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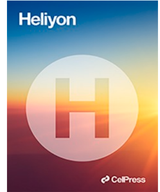

The lncRNA FENDRR inhibits colorectal cancer progression via interacting with and triggering GSTP1 ubiquitination by FBX8

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A R T I C L E I N F O

Keywords:
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A B S T R A C T

Background: Colorectal cancer (CRC) is characterized by its aggressiveness and high fatality rate. Long noncoding RNAs (lncRNAs) as molecular scaffolding in CRC have received little attention.
Methods: The TCGA database was used to find putative anti-oncogenic lncRNAs in CRC. The effect of FENDRR on CRC was evaluated using the colony formation assay, transwell assays, and wound healing assays, and FENDRR expression was validated by qRT-PCR. The location of the FENDRR binding proteins was determined by an RNA pull-down experiment, and the retrieved proteins were recognized by mass spectrometry. RNA immunoprecipitation (RIP) studies were used to demonstrate the interaction of GSTP1, FBX8, and FENDRR. Co-IP and immunofluorescence were utilized to confirm the connection between GSTP1 and FBX8. To determine the precise signaling pathways implicated in the action of FENDRR in CRC, we performed next-generation sequencing (NGS) on CRC cells transfected with a vector overexpressing FENDRR.
Results: The expression of FENDRR was significantly downregulated in CRC tissue and cells. The results of the function experiments showed that overexpression of FENDRR reduced CRC cells' ability to proliferation, invasion, migration and tube formation. In terms of mechanism, FENDRR could bind both GSTP1 and FBX8, act as a molecular scaffold, and utilize FBX8 to regulate the stability of GSTP1's protein. Additionally, the outcomes of NGS and qRT-PCR demonstrated that the expression of genes linked to the HIF-1 pathway was down-regulated following FENDRR overexpression. Lastly, rescue tests demonstrated that overexpression of GSTP1 in CRC cells could completely restore the inhibition induced by FENDRR.
Conclusion: In this study, we found that the molecular scaffolding protein FENDRR regulates the ubiquitination of GSTP1 and the suppression of the HIF-1 signaling pathway in the development of CRC. Our research provides more evidence of FENDRR's crucial role in the emergence of CRC and identifies it as a potential therapeutic target for CRC patients.

1. Introduction

The third most common cancers in the world, the incidence of colorectal cancer (CRC) is increasing year by year, and the cause of

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death ranks the fourth [1]. After comprehensive treatment, which may include surgery and chemotherapy, the 5-year survival rate for CRC patients is good and can range from 50% to 80% for people who are in the early stages of operable disease. However, for patients at a late stage with distant metastases, the surgical prognosis is not favorable, and the 5-year survival rate is just 31%–39% [2]. Previous studies have shown a direct link between genetic alterations and the emergence of CRC. For instance, 15% of CRC is brought on by the loss of function of the mismatch repair (MMR) genes, specifically hMSH2 and hMSH1 [3]. The RAS gene cluster, of which K-RAS is a subset, contains some of the most frequently altered cancer genes in CRC [4]. Cancer research is also looking at whether changes in lncRNA and other non-coding RNAs (ncRNAs) might serve as biomarkers or therapeutic targets.

LncRNAs are noncoding RNA molecules with more than 200 base pairs. Long non-coding RNAs (lncRNAs), which predominately exist in the nucleus and cytoplasm, are produced through variable splicing [5]. Although most lncRNAs do not have the required open reading frames to encode proteins [6] Some lncRNAs contribute to a number of biological processes and so have a crucial regulatory role in the development and spread of malignancies [7]. Recent research has shown that long noncoding RNAs (lncRNAs) play an important role in physiological and pathological processes, including cell proliferation, differentiation, and apoptosis, and can aid in the development of malignancies [8]. Studies have demonstrated a correlation between the expression of LINC00978 and the pathological stage of CRC, with the expression of LINC00978 being significantly higher in CRC tissues than in paracancer tissues. In the CRC, the sensitivity and specificity of the LINC00978 diagnosis are 72.7% and 81.0%, respectively, with an area under the ROC of 0.810. These results suggested that the CRC diagnosis accuracy of LINC00978 is very high [9]. Additionally, the SNHG17-Trim23-PES1 axis and the SNHG17-miR-339-5p-FOSL2-SNHG17 positive feedback loop are also associated with the acceleration of CRC and may be exploited as therapeutic targets [10]. These findings demonstrate how important lncRNA regulatory roles they play in CRC.

In this study, it was discovered that FENDRR can regulate GSTP1's ubiquitination and the deactivation of the HIF-1 signaling pathway, consequently regulating the growth of CRC tumors. Our research provides more evidence of FENDRR's crucial role in the emergence of CRC and raises the possibility that CRC patients may benefit from using it as a therapeutic target.

2. Materials and methods

2.1. Clinical tissues and cell lines

The Shanghai Tongren Hospital supplied 74 pairs of freshly collected CRC samples and their matched paracancerous normal colorectal tissues. All tissues were initially separated, and they were all maintained in liquid nitrogen for storage. This work has received permission from the Shanghai Tongren Hospital Ethics Committee (K2023-001-01). Human colorectal cell lines (FHC, HCT116, SW480, SW620, and LOVO) were obtained from the American Type Culture Collection (ATCC, USA) and grown in DMEM or RPMI 1640 supplemented with 10% fetal bovine serum (FBS), 1% penicillin/streptomycin, and maintained at 37° Celsius with 5% carbon dioxide. (ATCC, USA).

2.2. Plasmid and transfection

We acquired control siRNA (siSCR) and FENDRR's scrambled siRNA (siFENDRR) from GenePharma (Shanghai, China). FENDRR was overexpressed using pcDNA3.1FENDRR (pcDNA/FENDRR). SiRNA or plasmids were transfected into cells using Lipofectamine 3000 (Thermo Fisher Scientific). Cells were transfected with over expression plasmid using Lipofectamine 2000 (12,566,014, Invitrogen, Shanghai, China) following the manufacturer's protocols. To generate the lentivirus, 293FT cells were co-transfected with pcDNA3.1-FENDRR for over expression. Forty-eight hours after transfection, the lentiviral supernatants were collected and fltered through a 0.45-μm flter. The lentiviruses were added to media containing 8 μg/ml polybrene (Sigma, St. Louis, MO, USA) and transduced into CRC cells according to the manufacturer's instructions. Stable cells were selected for at least 1 week using G418 (10,131,035, Invitrogen, Shanghai, China).

