To Identify and Verify the Role and Crucial Genes of Methionine Restriction in Inhibiting the Progression of Colon Cancer Cells

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Research Article

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Abstract

Purpose: Study the potential mechanism of methionine restriction (MR) inhibiting colon cancer (CC).

Materials and Methods: The differential expression genes (DEGs) of the CC cell line HCT116 after MR restriction treatment in the GSE72131 and GSE103602 data sets were identified, and functional enrichment analysis was performed. Furthermore, the DEGs of CC in the TCGA database were identified, and the MR-related DEGs were combined to establish transcriptional regulation (TR), competing endogenous RNA (ceRNA), and protein-protein interaction (PPI) networks, and identify key genes from it. Then, in vitro experiments were performed to analyze the effects of MR on the proliferation, invasion, migration, and apoptosis of HCT116 cells, and to verify the effects of MR on the expression of key genes identified.

Results: In HCT116 cells, 1181 genes regulated by MR were identified, and their functional enrichment suggests that they are closely related to tumor progression. Combined with the DEGs of CC, a total of 330 dysregulated genes of CC were affected by MR. 6 hub genes (E2F1, MIR17HG, FANCI, HJURP, KPNA2, KIF15) were obtained from the TR, ceRNA, and PPI network constructed based on this, and the results were consistent with the analysis by rt-PCR. In vitro studies have shown that MR can inhibit the proliferation and metastasis of CC cells and promote apoptosis.

Conclusion: MR may inhibit the progress of CC by down-regulating the expression of E2F1, MIR17HG, FANCI, HJURP, KPNA2, and KIF15.

Background

Colon adenocarcinoma is a very common gastrointestinal tumor. According to statistics, its incidence ranks third among all tumors, but its mortality rate ranks second^[1]. The specific symptoms of colon cancer are not obvious, the occurrence and development are secret, and it is usually difficult to detect, resulting in the diagnosis of patients in the middle and advanced stages^[2]. The development and progress of colon cancer is a multi-step process in which accumulated genetic changes can play an important role. Although considerable progress has been made in surgery, chemotherapy, radiotherapy, and targeted drugs, there is no actual achievement in terms of the overall survival of colon cancer patients^[3]. Therefore, it is necessary to find new ideas to prevent and treat colon cancer.

There are significant differences in the material metabolism between tumor cells and normal cells, and there is great potential for anti-tumor therapy in response to these differences^[4]. Studies have found that most tumor cells cannot grow in methionine-deprived media, and normal cells can grow normally under methionine-deprived conditions^[5]. This may be due to the increase in protein synthesis and transmethylation in tumor cells. The requirement for methionine far exceeds that of normal cells. At the same time, due to the low activity of methionine synthase in tumor cells, it cannot use homocysteine to synthesize enough endogenous methionine like normal cells^[6]. Studies have shown that the restriction

of methionine provided in the intestines for an average of 17 weeks is safe and feasible for patients with advanced metastatic cancer^[7]. The short-term methionine restriction obtained through total parenteral nutrition can improve the efficacy of 5-fluorouracil in patients with advanced gastric cancer^[8]. These pieces of evidence indicate that restricting colon cancer cells' access to methionine is a promising treatment.

This study used Gene Expression Omnibus (GEO) and The Cancer Genome Atlas (TCGA) databases to comprehensively analyze the effect of methionine restriction on the expression of colon cancer genes, and preliminarily explore its anti-tumor mechanism. It also confirmed that the methionine restriction ability inhibited the proliferation, metastasis, and invasion of colon cancer HCT116 cells and promoted apoptosis. Finally, a variety of statistical and bioinformatics methods were used to identify and verify the key genes that affect the progression of colon cancer by methionine restriction.

Materials And Methods

Data collection and processing

Download the GSE72131 and GSE103602 data sets from the GEO database. They included data on the treatment of colon cancer HCT116 cells with methionine restriction. Use the DESeq2 R package and the Edge R package to analyze the two data sets separately, and use $|\text{Log2Fold Change (FC)}| > 1$, False Discovery Rate (FDR) < 0.05 as the screening conditions, to identify the differentially expressed genes. Use the Venn diagram to obtain the common methionine starvation differentially expressed genes in the two data sets, and perform enrichment analysis and other related analyses. Download the colon cancer IlluminaHiSeq dataset from UCSC Xena (<https://xena.ucsc.edu>), extract matching colon cancer samples, and use the limma R package^[9] to identify genes that are differentially expressed in colon cancer. Furthermore, we used the Venn diagram to analyze the effect of methionine restriction treatment on the differentially expressed genes in colon cancer.

