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Research

Elevated BEAN1 expression correlates with poor prognosis, immune evasion, and chemotherapy resistance in rectal adenocarcinoma

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Abstract

Background The BEAN1 gene, primarily studied in neurodegenerative diseases, has been scarcely studied in the context of cancers. Our research examines BEAN1 expression specifically in rectal adenocarcinoma (READ) and its association with prognosis, immune evasion, and chemotherapy resistance.

Methods Data from TCGA and GEO were analyzed to assess BEAN1 levels across various cancer types, with particular emphasis on READ. Functional enrichment, immune infiltration, and treatment response analyses were conducted, followed by validation using patient tissue samples.

Results READ tissues exhibited a marked increase in BEAN1 expression compared to normal tissues. Elevated BEAN1 levels were associated with reduced overall survival and increased immune suppression, characterized by elevated M2 macrophage infiltration and reduced CD8+ T cell presence. BEAN1 expression was also linked to higher immune checkpoint genes expression and resistance to immune checkpoint inhibitors and 5-fluorouracil.

Conclusion This research offers initial evidence that BEAN1 is linked to unfavorable prognosis, immune escape, and resistance to chemotherapy in READ. BEAN1 appears to be a promising new biomarker and potential therapeutic target, warranting further investigation into its potential clinical applications in improving treatment outcomes for READ patients.

Keywords BEAN1 · Rectal adenocarcinoma · Immune infiltration · M2 macrophage · Drug resistance

1 Introduction

Rectal cancer is a prevalent form of colorectal cancer (CRC), representing a significant burden on global health due to its high incidence and mortality rates. READ arises from the epithelial lining of the rectal mucosa and constitutes more than 90% of rectal cancer cases [1]. In the United States, the incidence of rectal cancer is estimated to be 46,050 cases in 2023 [2], representing 3.2% of total cancer deaths [3]. Additionally, the incidence of rectal cancer is increasing, particularly in Western countries,

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due to contributors including alcohol intake, meat consumption, smoking, lack of physical activity, and obesity. Researches have also indicated that non-modifiable risk factors for READ encompass male sex, advanced age, genetic susceptibility, inflammatory bowel disease, and exposure to radiation. The management of READ remains challenging due to its complex biology and variable response to treatment. Despite advances in surgical techniques, chemotherapy, and radiotherapy, the outlook for READ patients is still bleak, with a 5-year overall survival rate of just 67% [4]. Discovering new biomarkers and therapeutic targets is crucial for improving the outcomes of READ patients.

The tumor microenvironment (TME) is crucial in the initiation, progression, and spread of READ. The components of the TME include all non-malignant stromal cells within the tumor, such as fibroblasts, platelets, immune cells, and endothelial cells [5]. The TME is a unique environment that evolves with tumor progression. Current research suggests that overlooking the intricate alterations in the TME during tumor progression is a major factor contributing to the failure of current targeted therapies against tumor cells [6]. Hence, innovative therapeutic approaches focusing on the TME hold promise for benefiting patients with READ in the context of tailored medical treatments. Tumor-associated macrophages (TAMs) constitute a significant portion of the immune cells within the TME. They release cytokines and chemokines, working in concert with inflammatory processes, playing crucial roles in promoting the initiation, invasion, spread, metastasis, immune suppression, angiogenesis, and drug resistance of READ [7]. Various TAM subtypes have unique roles and can undergo dynamic changes in reaction to diverse cues from cancer cells or the TME. A lot of research has demonstrated in READ, TAMs are linked to an unfavorable prognosis [8].

The development of the TME is also driven by alterations in the extracellular matrix (ECM), forming the vast majority of the bulk of the tumor stroma. The ECM is composed of various molecules such as collagens, elastins, laminins, fibronectins, and matrix metalloproteinases that are capable of breaking down these protein fibers and is pivotal in tissue remodeling [9]. Alterations in the ECM and the development of the TME eventually result in the progression, invasion, and spread of READ. Collagen can be altered by cancer cells, and with tumor progression, abnormal remodeling of collagen results in an overabundance, changes in ratio and arrangement, thereby regulating tumor proliferation, infiltration, and eventually the spread of metastatic disease [10]. Collagen can also inhibit the anti-tumor immune response by reducing the production of chemokines by immune cells [11]. Patients with CRC exhibiting upregulated combined expression of collagen and collagenase genes have shorter overall survival (OS) [12].

Brain Expressed Associated With NEDD4 1 (BEAN1) is one of the multiple proteins interacting with the ubiquitin protein ligase family member NEDD4, possessing a common PY motif that can bind to the WW domain of NEDD4 [13]. BEAN1 gene mutations are closely associated with spinocerebellar ataxia type 31. Earlier research has primarily concentrated on the function of BEAN1 in neurodegenerative diseases [14, 15]. However, beyond these studies, there has been no significant research exploring BEAN1's role in other diseases, particularly in cancers. This represents a critical gap in the current literature, as understanding BEAN1's function in cancer could reveal new avenues for diagnosis and treatment.

In this research, we initially undertook a thorough and systematic investigation of BEAN1 expression and its prognostic value in pan-cancer, with a particular focus on READ, which is the inaugural study of BEAN1 in tumors. Subsequently, by integrating data from The Cancer Genome Atlas (TCGA) and Gene Expression Omnibus (GEO) databases, and validating our findings in a cohort of READ patients, we found that BEAN1 is significantly upregulated in multiple tumors, including READ, and is associated with poor prognosis. BEAN1 is positively correlated with pathways related to the extracellular matrix and the infiltration of M2 macrophages. Additionally, READ patients with high BEAN1 expression exhibit resistance to immune checkpoint inhibitors (ICIs) and 5-fluorouracil (5-FU). Our research provides new insights into BEAN1 as a biomarker and therapeutic target for READ and other tumors.

2 Materials and methods

2.1 Patient samples

50 READ patients from the XJCH-READ cohort recruited at the Affiliated Cancer Hospital of Xinjiang Medical University between 2010 and 2015, were included in this study, which was authorized by the Ethics Committee of the Affiliated Cancer Hospital of Xinjiang Medical University in accordance with the Declaration of Helsinki (Approval Number: G-2021005). And informed consent was obtained from all subjects and/or their legal guardian(s). The selection of patients was based on the following inclusion and exclusion criteria. Inclusion Criteria: Patients diagnosed with READ confirmed by pathological examination; Adult patients aged between 18 and 75 years; Availability of sufficient tumor tissue and adjacent normal tissue for protein extraction and analysis; Detailed clinical and prognostic information

available for research purposes. Exclusion Criteria: Patients with a history of other malignancies; Patients who received treatment for other types of cancer prior to or during the study period; Patients with severe comorbidities (e.g., significant cardiovascular disease or uncontrolled diabetes) that could potentially affect the study results; Patients who did not provide informed consent. READ tissues and adjacent normal tissues were collected from these patients for protein extraction. Clinical and prognostic information for these patients was also collected to assess the association between BEAN1 protein expression and patient outcomes.

2.2 Protein extraction and western blotting

Proteins from READ or adjacent normal tissues were isolated with a lysis buffer and measured using the BCA protein assay. Western blotting was conducted following previously described methods [16]. In short, each lane was loaded with 30 μg of protein per sample and then separated on a 10% SDS-PAGE. Following electrophoresis, transfer, and blocking, the PVDF membranes were incubated overnight at 4 °C with the appropriate primary antibodies, including anti-BEAN1 (#ABIN657309, Antibodies Online, Pottstown, USA) and anti-GAPDH (#2118, Cell Signaling Technology, Danvers, USA). Secondary antibodies, anti-Rabbit and anti-Mouse, which were purchased from Beyotime Biotechnology (Shanghai, China), were then applied at room temperature for 1 h. The immunoblots were visualized using ECL.

2.3 Data processing

Transcriptomic and clinical data for Adrenocortical carcinoma (ACC), Bladder urothelial carcinoma (BLCA), Breast invasive carcinoma (BRCA), Cervical squamous cell carcinoma and endocervical adenocarcinoma (CESC), Cholangio carcinoma (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 Lower Grade Glioma (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), READ, Sarcoma (SARC), Skin Cutaneous Melanoma (SKCM), Stomach adenocarcinoma (STAD), Testicular Germ Cell Tumors (TGCT), Thyroid carcinoma (THCA), Thymoma (THYM), Uterine Corpus Endometrial Carcinoma (UCEC), Uterine Carcinosarcoma (UCS), Uveal Melanoma (UVM) and adjacent normal tissues were obtained from TCGA database with RNA-seq data normalized to TPM format [17]. mRNA expression data from the GSE20842, GSE123390, GSE15781, GSE35452, GSE79330, GSE133057, GSE56699, and GSE87211 datasets of GEO database were downloaded from the NCBI database for external validation of DPF2 expression differences and survival analysis [18]. Single-cell RNA sequencing data from the GSE146771 dataset were used to validate BEAN1 expression in immune cells within the tumor microenvironment. BEAN1 expression levels were compared among different cell types, including tumor cells, stromal cells, and various immune cells.

