Exploration of the mechanism of colorectal cancer metastasis using microarray analysis

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Abstract. The aim of the present study was to investigate the mechanism of metastasis in colorectal cancer (CRC) using microRNA (miRNA) and mRNA expression profiles. The mRNA and miRNA expression profiles of the GSE2509 and GSE56350 datasets were obtained from the Gene Expression Omnibus database. The differentially expressed genes (DEGs) and differentially expressed miRNAs (DEMs) were identified using the limma software package. The Database for Annotation, Visualization and Integrated Discovery was used to perform Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis of the DEGs. The predicted target genes associated with the DEMs were identified using the miRWalk database and the enrichment analysis was conducted using the clusterProfiler package. The miRNA-gene molecular interaction network was visualized using the Cytoscape software platform. A total of 544 DEGs and 42 DEMs were identified. DEGs were annotated in 320 GO terms and 11 KEGG pathways. Overall, 366 miRNA-gene pairs were identified and the miRNA-gene network was visualized. Furthermore, the predicted target genes were mainly classified in 12 pathways. The results of the present study suggest that fibronectin type III domain-containing 3B, cysteine rich transmembrane BMP regulator 1 and forkhead box J2 may be potential therapeutic and prognostic targets of metastatic CRC. In addition, pathways in cancer, the Wnt signaling pathway and extracellular matrix-receptor interaction may play a critical role in CRC metastasis.

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Introduction

Colorectal cancer (CRC) is the third most common malignancy worldwide and the third leading cause of cancer-associated mortality in the United States (1,2). It is estimated that ~1.3 million new cases are diagnosed and ~0.7 million people succumb to the disease each year worldwide (3). According to recent statistics, it was estimated that 134,490 new cases and 49,190 fatalities would occur in America in 2016 (1). The incidence and mortality rates for men and women have improved in the last decades as a result of advances in screening and clinical treatment (4,5). Metastasis, the most common cause of cancer-associated mortality, is a multi-step process through which tumor cells spread from their primary site and form secondary growths at a distance (6). Metastasis is among the six initially described hallmarks of cancer and is a major cause of CRC-associated mortality (7,8). The 5-year survival rate of early-stage CRC ranges between 60 and 95%; however, for patients with metastatic tumors, the survival rate ranges from 10 to 35% (9-11). Despite improvements in diagnosis and treatment, ~90% of CRC-associated mortalities are due to metastases and it has been estimated that ~50% of all patients diagnosed with CRC eventually succumb to metastatic disease (12,13). Therefore, it is critical to investigate the mechanism of metastasis in CRC and identify novel molecular therapeutic and prognostic targets. To date, some progress has been made. Epithelial-mesenchymal transition was demonstrated to serve a pivotal and intricate role in promoting CRC metastasis (6,14). In addition, certain genes and proteins were found to be associated with CRC metastasis, including semaphoring 3F, stromal interaction molecule 1, forkhead box C2 and hes family BHLH transcription factor 1 (14-17), as well as Cyclin b1, Angiopoietin-like 4 and p21-activated kinase 1 and 4 (18-20). However, one study demonstrated that metastasis occurs through a multistep cascade of events, but that it was inefficient as a whole process (8). Even though mutations associated with metastasis have been investigated in the past, only a limited number of such genetic alterations have been identified and the underlying molecular mechanism remains unclear (21). The present study analyzed the microRNA (miR/miRNA) and mRNA expression profiles of CRC samples

in order to investigate the mechanism of metastasis in CRC and identify novel molecular biomarkers.

Materials and methods

mRNA and miRNA expression data. The mRNA and miRNA expression profiles of the GSE2509 (22) and GSE56350 (23) datasets were obtained from the Gene Expression Omnibus database (www.ncbi.nlm.nih.gov/geo/). GSE2509 contains the mRNA profile of 3 primary CRC samples and 3 samples with lymph node metastasis. These data were analyzed with the GPL96 [HG-U133A] Affymetrix Human Genome U133A Array platform version 2.0 (Affymetrix; Fisher Scientific, Inc., Waltham, MA, USA). Additionally, 46 primary CRC samples and 43 CRC samples with lymph node metastasis were obtained from the miRNA profile of GSE56350. Detection of the miRNA profile was performed using the PL16744 OSU-CCC Human and Mouse MicroRNA Microarray platform version 4.0 (Comprehensive Cancer Center, The Ohio State University, Columbus, OH, USA).