Gene Ontology and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis

GO contains the representation of the essence of the knowledge system in the biological field. The ontology is usually composed of a set of categories (or terms or concepts), and there is a relationship between them. It describes our understanding of the biological field from three aspects (GO domains): Molecular Function (MF), Cellular Component (CC), and Biological Process (BP). KEGG is used to understand the advanced functions of biological systems (such as cells, organisms, and ecosystems) from molecular-level information (especially large-scale molecular data sets generated through genome sequencing and other high-throughput). To understand the molecular mechanism and related biological signal changes in colon cancer cells after methionine restriction. We used the "clusterprofiler" R package to perform GO and KEGG functional enrichment analysis on methionine starvation differentially expressed genes.

Construction of transcription regulation and competitive endogenous RNA (ceRNA) network

In order to understand the possible mechanism of methionine restriction affecting differentially expressed genes in colon cancer, we performed transcriptional regulation and ceRNA network construction on the obtained genes. For the transcriptional regulatory network, use DAVID6.8 to enrich the potential transcription factors of all genes, and consider the transcription factors with Bonferroni < 0.05 as meaningful^[10]. For the ceRNA network, we use mircode to compare the obtained genes to obtain the ceRNA network and identify key long-chain non-coding RNAs and microRNAs^[11]. Both networks are visualized using cytoscape 3.6.1.

Protein interaction network (PPI) construction and screening of independent prognostic candidate genes

for gastric cancer while removing collinearity and avoiding overfitting.

Cell culture and processing

The human HCT116 cell line (Wuhan Cell Bank, Chinese Academy of Sciences, Wuhan, China) contains 10% fetal bovine serum (Gibco, USA) and 100IU/mL penicillin (Solarbio, China) in McCoy's 5A (Boster, China) 1640. For methionine-restricted cells, they were cultured in McCoy's 5A (Boster, China) with MET-. All cells were cultured in a humidified atmosphere containing 5% CO₂ at 37°C.

Cell proliferation assay

Evaluation of colon cancer cell proliferation by 5-Ethynyl-2'-deoxyuridine (EDU) Cell proliferation detection and plate cell clone formation. First, staining was performed according to the instructions of the EDU commercial kit (US Everbright, China), and the treated cells were subjected to EDU measurement using a fluorescence microscope (Olympus, Japan). For colony formation analysis, 350 cells per well were cultured in a 6-well plate for 2 weeks and stained with GIMSA application staining solution (Solarbio, China) for 30 minutes to calculate the total number of colonies.

Flow cytometry apoptosis detection

Use Annexin V-APC and PI Apoptosis Detection Kit (US Everbright, China) to detect cells in the early and late stages of apoptosis. The processed cells were stained according to the instructions and analyzed in the Becton Dickinson FACS calibur instrument. Count the cells with early apoptosis-positive for Annexin V-APC and late apoptosis with PI-positive, and analyze the effect of methionine restriction on cell apoptosis.

Cell migration and invasion experiments

The wound-healing assay measures cell level migration. When the cells are close to 95% confluence, use 200μl of washing head to scrape the monolayer vertically. After washing away the cell debris with PBS,

an inverted microscope (Olympus, Japan) was used to analyze the wound gap size at 0, 24, and 48 hours after the injury to evaluate the migration ability.

For the determination of the vertical migration of cells, the cells were suspended in different serum-free media, and 2×10^4 cells were seeded in the chamber of the Transwell plate. For the cell invasion assay, the upper chamber was pre-coated with Matrigel (BD Biosciences, USA) for 2 hours, and the remaining steps were the same as the vertical migration assay. The lower chamber was supplemented with 800 μ l of serum-containing medium and cultured for 72 hours. At room temperature, the cells invaded into the lower chamber were fixed with methanol for 15 min, stained with 0.1% (w/v) crystal violet, and analyzed by inverted microscope imaging.

