hospital of Wenzhou Medical University in Wenzhou, China.

Outcome Measures • The research team assessed the expression of RAB35 in various tumor tissues and performed correlation analyses between RAB35 expression and prognosis, molecular subtypes, immunological subtypes, immune-associated cell infiltration, the tumor immune microenvironment, and drug sensitivity in pan-cancer.

Results • RAB35 exhibited significant differential expression for 21 cancer types. It demonstrated high sensitivity and specificity in diagnosing eight cancer types, showed distinct expression patterns in various molecular subtypes for six cancer types, and found different immune subtypes for eight cancer types. The abnormal expression of RAB35 was significantly related to overall survival (OS) for nine cancer types, progress free interval (PFI) for five cancer types, and disease-specific survival (DSS) for five cancer types. Its abnormal expression was closely associated with the immune microenvironment and multiple immune cells. Furthermore, it was related to the drug sensitivity for various drugs and might be associated with chemotherapy resistance.

Conclusions • RAB35 showed significant differential expression in various cancers and was significantly related to the prognosis of cancer patients, the immune microenvironment, multiple immune cells, drug sensitivity to various drugs, and chemotherapy resistance. It may serve as a potential biomarker and therapeutic target for cancer treatment.(*Altern Ther Health Med.* 2023;29(8):452-460).

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Ras-associated binding (RAB) proteins are small guanosine triphosphate (GTP)ases belonging to the Ras superfamily, which consists of about 70 members in humans.[1] They play diverse roles in almost all basic cellular processes, including proliferation, migration, differentiation, apoptosis, polarity, adhesion, survival, morphology, cell maintenance, and cell division.[2] They are frequently dysregulated in cancer,[3,4] and they exert significant effects on cancer-cell function by disrupting the homeostasis of cell-membrane transport, key cell-signaling pathways, and special secretory pathways.[5]

Similar to other small GTP enzymes, RAB35 is involved in numerous cellular processes.[6] The RAB35-p85/phosphatidylinositol 3-kinase (PI3K) axis is particularly crucial for promoting targeted chemotactic migration and convergent migration of various cells.[7]

Gibieza and Petrikaite in recent years found that RAB35 can function as an oncogene that plays a role in cancer invasion, metastasis, and immune evasion.[2] When RAB35 mutates, tumor cells survive in the absence of growth factor signals, considerably promoting tumorigenesis.[6,8,9]

RAB35 Expression

Gopinath et al found that knocking down RAB35 can increase the capacity of cells' self-renewal, invasiveness, and migration; promote growth of glioblastomas; and reduce the survival rate of hosts.[10]

Liang et al found that RAB35 was significantly highly expressed in hepatocellular carcinoma and that a long noncoding RNA (lncRNA), the homeobox (HOX) transcript antisense RNA (HOTAIR), can regulate the changes in RAB35 and in "synaptosomal associated protein of 23 kDa" (SNAP23) expression and localization by significantly influencing multivesicular-body motility in hepatocellular carcinoma cells through the secretion of hepatocellular carcinoma exosomes.[11]

Wen et al found that reduced expression of RAB35, which miR-185-5p regulates, is significantly related to the proliferation, migration, and invasion of non-small cell lung cancer (NSCLC) cells, mediated by tumor cell-derived exosomes.[12] Duan et al and Zheng et al found that RAB35's activation, which stimulation of endosome-localized epidermal growth factor (EGF) can induce, can promote the migration or proliferation of NSCLC and cervical cancer cells, respectively.[13,14]

Zhang et al found that RAB35 was also significantly highly expressed in human gastric carcinoma BGC-823 cells.[15] Zhu et al found that the activation of RAB35, as regulated by "wingless-related integration site 5a" (Wnt5a), significantly relates to cell migration and invasion in breast cancer.[16] Deng et al also found that RAB35 can promote the migration and invasion of various tumor cells, including breast cancer.[17] Tang found that MicroRNA-720 regulated in-vitro cell migration in cervical cancer and that RAB35 could promote cervical cancer.[18] Ye et al and Klinkert et al found that "differentially expressed in normal cells and neoplasia (DENN) domain containing 1A" (DENND1A) could activate RAB35 to regulate the migration and invasion of gastric cancer cells.[19,20]

In addition, RAB35 is upregulated in ovary adenocarcinoma cells (OVCAR3), a human ovarian cancer cell line; in the OSEC2 cell line, and in ovarian, epithelial cancer tissues treated with androgen.[21] It's also downregulated in other human tumors, such as glioma and squamous cell carcinoma.[22]

Survival Predictions

Ye et al examined the correlation between the expression of RAB35 and the prognosis of tumor patients and found that the expression of RAB35 was significantly related to overall survival (OS), progression-free interval (PFI) survival, and disease specific survival (DSS) in pan-cancer.[23]

PI3K, Akt, and p53

Wheeler et al found that RAB35's two somatic mutations—A151T and F161L—can activate PI3K and protein kinase B (Akt), causing cells to display a phenotype similar to Kirsten rat sarcoma (KRAS) allele mutations; consequently, they become carcinogenic and promote cancer cell survival and suppress apoptosis.[9] Abe et al found that RAB35 can inhibit the transcriptional activity of p53 that p53-related protein kinase induces, thereby promoting tumor-cell development.[24]

Current Study

The findings above collectively highlight the pivotal role of RAB35 in tumor progression and its potential as a tumor marker gene and a predictor of the survival status of cancer patients and a potential survival marker.

Oncogenic RAB35 participates in cancer invasion, metastasis, and immune evasion and shows different expression levels in various tumors. However, systematic studies on the expression and biological function of RAB35 in pan-cancer and its value as a biomarker in diagnosis and prognosis assessment have yet to be performed.

The current study intended to explore the potential value of RAB35 as a molecular biomarker in pan-cancer.

METHODS

The research team performed a genetic evaluation of RAB35. The study took place at the Second Affiliated Hospital of Wenzhou Medical University in Wenzhou, China.

The research team performed all procedures in this study in accordance with the institutional review board of the Second Affiliated Hospital of Wenzhou Medical University. The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding author/s.

Outcome measures. The research team assessed the expression of RAB35 various tumor tissues in pan-cancer and performed correlation analyses between RAB35 expression and prognosis, molecular subtypes, immunological subtypes, immune-associated cell infiltration, the tumor immune microenvironment, and drug sensitivity in pan-cancer.

Data processing and RAB35 expression analysis. The occurrence of significant differential expression for RAB35 in various tumors suggests its potential value in the diagnosis, prognosis, and treatment of cancer. Using the Human Protein Atlas (HPA) database (https://www.proteinatlas.org.), the research team constructed an expression plot of RAB35 messenger RNA (mRNA). The team downloaded the RNA-seq data and relevant clinical information from The Cancer Genome Atlas (TCGA) database and the Genotype-Tissue Expression (GTEx) database using the University of California Santa Cruz's (UCSC) XENA database (https://xenabrowser.net/datapages/). The team also used the Functional ANnoTation of the Mammalian Genome (FANTOM5) database (https://fantom.gsc.riken.jp/) to explore differential expression.

