SIN-CHN SCIENTIFIC PRESS PTE.LTD.

Molecular & Cellular Biomechanics 2025, 22(2), 1257.
https://doi.org/10.62617/mcb1257

Article

FHOD1 is a promising biomarker for diagnosis, prognosis, and immunotherapy in colorectal cancer

Yanan Li[1], Hua Zuo[1,2], Zhihong Liu[1], Ziyu Wu[1], Zeyang Zhou[1], Jianbo Deng[1], Xiaodong Yang[1,*]

[1] Department of Gastrointestinal Surgery, The Second Affiliated Hospital of Soochow University, Suzhou 215004, China
[2] Department of General Surgery, Renhe Hospital, Shanghai 200431, China
*** Corresponding author:** Xiaodong Yang, yangxiaodong2366@suda.edu.cn

CITATION

Li Y, Zuo H, Liu Z, et al. FHOD1 is a promising biomarker for diagnosis, prognosis, and immunotherapy in colorectal cancer. Molecular & Cellular Biomechanics. 2025; 22(2): 1257. https://doi.org/10.62617/mcb1257

ARTICLE INFO

Received: 27 December 2024
Accepted: 23 January 2025
Available online: 7 February 2025

Abstract: FHOD1 is a crucial regulator of cellular actin dynamics, and growing evidence suggests its involvement in tumorigenesis. Nevertheless, the precise function of FHOD1 in colorectal cancer (CRC) is still not well-defined. FHOD1 expression was analyzed using TIMER 2.0, and its prognostic value was assessed using the Kaplan-Meier Plotter. Functional analysis was performed via LinkedOmics, and its role in immune infiltration was investigated using the TISCH2 database and GSVA package. Drug sensitivity related to FHOD1 was evaluated with R software. Additionally, the CCK-8 assay, colony formation assay, wound-healing assay, and Transwell migration assay were used to evaluate the impact of FHOD1 on the proliferation and migration of colorectal cancer cells. Our study proved that FHOD1 expression was substantially higher in CRC tissues than in normal tissues, correlating with poorer patient prognosis. Functional analysis indicated that FHOD1 was involved in immune-related processes and the tumor microenvironment, particularly affecting numerous types of immune cells, such as natural killer cells and T cells. FHOD1 expression was positively associated with sensitivity to multiple chemotherapeutic agents. Finally, knockdown of FHOD1 in HCT116 and RKO NL cell lines impaired cell proliferation and migration, highlighting its potential as a target for treatment in managing CRC. In conclusion, these findings underscore the importance of FHOD1 in CRC progression and treatment strategies.

Keywords: FHOD1; colorectal cancer; prognosis; immune infiltration; tumor microenvironment; drug sensitivity; cell proliferation; migration

1. Introduction

Colorectal cancer (CRC) ranks as the third most common type of cancer across the globe, responsible for about 9.6% of all cancer deaths. It is also the second most significant cause of cancer-related fatalities, making up 9.3% of the total cancer mortality rate [1]. To improve early diagnostic rates, enhance immunotherapy efficacy, and extend patient survival, it is essential to identify reliable biomarkers for the development of novel therapeutic targets.

FHOD1 functions as a nucleation, capping, and bundling protein for actin filaments, and plays a critical role in regulating cellular actin dynamics. By binding actin filaments, it contributes to maintaining cell shape, migration, and cellular protrusion [2,3]. Emerging research indicates that FHOD1 significantly influences the migration, invasion, and stress response of various cancer cell types [4–12]. For example, FHOD1 is overexpressed in breast cancer, where it regulates cell migration and invasion while demonstrating a negative correlation with patient prognosis [4–6]. Moreover, it is upregulated during epithelial-mesenchymal transition (EMT) in squamous cell carcinoma [7] and contributes to melanoma tumor proliferation and

growth [8]. Furthermore, FHOD1 is involved in glioblastoma cell migration [9] and promotes cellular proliferation and invasion in gastric cancer, with elevated mRNA expression correlating with reduced overall survival in gastrointestinal cancers [10,11]. Taken together, these studies underscore FHOD1's involvement in various malignancies and its promise as a treatment target. However, the precise role of FHOD1 in CRC remains unclear, particularly regarding its implications for prognosis and immune infiltration.

The tumor microenvironment (TME) functions as a sanctuary for tumor cells and significantly influences tumor growth and metastatic progression. Key components of the TME comprise stromal cells and immune cells, with immune cell infiltration into the peritumoral stroma displaying distinct characteristics across many solid tumors [12]. Immune cells such as tumor-associated macrophages and neutrophils can be activated within the TME, thereby further supporting tumor growth and progression [13]. Consequently, cancer therapies targeting the TME have garnered substantial interest in both research and clinical practice [14].

This research explores the relationship between FHOD1, prognosis, and immune infiltration in CRC, thereby providing a vital molecular foundation for early diagnosis and immunotherapy strategies. We utilized bioinformatic methods to analyze FHOD1 expression in colorectal tumors and matched peritumoral tissues. Additionally, we validated the correlation of FHOD1 expression with survival outcomes and clinicopathological variables in CRC patients. We conducted enrichment analysis on genes co-expressed with FHOD1 to elucidate their biological functions. Immune-related analyses clarified the association between FHOD1 and the immune microenvironment, and we assessed drug sensitivity linked to FHOD1. In addition, we validated the FHOD1's biological function in vitro. The findings suggest that FHOD1 may be a promising biomarker for CRC diagnosis and prognosis, potentially providing an advanced therapeutic strategy for its treatment.

2. Materials and methods

2.1. Raw data download, process, and analysis

We downloaded fragments per kilobase million (FPKM) gene-level data from the TCGA database (https://portal.gdc.cancer.gov/) [15]. These outcomes were subsequently transformed into transcripts per million (TPM) using R (version 4.2.2). Additionally, pan-cancer normalized gene expression data were obtained from the UCSC Xena platform (https://xena.ucsc.edu/) [16]. Differential analysis and visualization were conducted on 50 paired colorectal cancer samples from the TCGA database.

2.2. mRNA expression analysis

The TIMER2.0 (http://timer.cistrome.org/) provides insights into tumor immunology [17]. It evaluates gene expression, its link to prognostic, and immune infiltration across diverse cancer types. We employed this database to examine FHOD1 expression in both normal and tumor samples from multiple malignancies.

2.3. Prognostic analysis

To assess the impact of FHOD1 on patient prognosis, Kaplan–Meier and Cox regression analyses were conducted utilizing the "survminer" and "survival" packages in R. We specifically examined overall survival (OS), disease-specific survival (DSS), and progression-free interval (PFI) as key prognostic indicators. The hazard ratio (HR) for FHOD1 expression was first estimated with a univariate Cox proportional hazards model, followed by adjusted HR calculation through a multivariate Cox model. A $p < 0.05$ was established as the threshold for statistical significance.

2.4. The function and enrichment analysis

LinkedOmics (www.linkedomics.org/login.php) was employed to conduct gene co-expression analysis of FHOD1 [18]. Spearman's test identified connection between FHOD1 and co-expressed genes. The LinkInterpreter cohort was utilized to conduct pathway analyses of differentially expressed genes, while gene set enrichment analysis (GSEA) method was utilized to carry out KEGG and GO analyses [19,20].

2.5. Immune infiltration analysis

The Tumor Immune Single-Cell Hub 2 (TISCH2) database (http://tisch.comp-genomics.org/) delivers comprehensive single-cell RNA sequencing (scRNA-seq) data, meticulously cataloging the diversity of immune cell populations across a wide range of cancer types [21]. This resource serves as a goldmine for researchers aiming to delve into the intricate interplay between immune cells and tumor microenvironments. The single-sample gene set enrichment analysis (ssGSEA) approach from the GSVA R package was employed to investigate the association between FHOD1 expression and 24 immune cell types in CRC.