Western Blot analysis

The cells were lysed in RIPA (Solarbio, China) containing protease inhibitors (Boster, China) for 20 min on ice. The bicinchoninic acid (BCA) protein content kit (Solarbio, China) to determine protein concentration. 40 μ g total protein per well was separated on 10% polyacrylamide gels and transferred to polyvinylidene fluoride (PVDF) membrane (Merck, Germany). The membrane was blocked with 5% BSA for 1 hour at room temperature. The PVDF membrane was combined with GAPDH (Proteintech, USA, Cat No. 60004-1-Ig), B-cell lymphoma/leukemia-2 (Proteintech, USA, Cat No. 12789-1-AP), BCL2 Associated X (Proteintech, USA, Cat No. 50599-2-Ig), Caspase-3 (Proteintech, USA, Cat No. 66470-2-Ig), N-cadherin (Abcam, USA, Cat No. ab76057), E-cadherin (Abcam, USA, Cat No. ab231303), Vimentin (Abcam, USA, Cat No. ab137321) was incubated overnight. After washing with Tris-buffered saline Tween, the membrane was probed with horseradish peroxidase-conjugated goat anti-rabbit IgG or goat anti-mouse IgG (Boster, China) for 1 hour at room temperature. The band was detected using Super ECL Plus (US Everbright, China). The protein expression results are expressed relative to the GAPDH band density.

Expression verification of key genes

We first used The Human Protein Atlas (HPA, <https://www.proteinatlas.org>) database to verify the expression of the obtained key gene proteins in colon cancer^[12]. Trizol reagent (Thermo Fisher, USA) was used to extract the total RNA according to the instructions. After the concentration and purity of the RNA were qualified, the reverse transcription kit RR047a (Takara, Japan) was used for reverse transcription according to the instructions. ACTB was used as the internal reference gene. The expression level of the target gene was analyzed by RR820 Kit (Takara, Japan) in the ABI7900-HT system (Thermo Fisher, USA). All primers used were synthesized by Shanghai Sheng Gong Company of China, and the primer sequences are shown in Table 1.

Table 1
The primer sequence used in this study.

Gene ID	Forward primer sequence (5' – 3')	Reverse Primer sequence (5' – 3')
ACTB	CACCATTGGCAATGAGCGGTTC	AGGTCTTTGCGGATGTCCACGT
E2F1	GGACCTGGAACTGACCATCAG	CAGTGAGGTCTCATAGCGTGAC
MIR17HG	GGGCCTCCGGTCGTAGTAA	AACAGGTTTCCCTCCGTCGC
FANCI	CCACCTTTGGTCTATCAGCTTC	CAACATCCAATAGCTCGTCACC
HJURP	CAGATGGGGTGGACAACAC	TCTTCCATCCTGTAAGACGTG
KPNA2	CTGTTGGCTCTCCTTGCACTTC	GCAGGATTCTTGTTGCGGCAAAG
KIF15	AGGAATCTGTATTCGCAACTGTG	ACTTCGTGGGATTACTCCTCTC

Results

Identify gene expression affected by methionine restriction

The effects of methionine restriction on gene expression were identified from GSE72131 and GSE103602 through the "Edge" package and the "DESeq" package. From GSE72131, 1477 up-regulated and 1115 down-regulated genes were identified (Fig. 1A). 1877 dysregulated genes were identified from GSE103602, including 1110 up-regulated and 767 down-regulated (Fig. 1B). The intersection of the two data sets identified a total of 649 co-upregulated and 532 co-downregulated genes (Fig. 1C), which are considered to be differentially expressed genes affected by methionine restriction. In addition, we also analyzed the dysregulated genes in colon cancer, and there were a total of 1,532 up-regulated and 2,986 down-regulated genes (Fig. 1D). To identify the effect of methionine starvation on these differentially expressed genes in colon cancer, we performed a Venn diagram analysis on the two. Finally, 330 genes were identified (Fig. 1E), and cluster analysis of these genes in colon paired samples was performed using heat maps (Fig. 1F).

