MODESTUM
OPEN ACCESS

Original Article

Electron J Gen Med 2019;16(1):em100
ISSN:2516-3507
https://doi.org/10.29333/ejgm/93467

Prediction of biomarker miRNAs signature in colorectal cancer metastasis to liver cancer

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ABSTRACT

Introduction: microRNAs (miRNAs) are frequently dysregulated in colorectal cancer (CRC) primary tumors vs. metastasic to the liver.
Objective: Our aim was to prediction of biomarker miRNAs signature of CRC Metastasis to liver cancer.
Material and Methods: mRNA and miRNA expression profiles of CRC primary tumors to metastases formed in the liver were downloaded from NCBI Gene Expression Omnibus (GEO) database. miRNAs targets were predicted using Targetscan algorithm. The Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway and Gene Ontology (GO) enrichment analysis were performed using DAVID online tool, since mRNA expression profile of liver and colon cancer primary tumors vs. metastasis to the liver were used in background of these analysis.
Results: 43 and 58 down- and up-regulated miRNAs were obtained from GSE98406 (p-value < 0.05). mRNA expression profile include of 1,048 mRNAs differentially expressed in metastatic and non-metastatic CRC to liver from GSE40367 (p-value < 0.05). The some of the down-regulated miRNAs were significantly enriched in migration signaling and cancer stem cell signaling. Moreover, the some of the up-regulated miRNAs were significantly enriched in negative regulation of cell migration.
Conclusions: Some of the miRNAs are many number of target genes that some of these are oncogenes and tumor suppressor genes. It is concluded that differentially expressed miRNAs in metastatic vs. non-metastatic CRC to the liver take part in cell migration and cancer stem cell signaling pathways.

Keywords: miRNA expression data, mRNA expression data, signaling pathway, colorectal cancer, liver cancer

INTRODUCTION

MicroRNAs (miRNA) are short, 18–25 nucleotide-long, noncoding RNA molecules that regulate gene expression by suppressing mRNA translation whose dysregulation has been implicated in most of the cancers (1). miRNAs bind to the 3′ noncoding region of the target mRNA and inhibit the expression of multiple target genes (2). Generally, oncogenic miRNAs (oncomiRs) are overexpressed while tumor-suppressive miRNAs are underexpressed in cancers (3). When these oncomiRs or tumor-suppressor miRNAs are stimulated or inhibited, respectively, cancer cell proliferation, metastasis, and/or survival may be significantly induced (3).

The incidence of colorectal cancer (CRC) is one of the highest rates of morbidity and mortality worldwide (4). Many studies are actively pursuing molecular biological analyses of the mechanisms involved in progression of colon cancer (4).

Biomarkers, such as coding and non-coding RNA are more remarkable and play significant roles in many biological processes (5). Many additional researches demonstrated that miRNAs are one of the most important types of cancer biomarkers and are therapeutic targets for CRC (5). Dysregulation of miRNAs were identified in CRC liver metastasis compared to primary CRC (6).

The biggest problem of cancer treatment is the spread of malignant cells from a primary tumor to distant sites or metastasis that is the most common cause of cancer-related mortality (7). Metastasis involves several continuous steps through which cancer cells disseminate and spread from a primary tumor to distant sites and forming secondary tumors in other tissues (7). Many of miRNAs play a critical role in pathological conditions, including tumorigenesis and metastasis

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Received: 11 Mar 2018, Accepted: 12 May 2018

Table 1: *Description of microarray datasets*

Dataset	Type of samples	Group	Tissue	Number of samples
Series GSE98406	miRNA	Non-metastatic	Colon/liver	14
Series GSE98406	miRNA	Metastatic	Colon/liver	28
Series GSE40367	mRNA	Non-metastatic	tumor endothelium from colon adenocarcinoma tissue	8
Series GSE40367	mRNA	metastatic	tumor endothelium from liver metastasis	7

(8). Specific metastasis-regulating miRNAs, "metastamirs", govern molecular processes and pathways in malignant progression (8). However, research on the functions and mechanisms of microRNAs (miRNAs) in metastasis has only recently begun (8). So far, it has not been investigated CRC liver metastasis is being regulated by which important miRNAs. Therefore, our aim was to predict the miRNAs and molecular mechanisms of CRC liver metastasis.