2.4 Immunohistochemistry (IHC)

IHC data of BEAN1 in READ and COAD specimens were obtained from the Human Protein Atlas (HPA) database, and IHC staining intensity was compared between READ and COAD tissues.

2.5 Differentially expressed gene analysis (DEG)

Based on the median BEAN1 expression levels in the TCGA-READ cohort, samples were stratified into high and low BEAN1 expression groups. DEG analysis was performed using the 'DESeq2' package in R [19], with thresholds set at an adjusted p value < 0.05 and $|\log2FoldChange| > 1$. The resulting DEGs were subsequently visualized using volcano plots.

2.6 Functional enrichment analysis

Gene Ontology (GO), Kyoto Encyclopedia of Genes and Genomes (KEGG), and Gene Set Enrichment Analysis (GSEA) were performed on the differentially expressed genes (DEGs) using the 'clusterProfiler' package. Single-sample GSEA (ssGSEA) was conducted using the 'GSVA' package with the parameter method set to 'ssgsea'. For GSEA analysis, the gene sets c2.cp.all.v2022.1.Hs.symbols.gmt and c5.all.v2022.1.Hs.symbols.gmt were utilized [20]. Significance was determined by an adjusted p value < 0.05 and a False Discovery Rate (FDR) < 0.25. The results were visualized using the ggplot2 package.

2.7 Immune infiltration analysis

Immune infiltration analysis was conducted using the "immuneeconv" R package, integrating several advanced algorithms including quanTIseq, CIBERSORT, MCP-counter, EPIC, and ssGSEA. Immune cell fractions in tumor samples were estimated using these algorithms, with cross-validation across different methods to ensure consistency. The relationships between BEAN1 expression levels and specific immune cell types, such as CD8+ T cells and M2 macrophages, were assessed using Spearman's rank correlation coefficients. The normality assumption was checked for immune cell fraction data, and non-parametric methods were applied when necessary. Immune infiltration scores were normalized using log2(TPM + 1) transformation, and p values were adjusted for multiple comparisons using the Benjamini–Hochberg procedure to control the FDR. Cross-validation of the immune infiltration results was performed across multiple algorithms, and external validation was conducted with independent datasets to confirm the robustness of the analysis.

2.8 Survival analysis

Kaplan–Meier survival analysis was performed to evaluate the prognostic significance of BEAN1 expression in READ. OS and recurrence-free survival (RFS) were analyzed using the "survival" and "survminer" packages in R. Univariate Cox regression analysis was conducted to determine the hazard ratios (HRs) for high BEAN1 expression in relation to OS, disease-specific survival (DSS), and disease-free interval (DFI). Specifically, Kaplan–Meier survival curves were generated to assess survival among different BEAN1 expression groups, with the log-rank test used to compare survival distributions between high and low BEAN1 expression groups. The proportional hazards assumption was tested using Schoenfeld residuals, and stratified analyses were conducted when this assumption was violated. HRs and 95% confidence intervals (CIs) were calculated using univariate and multivariate Cox proportional hazards models, adjusted for relevant covariates such as age, gender, tumor stage, and treatment regimen. To ensure the robustness of the findings, internal validation was conducted using bootstrap resampling (1000 iterations), and external validation was performed with independent datasets from the GEO database and XJCH-READ cohort.

2.9 Treatment response analysis

The ESTIMATE algorithm was used to compare immune, stromal, and ESTIMATE scores between high and low BEAN1 expression groups in READ. The expression of ICGs (CD274, HAVCR2, PDCD1LG2, SIGLEC15) was compared between the two groups. The TIDE algorithm was used to predict the response of BEAN1 to ICIs in READ. Stemness and drug sensitivity scores were assessed to evaluate the response of BEAN1 to chemotherapy, including the calculation of mRNAsi scores and 5-FU half-maximal inhibitory concentration (IC50) scores. The mRNAsi was calculated using the one-class logistic regression (OCLR) algorithm. Based on the mRNA expression signature, the gene expression profile included 11,774 genes. The same Spearman correlation was applied to the RNA expression data. The minimum value was subtracted, and the result was divided by the maximum value to map the dryness index to the range [0,1]. The chemotherapeutic response for each sample was predicted using the largest publicly available pharmacogenomics database, the Genomics of Drug Sensitivity in Cancer (GDSC). The prediction process was implemented with the R package "pRRophetic." IC50 for each sample was estimated using ridge regression, with all parameters set to their default values. Batch effects were corrected using the "combat" method, and tissue types were taken into account. Duplicate gene expression values were summarized as mean values.

2.9.1 Statistical analysis

All statistical analyses were conducted using R software (version 4.0.2). Continuous variables were compared between groups using the Student's t-test or Wilcoxon rank-sum test, as appropriate. Categorical variables were compared using the chi-squared test or Fisher's exact test. p values < 0.05 were considered statistically significant. ns: $p \geq 0.05$; *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

3 Results

3.1 Expression and prognostic significance of BEAN1 in *pan-cancer*

We initially examined the expression of BEAN1 across 33 types of cancer using data from TCGA. As illustrated in Figs. 1A and 1B, BEAN1 expression is significantly dysregulated in cancer tissues compared to adjacent normal tissues. Specifically, BEAN1 is highly expressed in BLCA, CHOL, COAD, KIRC, KIRP, LUAD, PRAD, READ, STAD, and THCA, while it is lowly expressed in BRCA, LUSC, and UCEC, indicating heterogeneity in BEAN1 expression patterns across different cancer types. Given the distinct expression patterns of BEAN1 in various cancers, we further explored its prognostic significance in these malignancies. Kaplan–Meier survival analysis revealed that high BEAN1 expression is associated with poorer OS in BLCA (Fig. 1C), KIRC (Fig. 1D), LUAD (Fig. 1E), LUSC (Fig. 1H), and READ (Fig. 1J), but with better OS in UCEC (Fig. 1M). In terms of disease-specific survival (DSS), high BEAN1 expression predicts poor prognosis in LUAD (Fig. 1F) and READ (Fig. 1K). Additionally, high BEAN1 expression correlates with shorter progression-free interval (PFI) in LUAD (Fig. 1G), LUSC (Fig. 1I), and READ (Fig. 1L), but with longer PFI in UCEC (Fig. 1N).

Univariate Cox regression analysis further demonstrated that high BEAN1 expression is associated with poor OS in KIRC (HR 1.408, 95% CI 1.043–1.902), LGG (HR 1.592, 95% CI 1.129–2.245), LUAD (HR 1.367, 95% CI 1.025–1.824), READ (HR 2.854, 95% CI 1.235–6.596), and SARC (HR 1.989, 95% CI 1.327–2.980) (Fig. 1O). For DSS, high BEAN1 expression predicts poorer outcomes in LGG (HR 1.559, 95% CI 1.086–2.239), LUAD (HR 1.444, 95% CI 1.002–2.082), READ (HR 3.044, 95% CI 1.050–9.755), and SARC (HR 2.031, 95% CI 1.302–3.170) (Fig. 1P). Regarding DFI, high BEAN1 expression is associated with worse DFI in COAD (HR 1.579, 95% CI 1.109–2.246), LUAD (HR 1.411, 95% CI 1.082–1.839), PRAD (HR 1.520, 95% CI 1.008–2.292), READ (HR 2.423, 95% CI 1.206–4.868), SARC (HR 1.593, 95% CI 1.144–2.218), and STAD (HR 1.438, 95% CI 1.003–2.062), but better DFI in KIRP (HR 0.409, 95% CI 0.235–0.714) (Fig. 1Q). These findings suggest that BEAN1 is abeerrantly expressed in various cancers and impacts prognosis.

3.2 High expression of BEAN1 in READ indicates poor prognosis

Given that TCGA data suggest high BEAN1 expression is associated with poor prognosis in several cancers, including READ, we validated these findings using multiple READ sequencing datasets from the GEO and our own samples. The GEO datasets used are detailed in Table 1. All three independent GEO datasets for READ showed significantly upregulated BEAN1 expression in READ tissues compared to paired normal tissues (Fig. 2A–C), consistent with the TCGA results. Additionally, BEAN1 expression was significantly higher in perioperative chemoradiotherapy-responsive READ patients compared to non-responders (Fig. 2D). BEAN1 expression was also significantly higher in Wnt mutation-positive (Wnt+) READ tissues compared to Wnt-patients (Fig. 2E). Kaplan–Meier survival curves showed that high BEAN1 expression was associated with worse OS (Fig. 2F, G, I) and worse RFS (Fig. 2H, J) in three independent GEO READ cohorts.