2.6. Drug sensitivity analysis

Chemosensitivity is a key factor influencing the effectiveness of chemotherapy in patients with CRC. We leveraged drug sensitivity information sourced from the CellMiner database (https://discover.nci.nih.gov/cellminer/home.do) [22], which provides comprehensive drug responses across 60 human cancer cell lines. This database allows the exploration of correlations between FHOD1 expression and drug sensitivity, facilitating identification of potential therapeutic targets. Data analysis was carried out using R software (version 4.2.2) with packages such as "impute" for handling missing data, "limma" for differential expression analysis, and "ggplot2" for visualizing the results. This approach enabled a more robust evaluation of drug sensitivity patterns in relation to FHOD1 expression across different cell lines.

2.7. Cell culture and transfection

The human CRC cell lines HCT116 and RKO NL (Procell, Wuhan, China) were maintained in DMEM (Biosharp, Hefei, China) containing 10% FBS (Vazyme, Nanjing, China) at 37 ℃ under 5% CO_2. An appropriate number of cells were seeded onto culture plates using Opti-MEM medium (Gibco) for culture. Small interfering

RNA (siRNA) (stB0010925A-C, RIBOBIO, China) and in vitro siRNA transfection reagent (Yeasen, Shanghai, China) were mixed at the recommended ratio and added to Opti-MEM. Subsequent experiments were performed 48 hours post-transfection

2.8. RNA isolation and quantitative real-time PCR (qRT-PCR)

Total RNA was extracted by TRI reagent (Sigma, St.Louis,USA). The high-quality RNA obtained was then converted into complementary DNA (cDNA) using cDNA Synthesis SuperMix (Yeasen, Shanghai, China). For the qPCR process, SYBR Green Master Mix (Yeasen, Shanghai, China) was consumed. Endogenous GAPDH expression served as a control for standardized quantification. The primer sequences were listed below:

FHOD1:
Forward, 5′-CCTCAGCTGACACCTCCAG-3′;
Reverse, 5′-CAGCGCAACCTGCTTCTC-3′.
GAPDH:
Forward: 5′-GCACAGTCAAGGCCGAGAAT-3′;
Reverse: 5′-GCCTTCTCCATGGTGGTGAA-3′.

2.9. Western blot

Protein was extracted from cells using RIPA lysis buffer supplemented with protease inhibitors (MCE, Shanghai, China). After electrophoretic separation, the proteins were transferred onto a PVDF membrane (Millipore, Massachusetts, USA). Following a 2-h blocking with 5% non-fat milk, the membrane was rinsed with PBST and treated with the corresponding antibodies. Protein expression was analyzed using ECL ultra-sensitive reagent (Yeasen, Shanghai, China) on a chemiluminescence imaging system (Tanon, Shanghai, China). The primary antibodies employed were mouse antiGAPDH (1:1000; Proteintech, Wuhan, China), mouse anti-FHOD1 (1:1000; Santa Cruz, USA).

2.10. CCK8 assay and colony formation assay

Per well of the 96-well plate contained approximately 1000 cells. Cell viability was calculated with the CCK-8 solution (MCE, Shanghai, China). The optical density (OD) value per well was recorded at 450 nm. Approximately 800 cells were grown in each well of the 6-well plate, with culture medium being refreshed every three days. After 14 days of incubation, the cells were incubated in 4% formaldehyde (Beyotime, Jiangsu, China) for 30 min and then in 0.1% crystal violet (Yeasen, Shanghai, China) for additional 30 min. Cell colonies were quantified with ImageJ software.

2.11. Wound healing assay and transwell migration assay

When the cell density in the 6-well plate reached 80%–90%, appropriate scratches were made using a 100μL pipette tip, followed by imaging under a microscope. After 48 h of culture, images were captured again to measure and document cell migration distances. For transwell migration assays, 100μL of serum-free medium containing 1×10^5 cells was added to the upper chamber, while 500μL

of medium containing 20% FBS was added to the lower chamber. Following 24 h of incubation, cells were incubated in 4% paraformaldehyde for 30 min and then in 0.1% crystal violet for another 30 min before being visualized under a microscope. All experimental results were analyzed using ImageJ software.

2.12. Statistical analysis

Each statistical analysis was performed using GraphPad Prism 8.0 (GraphPad, CA, USA). Unpaired *t*-tests were used to compare two groups. The screening criterion for all data was $p < 0.05$. Each experiment was conducted a minimum of three times.

3. Results

3.1. Expression level of FHOD1 in CRC

At first, to evaluate variances in FHOD1 expression between tumor and normal tissues, we analyzed the FHOD1 expression levels across various cancer types using the TIMER 2.0 database. The findings indicated that FHOD1 levels were notably elevated in COAD (colon adenocarcinoma), ESCA (esophageal carcinoma), LIHC (liver hepatocellular carcinoma), READ (rectum adenocarcinoma), STAD (stomach adenocarcinoma), ect. relative to corresponding normal tissues. In contrast, FHOD1 expression was significantly lower in KICH (kidney chromophobe), LUSC (lung squamous cell carcinoma) and PAAD (pancreatic adenocarcinoma) relative to corresponding normal tissues (**Figure 1a**). Besides, a significant upregulation of FHOD1 expression was identified in five cancer types, specifically in paired tumor tissues relative to the adjacent normal tissues. (**Figure 1b**). Both unpaired and paired expression data analyses indicated that FHOD1 expression was markedly higher in CRC samples relative to normal samples (**Figure 1c,d**).

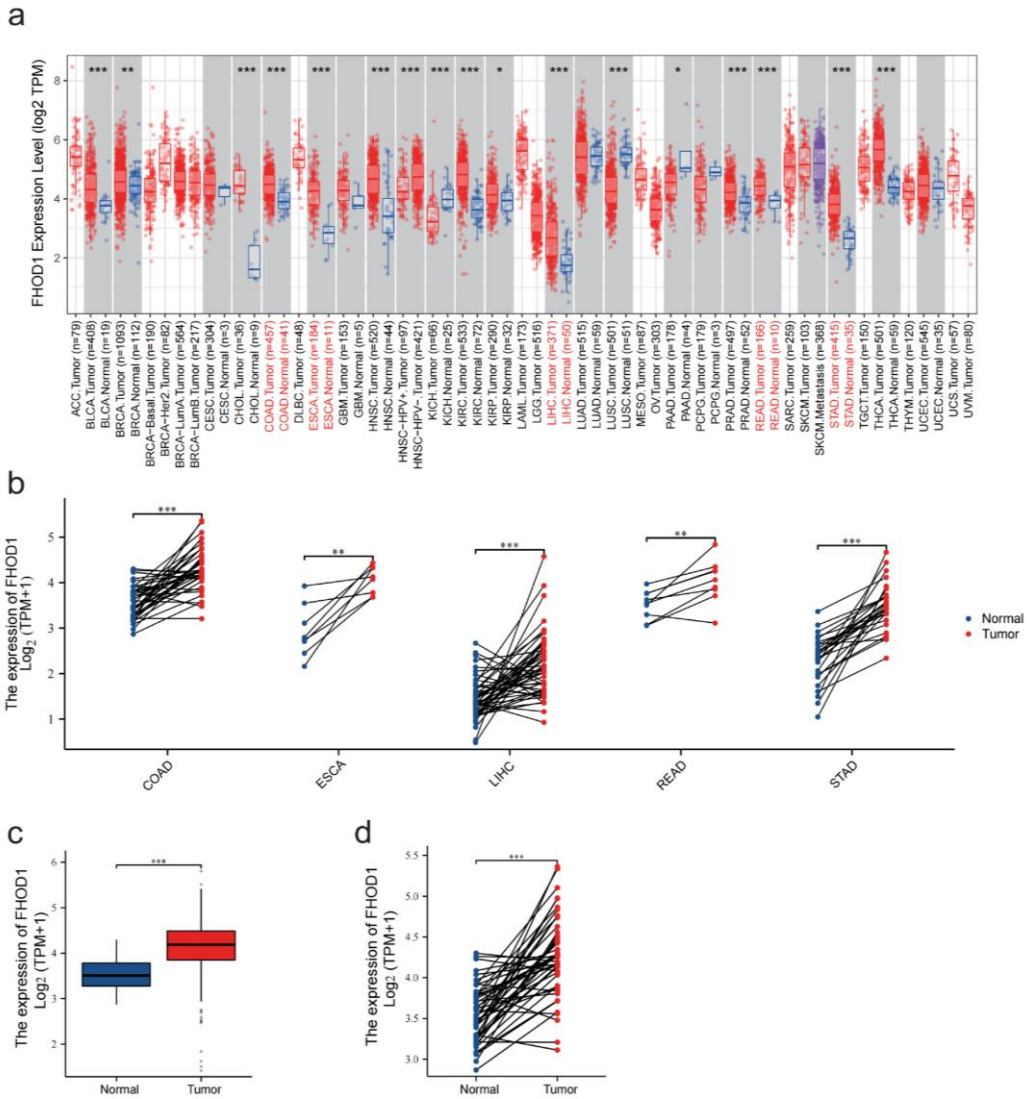

Figure 1. FHOD1 expression profiles in CRC: **(a)** Expression of FHOD1 across multiple cancer types; **(b)** Evaluation of FHOD1 expression by comparing tumor tissues with corresponding normal tissues; **(c,d)** Unpaired and paired expression data analyses indicated that FHOD1 expression was markedly higher in CRC.

