Function Study of RNF43 in Colon Cancer and CRISPR Detection of its Mutation

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Research Article

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Abstract

Purpose

In the present study, we aimed to study the expression levels and functional role of RNF43 in colon cancer, and provide a new theoretical basis and research direction for assessing the risk of colon cancer.

Methods

We used bioinformatics to analyze the expression and effect of RNF43 in colon cancer. Cell proliferation, migration and invasion were detected by CCK-8, Transwell and wound healing experiments. The CRISPR-cas12a was used to detect the mutation sites of RNF43. Meanwhile, Sanger sequencing was used to recheck.

Results

RNF43 is up-regulated in colon cancer and participated in the signal pathway in tumors. *In vitro* cell experiments revealed that RNF43 inhibits the proliferation, migration and invasion of colon cancer cells. Finally, rs786205215 and rs1567880628 were found to have higher mutation frequencies and the results of the two detection methods are completely consistent.

Conclusion

Studies have shown that rs786205215 and rs156788062 of RNF43 may be used as indicators for colon cancer risk prediction, and CRISPR-cas12a system can be identified as one of the detection methods of SNP sites.

Introduction

Colon cancer is a common malignant tumor of the digestive system cancer worldwide, and its incidence is third among all of the cancers types [1]. The high mortality rate of colon ranks second in the world [2]. The pathogenesis of colon is really complicated. It is characterized by high malignancy and low survival rate, threatening human life and health at all times [3]. Therefore, it is of great significance to determine the pathogenesis of colon cancer to accurately assess the progress and prognosis of colon cancer.

RNF43, a member of the RING finger E3 ubiquitin ligase family, was originally identified as an oncoprotein with upregulated expression on colorectal [4] and have been discovered to inhibit the Wnt signaling at the level of Frizzled (FZD) receptors, thus it usually serve as negative regulator of Wnt signaling [5, 6]. Meanwhile, RNF43 overexpression leads to a significant increase in the growth rate of colorectal cancer cell, while siRNA inhibits the expression of RNF43 and exerts an inhibitory role in the cell growth [7]. These findings suggested that RNF43 was involved in the carcinogenesis of colorectal cancer. In addition, a large

number of researches have reported that RNF43 was mutated in various types of solid tumor, such as ovarian carcinoma [8], intraductal papillary mucinous neoplasm [9] and early gastric cancer [10], suggesting that loss of RNF43 function is one of crucial events in the tumors. Notably, RNF43 was found to have a high frequency of mutations in gastric cancer, which contributes to the development of gastric malignant tumors [6]. There are insufficient researches showing that RNF43 has mutations in HCC, affecting the progress of HCC.

In this study, for better understanding, the role of RNF43 in the pathogenesis of colon cancer, the expression of RNF43 in colon cancer tissues was received from Oncomine and TCGA database. Meanwhile, it was found that high expression of RNF43 was closely related to clinical feature information. Subsequently, the results of functional enrichment analysis suggested that RNF43 participate in the regulation of signal pathways in colon cancer, which may affect the progression of colon cancer. Next, *in vitro* experiments were conducted to investigate the role of RNF43 on proliferation, migration and invasion of colon cancer. Furthermore, we found that there was a high frequency of RNF43 mutation in colon cancer by bioinformatics analysis. Finally, the mutation of RNF43 in clinical samples of colon cancer was detected by CRISPR-cas12a technique.

Materials And Methods

Oncomine and the cancer genome atlas (TCGA) database analysis

Oncomine (<http://www.oncomine.org>), as an online microarray database, was used to assess the mRNA expression levels of RNF43 in colon cancer. We screened for relevant studies on RNF43 expression in colon cancer tissues and normal tissues. With “Cancer VS. Normal” analysis as the primary filter. Furthermore, the mRNA expression profiles and corresponding clinical characteristic were acquired from the Cancer Genome Atlas (TCGA) database (<https://portal.gdc.cancer.gov>).