We further validated BEAN1 expression at the protein level in READ and CRC tissues using IHC from the HPA database. The analysis revealed significantly higher IHC staining intensity of BEAN1 in READ tissues compared to CRC tissues (Fig. 2K). Finally, in our XJCH-READ cohort, whose clinical characteristics are shown in Table 2, BEAN1 protein levels were significantly upregulated in READ tissues compared to paired normal tissues (Fig. 2L), and high BEAN1 expression was associated with poor prognosis in READ patients (Fig. 2M). These results indicate that high BEAN1 expression in READ is associated with poor prognosis, suggesting that BEAN1 may play a significant role in the pathogenesis of READ.

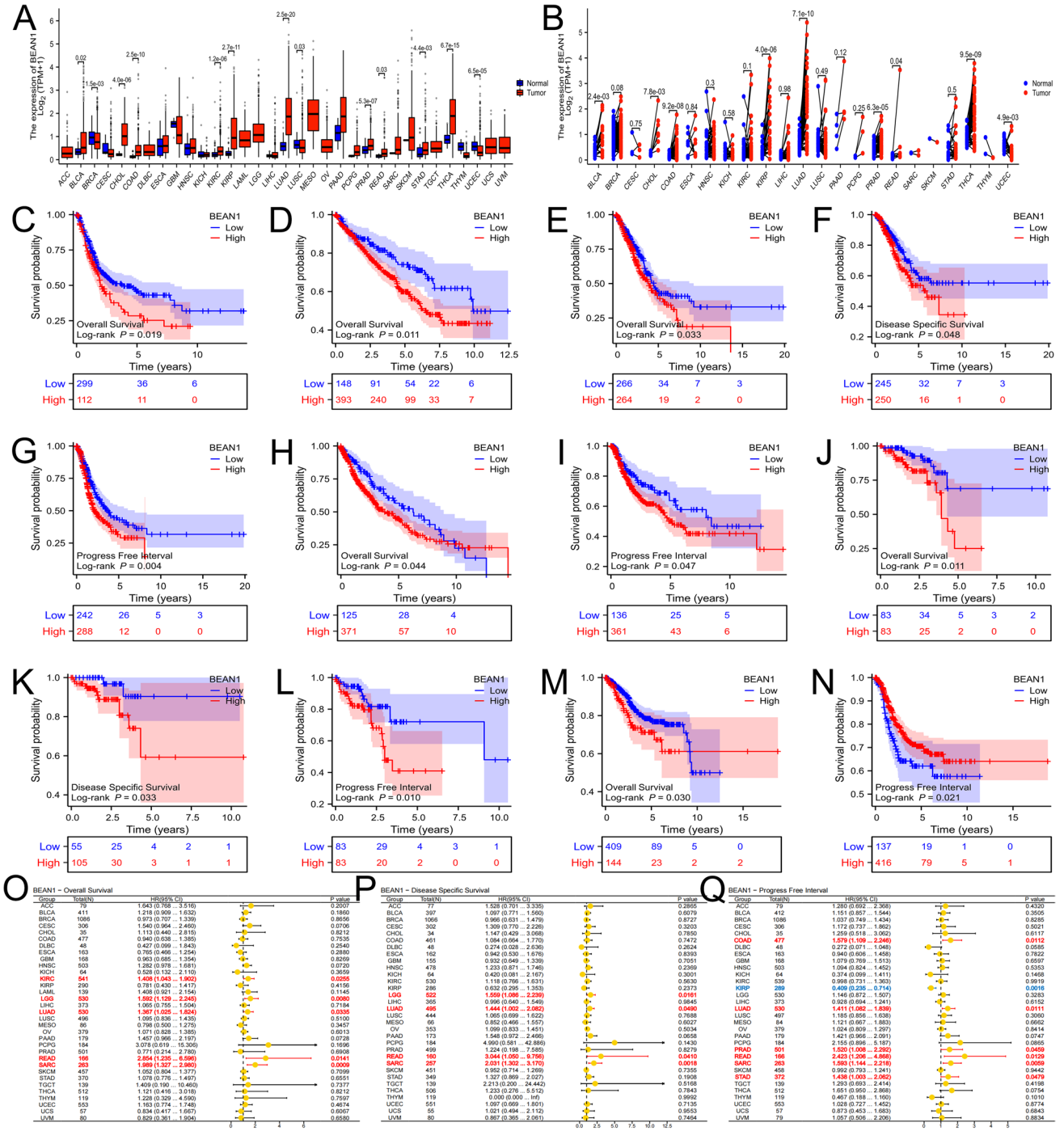


Fig. 1 Pan-cancer analysis of BEAN1 expression and prognosis based on TCGA. **A** BEAN1 expression in pan-cancer; **B** Paired BEAN1 expression in pan-cancer; **C** Impact of BEAN1 expression on OS in BLCA; **D** KIRC; **E** LUAD; **F** DSS in LUAD; **G** PFI in LUAD; **H** LUSC; **I** PFI in LUSC; **J** READ; **K** DSS in READ; **L** PFI in READ; **M** UCEC; **N** PFI in UCEC; **O** Prognostic significance of BEAN1 in pan-cancer for OS; **P** DSS; **Q** PFI

3.3 Identification of BEAN1-related differentially expressed genes and enriched pathways in READ, mainly ECM, Wnt and TGF-β

Patients within the TCGA-READ cohort were divided into low and high expression groups according to BEAN1 expression levels. The clinical characteristics of the two groups are shown in Table 3. A total of 743 transcriptome genes

Table 1 Basic information of READ-related GEO datasets used in the study

GEO accession	Platform	Patient	Sample	Analysis
GSE20842	GPL4133	Locally advanced rectal cancer	65 tumors *VS* 65 mucosas	Expression
GSE123390	GPL17586	Locally advanced rectal cancer	28 tumors *VS* 5 mucosas	Expression
GSE15781	GPL2986	Rectal cancer	13 tumors *VS* 10 mucosas	Expression
GSE35452	GPL570	Rectal cancer	22 chemoradiotherapy responders *VS* 24 non-responders	Expression
GSE79330	GPL4133	Early-onset rectal cancer	23 Wnt + *VS* 28 Wnt -	Expression
GSE133057	GPL6102	Rectal cancer	32 Overall survival	Prognosis
GSE56699	GPL14951	Rectal cancer	60 Overall survival 60 Recurrence-free survival	Prognosis
GSE87211	GPL13497	Rectal cancer	195 Overall survival 195 Recurrence-free survival	Prognosis
GSE146771	GPL20301	Colorectal cancer	10	scRNA-seq

showed differential expressions between the BEAN1 low and high expression groups, with 598 genes upregulated and 145 genes downregulated (adjusted p value < 0.05 and |log2FoldChange|> 1) (Fig. 3A). Next, we conducted GO and KEGG pathway analyses on the upregulated and downregulated BEAN1-DEGs. A bubble map revealed that the upregulated DEGs were enriched in ossification, extracellular structure and matrix organization for biological process (BP) analysis; collagen-containing extracellular matrix, blood microparticle, and endoplasmic reticulum lumen for cellular component (CC) analysis; and heparin binding, glycosaminoglycan binding, and extracellular matrix structural constituent for molecular function (MF) analysis. For KEGG pathway analysis, the upregulated DEGs were enriched in malaria, complement and coagulation cascades, and ECM-receptor interaction pathways. Conversely, the downregulated DEGs were enriched in nucleosome assembly, DNA replication-dependent chromatin organization, and DNA replication-dependent chromatin assembly for BP analysis; protein-DNA complex, DNA-packaging complex, and nucleosome for CC analysis; and protein heterodimerization activity for MF analysis. KEGG pathway analysis for the downregulated DEGs revealed enrichment in systemic lupus erythematosus, neutrophil extracellular trap formation, and alcoholism (Fig. 3B).

Additionally, GSEA results from the KEGG database demonstrated that high BEAN1 expression was strongly associated with processes like the matrisome, ECM glycoproteins, TGF-β signaling pathway, and Wnt signaling pathway (Fig. 3C-G). Spearman correlation analysis using the ssGSEA algorithm indicated that the expression of BEAN1 in READ was strongly and positively correlated with pathways such as collagen formation, ECM-related genes, degradation of ECM, epithelial-mesenchymal transition (EMT) markers, angiogenesis, TGF-β, glycosaminoglycan biosynthesis heparan sulfate/heparin, glycosphingolipid biosynthesis ganglio series, glycosaminoglycan biosynthesis chondroitin sulfate/dermatan sulfate, and glycosphingolipid biosynthesis globo and isoglobo series (Fig. 3E-P). These findings align with the results of GO/KEGG and GSEA analyses, suggesting that BEAN1-associated pathways in READ are predominantly enriched in ECM,Wnt and TGF-β.