3.2. Prognostic value of FHOD1 in CRC

Kaplan-Meier survival curve analysis displayed that CRC patients exhibiting elevated FHOD1 expression had lower overall survival (OS) and disease-specific survival (DSS) compared to those in the low-expression group. ($p < 0.05$) (**Figure 2a,b**). However, no notable statistical distinction was observed in the progression-free interval (PFI) between CRC patients exhibiting elevated FHOD1 expression and those with reduced expression ($p = 0.074$) (**Figure 2c**). Further subgroup analysis of multiple clinical features showed that OS was shorter in the group with high FHOD1 expression compared to the group with low FHOD1 expression in the following cases: Pathologic T stage: T3&T4 ($p = 0.031$), Pathologic N stage: N0 ($p = 0.024$), age > 65 ($p = 0.016$), male ($p = 0.032$). However, the OS rate was statistically similar in cases of pathologic M stage: M0 ($p = 0.068$), CEA level: > 5 ($p = 0.052$) (**Figure 2d–i**). The above results indicated that FHOD1 may be a good prognostic factor for CRC.

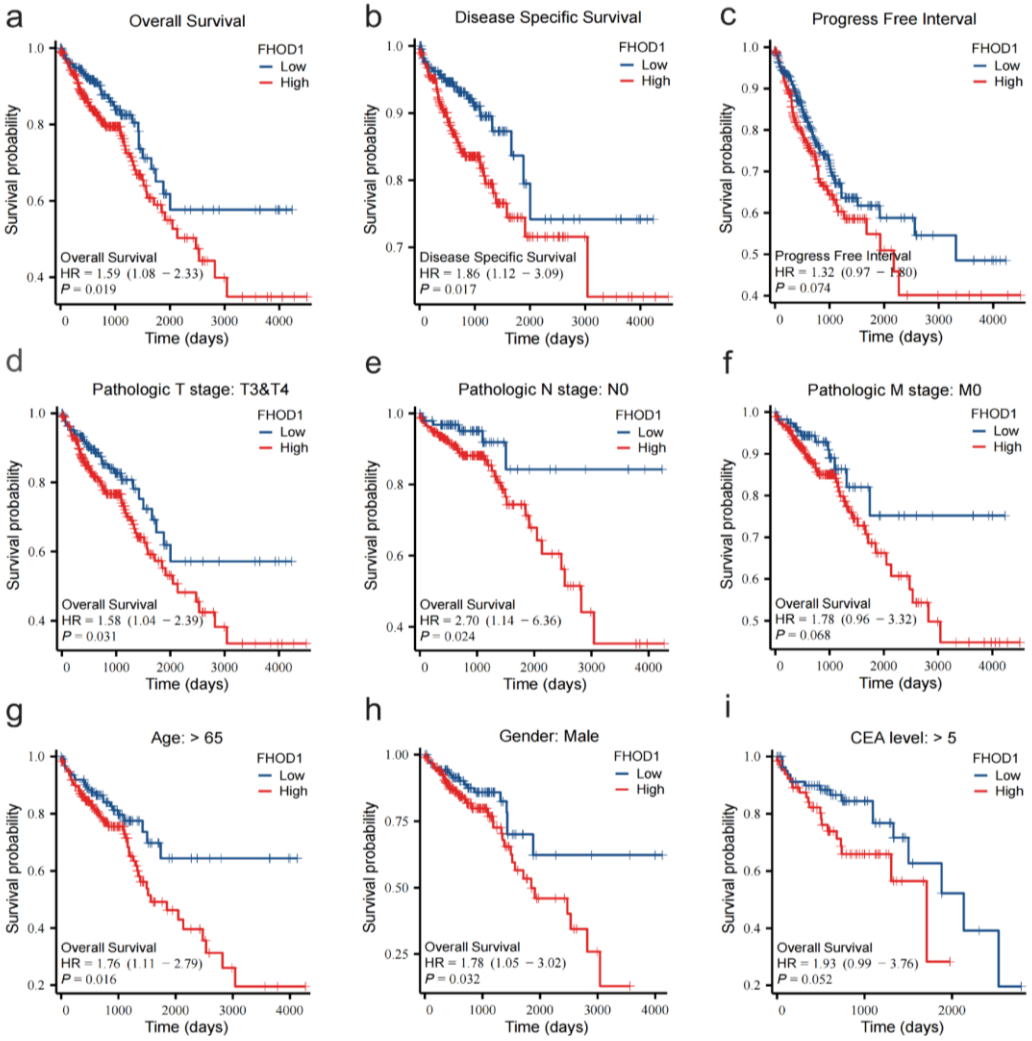

Figure 2. The prognostic analysis of FHOD1 in CRC: **(a–c)** The OS, DSS, PFI curve in CRC patients with high and low expression of FHOD1; **(d–i)** Subgroup analysis based on T3&T4, N0, M0, Age > 65, Male, CEA level: > 5.

3.3. Functional enrichment analysis of FHOD1 co-expressed genes in CRC

We employed the LinkedOmics to evaluate the FHOD1 mode of co-expression in the CRC cohort. The volcano map indicated the genes positively and negatively related to FHOD1 (**Figure 3a**). The heatmap illustrated 50 significant genes that were positively or negatively correlated with FHOD1 (**Figure 3b,c**). According to the GSEA-annotated GO terms, FHOD1 co-expression genes were mainly associated with the intestinal immune network for IgA production, osteoclast differentiation, hematopoietic cell lineage, graft-versus-host disease, staphylococcus aureus infection, Th1 and Th2 cell differentiation, autoimmune thyroid disease, Th17 cell differentiation, etc (**Figure 3d**). Besides, KEGG analysis illustrated that these genes were primarily associated with the adaptive immune response, leukocyte apoptotic process, interleukin-2 production, regulation of GTPase activity, T cell activation, interferon-gamma production, leukocyte cell-cell adhesion, collagen metabolic process, etc (**Figure 3e**).

