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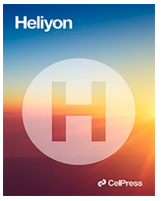

Research article

# Comprehensive analysis of ADGRE5 gene in human tumors: Clinical relevance, prognostic implications, and potential for personalized immunotherapy

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ABSTRACT

*Purpose:* The Adhesion G protein receptor E5 (ADGRE5) gene is involved in a wide range of biological functions in human tumors; however, its specific molecular mechanism and significance in the analysis of human tumors have not yet been determined. Here, we provide a comprehensive genomic architecture of ADGRE5 in the tumor immune microenvironment and its clinical relevance across a broad range of solid tumors.

*Methods:* In this study, we used publicly available bioinformatics databases, with a primary focus on The Cancer Genome Atlas (TCGA) database and GTEx data, to conduct a comprehensive analysis of the impact on patient prognosis associated with ADGRE5.

*Results:* Statistics of more than 30 solid tumors from TCGA and Cancer Cell Line Encyclopedia (CCLE) were examined. ADGRE5 was differentially expressed in several cancers and was significantly associated with survival outcomes. Higher ADGRE5 levels were associated with worse prognosis in adrenocortical carcinoma, low grade glioma of the brain (LGG), lung squamous cell carcinoma, liver hepatocellular carcinoma, and uveal melanoma (UVM). Additionally, ADGRE5 was found to be an independent risk factor for LGG and UVM. The clinical relevance of ADGRE5 in tumor immunogenicity was further investigated. The expression level of ADGRE5 was not only strongly associated with tumor infiltration, such as tumor-infiltrating immune cells and immune subtypes, but also with tumor mutation burden, pyroptosis, and epithelial-mesenchymal transition in various types of cancer ($P < 0.05$). Furthermore, we noted that ADGRE5 exhibited a positive association with targeted drug sensitivity and conversely, a negative association with traditional chemotherapeutic drug sensitivity. Thus, ADGRE5 is expected to be a guiding marker gene for clinical prognosis and personalized tumor immunotherapy.

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## 1. Introduction

The advent of immunotherapy has transformed cancer treatment, ushering in a new era of tumor immunology. Various approaches, such as adoptive cell transfer and immune checkpoint inhibitors, have shown remarkable clinical responses; however, their efficacy varies among different cancer types and patient subsets. Pan-cancer analyses, which explore molecular aberrations across diverse cancers [1], offer insights into shared biological processes, differences, and emerging themes in tumorigenesis [2].

Adhesion G protein-coupled receptor E5 (ADGRE5), also known as cluster of differentiation 97 (CD97), belongs to the seven-transmembrane epidermal growth factor subfamily of class B G protein-coupled receptors (GPCRs) [3,4]. Despite its association with gastric, colorectal, and thyroid cancers, the specific role of ADGRE5 in carcinogenesis remains elusive [4–7]. Recent research has indicated that ADGRE5 plays a crucial role in tumor dedifferentiation, migration, invasion, and metastasis, positioning it as a significant contributor to various human malignancies [8].

While the link between ADGRE5 function and cancer initiation is not fully understood, it has become evident that ADGRE5 plays a critical role in mediating the impact of cancer on the human body. This study aimed to fill the existing research gap by conducting a comprehensive pan-cancer analysis of ADGRE5, exploring its differential expression across various tumors and assessing its prognostic value, clinical correlations, and implications for immunotherapy.

## 2. Methods

### 2.1. Data download

Transcripts per million data for normal and tumor tissues were downloaded from The Cancer Genome Atlas (TCGA) (https://portal.gdc.cancer.gov/) and GTEx (https://gtexportal.org/). To assess variations in ADGRE5 expression, we applied log transformation. The analyzed cancers included adrenocortical carcinoma (ACC), bladder urothelial carcinoma (BLCA), breast invasive carcinoma (BRCA), cervical squamous cell carcinoma and endocervical adenocarcinoma (CESC), cholangiocarcinoma (CHOL), colon adenocarcinoma (COAD), rectum adenocarcinoma esophageal carcinoma (READ), 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), low grade glioma of the brain (LGG), liver hepatocellular carcinoma (LIHC), lung adenocarcinoma (LUAD), lung squamous cell carcinoma (LUSC), mesothelioma (MESO), ovarian serous cystadenocarcinoma (OV), pancreatic adenocarcinoma (PAAD), pheochromocytoma and paraganglioma (PCPG), prostate adenocarcinoma (PRAD), rectal adenocarcinoma (READ), sarcoma (SARC), skin cutaneous melanoma (SKCM), stomach adenocarcinoma (STAD), stomach and esophagus cancer (STES), testicular germ cell tumors (TGCT), thyroid carcinoma (THCA), thymoma (THYM), uterine corpus endometrial carcinoma (UCEC), and uveal melanoma (UVM).

UALCAN (http://ualcan.path.uab.edu/index.html) [9]database, based on the Clinical Proteomic Tumor Analysis Consortium (CPTAC) and the International Cancer Proteogenome Consortium datasets, provides protein expression of 13 types of tumors (colorectal cancer, breast cancer, ovarian cancer, clear cell renal cell carcinoma, uterine corpus endometrial carcinoma, gastric cancer, glioblastoma, pediatric brain tumors, head and neck squamous cell carcinoma, lung adenocarcinoma, lung squamous cell carcinoma, liver cancer, pancreatic cancer, and prostate cancer). Selecting the "pan-cancer view" module enables visualization of the protein expression profiles of 10 tumors and their corresponding paracancerous tissues. We selected the "CPTAC" module, entered the target gene ADGRE5, and selected the "pan-cancer view" module. The results showed the protein expression of ADGRE5 in the cancer and adjacent tissues of 10 tumors.

We inputted the target gene, selected the species as human, and downloaded the expression profile of ADGRE5 in tumor and normal cells using the BioGPS database (http://biogps.org/#goto=welcome) [10].

### 2.2. Clinical features and prognosis analysis

We selected the Kaplan–Meier Survival Analysis" module of Gene Expression Profiling Interactive Analysis version2 (GEPIA2) (http://gepia2.cancer-pku.cn/) [11] to visualize the Kaplan–Meier (K-M) plot of overall survival (OS) and disease-free survival (DFS) of ADGRE5 in pan-cancer. Then, we selected the "Expression DIY" module to visualize the correlation of ADGRE5 with Stage in pan-cancer. The association between ADGRE5 expression and prognosis in LGG and UVM was explored via univariate and multivariate COX regression analyses.

### 2.3. Immune infiltration

Using the Tumor Immune Estimation Resource version 2 (TIMER2) database (http://timer.cistrome.org/) [12], the immune module was selected, and the MCPcounter, XCELL, Extended Polydimensional Immunome Characterization (EPIC), and the Tumor Immune Dysfunction and Exclusion (TIDE) algorithms were used to visualize the association of ADGRE5 with immune cells in pan-cancer. The correlation between ADGRE5 expression levels and immune cell infiltration in LGG and UVM was explored by calculating the immune cell enrichment score of each sample using the GSVA package [13] and the single-sample gene set enrichment analysis (ssGSEA) algorithm [14].

Through the TISIDB database(http://cis.hku.hk/TISIDB/index.php) [15], the target gene was entered and "subtype" was selected to visualize the expression differences between ADGRE5 immune subtypes and molecular subtypes in different tumors.