2.3. Total RNA extraction and quantitative real-time PCR (qRT-PCR)

Total mRNA was isolated from tissues or cells using the Trizol (Invitrogen, USA) reagent, as per the manufacturer's instructions. (Life Technologies, CA, USA). CDNA was produced using a kit for an experiment involving inverse transcription. (Invitrogen, USA). Finally, qRT-PCR was carried out using the SYBR green PCR mix. (Takara). Internal standard controls like GAPDH or U6 were applied. Expression levels were calculated using a $2^{-\Delta\Delta ct}$ method.

2.4. Western blot

Total protein was extracted from SW480 and SW620 cells using RIPA buffer, then separated by SDS-PAGE, and finally transferred to a PVDF membrane. (Millipore, USA). After being blocked overnight at 4 °C with 5% skim milk powder, membranes were treated with primary antibodies (FBX8, 1:1000; GSTP, 1:1000). Membranes were rinsed three times the next day, and then treated with an HRP-labeled secondary antibody. Finally, the bands were analyzed using chemiluminescence. (beyotime, China).

2.5. Immunofluorescence staining

After being fixed with 2% paraformaldehyde (PFA, Solarbio, Beijing, China), cells were immediately penetrated for 15 min with

0.5% Triton X-100. After three washes with PBS, cells were incubated overnight at 4° Celsius with anti-FBX8 or anti-GSTP1 antibodies. The following day, cells were incubated with a fluorescent secondary antibody, and DAPI was utilized to counterstain the nuclei. Finally, images were captured using a laser confocal microscope.

2.6. Boinformatics analysis of online databases

The National Cancer Institute's Genomic Data Commons were utilized to download TCGA-COAD, which was then used to analyze the mRNAs that mattered most from the expression data of tumor samples and healthy tissues (Sangerbox, China).

2.7. RNA pulldown and mass spectrometry

In brief, after the FENDRR RNA-pulldown MS2 plasmid was created, it was transfected into CRC cells. Using protein A/G-agarose from Santa Cruz and anti-GST from Abcam in the United States, cells were collected 48 h after transfection and precipitated at 4 °C overnight. The beads were cleaned the next day, and using mass spectrometry, proteins were retrieved and identified.

2.8. Co-immunoprecipitation (Co-IP)

After being blocked with IgG or protein A + G agarose for 2 h, CRC cell extracts were incubated overnight at 4 °C with anti-FBX8 or anti-GSTP1 antibodies. The next day, proteins that had been separated by SDS-page were treated with primary antibodies. Last but not least, a kit for HRP-enhanced chemiluminescence was used to detect protein bands (beyotime, China).

2.9. RNA immunoprecipitation (RIP) assay

The RIP experiment was performed using a Magna RIPTM RNA Binding. Protein immunoprecipitation kit from Millipore, Billerica, Massachusetts, as directed by the manufacturer. The previously published method of measuring the expression levels of FENDRR was RT-qPCR.

2.10. Cell functional assay

A colony formation experiment involved the cultivation of cells for two weeks. Colonies were meticulously counted after being stained with 0.1% crystal violet.

For the cell invasion and migration test, either Matrigel or no Matrigel was added to the upper chamber of a Transwell membrane filter (Corning, NY, USA) (Corning). Following incubation, cells were fixed with 10% formaldehyde and stained with 0.1% crystal violet (Solarbio, Beijing, China).

Human umbilical vein endothelial cells (HUVECs) (Procell) were suspended in conditioned medium and seeded into 24-well plates coated with Matrigel for the tube formation experiment. After incubation, tube formation was observed under a microscope.

2.11. Xenograft tumor models

All animal experiments were approved by the Shanghai Tongren Hospital Ethics Committee. 10 BALB/c mice were randomly divided into two groups (siSCR and siFENDRR). SW480 cells were injected at a density of 1×10^6 cells per injection into BALB/c mice to create tumor xenograft models. One month after that, the mice were euthanized by an intraperitoneal injection of 100 mg/kg pentobarbital sodium (Sigma, St. Louis, MO, USA). The tumor was taken out, measured, and documented. Volume is equal to $0.5 \times (\text{length} \times \text{width}^2)$.

2.12. RNA-sequencing

In brief, SW480 cells were seeded to a 6-well plate at a density of 1×10^6 cells per well. Twenty-four hours later, cells were transfected with a FENDRR overexpression plasmid with the help of lipofectamine 2000 reagent (Invitrogen) according to the manufacturer's instructions. Cells were collected after 48 h for further RNA sequencing by Aksmics company (Shanghai, China).

2.13. Statistical analysis

GraphPad Prism 7 was used to show the results of the statistical analysis as the mean and standard deviation (SD). Student's t-test was used to examine the disparities between the two groups ($^{n.s.}P > 0.05$; $^{***}P < 0.001$).

3. Results

3.1. FENDRR was down-regulated in CRC

In order to discover possible anti-oncogenic lncRNAs, we first analyzed the expression profiles of DElncRNAs in CRC and adjacent

normal tissues using the TCGA database. Fig. 1A–B shows that, in contrast to normal tissues, CRCs considerably downregulated 1350 lncRNAs and dramatically elevated 596 lncRNAs (foldchange >2, $p<0.05$, Additional file 1: Dataset 1). In comparison to normal control tissue, the expression of FENDRR was significantly reduced in CRC tissue. (Fig. 1C). The association between CRC, the lower level of FENDRR was remarkably connected to poor overall survival of CRC. (Fig. 1D). Moreover, the results of RNA subcellular separation demonstrated that FENDRR is mostly located in the cytoplasm of CRC cells. (Fig. 1E). Afterwards, qRT-PCR was used to verify FENDRR expression in CRC and adjacent normal tissues. We discovered that FEDNRR was significantly downregulated in CRC tissues, as illustrated in Fig. 1F. Furthermore, we discovered that both CRC as well as normal colon epithelial (FHC) cell lines expressed FENDRR. When compared to FHC, the majority of CRC cells showed a reduced expression of FENDRR. (Fig. 1G). To achieve FENDRR overexpression or knockdown, either siRNAs or FENDRR overexpression vectors were generated and transfected into SW480 and SW620 cells. (Fig. 1H).