Data processing and differential expression analysis. The mRNA profile data were converted into recognizable format in R and then were normalized using the Robust Multi-Array Average algorithm from *Affy* version 1.40.0 package (http://www.bioconductor.org/packages/2.13/bioc/html/affy.html) (24). For the miRNA profile, the original expression value matrix was obtained and normalization was conducted using the preprocessCore function package version 3.5 (http://www.bioconductor.org/packages/release/bioc/html/preprocessCore.html) (25). Subsequently, the differentially expressed genes (DEGs) and differentially expressed miRNAs (DEMs) were identified in the CRC samples with lymph node metastasis compared with the primary CRC samples using the limma version 3.18.13 software package (http://www.bioconductor.org/packages/2.13/bioc/html/limma.html) (26). $P<0.05$ and $|\log_2(\text{fold-change})| > 0.2$ were used as threshold criteria.

Functional and pathway enrichment analysis of DEGs. The Database for Annotation, Visualization and Integrated Discovery version 6.8 (https://david.ncifcrf.gov/) (27) was used to perform Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis of DEGs. GO terms and KEGG pathways with $P<0.05$ were selected.

Construction of the miRNA-gene network. The miRWalk version 2.0 database (mirwalk.uni-hd.de/) is a publicly available comprehensive resource containing the predicted and the experimentally validated miRNA-target interaction pairs (28). The DEM-associated predicted target genes were selected when they were included in at least four out of five databases (miRanda-rel2010, miRDB version 4.0, miRWalk version 2.0, RNA22 version 2.0 and TargetScan version 6.2). Subsequently, the overlapping target genes were identified and the miRNA-gene pairs and DEMs were selected. The miRNA-gene network was formed and visualized using the Cytoscape version 3.5.1 (29) software (http://www.cytoscape.org/download.php).

Functional enrichment analysis of predicted target genes regulated by DEMs. The clusterProfiler version 3.5 (http://www.bioconductor.org/packages/release/bioc/html/clusterProfiler.html), an R package, was used to perform biological-term classification and enrichment analysis of gene clusters (30). Following the identification of the predicted target genes regulated by DEMs, enrichment analysis was performed and the enriched pathways with a P-value of <0.05 were selected.

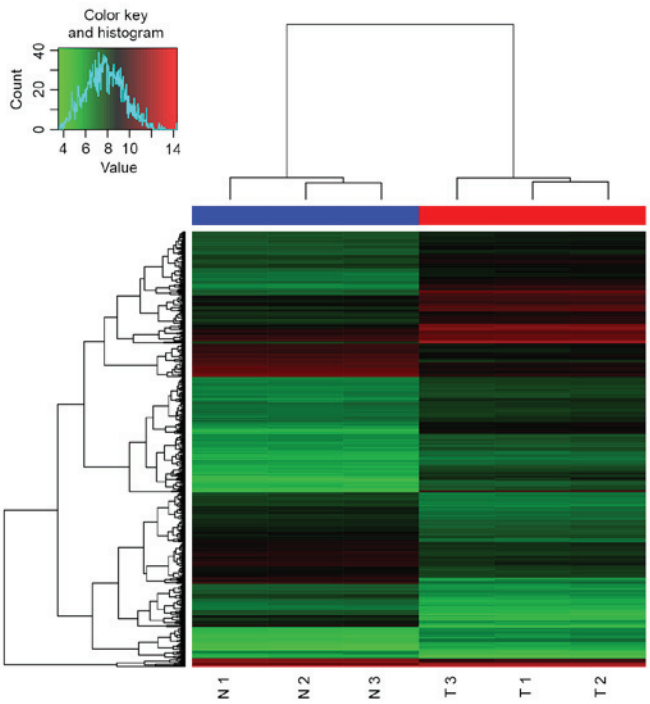

Figure 1. Heat map of differentially expressed genes.

Results

Differentially expressed mRNAs and differentially expressed miRNAs. A total of 544 DEGs (227 upregulated and 317 downregulated) were identified, and the heat map of hierarchical clustering is presented in Fig. 1. Additionally, 42 DEMs (25 upregulated and 17 downregulated) were identified. The 20 most significant DEGs and the 20 most significant DEMs are presented in Tables I and II, respectively.

Enriched GO terms and KEGG pathways of differentially expressed mRNAs. DEGs were enriched in 320 GO terms and 11 KEGG pathways. The 10 most significant enriched GO terms and the enriched KEGG pathways are presented in Tables III and IV, respectively.

miRNA-gene pairs and miRNA-gene network. A total of 366 miRNA-gene pairs among the overlapped genes with the DEMs were selected, and the miRNA-gene network was generated and analyzed. The network is presented in Fig. 2 and the 20 highest degree nodes are presented in Table V. The term 'degree' represented connections of one node with other nodes.

Enriched pathways of predicted target genes. In total 271, 237, 192, 275 and 120 genes were respectively regulated by each of the 5 miRNAs *hsa-miR-106a, hsa-miR-15a, hsa-miR-16,*

Table I. 20 most significant differentially expressed genes in colorectal cancer samples with lymph node metastasis.