MATERIALS AND METHODS

Data Sources

The mRNA expression profile of GSE40367 (in metastatic vs. non-metastatic CRC to liver) was obtained from the National Center for Biotechnology Information (NCBI) Gene Expression Omnibus (GEO) database (http://www.ncbi.nlm.nih.gov/geo), that is based on the GPL570 [HG-U133_Plus_2] Affymetrix Human Genome U133 Plus 2.0 Array. On the other hand, comparing miRNA Signature of CRC primary tumors compare to metastases formed in the liver of GSE98406 was obtained from the GEO database, based on Affymetrix Multispecies miRNA-3 array. Description of microarray datasets was shown in **Table 1**. The row data were analyzed with GEO2R.

miRNA Target Prediction

Target genes of all miRNAs were predicted by Targetscan database with context score <-0.2 (http://www.targetscan.org). The predicted targets that became in GSE40367 were used for analysis.

Pathway and Functional Enrichment Analyses

Kyoto encyclopedia of genes and genomes (KEGG) pathway (9) and gene ontology (GO) (10) functional analyses were performed to identify significantly enriched pathways and the biological processes of miRNAs target genes, respectively, using the online tool of the database for annotation, visualization and integrated discovery (DAVID, http://david.abcc.ncifcrf.gov/) (11). mRNAs list in series GSE40367 (p-value < 0.05) were uploaded into DAVID database as background tab. miRNAs target genes lists were uploaded into DAVID database. The entire human genome and mRNAs list in series GSE40367 were selected for the background control and we filtered out the specific gene expressed in metastatic vs. non-metastatic CRC to liver of series GSE40367 (p-value < 0.05).

RESULTS

Differentially Expressed mRNAs and miRNAs in Metastatic vs. Non-metastatic CRC to Liver

In this study we collected the miRNA expression profile (Accession: GSE98406) and mRNA expression profiling (Accession: GSE40367) in GEO database. miRNA expression profile include of 43 and 58 down- and up-regulated miRNAs, respectively. mRNA expression profile include of 1,048 mRNAs differentially expressed in metastatic and non-metastatic CRC to liver (p-value < 0.05). The microarray datasets were descripted in **Table 1** and miRNAs list and log FC for each miRNAs were shown in **Table 2**.

Table 2: *ID, names and fold change of up- and down-regulated miRNAs of metastatic vs. non-metastatic CRC to liver*