3.2. FENDRR suppressed cell proliferation, invasion and angiogenesis in CRC

First, we examined the impact of FENDRR on CRC cell proliferation using a colony formation experiment. Fig. 2A shows that FENDRR knockdown boosted colony formation in SW480 cells while FENDRR overexpression inhibited cell proliferation in SW620

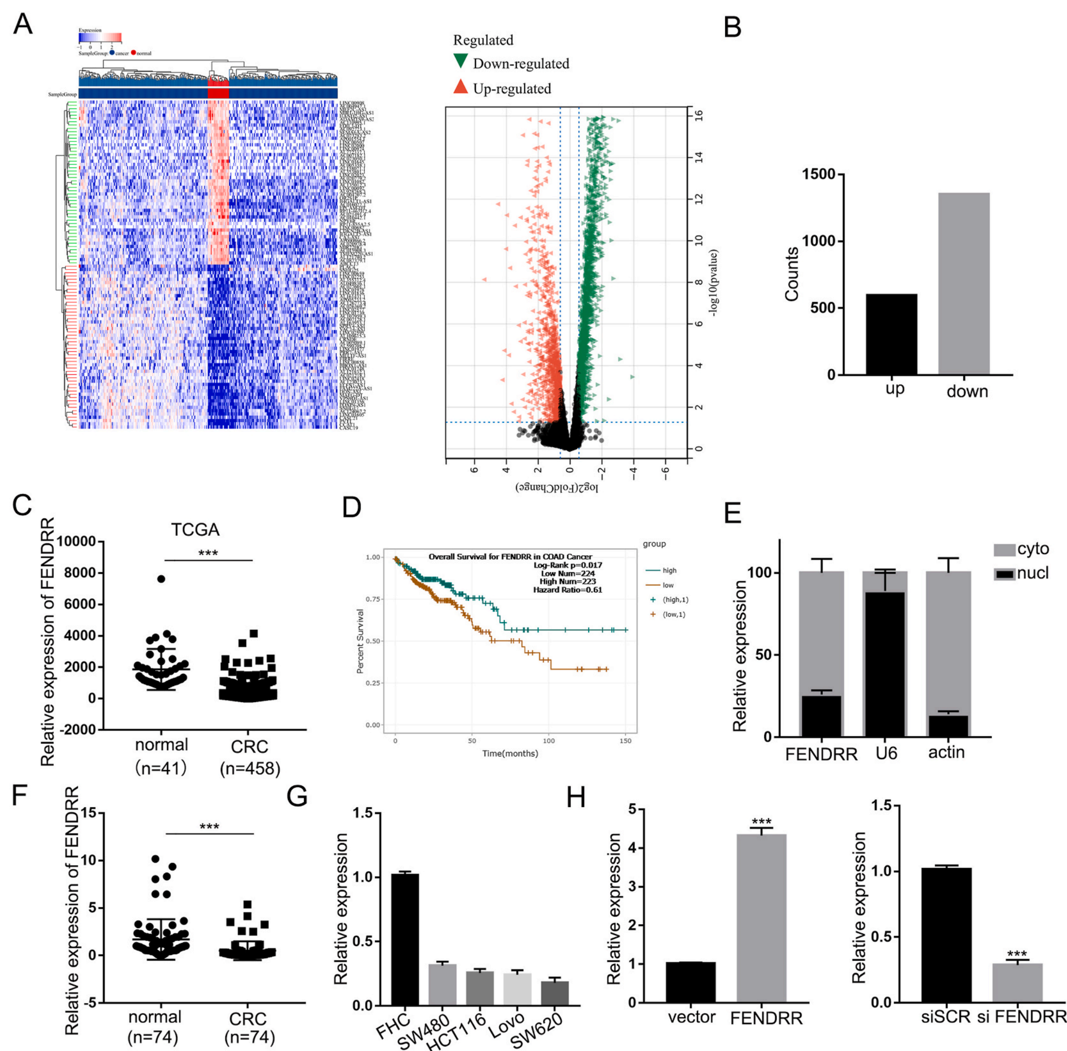

Fig. 1. FENDRR was down-regulated in CRC. (A) Differential analysis was performed to identify DElncRNAs in the TCGA-COAD dataset using the "limma" package. (B) The number of DElncRNAs in the TCGA-COAD dataset was shown. (C) The expression of FENDRR was observed to be down-regulated in tumor tissue in the TCGA-COAD dataset. (D) The effect of FENDRR expression on survival was conducted with Kaplan-Meier curves. (E) The localization of FENDRR in CRC cells was measured by subcellular fractionation. (F) The expression of FENDRR in clinical samples was measured by qRT-PCR. (G) The expression of FENDRR in CRC and paired normal cells was measured by qRT-PCR. (H) The expression of FENDRR in CRC cells was detected by qRT-PCR after FENDRR overexpression and knockdown.

cells. Additionally, it was shown using transwell assays to test that FENDRR overexpression also reduced the ability of SW620 cells to invasion and migration, while FENDRR knockdown markedly boosted the invasion and migration of SW480 cells compared to the control. (Fig. 2B–C). In addition, the results of the tube formation experiment revealed that when HUVECs were cultured in media preconditioned by SW480 cells with FENDRR knockdown, tube formation was significantly enhanced, whereas FENDRR overexpression in SW620 cells severely decreased tube formation. (Fig. 2D). To reveal the impact of FENDRR on CRC cell growth *in vivo*, xenograft experiments was performed. Fig. 2E reveals that tumor growth in the FENDRR knockdown group was significantly more rapid than that of the control group, with significantly greater tumor weights and volumes. The expression of cell proliferation markers including Ki67 and PCNA was likewise increased in response to FENDRR knockdown *in vivo*.