Gene	P-value	$\log_2$ FC
RNF128	4.07×10^{-15}	4.435925
CNN3	6.05×10^{-15}	4.696778
WNT5A	8.42×10^{-15}	-5.04177
TOX3	9.65×10^{-15}	5.640899
FZD10	1.13×10^{-14}	3.438707
IGFBP3	3.26×10^{-14}	-4.23336
AKR1C3	4.50×10^{-14}	4.643379
TRIP6	5.04×10^{-14}	-3.79486
NPC2	5.37×10^{-14}	-3.86253
KRT13	8.49×10^{-14}	-5.41489
KRT23	8.91×10^{-14}	-4.84643
NGFR	9.23×10^{-14}	-3.8684
KRT81	9.38×10^{-14}	-3.54854
CXCR4	1.25×10^{-13}	-3.41813
ANXA6	1.29×10^{-13}	-3.29244
MSX1	1.64×10^{-13}	-3.64506
EFNB2	1.76×10^{-13}	3.675191
SLC2A3	1.77×10^{-13}	-4.2354
IGFBP7	2.18×10^{-13}	-3.27699
GNG11	2.77×10^{-13}	-5.77814

FC, fold-change.

Table II. 20 most significant differentially expressed miRNAs in colorectal cancer samples with lymph node metastasis.

miRNA	P-value	$\log_2$ FC
hsa-miR-342-3p	5.2×10^{-10}	1.3803
hsa-miR-150	1.31×10^{-9}	1.5415
hsa-miR-155	2.73×10^{-7}	1.5434
hsa-miR-92b	6.19×10^{-7}	-1.6535
hsa-miR-375	4.04×10^{-6}	-1.8421
hsa-miR-142-5p	3.72×10^{-6}	1.752
hsa-miR-453	8.88×10^{-6}	-1.1169
hsa-miR-622	1.47×10^{-5}	-1.4782
hsa-miR-595	6.48×10^{-5}	-1.0289
hsa-miR-629	8.46×10^{-5}	-1.1146
hsa-mir-621	1.04×10^{-4}	-1.2234
hsa-miR-26a	1.19×10^{-4}	2.4369
hsa-miR-146a	1.51×10^{-4}	2.1241
hsa-miR-26b	1.56×10^{-4}	2.7188
hsa-miR-200b	2.05×10^{-4}	-1.1071
hsa-miR-146b-5p	2.18×10^{-4}	1.9222
hsa-miR-107	2.91×10^{-4}	2.1441
hsa-miR-560	3.29×10^{-4}	-1.4887
hsa-mir-766	3.33×10^{-4}	-1.1362
hsa-miR-103	4.97×10^{-4}	2.1098

miRNA/miR, microRNA; FC, fold-change; hsa, *Homo sapiens*.

Table III. 10 most significant enriched GO terms of differentially expressed microRNAs.

Category	GO ID	GO name	Count	P-value
BP	GO:0001944	Vasculature development	29	1.30×10^{-8}
BP	GO:0001568	Blood vessel development	28	3.08×10^{-8}
BP	GO:0016477	Cell migration	27	1.22×10^{-6}
BP	GO:0048514	Blood vessel morphogenesis	22	5.54×10^{-6}
BP	GO:0051674	Localization of cell	27	8.67×10^{-6}
BP	GO:0048870	Cell motility	27	8.67×10^{-6}
BP	GO:0042127	Regulation of cell proliferation	50	9.42×10^{-6}
BP	GO:0051094	Positive regulation of developmental process	25	1.40×10^{-5}
BP	GO:0006928	Cell motion	35	1.46×10^{-5}
BP	GO:0045597	Positive regulation of cell differentiation	22	1.95×10^{-5}

GO, Gene Ontology; BP, biological process.

hsa-miR-20a and *hsa-miR-29b*, respectively. Overall, 12 pathways were enriched and the results are presented in Fig. 3.

Discussion

In the present study, DEGs and DEMs were initially identified in colorectal cancer samples with lymph node metastasis compared with primary colorectal cancer samples. Subsequently, the functional and pathway enrichment analysis of DEGs and the predicted target genes regulated by DEMs was performed. The over-represented pathways were associated with pathways in cancer, the Wnt signaling pathway and extracellular matrix (ECM)-receptor interaction. The major enriched pathway of DEMs was pathways in cancer characterized by the lowest P-values (Table IV). Overall, ~12% of predicted target genes regulated by *hsa-miR-15a*, *hsa-miR-16* and *hsa-miR-20a* were associated with this pathway; suggesting that it serves a critical role in CRC metastasis.