Table 1 shows the number of cancer and normal cases per cancer type.

Correlation of RAB35 expression with survival prognosis and clinical features. The research team used Kaplan–Meier (K-M) plots to assess the relationship between RAB35 mRNA expression and survival outcomes: OS and PFI. The team used Cox regression to analyze the significant differences between high and low expression of RAB35. The team used the survival and survminer packages in the R software (R Foundation for Statistical Computing, Vienna, Austria).

Table 1. Number of Cases Per Cancer Type

Cancer	Normal	Tumor
ACC	128	77
BLCA	28	407
BRCA	292	1099
CESC	13	306
CHOL	9	36
COAD	349	290
DLBC	444	47
ESCA	666	182
GBM	1157	166
HNSC	44	520
KICH	53	66
KIRC	100	531
KIRP	60	289
LAML	70	173
LGG	1152	523
LIHC	160	371
LUAD	347	515
LUSC	338	498
MESO	-	87
OV	88	427
PAAD	171	179
PCPG	3	182
PRAD	152	496
READ	318	93
SARC	2	262
SKCM	813	469
STAD	210	414
TGCT	165	154
THCA	338	512
THYM	446	119
UCEC	101	181
UCS	78	57
UVM	-	79

Abbreviations: ACC, adrenocortical carcinoma; BLCA, bladder cancer; BRCA, breast cancer; CESC, cervical squamous cell carcinoma; CHOL, cholangiocarcinoma; COAD, colon adenocarcinoma; DLBC, lymphoid neoplasm diffuse large B cell lymphoma; ESCA, esophageal carcinoma; GBM, glioblastoma multiforme; HNSC, head and neck squamous cell carcinoma; KICH, kidney chromophobe; KIRC, kidney renal clear cell carcinoma; KIRP, kidney renal papillary cell carcinoma; LAML, acute myeloid leukemia; LGG, brain low-grade glioma; LIHC, liver hepatocellular carcinoma; LUAD, lung adenocarcinoma; LUSC, lung squamous cell carcinoma; MESO, mesothelioma; OV, ovarian cancer; PAAD, pancreatic adenocarcinoma; PCPG, pheochromocytoma and paraganglioma; PRAD, prostate adenocarcinoma; READ, rectum adenocarcinoma; SARC, sarcoma; SKCM, human skin cutaneous melanoma; STAD, stomach adenocarcinoma; TGCT, testicular germ cell tumors; THCA, thyroid cancer; THYM, thymoma; UCEC, uterine corpus endometrial carcinoma; UCS, uterine carcinosarcoma; UVM, uveal melanoma

Genetic alterations RAB35 in pan-cancer. Using the cBioPortal database, the research team analyzed the gene alternations of RAB35 in the TCGA pan-cancer datasets (http://www.cbioportal.org/).The team collected the data for the genetic alterations and mutations of RAB35 using the Oncoprint, Cancer Type Summary, and Mutations modules of the TCGA database.

Association of RAB35 expression with tumor immune microenvironment. The research team examined the association between RAB35 and the immune checkpoint genes using the R software's Gene Set Variation Analysis (GSVA) package. The team performed an analysis of immuno-infiltration using the gene set enrichment analysis (ssGSEA) algorithm.

The team used the GSEA's Molecular Signatures Database (MSigDB) (https://www.gsea-msigdb.org) to perform a biofunctional analysis of RAB35 to use in the Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis (https://www.genome.jp/kegg/). The research team performed further correlation analysis among the MisMatch Repair (MMR), Microsatellite (MSI), and Tumor Mutation Burden (TMB).

The team corrected the P values using the Benjamini & Hochberg method, with the conditions for the False Discovery rate (FDR) <0.25 and the p.Adjust <0.05, to create significant enrichment. To analyze the biological function of RAB35 in pan-cancer, the team used the R softwares' clusterProfiler package with GSEA.

Drug sensitivity of RAB35 in pan-cancer. The research team downloaded the data for NCI-60 compound activity and RNA-seq expression profiles using CallMiner (https://discover.nci.nih.gov/cellminer/home.do.) The team selected FDA-approved drugs or drugs in clinical trials for analysis and performed the analysis using the R softwares' impute, limma, ggplot2, and ggpubr packages.

Correlation between RAB35 and prognosis. The research team investigated the potential prognosis value of RAB35 using GEPIA2.

Genetic alteration analysis. The research team explored the cancer-case numbers, sites, and types of RAB35 modifications using the cBioPortal database (https://www.cbioportal.org/).

Outcome Measures

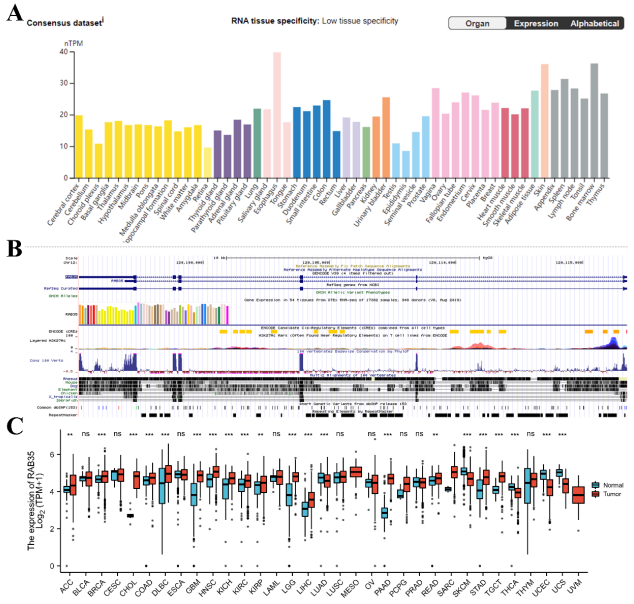
Differential Expression of RAB35. The research team investigated the expression of RAB35 in tumor tissues and normal tissues using the TCGA, GTEx, and FANTOM5 databases. The team evaluated expression in tissues for adrenocortical carcinoma (ACC); bladder cancer (BLCA); breast cancer (BRCA); cervical squamous cell carcinoma (CESC); cholangiocarcinoma (CHOL); colon adenocarcinoma (COAD); lymphoid neoplasm diffuse large B cell lymphoma (DLBC); esophageal carcinoma (ESCA); glioblastoma multiforme (GBM); head and neck squamous cell carcinoma (HNSC); kidney chromophobe (KICH); kidney renal clear cell carcinoma (KIRC); kidney renal papillary cell carcinoma (KIRP); acute myeloid leukemia (LAML); brain low-grade glioma (LGG); liver hepatocellular carcinoma (LIHC); lung adenocarcinoma (LUAD); lung squamous cell carcinoma (LUSC); mesothelioma (MESO); ovarian cancer (OV); oral squamous cell carcinoma (OSCC), pancreatic adenocarcinoma (PAAD); pheochromocytoma and paraganglioma (PCPG); prostate adenocarcinoma (PRAD); rectum adenocarcinoma (READ); sarcoma (SARC); human skin cutaneous melanoma (SKCM); stomach adenocarcinoma (STAD); testicular germ cell tumor (TGCT); thyroid cancer (THCA); thymoma (THYM); uterine corpus endometrial carcinoma (UCEC); uterine carcinosarcoma (UCS); and uveal melanoma (UVM).