The Wnt signaling pathway, which emerged as significantly associated with high BEAN1 expression, is known to be pivotal in the development and advancement of CRC. Our findings suggest that BEAN1 may contribute to the activation of the Wnt/β-catenin signaling cascade, which not only facilitates the proliferation and invasion of READ cell but also enhances metastatic potential by inducing EMT and activating cancer stem cells. Additionally, Wnt signaling has been associated with resistance to chemotherapy, potentially explaining the observed correlation between high BEAN1 expression and resistance to 5-FU. The TGF-β signaling pathway, also significantly associated with BEAN1 expression, plays a dual role in cancer. While TGF-β initially suppresses tumor growth in early stages, it later promotes tumor invasion and metastasis during cancer progression. TGF-β does this by promoting EMT, regulating immune suppression within the tumor microenvironment, and interacting with TAMs. In READ, the activation of TGF-β pathway also links to immune evasion and resistance to ICIs. Furthermore, our enrichment analysis revealed that pathways connected to ECM remodeling were significantly enriched in READ with high BEAN1 expression. Abnormal ECM remodeling is a hallmark of tumor progression, driving cancer development not only by facilitating the migration and invasion of tumor cells but also by modulating signaling within the tumor microenvironment to affect immune evasion and treatment response. Changes in the ECM, such as collagen deposition and degradation, have been associated with heightened malignancy aggressiveness and poor prognosis in cancer. By elaborating on the biological significance of these pathways, we further clarify

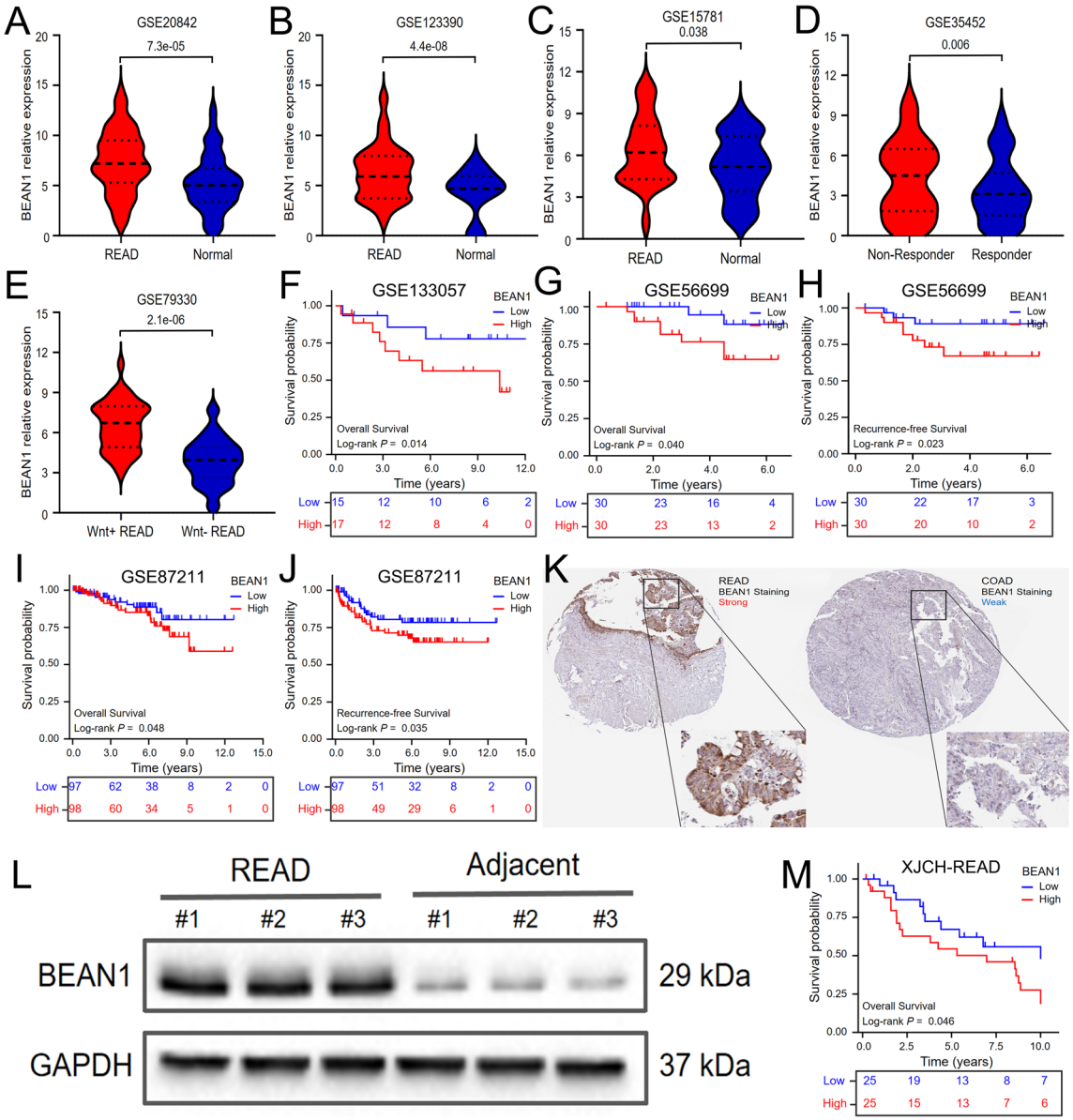


Fig. 2 Validation of BEAN1 expression and prognosis in READ. BEAN1 expression in READ tissues and paired normal tissues in **A** GSE20842, **B** GSE123390, and **C** GSE15781; **D** BEAN1 expression in perioperative chemoradiotherapy responders and non-responders in READ tissues; **E** BEAN1 expression in Wnt + and Wnt- READ tissues; Kaplan–Meier survival curves of BEAN1 in **F** overall survival in GSE133057, **G** overall survival, and **H** recurrence-free survival in GSE56699, **I** overall survival, and **J** recurrence-free survival in GSE87211; **K** Typical immunohistochemical images of BEAN1 high or low expression in READ/COAD tissues from the HPA database; **L** Validation of BEAN1 protein level expression in READ tissues and paired normal tissues in the XJCH-READ cohort; **M** Validation of BEAN1 impact on overall survival in the XJCH-READ cohort

the potential pathogenic mechanisms through which BEAN1 contributes to rectal adenocarcinoma, supporting its role as a novel therapeutic target. These findings not only elucidate how BEAN1 impacts tumor progression and treatment resistance but also provide valuable insights for future therapeutic strategies targeting these pathways.

3.4 BEAN1 is associated with immune infiltration in READ, especially M2 macrophages

Through correlation analysis, we identified the connection linking BEAN1 expression with various immune cell types determined by quanTIseq, CIBERSORT, MCP-counter, EPIC, and ssGSEA. Notably, quanTIseq showed that BEAN1 expression in READ was positively correlated with basophil recruiting ($r = 0.81$, $p < 0.001$), CD4 + T cells ($r = 0.45$, $p < 0.001$),

Table 2 Relationship between BEAN1 expression and clinicopathological features in XJCH-READ

Characteristics	BEAN1 expression		*p* value
	Low	High	
n	25	25	0.768
Age, n (%)			
< = 65	17	15	
> 65	8	10	
Gender, n (%)			1.0
Female	10	11	
Male	15	14	
BMI, n (%)			0.741
< = 25	7	5	
> 25	18	20	
Pathologic T stage, n (%)			0.142
T1	2	0	
T2	6	2	
T3	16	20	
T4	1	3	
Pathologic N stage, n (%)			0.030
N0	15	6	
N1	6	9	
N2	4	10	
Pathologic M stage, n (%)			0.602
M0	24	22	
M1	1	3	
Pathologic stage, n (%)			0.022
Stage I	6	0	
Stage II	10	7	
Stage III	7	13	
Stage IV	2	6	
Histological type, n (%)			0.276
Adenocarcinoma	24	23	
Mucinous adenocarcinoma	1	2	
History of colon polyps, n (%)			1.0
No	21	20	
Yes	4	5	
Anatomic neoplasm subdivision, n (%)			0.834
Rectosigmoid Junction	7	7	
Rectum	17	16	
Sigmoid Colon	1	2	

monocyte recruiting ($r = 0.38$, $p < 0.001$), and macrophages ($r = 0.35$, $p < 0.001$), and negatively correlated with Treg cell recruiting ($r = -0.75$, $p < 0.001$), Th1 cell recruiting ($r = -0.43$, $p < 0.001$), and CD8+ T cell recruiting ($r = -0.37$, $p < 0.001$) (Fig. 4A). CIBERSORT also showed the expression of BEAN1 in READ was positively correlated with eosinophils ($r = 0.30$, $p < 0.001$), B cell memory ($r = 0.28$, $p < 0.001$), and M2 macrophages ($r = 0.24$, $p < 0.001$), and negatively correlated with activated NK cells ($r = -0.42$, $p < 0.001$), resting mast cells ($r = -0.40$, $p < 0.001$), and CD4 + T cell memory ($r = -0.25$, $p < 0.001$) (Fig. 4B). MCP-counter analysis showed BEAN1 expression in READ was positively correlated with macrophages/monocytes ($r = 0.92$, $p < 0.001$), monocytes ($r = 0.50$, $p < 0.001$), and negatively correlated with neutrophils ($r = -0.29$, $p < 0.001$), myeloid dendritic cells ($r = -0.78$, $p < 0.001$), CD8+ T cells ($r = -0.33$, $p < 0.001$), and cytotoxicity score ($r = -0.32$, $p < 0.001$) (Fig. 4C). EPIC analysis showed that BEAN1 expression in READ was positively correlated with macrophages ($r = 0.52$, $p < 0.001$), NK cells ($r = 0.39$, $p < 0.001$), and CD4 + T cells ($r = 0.38$, $p < 0.001$), and negatively correlated with endothelial cells ($r = -0.33$, $p < 0.001$) (Fig. 4D). Furthermore, ssGSEA indicated that BEAN1 expression

