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Pan-cancer analysis reveals CHRM2 as a biomarker for cancer prognosis and immunotherapy

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ABSTRACT. Background: CHRM2 is a member of the muscarinic acetylcholine receptor gene, which plays an important role in many cancers. **Methods:** Differential expression of CHRM2 across cancers was analyzed using The Cancer Genome Atlas (TCGA) database, and survival analysis of CHRM2 was performed using the Kaplan–Meier method. Spearman correlation analysis was used to study the correlation between CHRM2 and tumor mutation burden and microsatellite instability, and immune cell infiltration in tumor tissues were analyzed by the ESTIMATE and CIBERSORT algorithms. The biological role of CHRM2 in cancer was investigated by gene set enrichment analysis, and drug sensitivity analysis was conducted using the CellMiner database.
Results: CHRM2 expression was downregulated in most cancers, and low CHRM2 expression was associated with better prognosis, especially in BLCA and COAD. CHRM2 expression correlated significantly with clinical stage, TMB, MSI, and a variety of immune cells and immune factors. GSEA

showed that CHRM2 was enriched in many functions and pathways, and drug sensitivity analysis showed that CHRM2 correlated positively with several c-Met inhibitors.
Conclusion: CHRM2 may be a prognostic indicator, a potential biomarker and a therapeutic target for pan-cancer, especially BLCA and COAD.

Key words: CHRM2; Pan-cancer; Prognosis; Immune infiltration

INTRODUCTION

Cancer is the leading cause of death worldwide (Sung H et al., 2021). GLOBOCAN estimates that the number of cancer cases and deaths will rise to 270,000 new cases and 50,000 deaths by 2040 (Galván Morales MA, 2020). In the United States, 1,958,310 new cancer cases and 609,820 cancer deaths are expected to occur by 2023 (Siegel RL, 2023). Despite breakthroughs in new therapeutic approaches such as targeted therapy and immunotherapy, clinical outcomes for cancer patients remain unsatisfactory (Sung H et al., 2021).

Muscarinic acetylcholine receptors (mAchRs) are G-protein-coupled receptors located in the nervous system, heart and smooth muscle neurons that regulate a variety of biological processes by activating the EGFR pathway (Bock A, 2018). Muscarine receptors are divided into subtypes M1-M5, which are encoded by the cholinergic receptor muscarine 1-5 (CHRM1-5) genes (Oenema TA et al., 2010). The M2 acetylcholine receptor (M2R) encoded by the CHRM2 gene is widely distributed in the human body, especially in the nervous system and cardiovascular system, which can lead to slowing of the heart rate and a reduction in ventricular contractile force by regulating the rhythm and contraction of the heart (Hautala AJ et al., 2006). It also participates in regulating release of neurotransmitters, affecting learning, memory and other cognitive functions (Volpicelli LA, 2004; Nelson CP, 2006).

CHRM2 is closely associated with a variety of cancers. Studies have shown that CHRM2 correlates significantly with the clinical stage, immune invasion, immune response and important signaling pathways of endometrial cancer and may be used as a biomarker for prognosis of endometrial cancer (Chen F et al., 2022). However, the expression level of CHRM2 in colorectal cancer tissues is significantly lower than that in adjacent normal tissues (Li Y, 2022). Despite studies on the role of CHRM2 in cervical cancer, gastric cancer, prostate cancer, etc. (Ma Y et al., 2022; Chen L et al., 2012; Sun X et al., 2021) no relevant studies have systematically clarified the expression and significance of CHRM2 across cancers, and more studies are needed to determine whether CHRM2 can be used as a biomarker and therapeutic target for cancer.

MATERIALS AND METHODS

Data sources

The TCGA (The Cancer Genome Atlas) database (https://portal.gdc.cancer.gov/) is a large-scale cancer genomics database that is a collaboration between the National Cancer Institute (NCI) and the National Human Genome Research Institute (NHGRI) and covers multiple cancer types. In this study, mRNA expression profiles and related clinical data of 33 cancer samples (Supplementary Table S1) and corresponding normal samples were downloaded from the TCGA database using

UCSC Xena (http://xena.ucsc.edu/). The expression data were transformed using log2 (TPM) to eliminate missing data and duplicate values. The analysis was performed using R software v4.3.1.

Differential expression analysis

The expression level of CHRM2 in normal tissues and 33 tumor tissues was analyzed by the Wilcoxon test, and to determine whether there was differential expression between the normal and tumor groups, $P < 0.05$ was considered statistically significant.

Clinical significance analysis of CHRM2 in pan-cancer

Kaplan–Meier curves were plotted to show the overall survival (OS), disease-specific survival (DSS), disease-free interval (DFI), and progression-free interval (PFI) phases of 33 cancers. The correlation between CHRM2 and the clinicopathologic stage of each tumor was analyzed by plotting box plots.

TMB and MSI analysis

The tumor mutation burden (TMB) is the total number of somatic/acquired mutations in each coding region (Mut/Mb) of the tumor genome. Microsatellite instability (MSI) refers to the phenomenon of microsatellite instability due to changes in the length of microsatellite repeats in the genome, such as errors, deletions, or insertions. We used the Spearman rank correlation coefficient to analyze the correlation between CHRM2 and TMB and MSI and displayed the results in a radar chart.

Correlation analysis of CHRM2 with immune infiltration

The tumor immune estimation resources TIMER and CIBERSORT were used to estimate the degree of tumor immune invasion in different cancers. The matrix score and immune score of each case were calculated by ESTIMATE, and the relationship between CHRM2 and 22 types of immune cell phenotypes was explored by the CIBERSORT method. The expression commonalities of CHRM2 and several immune-related genes in pan-cancer were analyzed by the “limma” package.

GSEA enrichment analysis

GSEA enrichment analysis of CHRM2 based on TCGA data, including GO analysis and KEGG analysis, was used to predict biological processes and the signaling pathways in which it participates. This analysis was processed using the R package "clusterProfiler".

Drug sensitivity analysis

CellMiner database collects a large amount of cancer cell line data, including gene expression, protein expression, gene mutation, drug sensitivity, and the NCI-60 cell line is the most widely used cancer cell sample group in anticancer drug testing (Reinhold WC et al., 2014). We downloaded NCI-60 susceptibility data and RNA-seq gene expression data to explore the relationship between CHRM2 and antitumor drug sensitivity.