Molecular or immune subtypes. The research team analyzed the correlations between RAB35 and the molecular and immune subtypes in pan-cancer.

Diagnostic value. The research team analyzed the diagnostic value of RAB35 by receiver operating characteristic (ROC) curve.

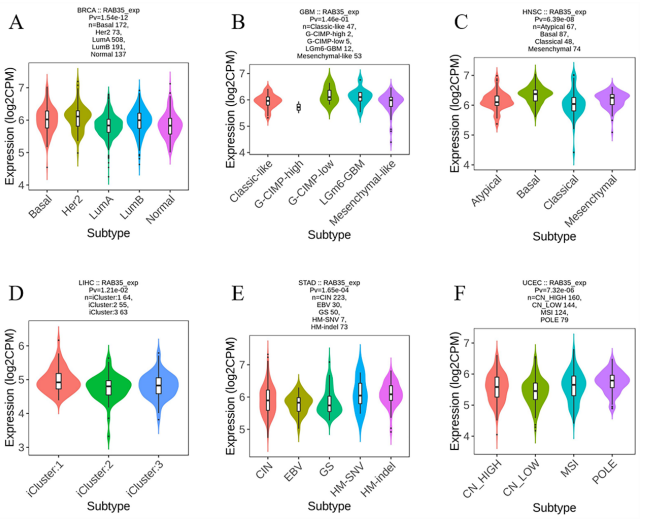
Prognostic value. According to the median of RAB35 mRNA expression, the team divided the data into high expression and low expression.

Figure 1. Differential Expression of RAB35. Figure 1A shows the consensus RAB35 tissue expression; Figure 1B shows the gene conservation analysis of RAB35; and Figure 1C shows the expression status of RAB35 in different tumor types.



Abbreviations: ACC, adrenocortical carcinoma; BLCA, bladder cancer; BRCA, breast cancer; CESC, cervical squamous cell carcinoma; CHOL, cholangiocarcinoma; COAD, colon adenocarcinoma; DLBC, lymphoid neoplasm diffuse large B cell lymphoma; ESCA, esophageal carcinoma; GBM, glioblastoma multiforme; HNSC, head and neck squamous cell carcinoma; KICH, kidney chromophobe; KIRC, kidney renal clear cell carcinoma; KIRP, kidney renal papillary cell carcinoma; LAML, acute myeloid leukemia; LGG, brain low-grade glioma; LIHC, liver hepatocellular carcinoma; LUAD, lung adenocarcinoma; LUSC, lung squamous cell carcinoma; MESO, mesothelioma; OV, ovarian cancer; PAAD, pancreatic adenocarcinoma; PCPG, pheochromocytoma and paraganglioma; PRAD, prostate adenocarcinoma; READ, rectum adenocarcinoma; SARC, sarcoma; SKCM, human skin cutaneous melanoma; STAD, stomach adenocarcinoma; TGCT, testicular germ cell tumor. THCA, thyroid cancer; THYM, thymoma; UCEC, uterine corpus endometrial carcinoma ; UCS, uterine carcinosarcoma; UVM, uveal melanoma

Figure 2. Correlations Between RAB35 Expression and Molecular Subtypes in TCGA Tumors. Figure 2A shows BRCA; Figure 2B shows GBM; Figure 2C shows HNSC; Figure 2D shows LIHC; Figure 2E shows STAD; and Figure 2F shows UCEC.



Abbreviations: BRCA, breast cancer; CIN ,chromosomal instability; CN-HIGH, copy number-high; CN-LOA, copy number-low; EBV, Epstein-Barr virus; G-CIMP-high, glioma CpG island methylator phenotype-high; G-CIMP-low, glioma CpG island methylator phenotype-low; GBM, glioblastoma multiforme; HER2, human epidermal growth factor receptor 2; HM-indel, hypermutated-insertion deletion mutation; HM-SNV, hypermutated-single-nucleotide variant predominant; HNSC, head and neck squamous cell carcinomas; LIHC, liver hepatocellular carcinoma; LumA, luminal A; LumB, luminal B; MSI, microsatellite instability; POLE, polymerase epsilon; STAD, stomach adenocarcinoma; TCGA, The Cancer Genome Atlas; UCEC, uterine corpus endometrial carcinoma

Genetic alterations. The research team analyzed the Genetic alterations of RAB35 to explore potential mutation sites of RAB35 in tumor progression.

Biomarkers and microenvironment. The research team performed further correlation analysis to identify significant correlations between RAB35 and various immune-associated cells in pan-cancer, according to the Tumor IMmune Estimation Resource (TIMER) database (http://cistrome.org/TIMER/). The team further examined the biological function of RAB35 in pan-cancer using Gene Set Enrichment Analysis (GSEA) by R software).

Drug sensitivity. The research team examined the relationship between RAB35 expression and drug sensitivity for 5-fluoro deoxy uridine, floxuridine, SNS-314, vinorelbine, bleomycin, fludarabine, temsirolimus, and 7-ethyl-10-hydroxycamptothecin.

Statistical Analysis

The research team analyzed the data using the R software (v3.6.3). The team: (1) performed the Wilcoxon rank-sum test and Spearman rank test to examine the differential expression and the correlation between groups, respectively; (2) performed the Log-rank test to analyze survival outcomes using Kaplan–Meier survival curves; and (3) used the Cox proportional-hazard regression model to calculate the hazard ratio (HR). $P < .05$ indicated significant differences.

RESULTS

Differential Expression

RAB35 is highly expressed in multiple human tissues, particularly in the esophagus, skin, and bone marrow (Figure 1A). Furthermore, RAB35 is relatively conserved in vertebrates (Figure 1B).

