Received: 19 February 2019 | Revised: 2 April 2019 | Accepted: 11 April 2019

DOI: 10.1002/jcp.28709

ORIGINAL RESEARCH ARTICLE

WILEY Journal of Cellular Physiology

miR-192/215-5p act as tumor suppressors and link Crohn's disease and colorectal cancer by targeting common metabolic pathways: An integrated informatics analysis and experimental study

Hu Zhao[1*] | Junqiu Chen[1*] | Jin Chen[1*] | Xuhui Kong[1*] | Hehuan Zhu[1] | Yongping Zhang[2] | Huiyue Dong[1] | Jie Wang[1] | Qun Ren[1] | Qinghua Wang[1] | Shushang Chen[1] | Zhen Deng[1] | Zhan Chen[1] | Qiang Cui[1] | Junqiong Zheng[3] | Jun Lu[1] | Shuiliang Wang[1] | Jianming Tan[1]

[1]Department of Urology, Fujian Provincial Key Laboratory of Transplant Biology, 900 Hospital of the Joint Logistics Team, Xiamen University, Fuzhou, Fujian, China

[2]Department of Neuro-oncology, University of Texas, MD Anderson Cancer Center, Houston, Texas

[3]Department of Oncology, Longyan First Hospital, Affiliated to Fujian Medical University, Longyan, Fujian, China

Correspondence
Hu Zhao, Jun Lu, Shuiliang Wang, and Jianming Tan, 156 Xierhuan Northern Road, 350025 Fuzhou, China.
Email: zhaohubear@163.com (H.Z.); junlu.heather@xmu.edu.cn (J.L.); shuiliang.wang@xmu.edu.cn (S.W.); tanjm156@xmu.edu.cn (J.T.)

Funding information
Military Medical Science project, Grant/Award Number: 15QNP025; Natural Science Foundation of Fujian Province, Grant/Award Numbers: 2012YZ0001-1, 2016J01582; National Nature Science Foundation of China, Grant/Award Numbers: 81402107, 81601618

Abstract

MicroRNAs have emerged as key regulators involved in a variety of biological processes. Previous studies have demonstrated that miR-192/215 participated in progression of Crohn's disease and colorectal cancer. However, their concrete relationships and regulation networks in diseases remain unclear. Here, we used bioinformatics methods to expound miR-192/215-5p macrocontrol regulatory networks shared by two diseases. For data mining and figure generation, several miRNA prediction tools, Human miRNA tissue atlas, FunRich, miRcancer, MalaCards, STRING, GEPIA, cBioPortal, GEO databases, Pathvisio, Graphpad Prism 6 software, etc. are extensively applied. miR-192/215-5p were specially distributed in colon tissues and enriched biological pathways were closely associated with human cancers. Emerging role of miR-192/215-5p and their common pathways in Crohn's disease and colorectal cancer was also analyzed. Based on results derived from multiple approaches, we identified the biological functions of miR-192/215-5p as a tumor suppressor and link Crohn's disease and colorectal cancer by targeting triglyceride synthesis and extracellular matrix remodeling pathways.

KEYWORDS
bioinformatics, colorectal cancer, Crohn's disease, microRNA (miR)-192/215-5p

1 | INTRODUCTION

Crohn's disease (CD) is one type of inflammatory bowel diseases (IBD) that can occur in any part of the gastrointestinal tract, commonly affecting ileum and beginning of the colon. Genetic risk factors and the immune system disorder make great contributions to CD progression. Recently, more and more evidence proved that inflammation was closely related to the development and malignant progression in the majority of human cancers (Todoric, Antonucci, & Karin, 2016). Studies revealed that CD raises the risk for colorectal cancer (CRC) compared with the general control population (Freeman, 2008; Hemminki, Li, Sundquist J., & Sundquist K., 2009).

*Hu Zhao, Junqiu Chen, Jin Chen, and Xuhui Kong contributed equally to this study.

However, until recently, there is some lack of knowledge about common mechanism involved in CD and CRC.

MicroRNAs (miRNAs), key noncoding RNA molecules, are determined to be involved in a myriad of human diseases including colon development and colonic carcinogenesis through RNA silencing and posttranscriptional regulation of gene expression. Furthermore, serum miRNAs can also be used as biomarkers in diagnosis, classification, and prognosis monitoring. miR-192/215 family is one of the important examples in tuning and regulating gene expressions of the CD and CRC processes. Fisher and Lin (2015) reported that IBD-associated miRNA miR-192-5p suppressed NOD2 expression and regulated inflammatory response in colonic epithelial cell lines (Chuang et al., 2014). Another investigation reported that miR-215-5p could be a candidate prognostic marker for progression from nonpenetrating CD to penetrating CD (Peck et al., 2015). Several studies also reported that miR-192/215-5p were frequently down-regulated and participated in colorectal cancer progression (Chiang et al., 2012; Jones et al., 2015; Xu, Zhu, Sun, & Xiao, 2016). However, few previous studies have been performed on miR-192/215-5p's role in both CD and CRC diseases.

With the deepening of life science research and the advent of the post genome era, the methods of biological studies have undergone tremendous changes (Kobeissy et al., 2014). Compared with traditional experimental means, data mining is helping us better and faster understanding the functional information behind massive data. Herein, a number of bioinformatics' tools and databases were used to better understand miR-192/215-5p's role in CD and CRC.

2 | MATERIALS AND METHODS

2.1 | Analysis of miRNAs sequences, secondary structures, and tissue expression profiles

miRNAs' seed sequences and consequential pairs of target regions were predicted by TargetScanHuman 7.1 (Agarwal, Bell, Nam, & Bartel, 2015). Stem-loop secondary structures were assessed by Centerfold, which based on generalized centroid estimator (McCaskill [BL] as the inference engine and weight of base pairs are 22). Expression of miRNAs in different normal tissues was determined in Human miRNA tissue Atlas (Ludwig et al., 2016).

2.2 | Biological analysis of experimentally validated and predicted genes of miRNAs

DIANA-TarBase v7.0 (http://diana.imis.athena-innovation.gr) (Vlachos et al., 2015), miRtarbase (http:// mirtarbase.mbc.nctu.edu.tw/; Chou et al., 2016), miRecored (http://c1.accurascience.com/ miRecords/; Xiao et al., 2009), and miRpathDB (https://mpd.bioinf.uni-sb.de/; Backes et al., 2017) were used to analyze experimentally validated miRNA: Gene interactions. Predicted miRNAs target gene lists were acquired from a classic predicting tool Targetscanhuman (http://www.targetscan.org/vert_71/) for its significant advantage of incorporating more complete information on the number of isoforms than other prediction tools (Agarwal et al., 2015). All experimentally validated and remaining predicted genes (referring to rule out all experimentally validated genes) of miRNAs were functionally enriched by FunRich_V3 software (Pathan et al., 2015).

2.3 | Analysis of miRNAs potential role in human cancers

Literature excavation of miRNAs in human cancers was carried out through web-based PubMed (https://www.ncbi.nlm.nih.gov/pubmed/) and miRCancer (http://mircancer.ecu.edu/; Xie et al., 2013). OncoLnc (http://www.oncolnc.org) and GEPIA (Gene Expression Profiling Interactive Analysis; http://gepia.cancer-pku.cn/) were used to analyze the survival rate for miRNAs ad target mRNAs in "The Cancer Genome Atlas" (TCGA) data (Tang et al., 2017). Experimentally validated targets with strong evidence were selected by MiRTargetLink data (https://ccb-web.cs.uni-saarland.de/mirtargetlink/; Hamberg et al., 2016). Competitive endogenous microRNA-target expression profiles were analyzed by miRtarbase (http://mirtarbase.mbc.nctu.edu.tw/; Chou et al., 2016). cBioportal (http://www.cbioportal.org/) was used to research target gene applications, mutations, and deletions in TCGA database (Gao et al., 2013).

2.4 | Analysis of the emerging role of miRNAs target genes in Crohn's disease

To get full map of miRNAs regulated genes network in Crohn's Disease, all predicted miRNAs target gene lists were acquired by five commonly predicting tools including TargetMiner (http://www.isical.ac.in/~bioinfo_miu/)(Bandyopadhyay & Mitra, 2009), TargetScanHuman, miRDB (http://www.mirdb.org/; Wang, 2016; Wong & Wang, 2015), RNA22-HSA (https://cm.jefferson.edu/; Miranda et al., 2006), and microRNA.org (http://www.microrna. org/; Betel, Wilson, Gabow, Marks, & Sander, 2008). Conserved miRNAs target gene lists were queried from Targetscanhuman prediction. Human Disease Database MalaCards (http://www.malacards.org/) was applied to obtain genes closely related to Crohn's Disease (Rappaport et al., 2013). Venn diagrams was used to calculate the intersections of gene lists mentioned above. Prediction of protein–protein interaction of intersected genes was used by String (clustering algorithms KMEANS; Szklarczyk et al., 2017).