Table 3 Relationship between BEAN1 expression and clinicopathological features in TCGA-READ

Characteristics	BEAN1 expression		*p* value
	Low	High	
n	83	83	
Pathologic T stage, n (%)			0.028
T1	6	3	
T2	20	8	
T3	53	60	
T4	4	10	
Pathologic N stage, n (%)			0.005
N0	52	32	
N1	20	25	
N2	10	23	
Pathologic M stage, n (%)			0.168
M0	69	57	
M1	9	14	
Pathologic stage, n (%)			0.033
Stage I	21	9	
Stage II	29	22	
Stage III	21	30	
Stage IV	9	15	
Primary therapy outcome, n (%)			0.683
PD&SD	3	6	
PR&CR	25	28	
Gender, n (%)			0.436
Female	40	35	
Male	43	48	
Race, n (%)			1.000
Asian&Black or African American	3	4	
White	39	42	
Age, n (%)			0.535
≤ 65	43	39	
> 65	40	44	
Weight, n (%)			0.485
≤ 90	27	28	
> 90	8	12	
Height, n (%)			0.964
< 170	15	17	
≥ 170	19	22	
BMI, n (%)			0.719
≤ 25	10	10	
> 25	24	29	
Histological type, n (%)			0.135
Adenocarcinoma	77	70	
Mucinous adenocarcinoma	4	9	
Residual tumor, n (%)			0.573
R0	62	60	
R1&R2	6	8	
CEA level, n (%)			0.064
≤ 5	35	30	
> 5	17	30	
Perineural invasion, n (%)			1.000

Table 3 (continued)

Characteristics	BEAN1 expression		*p* value
	Low	High	
No	20	20	
Yes	7	7	
Lymphatic invasion, n (%)			0.319
No	45	39	
Yes	29	35	
History of colon polyps, n (%)			0.560
No	57	58	
Yes	14	18	
Colon polyps present, n (%)			0.249
No	25	37	
Yes	7	5	
Anatomic neoplasm subdivision, n (%)			0.768
Rectosigmoid Junction	23	26	
Rectum	57	53	
Sigmoid Colon	2	3	

PD Progressive disease, *SD* Stable disease, *PR* Partial response, *CR* Complete response

in READ was associated with infiltration levels of macrophages ($r = 0.287$, $p < 0.001$), neutrophils ($r = 0.258$, $p < 0.001$), dendritic cells ($r = 0.227$, $p = 0.008$), mast cells ($r = 0.222$, $p = 0.005$), and M2 macrophages ($r = 0.189$, $p < 0.001$) (Fig. 4E).

Given BEAN1's association with immune infiltration in READ, particularly macrophages, we utilized single-cell sequencing data of CRC from the GSE146771 dataset to validate BEAN1 expression in immune cells within the tumor microenvironment. As shown in Fig. 4F–H, BEAN1 was significantly higher in macrophages compared to tumor cells, stromal cells, and other immune cells. Since the polarization of macrophage is intimately connected with the progression of READ, we examined the association linking BEAN1 and macrophage markers. BEAN1 was strongly positively correlated with macrophage marker CD11b (ITGAM) ($r = 0.273$, $p < 0.001$) (Fig. 4I), strongly and inversely associated with M1 macrophage polarization marker NOS2 ($r = -0.206$, $p = 0.008$) (Fig. 4J), and significantly positively correlated with M2 macrophage polarization markers CD163 ($r = 0.2$, $p = 0.01$) (Fig. 4L) and CD206 (MRC1) ($r = 0.277$, $p < 0.001$) (Fig. 4M). M1 macrophages were associated with better prognosis (Fig. 4K). In contrast, M2 macrophages were linked to worse prognosis in READ patients (Fig. 4N). These data suggest that BEAN1 is related to immune infiltration in READ, particularly M2 macrophages, and may influence READ through tumor immunology mechanisms.

3.5 BEAN1 influences treatment response and promotes drug resistance in READ

BEAN1 expression is closely associated with macrophages, especially M2 macrophages. Macrophages are a significant component of the TME, contributing to immune suppression, resistance to ICIs, and chemotherapy resistance. Therefore, we explored the impact of BEAN1 on treatment response in READ. First, we employed the ESTIMATE algorithm and contrasted immune, stromal, and ESTIMATE scores across the low and high BEAN1 expression READ groups. As shown in Fig. 5A–C, the high group had higher scores, indicating a significant positive correlation, suggesting that BEAN1 might influence READ treatment response through the immune system. Next, we compared ICGs expression between low and high BEAN1 expression groups. The high group had significantly higher expressions of CD274, HAVCR2 PDCD1LG2, and SIGLEC15, indicating a stronger immune evasion capability and a more pronounced immunosuppressive microenvironment in READ patients with high BEAN1 expression (Fig. 5D). The TIDE algorithm predicted the response of BEAN1 to ICIs in READ. BEAN1 high expression group had higher TIDE scores and a significantly lower number of responders compared to the low expression group, indicating an association between BEAN1 and poor ICI response (Fig. 5E).

Chemotherapy is a common treatment for READ, and tumor stemness is associated with chemotherapy resistance. Therefore, we assessed stemness and drug sensitivity scores to evaluate the response of BEAN1 in READ to chemotherapy. These results showed BEAN1 high expression patients had higher mRNAsi scores (Fig. 5F), indicating a correlation