Cosmic Analysis

The Catalogue Of Somatic Mutations In Cancer (COSMIC) database (<https://cancer.sanger.ac.uk/cosmic>) [11], an online resource preserving and providing genes mutation data, is the global largest and most comprehensive resource for exploring the impact of somatic mutation in human cancer. We obtained mutation information for RNF43 in colon cancer after typing a gene name and multiple parameters in the search bar.

Cbioportal Analysis

The cBio Cancer Genomics Portal (<http://cbioportal.org>) [12] is an open-access resource for interactive exploration of multidimensional cancer genomics data sets. The RNF43 gene mutation and expression

information in colon cancer were obtained from cBioPortal database. A mutation analysis was performed in colon cancer studies, including mutation, amplification and deletion. All searches were carried out according to the cBioPortal's online instructions.

Functional Analysis And Ppi Network Construction

In order to determine which channels downstream pathways RNF43 goes through to play its role thereby affecting the development of colon cancer, UALCAN (<http://ualcan.path.uab.edu/>) and GEPIA databases were utilized to calculate genes related to expression of RNF43. Genes with an absolute value of R greater than 0.5 were extracted and the intersection was taken to obtain 141 intersection genes. In addition, functional enrichment analysis of the interaction proteins was performed using Funrich (<http://www.funrich.org>) [13], which is an open access, standalone functional enrichment and network analysis tool. Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis of 141 genes were performed using Database for Annotation, Visualization and Integrated Discovery (DAVID) (<https://david.ncifcrf.gov/>). Differences were considered to be significant at $P < 0.05$. The functional analysis of related genes that interact with RNF43 gene was conducted with Funrich software. The STrinG interaction proteins were imported into FunRich, and significantly enriched pathways was analysed. Moreover, Search Tool for the retrieval of interacting Genes (STRING; available at <http://string-db.org/>) [14] provides and collects protein-protein association data. In order to assess protein-protein interactions of genes, Cytoscape software, the Search Tool for the Retrieval of Interacting Genes (STRING) (<https://string-db.org/>) was used to analyze PPI relationship. Cytoscape software was used for visualization, in which the Mcode plug-in was used for module analysis and the top three modules were selected as important modules.

Cell Lines And Cell Culture

FHC, HCT-116, SW620, SW480 and HT29 cell lines were purchased from the American Type Culture Collection (ATCC, USA). All cells were cultured in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum (FBS) (Gibco, Grand Island, NY, USA) at 37°C in a 5% CO₂ atmosphere.

Transient transfection of small interfering RNA (siRNA)

To knockdown the RNF43 expression, transient transfection of small interfering RNA (siRNA) was performed, using the Lipofectamine 3000 Transfection Reagent (Thermo Fisher Scientific). Following 4 h transfection, the cells were recovered using fresh culture medium.

Cell Counting Kit-8 (Cck-8) Assays

Cell proliferation was performed via the Cell Counting Kit-8 (CCK-8) assay (Dojindo, Tokyo, Japan). The cells (3×10^3 cells/well) were seeded at a 96-well plate. At time points of 0, 24, 48 and 72 h, 10 μ L of CCK-8

was added in each well and the cells were incubated at 37°C for 1 h in an incubator. The number of viable cells was determined by measuring the absorbance at 450 nm using a microplate reader (Model 680; Bio-Rad Laboratories, Hercules, CA, USA). All of the experiments were performed three times.

Wound Healing Assays

A wound healing assays were performed to test cell lateral migration. Briefly, transfected cells were seeded in 6-well plates, and incubated to 80% confluency. Then, the cells were scraped across the surface of wound with a 100 µL pipette. After an incubation at 37°C of 24 h and the scratches were observed by a microscope.

Transwell Assay

Transfected cells were plated into the upper chamber with Matrigel (BD Biosciences), coated with the serum-free medium. While the bottom chamber were filled with 600 µL medium containing 10% fetal bovine serum. After 12h incubation, the cells on the upper chamber were removed using cotton swabs, and migrated cells were fixed with 1.0% crystal violet. Images were taken with a microscope.

