Integrated Multi-Omics Analysis of Colorectal Cancer Reveals Mir-20a as a Regulator of Fatty Acid Metabolism in Consensus Molecular Subtype 3

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Research

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Abstract

Background

microRNAs (miRNAs) serve important roles in metabolism. The consensus molecular subtype (CMS) 3 of colorectal cancer (CRC) is characterized by activated fatty acid (FA) metabolism. We aimed to identify essential miRNAs of CMS3-CRC and analyze the regulatory role in the FA metabolism.

Methods

The RobustRankAggreg method by integrating multi-omics data including genome, epigenome, transcriptome and interactome, was applied to filter out functional genes (Fgenes). The backward derivation approach based on Fgenes and miRNA-gene interactions was further applied to identify functional miRNAs (Fmirs). Nine human CRC cell lines with different CMSs were investigated. RT-qPCR, western blotting and immunofluorescence were performed to examine the effect of miR-20a on FA synthesis and Wnt/ β -catenin signaling. The effect of miR-20a on cell proliferation and metastasis were studied by clone-formation, EdU assay, wound healing and transwell assay.

Results

We identified 12 Fmirs by integrating multi-omics features in CMS3-CRC. These Fmirs exhibited significantly enriched CRC driver miRNAs and significant impacts on CMS3-CRC cell growth. Beyond the findings, miR-20a was significantly correlated with Wnt/ β -catenin signaling and participated in FA metabolism subpathway. In vitro assays combined with bioinformatics analyses demonstrated that elevated miR-20a up-regulated FA synthesis enzymes *FASN*, *ACAC* and *ACLY* via Wnt/ β -catenin signaling, and finally promoted proliferative and migration of CMS3-CRC cells.

Conclusions

Overall, our study revealed that miR-20a promoted progression of CMS3-CRC by regulating FA metabolism and served as a potential target for preventing tumor metastasis.

Background

Colorectal carcinoma (CRC) ranks the second leading cause of cancer-related deaths worldwide, with high heterogeneity at the molecular level[1]. The consensus molecular subtypes (CMSs) of CRC were proposed and present with differences in molecular characteristics and clinical outcomes[2]. Strikingly, CMS3-CRC is characterized by prominent metabolic activation. Metabolic alterations play a major role in the growth and progression of cancers[3]. Among these abnormalities, disorders of lipid metabolism particularly fatty acid (FA) metabolism, cannot be ignored[4, 5]. FA metabolism maintains energy homeostasis in cancer and is regarded as a new therapeutic strategy[6].

A microRNA (miRNA) is a small non-coding RNA molecule that functions as post-transcriptional gene regulators[7]. miRNAs serve important roles in several physiological conditions, including cell differentiation, development, apoptosis, immune response, hematopoiesis, cell death and proliferation [8, 9]. In recent years, several miRNAs have been identified to regulate FA metabolism in cancers[10]. It has been demonstrated that miR-185 and miR-342 control lipogenesis in prostate cancer cells by down-regulating fatty acid synthase[11]. However, many researchers seem to focus more on the regulation of miRNAs on FA metabolism in other cancer types than CRC. The relationship between miRNAs and FA metabolism in CRC is still unclear.

Multi-omics datasets represent distinct aspects of the central dogma of molecular biology, emphasizing the complementarity of underlying biology[12]. Previously, we proposed to prioritize functional gene regulators in CMSs by integrating genome, epigenome, transcriptome and interactome data, which play central roles in the corresponding subtypes[13]. As the lack of multi-omics data for miRNAs, we only focused on protein-coding gene regulators. Herein, we proposed to prioritize functional miRNAs (Fmirs) by identifying functional genes (Fgenes) in CMS3-CRC and constructing miRNA-Fgene interaction network. We found that Fmirs were enriched with driver miRNAs of CRC and mediated FA metabolism subpathways. Function assays showed miR-20a led to an increase in FA synthesis genes, and regulated proliferation and migration of CMS3-CRC via Wnt signaling pathway.