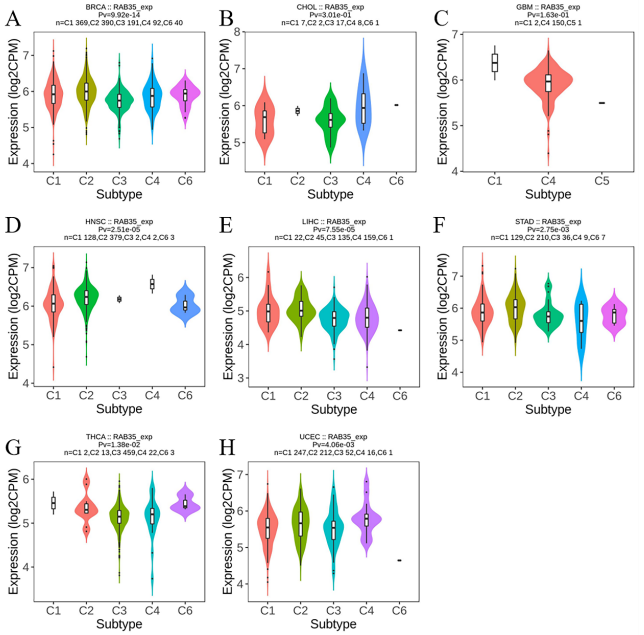
Figure 1C shows the expression of RAB35 in tumor tissues and normal tissues from the TCGA and GTEx databases. For the TCGA's tumors, RAB35 expression was upregulated compared to normal tissue in 16 cancer types: (1) ACC, (2) BRCA, (3) CHOL, (4) COAD, (5) DLBC, (6) GBM, (7) HNSC, (8) KICH, (9) KIRC, (10) KIRP, (11) LGG, (12) LIHC, (13) PAAD, (14) READ, (15) STAD, and (16) TGCT.

By contrast, RAB35 was downregulated in five cancer types: (1) LUAD, (2) SKCM, (3) THCA, (4) UCEC, and (5) UCS.

Molecular and Immune Subtypes

For the correlations between RAB35 expression and molecular or immune subtypes in pan-cancer, RAB35 was

Figure 3. Correlations Between RAB35 Expression and Immune Subtypes in TCGA Tumors. Figure 3A shows BRCA; Figure 3B shows CHOL; Figure 3C shows GBM; Figure 3D shows HNSC; Figure 3E shows LIHC; Figure 3F) shows STAD; Figure 3G shows THCA; and Figure 3H shows UCEC.

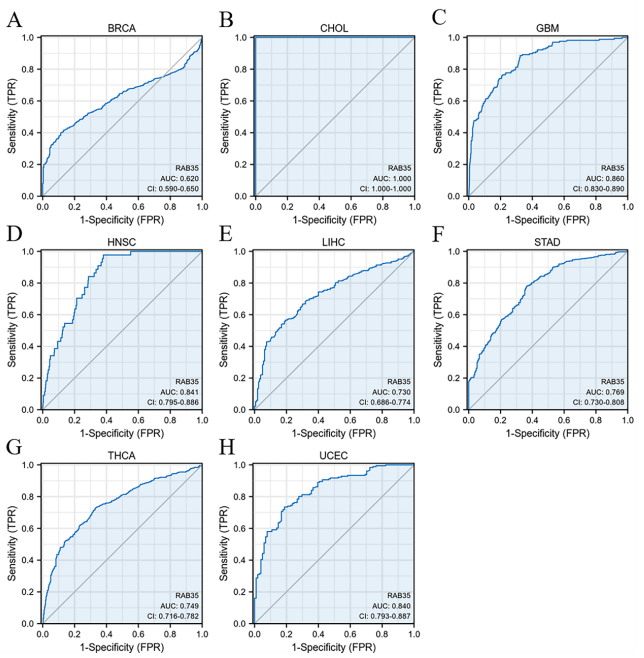


Abbreviations: BRCA, breast cancer; C1, wound healing; C2, IFN-gamma dominant; C3, inflammatory; C4, lymphocyte depleted; C5, immunologically quiet; C6, TGF-b dominant; CHOL, cholangiocarcinoma; GBM, glioblastoma multiforme; HNSC, head and neck squamous cell carcinoma; LIHC, liver hepatocellular carcinoma; STAD, stomach adenocarcinoma; TCGA, The Cancer Genome Atlas; THCA, thyroid cancer; UCEC, uterine corpus endometrial carcinoma

expressed differentially in various molecular subtypes of six cancer types (Figure 2): (1) BRCA—basal, human epidermal growth factor receptor 2 (HER2), luminal A (LumA), and luminal B (LumB); (2) GBM—classic-like, glioma CpG island methylator phenotype-high (G-CIMP-high), glioma CpG island methylator phenotype-low (G-CIMP-low), LGm6-GBM, and mesenchymal-like; (3) HNSC—atypical, basal, classical, and mesenchymal; (4) LIHC—iCluster.1, iCluster.2, and iCluster.3; (5) STAD—chromosomal instability (CIN), Epstein-Barr virus (EBV), GS, hypermutated-single-nucleotide variant predominant (HM-SNV), and hypermutated-insertion deletion mutation (HM-indel); and (6) UCEC—copy number-high (CN_HIGH), copy number-low (CN_LOW), microsatellite instability (MSI), and polymerase epsilon (POLE).

In addition, the RAB35 expression was significantly correlated with different immune subtypes (Figure 3): (1) BRCA—wound healing (C1), interferon gamma (IFN-γ) dominant (C2), inflammatory (C3), lymphocyte depleted (C4,) and transforming growth factor-beta (TGF-β) dominant (C6); (2) CHOL—C1, C2, C3, C4, and C6; (3) GBM—C1, C4, and immunologically quiet (C5); (4) HNSC—C1, C2, C3, C4, and C6; (5) LIHC—C1, C2, C3, C4, and C6; (6) STAD—C1, C2, C3, C4, and C6; (7) THCA—C1, C2, C3, C4, and C6; and (8) UCEC—C1, C2, C3, C4, and C6.

Figure 4. Receiver Operating Characteristic (ROC) Curve of RAB35 Expression in Pan-cancer. Figure 4A shows BRCA; Figure 4B shows CHOL; Figure 4C shows GBM; Figure 4D shows HNSC; Figure 4E shows LIHC; Figure 4F shows STAD; Figure 4G shows THCA; and Figure 4H shows UCEC.



Abbreviations: BRCA, breast cancer; CHOL, cholangiocarcinoma; GBM, glioblastoma multiforme; HNSC, head and neck squamous cell carcinoma; LIHC, liver hepatocellular carcinoma; STAD, stomach adenocarcinoma; THCA, thyroid cancer; UCEC, uterine corpus endometrial carcinoma

Table 2. Receiver Operating Characteristic (ROC) Curve of RAB35 Expression in Pan-cancer