2.5 | Analysis of miRNAs target genes in both Crohn's disease and colorectal cancer

To screen different gene expressions in biopsies of CRC and CD, GSE4183 Affymetrix array data were queried from NCBI and analyzed with specific software Expression Console (EC) and Transcriptome Analysis Console (TAC). Obtained differentially expressed genes (DEGs) in both samples were intersected with miRNAs predicted genes by Interactivenn (http://www.interactivenn.net/; Heberle, Meirelles, da Silva, Telles, & Minghim, 2015), and

physical interaction and biological pathways of intersected genes were then analyzed by String as described above. Intersected genes were also inputted on The Matrisome Project (http://matrisomeproject.mit.edu/) to further determine the components of extracellular matrix (ECM) proteins (Naba et al., 2012).

2.6 | Confirmation of miRNAs target genes in both diseases

CRC miRNAs array GSE83924 and CD miRNAs sequencing GSE84779 data series were downloaded from NCBI and analyzed to determine miRNAs expressions in CRC and CD, respectively. GEPIA was used to assess corresponding mRNA expressions in TCGA Colon Adenocarcinoma (COAD) and Rectum Adenocarcinoma (READ) data as previously described. GSE84779 data was also used to analyze corresponding mRNA expressions in CD clinical samples. Commonly used pathway editor PathVisio (http://www.pathvisio.org/) was applied to draw miRNAs target pathways in both diseases (Kutmon et al., 2015).

3 | RESULTS

3.1 | Mature hsa-miR-192-5p and has-miR-215-5p with identical seed sequence are specifically and highly expressed in colon tissues

"miR-192" and "miR-215" are common names used in vast majority of studies, but it should be noted that mature miRNAs (miR-192-3p/5p and miR-215-3p/5p, respectively) originated from 3′ or 5′ end of the hairpin structure are the real functional units. Multiple studies demonstrated that the stability of mature strands may largely determine its ability to enter the RISC complex to perform RNA silencing. So, the first issue that needs to address is the expression abundance of both mature miRNAs in various human organs.

PubMed searching of the words "miR-192-3p or miR-215-3p" only yielded five published articles, whereas "miR-192-5p or miR-215-5p" produced 60 results. And based on miRBase, miR-192/215-5p are more abundant than miR-192/215-3p. Moreover, as showed in Figure 1a (upper), there are only two nucleotides differences between *Homo sapiens* mature sequences of miR-192 and miR-215. They also have identical seed sequences and common target genes (YY1 as one example displayed in Figure 1a lower). All the clues mentioned above suggest that miR-192/215-5p are worthier of further analysis. Stem-loop structure of precursors is critical for Drosha and Dicer processing from precursor miRNA into miR-5p or -3p, so RNA secondary structure of pre-miR-192/215 was also predicted by CentroidFold and presented in Figure 1b. Tissue-specific miRNAs (TS miRNA) play an important role in tissue development, function, and related disease. Following human miRNA tissue atlas analysis, we found that miR-192/215-5p are highly specific expressed in human colon tissues, whereas quartile normalized data of miR-192-3p reduced 60 times more compared with that of miR-192/215-5p, and no data available for miR-215-3p for its too low abundance to count (Figure 1c). In summary, given the colon-specific expression of microRNAs, miR-192/215-5p may play an essential role in development of colon tissue-related diseases.

3.2 | Computational analysis of biomolecular networks among experimental validated and TargetscanHuman predicted target genes of miR-192/215-5p

miRNA exerts its biological functions through translation inhibition and degradation of target mRNAs. Thus, it is necessary to perform enrichment analysis of miR-192/215-5p target genes, which can provide specific clues for our subsequent functional research. miR-192/215-5p experimental validated target genes and TargetScanHuman predicted genes were listed at Table 1 and specifics were detailed in **Table S1**. Then gene functional enrichment analysis was carried out by FunRich software. Biological pathway results showed that experimental validated genes might be associated with "cell cycle," "DNA replication," "different mitotic phases," "ATM and Wnt pathways," etc. (Figure 2a). Biological process such as regulation of "regulation of nucleobase," "DNA repair," "regulation of cell cycle," and "microtubule-based process" are statistically enriched for validated genes (Figure 2b). COSMIC (the Catalogue of Somatic Mutations in Cancer), provides comprehensive somatic mutations in different cancer types. Comparative enrichment analysis for validated and predicted genes in COSMIC showed that genes were both significantly enriched in multiple cancer types including large intestine, lung, breast, liver, kidney, urinary tract, etc. (Figure 2c). More details can be found in **Table S**2 and **Table S**3. Overall, these results suggest that experimental validated and TargetScanHuman predicted target genes of miR-192/215-5p may be involved in several different biological pathways and processes, and probably participate in multiple tumor progression.

3.3 | miR-192/215-5p act as tumor suppressor in various human cancers

Previous results of Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis on experimental validated and predicted remaining target genes showed that several enriched pathways directly or indirectly correlated with cancers including colorectal cancer, prostate cancer, thyroid cancer, proteoglycans in cancer, and so forth. PubMed searching results revealed that 40.8% (145/308; "miR-192"/"miR-192 and cancer") and 67.6% (75/111; "miR-215"/"miR-215 and cancer") research are related to cancers for miR-192 and miR-215. miRCancer, a microRNA-cancer association database based on literature text mining, provides comprehensive miRNA expressions profiles in human cancers. Using this database, we found that miR-192 and miR-215 are dysregulated in several human cancers including bladder cancer, chronic lymphocytic leukemia, colorectal cancer, ovarian cancer, gastric cancer, and so forth (**Table S4**). All data indicated that miR-192/215-5p may play an important role in progression of human cancers.

OncoLnc links TCGA survival data to miRNA expression levels. First, survival analysis of miR-192/215-5p on pan-cancer was performed. The

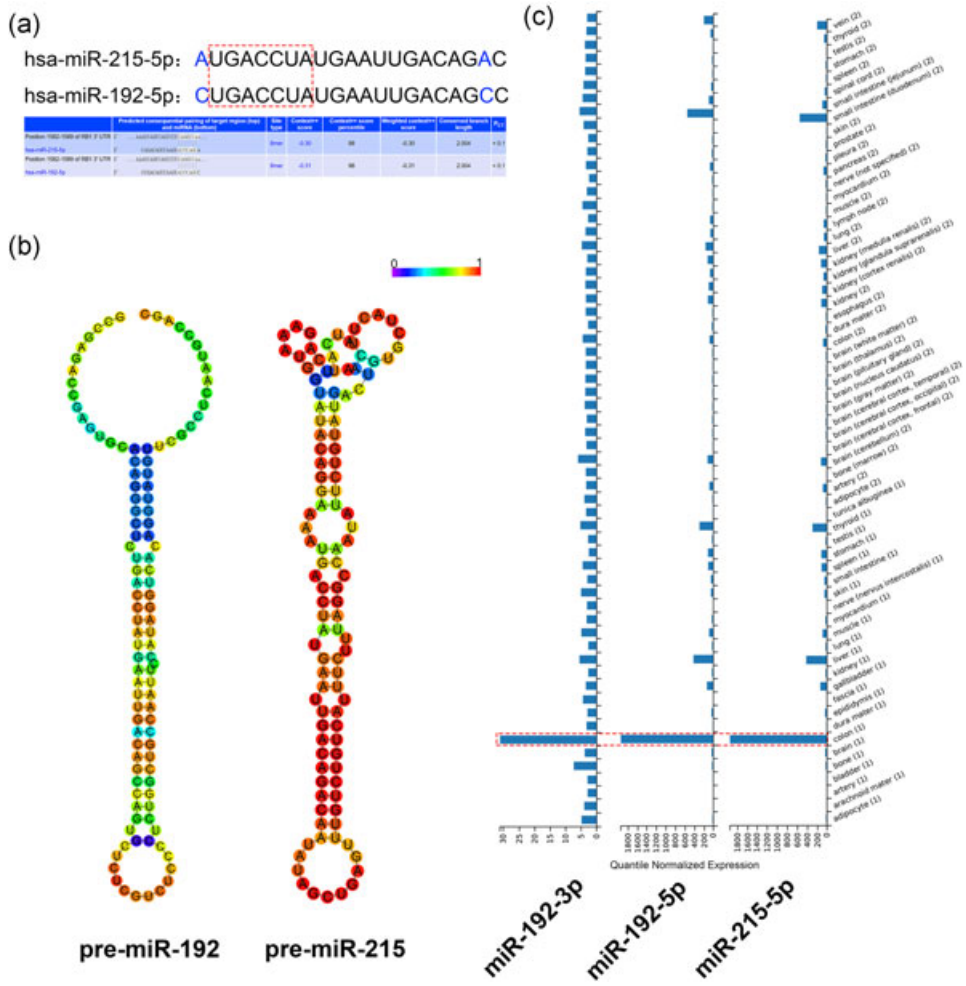


FIGURE 1 Mature sequences, secondary structures, and expression profiles of miR-192/215-5p. (a) Mature sequences comparison of both miRs, sequences enclosed in red frames represented common seed sequences, nucleotides in blue fonts were the diverging nucleotides between two miRs. The gene of RB1 was used as a typical example to illustrate base-complementation in both miRs. (b) Predicted secondary structures of human pre-miR-192/215. (c) miR-192/215-5p were specifically and highly expressed in colon tissues according to human miRNA tissue atlas analysis (red dashed frame) [Color figure can be viewed at wileyonlinelibrary.com]