Up-regulated miRNAs			Down-regulated miRNAs		
miRNA_ID	**P.Value**	**logFC**	**miRNA_ID**	**P.Value**	**logFC**
hsa-mir-200c	4.87e-08	3.4462	hsa-mir-486	7.67e-11	-1.186
hsa-mir-182	1.38e-07	1.738	hsa-mir-451	4.54e-08	-1.09
hsa-mir-501	2.13e-07	0.2092	hsa-mir-193a	8.33e-06	-0.8401
hsa-mir-1246	3.64e-06	1.6368	hsa-mir-1280	2.05e-05	-0.9676
hsa-mir-3651	7.03e-06	0.1843	hsa-mir-4454	6.07e-05	-0.2395
hsa-mir-18a	1.00e-05	1.2815	hsa-mir-4287	6.71e-05	-0.1317
hsa-mir-203	1.33e-05	1.4432	hsa-mir-630	1.00e-04	-0.0809
hsa-mir-18b	1.44e-05	0.5232	hsa-mir-603	1.26e-04	-0.4742
hsa-mir-106b	1.65e-05	0.6524	hsa-mir-1911	1.29e-04	-0.1571
hsa-mir-183	1.77e-05	0.4473	hsa-mir-3199-1	1.47e-04	-0.0569
hsa-mir-421	1.93e-05	0.4535	hsa-mir-3128	1.76e-04	-0.6054
hsa-mir-21	2.16e-05	1.4669	hsa-mir-4742	1.84e-04	-0.4358
hsa-mir-512-1 // hsa-mir-512-2	2.29e-05	0.1037	hsa-mir-4796	1.88e-04	-0.0729
hsa-mir-501	2.91e-05	0.1166	hsa-mir-720	2.96e-04	-0.9787
hsa-mir-552	3.50e-05	0.525	hsa-mir-122	3.27e-04	-0.0989
hsa-mir-224	3.60e-05	0.391	hsa-mir-887	3.41e-04	-0.0802
hsa-mir-200a	5.19e-05	1.5269	hsa-mir-4775	3.44e-04	-0.0863
hsa-mir-1179	6.14e-05	0.0676	hsa-mir-378	3.58e-04	-1.1897
hsa-mir-200b	7.16e-05	1.4889	hsa-mir-488	3.73e-04	-0.0933
hsa-mir-155	7.55e-05	1.1209	hsa-mir-3201	4.12e-04	-0.8948
hsa-mir-1291	1.09e-04	0.0895	hsa-mir-139	4.22e-04	-0.5518
hsa-mir-652	1.14e-04	0.7115	hsa-mir-193b	4.79e-04	-0.6085
hsa-mir-93	1.14e-04	0.2957	hsa-mir-548n	6.38e-04	-0.114
hsa-mir-141	1.21e-04	1.4191	hsa-mir-551b	6.49e-04	-0.8187
hsa-mir-660	1.73e-04	0.535	hsa-mir-4468	6.70e-04	-0.0782
hsa-mir-1248	1.93e-04	0.3049	hsa-mir-378f	6.87e-04	-0.0856
hsa-mir-210	2.33e-04	1.1209	hsa-mir-4686	6.88e-04	-0.0988
hsa-mir-17	2.90e-04	1.8819	hsa-mir-4668	7.10e-04	-1.1861
hsa-mir-146a	2.95e-04	1.1806	hsa-mir-320c-1 // hsa-mir-320c-2	8.57e-04	-0.6253
hsa-mir-200c	3.08e-04	0.1468	hsa-mir-378c	8.67e-04	-0.8827
hsa-mir-181b-1 // hsa-mir-181b-2	3.08e-04	0.9151	hsa-mir-4744	9.41e-04	-0.0747
hsa-mir-3651	3.68e-04	1.0025	hsa-let-7c	9.54e-04	-0.0733
hsa-mir-503	3.93e-04	0.4039	hsa-mir-18b	1.09e-03	-0.0609
hsa-mir-130b	4.20e-04	0.5855	hsa-mir-3545	1.11e-03	-0.0781
hsa-mir-181d	4.32e-04	0.5109	hsa-mir-548i-1 // hsa-mir-548i-2 // hsa-mir-548i-3 // hsa-mir-548i-4	1.22e-03	-0.0622
hsa-mir-362	4.46e-04	0.5768	hsa-mir-3910-1	1.22e-03	-0.3205
hsa-mir-500a	4.51e-04	0.4431	hsa-mir-4727	1.26e-03	-0.1704
hsa-mir-100	5.48e-04	0.0625	hsa-mir-204	1.26e-03	-0.069
hsa-mir-532	5.80e-04	0.7376	hsa-mir-548ai	1.28e-03	-1.1277
hsa-mir-21	5.84e-04	0.1499	hsa-mir-378d-1	1.30e-03	-0.1319
hsa-mir-106b	6.36e-04	0.0988	hsa-mir-320b-2	1.45e-03	-0.0829
hsa-mir-93	6.56e-04	1.0261	hsa-mir-628	1.47e-03	-0.5139
hsa-mir-25	6.80e-04	0.7632	hsa-mir-518e	1.47e-03	-0.0889
hsa-mir-221	7.01e-04	1.3834			
hsa-mir-222	7.45e-04	1.3807			
hsa-mir-106a	7.91e-04	1.6868			
hsa-mir-20a	8.78e-04	1.6132			
hsa-mir-1301	9.96e-04	0.2818			
hsa-mir-339	1.04e-03	0.3614			
hsa-mir-4711	1.13e-03	0.0667			
hsa-mir-500a	1.17e-03	0.3682			
hsa-mir-1244-1 // hsa-mir-1244-2 // hsa-mir-1244-3	1.20e-03	0.1371			
hsa-mir-1247	1.29e-03	0.0922			
hsa-mir-17	1.34e-03	0.3437			
hsa-mir-19a	1.39e-03	0.3013			
hsa-mir-20b	1.39e-03	1.0308			
hsa-mir-378d-2	1.41e-03	0.0574			
hsa-mir-1290	1.43e-03	0.4762			