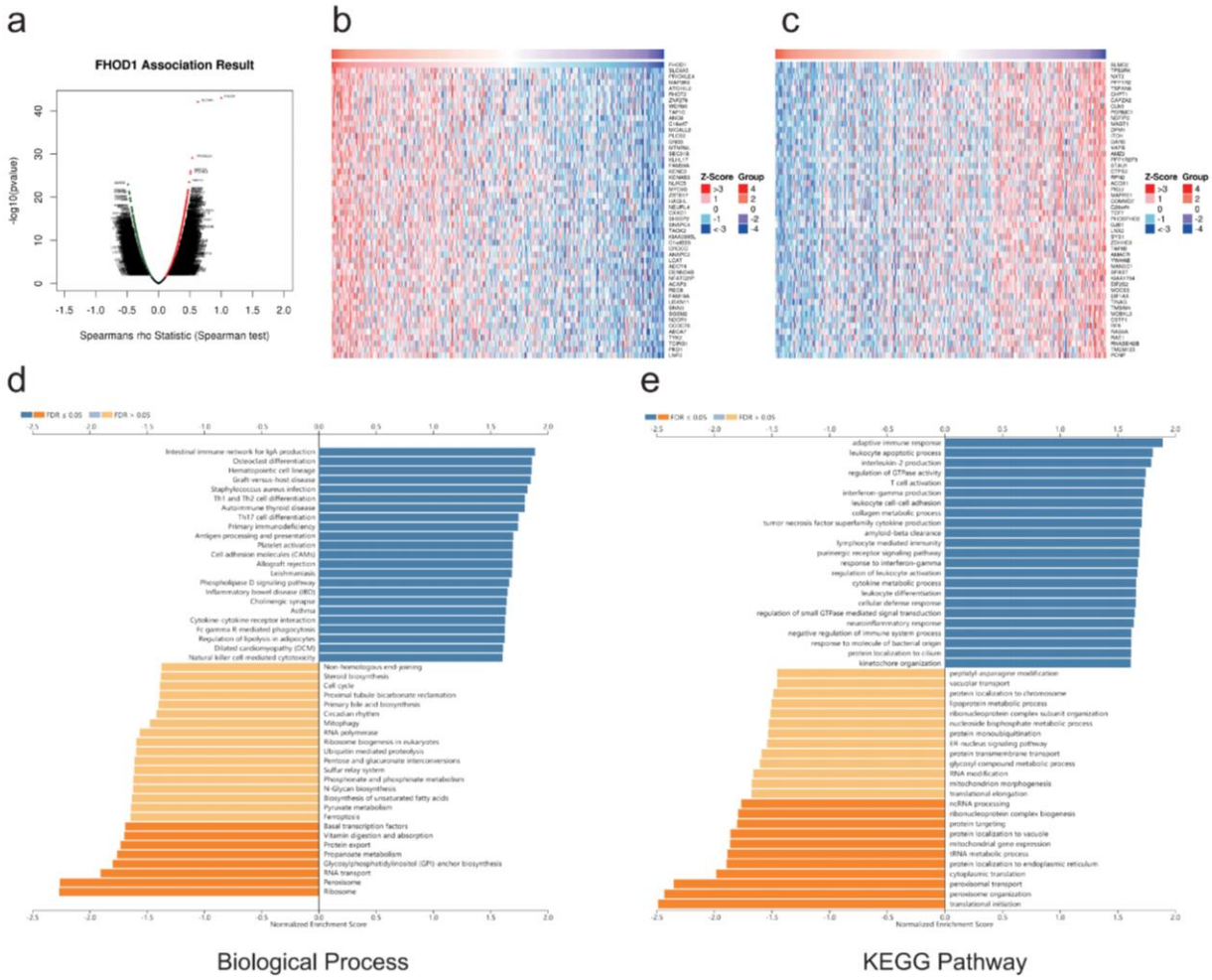

Figure 3. The enrichment analysis of FHOD1 in CRC: **(a)** Volcano plot displayed genes positively and negatively associated with FHOD1 expression; **(b,c)** Heat maps respectively presented the top 50 genes positively and negatively correlated with FHOD1; **(d,e)** Biological Process and KEGG Pathway analysis of FHOD1 co-expression genes.

3.4. The influence of FHOD1 on tumor microenvironment in CRC

Single-cell data analysis from the TISCH2 database indicated that FHOD1 was overexpressed in proliferating T cells, suggesting that FHOD1 might play an important role in proliferating T cells (**Figure 4a**). We further analyzed two colorectal cancer datasets: CRC-GSE139555 and CRC-GSE166555. The results indicated that FHOD1 expression was widely distributed across various immune cell types in the colorectal cancer microenvironment, such as B cells, conventional $CD4^+$ T(CD4Tconv) cells, $CD8^+$ T cells, Mast cells, monocytes or macrophages (Mono/Macro), etc (**Figure 4b-e**).

Subsequently, we employed the ssGSEA algorithm to assess the relationship between 24 immune cell types and FHOD1 expression in CRC. Among them, the top 10 immune cells positively correlated with FHOD1 expression included natural killer (NK) cells, $CD56^{bright}$ NK cells, regulatory $CD4^+$ T (Treg) cells, interstitial dendritic cells (IDC), $CD56^{dim}$ NK cells, CD8 T cells, Cytotoxic cells, Dendritic cells (DC), T follicular helper (TFH) cells and Mast cells. In contrast, those negatively correlated with FHOD1 expression were T helper cells and central memory T (Tcm) cells (**Figure 5a**). In addition, box plots specifically illustrated correspondence between

four distinct types of immune cells and the expression of FHOD1, including NK cells (R = 0.419), CD56^{bright} NK cells (R = 0.404), Treg cells (R = 0.281) and CD56^{dim} NK cells (R = 0.254) (**Figure 5b–e**). Above findings indicate that FHOD1 expression might inhibit the immune process of tumors and promote the immune escape of colon cancer cells.

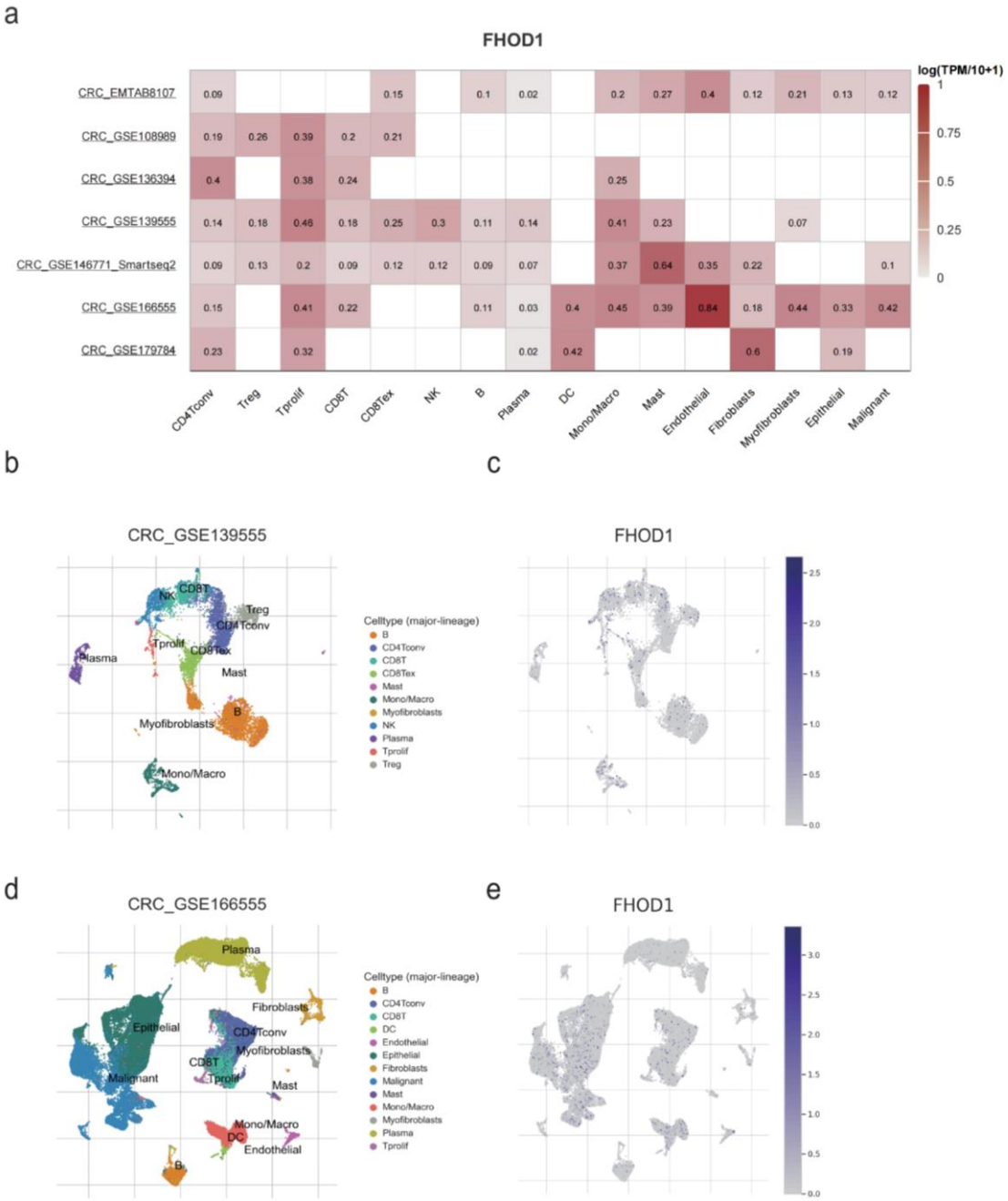

Figure 4. Association of FHOD1 with immune infltration levels in CRC: **(a)** FHOD1 expression across various immune cells by using the TISCH2 database; **(b–e)** The composition of cell types and single-cell expression profile of FHOD1 across CRC-GSE139555 and CRC-GSE166555 groups.