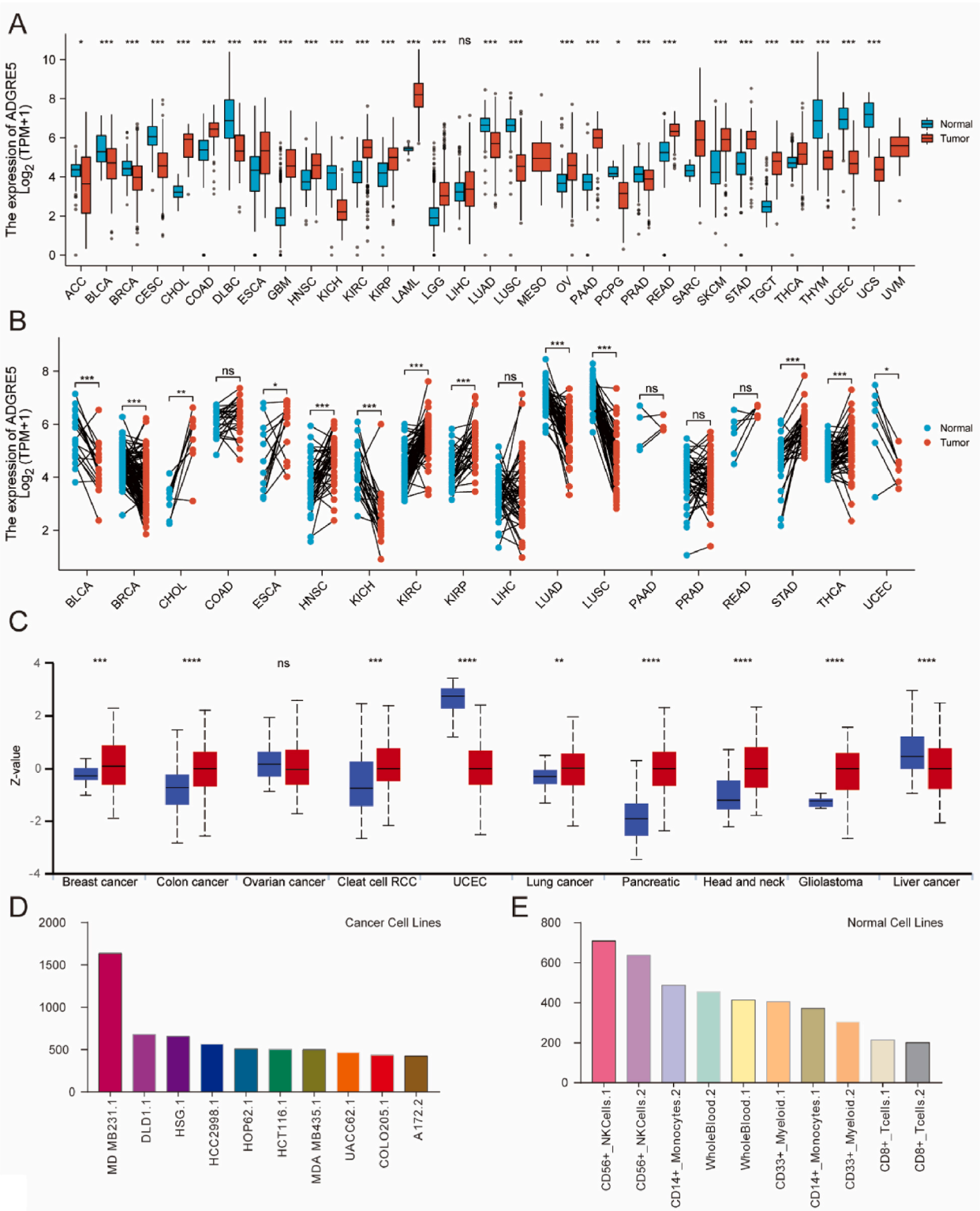


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**Fig. 1.** ADGRE5 is highly expressed in the vast majority of tumors
A. Differential expression of ADGRE5 transcript levels among 33 tumors based on TCGA and GTEx databases. B. Differential expression of ADGRE5 transcript levels in cancer and paired paracancerous tissues in 18 tumors based on the TCGA database. C. Differential expression of ADGRE5 protein levels in 10 tumors based on the CPTAC database. D. Expression levels of ADGRE5 in 10 tumor cells based on the BioGPS database. E. Expression levels of ADGRE5 in 10 normal cells based on the BioGPS database. ($P < 0.05$ indicates statistical significance, $P < 0.05$*, $P < 0.01$**, $P < 0.001$***, $P < 0.0001$****).

### 2.4. Mutation signature

The gene mutation map and mutation sites of ADGRE5 were visualized using cBioPortal (version 3.6.20) (https://www.cbioportal.org/) [16].

Tumor Mutation Burden (TMB) was defined as the total number of somatic gene-coding errors, base substitutions, and gene insertion or deletion errors were detected per million bases. It is an indicator of the frequency of gene mutations and is closely related to the evaluation of the effects of immunotherapy [17]. Microsatellites, short tandem repeat DNA sequences in the genome, are generally composed of 1–6 nucleotides and arranged in tandem repeats. Microsatellite Instability (MSI) is characterized by the emergence of novel microsatellite alleles at specific loci within tumors, resulting from the insertion or deletion of repetitive units. DNA mismatch repair is a crucial process. MSI is an important clinical tumor marker [18]. In the "Cancer Types Summary" module, the TMB and MSI data in TCGA pan-cancer were downloaded, and the correlation analysis showed the correlation of ADGRE5 with TMB and MSI in pan-cancer.

### 2.5. Correlation analysis of tumor characteristics

Pyroptosis [19], represents a recent paradigm of inflammatory cell demise, which exerts influence on tumor proliferation, invasion, and migration, thereby assuming a pivotal role in tumorigenesis and progression. Epithelial-mesenchymal transition (EMT) [20] is a process by which epithelial cells acquire mesenchymal characteristics. In cancer, EMT is significantly associated with multiple tumor characteristics including proliferation, invasion, metastasis, and treatment resistance [20]. To fully understand the role of ADGRE5 in tumors, we used Pearson's correlation analysis to explore the correlation of ADGRE5 expression with pyroptosis and EMT in pancreatic cancer.

### 2.6. Functional enrichment analysis

The STRING website (https://cn.string-db.org/cgi/about) [21] was used to construct a protein-protein interaction network, the "protein by name" module was selected, and the following parameters were selected: Active interaction sources: co-expression, measurement of network edges: evidence, maximum number of actors: 50, minimum required interaction score: low confidence (0.15).

The top 100 genes significantly associated with ADGRE5 in pan-cancer were downloaded using the GEPIA2 (http://gepia2.cancer-pku.cn/) "correlation analysis" module, and Cytoscape [22] was used to visualize the P-value ranking of the top 30 significantly associated genes.

Gene Ontology (GO) [23] analysis is a prevalent approach employed in extensive-scale investigations of functional enrichment, encompassing biological processes, molecular functions, and cellular components. Kyoto Encyclopedia of Genes and Genomes (KEGG) [24] serves as a widely utilized repository for housing data related to genomes, biological pathways, diseases, and pharmaceuticals. Differential genes were subjected to GO annotation analysis and KEGG pathway enrichment analysis using the cluster Profiler R [25] software package, and a cut-off value of false discovery rate $<0.05$ was considered statistically significant.

### 2.7. Drug sensitivity (CellMiner)

The mRNA expression profiles and drug activities of the target genes were downloaded from the CellMiner database (https://discover.nci.nih.gov/cellminer/) [26]. CellMiner is a web-based tool that contains genomic and pharmacological information that allows researchers to leverage transcript and drug response data from the NCI-60 cell line compiled by the National Cancer Institute. The transcriptional expression levels of the drug responses of 22,379 genes, 360 miRNAs, and 20,503 compounds are available on the CellMiner website. The correlation between target gene expression and compound sensitivity was calculated using Pearson's correlation analysis. Statistical significance was set at $P < 0.05$.

### 2.8. Statistical analysis

All data calculations and statistical analyses were performed using the R program (https://www.r-projec t.org/, version 4.0.2). To compare two groups of continuous variables, the statistical significance of normally distributed variables was estimated using the independent Student's *t*-test, and differences between non-normally distributed variables were analyzed using the Mann–Whitney *U* test (i.e., Wilcoxon rank sum test). Statistical P values were two-tailed, and statistical significance was set at $P < 0.05$.