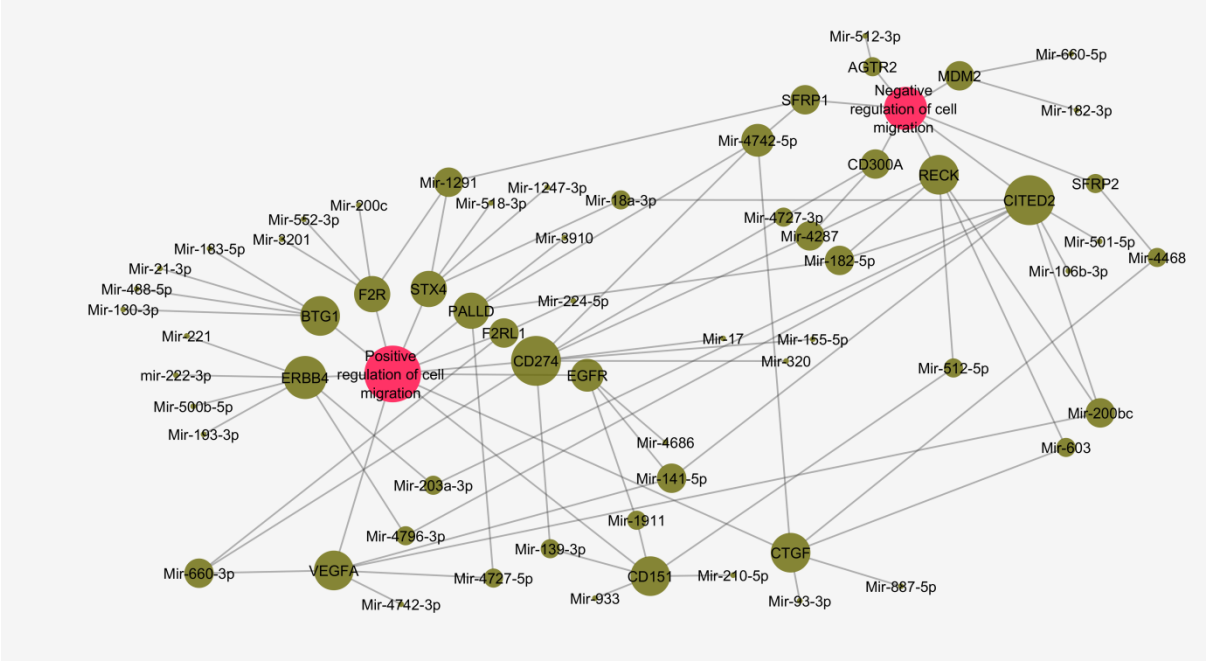

Figure 1: *Overall survival time by stage*

Identification of miRNAs Target Genes

Target genes of miRNAs were identified by Targetscan database (with total context score <−0.2). The number of miRNAs target genes were 30,630 and 24,435 for up- and down-regulated miRNAs in metastatic vs. non-metastatic CRC to liver, respectively.

Biological Process Analysis

In order to investigate the roles of miRNAs target genes, gene ontology (GO) biological processes (BP) module of the DAVID database were used. The GO BP results indicate that the some of the target genes of some down-regulated miRNAs are enriched to leukocyte migration, fibroblast migration, neuron migration, germ cell migration and regulation of endothelial cell migration. For example, ATP1B3, SLC16A1, KITLG and CD44, the target genes of mir-603, mir-4727-5p, mir-3201 and mir-204-3p, respectively, take part in leukocyte migration. The some of the results were shown in **Figure 1**.

In contrast, the some of the target genes of many up-regulated miRNAs take part in negative regulation of cell migration. For instance, mir-200bc, mir-182-5p, mir-501-5p, mir-203a-3p, mir-501-5p and mir-141-5p involved in negative regulation of cell migration by targeting CITED2.

Many of target genes were targeted by both up- and down-regulated miRNAs and involved in both negative and positive cell migration. The complete GO BP results, miRNAs and their target genes were shown in **Table 3**.

Table 3: *MiRNAs, their target genes and GO BP results of up- and down-regulated miRNAs of metastatic vs. non-metastatic CRC to liver*

miRNA	Regulation	Target Genes	Biological Process
Mir-182-5p	Up	ATPase Na+/K+ transporting subunit beta 3(ATP1B3)	leukocyte migration
Mir-603	down		
Mir-224-5p	up		
Mir-155-3p	up		
Mir-1301-3p	up		
Mir-200bc	Up	fibronectin 1(FN1)	
Mir-183-5p	up		
Mir-155-3p	up		
Mir-146a-3p	up		
Mir-887-5p	Down	solute carrier family 16 member 1(SLC16A1)	
Mir-21-3p	up		
Mir-4727-5p	down		
Mir-1291	up		
Mir-1911	Down	KIT ligand(KITLG)	
Mir-3201	Down		
Mir-320	Down		
Mir-4727-3p	Down		
Mir-200c	Up		
Mir-200ab	Up		
Mir-1911	Down	CD44 molecule (Indian blood group)(CD44)	
Mir-488-5p	Down		
Mir-18-3p	Down		
Mir-204-3p	Down		
Mir-518d	Down		
Mir-18b-3p	Up		
Mir-652-5p	Up		
Mir-3201	Down	CD48 molecule(CD48)	
Mir-18-3p	Down		
Mir-4727-5p	Down		
Mir-548ai	Down		
Mir-3651	Up		
Mir-18a-3p	Up		
Mir-18b-3p	Up		
Mir-21-3p	Up		
Mir-3651	Up		
Mir-193-5p	Down	CD34 molecule(CD34)	
Mir-3651	Up		
Mir-203a-5p	Up		
Mir-552-3p	Up		
Mir-3651	up		
Mir-4727-5p	Down	promyelocytic leukemia(PML)	fibroblast migration
Mir-1291	Up		
Mir-193a	Down	BTG anti-proliferation factor 1(BTG1)	cell migration
Mir-488-5p	Down		
Mir-4646-5p/mir-204-3p	Down		
Mir-183-5p	Up		
Mir-21-3p/mir-3591-3p	Up		
Mir-130-3p	Up		
Mir-4742-3p	Down	vascular endothelial growth factor A(VEGFA)	
Mir-4727-5p	Down		
Mir-200bc	Up		
Mir-141-5p	Up		
Mir-660-3p	Up		
Mir-4742-5p	Down	palladin, cytoskeletal associated protein(PALLD)	
Mir-3910	Down		
Mir-4727-5p	Down		
Mir-182-5p	up		
Mir-518-3p	Down	syntaxin 4(STX4)	
Mir-18a-3p	Up		
Mir-1291/mir-6775	Up		
Mir-1247-3p	up		
Mir-193-3p	Down	erb-b2 receptor tyrosine kinase 4(ERBB4)	
Mir-4796-3p	Down		
Mir-203a-3p	Up		
Mir-500b-5p	Up		
Mir-221/mir-222-3p	Up		