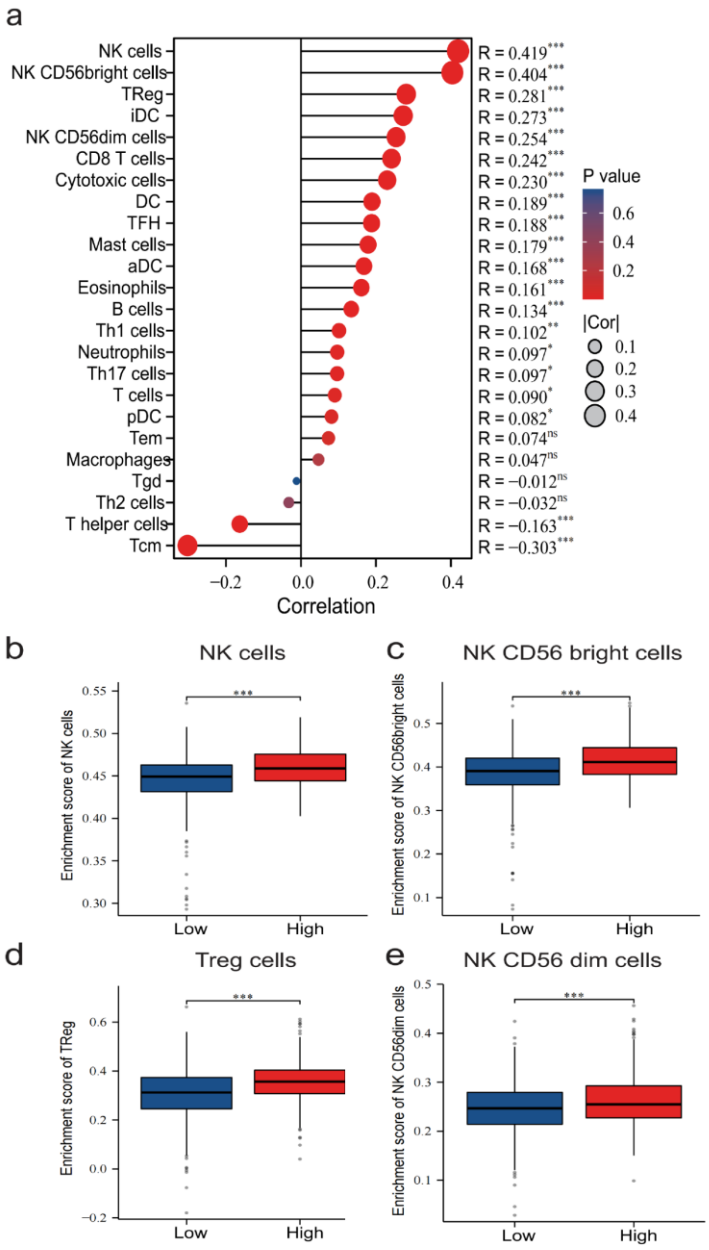

Figure 5. Relationship between FHOD1 and immune cells in CRC: **(a)** Relationships between the expression of FHOD1 and 24 types of immune infiltration cells. The size of the dots shows the absolute value of the Spearman R; **(b–e)** Relationships between FHOD1 expression and immune cell subsets.

3.5. FHOD1 influenced various drug sensitivity in CRC

Correlation analysis displayed that FHOD1 presented positive association with sensitivity to Raltitrexed (Cor = 0.436), Gemcitabine (Cor = 0.430), *R*-306465 (Cor = 0.402), 5-Fluoro deoxy uridine 10 (Cor = 0.392), Triethylenemelamine (Cor = 0.376), LMP-400 (Cor = 0.366), Pemetrexed (Cor = 0.359), Thiotepa (Cor = 0.358), Uracil mustard (Cor = 0.349), Cytarabine (Cor = 0.348), Gemcitabine elaidate (Cor = 0.342), Vorinostat (Cor = 0.339), Cladribine (Cor = 0.334), Cisplatin (Cor = 0.332). In contrast, FHOD1 expression was negatively correlated with sensitivity to LXS-196 (Cor = −0.355) and SM-406 (Cor =−0.339) (**Figure 6a–p**).

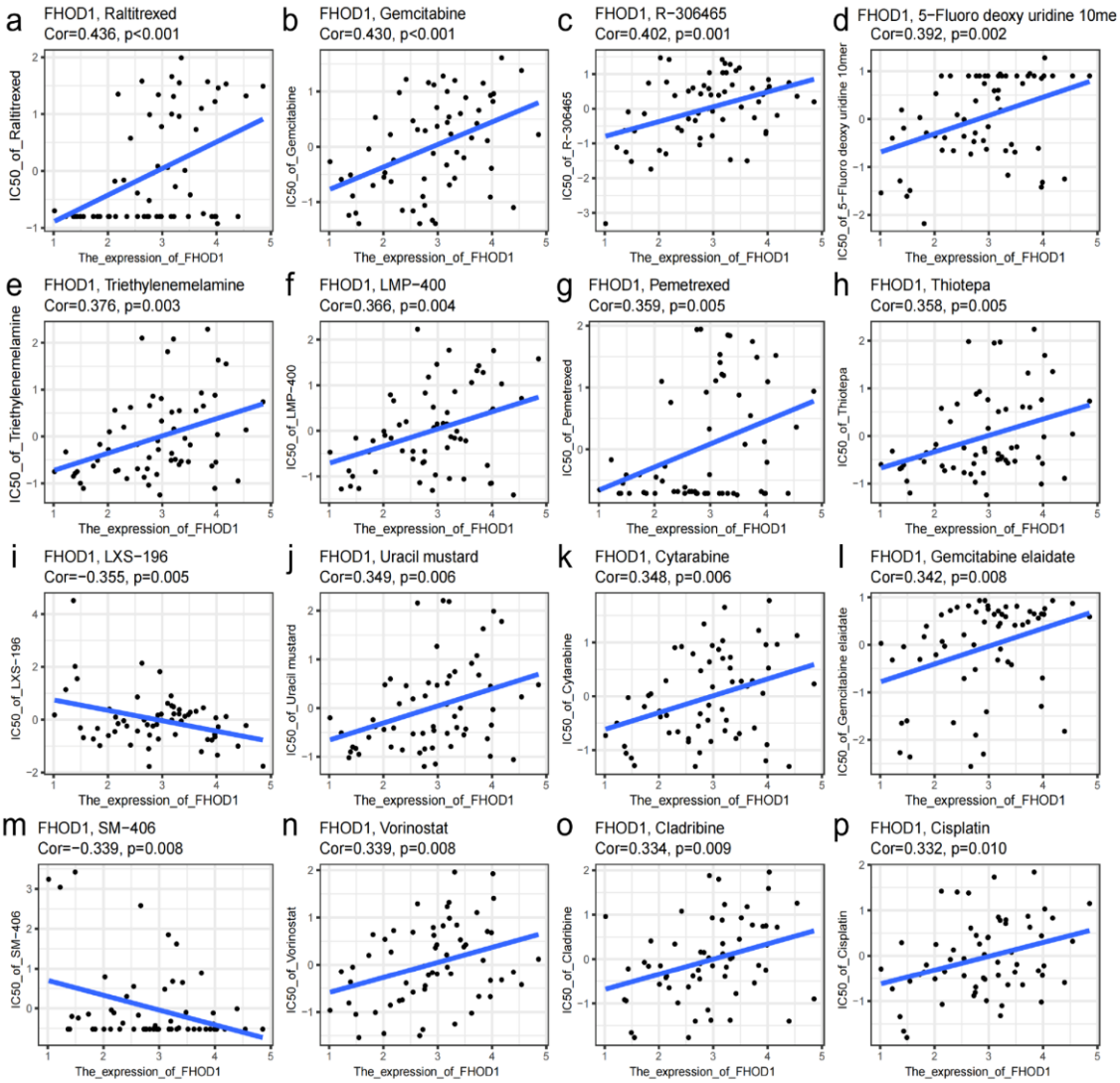

Figure 6. Drug sensitivity analysis of FHOD1 in CRC: **(a–p)** An illustration of the relationship between FHOD1 expression and predicted drug response.