Wnt signaling is involved in embryonic development (31) and a number of studies demonstrated that aberrant Wnt

Table IV. Enriched KEGG pathways of differentially expressed microRNAs.

Category	Pathway name	Count	P-value
KEGG_PATHWAY	hsa05200: Pathways in cancer	24	0.00217
KEGG_PATHWAY	hsa04360: Axon guidance	12	0.008095
KEGG_PATHWAY	hsa04310: Wnt signaling pathway	13	0.009973
KEGG_PATHWAY	hsa05222: Small cell lung cancer	9	0.012226
KEGG_PATHWAY	hsa04115: p53 signaling pathway	8	0.012507
KEGG_PATHWAY	hsa05217: Basal cell carcinoma	7	0.015538
KEGG_PATHWAY	hsa04916: Melanogenesis	9	0.030049
KEGG_PATHWAY	hsa04060: Cytokine-cytokine receptor interaction	17	0.033293
KEGG_PATHWAY	hsa05210: Colorectal cancer	8	0.035683
KEGG_PATHWAY	hsa04512: ECM-receptor interaction	8	0.035683
KEGG_PATHWAY	hsa04540: Gap junction	8	0.046579

KEGG, Kyoto Encyclopedia of Genes and Genomes; hsa, *Homo sapiens*; ECM, extracellular matrix.

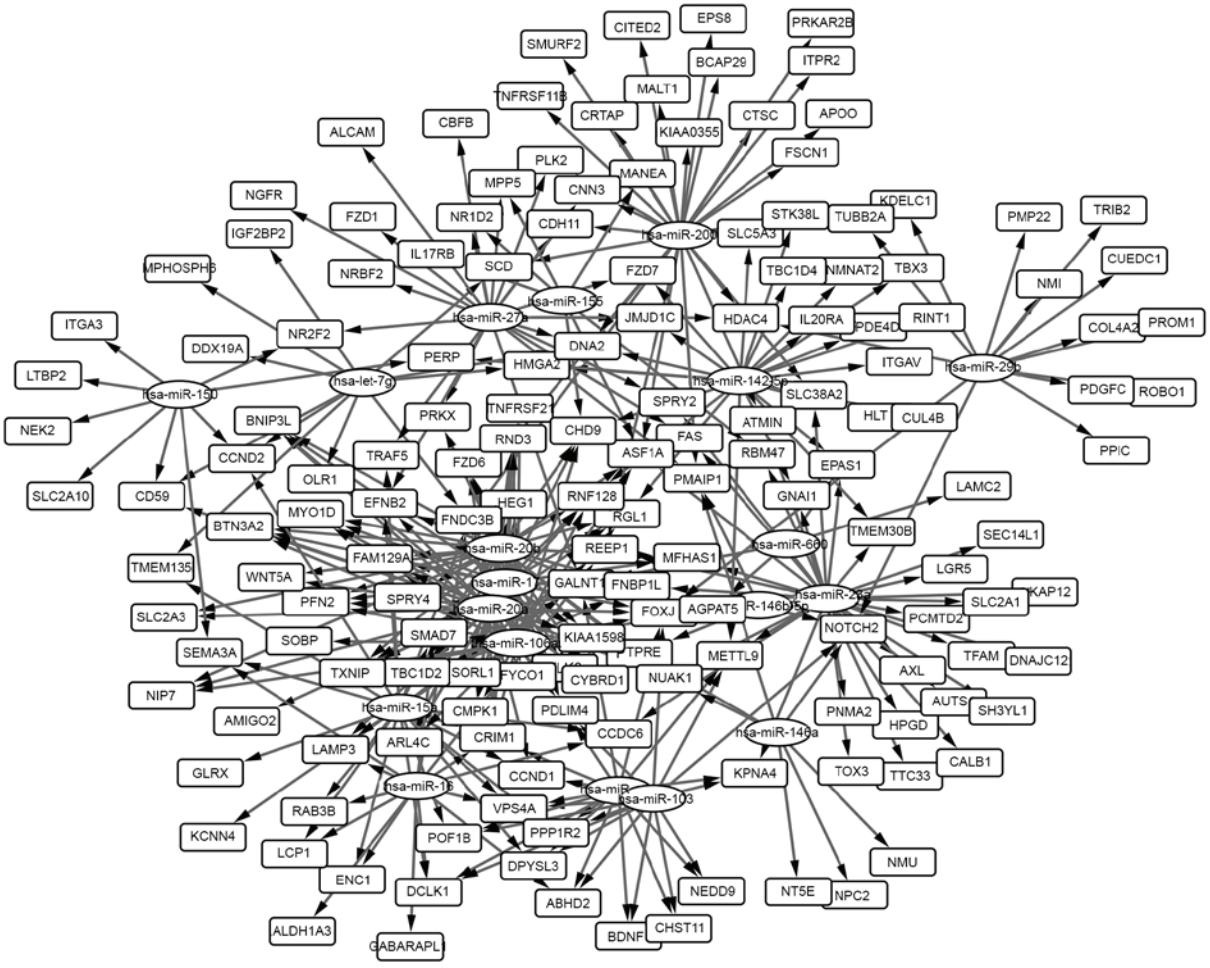

Figure 2. miRNA-gene network of predicted target genes regulated by differentially expressed miRNAs. miRNA, microRNA.