result showed that high expressions of miR-192/215-5p were associated with long survival in cervical squamous cell carcinoma and endocervical adenocarcinoma (CESC), kidney renal clear cell carcinoma (KIRC), and patients with bladder urothelial carcinoma (BLCA; Figure 3a). Tumor suppressor miRNAs are at decreased levels in cancerous tissues. To further understand the specific mechanism of miR-192/215-5p's tumor suppressor role on cancer, four experimental validated target genes (RB1, WNK1, ALCAM, and ACVR2B) from miRTargetLink Human database were chosen for coexpression profile analysis (Hamberg et al., 2016). Previous studies showed that expression of miR-192-5p and

TABLE 1 Analysis of experimentally validated target genes of miR-192/miR-215-5p

Database	miRs	Sum	Repetition	Remaining	Combination 1	Combination 2
Tarbase	miR-192-5p	2,003	754	1,251	1,370	1,685
	miR-215-5p	819	49	770		
miRTARBASE	miR-192-5p	1,001	44	957	970	
	miR-215-5p	751	21	730		
miRecored	miR-192-5p	3	0	3	5	
	miR-215-5p	4	0	4		
miRpathDB	miR-192-5p	939	84	855	1,152	
	miR-215-5p	730	0	730		

Combination 1: Combine the target genes of both miRs and remove duplicates; Combination 2: Combine all target genes of all database and remove duplicates.

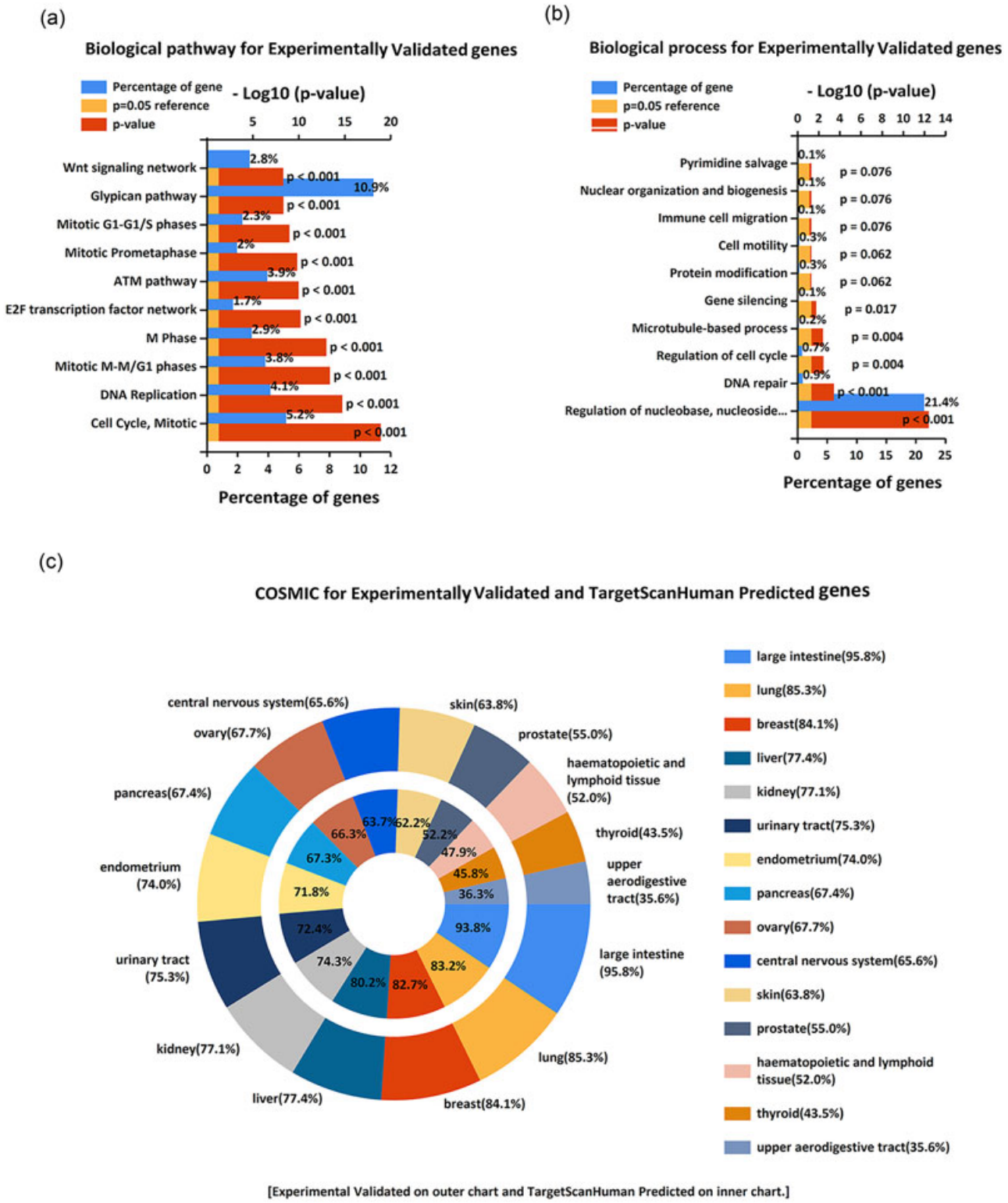


FIGURE 2 Funrich enrichments and COSMIC analysis of miR-192/215-5p targeted genes. (a,b) Biological pathway and biological process enrichments for experimentally validated genes of miR-192/215-5p. Statistical significance was accepted at the $p < 0.05$ level. (c) COSMIC analysis of both experimental validated and TargetscanHuman predicted genes of miR-192/215-5p [Color figure can be viewed at wileyonlinelibrary.com]

miR-215-5p were both downregulated in liver hepatocellular carcinoma (LIHC) and head and neck squamous cell carcinoma (HNSC), respectively (Chen et al., 2010; Lian et al., 2016). And consistently, four target genes were remarkably highly expressed in corresponding TCGA database (Figure 3b). Due to space limitations, ALCAM and WNK1 were further studied for somatic mutations, DNA amplification and survival analysis in several types of cancers. We found that although somatic mutations of ALCAM and WNK1 is one of the critical factors in development of