Methods

Data source

We downloaded publicly available CRC gene expression datasets from Gene Expression Omnibus (GEO, <https://www.ncbi.nlm.nih.gov/gds/>) and The Cancer Genome Atlas (TCGA, <https://portal.gdc.cancer.gov/>) (Additional file 1: Table S1). We collected a set of 3,360 CRC tissue samples from 21 datasets measured by eight platforms and another set of 618 adjacent normal tissue samples from 23 datasets measured by six platforms. Only 12,401 genes that are shared in all these datasets were used. Especially for CRC samples, we only used the datasets containing more than 50 samples to avoid the influence of small sample bias. Data were preprocessed as previously described[14].

We also downloaded 619 miRNA samples, 536 mutation samples analyzed by MuTect2 algorithm (.maf files), 617 copy number alteration (CNA) samples and 295 DNA methylation samples from the TCGA project (TCGA-COAD and TCGA-READ).

Classification of colorectal cancer tissue samples

The random forest classifyCMS function in the R package CMSclassifier (<https://github.com/Sage-Bionetworks/CMSclassifier>) was used to assign CMSs to CRC samples based on the gene expression profile of each tissue dataset[2]. Microarray data for all datasets were obtained in the form of normalized

expression values on the log2 scale. For RNAseq data, we used log2 transformed FPKM matrix after adding 1 to avoid undefined values.

Classification of colorectal cancer cell lines

The gene expression data of 57 large intestine cell lines were download from the Cancer Cell Line Encyclopedia (CCLE) database (<https://portals.broadinstitute.org/ccle>). The CMScaller algorithm was used to assign CMSs to CRC cell lines (<https://github.com/Lothelab/CMScaller>). CMS cell lines were also supplemented with the reported results[15].

Manually curation of gene interaction

Protein–protein interaction network (PPIN) data were downloaded from STRING v11.0 (<https://string-db.org/>)[16]. We used interactions with the organism of “Homo sapiens” and score more than 0.9 with high confidence. TF–gene interactions assayed by chromatin immunoprecipitation followed by sequencing (ChIP-seq) were also downloaded from the ChIPBase v2.0 database (<http://rna.sysu.edu.cn/chipbase/>)[17].

PPI and TF–gene interactions in the context of CMS3-CRC were filtered based on their expression correlation measured by the Pearson correlation analysis. We only retained the significantly co-expressed interactions with the threshold of adjusted $P < 0.05$ and $|\rho| > 0.3$. “ ρ ” represents the Pearson correlation coefficient.

miRNA-mediated gene regulation

miRNA–gene interactions were all experimentally validated and collected from public databases, including TarBase[18], miRTarBase[19], and miRecords[20]. miRNA–gene regulations in the context of CMS3-CRC were filtered based on their expression correlation measured by the Pearson correlation analysis with the threshold of $P < 0.05$ and $|\rho| > 0.3$.

We note that these target genes of miRNAs were derived from TCGA data. To avoid biased results, we took the target genes as a gene signature for each miRNA and performed an evaluation step (sigQC) to ensure that the signature used has suitable properties across different datasets. Here, we present radar plots summarizing the gene signature quality control metrics implemented by the R package sigQC[21].

Identification of functional miRNAs

We identified Fmirs by firstly identifying Fgenes. Based on the individual-level differentially expressed genes calculated by RankComp algorithm[22]. We identified genes with significantly upregulated or downregulated patterns and defined them as CMS-related genes. Then, we proposed a computational method to prioritize CMS-related genes and identify Fgenes in CMS3-CRC by integrating multi-omics features[13]. Briefly, for each CMS-related gene in CMS3-CRC, we calculated the scores of seven features that characterized the genomic and transcriptomic activity, regulation by co-expressed TFs, and central

roles in PPIN of gene regulators. The aggregated score for each gene was further calculated by integrating the seven separate ranks in CMS3-CRC, using the robust rank aggregation method in R package RobustRankAggreg[23]. Then, we utilized the most effective feature and identified top-ranked 100 genes as the Fgenes.