3.3. FENDRR interacted with GSTP1

It is well known that lncRNAs interact with binding factors to affect their target genes. Therefore, we performed an RNA pull-down

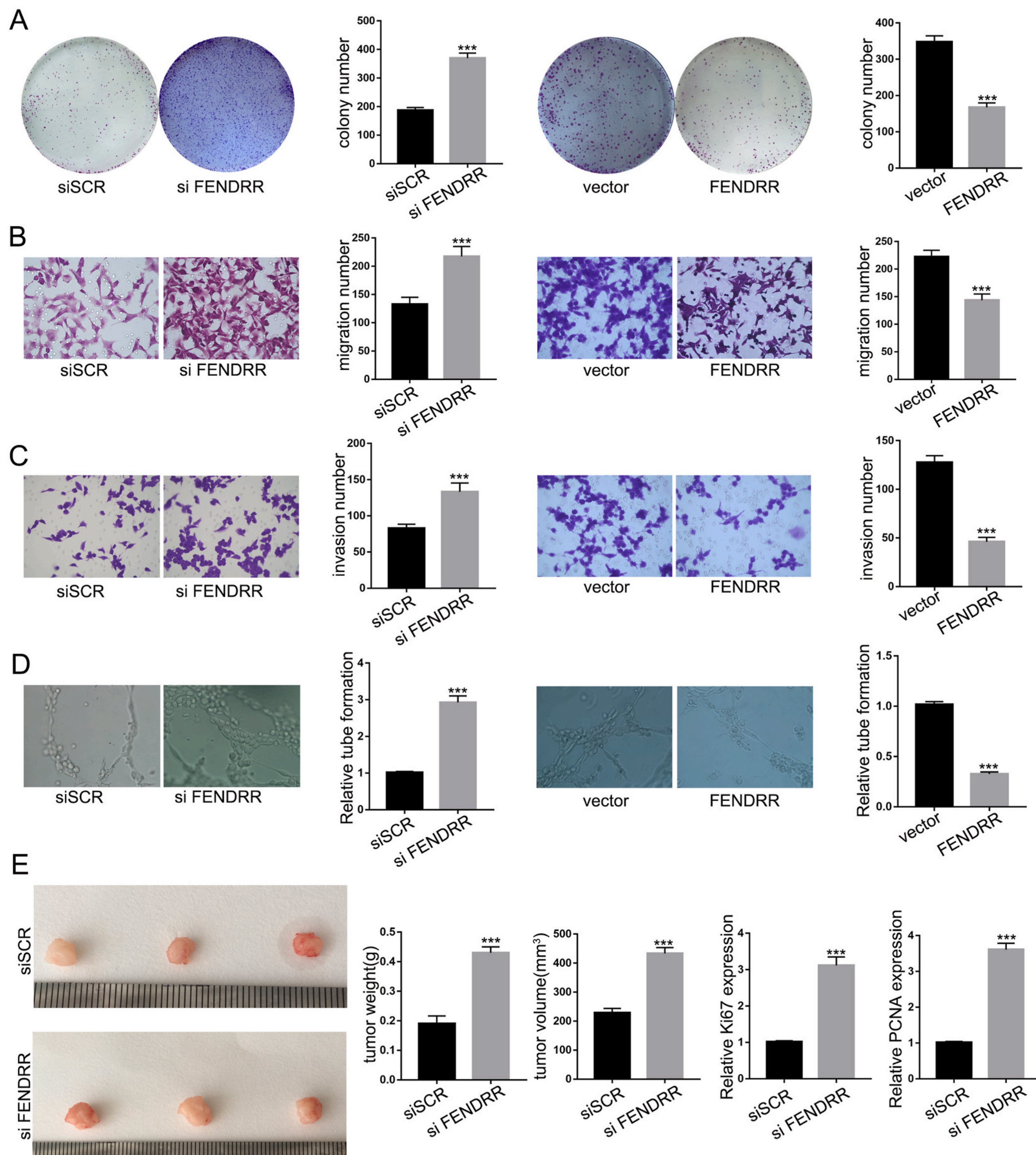

Fig. 2. FENDRR suppressed cell proliferation, invasion and angiogenesis in CRC. (A) Colony formation assay was performed to measure cell proliferation ability. (B-C) Transwell migration and matrigel invasion assays were used to detect the effect of FENDRR on cell migration and invasion. (D) The effects of FENDRR on tumor angiogenesis was detected by tube formation assay. (E) The effects of FENDRR on colorectal tumorigenesis were evaluated using the xenograft mouse model.

test to identify the proteins that bind to FENDRR. The proteins that were retrieved were discovered using mass spectrometry. As can be observed in Fig. 3A, mass spectrometry analysis allowed the identification of several proteins. The one that drew our attention the most was GSTP1. When compared to normal tissues, the CRC had unusually higher levels of GSTP1 expression, according to the TCGA database's expression analysis. (Fig. 3B). In order to pinpoint the particular region of the protein that interacted with GSTP1, the secondary structure of FENDRR with the lowest free energy (MFE) was predicted using the online tool RNAfold. (Fig. 3C). Based on the secondary structure, two shortened FENDRR probes representing two different stem-loops were constructed. Fig. 3D shows that whereas stem-loop #1 barely bound to GSTP, stem-loop #2 successfully drew down endogenous GSTP1 protein. The results of the TCGA correlation analysis, which was then used to ascertain the relationship between FENDRR and GSTP1, showed that the expression of FENDRR did not correlate with GSTP1 mRNA. (Fig. 3E). Similarly, neither FENDRR knockdown nor overexpression had an impact on the quantity of GSTP1 mRNA. (Fig. 3F). However, Western blot analyses revealed that after FENDRR overexpression, the level of GSTP1 protein was down-regulated, indicating that FENDRR regulated the protein stability of GSTP1. (Fig. 3G).