RESULTS

Differential expression of CHRM2 between tumor and normal tissue samples

Expression of CHRM2 in tumor and normal tissue samples was analyzed using TCGA data. We found significant differences in expression levels of CHRM2 in tumor groups and normal groups for 14 types of cancer, including BLCA, BRCA, CESC, CHOL, COAD, ESCA, GBM, LIHC, LUAD, LUSC, PRAD, READ, STAD and UCEC. CHRM2 expression in cancers was significantly lower than that in paired normal tissues ($p < 0.05$) (Figure 1A-1B).

Clinical value of CHRM2 in pan-cancer

We studied the correlation between CHRM2 expression and prognosis in patients with different tumors. Survival indicators included OS, DSS, DFI and PFI. Kaplan–Meier survival analysis showed that low CHRM2 expression was associated with better OS in BLCA and COAD (Figure 1C-1D). The same was true for DSS in BLCA and COAD (Figure 1E-1F). In LIHC, high CHRM2 expression was associated with poor DFI (Figure 1G); for PFI, low CHRM2 expression was associated with good prognosis in COAD (Figure 1H) and poor prognosis in MESO (Figure 1I).

Next, we analyzed expression of CHRM2 in patients with stage I, II, III, and IV cancer and found that CHRM2 correlated with the clinicopathological stages of BLCA, COAD, LUSC, PAAD, and STAD (Figure 1J-1N), with significant differences between early and late stages of cancer.

Correlation of CHRM2 expression with TMB and MSI

In the process of studying tumor characteristics, it is crucial to focus on TMB and MSI, which are important indicators for evaluating tumor genetic characteristics and predicting patient response to immunotherapy. Through the correlation analysis of CHRM2, we observed that it correlated positively with TMB in CESC, PCPG and THCA and negatively in BLCA, COAD, GBM, LGG, LIHC, LUAD, PAAD, and STAD (Figure 1O). In addition, CHRM2 correlated positively with MSI in CESC. In LIHC, SARC and STAD, CHRM2 correlated negatively with MSI (Figure 1P). This finding suggests that CHRM2 plays a different role in regulation of TMB in different cancer types, possibly involving different functions of CHRM2 in cell proliferation or DNA repair.

CHRM2 expression and immune infiltration

To further study the relationship between CHRM2 and the human immune system, we analyzed the relationship between CHRM2 expression and the tumor immune microenvironment according to the TIMER database and found expression of CHRM2 correlated positively with the matrix score in BLCA, COAD, ESCA, LIHC, PRAD, READ, STAD and TGCT and negatively with the matrix score in LGG and SARC. In addition, expression of CHRM2 correlated positively with the immune score in BLCA and STAD. In LGG and SARC, CHRM2 expression correlated negatively with the immune score (Supplementary Figure S1). This reflects the versatility of CHRM2 in regulating the tumor microenvironment, particularly its potential role in influencing regulation of immune cell infiltration.

Through CIBERSORT analysis, we thoroughly studied the association between CHRM2 and immune cell infiltration in the background of pan-cancer. CHRM2 was found to be significantly