For miRNA, we directly analyzed the 1,881 miRNAs measured by the TCGA-COAD and TCGA-READ project. We identified Fmirs using a backward derivation approach. Firstly, we identified significantly co-expressed miRNA and genes for CMS3-CRC among the collected miRNA-gene interactions. Then, miRNAs whose target genes were significantly intersected with Fgenes were defined as Fmirs. The hypergeometric test was used to calculate the significance as follows:

$$\Pr(i) = 1 - \sum_{x=0}^{F_i-1} \frac{\binom{T_i}{x} \binom{N-T_i}{F-x}}{\binom{N}{F}}$$

Where N is the number of target genes that overlap with all detected genes in the expression profiles, F is the number of target genes that overlap with Fgenes, T_i is the number of target genes of miRNA i , and F_i is the number of target genes for miRNA i that overlap with Fgenes.

Manually curation of CRC driver miRNAs

A panel of oncogenic and tumor-suppressive miRNAs was collected from the public databases miRCancer[24] and OncomiRDB[25]. Briefly, the microRNA–cancer association in miRCancer was extracted using the text mining algorithm against PubMed based on 75 rules. The entries of CRC-related miRNA regulations in OncomiRDB were manually curated from abstracts with direct experimental evidence. Finally, we retained miRNAs associated with CRC and collected 250 CRC-related miRNAs.

Subpathway analysis

The R package “Subpathway-GMir” was performed to identify the metabolism subpathways mediated by miRNAs[26]. We mapped the Fmirs and significantly co-expressed target genes as nodes into the reconstructed metabolism pathway graphs obtained from the package. Then, we identified miRNA-mediated FA metabolism subpathways based on the “lenient distance” similarity method. It evaluated the significance of candidate subpathways using the hypergeometric method. The miRNA–target interactions for CMS3-CRC were collected as described above.

Cell culture and Transfection

Human colon epithelial cell line NCM460 and human CRC cell lines Lovo, SW48, T84, SW948, LS174T, HT29, HCT116 and SW480 were purchased from Procell Life Sciences Co. Ltd., (Wuhan, Hubei, China). Cell lines NCM460 was cultured in RPMI-1640 (Gibco) medium, T84 in DMEM/F12 (Gibco) medium, Lovo in F12K (Gibco) medium, LS174T in MEM, HT29 and HCT116 in (Gibco) McCoy’s 5A, SW48, SW948 and SW480 in L15 (Gibco) medium.

All media for the human cell lines were supplemented with 10% FBS and 1% penicillin/streptomycin antibiotics. SW48, SW948 and SW480 cell lines were cultured in 100% air at 37 °C. Other cell lines were cultured under 95% air-5% CO₂ at 37 °C. All cell lines were validated by STR DNA finger-printing. Experiments were carried out within 6 months after acquisition of the cell lines. In addition, mycoplasma contamination was ruled out using a PCR- based method.

HT29 and LS174T cells were transfected with miR-20a mimic and negative control (NC) or miR-20a inhibitor and inhibitor NC (inNC) respectively using Lipofectamine 2000 (Invitrogen, USA). After 48 h transfection, these cells were harvested for further experiments.

Western Blotting

The cells were washed with ice-cold PBS and lysed with RIPA buffer containing protease inhibitors. After mixed with SDS sample buffer, the proteins were heated to 99 °C for 10 min, separated on 10% SDS–polyacrylamide gels, transferred to PVDF mem-branes, and probed with antibodies against β -catenin (1:1000, CST, USA) and β -actin (1:3000, Proteintech, China) at 4 °C overnight and blots were visualized using gel imaging systems (Bio-Rad). ECL was used to visualize the immunoblot signals.