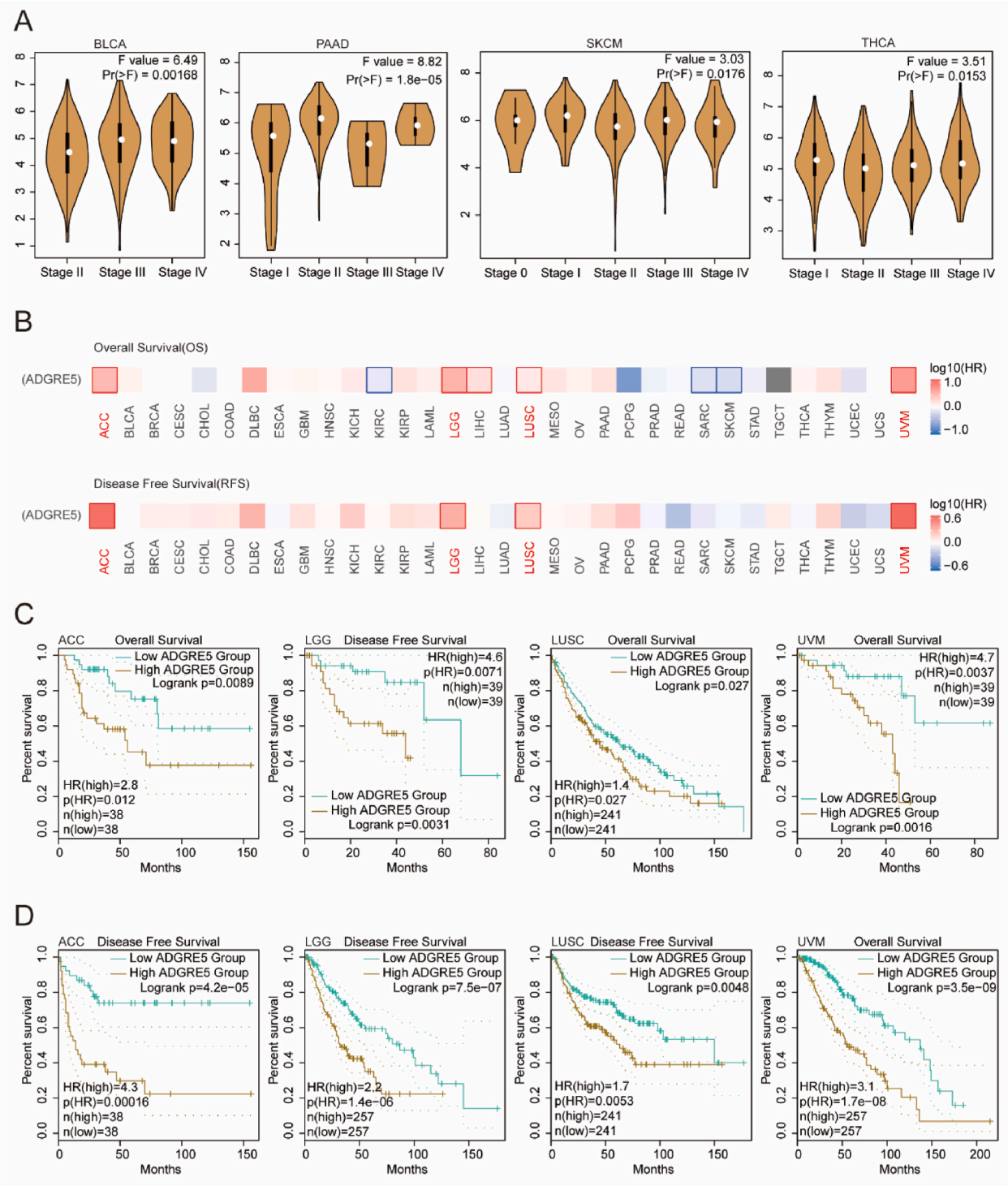


**Fig. 2.** ADGRE5 is associated with prognosis in various tumors

A. The expression differences of ADGRE5 transcript levels in different stages in BLCA, PAAD, SKCM, and THCA. B. Heatmap of overall OS and DFS of ADGRE5 in pan-cancer based on GEPIA2 database. C. Kaplan–Meier plots showed lower OS in the ADGRE5 high expression group. D. Kaplan–Meier plots showed lower DFS in the ADGRE5 high expression group. ($P < 0.05$ indicates statistical significance).

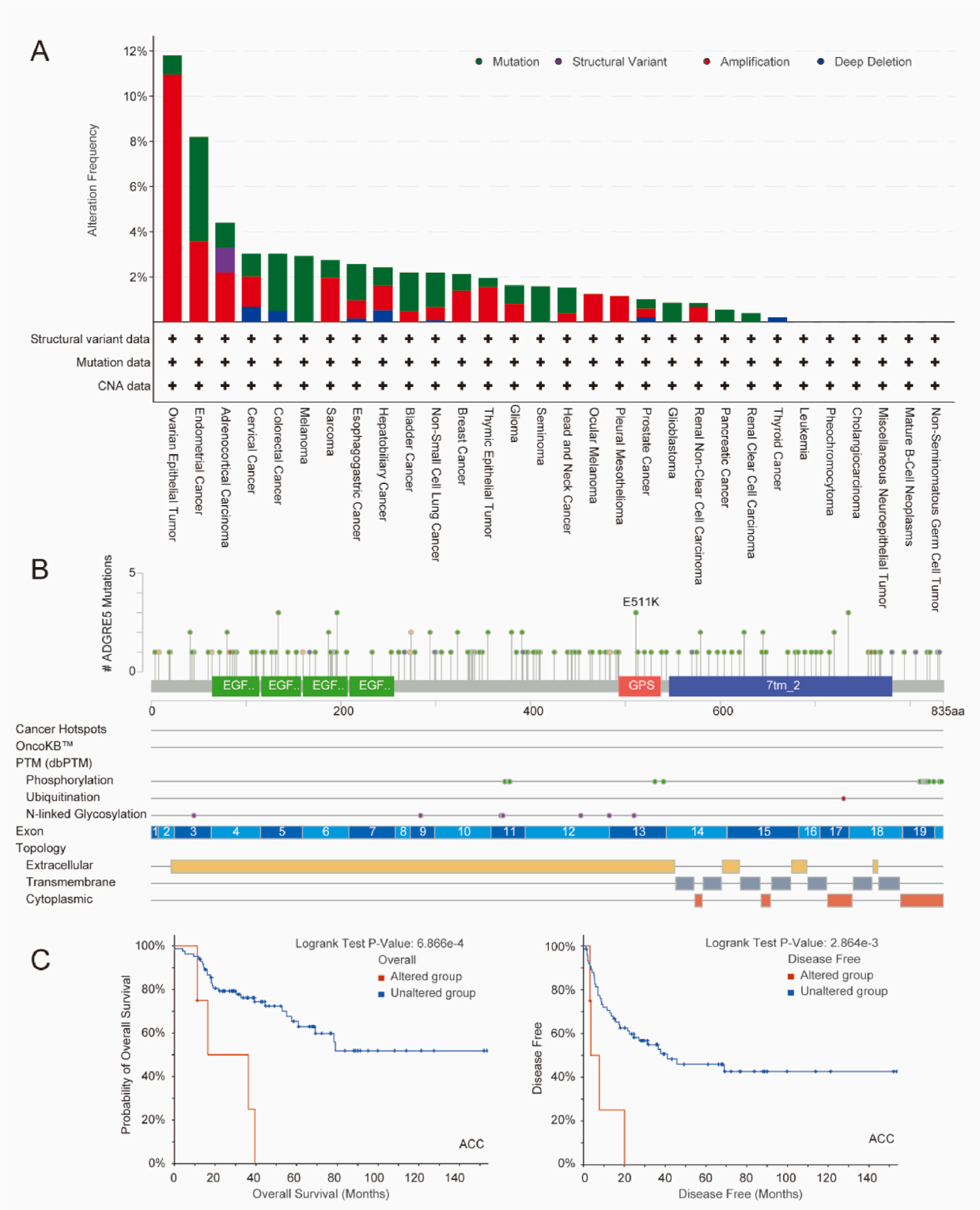


**Fig. 3.** Gene mutation of ADGRE5 exist in various tumors

A. Based on the cBioPortal database, the type and frequency of ADGRE5 gene mutation in pan-cancer is displayed. B. ADGRE5 gene mutation sites in pan-cancer. C. ADGRE5 mutation group was significantly associated with poor prognosis in ACC (Log-rank $P < 0.05$ indicates statistical significance).

## 3. Result

### 3.1. *ADGRE5 was highly expressed in the vast majority of tumors*

Based on the TCGA and GTEx databases, ADGRE5 was found to be abnormally expressed in various tumors. ADGRE5 was highly