Quantitative Rt-pcr Assay (Rt-qpcr)

Quantitative RT-PCR assay (RT-qPCR)

Total RNA of colon cancer cells was extracted by using TRIzol (Invitrogen) according to the manufacturer's instructions and reverse-transcribed to cDNA by using PrimeScript™ RT reagent kit (Takara). Data were collected and quantitatively analyzed on an Mx3005p quantitative PCR system. All of the primers were synthesized by Sangon Biotech. Results were analyzed by the $2^{-\Delta\Delta Ct}$ method. The PCR primer used were as follows: RNF43 forward, 5'-CCGACACCCACAGAGGAA-3'; RNF43 reversed, 5'-GAGGAGTCAGGCACAACCA-3'; GAPDH forward, 5'-GCTCTCTGCTCCTCCTGTTC-3'; GAPDH reversed, 5'-CGACCAAATCCGTTGACTCC-3'.

Crispr-cas12a System Detection

Whole blood collection: Blood was collected using EDTA blood collection tube and was full mixed when collected. Total DNA of cells can be extracted with the boold/cell/tissue genomic DNA extraction kit (Tiangen Biochemical Technology Co., Ltd., number: DP302-02). It can be preserved at -80°C or in liquid nitrogen.

Primer design and crRNA preparation

The wild type and mutant templates were designed according to the two mutation detection sites of the gene *RNF43*, and the amplification primers were designed. At the same time, the crRNA was designed for these mutation regions and the required oligonucleotide (crDNA) was synthesized. crRNA and cr-T7-F were

annealed according to the double stranded target step to prepare the transcription template. The hiscribe T7 quick high yield RNA synthesis Kit (NEB) without enzyme was used to incubate the transcription template at 37°C for 16 h. After the completion of the reaction, 2uL DNase 1 was added to eliminate the unreacted template in the system and required crRNA was purified. Wild type and mutant template sequences, amplification primers and crdna were synthesized by TianYiHuiYuan Biotechnology Co., Ltd. (Table 1)

Verification of CRISPR-cas12a fluorescence detection system

The 50-100ng template DNA was added to the detection reagent mixture (NEBuffer 3.1containing 0.75μM crRNA, 1.5uM Fncas12a, 50pM fluorescence probe and 3μL). The total volume 50μL was reacted at 37°C for 1 h to obtain the fluorecence value of Fncas12a protein and analyze the fluorecence curve. The whole process of the reactions was carried out at a constant temperature of 37–42°C.

Clinical sample detection: Conventional primers were used for PCR, and 50ng DNA samples were used as templates, following the procedure: 95°C for 5 min; 95°C for 3 min, 56°C for 10 s, 72°C for 20 s, 30 cycles; 72°C for 5 min. After the electrophoresis detection was qualified, the amplified product 1–5μL was taken for CRISPR-cas12a fluorescence detection. Next the remaining products were detected by Sanger sequencing.

Statistical analysis

Student's *t*-test (two-tailed) was utilized to compare the means between two groups. mRNA expression data were presented as fold change, and *P*-values < 0.05 was accepted as indicative of significant differences.

Results

RNF43 is highly expressed in colon cancer and is related to clinicopathological characteristics

Oncomine database was used to analyze (<http://www.oncomine.org>) [15] the RNF43 expression levels on the various types of cancer. Oncomine analysis results showed that RNF43 is differentially expressed in colorectal cancer tissues (Fig. 1a). Furthermore, expression level of RNF43 was analyzed in colon cancer through the TCGA online analysis tool. It is worth noting that the analysis result of RNF43 is differentially expressed in colon cancer tissues which is consistent with the that of Oncomine database (Fig. 1b). Analysis of data from TCGA revealed that RNF43 is up-regulated in colon cancer tissues compared with normal tissues (Fig. 1c). Subsequently, the expression of RNF43 in colon cancer cell lines and its relationship with clinical characteristics were analyzed through the Broad Institute Cancer Cell Line Encyclopedia (CCLE) database. We found that individual cancer stages (Fig. 2b, $P < 0.05$) and nodal metastasis status (Fig. 2c, $P < 0.05$) were related to the expression level of RNF43 through TCGA database analysis.