GO and KEGG function enrichment analysis

To understand the influence of methionine restriction on the molecular mechanism of colorectal cancer, the obtained methionine restriction-regulated genes were enriched and analyzed. For 649 up-regulated genes, BP is enriched in the extracellular matrix organization, extracellular structure organization, response to nutrient levels and I-kappaB kinase/NF-kappaB signaling, etc.; CC is enriched in the collagen-containing extracellular matrix, collagen trimer, and other items; MF is enriched in extracellular matrix structural constituent, protein tyrosine/threonine phosphatase activity, extracellular matrix structural constituent conferring tensile strength, MAP kinase tyrosine/serine/threonine phosphatase activity and MAP kinase phosphatase activity (Fig. 2A, Supplementary Table 1). KEGG is enriched in Cytokine-cytokine receptor interaction, Viral protein interaction with cytokine and cytokine receptor, IL-17 signaling

pathway, Starch and sucrose metabolism, etc. (Fig. 2C). For 532 down-regulated genes, BP is enriched in DNA replication, DNA conformation change, DNA-dependent DNA replication, negative regulation of cell cycle process, and other items; CC is mainly enriched in chromosome regions, mitochondria, and other regions; MF is mainly enriched in DNA -dependent ATPase activity, ATPase activity, ubiquitin-like protein ligase binding and other items (Fig. 2B, Supplementary Table 1). KEGG is enriched in DNA replication, cell cycle, Pyrimidine metabolism, Drug metabolism-other enzymes, Glycine, serine and threonine metabolism, and other pathways (Fig. 2D).

Transcription regulation and ceRNA network

We constructed a transcriptional regulatory network for differentially expressed genes regulated by methionine restriction in colon cancer. For the obtained 330 genes, we have identified 5 types of transcription factors (ROAZ, E2F, ATF, TAL1BETAITF2, AP1, ROAZ) and 298 downstream coding genes. It is worth noting that the differential genes we identified include E2F family genes. E2F1 (Fig. 3A). In addition, we found that these 330 genes contained 2 LncRNAs (MIR17HG and SNHG4), 325 protein-coding genes, and 3 pseudogenes. We constructed a ceRNA network based on this and identified 5 microRNAs downstream of MIR17HG (has-mir-23c, has-mir-23b-3p, has-mir-23a-3p, has-mir-203a, has-mir-455- 5p, has-mir-425-5p) and 190 microRNA target genes (Fig. 3B).

Construction of PPI network and identification of independent prognostic genes

Upload 325 encoded proteins to String, and remove the independent and unconnected proteins from the overall network to obtain a network of 256 genes and 1321 edges (Fig. 4A). Using MCODE, a key network with $k = 15.6$ was identified from the network, which contained 41 genes and 675 edges (Fig. 4B). For these 41 candidate genes, we first used Lasso regression to remove collinearity and identified 5 (MAD2L1, FANCI, KPNA2, KIF15, HJURP) prognostic-related genes (Fig. 5A). Furthermore, using multivariate Cox regression analysis, 4 (FANCI, KPNA2, KIF15, HJURP) independent prognostic genes were identified from these 5 genes(Fig. 5B).

The effect of methionine restriction on the proliferation and apoptosis of colon cancer cells

We first used EDU staining to analyze the effect of methionine restriction on the proliferation of colon cells, and the results showed that the cell proliferation ability of the methionine restriction group was weaker than that of the control group (Fig. 6A). The cell plate clone formation experiment showed that the methionine-restricted clone formation rate was significantly lower than that of the control group (Fig. 6B). We also analyzed the apoptosis rate. After methionine restriction, the apoptosis rate was significantly higher than that of the control group (Fig. 6C). By analyzing the expression of apoptosis-related proteins

(Fig. 6D), the expression of Bax and Caspase-3 was significantly increased while the expression of Bcl-2 was decreased, which also confirmed that methionine restriction can induce apoptosis.