signaling serves an important role in CRC, regulating several cellular processes, including cell migration and metastasis (32,33). Hu *et al* (34) reported that CXCR4 promotes CRC progression and epithelial-mesenchymal transition by activating the Wnt/β-catenin signaling pathway. A study by Ting *et al* (35) indicated that the genetic interaction profile of Wnt pathway genetic variants may increase the prognostic value of outcome prediction for CRC patients. Therefore, it was indicated that the Wnt signaling pathway may serve an important role in the processes of cell migration and metastasis, and that certain genes in this pathway may serve as potential metastatic biomarkers for CRC.

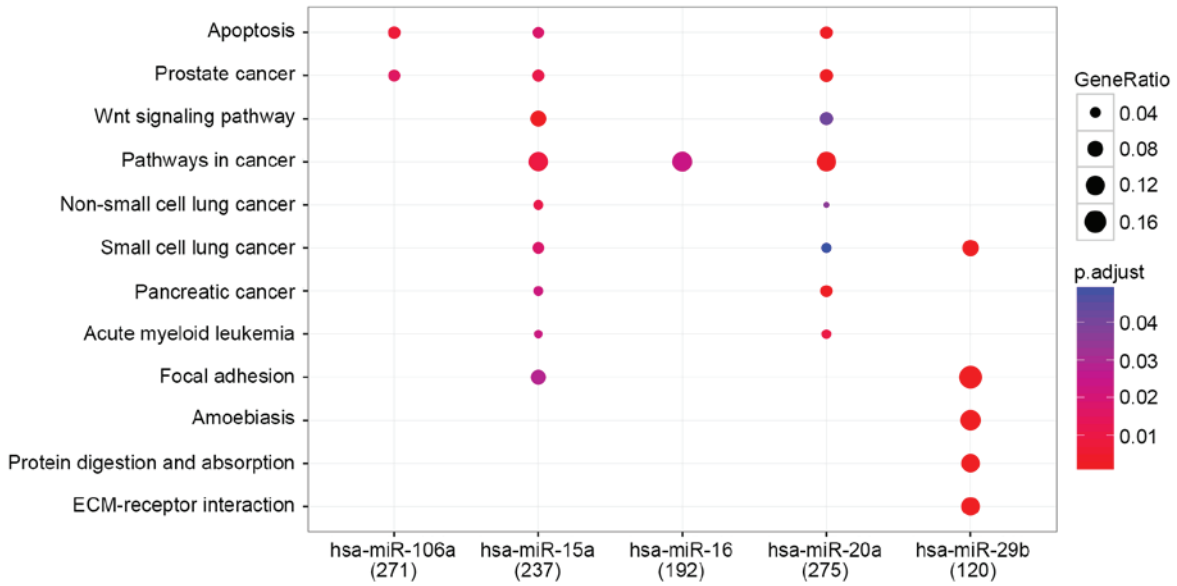

Figure 3. Enriched pathways of predicted target genes regulated by, differentially expressed miRNAs. miRNA/miR, microRNA; ECM, extracellular matrix.

Table V. 20 highest degree nodes in the miRNA-gene network.

Node	Degree
hsa-miR-20b	38
hsa-miR-20a	36
hsa-miR-106a	33
hsa-miR-23a	32
hsa-miR-200b	30
hsa-miR-17	26
hsa-miR-142-5p	24
hsa-miR-15a	23
hsa-miR-27a	22
hsa-miR-107	17
hsa-miR-103	15
hsa-miR-16	15
hsa-miR-29b	14
hsa-let-7g	11
hsa-miR-150	9
FNDC3B	8
CRIM1	7
FOXJ2	7
hsa-miR-146a	7
hsa-miR-155	7

hsa, *Homo sapiens*; miRNA/miR, microRNA; *FNDC3B*, fibronectin type III domain-containing 3B; *CRIM1*, cysteine rich transmembrane BMP regulator 1; *FOXJ2*, forkhead box J2.