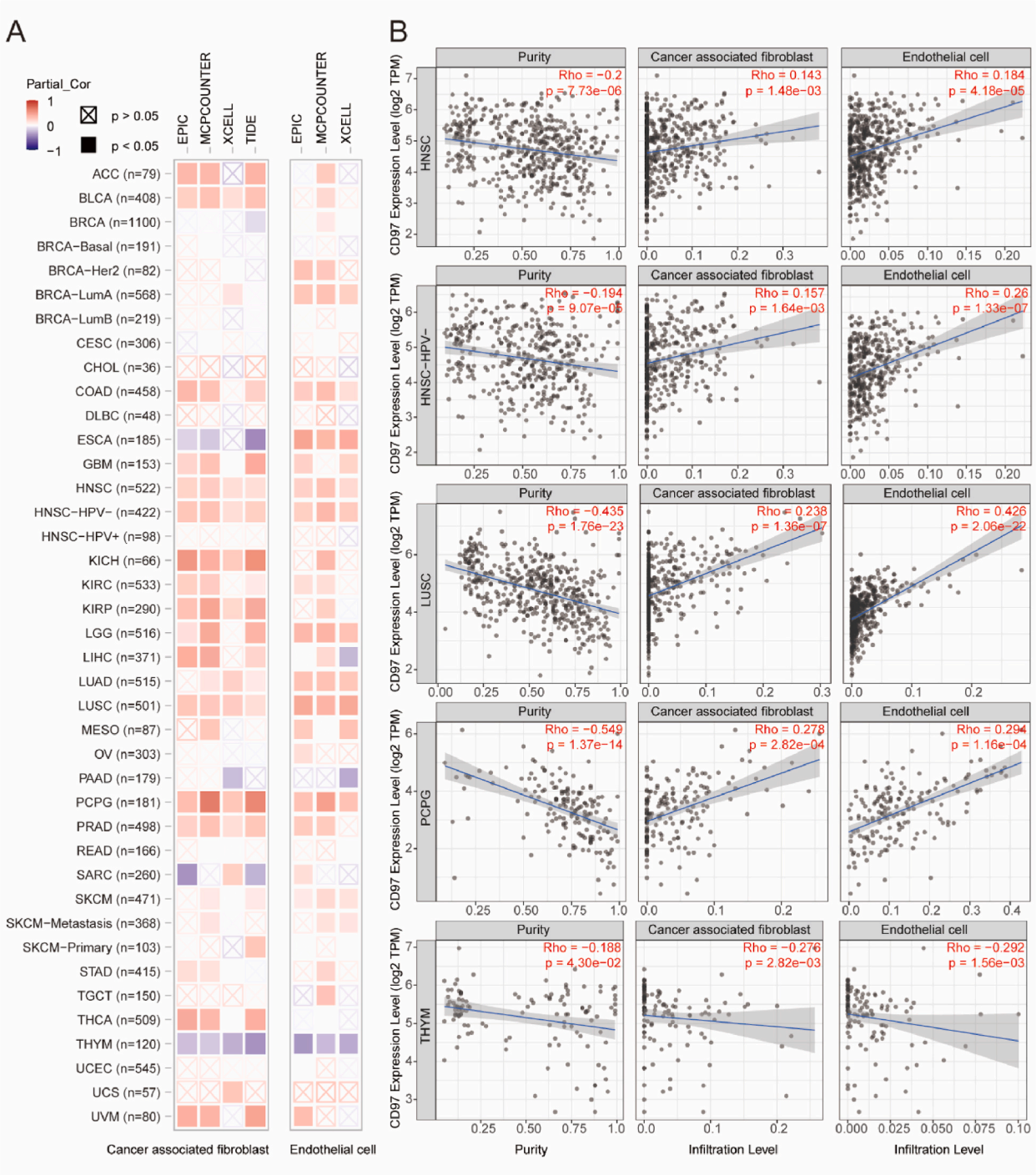


**Fig. 4.** ADGRE5 expression is significantly associated with immune infiltration
A. Correlation of ADGRE5 with CAF and EC in pan-cancer based on TIMER database. B. Correlation of ADGRE5 with CAF and EC in HNSC, HNSC-HPV-, LUSC, PCPG, and THYM based on the TIMER database. ($P < 0.05$ indicates statistical significance).

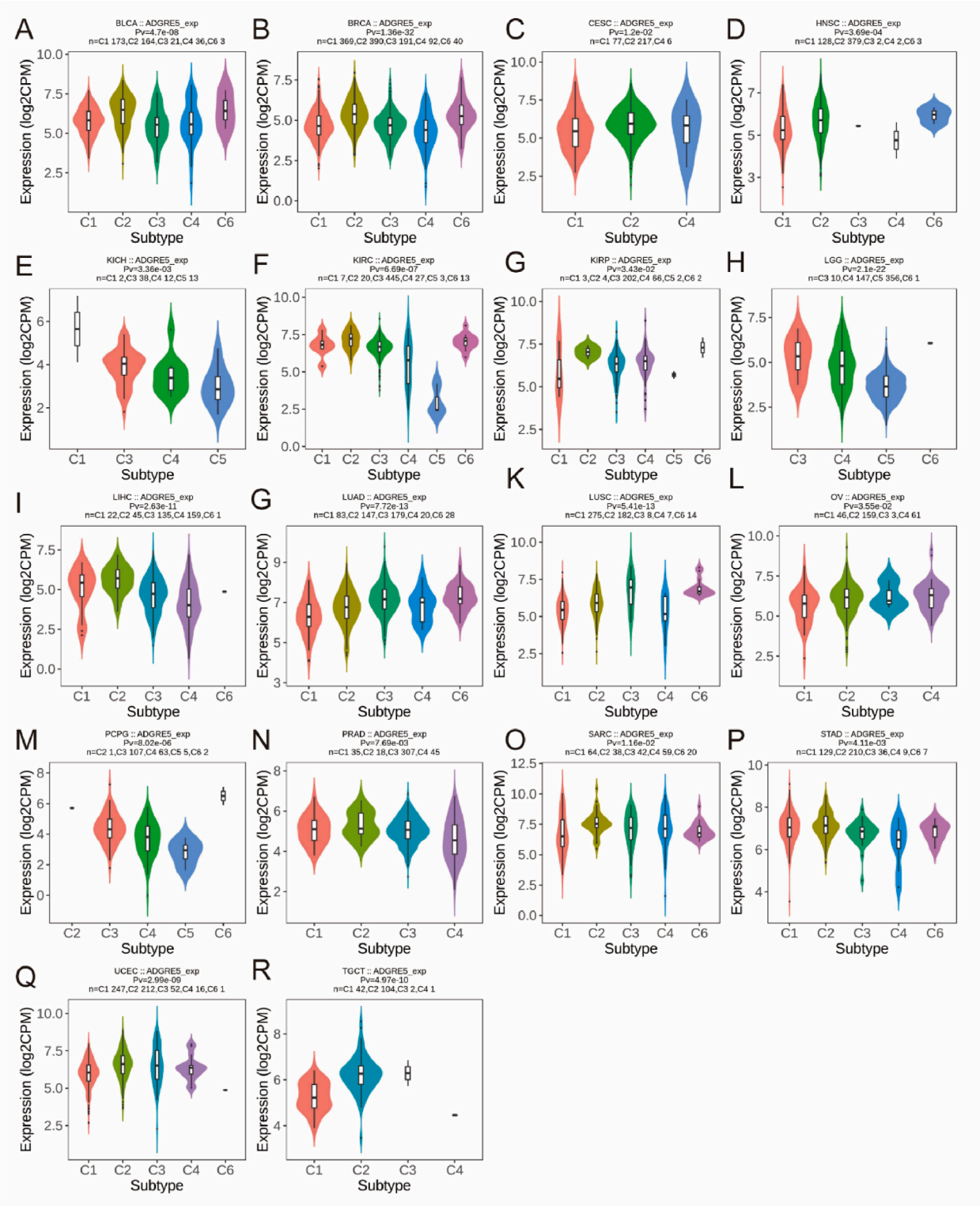


**Fig. 5.** ADGRE5 is differentially expressed in different immune molecular subtypes of tumors (BLCA(A), BRCA(B), CESC(C), HNSC(D), KICH(E), KIRC(F), KIRP(G), LGG(H), LIHC(I), LUAD (G), LUSC(K), OV(L), PCPG(M), PRAD(N), SARC(O), STAD(P), UCEC(Q), TGCT(R)). ($P < 0.05$ indicates statistical significance).

expressed in CHOL, COAD, ESCA, GBM, HNSC, KIRC, KIRP, LAML, LGG, OV, PAAD, READ, SKCM, STAD, TGCT, and THCA, whereas it was weakly expressed in ACC, BLCA, BRCA, CESC, DLBC, KICH, LUAD, LUSC, PCPG, PRAD, THYM, UCEC, and UCS ($P < 0.05$) (Fig. 1A).

Among 18 tumors and their paired adjacent tissues in TCGA, ADGRE5 was highly expressed in CHOL, ESCA, HNSC, KIRC, STAD, and THCA, but lowly expressed in BLCA, BRCA, KICH, LUAD, LUSC, and UCEC ($P < 0.05$) (Fig. 1B).

According to the UALCAN database, analysis of protein expression across 10 tumors revealed that ADGRE5 exhibited pronounced expression in BRCA, COAD, KIRC, lung cancer (LC), PAAD, HNSC, and GBM, while demonstrating comparatively lower expression levels in UCEC and LIHC ($P < 0.05$) (Fig. 1C).

Using the BioGPS database, we compared the expression levels of ADGRE5 in various cancer and normal cell lines and found that it was highly expressed in various tumor cells. We visualized ADGRE5 expression levels in the top 10 cancer and normal cell lines (Fig. 1D and E).