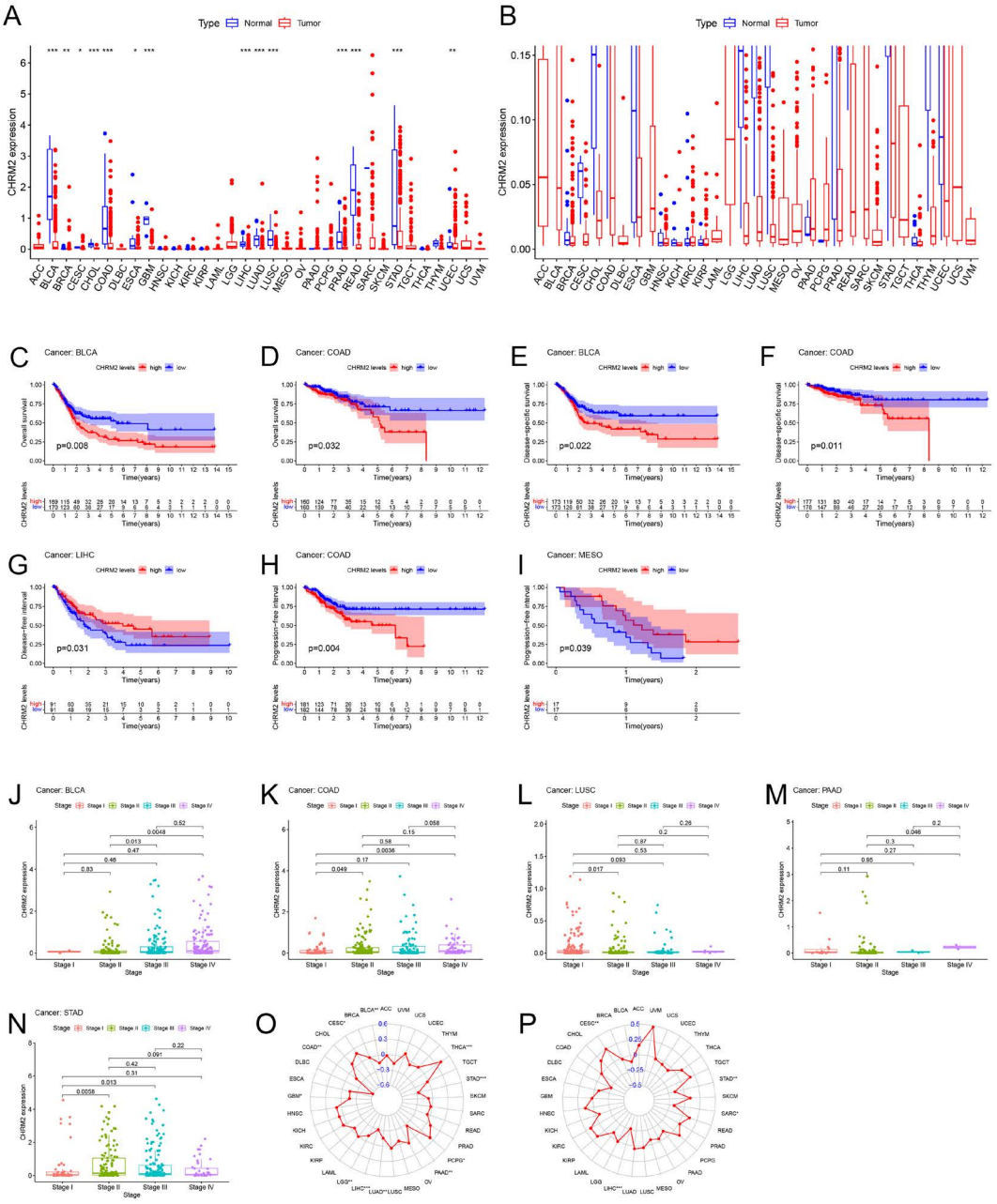


Figure 1. (A-B) Analysis of differential expression of CHRM2 between tumor and normal groups in pan-cancer. (C-I) Kaplan-Meier survival curves of OS, DSS, DFI, and PFI of CHRM2 in pan-cancer. (J-P) Relationship of CHRM2 expression to clinicopathologic stage, TMB, and MSI (O. TMB; P. MSI. P<0.05 was considered statistically significant. *P<0.05, **P<0.01, ***P<0.001).

associated with a variety of immune cells in 10 types of cancer: BLCA, CHOL, LIHC, LUAD, PAAD, PRAD, SARC, STAD, TGCT, and UCEC (Figure 2). The results showed that naive B cells correlated positively with CHRM2 expression in BLCA and LIHC; memory B cells were correlated negatively with CHRM2 expression in PAAD. Activated dendritic cells correlated negatively with CHRM2 expression in BLCA and UCEC, while resting mast cells correlated positively with CHRM2 expression in BLCA, LUAD and STAD. Mast cell activation correlated negatively with CHRM2 in STAD. M0 macrophages correlated negatively with CHRM2 in PAAD. PRAD and STAD and correlated positively with CHRM2 in TGCT. In addition, CHRM2 and CD8 T cells correlated negatively in BLCA but positively in PAAD and STAD. CHRM2 correlated positively with resting memory CD4 T cells in SARC and negatively with memory CD4 T cells activated in TGCT. CHRM2 correlated negatively with regulatory T cells in CHOL and LUAD and positively with eosinophils in LUAD. CHRM2 correlated positively with monocytes in STAD and negatively with neutrophils and resting NK cells.

We also conducted comprehensive analysis of BTLA, CD200, TNFRSF14 and 47 other key immune-related genes in the immune system (Supplementary Figure S2). We found that in 32 types of cancer, except for PCPG, CHRM2 and this series of immune-related genes jointly affected the tumor process. CHRM2 correlated positively with immune genes in BLCA, COAD, ESCA and PRAD and mostly negatively with immune genes in GBM, LGG, SARC and TGCT. This finding hints at the key role of CHRM2 in regulating immune gene networks. Future studies should further explore the molecular interaction mechanism between CHRM2 and these immune-related genes and its potential application in cancer therapy.

Enrichment analysis of CHRM2 in pan-cancer

We investigated the biological function of CHRM2 across cancers by GSEA. GO analysis showed that CHRM2 mainly inhibits migration of endothelial and epithelial cells and formation of blood vessels and promotes aging of cells in BLCA (Figure 3A). In COAD, differentiation of vascular-associated smooth muscle cells was negatively regulated, and cell senescence was positively regulated (Figure 3B). In SARC, it is mainly involved in activation of granulocytes, natural killer cells, T cells, chemokines, and molecular functional maturation of major histocompatibility complex proteins (Figure 3C). KEGG enrichment analysis showed that CHRM2 negatively regulates SARC signaling pathways but positively regulates STAD and TGCT signaling pathways. In SARC, CHRM2 is primarily involved in the biological pathways of allograft rejection, graft-versus-host disease (GVHD)-associated pathways, IgA production in the intestinal immune network, and primary immunodeficiency-associated pathways (Figure 3D). In STAD, we found that CHRM2 is associated with amyotrophic lateral sclerosis and arrhythmogenic right ventricular cardiomyopathy, as well as calcium signaling and olfactory transduction (Figure 3E). In TGCT, high expression of CHRM2 is mainly associated with metabolism-related activities, including drug metabolism of cytochrome P450, metabolism of retinol and porphyrins, and biosynthesis of steroid hormones (Figure 3F).

Drug sensitivity

We used the CellMiner database to conduct correlation analysis between CHRM2 and drug sensitivity and found that CHRM2 expression correlated positively with sensitivity to seven antitumor drugs (Figure 3G-3M). Specifically, various tumor cells with high expression of CHRM2 are more sensitive to PF−04217903, SGX−523, JNJ−38877605, AMG−458, LMP−400, BMS−777607, and MK−8033.