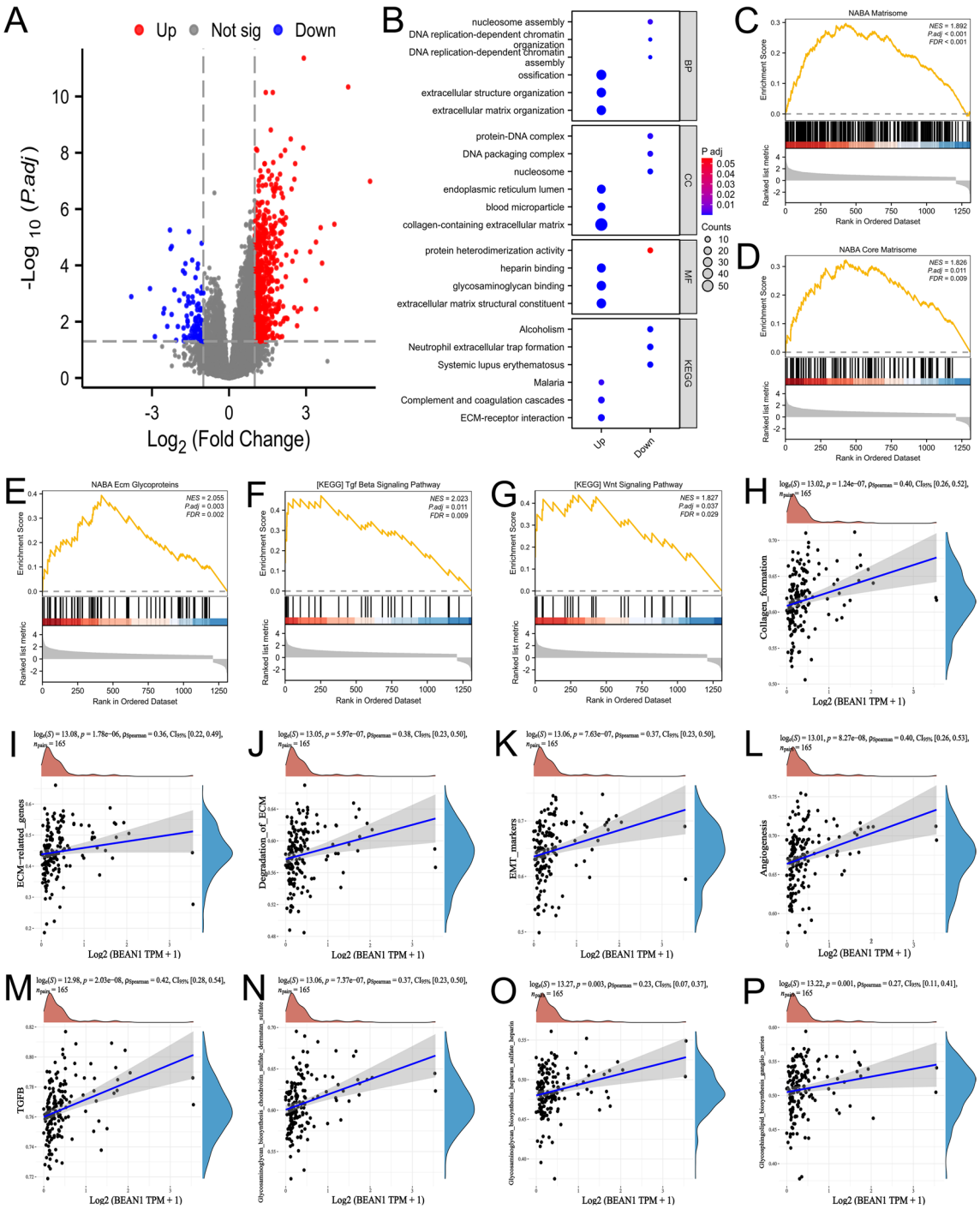


Fig. 3 Identification of BEAN1-related differentially expressed genes and enriched pathways in READ. **A** Volcano plot showing differentially expressed genes in READ based on BEAN1 expression (adjusted *p* value < 0.05, |log2FoldChange| > 1); **B** GO and KEGG Pathway enrichment analyses for upregulated and downregulated DEGs; **C**–**G** GSEA enrichment plots showing correlated pathways of BEAN1; (H-P) Spearman correlation analysis between BEAN1 expression and pathway scores in READ

between BEAN1 and tumor stemness. Additionally, BEAN1 high expression group had higher IC50 scores for 5-FU (Fig. 5G), indicating an association between BEAN1 and resistance to 5-FU.

4 Discussion

This study represents the first exploration of BEAN1 expression and function in cancer, demonstrating abnormal expression of BEAN1 in 13 types of cancer and its correlation with prognosis in six cancer types, serving as either a risk factor or a protective factor for prognosis. Specifically, in READ, multiple datasets confirmed a significant upregulation of BEAN1 expression in comparison with the adjacent tissues. High BEAN1 expression was linked to poorer OS and RFS in READ patients, promoting resistance to ICIs and 5-FU chemotherapy, suggesting that BEAN1 could serve as an indicator of prognosis and highlighting its promise as a therapeutic target in READ. Given the current limitations of READ treatment, particularly the variable response to chemotherapy and immunotherapy, targeting BEAN1 may offer a novel approach to improve patient outcomes. Our study is among the first to explore the role of BEAN1 in READ, a gene that has primarily been studied in the context of neurodegenerative diseases such as spinocerebellar ataxia type 31. Previous research has not extensively examined BEAN1 in cancer, making our findings particularly novel and significant. In comparing our results with existing literature, we find that while BEAN1 has been implicated in neurodegenerative processes, there is little to no prior evidence of its involvement in cancer biology. This contrasts with our study, which identifies BEAN1 as a key player in promoting poor prognosis, immune evasion, and chemotherapy resistance in READ. Specifically, our findings suggest that BEAN1 interacts with critical pathways like Wnt/β-catenin and TGF-β, which are well-known contributors to cancer progression and treatment resistance.

TME is vital in cancer progression and treatment resistance. Our analysis indicates in READ, BEAN1 expression is positively correlated with the infiltration of macrophages, especially M2 macrophages, and negatively linked to the infiltration of CD8+ T cells. Macrophages can differentiate into two separate polarization forms: M1 macrophages, which are classically activated (pro-inflammatory), and M2 macrophages, which are alternatively activated (anti-inflammatory) [21]. M1 macrophages can promote Th1 responses, phagocytize, and eliminate target tumor cells. Research has demonstrated cytokines such as IFN-γ can induce M1 macrophages. M1 TAMs release reactive oxygen species, IL-6, and other inflammatory mediators within the TME, contributing to immune reactions and exerting anti-tumor immunity [22]. Conversely, M2 macrophages, induced by IL-4, TGF-β, or glucocorticoids, release molecules that reduce inflammation such as IL-10, promoting angiogenesis, tissue remodeling, wound repair, and tumorigenesis [23]. TAMs predominantly exhibit an M2-like phenotype within the TME, promoting tumor immunosuppression by either facilitating cancer blood vessel formation or indirectly inducing interactions among immune cells [24]. Gastrointestinal macrophages are the most abundant, attracting monocytes from bloodstream to cancerous areas under external stimuli, polarizing them into TAMs [25], which engage alongside cancer cells through exosomes or secretion of various signaling molecules, promoting tumor cell growth, infiltration, movement, and blood vessel formation. Via numerous signaling routes, cancer cells employ TAMs to facilitate the expansion and advancement of CRC, with TAMs tending to transform into a pro-tumor M2 phenotype.

Our results further demonstrate that BEAN1 is associated with an immunosuppressive microenvironment in READ, characterized by high levels of M2 macrophage infiltration, higher expression of immune checkpoints, and lower levels of CD8+ T cell infiltration. TAMs suppress T cells both directly and indirectly. Previous research has demonstrated TAMs highly express ligands such as PD-L1, CTLA-4, and B7-H1, inhibiting the cell-killing activities of T lymphocytes, natural killer and NKT cells, thereby diminishing the body's capacity to eliminate CRC cells [26]. TAMs control T cell attraction and limit T cell distribution, further indirectly inhibiting T cell activity [27]. TAMs attract Tregs through the release of chemokine CCL2, inhibiting T cells' immune defense against tumors, interfering with interactions among immune cells, and leading to an immunosuppressive environment in CRC [28]. Macrophages secrete IL-17, enhancing the accumulation of G-MDSCs and increasing the percentage of Th17 cells, ultimately advancing tumor progression [29]. Additionally, TAMs generate IL-10, inhibiting cytotoxic lymphocyte stimulation via reducing co-localization of CD8 and T cell receptor [30]. TAMs express high levels of CD206, inhibiting CD45 phosphatase activity, leading to impaired cytotoxicity of CD8+ T cells [31]. TAMs inhibit the expansion of T cells via arginine breakdown controlled by arginase 1, and other free radicals [32]. TAMs promote a tumor-friendly and immune-inhibitory tissue environment during CRC progression through direct inhibition of CD8+ T cell cytotoxicity via arginase 1 release [29]. Furthermore, TAMs in CRC exert immunosuppressive effects via various metabolic routes and engagements with gut flora [33].

Our study indicates that in READ, BEAN1 is associated with pathways related to ECM remodeling, EMT, angiogenesis, Wnt, and TGF-β signaling. ECM remodeling is a hallmark of tumor progression, promoting invasion and metastasis.