Real Time-quantitative PCR (RT-qPCR)

Total RNA of HT29 and LS174T cells were extracted using total RNA extraction kit (Thermo Fisher Scientific, Waltham, USA). 1 μ g RNA was used for reverse transcription into complementary DNA (cDNA) using the PrimeScript RT-PCR Kit (Takara, Tokyo, Japan). Real-time polymerase chain reaction (RT-PCR) was conducted using the SYBR Premix Ex-Taq II kit (Takara, Tokyo, Japan) on the Quant Studio 3 Real-Time PCR System (Applied Biosystems, CA, USA). The fold changes were determined using the $2^{-\Delta\Delta CT}$ method. All primers used were as follows: Quantitative-FASN, forward 5'-AAGGACCTGTCTAGGTTTGGATGC-3', reverse 5'-TGGCTTCATAGGTGACTTCCA-3', Quantitative-ACACA, forward 5'-ATGTCTGGCTTGACCTAGTA-3' reverse 5'-CCCCAAAGCGAGTAACAAATTCT-3', Quantitative-ACLY, forward 5'-TCGGCCAAGGCAATTTTCAGAG-3' reverse 5'-CGAGCATACTTGAACCGATTCT-3', Quantitative-CPT1A, forward 5'-TCCAGTTGGCTTATCGTGGTG-3' reverse 5'-TCCAGAGTCCGATTGATTTTTTGC-3', Quantitative-Bactin, forward 5'-CATGTACGTTGCTATCCAGGC-3' reverse 5'-CTCCTTAATGTCACGCACGAT-3', Quantitative-U6, forward 5'-CTCGCTTCGGCAGCACATATACT-3' reverse 5'-ACGCTTCACGAATTTGCGTGTC-3', Quantitative-miR-20a, forward 5'-TAAAGTGCTTATAGTGCAG-3' reverse 5'-GTCGTATCCAGTGCAGGGTCCGAGGT-3', Reverse Transcription-miR-20a, 5'-GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACCTACCT-3', Reverse Transcription-U6, 5'-AAAATATGGAACGCTTCACGAATTTG -3'.

Clone-formation assay, EdU assay, Transwell assay and Wound-healing assay

For the clone formation assay, HT29 and LS174T cell lines were transfected with mimic/NC (inhibitor/inNC) on day 1. Digest the cells the next day and seed 200 cells into a six-well plate, followed by the addition of 2 ml of 10% FBS DMEM for 14 days. After 14 days, the cells were fixed in 4%

paraformaldehyde and stained with crystal violet for 30 min at room temperature. Colonies consisting of > 50 cells were counted. For EdU assay, cells were plated in 12-well plates and performed transfection. After 48h, 5-ethynyl-2'-deoxyuridine (EdU) assay (BeyoClick™ EdU-488 Kit, Beyotime, Shanghai, China) were performed to analyze the cell proliferation. The cells were incubated with 10μM EdU solution for 2 h and fixed with 4% paraformaldehyde. And the cells were washed with PBS for 3 times and 0.5% TritonX-100 once. Then, the cells were stained with 100 μL 1 × DAPI solution. After washed with 100μL PBS for 3 times, images were obtained from fluorescence microscope for further calculation of proliferation rates.

Transwell assays were conducted using an 8 μm pore Transwell filter (Corning, US). HT29 or LS174T cells were seeded in the upper chamber with serum-free medium. And medium with 10% FBS was added to the lower chamber. After incubation for 48 h, the Transwell filter was washed, fixed with 4% paraformaldehyde and in turn stained with 0.1% crystal violet staining solution.

For wound-healing assay, HT29 or LS174T cells were seeded on six-well plates. Then, a wound across the wall was introduced. After washing with PBS, the plate was incubated with serum-free medium for 24 h. Cell migration was observed and photographed by microscopy.