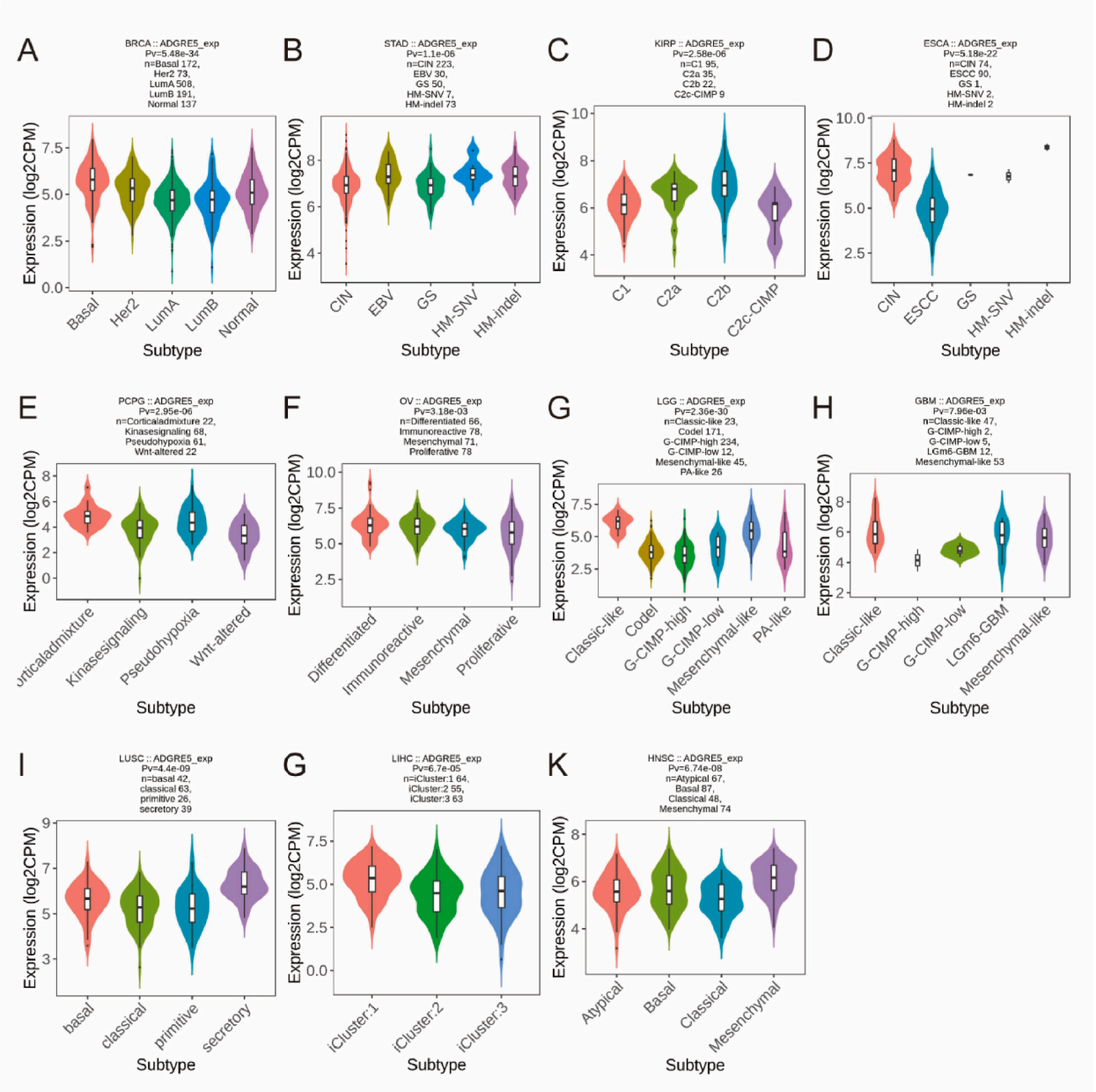


**Fig. 6.** ADGRE5 is differentially expressed in different molecular subtypes of tumors (BRCA (A), STAD (B), KIRP (C), ESCA (D), PCPG (E), OV (F), LGG (G), GBM (H), LUSC (I), LIHC and (G), HNSC (K)) ($P < 0.05$ indicates statistical significance).

### 3.2. ADGRE5 was associated with prognosis in various tumors

Based on GEPIA2, we found that ADGRE5 expression was significantly associated with the BLCA, PAAD, SKCM, and THCA stages (Fig. 2A).

Overall Survival (OS) results showed that increased ADGRE5 expression in ACC, LGG, LUSC, LIHC, and UVM was significantly associated with poor prognosis, whereas low ADGRE5 expression in KIRC, SARC, and SKCM was significantly associated with poor prognosis (Fig. 2B). DFS results showed that high ADGRE5 expression in ACC, LGG, LUSC, and UVM was significantly associated with poor prognosis (Fig. 2B). We visualized results that were significantly correlated with both OS (Fig. 2C) and DFS (Fig. 2D), and a Log-rank P value $< 0.05$ was deemed indicative of a significant difference.

### 3.3. Gene mutation of ADGRE5 existed in various tumors

Using cBioPortal, we explored ADGRE5 mutations in different tumors based on the TCGA database. The top three tumors with the


**Fig. 7.** Correlation between ADGRE5 and tumor markers

A. Radar plot showing the correlation of ADGRE5 with TMB and MSI in pan-cancer. B. Heat map showing the correlation of ADGRE5 with MMR-related molecules in pan-cancer. C. Heat map showing the association of ADGRE5 with immune checkpoint-related molecules in pan-cancer. D. Heat map showing the association of ADGRE5 with pyroptosis-related molecules in pan-cancer. E. Heat map showing the association of ADGRE5 with EMT-related molecules in pan-cancer ($P < 0.05$ indicates statistical significance).

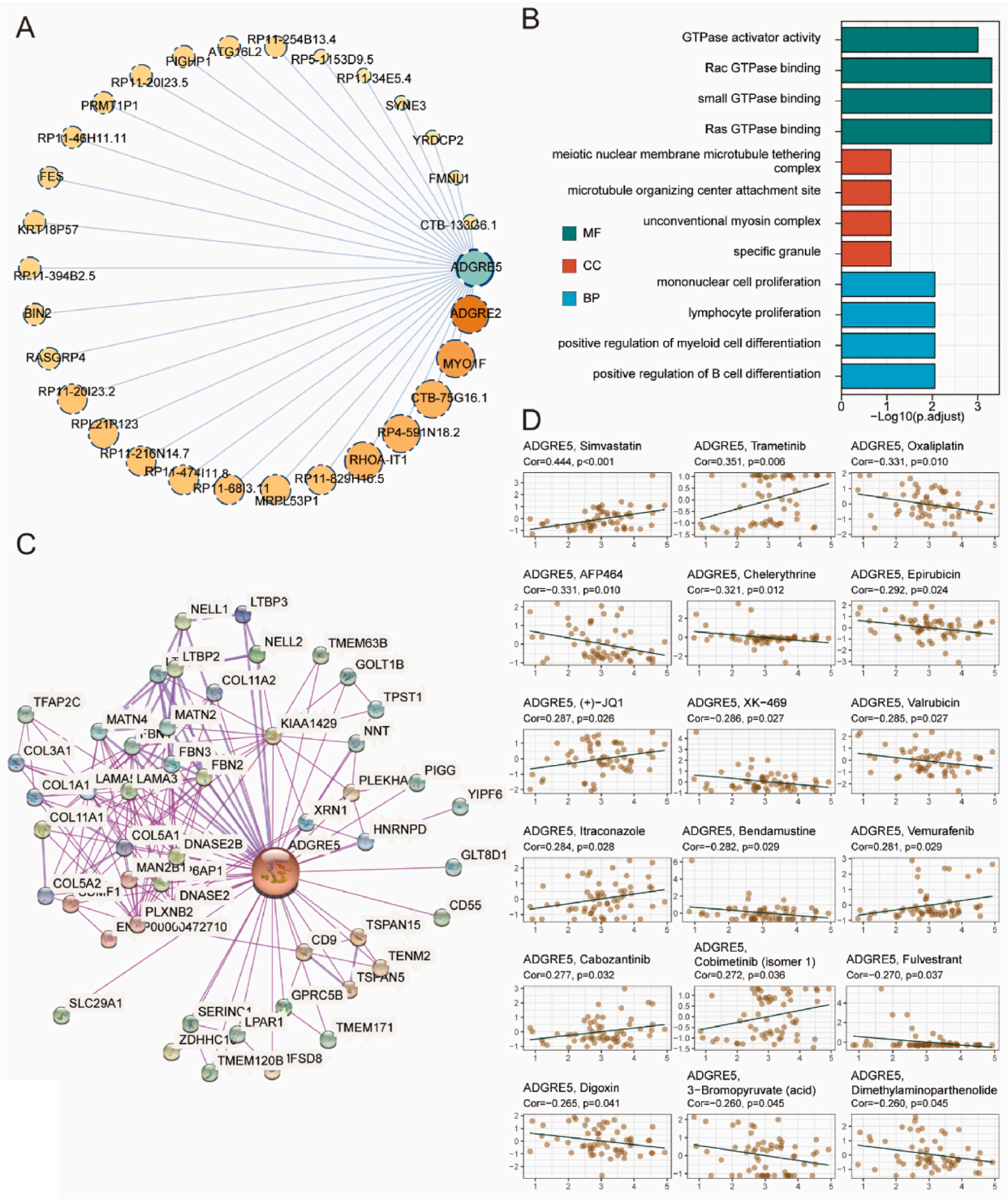


**Fig. 8.** ADGRE5 function and drug sensitivity

A. Thirty molecules significantly associated with ADGRE5 in pan-cancer based on GEPIA2. B. ADGRE5 was highly correlated with molecular GO function. C. ADGRE5-based PPI network construction in pan-cancer. D. ADGRE5 chemotherapeutic drug sensitivity analysis, ranked according to P-value ($P < 0.05$ was considered statistically significant).

highest mutation frequencies were ovarian epithelial tumors (amplification: 64 cases; mutation: 5 cases; Alteration Frequency: 11.0%), UCEC (amplification: 21 cases; mutation: 27 cases; Alteration Frequency: 8.2%), and ACC (amplification: 2 cases; mutation: 1 case; Structural Variant: 1 case; Alteration Frequency: 2.2%) (Fig. 3A). A total of 145 ADGRE5 mutations were detected in the TCGA tumor samples, including 119 missense mutations, 12 truncation mutations, 3 in-frame mutations, 10 splicing mutations, and 1 fusion mutation (Fig. 3B). Simultaneously, we explored the correlation between ADGRE5 mutation and prognosis, and the results indicated a significant association between ADGRE5 mutation and unfavorable prognosis in ACC OS and DFS analysis (Fig. 3C).