3.6. The impact of FHOD1 on CRC cell proliferation and migration.

Under the condition of relative expression levels, we knocked down FHOD1 by introducing specific siRNAs in HCT116 and RKO NL colorectal cancer cells, followed by qRT-PCR analysis (**Figure 7a,b**) and western blot analysis (**Figure 7c,d**). CCK-8 assays showed suppression of FHOD1 in HCT116 and RKO NL cells inhibited cell proliferation (**Figure 8a,b**). Colony formation assays revealed FHOD1 knockdown reduced the colony number of HCT116 and RKO NL cells (**Figure 8c,d**). Wound healing and Transwell migration assays revealed FHOD1 knockdown impaired the migratory potential of HCT116 and RKO NL cells (**Figure 9a–d**).

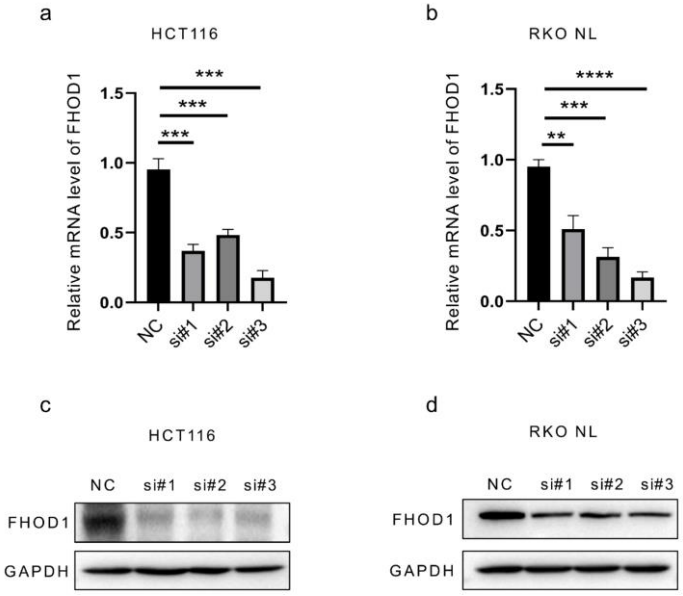

Figure 7. Knockdown of FHOD1 in CRC cells: **(a,b)** Transfection efficiency of FHOD1 siRNA in HCT116 and RKO NL by qRT-PCR; **(c,d)** Transfection efficiency of FHOD1 siRNA in HCT116 and RKO NL by Western blot.
$^{**}p < 0.01$,$^{***}p < 0.001$,$^{****}p < 0.0001$.

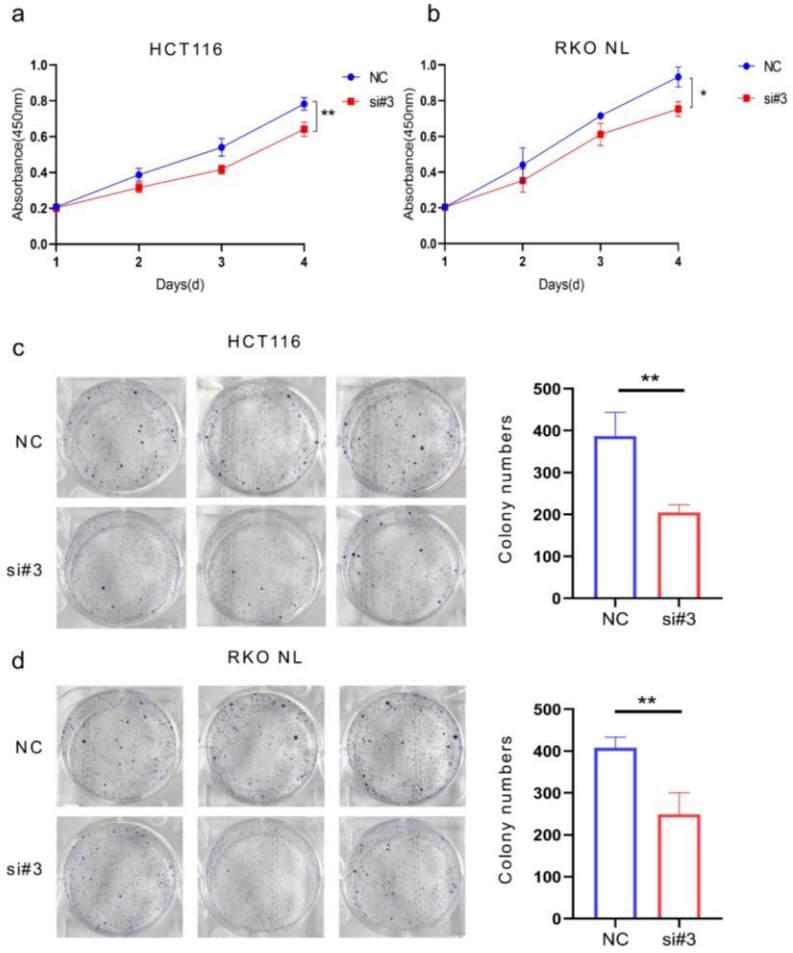

Figure 8. FHOD1 promoted colorectal cancer cell proliferation in vitro: **(a,b)** The CCK8 assays to detect the function of FHOD1 on cancer cell proliferation; **(c,d)** The colony formation assays to detect the function of FHOD1 on cancer cell colony formation ability.
$^{*}p < 0.05$, $^{**}p < 0.01$.