Immunofluorescence analysis

The cells were seeded on poly-lysine-coated coverslips for 24 h, fixed with 4% paraformaldehyde for 15 min, and permeabilized in 1% Triton X-100 for 10 min. The cells were then blocked with 5% BSA in PBS for 30 min at room temperature and incubated in antibodies overnight at 4°C. The coverslips were washed three times with PBS buffer, and the secondary antibody was applied for 1 h at 37°C. The coverslips were then mounted onto glass slides using an antifade mounting medium (Beyotime, China). Cells were then examined under a laser scanning confocal microscope (Zeiss LSM 510 Meta).

Results

Identification of functional miRNAs in CMS3-CRC tumors

In order to find out the functional miRNAs (Fmirs), we initially identified Fgenes in CMS3-CRC by integrating multi-omics features. We firstly summarized CMS3-reglated genes that were significantly frequently up- or down-regulated in CMS3-CRC tumors from the individual-level genes identified by RankComp algorithm (Cumulative binomial model, adjusted $P < 0.05$). Fgenes were defined as the top-ranked 100 CMS3-related genes with highest aggregated multi-omics scores (Additional file 2: Table S2). For CMS3-CRC cell line HT29, Fgenes showed significantly lower dependency scores than other genes using RNAi data (Figure 1A), indicating their critical roles for cancer cell growth. Besides, Fgenes were significantly enriched with tumor driver genes identified by TUSON explorer[27] (Additional file 3: Figure S1A), and can separate CMS3-CRC tumors from normal samples (Additional file 3: Figure S1A). All results suggested that Fgenes were involved in the essential function of CMS3-CRC tumors.

Then, we identified significantly correlated miRNA-gene interactions in CMS3-CRC using paired miRNA and mRNA expression data from TCGA (Pearson correlation analysis, $P < 0.05$ and $|\rho| > 0.3$). Taking the significant interacting genes as a gene set signature for each miRNA, we found that CRC datasets are comparable in quality for the application of the gene signature using sigQC (Figure 1B, taken miR-20a as an example). Thus, we identified Fmirs based on the identified miRNA-gene interactions using TCGA data. The target genes of 12 miRNAs were enriched with Fgenes and these miRNAs were defined as Fmirs (Hypergeometric test, $P < 0.05$), 11 of which were significantly deregulated in CMS3-CRC compared to normal samples. Hierarchical clustering analysis found that Fmirs can separate CMS3-CRC tumors from normal samples (Figure 1C). Moreover, Fmirs were significantly enriched with driver miRNA of CRC collected from miRCaner and OncomiRDB databases (Hypergeometric test, $P < 0.05$, red in Figure 1C). Besides, targets of several Fmirs such as miR-363, miR-20a and miR-148a, were significantly overrepresented at the top of gene list in ascending order in the RNAi and CRISPR screened HT29 cell lines (GSEA, $P < 0.05$, Figure 1D), indicating their critical roles for cancer cell growth.

miR-20a promoted FA synthesis metabolism in CMS3-CRC cells

Accumulating evidence suggests that alterations in lipid metabolism represented by FA metabolism, contribute to overall metabolic reprogramming in cancer cells[28, 29]. CMS3-CRC had significantly higher enrichment score (ES) in FA metabolism pathway than the other subtypes (Figure 2A). Then, we identified FA metabolism subpathway mediated by Fmirs in CMS3-CRC. The subpathway contained 34 consecutive differential molecules, including six miRNAs and 28 genes (Figure 2B). For the six miRNAs, all except miR-363 showed significantly higher expression in CMS3-CRC tumors compared with normal samples. Particularly, miR-148a and miR-20a were also significantly over-expressed in CMS3-CRC than at least two CMSs (Figure 2C). miR-148a is associated with lipid metabolism in the liver[30], and its target Fgene *MMP7* participates in esophageal cancer metabolism[31]. The target Fgene *FOXQ1* of miR-20a can regulate liver metabolism[32]. Thus, miR-148a and miR-20a may be essential for FA metabolism in CMS3-CRC and were selected for further analysis.