### 3.4. ADGRE5 expression showed a significant correlation with immune infiltration

Using the TIMER2 database, we explored the association of aberrantly expressed ADGRE5 with tumor microenvironment stromal cells in pan-cancer using several methods to predict immune infiltration (EPIC, MCPcounter, XCELL, and TIDE). We showed that all four methods were significantly correlated with each other. The results indicated a significant correlation between ADGRE5 and cancer-associated fibroblasts (CAF) as well as endothelial cells (CE) across multiple tumor types. ADGRE5 showed a significant positive correlation with CE in BLCA, HNSC, HNCS-HPV-, KICH, KIRP, LUSC, PCPG, and PRAD but a significant negative correlation with THYM. ADGRE5 was positively correlated with CAF in BRCA-LumA, COAD, ESCA, HNSC, HNSC-HPV-, LGG, LUAD, LUSC, PCPG, and SKCM but was also negatively correlated with THYM (Fig. 4A). ADGRE5 expression was significantly associated with HNSC, HNSC-HPV-, LUSC, PCPG, and THYM (Fig. 4B).

Using the TISIDB database, we explored the differences in ADGRE5 expression in the immune subtypes of different tumors. The immune subtypes were divided into C1 (wound healing), C2 (IFN -gamma dominant), C3 (inflammatory), C4 (lymphocyte depleted), C5 (immunologically quiet), and C6 (TGF-b dominant). The results showed that ADGRE5 expression was significantly different in different immune subtypes of various tumors, such as BLCA, BRCA, CESC, HNSC, KICH, KIRC, KIRP, LGG, LIHC, LUAD, LUSC, OV, PCPG, PRAD, SARC, STAD, UCEC, and TGCT (Fig. 5A-R). Simultaneously, we investigated the differences in ADGRE5 expression in the molecular subtypes of different tumors. There were significant differences in ADGRE5 expression among the different molecular subtypes of BRCA, STAD, KIRP, ESCA, PCPG, OV, LGG, GBM, LUSC, LIHC, and HNSC (Fig. 6A–K).

The Tumor Mutational Burden (TMB) represents the number of mutations in tumor samples. Correlation analysis was used to explore the relationship between ADGRE5 and TMB in different tumors. Statistical significance was set at $P < 0.05$. The results showed a significant positive correlation for STAD, PRAD, KIRC, and ESCA and a significant negative correlation for TGCT, LUAD, LIHC, and CHOL. Microsatellite Instability (MSI) is an important tumor marker related to mutations, and the correlation results showed that ADGRE5 was significantly positively correlated with MSI in UVM, STAD, and ESCA and showed a significant negative correlation with UCS, UCEC, TGCA, HNSC, and PRAD (Fig. 7A). Mismatch repair (MMR) abnormalities promote MSI and tumor development. Through correlation analysis, we investigated the relationship between ADGRE5 and MMR-related molecules, revealing a significant correlation between ADGRE5 and MMR across various tumor types (ACC, KIRC, LIHC, SKCM, THCA, and UCEC) (Fig. 7B). The correlation between ADGRE5 expression and immune checkpoint genes was also investigated. The results indicated that ADGRE5 was significantly associated with immune checkpoints across multiple tumor types (BLCA, BRCA, DLBC, HNSC, KICH, KIRC, KIRP, LGG, LIHC, LUAD, LUSC, PCPG, PRAD, SKCM, STAD, TGCT, THCA, UCEC, and UVM) (Fig. 7C).

To further understand the role of ADGRE5 in different tumors, we simultaneously evaluated the correlation among ADGRE5, pyroptosis, and EMT. The results showed that ADGRE5 expression was significantly correlated with pyroptosis in various tumors (BLCA, HNSC, KICH, KIRC, LGG, LIHC, LUAD, PAAD, PCPG, PRAD, SKCM, STAD, THCA, and UVM). Moreover, ADGRE5 expression was highly correlated with EMT in a variety of tumors (ACC, BLCA, BRCA, HNSC, KICH, KIRC, KIRP, LGG, LIHC, LUAD, LUSC, PAAD, PCPG, PRAD, SKCM, THCA, UCEC, and UVM). (Fig. 7D and E).

### 3.5. Functional enrichment analysis of ADGRE5

GEPIA2 was used to explore the top 100 genes significantly related to ADGRE5 in the pan-cancer analysis. We visualized the top 30 related genes, of which the top three were ADGRE2, MYO1F, and CTB-75G16.1 (Fig. 8A). Additionally, we explored their potential functions. GO functional analysis revealed that it was mainly enriched in GTPase-related molecules, microscopy-related processes, and immune cell regulatory processes (Fig. 8B). KEGG functional analysis indicated that no related pathways were enriched. STRING was used to construct a protein-protein interaction (PPI) network based on ADGRE5 (Fig. 8C).

### 3.6. ADGRE5 and drug sensitivity

To further investigate the ADGRE5-sensitive drugs, we explored the chemotherapeutic drug sensitivity of the predicted gene sets using CellMiner. Chemotherapeutic drugs were screened using clinical trials and FDA (US Food and Drug Administration) approval as thresholds, and the Pearson correlation coefficient between the predicted gene set and chemotherapeutic drugs was calculated. Significantly associated drugs were visualized and ranked according to their p-values (Fig. 8D). Our findings revealed a positive correlation between ADGRE5 and simvastatin, trametinib, itraconazole, vemurafenib, cabozantinib, and cobitinib. Conversely, ADGRE5 demonstrated a negative correlation with oxaliplatin, AFP464, chelerythrine, epirubicin, (+)-JQ1, XK469, valrubicin, Binda mustard, fulvestrant, digoxin, bromopyruvate, and dimethylamino parthenolide. ADGRE5 expression was positively correlated with sensitivity to targeted agents and negatively correlated with sensitivity to conventional chemotherapeutic agents.

### 3.7. ADGRE5 was an independent risk factor for LGG

Pan-cancer analysis showed that ADGRE5 expression was abnormally elevated in LGG and that high ADGRE5 expression was associated with poor prognosis. Therefore, we explored the possibility of using ADGRE5 as a prognostic marker for LGG. There were