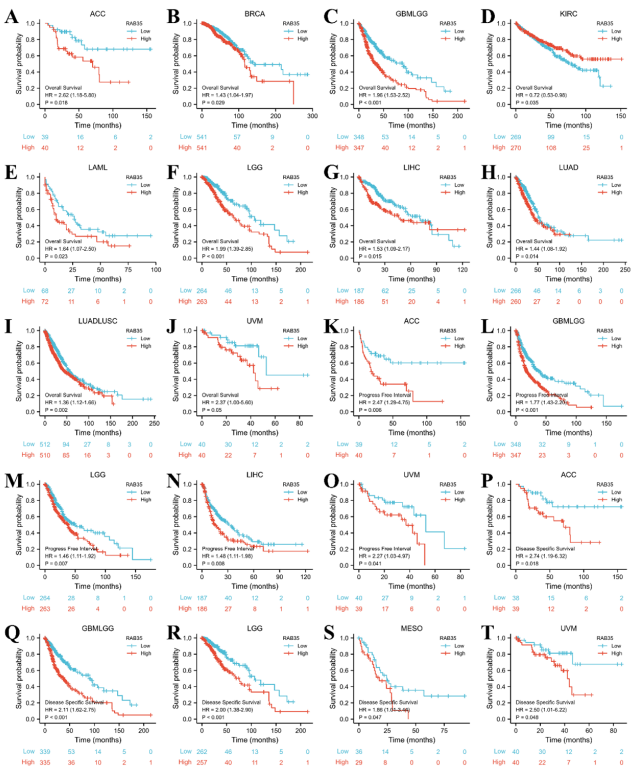
Cancer	Normal	Tumor	AUC	CI
BRCA	292	1099	0.62	0.590-0.650
CHOL	9	36	1.00	1.000-1.000
GBM	1157	166	0.86	0.830-0.890
HNSC	44	502	0.841	0.795-0.886
LIHC	160	371	0.73	0.686-0.774
STAD	210	414	0.769	0.730-0.808
THCA	338	512	0.749	0.716-0.782
UCEC	101	181	0.84	0.793-0.887

Abbreviations: BRCA, breast cancer; CHOL, cholangiocarcinoma; GBM, glioblastoma multiforme; HNSC, head and neck squamous cell carcinoma; LIHC, liver hepatocellular carcinoma; STAD, stomach adenocarcinoma; THCA, thyroid cancer; UCEC, uterine corpus endometrial carcinoma

Diagnostic Value

Figure 4 shows that RAB35's diagnostic value was accurate (AUC>0.7) and Table 2 show that RAB35 had a high AUC in predicting multiple tumors: (1) BRCA—AUC = 0.620, 95% confidence interval (CI): 0.590-0.650); (2) CHOL—AUC = 1.000, 95% confidence interval (CI): 1.000-1.000); (3) GBM—AUC = 0.860, CI: 0.830–0.890); (4) HNSC—AUC = 0.841, CI: 0.795–0.886); (5) LIHC—AUC = 0.730, CI: 0.686–0.774); (6) STAD—AUC = 0.769, CI: 0.730–0.808); (7) THCA—AUC = 0.749, CI: 0.716–0.782); and (8) UCEC— AUC = 0.840, CI: 0.793–0.887).

Figure 5. Survival Period Analysis in Patients With Different TCGA Tumor Types. Figures 5A-5J show the correlation between RAB35 expression and overall survival (OS); Figures 5K-5O show the correlation between RAB35 expression and the progress free interval (PFI). Figures 5P-5T show the correlation between RAB35 expression and disease specific survival (DSS).



Abbreviations: ACC, adrenocortical carcinoma; BRCA, breast cancer; GBMLGG, glioblastoma multiforme and low-grade glioma; KIRC, kidney renal clear cell carcinoma; LAML, acute myeloid leukemia; LGG, brain low-grade glioma; LIHC, liver hepatocellular carcinoma; LUAD, lung adenocarcinoma; LUADLUSC, lung adenocarcinoma and lung squamous cell carcinoma; MESO, mesothelioma; TCGA, The Cancer Genome Atlas; UVM, uveal melanoma

Prognostic Value

Lower expression of RAB35 was significantly correlated with shorter OS for patients with KIRC—HR = 0.72 (0.53-0.98), *P* = .035 (Figure 5, Table 3). Higher expression of RAB35 significantly correlated with shorter OS in patients: (1) with ACC—HR = 2.62 (1.18-5.80), *P* = .018; (2) with BRCA—HR = 1.43 (1.04-1.97), *P* = .029; (3) with glioblastoma multiforme and low-grade glioma (GBMLGG)—HR = 1.96 (1.53-2.52), *P* < .001; (4) with LAML—HR = 1.64 (1.07-2.50), *P* = .023; (5) with LGG—HR = 1.99 (01.39-2.85), *P* < .001; (6) with LIHC—HR = 1.53 (1.09-2.17), *P* = .015; (7) with LUAD—HR = 1.44 (1.08-1.92), *P* = .014; (8) with lung adenocarcinoma and lung squamous cell carcinoma glioma (LUADLUSC)—HR = 1.36 (1.12-1.66), *P* = .002. RAB35 and UVM were related but the relationship didn't reach statical significance—HR = 2.37 (1.00-5.60), *P* = .05.

A higher RAB35 was significantly correlated with a shorter PFI for patients: (1) with ACC—HR = 2.47 (1.29-4.75), *P* = .006; (2) with GBMLGG—HR=1.77 (1.43-2.20), *P* < .001; (3) with LGG—HR = 1.46 (1.11-1.92), *P* = .007; (4) with LIHC—HR = 1.48 (1.11-1.98), *P* = .008; and (5) with UVM—HR = 2.27 (1.03-4.97), *P* = .041 (Figures 5K-5O).

A higher RAB35 was significantly correlated with a shorter DSS for patients: (1) with ACC—HR = 2.74 (1.19-6.32), *P* = .018; (2) with GBMLGG—HR = 2.11 (1.62-2.75), *P* < .001; (3) with LGG—HR = 2.00 (1.38-2.90), *P* < .001; (4) with mesothelioma (MESO)—HR = 1.86 (1.01-2.90), *P* < .001; and (5) with UVM—HR = 2.50 (1.01-6.22), *P* = .048 (Figures 5P-5T).

Table 3. Survival Period Analysis for Different TCGA Tumor Types

Cancer	Survival Period	HR	*P* value
ACC	OS	2.62 (1.18-5.80)	.018[a]
BRCA	OS	1.43 (1.04-1.97)	.029[a]
GBMLGG	OS	1.96 (1.53-2.52)	<.001[c]
KIRC	OS	0.72 (0.53-0.98)	.035[a]
LAML	OS	1.64 (1.07-2.50)	.023[a]
LGG	OS	1.99 (1.39-2.85)	<.001[c]
LIHC	OS	1.53 (1.09-2.17)	.015[a]
LUAD	OS	1.44 (1.08-1.92)	.014[a]
LUADLUSC	OS	1.36 (1.12-1.66)	.002[b]
UVM	OS	2.37 (1.00-5.60)	.05
ACC	PFI	2.47 (1.29-4.75)	.006[b]
GBMLGG	PFI	1.77 (1.43-2.20)	<.001[c]
LGG	PFI	1.46 (1.11-1.92)	.007**
LIHC	PFI	1.48 (1.11-1.98)	.008[b]
UVM	PFI	2.27 (1.03-4.97)	.041[a]
ACC	DSS	2.74 (1.19-6.32)	.018[a]
GBMLGG	DSS	2.11 (1.62-2.75)	<.001[c]
LGG	DSS	2.00 (1.38-2.90)	<.001[c]
MESO	DSS	1.86 (1.01-3.44)	.047[a]
UVM	DSS	2.50 (1.01-6.22)	.048[a]