Table 3 (continued): *MiRNAs, their target genes and GO BP results of up- and down-regulated miRNAs of metastatic vs. non-metastatic CRC to liver*

miRNA	Regulation	Target Genes	Biological Process
Mir-1911	Down	epidermal growth factor receptor(EGFR)	cell migration
Mir-4686	Down		
Mir-141-5p	Up		
Mir-603	Down	connective tissue growth factor(CTGF)	
Mir-4742-5p	Down		
Mir-887-5p	Down		
Mir-4468	Down		
Mir-93-3p	Up		
Mir-224-5p	Up	F2R like trypsin receptor 1(F2RL1)	
Mir-660-3p	Up		
Mir-200c	Up	coagulation factor II thrombin receptor(F2R)	
Mir-552-3p	Up		
Mir-1291	Up		
Mir-3201	Down		
Mir-4287	Down	CD274 molecule(CD274)	
Mir-4742-5p	Down		
Mir-139-3p	Down		
Mir-320	Down		
Mir-4727-3p	Down		
Mir-17	Up		
Mir-155-5p	Up		
Mir-660-3p	Up		
Mir-1911	Down	CD151 molecule (Raph blood group)(CD151)	
Mir-139-3p	Down		
Mir-933	Down		
Mir-512-5p	Up		
Mir-210-5p	Up		
Mir-4287	Down	CD300a molecule(CD300A)	negative regulation of eosinophil migration,
Mir-4727-3p	Down		
Mir-4796-3p	Down	Cbp/p300 interacting transactivator with Glu/Asp rich carboxy-terminal domain 2(CITED2)	negative regulation of cell migration
Mir-200bc	Up		
Mir-182-5p	Up		
Mir-501-5p	Up		
Mir-18a-3p	Up		
Mir-203a-3p	Up		
Mir-106b-3p	Up		
Mir-501-5p	Up		
Mir-141-5p	Up		
Mir-4468	Down	secreted frizzled related protein 2(SFRP2)	
Mir-4287	Down	reversion inducing cysteine rich protein with kazal motifs(RECK)	
Mir-603	Down		
Mir-200bc	Up		
Mir-182-5p	Up		
Mir-512-5p	Up		
Mir-4742-5p	Down	secreted frizzled related protein 1(SFRP1)	
Mir-1291	Up		
Mir-512-3p	Up	angiotensin II receptor type 2(AGTR2)	
Mir-182-3p	Up	MDM2 proto-oncogene(MDM2)	
Mir-660-5p	Up		
Mir-1291	up	doublecortin like kinase 1(DCLK1)	neuron migration
Mir-1911-5p	Down	myocyte enhancer factor 2C(MEF2C)	
Mir-122-3p	Down		
Mir-200c	Up		
Mir-182-5p	Up		
Mir-501-5p	Up		
mir-501-3p	Up		
Mir-1911	down	fibroblast growth factor receptor 1(FGFR1)	
Mir-320	down	delta/notch like EGF repeat containing(DNER)	
Mir-603	Down	cyclin dependent kinase 5 regulatory subunit 1(CDK5R1)	
Mir-183-5p	Up		
mir-660-3p	Up		
Mir-1911	Down		
Mir-3201	Down		
Mir-320	Down		
Mir-4727-3p	Down		