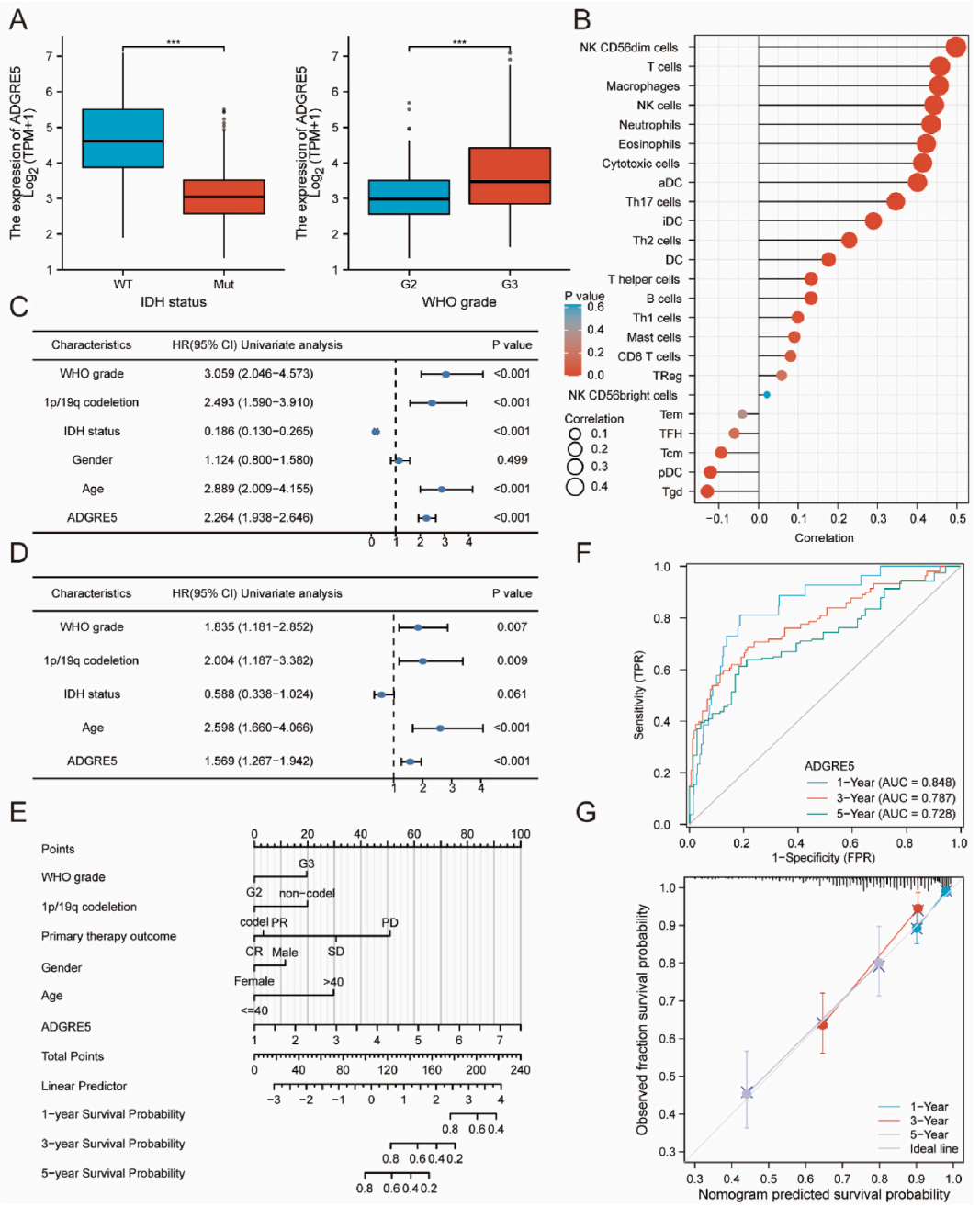


**Fig. 9.** ADGRE5 is an independent risk factor for LGG

A. Box plots illustrated the variations in ADGRE5 expression across different IDH stages and WHO grades.B. Bubble plot depicted the correlation between ADGRE5 and 23 immune cell types. C. Univariate Cox regression analysis revealed that ADGRE5 posed as a risk factor for LGG. D. Multivariate Cox regression analysis demonstrated that ADGRE5 acted as an autonomous risk factor for LGG. E. The nomogram indicated that ADGRE5 had the highest contribution among ADGRE5 and other clinical features (age, sex, 1p/19q codeletion, IDH stage, and WHO grade). F. The time ROC curve suggested that ADGRE5 transcript level expression was a prognostic indicator, and 1 year, 3 years, and 5 years had good diagnostic value (area under the curve AUC>0.7). G. 1-year, 3-year, and 5-year calibration curve results suggest that ADGRE5 transcript level expression is a prognostic indicator with high accuracy ($P < 0.05$ indicates statistical significance, $P < 0.05$*, $P < 0.01$**, $P < 0.001$***, $P < 0.0001$****).

significant differences in the expression levels of ADGRE5 in the LGG among the different groups of clinical characteristics (IDH status, WHO grade) (Fig. 9A). ADGRE5 expression was significantly increased in IDH wild-type (WT) and G3 stages, and WT and G3 were associated with a poor prognosis. Based on ssGSEA analysis, we explored the correlation of ADGRE5 with 28 immune cells in LGG and found that ADGRE5 was significantly positively correlated with aDC, B cells, cytotoxic cells, eosinophils, iDC, macrophages,

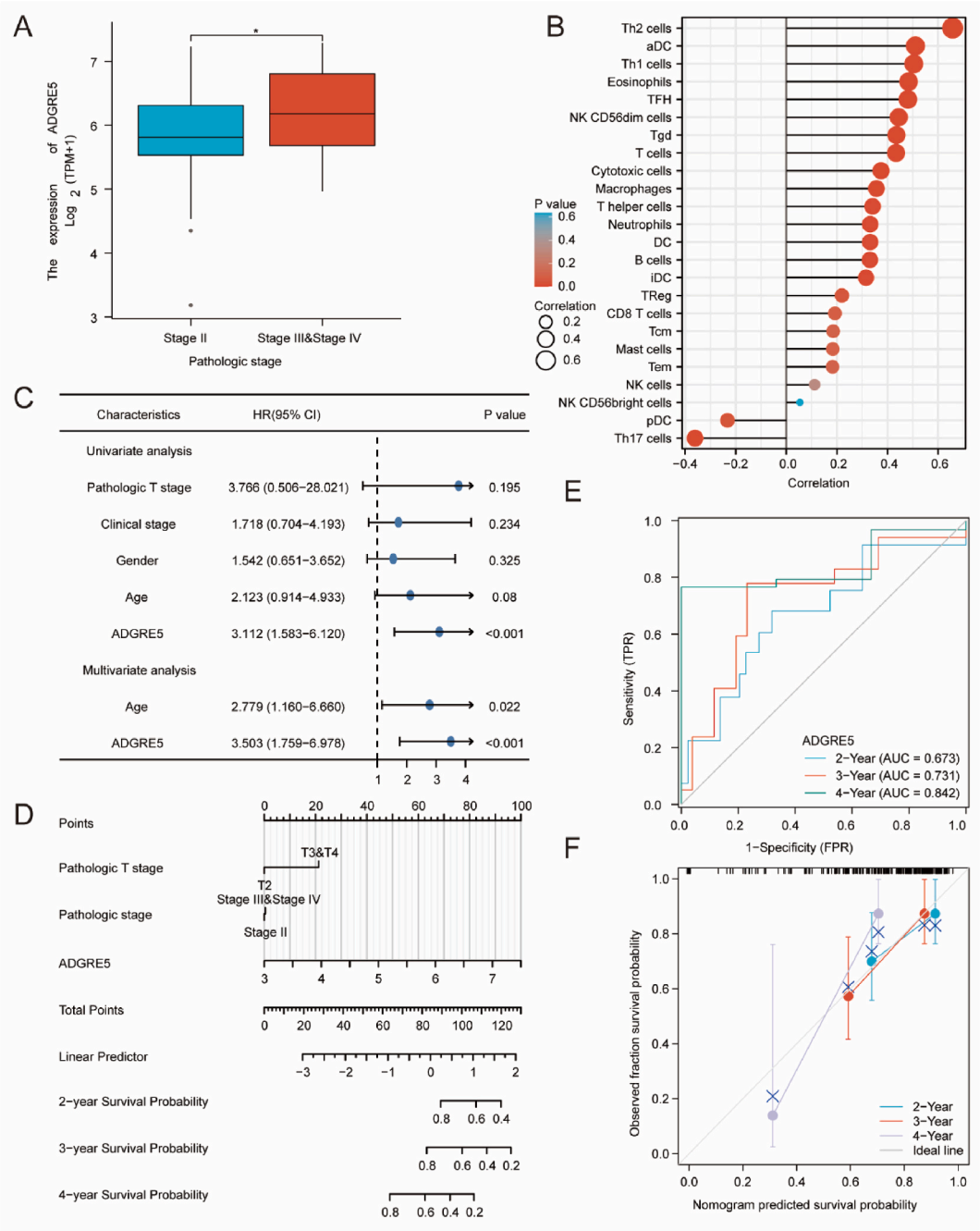


**Fig. 10.** ADGRE5 is an independent risk factor for UVM

A. Box plots illustrated the variations in ADGRE5 expression across different stages. B. Bubble plot depicted the correlation between ADGRE5 and 23 immune cell types. C. Both univariate and multivariate COX regression analyses indicated that ADGRE5 served as a independent risk factor for UVM. D. The nomogram showed that ADGRE5 had the highest contribution value among ADGRE5 and other clinical features (age, sex, clinical stage, and pathologic T stage). E. The time RCO curve suggests that ADGRE5 transcript level expression is a prognostic indicator, and it has a good diagnostic value at 2, 3, and 4 years (AUC 0.67–0.842). F. 2-year, 3-year, and 4-year calibration curve results suggest that ADGRE5 transcript level expression is a prognostic indicator with high accuracy ($P < 0.05$ indicates statistical significance, $P < 0.05$*, $P < 0.01$**, $P < 0.001$***, $P < 0.0001$****).

neutrophils, NK CD56dim cells, NK cells, T cells, Th17 cells, Th2 cells, DC, T helper cells, Th1 cells, and mast cells, but significantly negatively correlated with pDC and Tcm (Fig. 9B). To explore the role of ADGRE5 in the prognosis of LGG, we performed univariate and multivariate Cox analyses by combining the clinical characteristics (WHO grade, 1p/19q primary therapy outcome, sex, and age). The results showed that ADGRE5 was an independent risk factor (multivariate COX regression HR = 1.569, 95% CI [1.267–1.942]; $P < 0.001$) (Fig. 9C and D). Using nomograms, we found that ADGRE5 expression contributed the most to the prediction of patient survival risk compared with age, sex, WHO grade, and 1p/19q primary therapy outcomes (Fig. 9E). The time-dependent ROC curve results showed that the 1-year AUC value was 84.8%, the 3-year AUC value was 78.7%, and the 5-year AUC value was 72.8%, proving that ADGRE5 was used as a prognostic indicator with good prediction accuracy (Fig. 9F). Next, we used a calibration curve to test the prediction accuracy of the model. The findings indicated that the predictive accuracy for the 1-year, 3-year, and 5-year survival rates was notably high (Fig. 9G).

### *3.8. ADGRE5 was an independent risk factor for UVM*

The results of pan-cancer analysis showed that ADGRE5 was significantly associated with poor prognosis in UVM. Therefore, we investigated the possibility of using ADGRE5 as a prognostic marker for UVM. The expression levels of ADGRE5 in the UVM differed significantly at different stages (Fig. 10A). The later the stage, the higher was the expression of ADGRE5. Based on ssGSEA, we explored the correlation of ADGRE5 with 28 immune cells in the UVM and found that ADGRE5 was significantly positively correlated with aDC, eosinophils, NK CD56dim cells, T cells, T helper cells, TFH, Th1 cells, Th2 cells, cytotoxic cells, Tgd, B cells, DCs, macrophages, neutrophils, and iDCs and significantly negatively correlated with Th17 cells and pDCs (Fig. 10B). To explore the role of ADGRE5 in the prognosis of LGG, we performed univariate and multivariate Cox analyses by combining clinical characteristics (WHO grade, 1p/19q primary therapy outcome, sex, and age), and the results indicated that ADGRE5 served as an independent risk factor (multivariate COX regression HR = 3.503, 95% CI [1.759–6.978]; $P < 0.001$) (Fig. 10C). According to the distribution of the sample survival data, we used 2-year, 3-year, and 4-year survival nodes for follow-up survival analysis. Using a nomogram, we found that compared to the pathologic T stage and pathologic stage, ADGRE5 transcript expression contributed the most to the survival risk of patients (Fig. 10D). The time-dependent ROC curve results showed that the 2-year AUC value was 67.3%, 3-year AUC value was 73.1%, and 4-year AUC value was 84.2%, proving that the prediction accuracy was good (Fig. 10E). Next, we used a calibration curve to test the prediction accuracy of the model. The results demonstrated that the predictive accuracy for the 2-year, 3-year, and 4-year survival rates was considerable (Fig. 10F).