Using CMScaller algorithm, we assigned CMSs to CRC cell lines. The expression of miR-148a and miR-20a was measured by RT-qPCR in the four CMSs cell lines and human colon epithelial cell line (Figure 2D). It was demonstrated that miR-20a was upregulated in CMS3-CRC cell lines HT29 and LS174T, when compared with colon epithelial cell line NCM460, which is consistent with the results identified in tissues. Moreover, miR-20a was also upregulated in CMS3-CRC cell lines in comparison with the other subtypes. The results were not observed for miR-148a (Additional file 4: Figure S2). To explore whether the miR-20a was involved in abnormal FA metabolism in CMS3-CRC tumors, we first investigated miRWalk and TargetScan database, and found potential complementary base pairing between miR-20a and key FA metabolism enzymes including *FASN*, *ACAC* and *ACLY*. The three genes were responsible for de novo FA synthesis and over-expressed in CMS3-CRC.

The HT29 and LS174T cells were transfected with miR-20a mimic, miR-20a inhibitor or NC to analyze multiple FA synthesis related genes, including *FASN*, *ACAC* and *ACLY*. We observed that transfected miR-

20a mimic into HT29 and LS174T cell lines upregulated the expression of miR-20a (Figure 3A). Simultaneously, the expression of *FASN*, *ACAC* and *ACLY* were increased in response to miR-20a mimic versus NC (Figure 3B). The subsequent loss-of-function experiments revealed that HT29 and LS174T cells transfected with miR-20a inhibitor had diminished miR-20a, *FASN*, *ACAC* and *ACLY* expressions compared with inNC groups (Figure 3C-D). These results indicated that upregulation of miR-20a promoted FA synthesis in CMS3-CRC tumors.

miR-20a promotes FA synthesis via Wnt signaling pathway

Activation of the Wnt signaling pathway led to lipid accumulation in cells[33]. The target Fgene *FOXQ1* of miR-20a, which can regulate liver metabolism[32], is a marker for activation of Wnt signaling in solid tumors[34]. Thus, we inferred that miR-20a promoted FA synthesis via Wnt signaling pathway in CMS3-CRC tumors. Using ssGSEA, we calculated the ES of Wnt gene sets listed in MSigDB. The expression of miR-20a was significantly positively correlated with canonical Wnt signaling pathway (Pearson correlation analysis, $P = 0.0108$, $\rho = 0.2766$), but negatively correlated with non-canonical Wnt signaling pathway (Pearson correlation analysis, $P = 0.0176$, $\rho = -0.2585$). The canonical Wnt signaling pathway causes an accumulation of β -catenin in the cytoplasm and induce a cellular response. Therefore, we sought to determine whether Wnt participated in miR-20a induced FA synthesis through exploring the effects on β -catenin expression.

After transfecting miR-20a mimic, inhibitor, and paired NC, the expression of β -catenin was detected by western blotting (WB) and immunofluorescence (IF) in HT29 and LS174T cell lines. As expected, WB analysis showed that miR-20a mimic-treated cells expressed higher β -catenin compared with NC groups (Figure 4A), whereas lower β -catenin was detected in miR-20a inhibitor-treated cells (Figure 4B). We obtained similar results in IF staining assays (Figure 4C). miR-20a mimic-treated HT29 cells showed significantly higher β -catenin positive staining counts than the NC group. In contrast, miR-20a inhibitor-treated HT29 cells showed a reversed phenotype. The results suggest that miR-20a promotes Wnt/ β -catenin level in CMS3-CRC cell lines. Previous studies have reported that the activated Wnt signaling pathway can up-regulate the activity of FA synthesis in tumor. Therefore, we used LiCl (10mmol/L) to treat HT29 and LS174T cells to activate Wnt signaling pathway and analyze the expression of FA-related genes. The results showed that activating the Wnt signaling pathway can significantly promote the up-regulation of FA metabolism genes (*FASN*, *ACAC* and *ACLY*) in CMS3-CRC cell lines (Figure 4D).