Table 3 (continued): *MiRNAs, their target genes and GO BP results of up- and down-regulated miRNAs of metastatic vs. non-metastatic CRC to liver*

miRNA	Regulation	Target Genes	Biological Process
Mir-200c	Up	cyclin dependent kinase 5 regulatory subunit 1(CDK5R1)	neuron migration
Mir-200ab	Up		
Mir-933	Down	KIT proto-oncogene receptor tyrosine kinase(KIT)	germ cell migration
Mir-130b-5p	Up	SET domain containing 2(SETD2)	cell migration involved in vasculogenesis
Mir-3910	Down	SP100 nuclear antigen(SP100)	negative regulation of endothelial cell migration
Mir-200bc	Up		
Mir-603	Down	epithelial membrane protein 2(EMP2)	regulation of endothelial cell migration
Mir-183-5p	Up		
mir-660-3p	Up		
Mir-1911	Down	phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha(PIK3CA)	
Mir-3201	Down		
Mir-320	Down		
Mir-4727-3p	Down		
Mir-200c	Up		
Mir-200ab	Up		
Mir-3201	Down	fibroblast growth factor 1(FGF1)	
Mir-1301-3p	up		
Mir-4668	Down	forkhead box C2(FOXC2)	
Mir-548ai	Down		
Mir-887-5p	Down	forkhead box P1(FOXP1)	
Mir-183-5p	Up		
Mir-93-3p	Up		

Table 4: *MiRNAs, their target genes and KEGG pathway results of up- and down-regulated miRNAs of metastatic vs. non-metastatic CRC to liver*

miRNA	regulation	Target gene	Signaling pathway
Mir-4742-3p	Down	G protein subunit gamma 12(GNG12)	PI3K-Akt signaling pathway
Mir-887-5p	Down		
Mir-4468	Down		
Mir-548	Down		
Mir-4668-5p	Down		
Mir-4744	Down		
Mir-183-5p	Up		
Mir-512-5p	Up		
Mir-552-3p	Up		
Mir-503-3p	Up		
Mir-1244	Up		
Mir-1911	Down	KIT ligand(KITLG)	
Mir-3201	Down		
Mir-320	Down		
Mir-4727-3p	Down		
Mir-200c	Up		
Mir-200ab	Up		
Mir-933	Down	KIT proto-oncogene receptor tyrosine kinase(KIT)	
Mir-182-3p	Up	MDM2 proto-oncogene(MDM2)	
Mir-660-5p	Up		
Mir-5440-3p	Down	Raf-1 proto-oncogene, serine/threonine kinase(RAF1)	
Mir-4468	Down		
Mir-146a-3p	Up		

Signaling Pathways Analysis

We performed the KEGG pathways enrichment analysis for miRNAs target genes that up- or down-expressed in metastatic vs. non-metastatic CRC to liver. KEGG pathway indicated that some of the up-regulated miRNAs such as mir-182-3p, mir-660-3p, mir, mir-4711-3p and mir-25 take part in tight junction, gap junction and adherens junction. Some of the up-regulated miRNAs such as mir-1291 and mir 1244 involved in cancer stem cells signaling as MAPK signaling pathway and PI3K-Akt signaling pathway.

But some of the down-regulated miRNAs in metastatic vs. non-metastatic CRC to liver such as mir-4742-3p, mir-887-5p, mir-4468, mir-933, mir-1911 and mir-4668 involved in MAPK, Notch, Wnt and PI3K-Akt signaling pathway. Moreover, some of the down-regulated miRNAs act in cell junctions. The complete results of KEGG pathway for each up- and down-regulated miRNAs target genes were shown in **Table 4**.

Table 4 (continued): *MiRNAs, their target genes and KEGG pathway results of up- and down-regulated miRNAs of metastatic vs. non-metastatic CRC to liver*