## 4. Discussion

Numerous studies have shown that ADGRE5 has an aggressive phenotype that correlates with tumor grade, lymph node invasion, metastatic spread, and overall prognosis in various cancers, including thyroid cancers [4], gastric cancers [6], and prostate cancers [27], as well as glioma cells [28]. To our knowledge, there is currently no literature on the potential prognostic impact and biological function of ADGRE5 in a pan-cancer analysis. In this study, we investigated the expression and prognostic significance of ADGRE5 in human tumors. The association of ADGRE5 with tumor immune infiltration, immune subtype, TMB, MSI, and MMR was analyzed in multiple cancers to explore its immunogenicity. In our investigation, we observed elevated expression of ADGRE5 mRNA in CHOL, COAD, ESCA, GBM, HNSC, KIRC, KIRP, LAML, LGG, OV, PAAD, READ, SKCM, STAD, TGCT, and THCA. Correspondingly, at the protein level, ADGRE5 exhibited high expression in BRCA, COAD, KIRC, LC, PAAD, HNSC, and GBM. OS results showed that high ADGRE5 expression in ACC, LGG, LUSC, LIHC, and UVM was significantly associated with poor prognosis. We further independently analyzed LGG and UVM with high ADGRE5 mRNA and protein expression levels and poor prognosis. These results confirm the possibility of using ADGRE5 as a prognostic marker for LGG and UVM.

Accumulating evidence has recently demonstrated that, as important components of the tumor microenvironment (TME), CAF and EC can influence tumor initiation, progression, immune escape, and metastasis and serve as important determinants of immunotherapy response and clinical outcome [29–32]. Our findings are the first to identify an association between ADGRE5 expression and CAF and EC infiltration in different tumors. ADGRE5 expression displayed a significant correlation with both cell types across HNSC, HNSC-HPV, LUSC, PCPG, and THYM. In addition, ADGRE5 showed obvious differences between different immune subtypes in a variety of tumors.

MSI is linked to an elevated risk of cancers characterized by specific clinicopathological features, such as heightened TMB and infiltration of tumor-infiltrating lymphocytes. TMB stands as a promising biomarker for predicting the response to immune checkpoint blockade therapy [33]. Furthermore, Thomas et al. revealed that TMB can predict immune-related survival outcomes in patients with breast cancer [34]. These results suggest that higher somatic TMB and MSI are associated with increased immunotherapy efficiency and improved overall survival in most cancer histologies. To determine the potential of ADGRE5 in clinical immunotherapy, we analyzed the correlation between ADGRE5 expression and TMB, MSI, MMR, and immune checkpoints.

Our results showed that ADGRE5 was positively correlated with TMB in STAD, PRAD, KIRC, and ESCA and negatively correlated with TGCT, LUAD, LIHC, and CHOL. Combined with the above prognostic results, it is suggested that low ADGRE5 expression in KIRC was associated with poor prognosis, whereas high ADGRE5 expression in LIHC was associated with poor prognosis, which is consistent with the low TMB in both cancers. In addition, ADGRE5 and MSI was positively correlated with UVM, STAD, and ESCA, whereas they were negatively correlated with UCS, UCEC, TGCA, HNSC, and PRAD. A previous prognostic analysis suggested that the high expression of ADGRE5 in UVM was associated with a poor prognosis, combined with the high expression of MSI, suggesting that immunotherapy has good prospects in UVM and needs to be further developed. In ACC, KIRC, LIHC, SKCM, THCA, and UCEC, ADGRE5

was positively correlated with MMR, while in ESCA, LUAD, LUSC, and THYM ADGRE5 was negatively correlated with MMR. A previous prognostic analysis has suggested that high expression of ADGRE5 in LUSC is associated with poor prognosis, whereas ADGRE5 is negatively correlated with MMR in LUSC. Therefore, we believe that immunotherapy has good prospects for the treatment of LUSC, which is consistent with previous research results [35]. In addition, there was a significant correlation between ADGRE5 and Immune checkpoint genes in various tumors (BLCA, BRCA, DLBC, HNSC, KICH, KIRC, KIRP, LGG, LIHC, LUAD, LUSC, PCPG, PRAD, SKCM, STAD, TGCT, THCA, UCEC, and UVM). These findings suggest a close association between ADGRE5 and tumor immunotherapy.

Immune infiltration in the TME plays a key role in tumor development and affects the clinical outcomes of patients with cancer [36]. Therefore, we further comprehensively analyzed the tumor-infiltrating immune cells of LGG and UVM. The results showed that in the LGG, ADGRE5 was significantly positively correlated with NK CD56dim cells, T cells, macrophages, neutrophils, eosinophils, cytotoxic cells, aDC, Th17 cells, iDC, Th2 cells, DC, T helper cells, B cells, Th1 cells, and mast cells and negatively correlated with pDC and Tcm. This is consistent with the results of Safaee et al., who found that the expression of non-polarized M0 macrophages, immunosuppressive Treg cells, and resting NK cells increased in glioma cells with high ADGRE5 expression [37]. Under UVM, ADGRE5 was significantly positively correlated with Th2 cells, aDC, Th1 cells, eosinophils, TFH, NK CD56dim cells, Tgd, T cells, cytotoxic cells, macrophages, T helper cells, neutrophils, DC, B cells, and iDC and negatively correlated with Th17 cells and pDC. Therefore, these results reveal that the expression of ADGRE5 is closely related to the degree of immune invasion in cancer.

According to enrichment analysis, the top two genes related to ADGRE5s were ADGRE2 and MYO1F, both of which are related to inflammation and immunity. ADGRE2 can promote chemotaxis, degranulation, and adhesion of granulocytes and promote the release of inflammatory cytokines in macrophages, including IL-8 and TNF [38]. MYO1F plays an important role in host self-defense, mainly in innate immunity involving cell migration and phagocytosis. We explored the potential functions of ADGRE5, and GO functional analysis revealed that it was mainly enriched in GTPase-related molecules, microscope-related processes, and immune cell regulation. Therefore, the significance of ADGRE5 in tumor immunity is worth noting. Furthermore, we conducted a correlation analysis of tumor characteristics, revealing a significant association between ADGRE5 expression and pyroptosis as well as EMT across multiple tumor types.

We investigated and synthesized data from various databases to provide a comprehensive genomic structure describing the impact of ADGRE5 gene on the immune microenvironment of approximately 30 solid tumors. Our results suggest that ADGRE5 expression modulates the immune microenvironment and long-term clinical outcomes of different malignancies. In general, our pan-cancer investigations illustrate the potential of ADGRE5 in predicting the survival status of certain cancers.

However, this study had some limitations. First, while bioinformatics analysis has offered valuable insights into the role of ADGRE5 in malignancies, additional in vitro or in vivo experiments are necessary to validate our findings and enhance therapeutic efficacy. Furthermore, although ADGRE5 expression is associated with immune and clinical survival in human malignancies, it remains unclear whether ADGRE5 affects clinical survival through modulation of the immune pathway.

## 5. Conclusion

Our findings underscore the pivotal role of ADGRE5 in tumorigenesis and metastasis and elucidate its impact on tumor immunology, pyroptosis, and EMT in malignant tumors. Notably, ADGRE5 has surfaced as a promising standalone prognostic marker for a wide array of cancer patients, particularly individuals diagnosed with LGG and UVM, thus contributing to the refinement of cancer treatment precision. Prospective investigations into ADGRE5 expression and its interaction with the tumor immune microenvironment hold promise for delivering conclusive insights and advancing the development of immune-based cancer therapies.

## Consent for publication

Not Applicable.

## Data availability statement

The data associated with our study have not been deposited in a publicly available repository; however, they will be made available upon request.

## CRediT authorship contribution statement

**Xiangjian Zhang:** Writing – review & editing, Supervision, Project administration, Conceptualization. **Xinxin Zhang:** Writing – original draft, Investigation, Formal analysis, Data curation. **Qiuhui Yang:** Writing – original draft, Software, Resources, Methodology, Formal analysis. **Ruokuo Han:** Validation, Supervision, Software. **Walaa Fadhul:** Writing – original draft, Visualization, Investigation. **Alisha Sachdeva:** Writing – original draft, Resources, Investigation. **Xianbo Zhang:** Writing – review & editing, Supervision, Project administration, Methodology, Funding acquisition, 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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