[a]*P* < .05, indicating that a higher RAB35 was significantly correlated with a shorter OS for ACC, BRCA, KIRC, LAML, LIHC, and LUAD; with a shorter PFI for UVM; and with a shorter DSS for ACC, MESO, and UVM (*P* = .048)
[b]*P* < .01, indicating that RAB35 was significantly correlated with a shorter OS for LUADLUSC and with a shorter PFI for ACC, LGG, and LIHC
[c]*P* < .05, indicating that RAB35 was significantly correlated with a shorter OS for GBMLGG and LGG; with a shorter PFI for GBMLGG; and with a shorter DSS for GBMLGG and LGG

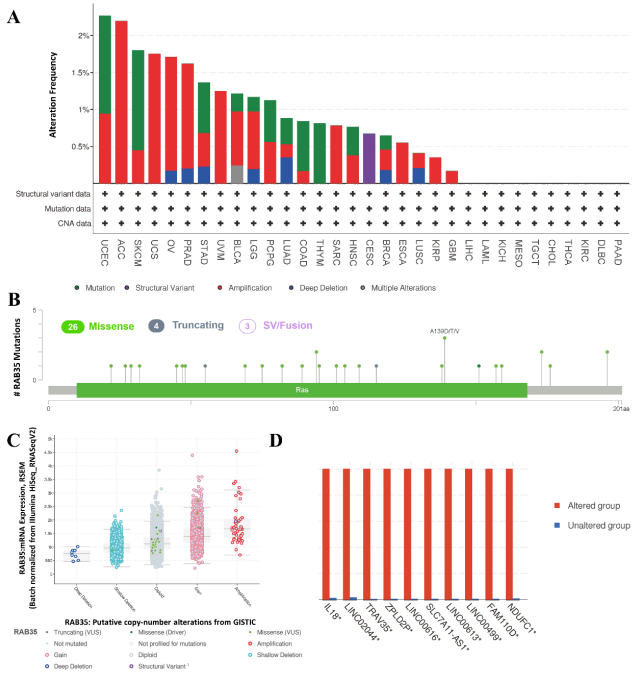
Abbreviations: ACC, adrenocortical carcinoma; BRCA, breast cancer; DSS, disease specific survival; GBMLGG, glioblastoma multiforme and low-grade glioma; KIRC, kidney renal clear cell carcinoma; LAML, acute myeloid leukemia; LGG, brain low-grade glioma; LIHC, liver hepatocellular carcinoma; LUAD, lung adenocarcinoma; LUADLUSC, lung adenocarcinoma and lung squamous cell carcinoma; MESO, mesothelioma; OS, overall survival; PFI, progress free interval; TCGA, The Cancer Genome Atlas; UVM, uveal melanoma.

Genetic Alterations

As Figure 6A shows, RAB35 exhibited the highest gene alteration frequency in patients with UCEC and ACC, and amplification, deep deletion, and mutation were the main forms of genetic alterations in RAB35. Regarding cancer-case numbers, sites, and types of RAB35, 33 RAB35 mutations and missense mutations were the major alteration types (Figure 6B).

Amplification, gain function, and diploid were the most frequent copy-number alterations of RAB35 (Figure 6C). Interleukin 18 (IL18), long intergenic non-protein coding RNA 02044 (LINC02044), zona pellucida like domain containing 2, pseudogene (ZPLD2P), LINC00616, solute carrier family 7 member 11-AS1 (SLC7A11-AS1), LINC00613, LINC00499, family with sequence similarity

Figure 6. The Genetic Alterations of RAB35. Figure 6A shows the alterations of changes in RAB35 in the TCGA pan-cancer dataset; Figure 6B shows the type, number and site of mutations in PNPO gene alterations; Figure 6C shows the types of RAB35 alterations in pan-cancer; and Figure 6D shows the frequency of altered genes of interest in the RAB35 altered and unaltered groups.



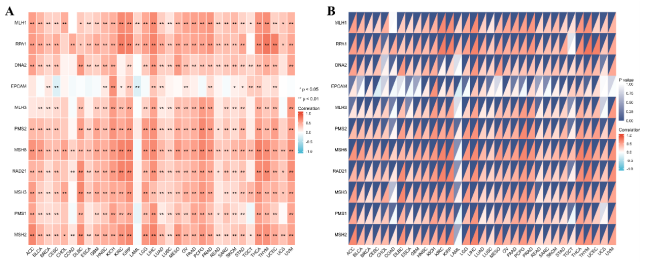
Abbreviations: ACC, adrenocortical carcinoma; BLCA, bladder cancer; BRCA, breast cancer; CESC, cervical squamous cell carcinoma; COAD, colon adenocarcinoma; CHOL, cholangiocarcinoma; DLBC, lymphoid neoplasm diffuse large B cell lymphoma; ESCA, esophageal carcinoma; FAM110D, family with sequence similarity 110 member D; GBM, glioblastoma multiforme; HNSC, head and neck squamous cell carcinoma; IL18, interleukin 18; KICH, kidney chromophobe; KIRC, kidney renal clear cell carcinoma; KIRP, kidney renal papillary cell carcinoma; LAML, acute myeloid leukemia; LGG, brain low-grade glioma; LIHC, liver hepatocellular carcinoma; LINC, long intergenic non-protein coding RNA; LUAD, lung adenocarcinoma; LUSC, lung squamous cell carcinoma; MESO, mesothelioma; NDUFC1, nicotinamide adenine dinucleotide + hydrogen (NADH): ubiquinone oxidoreductase subunit C1; OV, ovarian cancer; PAAD, pancreatic adenocarcinoma; PCPG, pheochromocytoma and paraganglioma; PRAD, prostate adenocarcinoma; SARC, sarcoma; SKCM, human skin cutaneous melanoma; SLC7A11-As1, solute carrier family 7 member 11-AS1; STAD, stomach adenocarcinoma; TGCT, testicular germ cell tumor; THCA, thyroid cancer; THYM, thymoma; TRAV35, T cell receptor alpha variable 35; UCEC, uterine corpus endometrial carcinoma; UCS, uterine carcinosarcoma; UVM, uveal melanoma; ZPLD2P, zona pellucida like domain containing 2, pseudogene

110 member D (FAM110D), and nicotinamide adenine dinucleotide + hydrogen (NADH): ubiquinone oxidoreductase subunit C1 (NDUFC1) were the most prevalent form in the altered group (Figure 6D).

Biomarkers and Microenvironment

The expression of RAB35 showed a positive correlation with MMR-related genes in most cancer types, particularly for ACC, DLBC, KIHC, KIRC, KIRP, LIHC, PCPG, THCA, and THYM (Figure 7).

Figure 7. Correlation Between RAB35 Expression and Mismatch Repair (MMR) Genes.