miRNA	regulation	Target gene	Signaling pathway
Mir-139-3p	Down	SGK2, serine/threonine kinase 2(SGK2)	
Mir-200c	Up	coagulation factor II thrombin receptor(F2R)	
Mir-552-3p	Up		
Mir-1291	Up		
Mir-3201	Down		
Mir-603	Down	collagen type IV alpha 3 chain(COL4A3)	
Mir-548	Down		
Mir-18a-3p	Up	collagen type XXVII alpha 1 chain(COL27A1)	
Mir-106b-3p	Up	cyclin E1(CCNE1)	
Mir-1179	Up		
Mir-3910	Down		
Mir-4468	Down	ephrin A3(EFNA3)	
Mir-210-3p	Up		
Mir-210-5p	Up		
Mir-141-5p	Up	eukaryotic translation initiation factor 4E family member 2(EIF4E2)	
Mir-3201	Down	fibroblast growth factor 1(FGF1)	
Mir-1301-3p	up		
		fibroblast growth factor 4(FGF4)	
Mir-1911	down	fibroblast growth factor receptor 1(FGFR1)	
Mir-200bc	Up	fibronectin 1(FN1)	
Mir-183-5p	up		
Mir-155-3p	up		
Mir-146a-3p	up		
Mir-887-5p	Down	interleukin 2 receptor subunit alpha(IL2RA)	
Mir-4727-5p	Down		
Mir-512-3p	Up		
Mir-329-3p	Up		
Mir-500a-3p	Up		
Mir-4742-3p	Down	lysophosphatidic acid receptor 1(LPAR1)	
Mir-3910	Down		
Mir-200bc	Up		
Mir-501-5p	Up		
Mir-3651	Up		
Mir-660-3p	Up		
Mir-503-3p	Up		
Mir-1911	Down	phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha(PIK3CA)	
Mir-3201	Down		
Mir-320	Down		
Mir-4727-3p	Down		
Mir-200c	Up		
Mir-200ab	Up		
Mir-4287	Down	toll like receptor 4(TLR4)	
Mir-630	Down		
Mir-3201	Down		
Mir-4742-3p	Down	vascular endothelial growth factor A(VEGFA)	
Mir-4727-5p	Down		
Mir-200bc	Up		
Mir-141-5p	Up		
Mir-660-3p	Up		
Mir-1291	Up	ECSIT signalling integrator(ECSIT)	MAPK signaling pathway
Mir-139-3p	Down	ELK1, ETS transcription factor(ELK1)	
Mir-660-5p	Up		
Mir-4742-3p	Down	G protein subunit gamma 12(GNG12)	
Mir-887-5p	Down		
Mir-4468	Down		
Mir-548	Down		
Mir-4668-5p	Down		
Mir-4744	Down		
Mir-183-5p	Up		
Mir-512-5p	Up		
Mir-552-3p	Up		
Mir-503-3p	Up		
Mir-1244	Up		
Mir-5440-3p	Down	Raf-1 proto-oncogene, serine/threonine kinase(RAF1)	
Mir-4468	Down		
Mir-146a-3p	Up		
Mir-3910	Down	arrestin beta 1(ARRB1)	

Table 4 (continued): *MiRNAs, their target genes and KEGG pathway results of up- and down-regulated miRNAs of metastatic vs. non-metastatic CRC to liver*

miRNA	regulation	Target gene	Signaling pathway
Mir-17	Up	calcium voltage-gated channel auxiliary subunit gamma 1(CACNG1)	
Mir-3128	Down		
Mir-1291	Up	calcium voltage-gated channel auxiliary subunit gamma 4(CACNG4)	
Mir-1247-3p	Up		
Mir-122-5p	Down	dual specificity phosphatase 2(DUSP2)	
Mir-4727-5p	Down		
Mir-1911	Down		
Mir-4686	Down	epidermal growth factor receptor(EGFR)	
Mir-141-5p	Up		
		fibroblast growth factor 1(FGF1)	
		fibroblast growth factor 4(FGF4)	
Mir-1911	down	fibroblast growth factor receptor 1(FGFR1)	
Mir-1911-5p	Down		
Mir-4686	Down		
Mir-4668-5p	Down		
Mir-548a	Down	mitogen-activated protein kinase kinase kinase 2(MAP3K2)	
Mir-203a-5p	Up		
Mir-183-5p	Up		
Mir-1244	Up		
Mir-887-5p	Down	mitogen-activated protein kinase kinase kinase kinase 1(MAP4K1)	
Mir-1911-5p	Down		
Mir-122-3p	Down		
Mir-628-5p	Down		
Mir-200c	Up	myocyte enhancer factor 2C(MEF2C)	
Mir-182-5p	Up		
Mir-501-5p	Up		
Mir-93-3p	Up	nuclear factor of activated T-cells 3(NFATC3)	
Mir-203a-5p	Up	ribosomal protein S6 kinase A2(RPS6KA2)	
Mir-106b-3p	Up		
Mir-193-3p	Down		
Mir-3201	Down		
Mir-4668-5p	Down	stathmin 1(STMN1)	
Mir-200ab	Up		
Mir-155-3p	Up		
Mir-1911	Down		
Mir-933	Down	RFNG O-fucosylpeptide 3-beta-N-acetylglucosaminyltransferase(RFNG)	Notch signaling pathway
Mir-93-3p	Up		
Mir-1911-5p	Down		
Mir-4742-5p	Down		
Mir-887-5p	Down		
Mir-488-5p	Down		
Mir-4686	Down		
Mir-4668-5p	Down	Wnt family member 2B(WNT2B)	Wnt signaling pathway
Mir-501-5p	Up		
Mir-18a	Up		
Mir-18b	Up		
Mir-501-5p	Up		
Mir-1179	Up		
Mir-652-5p	Up		
Mir-3910	Down	axin 2(AXIN2)	
Mir-1246	Up		
		frizzled class receptor 2(FZD2)	
Mir-93-3p	Up	nuclear factor of activated T-cells 3(NFATC3)	
Mir-182-3p	Up	LLGL1, scribble cell polarity complex component(LLGL1)	Tight junction
Mir-3201	Down		
Mir-660-3p	Up	myosin heavy chain 11(MYH11)	
Mir-4711-3p	Up		
		myosin heavy chain 3(MYH3)	
Mir-887-5p	down	myosin light chain, phosphorylatable, fast skeletal muscle(MYLPF)	
Mir-887-5p	Down		
Mir-4668-5p	Down		
Mir-3910	Down		
Mir-203a-5p	Up	claudin 11(CLDN11)	
Mir-421	Up		
Mir-501/mir-502	Up		
Mir-25-5p	Up		