3.4. *FENDRR regulated the protein stability of GSTP1 via FBX8*

According to earlier research, FBX8 destroyed GSTP1 through ubiquitination, which prevented the progression of CRC. We questioned whether direct interaction between FENDRR and FBX8 may affect GSTP1's protein stability. The specificity of contacts between FENDRR and FBX8 was examined using RNA immunoprecipitation (RIP) experiments in SW480 and SW620 cells. (Fig. 4A). With the aid of mass spectrometry, it has been demonstrated that FBX8 and FENDRR interact. Following that, immunofluorescence was used to confirm that GSTP1 and FBX8 were colocalized. (Fig. 4B). The results of co-IP provided more evidence in favor of the association between GSTP1 and FBX8. (Fig. 4C). TCGA study also showed that FBX8 was turned down and that its mRNA level had little physical relationship with GSTP1. (Fig. 4D). Contrary to FENDRR, FBX8 overexpression or knockdown changed the level of the protein

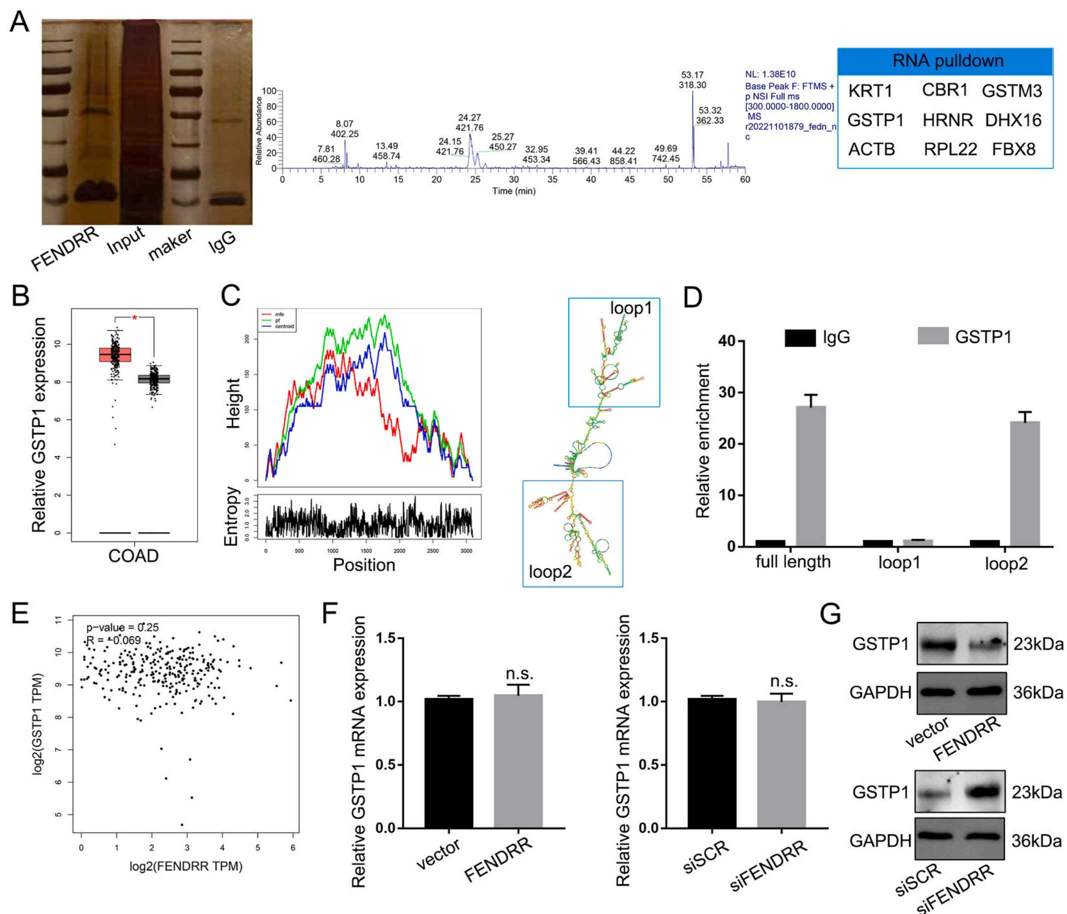

Fig. 3. FENDRR interacted with GSTP1. (A) Proteins retrieved from the FENDRR RNA pull-down assays were analyzed by SDS–PAGE with silver staining and validated by mass spectrometry. (B) The expression of GSTP1 was analyzed by TCGA database. (C) The secondary structure of FENDRR was predicted by bioinformatic analysis. (D) The interaction between FENDRR and GSTP1 was detected by RIP assay. (E) The relativity of FENDRR and GSTP1 mRNA was validated by TCGA analysis. (F) The expression of GSTP1 mRNA was measured by qRT-PCR after FENDRR overexpression or knockdown. (G) The protein level of GSTP1 was measured by Western blot after FENDRR overexpression or knockdown.