Abbreviations: ACC, adrenocortical carcinoma; BLCA, bladder cancer; BRCA, breast cancer; CESC, cervical squamous cell carcinoma; CHOL, cholangiocarcinoma; COAD, colon adenocarcinoma; DLBC, lymphoid neoplasm diffuse large B cell lymphoma; DNA2, DNA replication helicase/nuclease 2; EPCAM, epithelial cellular adhesion molecule; ESCA, esophageal carcinoma; GBM, glioblastoma multiforme; HNSC, head and neck squamous cell carcinoma; KICH, kidney chromophobe; KIRC, kidney renal clear cell carcinoma; KIRP, kidney renal papillary cell carcinoma; LAML, acute myeloid leukemia; LGG, brain low-grade glioma; LIHC, liver hepatocellular carcinoma; LUAD, lung adenocarcinoma; LUSC, lung squamous cell carcinoma; MESO, mesothelioma; MLH1, Mutl homolog 1; MLH3, Mutl homolog 3; MSH2, MutS homolog 2; MSH3, MutS homolog 3; MSH6, MutS homolog 6; OV, ovarian cancer; PAAD, pancreatic adenocarcinoma; PCPG, pheochromocytoma and paraganglioma; PMS1, PMS1 postmeiotic segregation increased 1; PMS2, postmeiotic segregation increased 2; PRAD, prostate adenocarcinoma; RAD21, RAD21 cohesion complex component; READ, rectum adenocarcinoma; RPA1, replication protein A1; SARC, sarcoma; SKCM, human skin cutaneous melanoma; STAD, stomach adenocarcinoma; TGCT, testicular germ cell tumor; THCA, thyroid cancer; THYM, thymoma; UCEC, uterine corpus endometrial carcinoma; UCS, uterine carcinosarcoma; UVM, uveal melanoma

RAB35 expression was significantly correlated with various immune-associated cells in pan-cancer, including cluster of differentiation 8 (CD8)+T cells in nine tumor types—BLCA, BRCA, COAD, GBMLGG, PAAD, SKCM, TGCT, UCEC, and UVM—and macrophages in 22 tumor types: BLCA, BRCA, CHOL, COAD, GBM, GBMLGG, HNSC, KIRC, LAML, LIHC, LUAD, OSCC, OV, PRAD, READ, SKCM, STAD, TGCT, THCA, THYM, UCEC, and UVM (Figure 8).

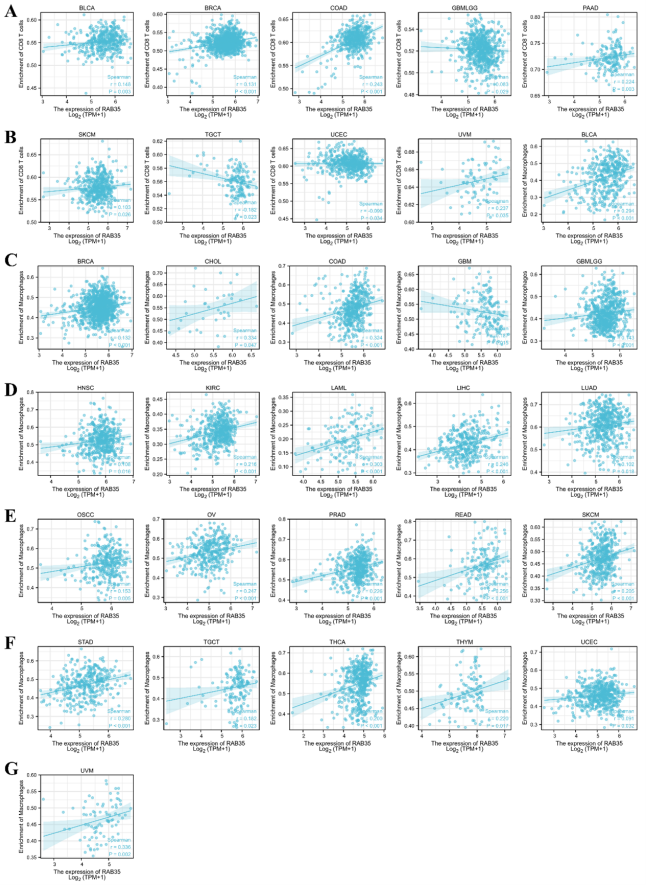
RAB35 expression was significantly correlated with natural killer (NK) cells in 18 tumor types, mast cells in eight tumor types, dendritic cells (DC) cells in 12 tumor types, B cells in 12 tumor types, and type 1 T helper (Th1) cells in 15 tumor types (Figure 9).

RAB35 positively regulated signaling pathways in KICH, LGG, TGCT, and UVM, and negatively regulated pathways in BRCA, COAD, LUAD, LUSC, MESO, SKCM, STAD, and UCEC (Figure 10). The most common signaling pathway of RAB35 was neutrophil degranulation, followed by G Protein-Coupled Receptor (GPCR) ligand binding.

Drug Sensitivity

The expression of RAB35 was positively related to 5-fluoro deoxy uridine, floxuridine, SNS-314, bleomycin, fludarabine, temsirolimus, and 7-ethyl-10-hydroxycamptothecin sensitivity (Figure 11A-11C and 11E-11H), but was negatively related to Vinorelbine sensitivity

Figure 8. Correlation Between RAB35 Gene Expression and Tumor Immune Microenvironment in TCGA Database. Figures 8A-8E show the immune-related, cellular infiltration analysis of RAB35 expression in pan-cancer.



Abbreviations: BLCA, bladder cancer; BRCA, breast cancer; CHOL, cholangiocarcinoma; COAD, colon adenocarcinoma; GBM, glioblastoma multiforme; GBMLGG, glioblastoma multiforme and low-grade glioma; HNSC, head and neck squamous cell carcinoma; KIRC, kidney renal clear cell carcinoma; LAML, acute myeloid leukemia; LIHC, liver hepatocellular carcinoma; LUAD, lung adenocarcinoma; OSCC, oral squamous cell carcinoma; OV, ovarian cancer; PAAD, pancreatic adenocarcinoma; PRAD, prostate adenocarcinoma; SKCM, human skin cutaneous melanoma; STAD, stomach adenocarcinoma; TGCT, testicular germ cell tumor; THCA, thyroid cancer; THYM, thymoma; UCEC, uterine corpus endometrial carcinoma; UCS, uterine carcinosarcoma; UVM, uveal melanoma

Figure 9. Correlation Between RAB35 Gene Expression and Tumor Immune Microenvironment in TCGA Database. (A-G) Immune-related cellular infiltration analysis of RAB35 expression in pan-cancer.