The effect of methionine restriction on the invasion and migration of colon cancer cells

The cell scratch test can reflect the horizontal migration ability of colon cancer, and the results show that the horizontal migration ability of methionine restricted cells is lower than that of the control group (Fig. 7A). We also used the Transwell method to evaluate vertical migration. Similarly, methionine restriction can inhibit the migration of colon cancer cells (Fig. 7B). In addition, we showed through invasion experiments that methionine restriction caused a decrease in invasion ability (Fig. 7C). Finally, we also analyzed the expression of metastasis-related proteins (Fig. 7D). After methionine restriction, the expression of N-cadherin and Vimentin decreased, while the expression of E-cadherin increased, which also showed that methionine restriction inhibited colon cancer metastasis.

To verify the expression of key genes affected by methionine restriction

In the transcriptional regulatory network, we identified the key transcription factor E2F1. In the ceRNA network, we found that MIR17HG is the core LncRNA of the network. By identifying candidate genes obtained from the key network of the PPI network, combined with Lasso regression and multivariate Cox regression, we identified 4 independent prognostic genes (FANCI, KPNA2, KIF15, HJURP). We first verified the protein expression of these genes by HPA. The results showed that the protein expression of E2F1, FANCI, HJURP, KPNA2 and KIF15 was significantly increased in colon cancer (Fig. 8A). Then, we use rt-PCR to verify the expression of these 6 genes after methionine restriction. The results showed that the expression of the above 6 genes was significantly down-regulated after methionine restriction, which was consistent with our previous analysis (Fig. 8B).

Discussion

Colon cancer usually occurs at the junction of the rectum and the sigmoid colon, mostly in people aged 40–50. With the development of society, people's diet and living habits have also undergone tremendous changes. Their preference for heavy flavors, high-sugar and high-fat diets, disordered hunger and fullness, unclean diet, and staying up late make the incidence of colon cancer more and more youthful^[13]. More than 30 years ago, Halpern et al. found that there is a methionine-dependent metabolic phenotype in tumor cells. Kano Y et al. subsequently found that except for lymphocytes, normal cells can grow normally under methionine starvation, which supports this theory^[14]. Studies on colon cancer have shown that supplementation of methionine in the diet can stimulate malignant changes in the intestinal tissues of mice^[15, 16]. Hanley et al. and Komninou et al. successively found that restricting methionine intake inhibited the progression of colorectal cancer^[17, 18]. At present, the drug for the metabolism of

methionine is methioninase, which can catalyze the α and γ -cleavage reactions of methionine to produce α -butyric acid, methyl mercaptan and ammonia. In the study of the drug, methioninase can cooperate with traditional chemotherapy drugs to treat colon cancer and show a better therapeutic effect^[19–22]. The study of Machover et al. showed that the possible mechanism of methioninase's tumor suppressor is that it can induce a decrease in the methylation level of the proto-oncogene cyclin dependent kinase inhibitor 2A and an increase in its expression. We studied the effect of methionine restriction at the cell level on colon cancer cells^[23]. The results show that methionine restriction can inhibit the proliferation, metastasis and invasion of colon cancer cells, and promote apoptosis, which also reflects the methionine-dependent phenotype of colon cancer cells.

Methionine restriction is a promising means of suppressing cancer, and there are few reports on the analysis of the potential mechanism of methionine inhibiting the growth of colon cancer. Using public data, we analyzed the genes that may be regulated by methionine restriction and performed an enrichment analysis on these genes. Among the up-regulated genes, we focus on MAP kinase phosphatase activity and protein tyrosine/threonine phosphatase activity. The MAPK cascade is a signal transduction component that plays a key role in converting extracellular stimuli into cellular responses through phosphorylation of different substrates. The MAPK signaling pathway controls various cellular processes, such as growth, differentiation, proliferation, survival and death. The activation of this signal depends on the simultaneous phosphorylation of both threonine (T) and tyrosine (Y) sites^[24]. For the study of methionine on MAPK signaling, Lin et al. used proteomics methods to find in gastric cancer that the expression of p38 increased after methionine restriction^[25]. P38 can induce cell cycle arrest that has undergone malignant transformation, and even enter a state of senescence or apoptosis, which is an important factor in inhibiting tumor formation and development. Among the down-regulated genes, we observed that they were enriched in glycolysis process, ATPase activity, Drug metabolism, etc. Tumor cells and normal cells also have differences in energy metabolism. They may preferentially generate large amounts of lactic acid through aerobic glycolysis to obtain more energy. By inhibiting the energy production of tumor cells, it can effectively play an anti-tumor effect. In the study of gastric cancer, methionine restriction can reduce the activity of aerobic glycolysis and induce tumor cell apoptosis^[26]. The reduction of tumor productivity leads to the inactivation of many ATPases. It is important that drug metabolism depends on a variety of ATP-dependent transport enzymes. Among them, P-gp glycoprotein plays an important role in tumor resistance, and it uses ATP to supply energy to metabolize cell-energy drugs. The study of Xin et al. found that methionine restriction induces down-regulation of the expression of resistance-related protein P-gp, which enhances the drug sensitivity of gastric cancer^[27].