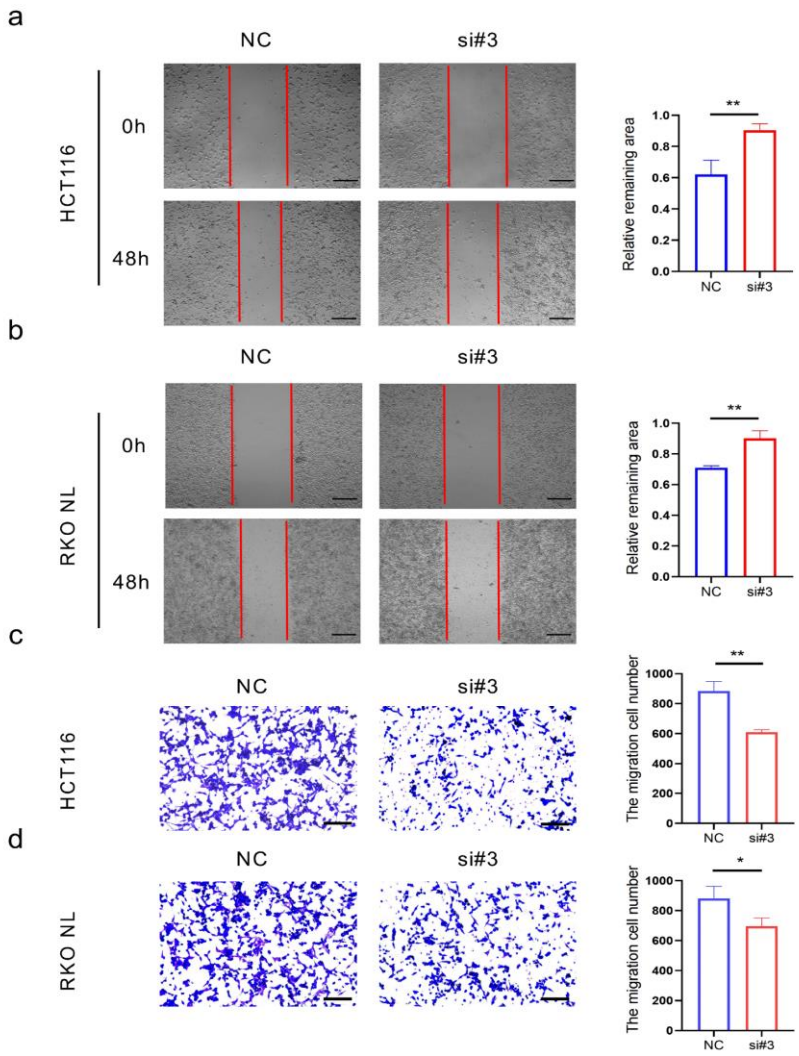

Figure 9. FHOD1 promoted CRC cell migration in vitro: **(a,b)** The wound healing assays; **(c,d)** Transwell migration assays to detect the function of FHOD1 on cancer cell migrative capacity.
*$p < 0.05$, **$p < 0.01$.

4. Discussion

Colorectal cancer (CRC) is recognized a highly complex and heterogeneous malignancy. Despite advances in various treatment modalities, the survival rate of patients with late-stage CRC remains relatively low. Therefore, there is an urgent need to discover new factors for predicting and effectively managing CRC as well as for designing novel therapeutic interventions [23]. FHOD1 is upregulated in several cancers including glioma, melanoma, squamous cell carcinoma, gastric cancer, and breast cancer, and its overexpression is associated with poor survival. Numerous studies have indicated that decreased expression of FHOD1 in cancer cells diminishes their proliferation, migration, and invasion capabilities [4,6,7,9,10]. Currently, the relationship between FHOD1 expression and CRC remains to be fully established. This research seeks to describe FHOD1's role in CRC and suggest that elevated FHOD1 expression correlates with unfavorable outcomes. Our study enhances the comprehension of FHOD1's potential mechanisms in tumor

immunology, and highlights a diagnostic and therapeutic target for personalized treatment of colorectal cancer.

To obtain reliable results, we examined FHOD1 expression across 24 tumor types and adjacent normal tissues, finding that FHOD1 is highly expressed in most tumors. Specifically, the CRC cohort exhibited higher FHOD1 expression compared to its adjacent normal tissues, which was further confirmed by paired sample testing. Therefore, monitoring the expression level of FHOD1 may be an effective method for diagnosing CRC. Prognostic analysis revealed that patients exhibiting low FHOD1 expression experienced significantly better survival rates compared to those with elevated FHOD1 levels. Low expression of FHOD1 has been reported to predict a good prognosis in HER2-positive breast cancer and gastric cancer [6,11].

To explore the biological functions of FHOD1, we constructed a co-expression system and conducted enrichment analysis, revealing FHOD1-related genes mainly affect onset and progression of CRC through various immune-related functions, such as regulating intestinal immune systems for IgA generation and adaptive immune responses. Dysregulation of intestinal immune systems for IgA generation is significant in lung metastases among CRC patients, highlighting its potential role in cancer progression [24]. Adaptive immune cells are involved in CRC progression and metastasis, and have a prognostic value [25]. Furthermore, research has characterized the adaptive immune response in microsatellite instability (MSI) CRC, showing that T lymphocyte density is higher in MSI CRC than in microsatellite stable (MSS) CRC [26].

TME is crucial to the advancement and treatment response of CRC [27]. Immune analysis revealed the role of FHOD1 within the CRC microenvironment and its relevance to the immune cells. Single-cell analysis indicated that FHOD1 expression was upregulated in proliferating T cells. Previous studies have emphasized the clonal expansion potential of proliferating CD4+ T cells as tumor-responsive T cells [28]. Further examination of FHOD1's connection with immune cell infiltration in CRC revealed a close association with "NK cells" and "Treg cells". A comprehensive analysis of the percentages of NK cells surface receptors and cytotoxic granules among various cancer patients, including CRC, highlights the significant role of NK cells characteristics among different cancer types in potential therapeutic interventions [29]. Peripheral blood NK cells have prognostic value in CRC patients, serving as independent predictors of survival [30]. NK cells and T cells contribute synergistically to eliciting CRC tumor antigen-specific immune responses [31]. Granzyme B-expressing Treg cells can accumulate in CRC and possess the potential to eliminate conventional T cells [32]. We propose that FHOD1 may regulate the TME primarily through modulating these immune cells, although further validation is necessary.

Drug responsiveness is a vital element in cancer study. Our results indicate that FHOD1 can enhance sensitivity to various chemotherapeutic drugs. This suggests that assessing FHOD1 expression levels can be a dependable indicator for clinical treatment. Consistent with bioinformatics analysis, our functional experiments confirmed that FHOD1 exerts a tumor-promoting effect in CRC cells. Following knockdown of FHOD1 in HCT116 and RKO NL cell lines, cell proliferation and migration were significantly inhibited.

In brief, we elucidated the correlation between FHOD1 expression and adverse outcomes in patients, underscoring its clinical significance. Unlike previous studies that primarily focused on FHOD1 expression and cellular functions in other cancer types, this study integrated an analysis of FHOD1's relationship with immune infiltration in CRC. Specifically, we explored associations between FHOD1 and various immune cell types involved in tumorigenesis. These findings underscored FHOD1's potential involvement in the tumor immune microenvironment and expanded the understanding of its contribution to tumor immunology. Above insights hold crucial clinical implications, FHOD1 could serve as a valuable prognostic biomarker, enabling more precise risk stratification of CRC patients. Additionally, targeting FHOD1 may represent a novel therapeutic approach to disrupt tumor growth and immune evasion. Beyond its immediate clinical relevance, our study establishes a foundation for further exploration into the molecular mechanisms underlying FHOD1 in CRC. Future research directions include examining FHOD1's interactions with specific immune cell subpopulations and its involvement in signaling pathways driving tumor progression.

However, this study has certain limitations that warrant further discussion. Our research primarily relied on data from public databases, which may introduce systematic biases. First, these data were sourced from various platforms, each with unique data collection and processing protocols, potentially affecting the comparability and accuracy of the datasets. Additionally, these datasets may not fully represent diverse patient populations, such as variations in race or clinical factors, thereby limiting the generalizability of our findings. The dependence on publicly available datasets also restricted our ability to directly validate the quality of the original data. Missing data, errors, or incomplete clinical information within these databases further compromise the reliability of our analyses.

While we successfully identified the association between FHOD1 expression and prognosis, as well as its role in the CRC tumor microenvironment, the evidence remains indirect. To address these limitations, further validation through in vivo and in vitro experiments is necessary, such as using CRC animal models to investigate the interactions between FHOD1 and immune cells and their effects on tumor growth and metastasis. Moreover, exploring the molecular mechanisms by which FHOD1 regulates key signaling pathways involved in CRC progression could provide valuable insights into its biological role.

5. Conclusion

In conclusion, we have elucidated the expression of FHOD1 in CRC and its association with clinical prognosis and immune cell infiltration. This contributes to understanding the potential role of FHOD1 in determining colorectal cancer prognosis, developing new therapeutic strategies, and extending overall survival in CRC patients.

Author contributions: Conceptualization, YL; methodology, YL; software, YL; validation, YL; formal analysis, YL, HZ; investigation, YL; resources, YL; data curation, YL and HZ; writing—original draft preparation, YL, HZ and ZL; writing—

review and editing, YL, HZ and ZL; visualization, YL; supervision, ZW, ZZ, JD; project administration, XY. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the project of Suzhou Technology Bureau (SLJ2022011), Nuclear Technology Application Innovation Team, General Hospital of Nuclear Industry (XKTJ-HTD2021004).