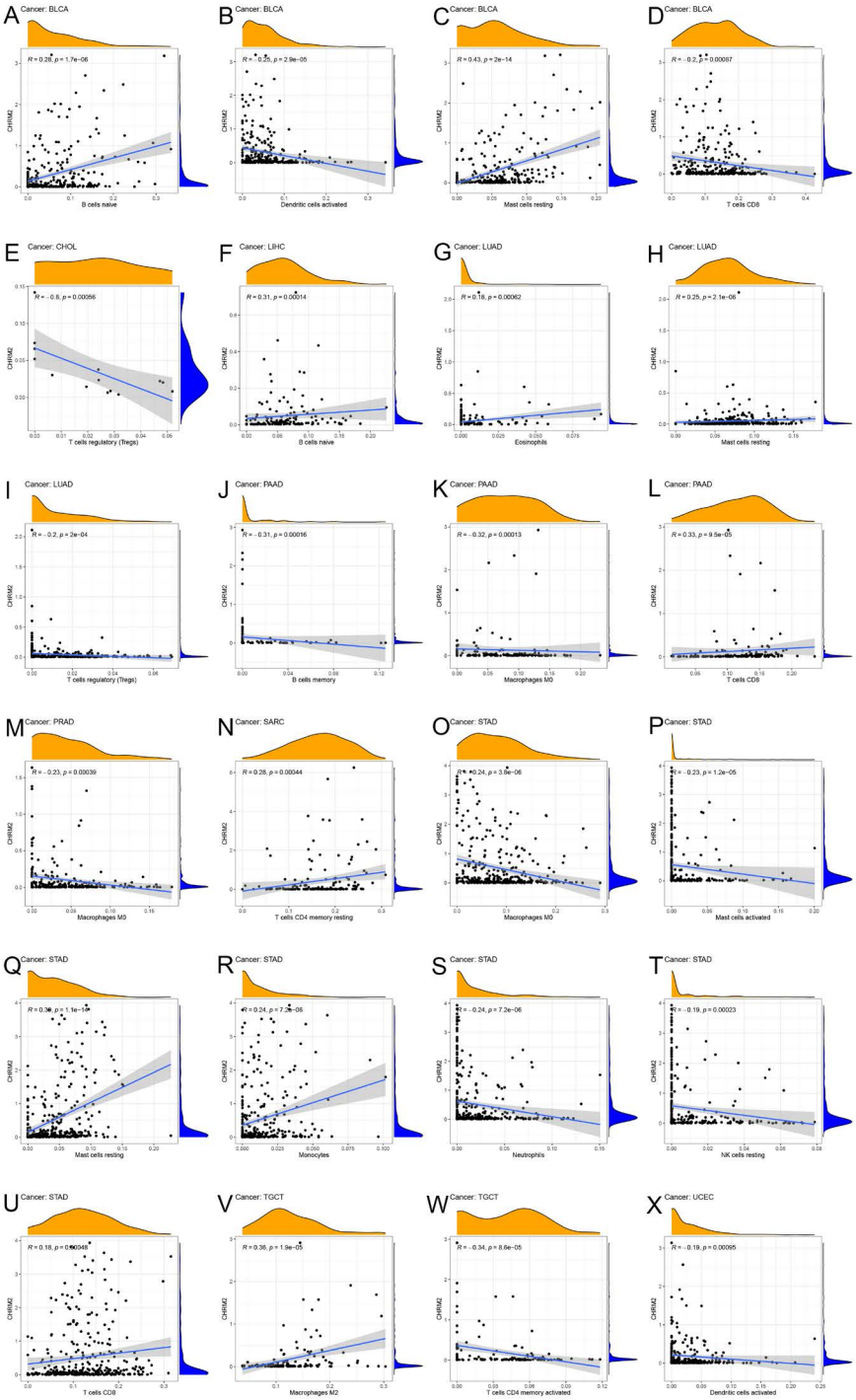
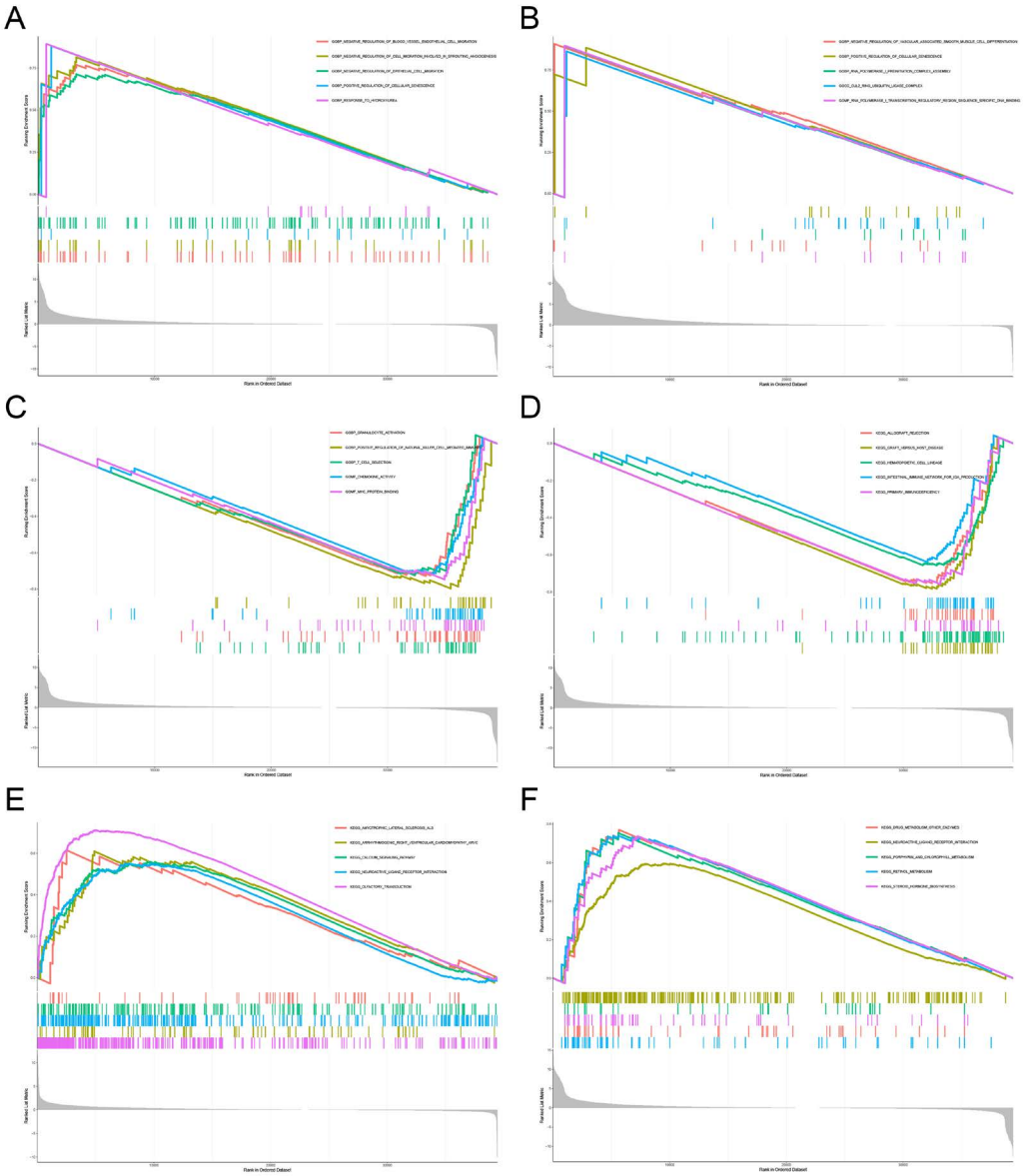