Genes Related To Rnf43 Set Enrichment Analysis And Ppi Network Analysis

Search Tool for the Retrieval of Interacting Genes (STRING, <http://string.embl.de/>) is an online analysis tool for investigating and collecting comprehensive information of interaction between proteins [16]. To further analyze the biological function of the DEGs, DAVID was used to process GO function and KEGG pathway enrichment analysis on overlapping genes related to RNF43, which were obtained from the UALCAN and GEPIA databases (Fig. 3a). Moreover, 141 genes were analyzed by DAVID for functional enrichment (Fig. 3b). The results showed that biological process is mainly enriched in regulation of insulin secretion, negative regulation of canonical Wnt signaling pathway, vesicle-mediated transport, actin nucleation, regulation of transcription from RNA polymerase II promoter; cellular component is mainly enriched in nucleus, cytosol, Trans-Golgi network, transcription factor complex; molecular function is mainly enriched in transcription factor activity, sequence-specific DNA binding, Poly (A) RNA binding, sequence-specific DNA binding, RNA polymerase II transcription factor activity, ligand-activated sequence-specific DNA binding, zinc ion binding (Fig. 3b). KEGG is mainly enriched in basal cell carcinoma, pathways in cancer, sulfur relay system, cAMP signaling pathway. Subsequently, the overlapping genes were imported into STRING to detect the interactive proteins with RNF43 expression and the PPI network of these genes were received (Fig. 3c). In addition, to further explore the association within the PPI network, we extracted the top three modules inside the PPI network with Cytoscape (Fig. 3d).

The Expression Levels Of Rnf43 In Colon Cancer

RT-qPCR was carried out to detect the RNF43 mRNA expression levels on colon cancer. As shown in Fig. 4a, RNF43 expression was higher in the colon cancer tissues than in adjacent tissues. Next, RNF43 expression level was determined in colon cancer cell lines ($P < 0.05$). The data of RT-qPCR showed that RNF43 has the highest expression in HCT-116 and HT-29 cell lines (Fig. 4b). Therefore, HCT-116 and HT-29 cell lines were selected for follow-up experiments. To explore the role of RNF43 in colon cancer, RNF was knocked down in HCT-116 and HT-29 cell lines transfected with two siRNAs targeting RNF43. As illustrated in Fig. 4c, the mRNA expression levels of RNF43 were significantly inhibited by si-RNF43-2. We chose si-RNF43-2 for follow-up research.

Rnf43 Knockdown Inhibits Cellular Proliferation, Migration And Invasion

To investigate the effect of RNF43 on the proliferation, migration and invasion of colon cancer cells, CCK-8 assay, the scratch wound healing assay and invasion assay *in vitro* were applied to assess the role of RNF43 on colon cancer. Proliferation of the cells was measured by CCK-8 assay. The results showed that si-RNF43-2 evidently inhibited growth of colon cancer cells (Fig. 5a). The effects of RNF43 knockdown on cell migration was examined by scratch wound healing assay. The data of scratch wound healing assay demonstrated that knockdown of RNF43 significantly restrained the migration in HCT-116 and SW620 cells

(Fig. 5b). Then, an invasion assay was carried out to further confirm the effect of RNF43 on invasion of HCT-116 and SW620 cells. Our results proved the significant repression of invasion in HCT-116 and SW620 cells by knockdown of RNF43 (Fig. 5c). In summary, these data indicated that knockdown of RNF43 suppressed the cells proliferation, migration and invasion abilities.