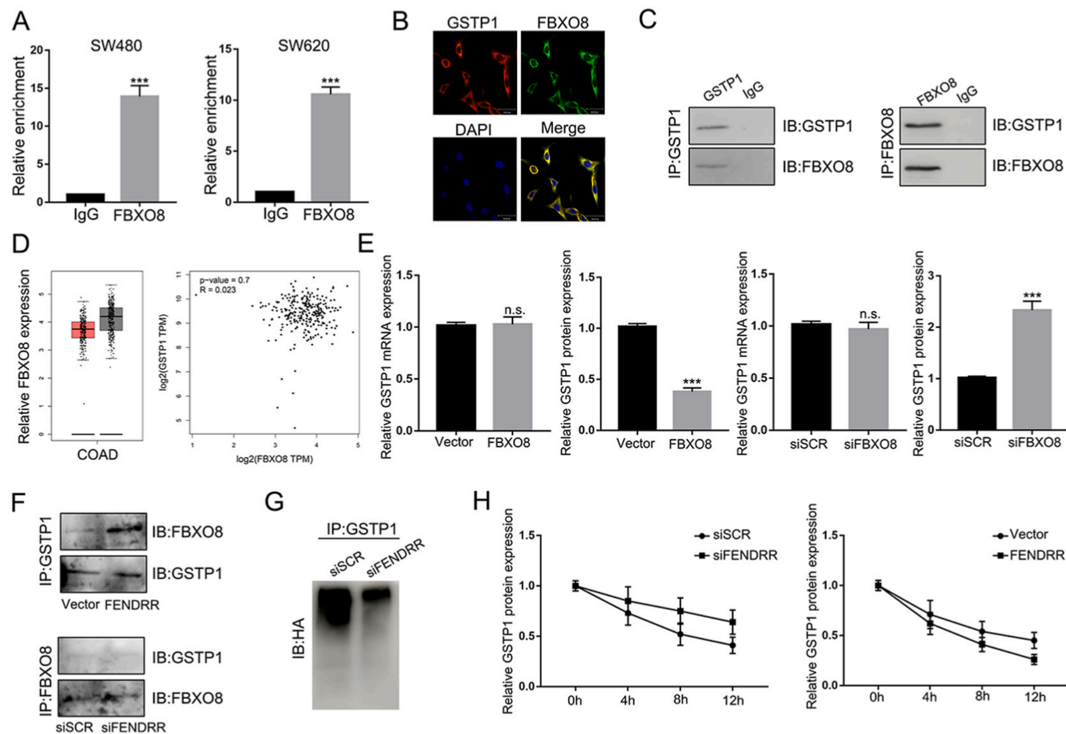

Fig. 4. FENDRR regulated the protein stability of GSTP1 via FBX8. (A) The interaction between FBX8 and FENDRR was validated by RIP assay. (B) The localization of GSTP1 and FBX8 was detected by immunofluorescence assay. (C) FBX8 or GSTP1 were immunoprecipitated from SW620 cell lysates and analyzed by SDS-PAGE. (D) The expression of FBX8 was analyzed by TCGA database. (E) The mRNA level of FBX8 was measured by qRT-PCR and the protein level was measured by Western blot. (F) The interaction between FBX8 and GSTP1 was detected by immunoprecipitation after FENDRR overexpression or knockdown. (G) GSTP1 were immunoprecipitated from the lysates of CRC cell treated with si FENDRR transfection and analyzed by Western blot to detect the ubiquitination. (H) The stability of GSTP1 proteins was detected by Western blot after CHX treatment.

but not the level of the GSTP1 mRNA. (Fig. 4E). FENDRR overexpression or knockdown led to the subsequent discovery of the interaction between GSTP1 and FBX8. Fig. 4F shows that, compared to the empty vector, the interaction between GSTP1 and FBX8 was significantly improved in the FENDRR overexpression group, but considerably decreased in the FENDRR knockdown group. In addition, GSTP1's ubiquitination decreased after FENDRR was silenced, even while GSTP1's protein stability increased. (Fig. 4G and H).

3.5. *FENDRR suppressed HIF-1 signaling pathway*

To determine the precise signaling pathways underpinning FENDRR's action in CRC, we performed next-generation sequencing (NGS) on CRC cells transfected with an overexpression vector of FENDRR or a negative control. We found 1393 down-regulated genes and 843 up-regulated genes (Fig. 5A, additional file 2: Dataset 2). It was discovered using RNA-seq data analysis using the KEGG and Gene Set Enrichment Analysis (GSEA) methods that overexpression of FENDRR significantly decreased the activation of HIF-1 signaling pathways. (Fig. 5B–C). As shown in Fig. 5D, NGS data showed that after FENDRR overexpression, the expression of genes linked to the HIF-1 pathway was down-regulated. After that, we used qRT-PCR to confirm it. (Fig. 5E). Then, qRT-PCR was used to measure the expression levels of a selection of metastasis-related genes that were chosen and positioned downstream of HIF-1. Fig. 5F shows that in CRC cells, FENDRR knockdown significantly decreased the expression of AK4, ENO1, HK2, and Twist1, whereas FENDRR overexpression may have increased these proteins' levels.

3.6. *Overexpression of GSTP1 rescues the inhibition role of FENDRR on cell proliferation and metastasis in CRC*

Rescue studies were performed to determine whether FENDRR's participation is related to its capacity to inhibit GSTP1. Colony formation in particular indicated that GSTP1 overexpression could completely reverse the growth inhibition induced by FENDRR in CRC cells, as illustrated in Fig. 6A. Additionally, the outcomes of the transwell assay demonstrated that overexpression of GSTP1 blocked the FENDRR-induced decrease in cell invasion. (Fig. 6B). The tube formation assay also showed that cells overexpressing FENDRR may be protected from having their angiogenesis abilities reduced by GSTP1. (Fig. 6C). Moreover, the effect of FENDRR knockdown on cell proliferation, invasion and angiogenesis abilities was also reversed by HIF-1A knockdown. (Fig. 6D–F). Together, we uncovered a brand-new molecular mechanism by which FENDRR regulates the growth of CRC, as shown in Fig. 6D. FENDRR could