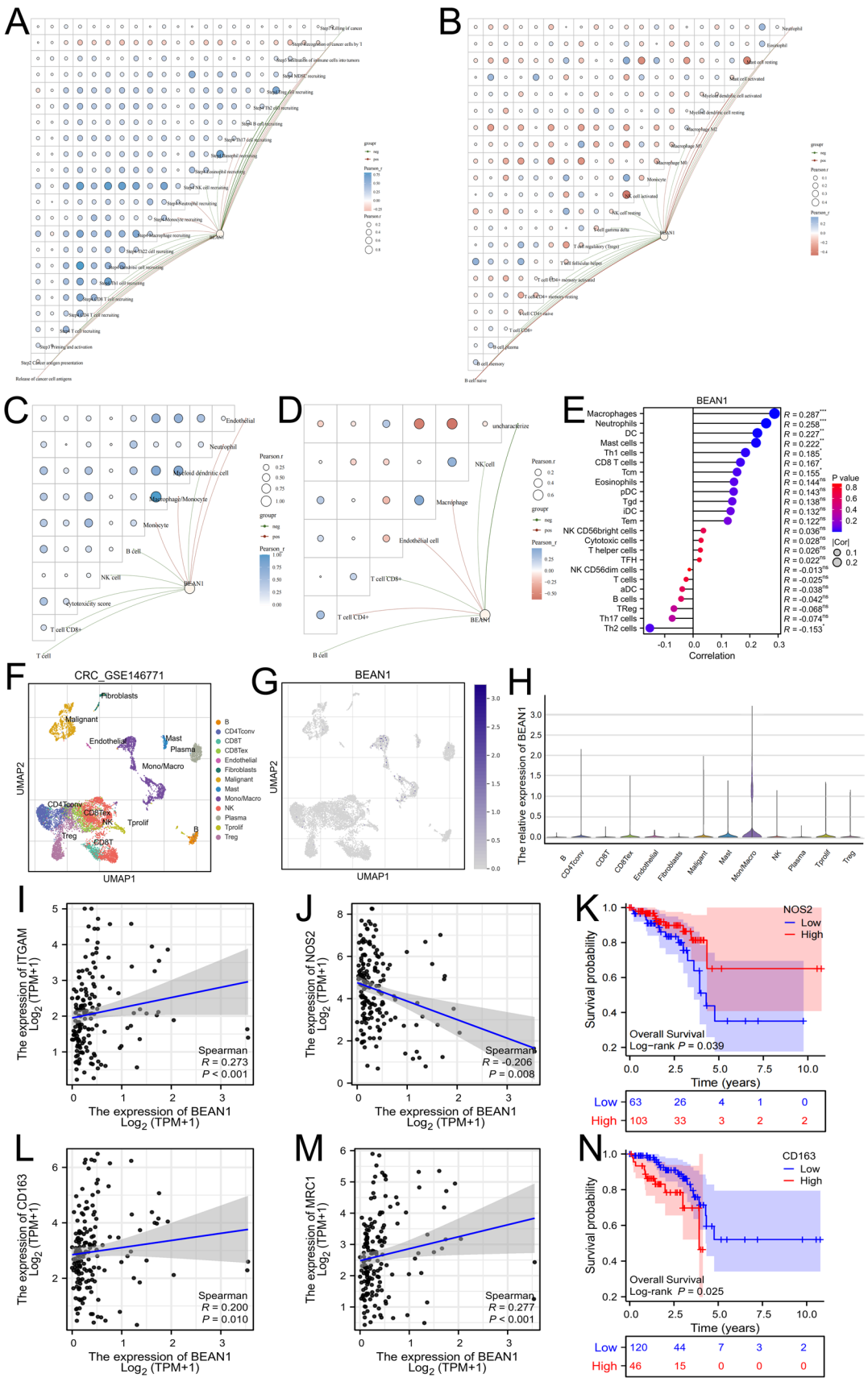
Fig. 4 The association of BEAN1 expression with the levels of infiltrating immune cells. **A** The relationship between BEAN1 expression and immune cell infiltration in READ based on quanTIseq. **B** CIBERSORT. **C** MCP-counter. **D** EPIC. **E** ssGSEA. **F**–**H** BEAN1 expression in tumor cells and the immune microenvironment based on CRC single-cell sequencing dataset GSE146771. **I**–**M** Correlation between BEAN1 and macrophage-related markers CD11b (ITGAM), NOS2, CD163, and CD206 in TCGA-READ. Kaplan–Meier curves depicting the impact of M1 macrophages (**K**) and M2 macrophages (**N**) on overall survival in READ patients

Collagen deposition, a core component of ECM, is characteristic of CRC [9]. Patients with upregulated expression of collagen and collagenase combinations have shorter OS, suggesting these genes as prognostic biomarkers for CRC [12]. Cancer cells can modify collagen to control tumor growth and invasion. In the TME, collagen plays a role in reducing the production of chemokines, thereby inhibiting anti-tumor immune responses. Type I collagen can modulate IL-18, facilitating faster cancer growth [34]. As the tumor progresses, abnormal collagen remodeling accompanies the process, primarily resulting in excessive collagen accumulation, altered ratios, and changes in alignment [35]. Proteoglycans (PGs) are complex molecules with a protein core covalently attached to glycosaminoglycan chains. PGs serve a crucial function in maintaining ECM structure and are implicated in cancer pathogenesis [36]. Chondroitin sulfate proteoglycan versican serves as an unfavorable prognostic marker and a significant subsequent target of HIF-1α driving CRC spread [37]. Serglycin, an uncommon type of intracellular PGs, is persistently produced and extensively secreted by invasive CRC cells [38]. The significant positive correlation between BEAN1 and collagen and glycosaminoglycan metabolic pathways suggests that BEAN1 may enhance ECM remodeling by upregulating collagen and glycosaminoglycan and altering their structures, thus creating a microenvironment conducive to tumor invasion and metastasis.

The classical Wnt/β-catenin pathway is an established promoter of CRC [39], which plays a pivotal role in READ pathogenesis and is closely associated with tumorigenesis, metastasis, and drug resistance. Elevated levels of WNT4 in the serum of CRC patients have been observed, with CRC tissues secreting WNT4 to induce EMT, activate fibroblasts, and promote blood vessel formation via the classical Wnt/β-catenin pathway, thus promoting the development of CRC [40]. Inhibiting Wnt/β-catenin has demonstrated to suppress growth and metastasis of both 5-FU sensitive and resistant CRC [41]. TCF7L2, a Wnt transcription factor, is highly expressed in initial rectal carcinomas unresponsive to radiation and chemotherapeutic treatments, mediating resistance to these treatments. The upregulation of GBP-2 could boost cytotoxic effects of Paclitaxel (PTX) on both PTX-sensitive and PTX-resistant CRC cells by inhibiting Wnt signaling [42].

TGF-β is released by various cells within the TME, including macrophages, and it regulates cancer cell growth, differentiation, and apoptosis. Elevated levels of TGF-β1 have been associated with CRC metastasis and poor prognosis [43]. Upregulation of TGF-β is also linked with genes indicative of increased EMT. TGF-β serves as a key factor during the recruitment and polarization of macrophages to an M2 phenotype. It can induce the expression of integrins and collagenase, thereby enhancing migration to the inflammatory phase of macrophages [44]. In mouse CRC models, CRC cell-derived collagen triple helix repeat containing 1 induces TAMs' M2 polarization through TGF-β signaling, further promoting liver metastasis [45]. Besides its role in recruiting and polarizing macrophages, TAMs can also release TGF-β, further promoting tumor progression, and under the mediation of TAM-derived TGF-β, the EMT of CRC cells is increased [46].

BEAN1's role in READ appears to be multifaceted, contributing to both immune evasion and chemotherapy resistance. These functions are likely mediated through its interactions with several key signaling pathways and its influence on the tumor microenvironment. One of the primary mechanisms through which BEAN1 may promote immune evasion is by modulating Wnt/β-catenin. BEAN1 may enhance Wnt signaling, leading to reduced infiltration of cytotoxic CD8+ T cells and an increased presence of immunosuppressive cells such as M2 TAMs. This shift in the immune landscape could enable the tumor to evade immune surveillance and contribute to resistance against immune checkpoint inhibitors [47–51]. Additionally, BEAN1 may influence chemotherapy resistance through its interaction with the TGF-β signaling pathway. TGF-β is a well-known mediator of EMT, a process that endows cancer cells with increased migratory and invasive capabilities, as well as resistance to chemotherapy [52–55]. BEAN1 could potentiate TGF-β signaling, thereby promoting EMT and enhancing the tumor's resistance to chemotherapeutic agents such as 5-FU. This interaction not only facilitates metastasis but also creates a tumor microenvironment that is more resistant to conventional therapies. Moreover, BEAN1's involvement in ECM remodeling further supports its function in altering TME. Changes in the ECM composition and structure can influence the behavior of surrounding stromal cells and immune cells, creating a niche that supports tumor growth and survival [56–60]. BEAN1 may drive ECM remodeling processes, leading to increased stiffness of the tumor matrix, which has been associated with enhanced drug resistance and poor therapeutic outcomes. In summary, BEAN1's contribution to immune evasion and chemotherapy resistance in READ likely involves its interactions with critical signaling routes like Wnt/β-catenin and TGF-β, as well as its role in ECM remodeling. These mechanisms not only help the tumor to evade immune detection but also to withstand the cytotoxic effects of chemotherapy, ultimately leading


A
B
C
D
E
BEAN1
Macrophages
Neutrophils
DC
Mast cells
Th1 cells
CD8 T cells
Tcm
Eosinophils
pDC
Tgd
iDC
Tem
NK CD56bright cells
Cytotoxic cells
T helper cells
TFH
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T cells
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Malignant
Endothelial
Mast
Plasma
Mono/Macro
CD4Tconv
CD8Tex
NK
Tprolif
Treg
CD8T
B
UMAP1
UMAP2
G
BEAN1
H
The relative expression of BEAN1
I
The expression of ITGAM Log2 (TPM+1)
Spearman
R = 0.273
P < 0.001
The expression of BEAN1 Log2 (TPM+1)
J
The expression of NOS2 Log2 (TPM+1)
Spearman
R = -0.206
P = 0.008
K
NOS2
Low
High
Survival probability
Overall Survival
Log-rank P = 0.039
Time (years)
Low 63 26 4 1 0
High 103 33 3 2 2
L
The expression of CD163 Log2 (TPM+1)
Spearman
R = 0.200
P = 0.010
M
The expression of MRC1 Log2 (TPM+1)
Spearman
R = 0.277
P < 0.001
N
CD163
Low
High
Survival probability
Overall Survival
Log-rank P = 0.025
Time (years)
Low 120 44 7 3 2
High 46 15 0 0 0