Rnf43 Mutation In Colon Cancer

There are researches showing that gene mutation may potentially affect gene expression through different channels [17] and each gene has multiple mutation types [18]. The gene mutation affecting RNF43 in colon cancer samples was analyzed using COSMIC online database. The results showed that RNF43 was found in the top 20 genes with colon cancer mutations and ranked 15th (Fig. 6a). Further analysis of the data revealed the mutation types and the percentage of mutant bases in RNF43. The mutation types of RNF43 are presented on the pie chart in Fig. 6b. What stands out in this figure is that the RNF43 mutation type is mainly missense substitution, accounting for 36% of all mutations. The base mutation of RNF43 is presented on the pie chart in Fig. 6c and there is 39% C > G mutation in the missense substitution. We further searched RNF43 gene alteration from cBioPortal (<http://www.cbioportal.org/>). Notably, the results in Fig. 7a showed that RNF43 mutation was analyzed in colon adenocarcinoma (CaseCCC, PNAS 2015), colon cancer (CPTAC-2 Prosperctive, cell 2019), colorectal adenocarcinoma (DFCI, cell reports 2016), colorectal adenocarcinoma (Genentech, Nature 2012), colorectal adenocarcinoma (TCGA, Firehose Legacy), colorectal adenocarcinoma (TCGA, Nature 2012), colorectal adenocarcinoma (TCGA, PanCancer Atlas), colorectal adenocarcinoma Triplets (MSKCC, Genome Biol 2014) and metastatic colorectal cancer (MSKCC, Cancer Cell 2018) 9 sample banks. Subsequently, several RNF43 genetic alterations were discovered in colon cancer, including mutation, deletion and amplification, the highest frequency is more than 15% and the lowest is less than 5% (Fig. 7b). The TCGA data were analyzed to determine the location of RNF43 in colon cancer samples. As shown in Fig. 7c, the type and frequency of RNF43 mutation are basically consistent with the results of cBioPortal database analysis (missense and truncating). The genetic aberration of RNF43 may also contribute to tumorigenesis, but further investigation is necessary.

Rnf43 Mutation Influences Drug Selection

To evaluate the effect of RNF43 mutation on drug selection of colon cancer, we required the result of RNF43 mutation affecting the half-maximal inhibitory concentration (IC₅₀) of colon cancer medicines for GDSC database. It was clearly observed that all types of cancer with RNF43 mutation were greatly sensitive to AZD4547 and AZD1332. Besides, we also found that RNF43 mutants treated with AZD4547 or AZD1332 exhibited lower IC₅₀ levels than that treated with AZD4547 or AZD1332 in colon cancer cell lines. The results revealed that colon cancer cells with RNF43 mutation was significantly suppressed by AZD4547 and ZD1332 ($P < 0.05$) (Fig. 8a and b).

Crispr-cas12a System Detects Rnf43 Mutations In Clinical Samples

To investigate whether RNF43 has mutation in clinical samples, we screened the SNP sites of RNF43 through the clinvar database and found that 14 of them were pathogenic, and rs786205215: c.394 G > A (p.R132*) and rs1567880628: c.-45 C > T (p.R113*) have high mutation frequency, so we used CRISPR-cas12a to detect mutations at these two sites in 40 clinical samples of colon cancer. We designed mutant crRNA for the mutated sequence of RNF43 gene RNF43: c.394 G > A (p.R132*) and RNF43: c.-45 C > T (p.R113*), and verified the cleavage efficiency of crRNA through target DNA. As illustrated in Fig. 9a, the fluorescence value of RNF43-R132* and RNF43-R133* mutant target DNA was significantly higher than that of wild-type target DNA ($P < 0.05$, $P < 0.05$). The CRISPR-cas12a system constructed by crRNA can be used to detect the presence of mutations at these two sites in clinical samples. Subsequently, we used the CRISPR-cas12a system to detect whether there were mutations at RNF43-R132* and RNF43-R133* sites in 40 clinical samples of colon cancer. The results revealed that the fluorescence values of 40 colon cancer patients were similar to those of wild-type target DNA molecules, indicating that the RNF43-R132* and RNF43-R113* of 40 colon cancer patients did not have mutations (Fig. 9b).