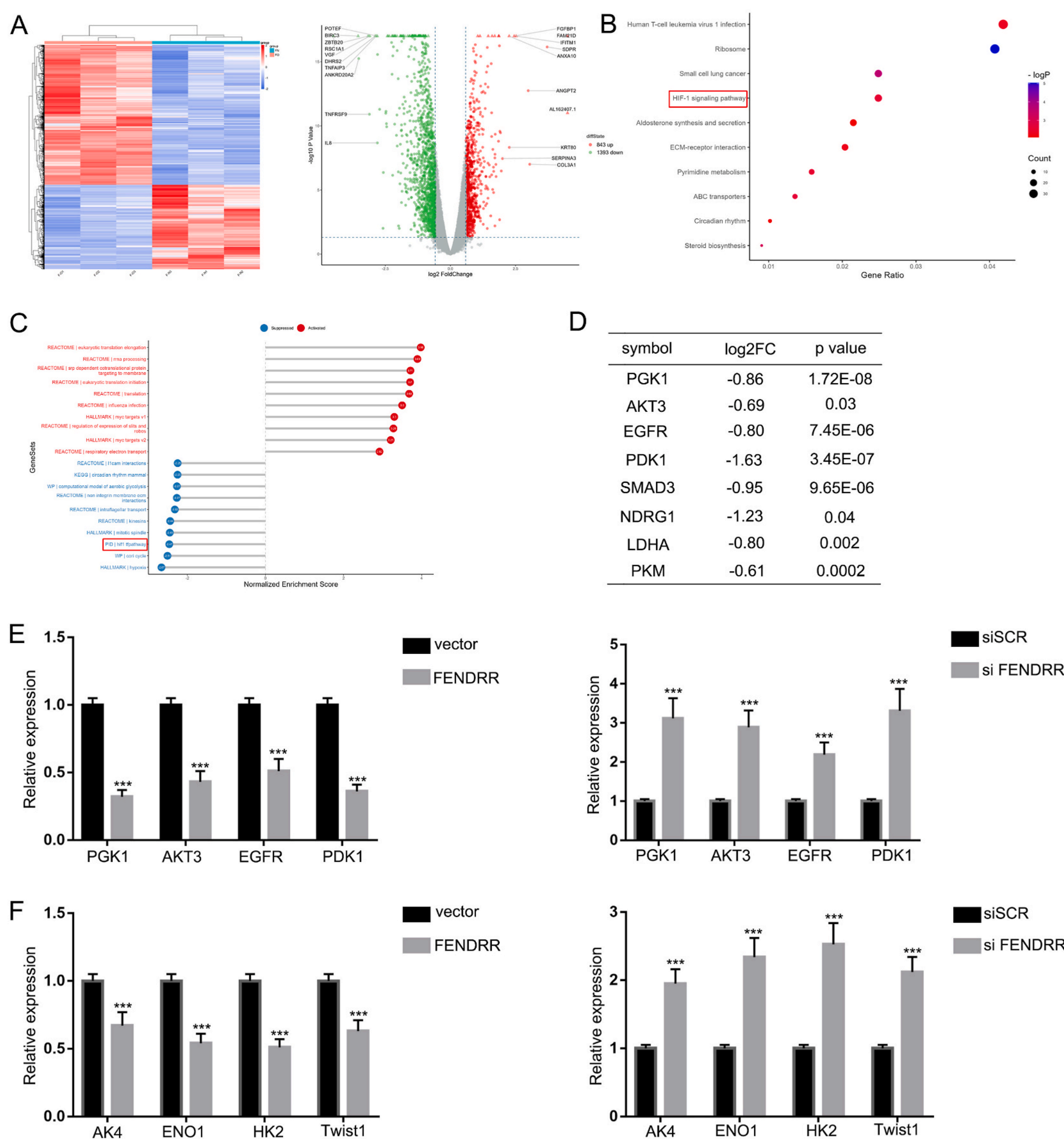

Fig. 5. FENDRR suppressed HIF-1 signaling pathway. (A) The Cluster heat map and Volcano map showing the differentially expressed genes after FENDRR overexpression in CRC cells. (B-C) The RNA-seq data was enriched by KEGG and GSEA. (D) The expression of HIF-1 pathway-related genes in RNA-seq data was shown. (E) The expression of HIF-1 pathway-related genes was measured by qRT-PCR. (F) The expression of HIF-1 pathway downstream targets was detected by qRT-PCR.

influence the stability of the GSTP1 protein via improving the interaction between FBX8 and GSTP1. In addition to preventing invasion and metastasis, FENDRR also slowed the development of CRC by inactivating the HIF-1 signaling pathway (Fig. 6G).

4. Discussion

CRC is the most typical malignant tumor in the globe [11]. LncRNAs, which control gene expression and have been linked to the development of CRC, are oncogenes or tumor suppressors. This study discovered that the expression of FENDRR was noticeably down-regulated in CRC tissue using the TCGA database and qRT-PCR analysis. Several CRC cell lines and healthy colon epithelial cell lines both expressed FENDRR at the same time, with most CRC cells showing a lower expression of FENDRR. After that, FENDRR was discovered to have an impact on CRC cells, and functional testing revealed that it prevented CRC cells from proliferating, invading, and undergoing angiogenesis both *in vitro* and *in vivo*. These results demonstrate a critical function for the tumor suppressor gene FENDRR in the pathogenesis of CRC, which is consistent with past studies [12]. However, it has been demonstrated in prior studies that FENDRR functions as a molecular sponge in the control of CRC. For instance, FENDRR suppresses CRC while boosting the expression of ING4 by interacting with miR-18a-5p [13].