Figure 2. Correlation between CHRM2 expression and immune cell infiltration.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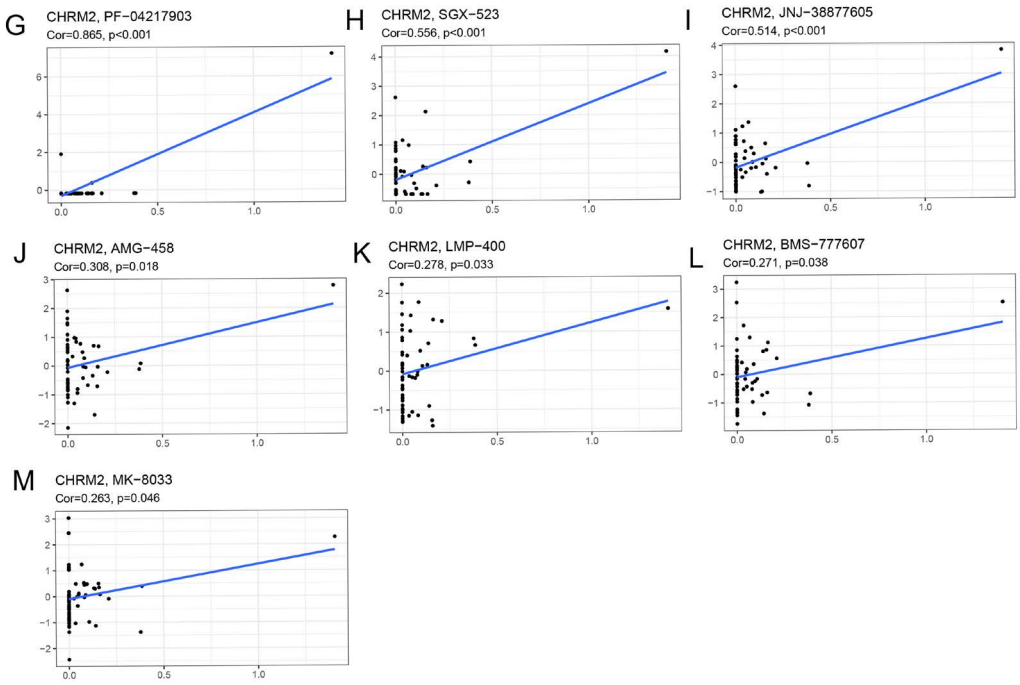

Figure 3. GSEA enrichment analysis of CHRM2 (A. GO analysis of BLCA; B. GO analysis of COAD; C. GO analysis of SARC; D. KEGG analysis of SARC; E. KEGG analysis of STAD; F. KEGG analysis of TGCT.) (G-M) Correlation between CHRM2 expression and drug sensitivity.

DISCUSSION

Cancer research is important for the medical field, and pan-cancer analysis can discover biomarkers suitable for diagnosis, treatment and prognosis assessment of various cancers. By pan-cancer analysis, we found that CHRM2 expression levels were significantly downregulated in 14 cancers. Studies have shown that the mRNA level of CHRM2 is significantly downregulated in liver cancer tissues (Ye C et al., 2020). Pacini L et al., found that the M2 muscarinic receptor could inhibit proliferation and migration of urothelial bladder cancer cells (Pacini L et al., 2014). Ferretti M et al., showed that M2 muscarinic receptors inhibit cell proliferation in human glioblastoma cell lines (Ferretti M et al., 2012). Espanol AJ et al., also found that the M2 receptor is involved in the rhythm of paclitaxel and cholinergic agonists, inhibiting progression of triple-negative breast tumors (Espanol AJ et al., 2020). However, other studies have shown that the expression level of M2R in the urothelium of patients with invasive bladder cancer is significantly increased (Wei W et al., 2018), which is somewhat controversial with this study. Downregulation of CHRM2 expression levels may lead to disturbance of the acetylcholine signaling pathway, and whether CHRM2 can be used as a therapeutic target or biomarker needs more in-depth study.

To investigate the relationship between CHRM2 expression and prognosis, we performed survival association analysis for each type of cancer using Kaplan–Meier survival curves. It was found that in BLCA and COAD, a low level of CHRM2 expression was associated with good OS

and DSS, suggesting that CHRM2 may act as a negative prognostic factor in these two cancers, playing a role in inhibiting tumor development. In LIHC, high levels of CHRM2 expression are associated with good DFI, suggesting that CHRM2 may act as a positive prognostic factor in patients with hepatocellular carcinoma, and its high expression is associated with better disease control and relapse-free survival. In addition, studies have shown that expression of the M2 receptor correlates with the grade of urothelial tumors in vivo, and we have observed that in the clinical staging of CHRM2, stage II of BLCA correlates with stage III and that stage II correlates with stage IV. COAD I is related to IV. Phase II of PAAD is related to phase IV. Stage I of STAD is related to stage III. These results indicate that CHRM2 is involved in the biological processes of tumor growth, spread and invasion and plays a certain role in late development of these cancers. Moreover, clinical stage is usually associated with patient prognosis, and the results indicate the potential clinical value of CHRM2 in evaluation of cancer prognosis, suggesting that it may serve as a predictor of disease progression and a potential target for therapeutic intervention.

Immunotherapy, such as anti-CTLA-4 and PD-1/PD-L1 inhibitors and CAR-T-cell therapy, is widely used in cancer treatment. However, due to the inconsistency of treatment response, immune resistance and immune-related side effects, only some patients have achieved good therapeutic effects (Merlano MC, 2020). TMB, MSI and the tumor immune microenvironment play a great role in tumor progression and treatment and are crucial to solving the problem of immunotherapy resistance (Baba Y et al., 2020; Badr NM, 2020). In our study, CHRM2 correlated positively with TMB in CESC, PCPG, and THCA and with MSI in CESC. High TMB and MSI make it easier for the immune system to recognize and attack cancer cells, thus boosting immune system activity, and they are often considered a potential predictor of immunotherapy, showing better efficacy against immunotherapies such as immune checkpoint inhibitors. MSI-High may be a marker in some inherited cancer syndromes, such as Lynch syndrome, which involves genetic defects in DNA instability (Baretti M, 2018).