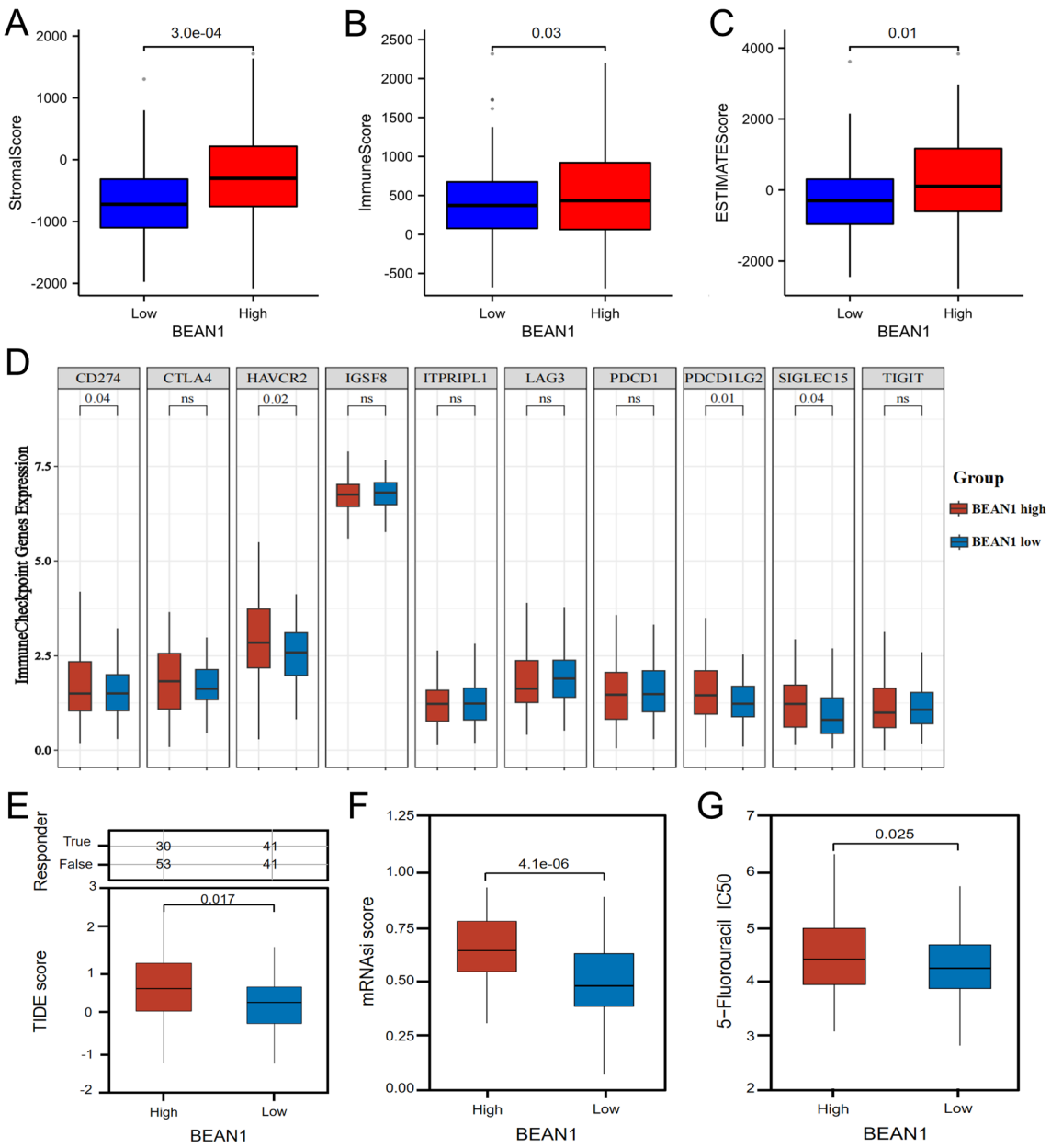

Fig. 5 Impact of BEAN1 on treatment response in READ. **A**–**C** The ESTIMATE algorithm was applied to determine the association of BEAN1 expression with stromal, immune, and ESTIMATE scores. **D** Immune checkpoint gene expression in high and low BEAN1 expression groups in TCGA-READ cohort. **E** Immune response statistics and TIDE score distribution between high and low BEAN1 expression groups. **F** Distribution of stemness scores between high and low BEAN1 expression groups. **G** Distribution of 5-FU IC50 scores between high and low BEAN1 expression groups

to poorer clinical outcomes. Further research into these pathways could uncover new therapeutic strategies that target BEAN1 to improve treatment efficacy in READ.

The findings of this study have significant therapeutic implications. BEAN1 is linked to an unfavorable outcome, immunosuppression, and chemotherapy resistance in READ, suggesting that BEAN1 could be a valuable target for novel therapeutic strategies. Inhibiting BEAN1 may help disrupt the immunosuppressive TME, enhance the efficacy of immune checkpoint inhibitors, and overcome chemotherapy resistance. Although our research offers new perspectives on the function of BEAN1 in READ, several limitations should be acknowledged to contextualize our findings. Firstly, the sample size of our READ patient cohort was relatively small, which may limit the generalizability of our results. Although we validated our findings using multiple independent datasets, a larger sample size would provide more robust statistical power and potentially reveal additional nuances in BEAN1's role in READ. Secondly, potential confounding variables,

such as the heterogeneity of the patient population with regard to genetic background, comorbidities, and treatment histories, may have impacted the results. While we attempted to control for these factors through statistical adjustments, residual confounding cannot be entirely ruled out. Future studies should aim to include a more homogeneous patient population or employ stratified analyses to better account for these variables. Thirdly, there are methodological limitations inherent to the use of publicly available datasets and retrospective analysis. For example, the integration of data from different platforms may introduce batch effects, and the study's backward-looking design could lead to selection bias. Prospective studies with standardized data collection methods are needed to validate our findings and mitigate these methodological concerns. To address these limitations, future research should focus on several key areas. First, large studies conducted across multiple centers with cohorts are necessary to validate the generalizability of our findings and to provide more comprehensive insights into BEAN1's role in READ. Additionally, prospective, longitudinal studies will allow for the assessment of BEAN1 expression over time and its correlation with clinical outcomes, thereby offering stronger evidence of causality. Furthermore, the study in vivo is crucial for elucidate the precise molecular mechanisms by which BEAN1 influences tumor progression, immune evasion, and chemotherapy resistance. These studies should explore BEAN1's interactions with specific signaling pathways and its role within the tumor microenvironment. Finally, validating these findings in diverse populations, across different ethnicities and geographic regions, will ensure the broader applicability of BEAN1 as a biomarker and therapeutic target. Additionally, developing specific inhibitors or antagonists against BEAN1 could provide new therapeutic avenues for READ and other cancers with high BEAN1 expression. Through following these lines of research, we could deepen the understanding of BEAN1's role in READ and explore its potential as a target for personalized therapeutic interventions.

5 Conclusion

This study identifies BEAN1 as a critical player in rectal adenocarcinoma (READ), significantly associated with poor prognosis, immune evasion, and chemotherapy resistance. BEAN1 promotes tumor progression and resistance to conventional therapies through interactions with the Wnt/β-catenin and TGF-β pathways. Its strong correlation with adverse clinical outcomes positions BEAN1 as a promising biomarker for anticipating disease progression and treatment response in READ. Additionally, BEAN1 holds significant potential as a therapeutic target. Targeting BEAN1 could disrupt key signaling pathways, enhancing the effectiveness of immunotherapy and chemotherapy. Our findings expand the understanding of BEAN1's role in cancer and provide a foundation for developing BEAN1-targeted therapies. In summary, BEAN1 is a possible marker and treatment target in READ, with the capacity to significantly influence patient prognosis and treatment strategies. Upcoming studies should concentrate on uncovering BEAN1's molecular mechanisms and assessing its therapeutic potential in clinical settings.

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Author contributions All authors contributed to the study conception and design. Experimental data: TS, CY and YPP; Data analysis: ZMW, ZLZ and XYZ; Writing-original draft: TS, CY, and XYZ; Experimental design: TS, CY, TXT and XYZ. All researchers attributed to this study have read and agreed to the manuscript's published version.

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Data availability The data is presented by the public database. The RNA-seq and scRNA-seq data or presented in this study are openly available at TCGA database (https://portal.gdc.cancer.gov/), and GEO databases (https://www.ncbi.nlm.nih.gov/geo/). The IHC data in this study is openly available at TCGA database (https://www.proteinatlas.org/).

Declarations

Ethics approval and consent to participate This study was authorized by the Ethics Committee of the Affiliated Cancer Hospital of Xinjiang Medical University in accordance with the Declaration of Helsinki (Approval Number: G-2021005). And informed consent was obtained from all subjects and/or their legal guardian(s).

Consent for publication All authors read the guidelines of the journal and agreed with consent for publication.

Competing interests The author(s) report no competing interests in this work. All methods were carried out in accordance with relevant guidelines and regulations.

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