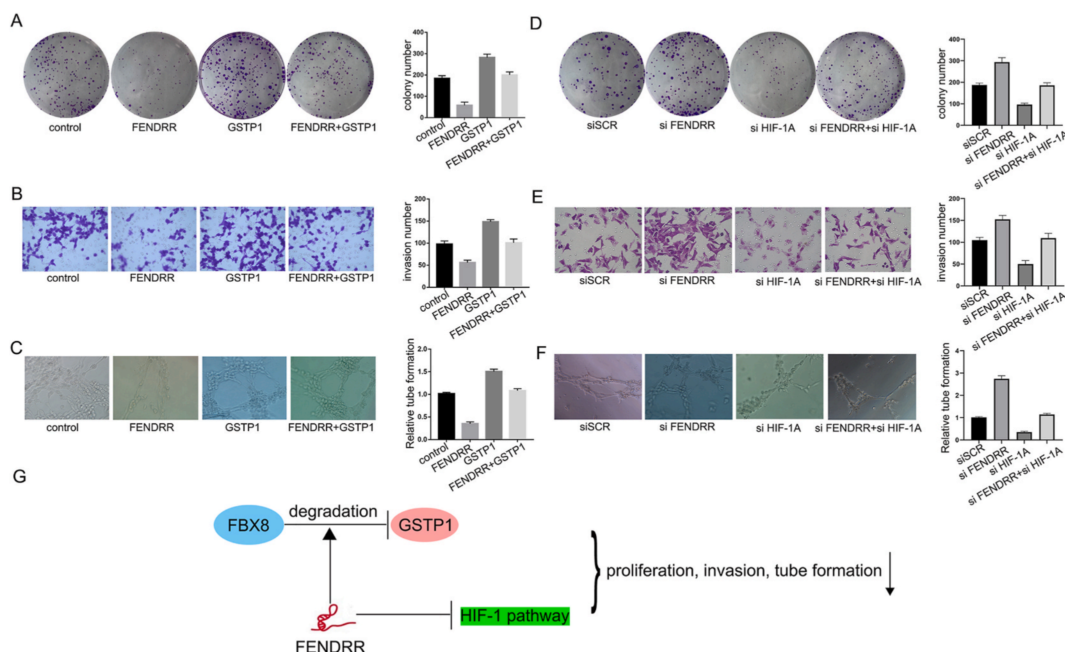

Fig. 6. Overexpression of GSTP1 rescues the inhibition role of FENDRR on cell proliferation and metastasis in CRC. (A) Colony formation assays for effects of GSTP1 re-expression on proliferation of CRC cells with FENDRR overexpression. (B-C) Effects of GSTP1 re-expression on invasion and tumor angiogenesis of CRC cells with FENDRR overexpression. (D) The proliferation of CRC cells was detected by colony formation assay after si FENDRR and si HIF-1A co-transfection. (E-F) Effects of HIF-1A knockdown on invasion and tumor angiogenesis of CRC cells with si FENDRR transfection. (G) Integrated model depicting the tumor-suppressing effect and molecular mechanism of FENDRR in CRC.

It is well known that lncRNAs interact with binding factors to affect their target genes. In this study, we demonstrated that FENDRR worked by interacting with binding proteins, enabling CRC formation for the first time. Therefore, to identify the proteins that FENDRR interacts to, we performed an RNA pull-down assay. We were able to identify several proteins using mass spectrometry. The protein GSTP1 in particular caught our attention. Previous research has shown that the abnormal expression of GSTP1 is associated with the emergence of a variety of tumor forms, including esophageal cancer, melanoma, lung cancer, and breast cancer [14–17]. GSTP1 may be a potential therapeutic target for the clinical treatment of CRC since it raises STAT3 expression in CRC cells, which encourages their expansion, invasion, and metastasis [18]. Here, the results of the RIP tests demonstrated the possibility of an interaction between GSTP1 and FENDRR, and the Western blot experiments demonstrated that GSTP1 protein levels were down-regulated after FENDRR overexpression. We did more research on the FENDRR-GSTP1 regulation system. Previous research found that FBX8 reduced GSTP1 by ubiquitination to inhibit CRC development [19]. Mass spectrometry has shown the interaction between FENDRR and FBX8, and immunofluorescence and co-IP have validated the colocalization of GSTP1 and FBX8. Additionally, GSTP1's ubiquitination decreased but its protein stability increased after FENDRR was deleted. These results demonstrate that FENDRR employed FBX8 to regulate the protein ubiquitination of GSTP1. In order to properly describe the underlying signaling pathways associated with FENDRR's effect on CRC, we also performed next-generation sequencing (NGS) on CRC cells transfected with the FENDRR overexpression vector and the negative control. Overexpression of FENDRR significantly decreased the activation of HIF-1 signaling pathways, according to data from RNA-seq investigations using KEGG enrichment analysis and GSEA. Previous studies found a strong correlation between the emergence of CRC and the activation of the HIF-1 signaling pathways. Since it can regulate the Met/ERK/ELK-1, HIF-1, and VEGF-A pathways by boosting miR-206, which in turn suppresses angiogenesis in CRC, CCL19 may be a novel therapeutic method for anti-vascular treatment of CRC [20]. Since HIF-1 is a potential biomarker for 5-FU-resistant CRC, targeting it in conjunction to 5-FU may be an effective therapeutic strategy [21]. ART1 may regulate GLUT1-dependent glycolysis in CRC and boost glucose uptake in CT26 cells via activating the PI3K/AKT/HIF1 pathway [22]. Here, we selected a number of downstream HIF-1-related genes and assessed their expression levels using qRT-PCR. While FENDRR overexpression might increase the amounts of these proteins, FENDRR knockdown significantly decreased the expression of AK4, ENO1, HK2, and Twist1 in CRC cells. According to our research, overexpression of FENDRR significantly reduces the activation of the HIF-1 signaling pathway and regulates the onset of CRC. Our findings revealed a hitherto unreported capability of FENDRR to regulate the ubiquitination of GSTP1 and the deactivation of the HIF-1 signaling pathway, hence controlling the growth of CRC tumors.

In potential clinical applications, the recent developments in clinical applications or therapeutic implications of targeting FENDRR, GSTP1, or the HIF-1 signaling pathway in CRC treatment is limit. However, this study demonstrates the potential clinical applications of FENDRR, GSTP1, or the HIF-1 signaling pathway in CRC. In addition, inhibitor products for GSTP1 and the HIF-1 signaling pathway have been developed to make them more feasible for clinical application. The potential challenges and limitations in translating their findings into clinical practice may include issues related to safety, efficacy, patient selection, and the need for further research.

Highlighting these limitations to provide a balanced view of the potential of their findings and to guide future research efforts.

However, our study also has a number of drawbacks. 1. More CRC patients should be enrolled in the study in order to examine the relationship between FENDRR and patient prognosis. 2. There might be additional researchable processes for how FENDRR influences CRC progression. We'll expand the sample size in the future to do further study.

5. Conclusion

In conclusion, we found that FENDRR controls the ubiquitination of GSTP1 and blocks the HIF-1 signaling pathway, which both significantly contribute to the development of CRC. Our research provides more evidence of FENDRR's crucial role in the emergence of CRC and identifies it as a potential therapeutic target for CRC patients.

Ethics approval statement

This work has received permission from the Shanghai Tongren Hospital Ethics Committee (K2023-001-01).

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Data availability statement

TCGA (https://www.cancer.gov/ccg/research/genome-sequencing/tcga) and GEO (https://www.ncbi.nlm.nih.gov/geo/) databases for the availability of the public data. All data generated or analyzed during this study are included in this published article. Please contact us by email (cky9920@163.com) about the experimental data and in additional file5.

CRediT authorship contribution statement

Jing Yang: Writing – original draft, Data curation. **Yuemei Hu:** Formal analysis. **Zhenyu Tan:** Data curation. **Feng Zhang:** Writing – review & editing. **Wentao Huang:** Software. **Kai Chen:** Supervision, Investigation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Not applicable.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2023.e23161.

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