CHRM2 and the stromal score correlated positively in eight cancers, such as BLCA and COAD, and negatively in LGG and SARC. As an increase in the stromal score is generally associated with an increase in tumor aggressiveness, the positive correlation of CHRM2 hints at its potential role in promoting tumor progression. In addition, a tumor with a low stromal score responds better to immunotherapy, while a high stromal score is generally associated with poor prognosis, as it indicates increased tumor aggressiveness and treatment resistance. For the immune score, CHRM2 correlated positively in BLCA and STAD and negatively in LGG and SARC. A high immune score reflects the presence of more immune cell infiltration in tumor tissue, and the immune system can recognize and remove cancer cells more effectively, while the presence of immune cells helps to inhibit the growth and spread of tumors such that patients have good prognosis. However, we observed a positive correlation between CHRM2 and stromal and the immune score in BLCA, but K–M analysis showed that patients with low CHRM2 expression had better survival prognosis. This indicates that CHRM2 is involved in regulation of both stromal and immune responses in the tumor microenvironment and that the role of CHRM2 may change at different stages of tumor development. CHRM2 promotes the immune response in the early stage of the tumor and is associated with immune escape in the late stage. Both the immune score and stromal score are complex indicators that are affected by many factors, and the positive correlation of a single indicator may not fully reflect the overall biological characteristics of a tumor. Through CIBERSORT analysis, we found that CHRM2 was associated with naive B cells, memory B cells, resting memory CD4 T cells, activated memory CD4 T cells, CD8 T cells, and T cells in 10 types of cancer regulatory cells (Tregs). M0 macrophages, M2 macrophages, activated dendritic cells, resting NK cells, resting mast cells, activated mast

cells, activated mast cells, eosinophils, monocytes and neutrophils correlated significantly. The results showed that expression of CHRM2 correlated negatively with infiltration levels of many immune cells, suggesting that low expression of CHRM2 provides an immunologically active tumor environment, which was more likely to respond positively to immunotherapy. However, when studying the co-expression relationship between CHRM2 and immune-related genes, it was found to correlate positively with most immune genes, such as BLCA and COAD, but negatively with CD8 T cells and activated dendritic cells in BLCA. This suggests that CHRM2 may be more positively related to inhibitory immune factors, high expression of CHRM2 can promote the escape of tumor immunity in some cases, and the immune response may be suppressed even in the presence of immune cell infiltration. Overall, this situation highlights the complexity of tumor biology, the possible complex regulatory role of CHRM2 in the immune response, and its specific mechanism of action and impact on patient prognosis, which still need more in-depth study.

Through GO analysis, we found that CHRM2 mainly inhibits generation of blood vessels and promotes aging of cells in BLCA and COAD, indicating that CHRM2 may participate in a mechanism of tumor antiangiogenesis, leading to the scarcity of blood vessels in the tumor microenvironment and affecting the blood supply and oxygen supply of the tumor. Moreover, promoting cell senescence will affect proliferation and survival of tumor cells, which is conducive to limiting the growth of tumor cells. This is somewhat similar to the role of epidermal growth factor receptor (EGFR), but EGFR is a factor that promotes tumor growth in BLCA and COAD (Daizumoto K, et al., 2018; Huang Z et al., 2019; Cheng WL, et al., 2021). In addition, the EGFR signaling pathway is a core driver of tumorigenesis and a major therapeutic target for BLCA and COAD (Sigismund S, 2018; Hong CS et al., 2020). Therefore, EGFR activators such as EGF and TGF-α may lead to upregulation of CHRM2 expression, and CHRM2 may regulate proliferation and growth of tumor cells by affecting the Ras/Raf/MAPK pathway, PI3K/AKT pathway or STAT pathway of EGFR (Figure 4). Simultaneous intervention in the CHRM2 and EGFR signaling pathways may have a certain impact on treatment of BLCA and COAD. Combined targeted therapy for CHRM2 and EGFR may have a synergistic effect and is expected to improve the therapeutic effect. Through KEGG enrichment analysis, although no CHRM2 pathway was found in BLCA and COAD, it was found to be involved in many functions and pathways in SARC, STAD and TGCT. These include primary immune deficiency-related pathways, calcium signaling, cytochrome P450 drug metabolism, retinol and porphyrin metabolism, and steroid hormone biosynthesis. These results suggest that CHRM2 is involved in the immune response, biosynthesis, signal transduction and metabolic activity in various cancers.

Drug sensitivity is at the heart of personalized cancer therapy and is critical to advancing precision medicine. Through the CellMiner database, we found that CHRM2 expression correlated positively with the sensitivity to several drugs, almost all of which were c-Met inhibitors. c-Met, a receptor tyrosine kinase, is associated with many types of cancer, such as lung, stomach, kidney, breast, pancreatic, head and neck cancers (Yu J et al., 2022; Yu S et al., 2013; Chen S et al., 2017; Mitra S et al., 2020; Sharma R, 2023). Activation of c-Met can trigger several signaling pathways, including the Ras/MAPK, PI3K/AKT, Wnt/β-catenin, STAT, and NF-κB pathways, which promote cell proliferation, growth, and metastasis. In BLCA and COAD, c-Met is considered to be a potential therapeutic target (Feng Y, 2022; Craig SG et al., 2021) and its abnormal activation is related to proliferation and invasion of cancer cells. Inhibition of c-Met may slow or prevent proliferation and spread of cancer cells by blocking the related pathways. Studies have shown that drugs can significantly inhibit migration and invasion of colon cancer cells by targeting c-Met and inhibiting Ras/Raf/mitogen activated extracellular signal-regulated kinase (MEK)/ERK signaling pathways