Elevated miR-20a promoted proliferation and migration of CMS3-CRC

Combined with the report that elevated FA synthesis promotes cancer cell proliferation and migration, we took on experiments to investigate miR-20a function during CRC tumorigenesis. GSEA was run to explore the potential role of miR-20a in CMS3 and showed that miR-20a played an important role in promoting cell cycle and DNA replicant (adjusted $P < 0.05$, Figure 5A). Besides, expression of miR-20a was significantly correlated with the proliferation marker *MKI67* (Pearson correlation analysis, $P = 1.72E-03$, $\rho = 0.34$, Figure 5B). The effects of miR-20a on cell proliferation of HT29 and LS174T cells were examined using clone-formation assay. The assay showed that elevated miR-20a expression promoted the clone

formation ability of HT29 cells compared with NC groups. In contrast, miR-20a inhibitor-treated HT29 cells showed a reversed phenotype. We repeated these experiments in LS174T cells and received coherent outcomes (Figure 5C). EdU assay showed that miR-20a promoted proliferation of HT29 and LS174T cells compared to NC groups (Figure 5D). While inhibition of miR-20a will reduce cell proliferation, suggesting that miR-20a had a critical role in CMS3-CRC tumor proliferation.

We performed wound healing assay and transwell assay to determine the effect of miR-20a on the migration ability of HT29 cell line. In the wound healing assay, cell motility was monitored at designated time points after scratches. The miR-20a overexpressed cells migrated toward the wound more quickly than the NC groups (Figure 6A). In contrast, miR-20a inhibitor-treated HT29 cells showed a reversed phenotype (Figure 6B). We repeated these experiments in LS174T cells and received coherent outcomes, suggesting that miR-20a had a unanimous pivotal role in CMS3-CRC oncogenesis, that is, inducing tumor migration in vitro (Figure 6C-D). A cell migration assay in a transwell system was performed to further assess the effect of miR-20a on HT29 cell migration. Our results showed that transfection with miR-20a mimic in HT29 cells effectively increased the migratory abilities (Figure 6E). In contrast, the migratory abilities exerted reversed trend in miR-20a inhibitor-treated HT29 cells.

Discussion

In recent years, the CMSs, which are emerging as critical factor for prognosis and treatment of CRC, have attracted wide attention. We have identified a core set of FGRs for each CMS by integrating multi-omics data. FGRs played critical roles in regulating immune microenvironment of CMSs. miRNAs are also gene regulators serving an important role in tumorigenic processes. Thus, we identified 12 Fmirs associated with CMS3-CRC based on Fgenes integrating from multi-omics features. Fmirs played a critical role in CMS3-CRC cell growth and FA metabolism pathway.

Consistent with our finding, the Fmir miR-20a has been documented to be highly expressed in CRC tissues versus normal mucosal tissues[35], and this upregulation in CRC has also been confirmed in serum and plasma[36]. Beyond these findings, we demonstrated that miR-20a promoted FA synthesis in CMS3-CRC. Activation of the Wnt signaling pathway led to lipid accumulation in cells[33]. We further found that miR-20a upregulated Wnt/ β -catenin signaling by systematically coupling bioinformatics analyses and in vitro experiments. β -catenin is a major component of cell-cell adhesion structures and functions as a controller of cell migration, colony formation and stem cell properties through translocation into nucleus[37, 38]. Aberrant β -catenin accumulation in the cytoplasm usually translocates to the nucleus and was associated with tumor relapse and metastasis in breast cancer patients[39]. The underlying mechanism needs to be further explored. Finally, we found that proliferation and migration abilities of the miR-20a overexpressed cells were significantly promoted, whereas the proliferation and migration abilities were lower in the miR-20a inhibitor groups.