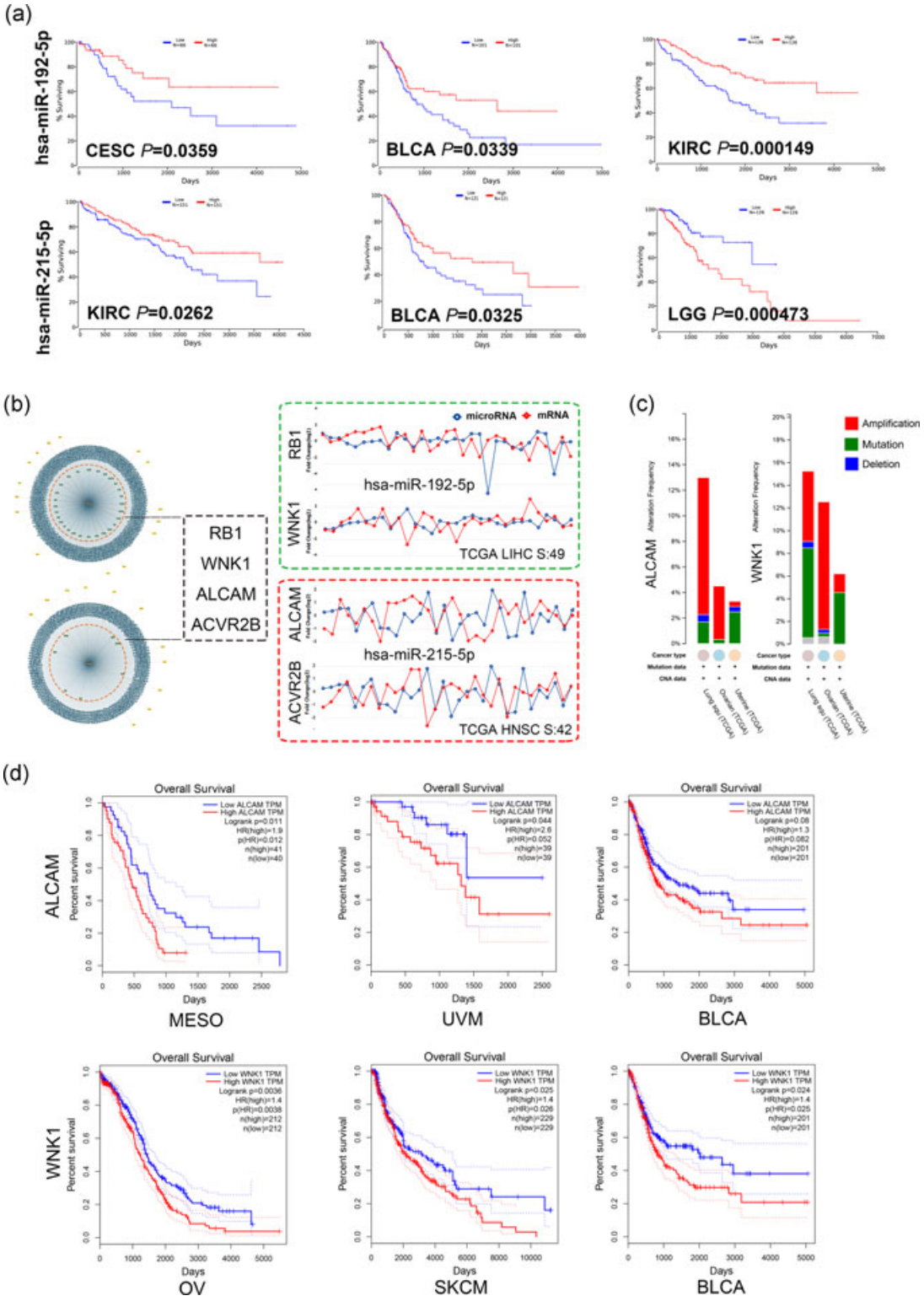


FIGURE 3 Tumor suppressor role of miR-192/215-5p in multiple human cancers. (a) OncoLnc survival analysis of miR-192/215-5p in different human cancers. BLCA, urothelial bladder carcinoma; CESC, cervical squamous cell carcinoma and endocervical adenocarcinoma; KIRC, kidney renal clear cell carcinoma; LGG, low grade glioma. (b) Four experimentally validated targets with strong evidence indicated by MiRTargetLink were selected to conduct coexpression analysis with both miRs in TCGA datasets. HNSC, head and neck squamous cell carcinoma; LIHC, liver hepatocellular carcinoma. (c) cBioportal analysis of ALCAM and WNK1 in three cancer types. (d) Survival analysis of ALCAM and WNK1 mRNA expressions in TCGA datasets. MESO: mesothelioma; OV: ovarian cancer; SKM: skin cutaneous melanoma; UVM: uveal melanoma [Color figure can be viewed at wileyonlinelibrary.com]

cancers (Figure 3c), high expression of miR-192/215-5p targeted ALCAM and WNK1 are significantly related to poor survival of several cancers (Figure 3d). From the results obtained so far, it seems that miR-192/215-5p act as tumor suppressors in various cancer types.

3.4 | Emerging role of miR-192/215-5p in Crohn's disease

There is now a general consensus that the pathogenesis of CD is a chronic and recurrent inflammatory disorder caused by comprehensive elements including genetic, epigenetic, and immunological factors. Multiple studies have revealed that dysregulated miRNAs expressions were closely related to the progression of CD, and CD-associated serum miRNA can be applied as a biomarker of CD (Fisher & Lin, 2015; Peck et al., 2015; Zahm et al., 2011). Chuang et al. (2014) reported that miR-192 suppressed NOD2 expression and immune response of muramyl dipeptide-mediated NF-κB activation in colonic epithelial HCT116 cells. By contrast, Zahm et al. (2014) drew the opposite conclusion in pediatric patients with CD. They found that miR-192 did not directly regulate human NOD2 by luciferase reporter activity assay (Figure 4a). As for miR-215, Peck et al. (2015) demonstrated that it can stratify different phenotypes of patients with CD according to disease behavior independent of the effect of inflammation. F. Wu et al. (2010) also found that miR-215 was significantly increased in the terminal ileum tissues of patients with active ileal CD. However, the exact mechanism of miR-192/215-5p regulating the progress of CD have yet to be fully defined.

MalaCards, an integrated database of human maladies, can provide hierarchical malady classification, integration, and disease set analyses(Rappaport et al., 2013). By searching of "Crohn's disease" on this website, 36 genes (including one elite gene, NOD2, presented a relevance score of 153.18) compiled from "GeneCards," "GeneTests," and "Disease" databases are closely related to CD (**Table S**5). And among its related pathways are the Jak/STAT signaling pathway, innate lymphoid cell differentiation pathways, and toxoplasmosis (see more on MalaCards). To directly address the role of miR-192/215-5p during the CD development, we want to further determine whether genes that targeted by miR-192/215-5p were significantly associated with CD. To get a more comprehensive and broader view of miRNA target genes, five miRNA target prediction tools including TargetMiner, TargetScanhuman, RNA22-HSA, miRDB, and microRNA.org were used in this section (Peterson et al., 2014). All predicted target genes are listed in Table S6. As showed in Figure 4b, miR-192/215-5p may target 15 (41.7%, 15/36) genes that involved in CD. Among these genes, four genes (*NOD2*, *IL6*, *DLG5*, and *FGFR1OP*) were validated, and two of which (*DLG5* and *FGFR1OP*) had conserved sites. Another 11 genes (*IL23R*, *SP140*, *ABCB1*, *IL1B*, *IL12RB2*, *NDUFA13*, *ATG16L1*, *TNFSF15*, *IL18BP*, *DUOX2*, and *TLR4*) had poorly conserved sites. In an effort to further confirm the fact that these genes were implicated in CD, functional protein association networks analysis by STRING was implemented (Szklarczyk et al., 2017) and results showed that gene ontology (GO; KEGG) analysis of 15 intersection genes are markedly related to inflammatory bowel disease, cytokine–cytokine receptor interaction and NOD-like receptor signaling (Figure 4c,d, Table S5). In summary, miR-192/215-5p may affect the course of CD disease partially by targeting inflammation-related genes.

3.5 | miR-192/215-5p may be involved in both colorectal cancer and Crohn's disease

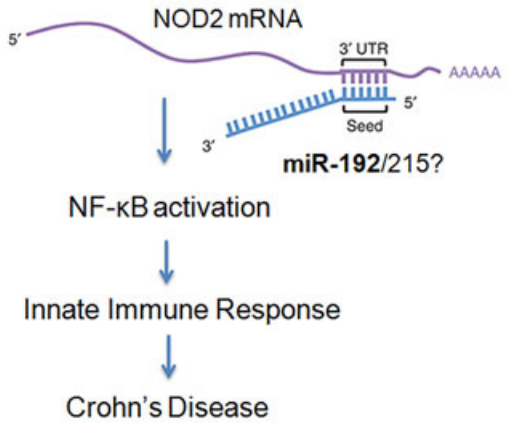
CD mainly affects the colon and terminal ileum. It is well accepted that patients with CD were at increased risk of CRC (standardized incidence ratios, 2.43) according to the Danish studies from Jess, Horvath-Puho, Fallingborg, Rasmussen, & Jacobsen (2013) report. A meta-analysis of 12 population based studies confirmed that cumulative risk of CRC among patients with CD was 2.9%, 5.6%, and 8.3% after 10, 20, and 30 years of the CD diagnosis, respectively (Wang & Fang, 2014). And in CD, occurrence of CRC may even predict a worse prognosis (Freeman, 2008). However, important issues relating to miRNAs regulation and their biological functions on progression of both diseases are still poorly understood.

To obtain different gene expressions in biopsies of CRC and CD, GEO series accession number GSE4183, which met our requirements, was retrieved from NCBI's Gene Expression Omnibus. A total of 54,613 genes were tested to compare gene expressions between CRC or CD and normal control, respectively. After routine EC and TAC processing, significantly DEGs data with a two-fold difference and adjusted p-value < 0.05 were generated (Table S7). Scatter plot, volcano plot, and heat map, which represents the distribution of significant genes are showed in Figure 5a,b. After merging same genes with different probe sets, 481 probe lists were determined as the most significantly dysregulated genes in CRC, and 845 more probe lists were found in CD compared with normal control. The next question is whether obtained DEGs can be directly targeted by miR-192-5p and miR-215-5p. Thus, intersections of lists between two DEGs and miR-192/215-5p target genes (TGs) derived from three sources were calculated (Figure 5c and Table S8). Because a probe set may correspond to multiple genes, the number of DEGs for CRC and CD slightly increased to 499 and 882, respectively. Encouragingly, 18.57% (57/307) DEGs (CRC + CD) may be targeted by both miRs, including 9.45% (29/307) DEGs, which have been experimentally validated (Figure 5d). In summary, the data obtained above support the hypothesis that miR-192/215-5p are involved in CRC and CD simultaneously.