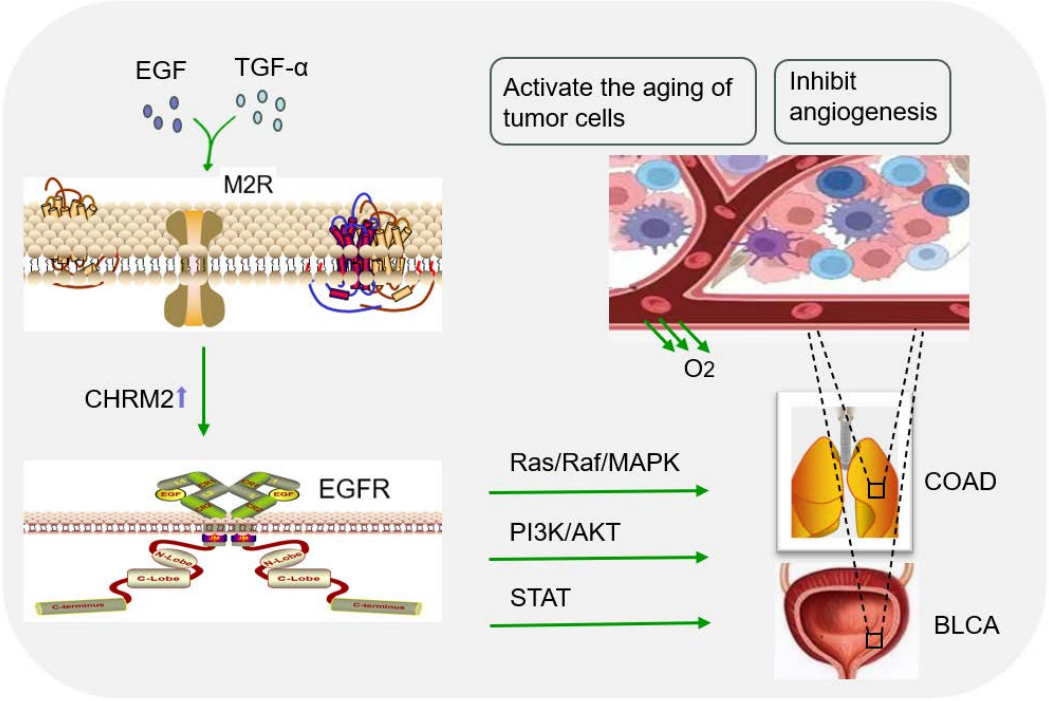

Figure 4. CHRM2 inhibits angiogenesis and activates cell aging in BLCA and COAD (In BLCA and COAD, EGFR activators such as EGF and TGF-α may lead to up-regulation of CHRM2 expression, and CHRM2 may inhibit angiogenesis and activate cell aging by affecting Ras/Raf/MAPK pathway, PI3K/AKT pathway or STAT pathway of EGFR.)

(Liu QQ et al., 2020). In addition, overexpression of c-Met in primary cancer tissues is associated with poor OS in human bladder cancer (Xu X, 2019). Although abnormal expression or activation of CHRM2 and c-Met may affect tumor development, no studies have shown a direct interaction between them.

This study is the first bioinformatic analysis of CHRM2 gene in pan-cancer and found that CHRM2 is down-regulated in multiple cancers and is associated with better prognosis, especially in BLCA and COAD. CHRM2 plays an important role in the tumor immune microenvironment and is associated with a variety of immune cells and immune factors. GSEA analysis and drug sensitivity analysis also indicate that CHRM2 may be a new target for cancer therapy, but the specific role of CHRM2 in each tumor needs further study.

CONCLUSION

CHRM2 may be a prognostic indicator, potential biomarker, and therapeutic target for pan-cancer, especially BLCA and COAD.

AUTHOR CONTRIBUTIONS

QH, ZS and HT conceived and designed the study. QH and ZS contributed to the experiment and analysis of the data. QH and HT wrote the first draft of manuscript. HX, CB, TL, KL, ZC, XY and YT critically revised the manuscript. All authors read and approved the final manuscript.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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DATA AVAILABILITY STATEMENT

All data human data used in study is publicly available. Data and materials can be provided upon reasonable request to the corresponding authors.

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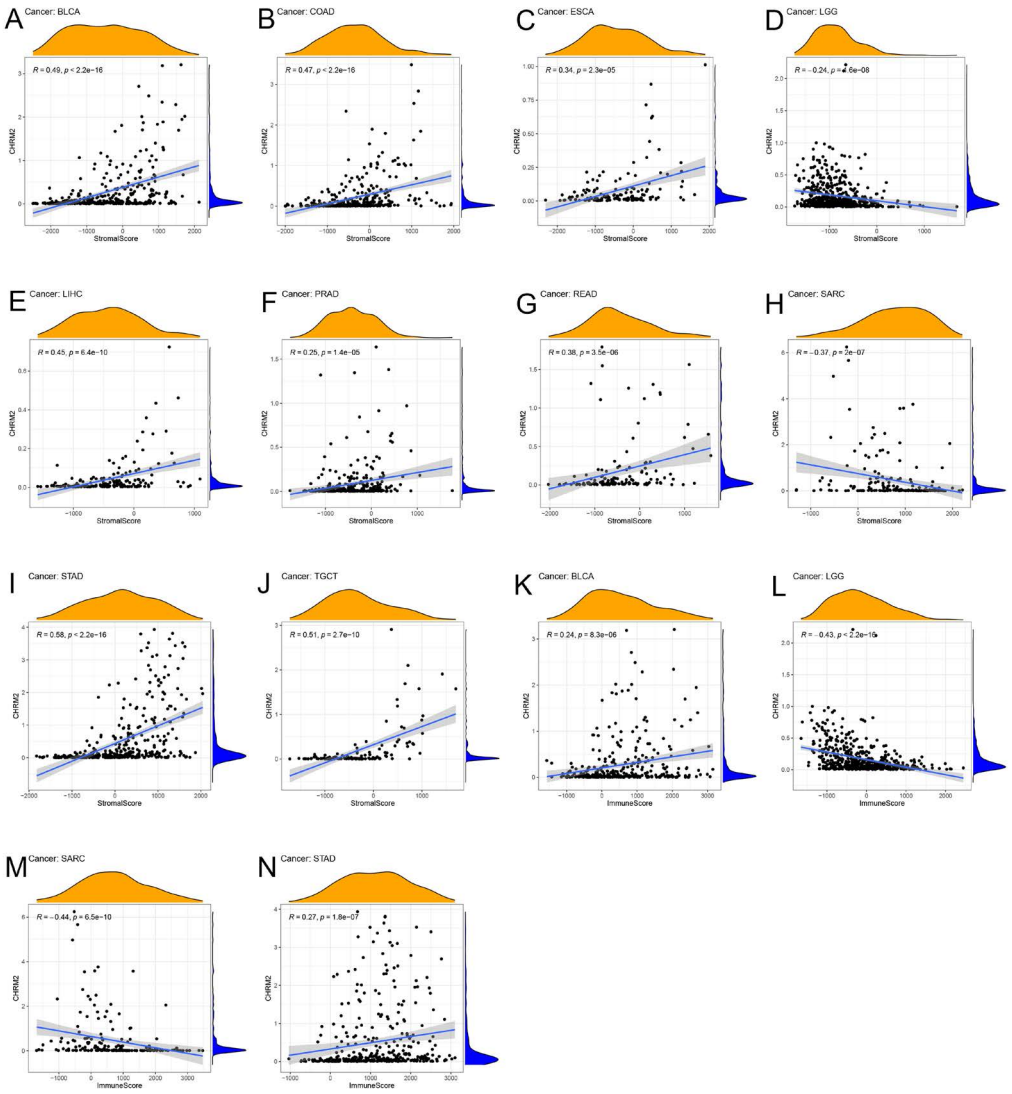
Supplementary Table S1