The ECM regulates tissue architecture and adipogenesis, which involves a complex mixture of structural and functional macromolecules, including glycosaminoglycans and fibrous proteins (36). One recent study revealed that twist-related protein 2 (Twist2) regulates the expression of integrin α-4 and CD44 antigen, two major proteins in the ECM-receptor interaction pathway (37). Furthermore, it was also demonstrated that the overexpression of Twist2 may be involved in cell growth regulation, apoptosis and motility, and that Twist2 may serve as a potential therapeutic target for the treatment of kidney cancer (37). Additionally, twist family BHLH transcription factor 2 was significantly overexpressed in several solid tumors and contributed to tumor progression (38). The results of the present study suggest that ECM-receptor interaction may be associated with CRC metastasis, however, further research is required to validate this association.

miRNA-gene network analysis revealed that fibronectin type III domain-containing 3B (*FNDC3B*), cysteine rich transmembrane BMP regulator 1 (*CRIM1*) and forkhead box J2 (*FOXJ2*) were the genes with the highest degree (Table V). It has been demonstrated that *FNDC3B* is a positive regulator of adipocyte differentiation, and that it suppresses the invasion and metastasis of melanoma cells (39). *FNDC3B* mutations were associated with rapid postnatal death and the inhibition of cellular proliferation, adhesion and migration (40). *FNDC3B* has been associated with the activation of several cancer pathways and tumor progression (41). *CRIM1* encodes the cysteine-rich motor neuron 1 protein (CRIM1), which has been characterized as a potential cancer biomarker (42). It has been reported that increased CRIM1 inhibits the proliferation and migration of vascular endothelial cells (43). Additionally, increased *CRIM1* expression has been reported in drug-resistant myeloid leukemia cells compared with drug-sensitive cells (44). *FOXJ2* serves an important role in the migration of glioma cells (45) and *FOXJ2* overexpression decreases the migration of breast cancer cells (46). Furthermore, abnormal expression of *FOXJ2* suppressed migration and invasion in extrahepatic cholangiocarcinoma, which was associated with an improved prognosis (47). *FNDC3B*, *CRIM1* and *FOXJ2* have been associated with tumor migration and prognosis. The findings of the present study suggest that they may also be associated with CRC metastasis.

In conclusion, the present study demonstrated that DEGs and predicted target genes of the DEMs are enriched

in pathways in cancer, the Wnt signaling pathway and ECM-receptor interaction, which may serve a critical role in the metastatic mechanism of CRC. Furthermore, *FNDC3B*, *CRIM1* and *FOXJ2* are proposed as potential biomarkers for metastatic CRC.