Data availability statement: The authors confirm that the data supporting the findings of this study are available within the article.

Ethics approval: Not applicable.

Conflict of interest: The authors declare no conflict of interest.

References

1. Bray F, Laversanne M, Sung H, et al. Global cancer statistics 2022: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA: A Cancer Journal for Clinicians. 2024; 74(3): 229-263. doi: 10.3322/caac.21834
2. Shi X, Zhao S, Cai J, et al. Active FHOD1 promotes the formation of functional actin stress fibers. Biochemical Journal. 2019; 476(20): 2953-2963. doi: 10.1042/bcj20190535
3. Antoku S, Schwartz TU, Gundersen GG. FHODs: Nuclear tethered formins for nuclear mechanotransduction. Frontiers in Cell and Developmental Biology. 2023; 11. doi: 10.3389/fcell.2023.1160219
4. Jurmeister S, Baumann M, Balwierz A, et al. MicroRNA-200c Represses Migration and Invasion of Breast Cancer Cells by Targeting Actin-Regulatory Proteins FHOD1 and PPM1F. Molecular and Cellular Biology. 2012; 32(3): 633-651. doi: 10.1128/mcb.06212-11
5. Heuser VD, Mansuri N, Mogg J, et al. Formin Proteins FHOD1 and INF2 in Triple-Negative Breast Cancer: Association With Basal Markers and Functional Activities. Breast Cancer: Basic and Clinical Research. 2018; 12. doi: 10.1177/1178223418792247
6. Peippo M, Gardberg M, Kronqvist P, et al. Characterization of Expression and Function of the Formins FHOD1, INF2, and DAAM1 in HER2-Positive Breast Cancer. Journal of Breast Cancer. 2023; 26(6): 525. doi: 10.4048/jbc.2023.26.e47
7. Gardberg M, Kaipio K, Lehtinen L, et al. FHOD1, a Formin Upregulated in Epithelial-Mesenchymal Transition, Participates in Cancer Cell Migration and Invasion. PLoS ONE. 2013; 8(9): e74923. doi: 10.1371/journal.pone.0074923
8. Peippo M, Gardberg M, Lamminen T, et al. FHOD1 formin is upregulated in melanomas and modifies proliferation and tumor growth. Experimental Cell Research. 2017; 350(1): 267-278. doi: 10.1016/j.yexcr.2016.12.004
9. Heuser VD, Kiviniemi A, Lehtinen L, et al. Multiple formin proteins participate in glioblastoma migration. BMC Cancer. 2020; 20(1). doi: 10.1186/s12885-020-07211-7
10. Mansuri N, Heuser VD, Birkman EM, et al. FHOD1 and FMNL1 formin proteins in intestinal gastric cancer: correlation with tumor-infiltrating T lymphocytes and molecular subtypes. Gastric Cancer. 2021; 24(6): 1254-1263. doi: 10.1007/s10120-021-01203-7
11. Jiang C, Yuan B, Hang B, et al. FHOD1 is upregulated in gastric cancer and promotes the proliferation and invasion of gastric cancer cells. Oncology Letters. 2021; 22(4). doi: 10.3892/ol.2021.12973
12. Hui L, Chen Y. Tumor microenvironment: Sanctuary of the devil. Cancer Letters. 2015; 368(1): 7-13. doi: 10.1016/j.canlet.2015.07.039
13. Kim J, Bae JS. Tumor-Associated Macrophages and Neutrophils in Tumor Microenvironment. Mediators of Inflammation. 2016; 2016: 1-11. doi: 10.1155/2016/6058147
14. Xiao Y, Yu D. Tumor microenvironment as a therapeutic target in cancer. Pharmacology & Therapeutics. 2021; 221: 107753. doi: 10.1016/j.pharmthera.2020.107753
15. Ganini C, Amelio I, Bertolo R, et al. Global mapping of cancers: The Cancer Genome Atlas and beyond. Molecular Oncology. 2021; 15(11): 2823-2840. doi: 10.1002/1878-0261.13056

16. Nassar LR, Barber GP, Benet-Pagès A, et al. The UCSC Genome Browser database: 2023 update. Nucleic Acids Research. 2022; 51(D1): D1188-D1195. doi: 10.1093/nar/gkac1072
17. Li T, Fu J, Zeng Z, et al. TIMER2.0 for analysis of tumor-infiltrating immune cells. Nucleic Acids Research. 2020; 48(W1): W509-W514. doi: 10.1093/nar/gkaa407
18. Vasaikar SV, Straub P, Wang J, et al. LinkedOmics: analyzing multi-omics data within and across 32 cancer types. Nucleic Acids Research. 2017; 46(D1): D956-D963. doi: 10.1093/nar/gkx1090
19. Kanehisa M, Furumichi M, Sato Y, et al. KEGG for taxonomy-based analysis of pathways and genomes. Nucleic Acids Research. 2022; 51(D1): D587-D592. doi: 10.1093/nar/gkac963
20. Gene Ontology Consortium. The Gene Ontology resource: enriching a GOld mine. Nucleic Acids Res; 2021.
21. Han Y, Wang Y, Dong X, et al. TISCH2: expanded datasets and new tools for single-cell transcriptome analyses of the tumor microenvironment. Nucleic Acids Research. 2022; 51(D1): D1425-D1431. doi: 10.1093/nar/gkac959
22. Luna A, Elloumi F, Varma S, et al. CellMiner Cross-Database (CellMinerCDB) version 1.2: Exploration of patient-derived cancer cell line pharmacogenomics. Nucleic Acids Research. 2020; 49(D1): D1083-D1093. doi: 10.1093/nar/gkaa968
23. Shin AE, Giancotti FG, Rustgi AK. Metastatic colorectal cancer: mechanisms and emerging therapeutics. Trends in Pharmacological Sciences. 2023; 44(4): 222-236. doi: 10.1016/j.tips.2023.01.003
24. Shen T, Liu JL, Wang CY, et al. Targeting Erbin in B cells for therapy of lung metastasis of colorectal cancer. Signal Transduction and Targeted Therapy. 2021; 6(1). doi: 10.1038/s41392-021-00501-x
25. Grizzi F. Prognostic value of innate and adaptive immunity in colorectal cancer. World Journal of Gastroenterology. 2013; 19(2): 174. doi: 10.3748/wjg.v19.i2.174
26. Boissière-Michot F, Lazennec G, Frugier H, et al. Characterization of an adaptive immune response in microsatellite-instable colorectal cancer. OncoImmunology. 2014; 3(6): e29256. doi: 10.4161/onci.29256
27. Trimaglio G, Tilkin-Mariamé AF, Feliu V, et al. Colon-specific immune microenvironment regulates cancer progression versus rejection. OncoImmunology. 2020; 9(1). doi: 10.1080/2162402x.2020.1790125
28. Yue JH, Li J, Xu QM. Multi-omics analyses of single cell-derived colorectal cancer organoids reveal intratumor heterogeneity and immune response diversity. bioRxiv; 2022.
29. Peng YP, Zhu Y, Zhang JJ, et al. Comprehensive analysis of the percentage of surface receptors and cytotoxic granules positive natural killer cells in patients with pancreatic cancer, gastric cancer, and colorectal cancer. Journal of Translational Medicine. 2013; 11(1). doi: 10.1186/1479-5876-11-262
30. Tang YP, Xie MZ, Li KZ, et al. Prognostic value of peripheral blood natural killer cells in colorectal cancer. BMC Gastroenterology. 2020; 20(1). doi: 10.1186/s12876-020-1177-8
31. Sconocchia G, Eppenberger S, Spagnoli GC, et al. NK cells and T cells cooperate during the clinical course of colorectal cancer. OncoImmunology. 2014; 3(8): e952197. doi: 10.4161/21624011.2014.952197
32. Sun B, Liu M, Cui M, et al. Granzyme B-expressing treg cells are enriched in colorectal cancer and present the potential to eliminate autologous T conventional cells. Immunology Letters. 2020; 217: 7-14. doi: 10.1016/j.imlet.2019.10.007