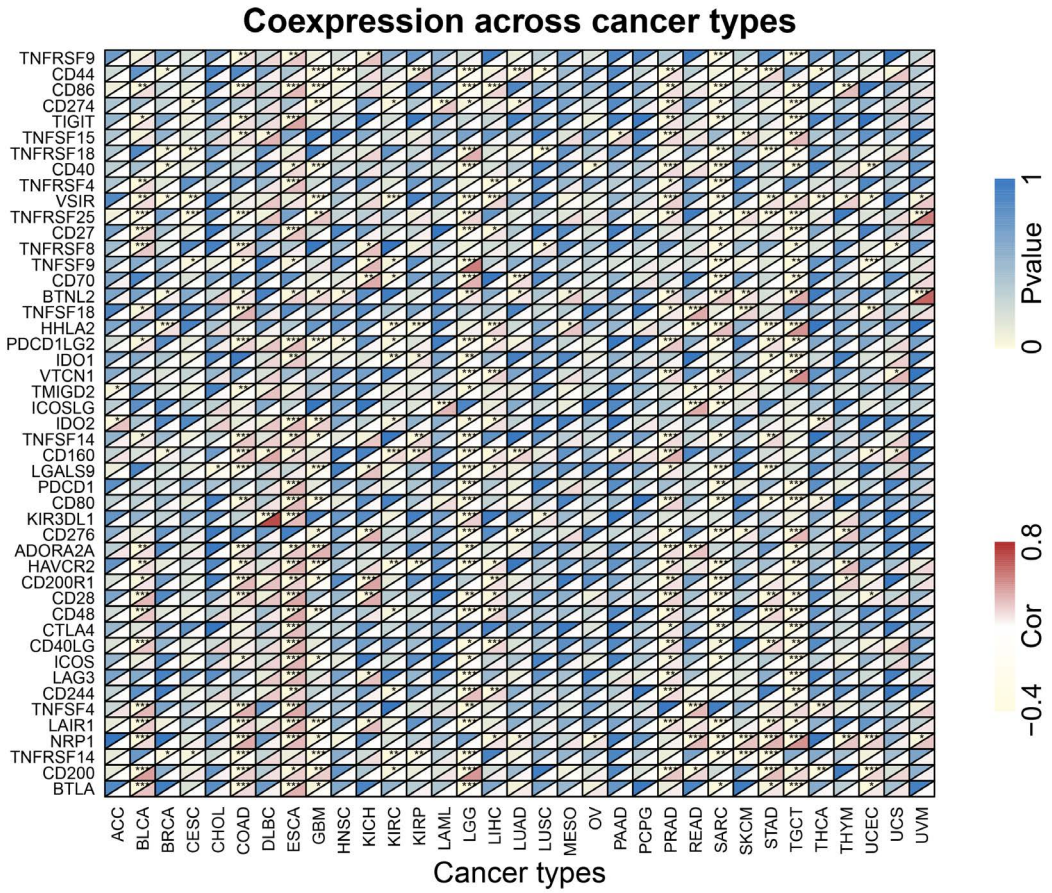
Table1. Abbreviation of 33 human cancers.

Abbreviation	Full name
ACC	Adrenocortical Carcinoma
BLCA	Bladder Urothelial Carcinoma
BRCA	Breast Invasive Carcinoma
CESC	Cervical Squamous Cell Carcinoma and Endocervical Adenocarcinoma
CHOL	Cholangiocarcinoma
COAD	Colon Adenocarcinoma
DLBC	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 Lower Grade Glioma
LIHC	Liver Hepatocellular Carcinoma
LUAD	Lung Adenocacinoma
LUSC	Lung Squamous Cell Carcinoma
MESO	Mesothelioma
OV	Ovarian Serous Cystadenocarcinoma
PAAD	Pancreatic Adenocarcinoma
PCPG	Pheochromocytoma and Paraganglioma
PRAD	Prostate Adenocarcinoma
READ	Rectum Adenocarcinoma
SARC	Sarcoma
SKCM	Skin Cutaneous Melanoma
STAD	Stomach Adenocarcinoma
TGCT	Testicular Germ Cell Tumors
THCA	Thyroid Carcinoma
THYM	Thymoma
UCEC	Uterine Corpus Endometrial Carcinoma
UCS	Uterine Carcinosarcoma
UVM	Uveal Melanoma

Supplementary Figures



Supplementary Figure S1. Correlation between CHRM2 expression and tumor immune microenvironment (A-J. Stromal score, K-N. Immune score.)



Supplementary Figure S2. Heat map of co-expression of CHRM2 with immune-related genes.