Sanger Sequencing Method Detects Rnf43 Mutations In Clinical Samples

To investigate the accuracy and sensitivity of CRISPR-cas12a system inspection results, Sanger sequencing was performed to detect the RNF43-R132* and RNF43-R113* sites of these 40 colon cancer samples to verify the reliability of the detection results of the CRISPR-cas12a fluorescence detection system. The results determined that there was no mutation detected on RNF43 gene RNF43: c.394 G > A (p.R132*) and RNF43: c.-45 C > T (p.R113*) sites (Fig. 10a). Interestingly, through further analysis of the sequencing results, it is found that there was a C > T mutation at the rs2257205 site near the RNF43: c.-45 C > T (p.R113*) site. Among 40 clinical samples, 35 cases had mutations, of which 2 cases were homozygous mutations and 33 cases were heterozygous mutations. The mutation rate was as high as 87.5% (Fig. 10b).

Discussion

Colon cancer is one of common malignant tumors, originates from the colon or rectum [19–21]. Single-nucleotide polymorphism (SNP) is considered as a common type of genetic variation in the genome. With the development of genome detection technology, SNP genotyping has become a powerful tool for clinical diagnosis and treatment of tumors. SNPs are the most abundant and widely-distributed mutations in genomes, which are also useful as genetic markers. There are sufficient studies suggesting that SNP has potential predictive significance risk of multiple tumors, such as lung cancer [22], breast cancer [23], prostate cancer [24].

RNF43, a member of E3 ubiquitin ligase family, was initially identified as an up-regulated tumor protein in colon cancer [25]. In the paper, RNF43 was found to be up-regulated in a variety of tumors via a comprehensive analysis of Oncomine and UALCAN databases, and RNF43 has the highest expression in colon cancer compared to other tumors. Simultaneously, we also got the results that RNF43 expression related to cancer staging and lymph nodal metastasis through CCLE database analysis. KEGG pathway enrichment plots show the enrichment of RNF43 in many colon cancer related pathways. We confirmed that RNF43 is up-regulated in colon cancer through RT-qPCR experiments. Besides, a series of *in vitro* experimental results showed that RNF43 inhibited the growth, migration and invasion of colon cancer cells. Regarding RNF43, it is mutated in various types of cancer and its loss of function affects the development of cancers [6, 26]. We found that RNF43 ranks 15th among the mutant genes of colon cancer by COSMIC database analysis in this paper. Moreover, through the screening of ClinVar database, 14 SNP sites of RNF43 were analyzed to be pathogenic, of which rs786205215: c.394 G > A (p.R132*) and rs1567880628: c.-45 C > T (p.R113*) have higher mutation frequencies than other SNP sites. Subsequently, the two mutation sites (rs786205215: c.394 G > A (p.R132*) and rs1567880628: c.-45 C > T (p.R113*)) of RNF43 were detected by CRISPR-cas12a fluorescence system in 40 clinical samples of colon cancer to lead a result that neither RNF43-R132* nor RNF43-R113* has mutation. Sanger sequencing detection results were consistent with the CRISPR-cas12a fluorescence system results, showing that the accuracy and sensitivity of the CRISPR-cas12a fluorescence system was not weaker than the Sanger detection. However, there were few detailed reports about the research of rs786205215 and rs1567880628 in RNF43 that can be used for colon cancer risk assessment. Interestingly, in this study, we were pleasantly surprised to find that RNF43 has rs2257205 C > T mutation. A research showed that a SNP (rs2257205) site of the RNF43 was associated with overall survival, indicating that RNF43 mutation affected the prognosis of colon cancer patients [27]. Thus, the research results of this article lay a new research direction for the index of colon cancer risk prediction.

In our study, both Sanger sequencing method and CRISPR cas12a fluorescence detection system were used to detect colon cancer SNP sites, and the results have confirmed the accuracy of the CRISPR-cas12a fluorescence detection system. CRISPR-Cas12a is the second type (Type V) CRISPR system for editing mammalian genomes, and has broad application prospects in basic research and biomedical applications.