Table 4 (continued): *MiRNAs, their target genes and KEGG pathway results of up- and down-regulated miRNAs of metastatic vs. non-metastatic CRC to liver*

miRNA	regulation	Target gene	Signaling pathway
Mir-4744	Down	claudin 14(CLDN14)	
Mir-660-3p	Up		
Mir-628-3p	Down	catenin alpha 1(CTNNA1)	
Mir-1179	Up		
Mir-122-3p	Down	RAB3B, member RAS oncogene family(RAB3B)	
Mir-501-5p	Up		
Mir-512-3p	Up		
Mir-660-3p	Up		
Mir-210-5p	Up		
Mir-146a-3p	Up		
Mir-5440-3p	Down	Raf-1 proto-oncogene, serine/threonine kinase(RAF1)	Gap junction
Mir-4468	Down		
Mir-146a-3p	Up		
Mir-1911	Down	epidermal growth factor receptor(EGFR)	
Mir-4686	Down		
Mir-141-5p	Up		
Mir-4468	Down	adenylate cyclase 2(ADCY2)	
Mir-501	Up		
Mir-628-3p	Down	catenin alpha 1(CTNNA1)	Adherens junction
Mir-1179	Up		
Mir-1911	Down	epidermal growth factor receptor(EGFR)	
Mir-4686	Down		
Mir-141-5p	Up		
Mir-1911	down	fibroblast growth factor receptor 1(FGFR1)	

DISCUSSION

According to the gene expression profile analysis between metastatic vs. non-metastatic CRC to liver groups, 58 up-regulated and 55 down-regulated miRNAs in metastatic vs. non-metastatic CRC to liver were obtained (P.Value < 0.05). On the other hand, 1,048 mRNAs differentially expressed in metastatic vs. non-metastatic CRC to liver were selected (P.Value < 0.05). After target prediction by targetscan algorithm, the miRNAs target genes that become in series GSE40367 were selected and GO BO and signaling pathways were analyzed by DAVID database. Since multiple miRNAs target the same gene, that determines the expression of miRNA target genes, one of the miRNAs targeted many numbers of mRNAs. Recent study indicated that hsa-mir-21 is an oncomir in CRC which in turn regulates the development of CRC (12). These results indicated that after up-regulation of miR-21 expression, the levels of AKT and PI3K protein expression significantly increased (12). Our results were shown that has-mir-21 upregulated in CRC and take part in cell migration. Evidences showed that has-mir-17 contributed to the proliferation and invasion of colorectal cancer and played a critical role in the proliferation and invasion of colorectal cancer (13). We showed that has-mir-17 were up-regulated in CRC and involved in cell migration (14). Moreover, levels of miR-21, and miR-155 increased significantly in human colon tumor samples, compared with normal tissues that have been associated with tumor growth (14) . In these studies, results indicated that has-mir-155 was up-regulated in CRC and take part in PI3K-Akt signaling pathway and cell migration. Jun Qin et al. suggested that has-mir-221 promotes CRC cell invasion and metastasis (15). In our study, has-mir-221 as an up-regulated miRNAs was enriched in some biological processes in cell migration. Our results were shown that some of the miRNAs are many number of target genes that some of these are oncogenes and some are tumor suppressor genes. Also, our results indicated that differentially expressed miRNAs in metastatic vs. nonmetastatic CRC to the liver take part in cell migration and cancer stem cell signaling pathways. The target genes of some of the miRNAs promote negative regulation of cell migration. The results of bioinformatics analysis indicated that many of up- and down-regulated miRNAs involve in cancer stem cell processing in metastatic vs. non-metastatic CRC to liver that were shown in **Table 3** and **4**.

In conclusion, our study showed that differentially expressed miRNAs in metastatic vs. non-metastatic CRC to the liver take part in cell migration and cancer stem cell signaling pathways.

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