Based on bioinformatics analysis, we identified that methionine restriction affects the important transcription factor E2F1 and the key lncRNA MIR17HG. In addition, four independent prognostic genes of colon cancer regulated by methionine restriction were identified based on Lasso and multivariate Cox regression, among which FANCI and HJURP can be considered as risk genes. E2F1 is a member of the E2F transcription factor family, which can bind to DNA with dimer partner (DP) protein through the E2 recognition site 5'-TTTC [CG] CGC-3' found in the promoter region of various genes. E2F1 is directly

involved in several types of cancer with poor prognosis, and has been shown to be a key cancer biomarker^[28, 29]. E2F1 not only promotes the proliferation and metastasis of rectal cancer, but also contributes to aerobic glycolysis, repressurization and oxidative metabolism, and promotes anabolic metabolism^[30]. MIR17HG is a miR-17-92 cluster host gene lncRNA, which participates in cell proliferation and growth by regulating cell growth phenotype. Studies on cervical cancer, non-small cell carcinoma and osteosarcoma have shown that MIR17HG acts as an oncogene in tumors, inhibiting its expression, resulting in tumor cell proliferation, metastasis, and drug resistance^[31–33]. Xu et al.'s research in colon cancer showed that MIR17HG is closely related to tumor immunity. First, it competitively sponges microRNA miR-375, thereby increasing the expression of nuclear factor kappa-B/ RELA, and then up-regulating programmed death receptor 1 expression, RELA can also directly bind to the MIR17HG promoter region to form a positive feedback loop to activate MIR17HG transcription^[34]. FANCI is a key gene to maintain chromosome stability and plays an important role in repairing DNA double-strand breaks by homologous recombination.

It repairs inter-strand DNA crosslinks through homologous recombination and induces FANCL to promote FANCD2 monoubiquitination, and participates in the recruitment of DNA repair sites^[35]. HJURP is a histone chaperone involved in the recruitment of de novo histone H3 variants CenH3 (CENP-A) and CENP-C in nucleosomes, which can regulate the amplification of centromeric chromatin and chromosomal stability^[36]. HJURP has been confirmed as a key prognostic-related gene in breast cancer, hepatocellular carcinoma, advanced serous ovarian cancer, colorectal cancer and prostate cancer^[37–41]. There are reports showing that HJURP promotes tumor cell proliferation. For example, Wang et al. showed that HJURP activates MDM2 transcription by regulating the recruitment of H3K4me2 in its promoter region to inhibit the expression of p53 and promote the growth of pancreatic cancer cells^[42]. Chen et al. found that HJURP destabilizes p21 through MAPK/ERK1/2 and Protein Kinase B/Glycogen Synthase Kinase-3 β pathways, thereby regulating nuclear cytoplasmic translocation and ubiquitin-mediated p21 degradation, and promoting tumor cell proliferation^[43]. In addition, studies have shown that HJURP can promote epithelial-mesenchymal transition of tumor cells by regulating the Wnt/ β -catenin pathway and SPHK1^[44, 45]. Not only that, studies by Yuan et al. and Cao et al. have successively confirmed that HJURP affects the oxidative stress and cell cycle arrest of tumor cells by regulating the peroxisome proliferator-activated receptor- γ /sirtuin1 feedback loop^[46, 47]. In our research, we confirmed that E2F1, MIR17HG, FANCI, and HJURP are highly expressed in colon cancer tissues. Moreover, its expression decreased significantly after methionine restriction, suggesting that methionine restriction may exert a tumor suppressor effect through the above pathways.