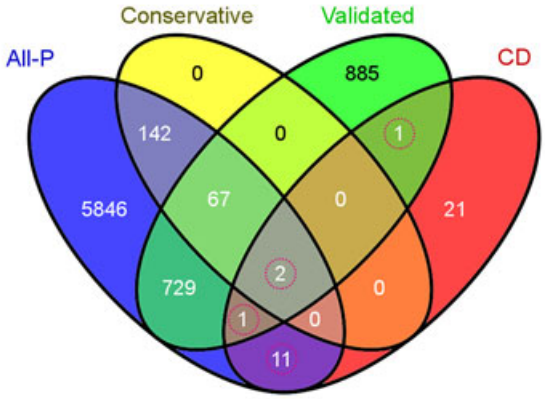
3.6 | miR-192/215-5p participate in progression of colorectal cancer and Crohn's disease by targeting extracellular matrix remodeling and fatty acid metabolism pathways

Following results from analysis of miR-192/215-5p targeted DEGs, we further wondered to examine possible pathways using STRING's gene interaction network analysis (von Mering et al., 2003). We found that three enriched pathways including ECM remodeling, fatty acid metabolism, and respiratory chain may be targeted by miR-192/215-5p and involved in CRC and CD diseases (Figure 6a). More details can be found in Table S9. To our excitement, ECM-related genes accounted for nearly

(a)



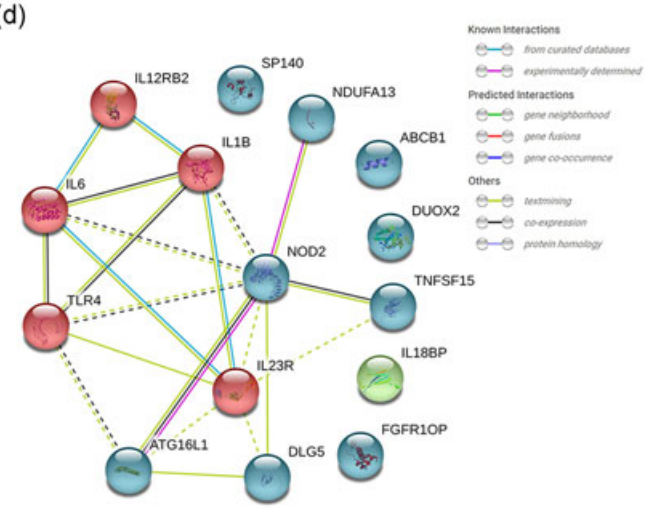
(b)



(c)

Conservative	Predicted	
DLG5	IL23R	IL18BP
FGFR1OP	SP140	DUOX2
Validated	ABCB1	TLR4
DLG5	IL1B	TNFSF15
FGFR1OP	IL12RB2	
IL6	NDUFA13	
NOD2	ATG16L1	

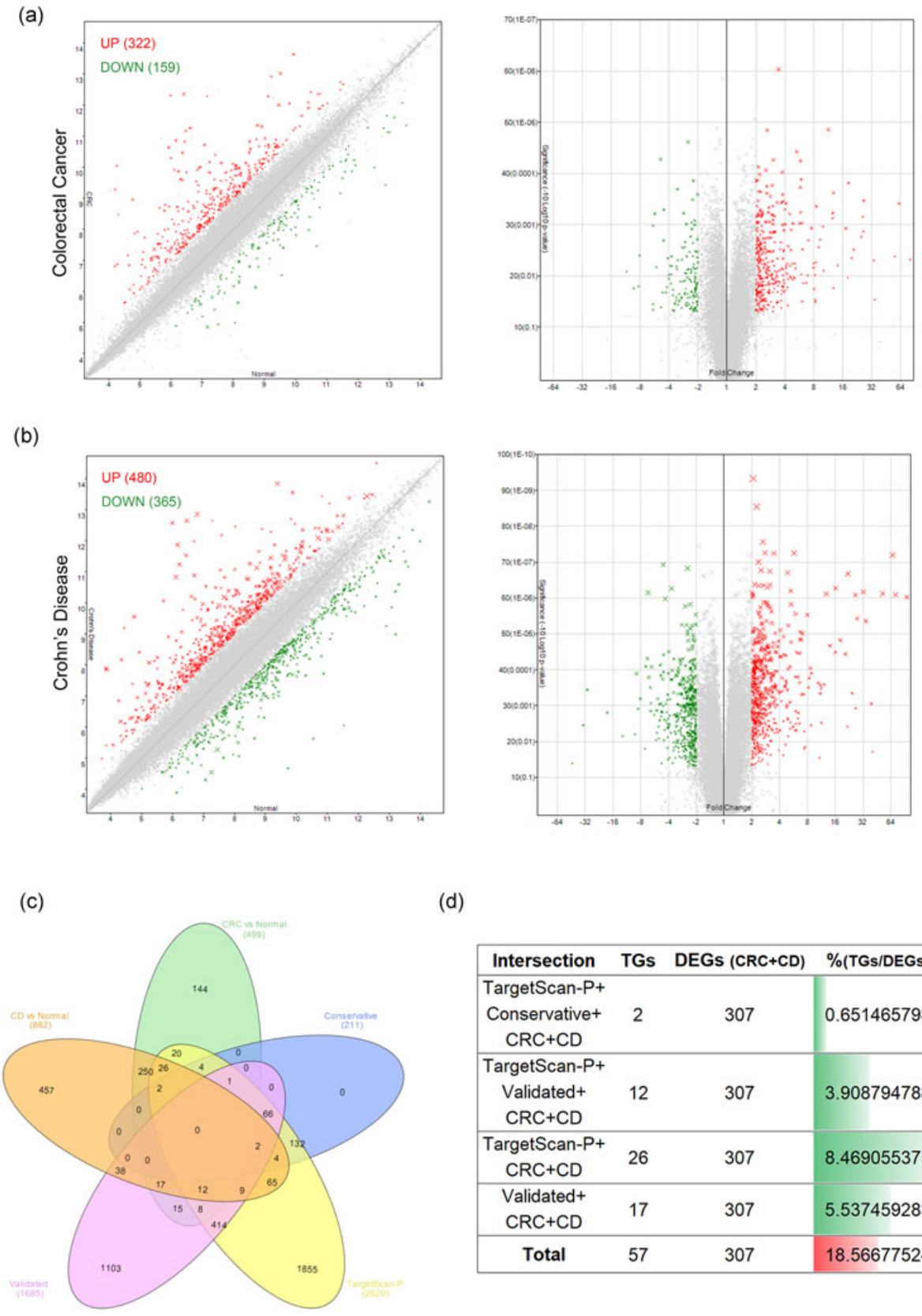
(d)



(e)

Annotated pathway (KEGG)	Count
Inflammatory bowel disease (IBD)	6
Cytokine-cytokine receptor interaction	5
NOD-like receptor signaling pathway	3
Legionellosis	3
Malaria	3
Tuberculosis	4
Pertussis	3
Salmonella infection	3
Rheumatoid arthritis	3
Chagas disease	3

FIGURE 4 Potential role of miR-192/215-5p in CD. (a) Depicted regulatory mechanism of miR-192/215-5p in CD (literature search). (b) Venn diagram of intersections of four sets, All-P: All predicted targets of five tools; conservative: All predicted targets with conserved sites by Targetscan 7.1; validated: Experimental validated genes; CD: MalaCards derived genes closely related to CD. The pink cycle indicated the intersection genes with 11 in All-P, 2 in validated and 2 in conservative. (c) Lists of 15 intersection genes in the Venn diagram B. (d) Protein–protein interactions network of 15 intersection genes predicted by String analysis. (e) KEGG pathway enrichment of 15 intersection genes. **Table S**5 for further details. CD: Crohn's disease; KEGG: Kyoto Encyclopedia of Genes and Genomes [Color figure can be viewed at wileyonlinelibrary.com]



Intersection	TGs	DEGs (CRC+CD)	%(TGs/DEGs)
TargetScan-P+ Conservative+ CRC+CD	2	307	0.651465798
TargetScan-P+ Validated+ CRC+CD	12	307	3.908794788
TargetScan-P+ CRC+CD	26	307	8.469055375
Validated+ CRC+CD	17	307	5.537459283
Total	57	307	18.56677524

FIGURE 5 Emerging role of miR-192/215-5p target genes in both CD and CRC. (c) Interactivenn diagram of intersections of five sets. Conservative: All predicted targets with conserved sites by TargetScan 7.1; validated: experimental validated genes; TargetScan-P: all predicted targets by TargetScan 7.1; CD versus Normal: DEGs in CD; CRC versus Normal: DEGs in CRC. (d) The number and portion of intersection genes represented by statistical table, total 57 target genes were dysregulated in both diseases (TGs, target genes). CD: Crohn's disease; CRC: colorectal cancer; DEGs: differentially expressed genes [Color figure can be viewed at wileyonlinelibrary.com]

quarter of DEGs. Thus, we analyzed 57 DEGs on The Matrisome Project, a database hosting ECM and ECM-associated genes, and found that 13 proteins were closely related to ECM, including seven core Matrisome proteins (Figure 6b).