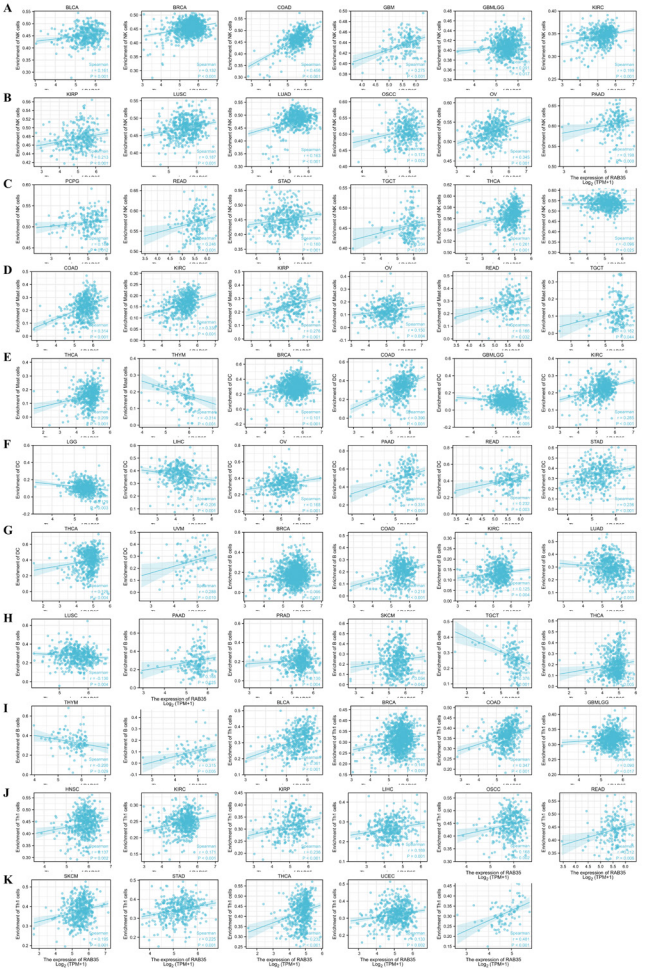
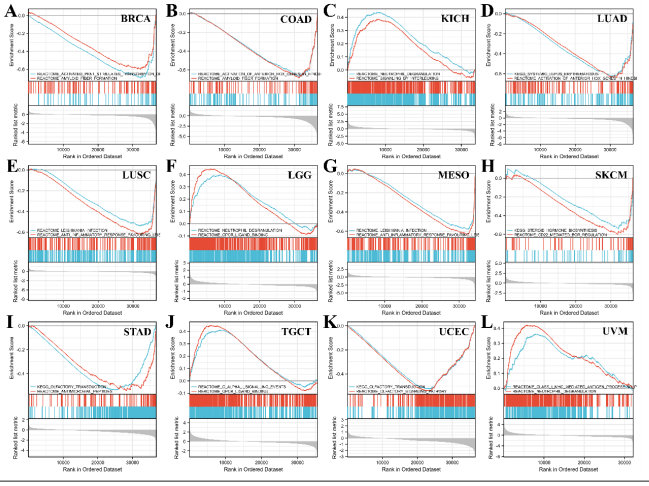


Figure 10. GSEA Analysis of the KEGG Signature of RAB35. Figure 10A shows BRCA; Figure shows 10B COAD; Figure 10C shows LUAD; Figure 10D shows LUSC; Figure 10E shows MESO; Figure 10F shows SKCM; Figure 10G shows STAD; and Figure 10H shows UCEC.



Abbreviations: BRCA, breast cancer; COAD, colon adenocarcinoma; GSEA, Genome Enrichment Analysis; KEGG, Kyoto Encyclopedia of Genes and Genomes; KICH, kidney chromophobe; LGG, brain low-grade glioma; LUAD, lung adenocarcinoma; LUSC, lung squamous cell carcinoma; MESO, mesothelioma; SKCM, human skin cutaneous melanoma; STAD, stomach adenocarcinoma; TGCT, testicular germ cell tumor; UCEC, uterine corpus endometrial carcinoma; UVM, uveal melanoma

(Figure 11D). The above indicates that RAB35 might be a promising target for the treatment for cancer.

DISCUSSION

RAB35 is an oncogene that plays a role in cancer invasion, metastasis, and immune evasion. The current study found that RAB35 is highly expressed in multiple human tissues, which was consistent with previous reports. [25] Furthermore, the study found that RAB35 was related to various molecular subtypes and immune subtypes in pan-cancer and showed some accuracy for the diagnosis of pan-cancer, which might indicate that RAB35 could be a potential marker for tumor diagnosis or treatment.

The current research team further performed a genetic variation analysis of RAB35 and found that it exhibited a high frequency of genetic alterations in pan-cancer, and amplification, deep deletion, and mutation were the main forms of genetic alterations. In addition, RAB35 was found to be positively correlated with MMR-related genes in pan-cancer and was significantly related to various immune-associated cells. More important, RAB35 was significantly related to various signaling pathways in pan-cancer. The research team speculates that RAB35 may be a potential therapeutic target based on the above findings.

The current research team further performed drug sensitivity analysis of RAB35 and found it to be correlated with many drug sensitivities, such as 5-Fluoro deoxy uridine, floxuridine, and temsirolimus. Therefore, the team hypothesizes that RAB35 expression can be associated with chemoresistance and played a role in chemotherapy.

However, the current study had some limitations: (1) it was primarily based on public data platforms and requires further clinical validation; (2) although the study demonstrated abnormal expression of RAB35 in various tumors and its correlation with tumor prognosis, the specific mechanisms remain unclear and require further confirmation.

CONCLUSIONS

RAB35 showed significant differential expression in various cancers and was significantly related to the prognosis of cancer patients, the immune microenvironment, multiple immune cells, drug sensitivity to various drugs, and chemotherapy resistance. It may serve as a potential biomarker and therapeutic target for cancer treatment.

AUTHORS' DISCLOSURE STATEMENT

The authors have no conflicts of interest to declare.

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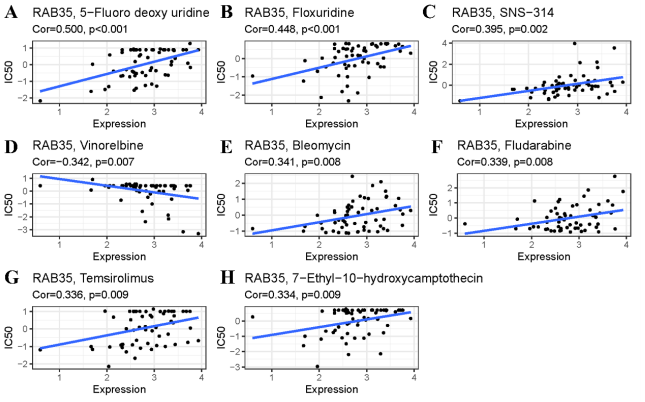
Figure 11. Drug Sensitivity Analysis of RAB35: The association of RAB35 expression and the sensitivity of drugs. Figure 11A shows 5-fluoro deoxy uridine; Figure 11B shows floxuridine; Figure 11C shows SNS-314; Figure 11D shows vinorelbine; Figure 11E shows bleomycin; Figure 11F shows fludarabine; Figure 11G shows temsirolimus; and Figure 11H shows 7-ethyl-10-hydroxycamptothecin.



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