In general, based on bioinformatics, we have analyzed the underlying mechanism of methionine restriction affecting colon cancer as a whole, and identified some key genes that methionine restriction affects the progression of colon cancer. Furthermore, we confirmed in vitro experiments that methionine restriction can inhibit the proliferation, metastasis and invasion of colon cancer cells, and induce apoptosis, and verified the expression of key genes after methionine restriction.

Conclusion

In summary, we have verified in vitro that methionine restriction inhibits the proliferation, metastasis and invasion of colon cancer. By means of bioinformatics, the potential molecular biological mechanisms affecting the progression of colon cancer were analyzed, and the key target genes regulated by methionine restriction were identified. Finally, the differences in the expression of these genes were verified by rt-PCR, which provides an idea for the follow-up study of methionine restriction therapy for colon cancer.

Declarations

Acknowledgments

None

Authors contributions

Liqiang Zhou and Zhiqing Chen designed the entire project and wrote the manuscript, and Chuan Liu supervised and supported the completion of the work.

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Data Availability Statement

All data can be obtained from the corresponding author.

Ethics statement

This study complies with the Declaration of Helsinki, and the clinical samples obtained were approved by the patient's informed consent.

Consent for publication

All authors agree to the publication of this study, and all personal data in this study have obtained the consent of the parties concerned.

Conflict of Interest

The author claims no conflict of interest

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Figures

Figure 1

Identifying that methionine restriction affects the genes expression of colon cancer. A. Genes regulated by methionine restriction in GSE72131; B. Genes regulated by methionine restriction in GSE103602; C. Methionine restriction co-regulated genes in GSE72131 and GSE103602; D. Dysregulated genes in colon cancer; E. Identification of methionine restriction regulating differentially expressed genes in colon cancer; F. The heat map shows that methionine restriction regulates the expression of differentially expressed genes in colon cancer in matched colon cancer tissues.

Figure 2

Enrichment analysis of methionine restriction regulatory genes. A. GO enrichment analysis of up-regulated genes; B. KEGG function enrichment analysis of up-regulated genes; C. GO enrichment analysis of down-regulated genes; D. KEGG function enrichment analysis of down-regulated genes.

Figure 3

Construction of transcriptional regulatory and ceRNA network based on methionine restriction regulation of differentially expressed genes in colon cancer. A. The transcriptional regulatory network screened out the key transcription factor E2F1; B. The ceRNA network identified the key lncRNA MIR17HG.

Figure 4

PPI network construction and key network identification. A. Establishment PPI network base on methionine restriction regulation of differentially expressed genes in colon cancer; B. MCODE identifies critical networks, k=15.6.

Figure 5

Lasso regression and multivariate Cox regression analysis identify independent prognostic genes from key modules. A. Lasso regression analysis; B. Multivariate Cox regression analysis identified 4 independent prognostic genes.

Figure 6

Methionine restriction inhibits the proliferation of colon cancer cells and promotes apoptosis. A. EDU showed that methionine restriction reduced the number of colon cancer cells proliferating; B. Plate cell clone formation experiments showed that methionine restriction decreased the proliferation ability of colon cancer cells; C. Flow cytometric detection showed that methionine restriction increased the apoptotic rate of colon cancer cells; D. Methionine restriction down-regulated the expression of Bcl-2 and increased the expression of Bax and caspase-3.

Figure 7

Methionine restriction inhibits the invasion and migration of colon cancer cells. A. Cell scraping experiments show that methionine restriction inhibits the horizontal migration of colon cancer cells; B. Transwell method shows that methionine restriction inhibits the vertical migration of colon cancer cells; C. Methionine restriction inhibits the invasiveness of colon cancer cells; D. Methionine restriction reduces the expression of N-cadherin and Vimentin and induces high expression of E-cadherin.

A

B

Figure 8

Methionine restriction down-regulated the expression of 6 key genes. A. HPA shows that the expression of six key genes is elevated in colon cancer; B. rt-PCR showed that methionine restriction decreased the expression of 6 key genes.

Supplementary Files

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