We first clarified the role of miR-192/215-5p in CRC clinical samples. By analyzing 14 CRC and 15 normal fresh frozen colonic biopsy samples in GSE83924 data series, we found that expression of both miRNAs was downregulated significantly with different degrees (Figure 6c, Table S10). We further checked the expression of miR-192/215-5p targeted genes involved in signaling pathway mentioned above. As we all know, the expression levels of miRNAs are contrary to their targeted genes. By using GEPIA RNA sequencing expression data (Tang et al., 2017), we found that genes involved in ECM remodeling and fatty acid metabolism were consistent with our previous results, whereas expression of genes related to respiratory chain were almost not altered (Figure 6D, Figure S3). Next, we analyzed miR-192/215-5p expression in 10 CD clinical samples and 12 normal fresh frozen biopsy samples of GSE84779 data series. Both miRNAs were found to be downregulated expression with distinctive differences in contrast with control by high throughput noncoding RNA sequencing (Figure 7a). Next expression of targeted genes involved in ECM remodeling and fatty acid metabolism was also determined in CD by analyzing GSE9686 expression profiling data series. As Figure 7b shows, expression of four genes including SCD ACSL1 COL1A2, and CYR61 were significantly increased whereas other genes had no statistical difference. As Figure 7c–e showed, ACSL1 was directly targeted by miR-192-5p and miR-215-5p. In summary, miR-192/215-5p indeed participate in progression of CRC and CD by targeting genes involved in extracellular matrix remodeling and fatty acid metabolism pathways.

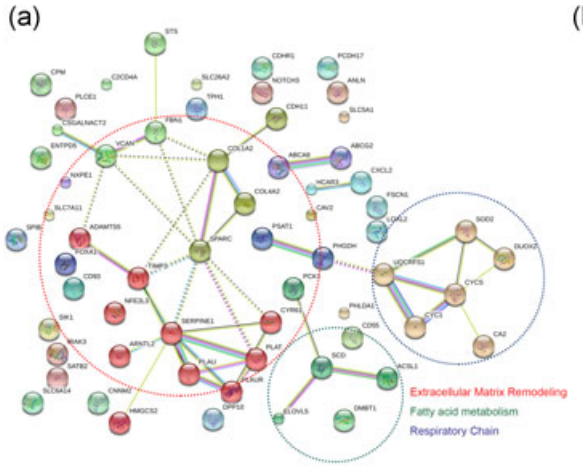
4 | DISCUSSION

In this investigation, we have demonstrated that miR-192/215-5p family were specifically distributed in colon tissues, and acted as tumor suppressor miRs in several cancers. In addition, we showed that miR-192/215-5p played an important role in both CD and CRC progression through common signaling pathways. Based on results above, we propose a molecular mechanism in which loss of miR-192/215-5p inhibition of triglyceride synthesis and ECM deposition signaling pathways lead to CD and CRC progression aggravation (Figure 8). This study demonstrates the biological functions of miR-192/215-5p in CD and CRC and supports the idea that dysfunction of miRs is important to the crosstalk between inflammation and cancer development.

miR-192-5p and miR-215-5p were studied as microRNA pairs in this study for their absolutely similar mature sequences (21 nucleotides in length with only two nucleotides difference) and identical seven base seed sequences, which are capable of carrying out their biological functions through base-complementation mechanism (see Figure 1a). Meanwhile, both miRs were highly expressed in colon tissues specifically and simultaneously (see Figure 1c), which provides a sound basis for their involvement in colon diseases. There are many similar instances like brain-specific miR-128b that participates in the formation of fear-extinction memory (Lin et al., 2011); muscles-specific miR-206 is involved in Duchenne muscular dystrophy by targeting multiple key mRNAs (Amirouche et al., 2017).

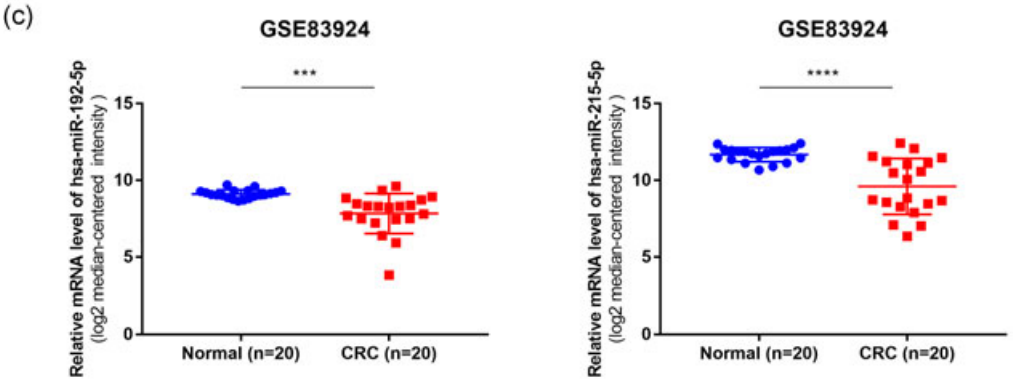
microRNA works depend on divergent biological functions of its target genes. Herein, enrichment of biological pathway or biological process for target genes may have important implications for further research. To conduct a comprehensive analysis of mir-192/215-5p target genes, experimentally validated and Targetscan predicted genes were enriched by FunRich software, respectively. Results revealed that for experimentally validated genes, cell cycle and cell mitosis occupied most of the proportion and "Wnt, ATM" ranked secondly following biological pathway analysis. 21.4% genes were implicated in "regulation of nucleobase, nucleoside, and nucleotide and nucleic acid metabolism" according to biological process analysis, which was consistent with function of microRNAs (see Figure 2a,b). For Targetscan predicted genes, multiple biological metabolic pathways such as "sodium/calcium exchangers" and biological process "cell-cell adhesion, transport" were enriched (see Figure 2c,d). COSMIC analysis of experimentally validated and Targetscan predicted genes further proved that these genes were significantly enriched in multiple cancer types. All the evidence pointed to human cancers, consistent with previous findings (Hou et al., 2015; Jin, Lu, Wen, Shen, & Wen, 2015; Xu & Fan, 2015). Survival analysis on patients with malignant cancers revealed that high expression of miR-192/215-5p are closely related to low-survival rates in several human cancers including CESC, BLCA, KIRC, and LGG (see Figure 3a). Coexpression analysis of miR-192/215-5p with four core validated target genes (RB1, WNK1, ALCAM, and ACVR2B), which were strongly associated with human cancers (Fernandez et al., 2016; Ku et al., 2017; Nissinen et al., 2016; Shyamasundar, Lim, & Bay, 2016), further presented negatively correlation between both miRs and target genes (see Figure 3b). In addition, survival rates of WNK1 and ALCAM were just the opposite of miR-192/215-5p (see Figure 3d). All indicated that miR-192/215-5p acted as tumor suppressors and may target several genes to interfere with the process of human cancers.

Several studies have reported that miR-192/215-5p exhibit altered expressions and are implicated in CD by targeting and repressing *NOD2* gene, an intracellular host pattern recognition receptor that could greatly increase the risk for the development of CD (Chuang et al., 2014; Wu et al., 2010; Wu et al., 2017). To get a comprehensive and deeper regulatory network of miR-192/215-5p in CD, we intersected their target genes with genes closely related to CD derived from MalaCards database. To our excitement, 41.7% (15/36) target genes may be involved in CD with four experimentally validated genes and two conservative genes, consistent with previous studies (Li et al., 2016; Ogura et al., 2001; Uemura et al., 2016; Yang et al., 2015). String enriched KEGG pathway analysis further revealed that "inflammatory bowel disease," "cytokine–cytokine receptor interaction," and "NOD-like receptor signaling" were the top three pathways (see Figure 4b–d). The above results strongly suggested that miR-192/215-5p may participate in CD divergently by targeting several genes.