References

1. Siegel RL, Miller KD and Jemal A: Cancer statistics, 2016. CA Cancer J Clin 66: 7-30, 2016.
2. Shike M, Winawer SJ, Greenwald PH, Bloch A, Hill MJ and Swaroop SV: Primary prevention of colorectal cancer. The WHO collaborating centre for the prevention of colorectal cancer. Bull World Health Organ 68: 377-385, 1990.
3. Matsuda T, Ono A, Kakugawa Y, Matsumoto M and Saito Y: Impact of screening colonoscopy on outcomes in colorectal cancer. Jpn J Clin Oncol 45: 900-905, 2015.
4. Sabatino SA, Lawrence B, Elder R, Mercer SL, Wilson KM, DeVinney B, Melillo S, Carvalho M, Taplin S, Bastani R, *et al*: Effectiveness of interventions to increase screening for breast, cervical and colorectal cancers: Nine updated systematic reviews for the guide to community preventive services. Am J Prev Med 43: 97-118, 2012.
5. Board PDQATE: Colon Cancer Treatment (PDQ®): Health professional version. In: PDQ Cancer Information Summaries. National Cancer Institute, Bethesda, MD, 2002.
6. Cao H, Xu E, Liu H, Wan L and Lai M: Epithelial-mesenchymal transition in colorectal cancer metastasis: A system review. Pathol Res Pract 211: 557-569, 2015.
7. Hanahan D and Weinberg RA: Hallmarks of cancer: The next generation. Cell 144: 646-674, 2011.
8. Christofori G: New signals from the invasive front. Nature 441: 444-450, 2006.
9. Kanthan R, Senger JL and Kanthan SC: Molecular events in primary and metastatic colorectal carcinoma: A review. Patholog Res Int 2012: 597497, 2012.
10. Siegel R, DeSantis C, Virgo K, Stein K, Mariotto A, Smith T, Cooper D, Gansler T, Lerro C, Fedewa S, *et al*: Cancer treatment and survivorship statistics, 2012. CA Cancer J Clin 62: 220-241, 2012.
11. Siegel R, Naishadham D and Jemal A: Cancer statistics, 2012. CA Cancer J Clin 62: 10-29, 2012.
12. Grothey A and Schmoll HJ: New chemotherapy approaches in colorectal cancer. Curr Opin Oncol 13: 275-286, 2001.
13. Fidler IJ: The pathogenesis of cancer metastasis: The 'seed and soil' hypothesis revisited. Nat Rev Cancer 3: 453-458, 2003.
14. Cui YM, Jiao HL, Ye YP, Chen CM, Wang JX, Tang N, Li TT, Lin J, Qi L, Wu P, *et al*: FOXC2 promotes colorectal cancer metastasis by directly targeting MET. Oncogene 34: 4379-4390, 2015.
15. Yuan R, Ke J, Sun L, He Z, Zou Y, He X, Chen Y, Wu X, Cai Z, Wang L, *et al*: HES1 promotes metastasis and predicts poor survival in patients with colorectal cancer. Clin Exp Metastasis 32: 169-179, 2015.
16. Zhang Z, Liu X, Feng B, Liu N, Wu Q, Han Y, Nie Y, Wu K, Shi Y and Fan D: STIM1, a direct target of microRNA-185, promotes tumor metastasis and is associated with poor prognosis in colorectal cancer. Oncogene 34: 4808-4820, 2015.
17. Zhou ZH, Rao J, Yang J, Wu F, Tan J, Xu SL, Ding Y, Zhan N, Hu XG, Cui YH, *et al*: SEMA3F prevents metastasis of colorectal cancer by PI3K-AKT-dependent down-regulation of the ASCL2-CXCR4 axis. J Pathol 236: 467-478, 2015.
18. Fang Y, Liang X, Jiang W, Li J, Xu J and Cai X: Cyclin b1 suppresses colorectal cancer invasion and metastasis by regulating e-cadherin. PLoS One 10: e0126875, 2015.
19. Li X, Chen T, Shi Q, Li J, Cai S, Zhou P, Zhong Y and Yao L: Angiopoietin-like 4 enhances metastasis and inhibits apoptosis via inducing bone morphogenetic protein 7 in colorectal cancer cells. Biochem Biophys Res Commun 467: 128-134, 2015.
20. Song B, Wang W, Zheng Y, Yang J and Xu Z: P21-activated kinase 1 and 4 were associated with colorectal cancer metastasis and infiltration. J Surg Res 196: 130-135, 2015.
21. Purnak T, Ozaslan E and Efe C: Molecular basis of colorectal cancer. N Engl J Med 362: 1246-1247, 2010.
22. Provenzani A, Fronza R, Loreni F, Pascale A, Amadio M and Quattrone A: Global alterations in mRNA polysomal recruitment in a cell model of colorectal cancer progression to metastasis. Carcinogenesis 27: 1323-1333, 2006.
23. Drusco A, Nuovo GJ, Zanesi N, Di Leva G, Pichiorri F, Volinia S, Fernandez C, Antenucci A, Costinean S, Bottoni A, *et al*: MicroRNA profiles discriminate among colon cancer metastasis. PLoS One 9: e96670, 2014.
24. Irizarry RA, Hobbs B, Collin F, Beazer-Barclay YD, Antonellis KJ, Scherf U and Speed TP: Exploration, normalization, and summaries of high density oligonucleotide array probe level data. Biostatistics 4: 249-264, 2003.
25. Wiberg AO, Liu L, Tong Z, Myslivets E, Ataie V, Kuo BP, Alic N and Radic S: Photonic preprocessor for analog-to-digital-converter using a cavity-less pulse source. Opt Express 20: B419-B427, 2012.
26. Diboun I, Wernisch L, Orengo CA and Koltzenburg M: Microarray analysis after RNA amplification can detect pronounced differences in gene expression using limma. BMC Genomics 7: 252, 2006.