As the basic genetic material of life, precise editing and rapid detection of DNA have always been highly valued. In recent years, with the discovery and development of the CRISPR system, people have rekindled new hopes for gene therapy; despite some potential safety risks and certain ethical disputes, the CRISPR system has begun to be applied in clinical research because of its relative accuracy and efficiency. Meanwhile, scientists began to introduce the CRISPR system into the field of rapid nucleic acid detection, hoping to bring breakthroughs to existing clinical diagnostic techniques. For example, CRISPR-Cas12a is utilized in the detection of African swine fever virus [28]. CRISPR-Cas12a-assisted recombination engineering can be used for plasmid genetic manipulation and plasmid solidification [29]. These studies reflect the application of CRISPR-Cas12a in multiple fields. Taken together, in this paper, CRISPR-Cas12a technology was used to detect the SNP site of RNF43, providing a new theoretical basis and research

direction for assessing the risk of colon cancer. However, the number of samples collected in this article is small, which is the limitation and deficiency of this study.

Declarations

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Conflict of Interest

Author Zhen Wang declares that he has no conflict of interest. Author Suping Shi declares that she has no conflict of interest. Author Chengling Bian declares that she has no conflict of interest. Author Feihu Yan declares that he has no conflict of interest.

Author Contributions

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Suping Shi, Feihu Yan and Chengling Bian. The first draft of the manuscript was written by Zhen Wang and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Data Availability

The datasets generated during the current study are available in Oncomine at [<http://www.oncomine.org>], TCGA at [<https://portal.gdc.cancer.gov>], COSMIC at [<https://cancer.sanger.ac.uk/cosmic>], cBioPortal at [<http://cbioportal.org>], UALCAN at [<http://ualcan.path.uab.edu/>], DAVID at [<https://david.ncifcrf.gov/>], STRING at [<http://string-db.org/>], GEPIA at [<http://gepia.cancer-pku.cn/>].

Ethical Approval

This article does not contain any studies with human participants or animals performed by any of the authors.

Informed consent

Not applicable.

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Tables

Table I Wild-type and mutant template sequences, isothermal amplification primers and crDNA sequences.

Primer	Sequence
RNF43-R132*-wild type	CCGAGCCTACCGCCCACTCGCTCCTCGGTCACGACAGGAGAAAC
RNF43-R132*-mut type	CCGAGCCTACCGCCCACTCACTCCTCGGTCACGACAGGAGAAAC
RNF43-R113*-wild type	GGCAGGGGCGGGGGGCCCCGTCGAGGACTCTCCAGCTTGACGATGCTG
RNF43-R113*-mut type	GGCAGGGGCGGGGGGCCCCGTTGAGGACTCTCCAGCTTGACGATGCTG
R132*-crDNA-R	TCGGATGGCGGGTGACTGAGATCTACAAGAGTAGAAATTACCCTATAGTGAGTCGTATTAATTTTC
R113*-crDNA-R	GCAGGGGTGGGGGGCCCCGTTATCTACAAGAGTAGAAATTACCCTATAGTGAGTCGTATTAATTTTC
cr-T7-F	GAAATTAATACGACTCACTATAGGG
Probe	5'-FAM-CTCACTACAGACGCACGCTA-BHQ1-3
PCR-R132*-F	CCAACCTCTACTGTGTGCCTCTGCAGGCTC
PCR-R132*-R	GCAAAGATGTGCTGTGGGCATAATCCTAGG
PCR-R113*-F	CCAAGGGAATCAGGTACATCACTCTTAGGA
PCR-R113*-R	GTACCTCCCTAGAAAAATGGAGAGGTATATG

Mut: mutation; F, forward; R, reverse.

Figures

Figure 1

High expression of RNF43 in colon cancer

(a) RNF43 mRNA expression in many different types of cancer relative to normal tissues from the Oncomine database. Red color indicated increased expression of RNF43 in cancer tissues compared with normal tissues (left column), while blue color indicated decreased expression of RNF43 in cancer tissues compared with normal tissues (right column). There was no analysis detected expression of RNF43 in cancer tissues and the columns were empty. (b) The data of RNF43 expression in a variety of tumors and normal tissues are obtained from UALCAN database. (c) RNF43 is up-regulated in colon cancer from UALCAN database. The data is represented in box format. The blue box represents normal tissues and red represents colon cancer tissues.

a

b

c

Figure 2

RNF43 has the highest expression in colon cancer cell lines and is related to tumor staging and lymph nodal metastasis

(a) RNF43 mRNA expression in colon cancer ranks first among all cancers from CCLE database. (b-c) Association between RNF43 expression and clinicopathologic characteristics, including individual cancer stages and nodal metastasis status.