(b)

Gene Symbol	Matrisome Division	Category
CYR61	Core matrisome	ECM Glycoproteins
DMBT1	Core matrisome	ECM Glycoproteins
FBN1	Core matrisome	ECM Glycoproteins
SPARC	Core matrisome	ECM Glycoproteins
COL1A2	Core matrisome	Collagens
COL4A2	Core matrisome	Collagens
VCAN	Core matrisome	Proteoglycans
ADAMTS5	Matrisome-associated	ECM Regulators
LOXL2	Matrisome-associated	ECM Regulators
PLAU	Matrisome-associated	ECM Regulators
SERPINE1	Matrisome-associated	ECM Regulators
TIMP3	Matrisome-associated	ECM Regulators
CXCL2	Matrisome-associated	Secreted Factors



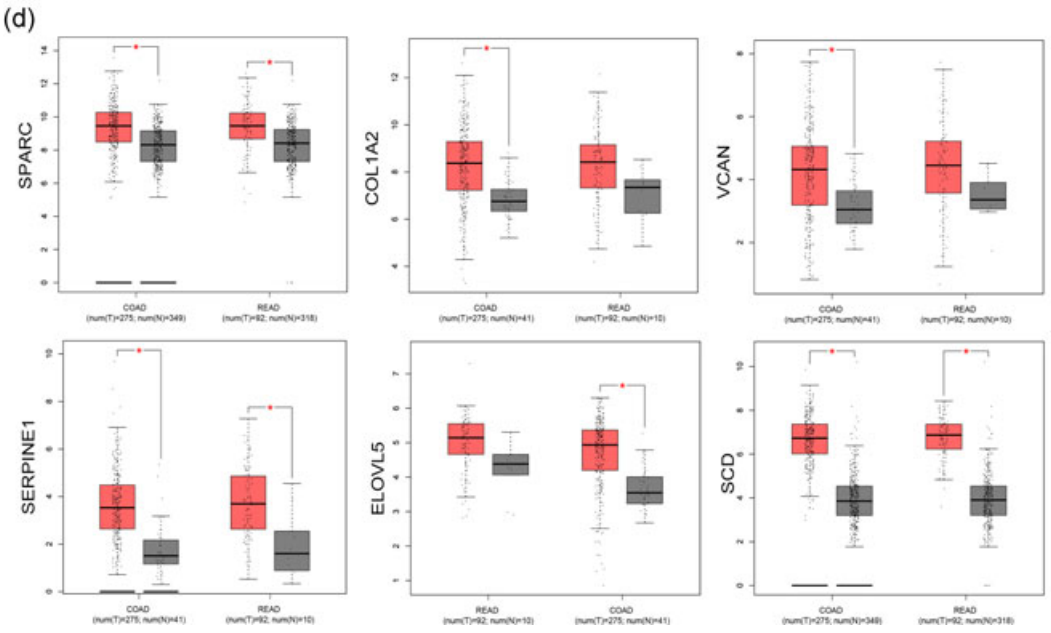


FIGURE 6 String enriched pathways are involved in CRC. (a) Biological network enrichment of 57 DEGs targeted by miR-192/215-5p revealed three statistically enriched KEGG pathways (big cycle with different color). (b) The Matrisome Project analysis of 57 DEGs, 13/57 DEGs are related to Matrisome. (c) miR-192/215-5p are downregulated in GSE83924 clinical CRC samples by microRNA array analysis. See more information on **Table S**10. (d) GEPIA analysis of enriched targeted DEGs demonstrates that ECM remodeling pathway and fatty acid metabolism pathway-related genes are highly expressed in TCGA CRC samples, whereas expressions of respiratory chain-related genes are not altered in TCGA CRC samples (Figure S2). CRC: colorectal cancer; DEGs: differentially expressed genes; ECM: extracellular matrix; KEGG: Kyoto Encyclopedia of Genes and Genomes [Color figure can be viewed at wileyonlinelibrary.com]

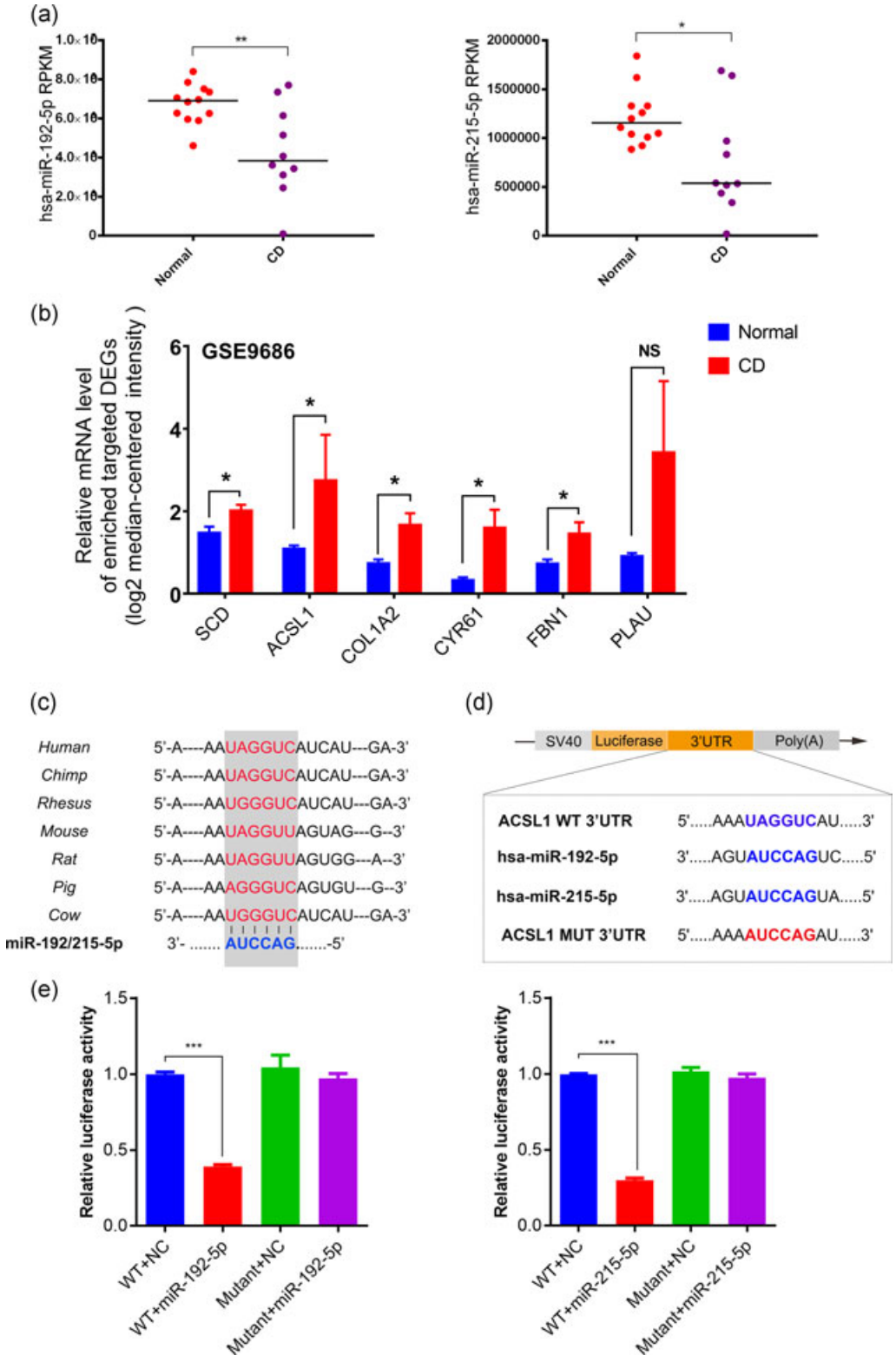


FIGURE 7 String enriched pathways also participate in CD. (a) miR-192/215-5p are downregulated in GSE84779 clinical CD samples (**Table S**11). (b) Enriched targeted DEGs expressions in GSE9686 11 CD samples compared with eight healthy normal tissues, SCD, ACSL1, COL1A2, and CYR61 are upregulated significantly, ANOVA p value < 0.05. Specific data could be found in **Table S**12. (c) Schematic diagram of ACSL1 3′-UTR, the corresponding mutant ACSL1 3′-UTR and *miR-192/215-5p* sequences. (d) Construction of luciferase reporter gene vector for human ACSL1. (e) The relative luciferase activities of HEK293T cells in each group. Data were presented as mean ± *SD* for three independent experiments, ***p < 0.001, compared with negative control. ANOVA: analysis of variance; CD: Crohn's disease; DEGs: differentially expressed genes [Color figure can be viewed at wileyonlinelibrary.com]