27. Dennis G Jr, Sherman BT, Hosack DA, Yang J, Gao W, Lane HC and Lempicki RA: DAVID: Database for annotation, visualization, and integrated discovery. Genome Biol 4: P3, 2003.
28. Dweep H, Gretz N and Sticht C: miRWalk database for miRNA-target interactions. Methods Mol Biol 1182: 289-305, 2014.
29. Shannon P, Markiel A, Ozier O, Baliga NS, Wang JT, Ramage D, Amin N, Schwikowski B and Ideker T: Cytoscape: A software environment for integrated models of biomolecular interaction networks. Genome Res 13: 2498-2504, 2003.
30. Yu G, Wang LG, Han Y and He QY: ClusterProfiler: An R package for comparing biological themes among gene clusters. OMICS 16: 284-287, 2012.
31. Heuberger J and Birchmeier W: Interplay of cadherin-mediated cell adhesion and canonical Wnt signaling. Cold Spring Harb Perspect Biol 2: a002915, 2010.
32. Tenbaum SP, Ordóñez-Morán P, Puig I, Chicote I, Arqués O, Landolfi S, Fernández Y, Herance JR, Gispert JD, Mendizabal L, *et al*: β-catenin confers resistance to PI3K and AKT inhibitors and subverts FOXO3a to promote metastasis in colon cancer. Nat Med 18: 892-901, 2012.
33. Qi J, Yu Y, Akilli Öztürk Ö, Holland JD, Besser D, Fritzmann J, Wulf-Goldenberg A, Eckert K, Fichtner I and Birchmeier W: New Wnt/β-catenin target genes promote experimental metastasis and migration of colorectal cancer cells through different signals. Gut 65: 1690-1701, 2016.
34. Hu TH, Yao Y, Yu S, Han LL, Wang WJ, Guo H, Tian T, Ruan ZP, Kang XM, Wang J, *et al*: SDF-1/CXCR4 promotes epithelial-mesenchymal transition and progression of colorectal cancer by activation of the Wnt/β-catenin signaling pathway. Cancer Lett 354: 417-426, 2014.
35. Ting WC, Chen LM, Pao JB, Yang YP, You BJ, Chang TY, Lan YH, Lee HZ and Bao BY: Common genetic variants in Wnt signaling pathway genes as potential prognostic biomarkers for colorectal cancer. PLoS One 8: e56196, 2013.
36. Mariman EC and Wang P: Adipocyte extracellular matrix composition, dynamics and role in obesity. Cell Mol Life Sci 67: 1277-1292, 2010.
37. Zhang HJ, Tao J, Sheng L, Hu X, Rong RM, Xu M and Zhu TY: Twist2 promotes kidney cancer cell proliferation and invasion by regulating ITGA6 and CD44 expression in the ECM-receptor interaction pathway. Onco Targets Ther 9: 1801-1812, 2016.
38. Ansieau S, Bastid J, Doreau A, Morel AP, Bouchet BP, Thomas C, Fauvet F, Puisieux I, Doglioni C, Piccinin S, *et al*: Induction of EMT by twist proteins as a collateral effect of tumor-promoting inactivation of premature senescence. Cancer Cell 14: 79-89, 2008.
39. Katoh D, Nishizuka M, Osada S and Imagawa M: Fad104, a positive regulator of adipocyte differentiation, suppresses invasion and metastasis of melanoma cells by inhibition of STAT3 activity. PLoS One 10: e0117197, 2015.
40. Nishizuka M, Kishimoto K, Kato A, Ikawa M, Okabe M, Sato R, Niida H, Nakanishi M, Osada S and Imagawa M: Disruption of the novel gene fad104 causes rapid postnatal death and attenuation of cell proliferation, adhesion, spreading and migration. Exp Cell Res 315: 809-819, 2009.
41. Cai C, Rajaram M, Zhou X, Liu Q, Marchica J, Li J and Powers RS: Activation of multiple cancer pathways and tumor maintenance function of the 3q amplified oncogene FNDC3B. Cell Cycle 11: 1773-1781, 2012.
42. Zeng H and Tang L: CRIM1, the antagonist of BMPs, is a potential risk factor of cancer. Curr Cancer Drug Targets 14: 652-658, 2014.
43. Nakashima Y, Morimoto M, Toda K, Shinya T, Sato K and Takahashi S: Inhibition of the proliferation and acceleration of migration of vascular endothelial cells by increased cysteine-rich motor neuron 1. Biochem Biophys Res Commun 462: 215-220, 2015.

44. Prenkert M, Uggla B, Tidefelt U and Strid H: CRIM1 is expressed at higher levels in drug-resistant than in drug-sensitive myeloid leukemia HL60 cells. Anticancer Res 30: 4157-4161, 2010.
45. Qiu X, Ji B, Yang L, Huang Q, Shi W, Ding Z, He X, Ban N, Fan S, Zhang J and Tian Y: The role of FoxJ2 in the migration of human glioma cells. Pathol Res Pract 211: 389-397, 2015.
46. Wang Y, Yang S, Ni Q, He S, Zhao Y, Yuan Q, Li C, Chen H, Zhang L, Zou L, *et al*: Overexpression of forkhead box J2 can decrease the migration of breast cancer cells. J Cell Biochem 113: 2729-2737, 2012.
47. Qiang Y, Wang F, Yan S, Zhang H, Zhu L, Chen Z, Tu F, Wang D, Wang G, Wang W and Chen Z: Abnormal expression of Forkhead Box J2 (FOXJ2) suppresses migration and invasion in extrahepatic cholangiocarcinoma and is associated with prognosis. Int J Oncol 46: 2449-2458, 2015.