Abbreviation: CCLE, Broad Institute Cancer Cell Line Encyclopedia

Figure 3

Functional enrichment analysis of genes related to RNF43

(a) Identification of differentially expressed genes of UALCAN and GEPIA database, $|R| > 0.5$ was used as the cut off criteria. (b) The significantly enriched GO categories were calculated. Red: Biological process; Yellow: Cellular component; Green: Molecular function; Blue: KEGG Terms. X axis : p values ($-\log_{10}$). P values are ordered from low to high. (c) Online tool of STRING database analysis of the PPI network for genes were obtained from UALCAN and GEPIA intersection. (d) Top three modules inside the PPI network were recognized via STRING software.

Abbreviation: PPI, protein-protein interactions; GO, gene ontology.

Figure 4

RNF43 expression in colon cancer cells and tissues

(a) RNF43 expression was detected by RT-qPCR in colon cancer tissues and adjacent tissues. **** $P < 0.0001$. (b) RNF43 expression was detected in different colon cancer cell lines (FHC, HCT-116, SW620, SW480 and HT29). **** $P < 0.0001$; ** $P < 0.01$. (c) Detection of transfection efficiency after interference with RNF43 by RT-qPCR. ** $P < 0.01$; * $P < 0.05$.

Figure 5

RNF43 inhibited cell proliferation, migration and invasion in colon cancer

(a) CCK-8 assay was performed to assess cell proliferation at 24h, 48h and 72 h after culture. **** $P < 0.0001$; *** $P < 0.001$. (b) The scratch wound healing assay was performed to determine the cell lateral migration. * $P < 0.05$. (c) Cell migration and invasion were measured at 24 h after culture. * $P < 0.05$.

a

b

Number of samples (%)

■ Nonsense substitution ■ Missense substitution ■ Synonymous substitution
 ■ Inframe insertion ■ Frameshift insertion ■ Inframe deletion
 ■ Frameshift deletion ■ Complex mutation ■ Other

c

Number of samples (%)

■ A>C ■ A>G ■ A>T ■ C>A ■ C>T ■ C>G ■ G>A ■ G>C ■ G>T ■ T>A ■ T>C ■ T>G

Figure 6

RNF43 is mutated in colon cancer

(a) The top 20 genes for colon cancer mutations include RNF43. RNF43 ranks 15th in the top 20 gene mutations. Blue represents samples with mutation, red represents all sample. All data from COSMIC database. (b) Distribution of RNF43 mutation in colon cancer. RNF43 mutation types, mainly including missense substitution (36%), frameshift deletion (33%) and nonsense substitution (13%). (c) The most common mutations in the mutation type of missense substitution are C>G (39%), G>A (21%), G>T (11%).

Abbreviation: COSMIC, Catalogue of Somatic Mutations in Cancer.

a

AZD4547

Figure 8

RNF43 mutation influences drug selection of colon cancer

GDSC database analysis results showed that colon cancer cells with RNF43 mutation, but not cancer of other types, was significantly inhibited by (a) AZD4547 and (b) AZD1332.

Figure 9

CRISPR-cas12a technology detects 40 clinical samples of colon cancer

(a) Fluorescence detection value of wild-type and mutant type target DNA of RNF43-R132* and RNF43-R113*. (b) The CRISPR-cas12a system detects RNF43-R132* and RNF43-R113* mutations in 40 clinical cases of colon cancer.

Figure 10

Sanger sequencing method detects RNF43 mutation of these 40 colon cancer samples

(a) Sanger detected mutations at RNF43: c.394 G>A (p.R132*) and RNF43: c.-45 C>T (p.R113*) in 40 clinical samples of colon cancer. **(b)** RNF43: c.-45 C>T (p.R113*) near the rs2257205 site with a C>T mutation was found by Sanger sequencing.