Conclusions

In conclusion, miR-20a could promote the proliferative and migration in CMS3-CRC via regulating fatty acid metabolism and Wnt signaling pathway, highlighting novel mechanisms associated with CMS3-CRC cancerogenesis.

Abbreviations

CMS (consensus molecular subtype), CRC (colorectal cancer), FA (fatty acid), miRNAs (microRNAs), Fmirs (functional miRNAs), Fgenes (functional genes), CNA (copy number alteration), CCLE (Cancer Cell Line Encyclopedia), ChIP-seq (chromatin immunoprecipitation followed by sequencing), NC (negative control), inNC (inhibitor NC), RT-PCR (Real-time quantitative polymerase chain reaction), EdU (5-ethynyl-2'-deoxyuridine), GEO (Gene Expression Omnibus), TCGA (The Cancer Genome Atlas), ES (enrichment score), WB (western blotting), IF (immunofluorescence).

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

The datasets analyzed during the current study are available in the databases TCGA and GEO.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

W.Z., K.S., C.L. and Y.Z. conceived the study. K.S., J.Z., H.X., R.Y., and K.L. collected public data and performed bioinformatics analysis. C.L., and Y.Y. cultured cells, collected study materials and performed experimental design and interpretation. K.S., C.L., W.Z. and Y.Z. wrote the manuscript. All authors read and approved the final manuscript.

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Figures

Figure 1

Identification of Fmirs. (A) Comparison of Fgenes and other genes. (B) SigQC for datasets. (C) Heatmap of Fmirs. (D) CRISPR and RNAi analysis.

Figure 2

miR-20a regulated FA metabolism. (A) ES of FA metabolism pathways among CMSs. (B) Fmirs and FA metabolism subpathway. (C) The expression of miR-20a among CMSs in tissues. (D) RT-qPCR analysis of the expression levels of miR-20a in 8 different CRC cell lines and one colon epithelial cell line.

Figure 3

The expression of FA genes. (A) RT-qPCR analysis of the expression of miR-20a in HT29 and LS174T cells transfected with miR-20a mimics and their NC groups. (B) RT-qPCR analysis of the expression of FA synthesis related genes in HT29 after transfected with miR-20a mimic. (C) RT-qPCR analysis of the expression of miR-20a in HT29 and LS174T cells transfected with miR-20a inhibitor groups and their NC groups. (D) RT-qPCR analysis of the expression of FA synthesis related genes in HT29 after transfected with miR-20a inhibitor. Statistical analysis was conducted using one-way ANOVA. (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$).

Figure 4

Correlation of miR-20a with Wnt signaling pathway. (A-B) Western blotting of β -catenin in HT29 and LS174T cells transfected with different miRNAs. (C) Representative photomicrographs of β -catenin immunofluorescence staining assay in HT29 cells. Bar = 50 μ m. (D) RT-qPCR analysis of the expression of FA synthesis related genes (FASN, ACAC and ACLY) in HT29 and LS174T after treated with LiCl (10mmol/L) for 24h. (** $P < 0.001$, ns, not significant).

Figure 5

miR-20a affect cell proliferation. (A) GSEA of cell cycle and DNA replicant pathway. (B) Correlation of miR-20a and MKI67 expression. Representative photomicrographs and quantifications of (C) clone formation assay and (D) Edu assay in HT29 and LS174T cells after transfection with miR-20a mimic, miR-20a mimic NC, miR-20a inhibitor, or miR-20a inNC for 14 days. (*P < 0.05, **P < 0.01, ***P < 0.001).

Figure 6

miR-20a promoted migration of CMS3-CRC cells. (A-D) Wound healing assay of HT29 and LS174T cells carrying different miRNAs. (E) Transwell assays of HT29 and LS174T cells carrying different miRNAs. Bar = 100µm. Mean ± SD are shown. (*P < 0.05, **P < 0.01, ***P < 0.001).

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