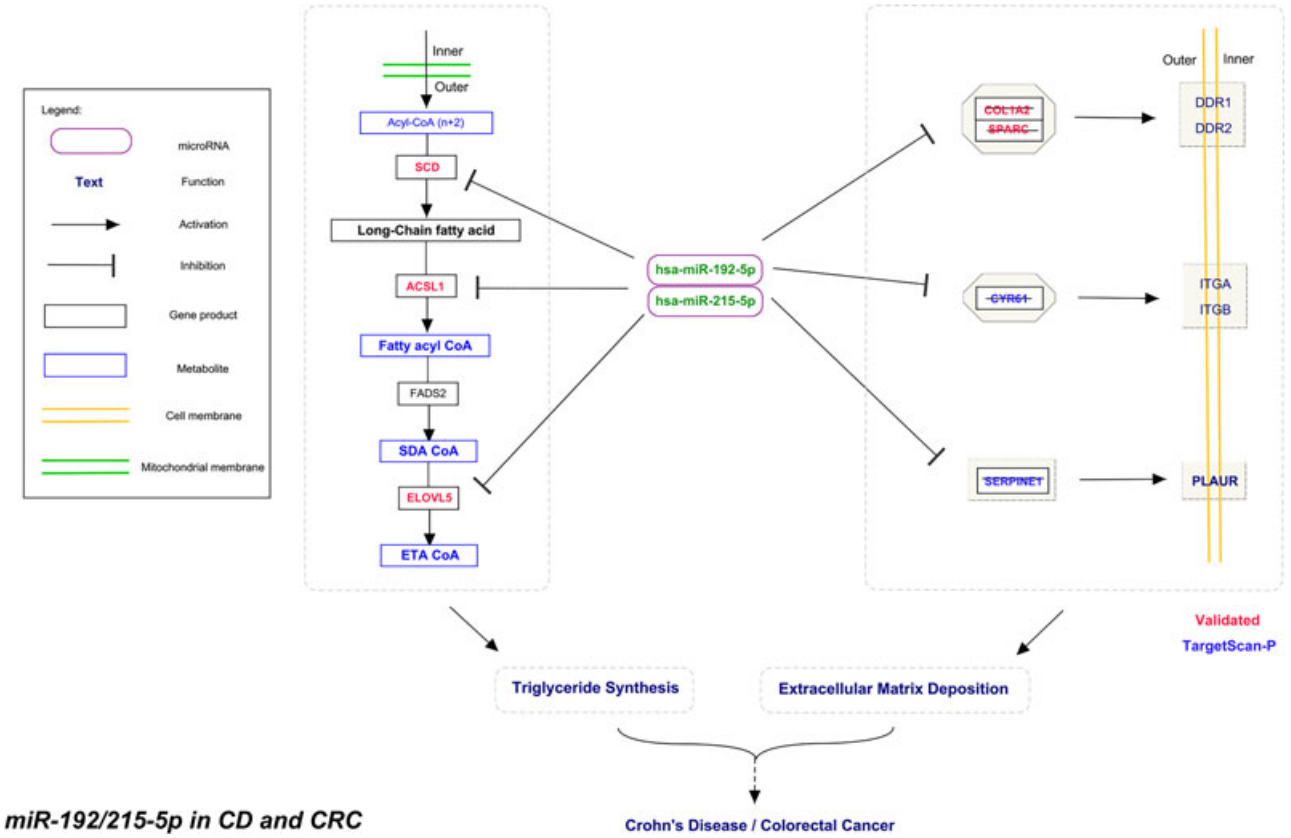


FIGURE 8 Depicted molecular mechanism of miR-192/215-5p in CD and CRC. Working model: Expression levels of miR-192/215-5p are downregulated in CD and CRC. Reduction of miR-192/215-5p levels release their inhibitions on triglyceride synthesis pathway and ECM remodeling pathway genes, which therefore promotes progression of CD and CRC. Red fonts represent validated targets; blue fonts represent TargetScan-P targets [Color figure can be viewed at wileyonlinelibrary.com]

Inflammation is reported to be closely related to various tumor progressions according to the majority of studies (Coussens, Zitvogel, & Palucka, 2013; Landskron, De la Fuente, Thuwajit, Thuwajit, & Hermoso, 2014). CD, as a disease of chronic and recurrent inflammatory disorders, has been proved to greatly increase the risk of CRC (Jess et al., 2013). However, there are few reports on the roles of microRNAs in both diseases (Bai et al., 2014). In light of this scientific issue, GEO data sets GSE83924 were analyzed (listed in Table S13), and we obtained 57 DEGs targeted by miR-192/215-5p (see Figure 5a–d) and three enriched signaling pathways including ECM remodeling, fatty acid metabolism, and respiratory chain were further enriched (see Figure 6a). To confirm the role of miR-192/215-5p in CRC, more GEO data series and GEPIA TCGA data were used and analyzed. We found that miR-192/215-5p were downregulated significantly with different degrees, consistent with previous studies. And several corresponding DEGs enriched in ECM remodeling and fatty acid metabolism pathways were upregulated. Among these DEGs, "SCD, COLIA2, ACSL1, and CYR61" and "SCD, COLIA2, SPARC, VCAN, SERPINE1, and ELOVL5" were overexpressed in CD and CRC, respectively. Among these genes, *SCD–ACSL1*–ELOVL5 axis plays an important role in regulating synthesis of triglyceride, whereas COLIA2, SPARC, CYR61, VCAN, and SERPINE1, recognized by DDRs (discoidin domain receptors), integrins, and PLAUR, are involved in extracellular matrix remodeling (see Figure 6c,d; Figure 7a,b).

SCD (stearoyl-CoA desaturase), one of the two confirmed common DEGs in CD and CRC, along with its downstream signaling pathway ACSL1-ELOVL5, catalyzes the de novo biosynthesis of oleate and palmitoleate, which are the major fatty acids found in triglycerides. Koutroubakis et al. (2000) reported that serum triglyceride levels in Greek patients with active CD were significantly higher in comparison with healthy controls (Agouridis, Elisaf, & Milionis, 2011), and Claudia Agnoli *et al.* conducted a case–cohort study on CRC participants and also found that plasma triglycerides concentration was associated with increased colorectal cancer risk in four Italian European Prospective Investigation into Cancer (EPIC) centers (Agnoli et al., 2014), which were consistent with our results above. Extracellular matrix exerts its physiological and pathological functions through recognizing receptors in cell surface. Consistent with several previous results, extracellular matrix receptors related ligands including COLIA2, CYR61, and SERPINE1 were upregulated in both diseases (Lau, 2011; Lussier, Sodek, & Beaulieu, 2001; Mazzoccoli et al., 2012; Small, Reid-Yu, McPhee, & Coombes, 2013), although a little studies have shown no change or inverse relationship (Koutroubakis et al., 2008; Lussier et al., 2001). Some limitations of the current study should be acknowledged. First, due to the limited fresh clinical samples, the study was analyzed only in public GEO or TCGA data. Second, expression of SPARC in CD was not consistent with previous study,

which could be partially attributed to the samples selection (Lawrance, Fiocchi, & Chakravarti, 2001). Despite its preliminary character, this study can clearly indicate that colon-specific miR-192/215-5p act as tumor suppressors and link Crohn's disease and Colorectal cancer by targeting triglyceride synthesis and extracellular matrix remodeling pathways.

5 | CONCLUSIONS

In conclusion, this study provides insight into the treatment of CD and CRC that miRNAs could be powerful therapeutic targets for its convergent and divergent role in regulating several signaling pathways.

ACKNOWLEDGEMENTS

We would like to thank TCGA, GEO, and Oncomine projects for providing high-quality clinical data on colon diseases.

FUNDING

This study was supported by the Natural Science Foundation of Fujian Province [grant numbers 2012YZ0001-1 and 2016J01582], National Nature Science Foundation of China [grant numbers 81402107 and 81601618], and Military Medical Science Project [grant number 15QNP025].

CONFLICT OF INTERESTS

Authors declare that they have no conflict of interests.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The study protocol was approved by the Ethics Committee of Fuzhou General Hospital, Xiamen University. Data collections and processions were performed according to policies of GEO and TCGA project.

AVAILABILITY OF DATA AND MATERIAL

All authors to ensure that all data are included in this article during this study.

AUTHORS' CONTRIBUTIONS

HZ, JQC, and JC contributed equally to this study. HZ, JQC, YPZ, and JQZ collected data; HZ, QR, YPZ, QHW, SLW, SSC, ZD, ZC, QC, and HHZ performed the computational analyses; HZ, JW, and JL wrote the manuscript; JL and JMT conceived and supervised the study.

ORCID

Hu Zhao http://orcid.org/0000-0003-2029-706X

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

How to cite this article: Zhao H, Chen J, Chen J, et al. miR-192/215-5p act as tumor suppressors and link Crohn's disease and colorectal cancer by targeting common metabolic pathways: An integrated informatics analysis and experimental study. *J Cell Physiol*. 2019;1–16. https://doi.org/10.1002/jcp.28709