Hindawi Publishing Corporation
ISRN Cell Biology
Volume 2014, Article ID 526075, 10 pages
http://dx.doi.org/10.1155/2014/526075

*Research Article*

# Identification of Unique miRNA Biomarkers in Colorectal Adenoma and Carcinoma Using Microarray: Evaluation of Their Putative Role in Disease Progression

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Received 9 June 2013; Accepted 7 July 2013; Published 22 April 2014

Academic Editors: D. Arnoult, A. Bruzzaniti, and C. Reynaud



MicroRNAs (miRNAs) are known to be dysregulated and play a key role in cancer progression. The present study aims to identify the miRNAs associated with colorectal adenoma and carcinoma to evaluate their role in tumor progression and metastasis using microarray. *In silico* analysis of miRNAs was performed on five different microarray data sets that represented the genes and miRNAs expressed in colorectal adenoma and carcinoma. We identified 10 different miRNAs that were common to both colorectal adenoma and carcinoma, namely, *miR9*, *miR96*, *miR135b*, *miR137*, *miR147*, *miR182*, *miR183*, *miR196b*, *miR224,* and *miR503*. Of these, *miR135b* and *miR147* were significantly downregulated in colorectal adenoma but upregulated in carcinoma. In addition, we studied the gene expression profile associated with colorectal adenocarcinoma and identified three genes, namely, *ZBED3*, *SLC10A3,* and *FOXQ1,* that were significantly downregulated in colorectal adenoma compared to carcinoma. Interestingly, of all the miRNAs and genes associated with colorectal adenocarcinoma, the myoglobin *(MB)* gene was identified to be under the direct influence of *miR135b,* showing an inverse relationship between them in adenoma and carcinoma. Most of the identified miRNAs and associated genes are involved in signaling pathways of cell proliferation, angiogenesis, and metastasis. The present study has identified putative miRNA targets and their associated gene networks which could be used as potential biomarkers of colon adenocarcinoma. Moreover, the association of *miR135b* and *MB* gene is very unique and can be considered as a lead candidate for novel cancer therapeutics.

## 1. Introduction

Colorectal cancer is one of the most common gastrointestinal cancers that shows a rising trend due to the dietary habits and lifestyle modifications. Accumulation of genetic and epigenetic aberrations predisposes the colonic epithelium to undergo gradual transformation with loss of cellular architecture and initiation of a benign adenoma, which subsequently develops into a malignant adenocarcinoma [1]. Successful screening methods have enabled early detection and hence reduction in the mortality rate associated with colorectal adenocarcinoma [2]. In addition to the oncogenes and the tumor suppressor genes involved in cancer initiation and progression, many other small molecules such as the oncoproteins, antisense RNAs, and microRNAs (miRNAs) are also implicated in cancer and its signaling mechanisms [3].

Among the small molecules involved in cancer progression and metastasis, the microRNAs (miRNAs) which are

Table 1: Overview of genome-wide transcriptomic and microRNA array data used for the study.

| Study | Platform | GEO accession | Species | Source | Description | Sample size |
|---|---|---|---|---|---|---|
| Galamb et al., 2010 [16] | Affymetrix HGU133 Plus 2.0 | GSE15960 | Homo Sapiens | mRNA | Colonic epithelial cells normal, adenoma, or colorectal cancer tissues | 18 |
| Sarver et al., 2009 [17] | Illumina Human v1 MicroRNA expression beadchip | GSE18392 | Homo Sapiens | miRNA | Macrodissected colon tumors and normal colon tissue | 145 |
| Arndt et al., 2009 [18] | Ambion Human_Mouse_Rat mirVANA miRNA Bioarray_1566V1 | GSE10259 | Homo Sapiens | miRNA | Colon cancer and normal tissues | 66 |
| Slattery et al., 2011 [19] | Agilent Human miRNA V3.0 Microarrays | NA | Homo Sapiens | miRNA | Colon and rectal tumors and normal tissue | 100 |
| Schetter et al., 2008 [20] | OSU-CCC MicroRNA Microarray Version 2.0 | GSE7828 | Homo Sapiens | miRNA | Colon tumor and adjacent nontumorous tissues | 168 |

The above evidence table depicts the authors of the study, high-throughput platform used, data repository accession number, species, source material, and the number of arrays used in each study.

21–25 nucleotides in length belong to the class of noncoding small RNA molecules that act as posttranslational regulators and are known to exert significant influence on most cancers [4–6]. Although the miRNAs constitute only 1–3% of the human genome, the fact that about 50% of the miRNA genes are located in the cancer associated loci indicates that these molecules are involved in either cancer progression or inhibition [5, 7]. Many different miRNAs such as *let-7, miR-24, miR-143,* and *miR-192* are involved in direct or indirect regulations of the KRAS or dihydrofolate signaling pathways of colorectal cancer cell proliferation [8–10]. Additionally, downregulations of *miR-143* and *miR-195* are reported to target the antiapoptotic Bcl-2 and hence induce apoptosis in colorectal cancer cell lines [11, 12]. As such the miRNAs are considered to hold great potential as diagnostic and prognostic markers of colorectal cancer [13].

Currently, there are about 2019 unique mature human miRNA sequences as revealed by the miRBase 19 (miRNA database of published miRNA sequences and annotation). Given the increasing numbers of miRNAs and the fact that they have diverse expression patterns and regulations ranging from developmental biology to cancer pathology [14], it becomes a practical difficulty to validate each miRNA in order to understand their biological targets. Though high-throughput technologies such as gene chip facilitate genome-wide expression analysis of genes either in normal or diseased states, this leads to generation of enormous data which again pose great difficulty in analysis. As such bioinformatics algorithms based analysis becomes inevitable to select lead candidates and targets which could be then taken up for further validation. Recently, Li et al., 2011 [15], have used partial least square regression analysis approach for predicting miRNA targets in human colon cancer and reported that they were able to detect more miRNA-mRNA targets than a simple correlation based association in colorectal cancer. However, comparisons between numerous studies on miRNA-mRNA associations are necessary for the validation of such technology based predictions before it can be considered as an effective diagnostic/prognostic indicator.

Given the scope of high-throughput technologies and the power of bioinformatics analysis, we have carried out an in-depth *in silico* analysis of miRNAs associated with colorectal adenoma and carcinoma. Using high-throughput gene expression as well as integrated network and pathway analyses strategies, we have critically dissected the differentially expressed miRNAs and associated gene networks in colorectal adenoma and carcinoma, respectively. We have also identified some of the predictive and therapeutic markers associated with adenoma and carcinoma and their potential to activate the adenoma-carcinoma sequel.

## 2. Methodology

Five different microarray data sets with genome-wide and miRNA expression associated with colorectal adenoma and carcinoma were used in this study. All data were obtained from recent studies on homosapiens, and the details are provided in Table 1.

## 3. Genome-Wide Transcriptomic and miRNA Analysis

Raw CEL files corresponding to gene expression data GSE15960 [16] were transferred to PARTEK Genomics Suite version 6.5 0 (Partek Inc., MO, USA) and normalized using GCRMA with quantile normalization to correct for variances in distribution patterns and GC nucleotide content. Principal component analysis (PCA) was performed on all probes to visualize high dimensional data. PCA was used to demonstrate quality control as well as overall variance in gene expression between the disease states. Analysis of

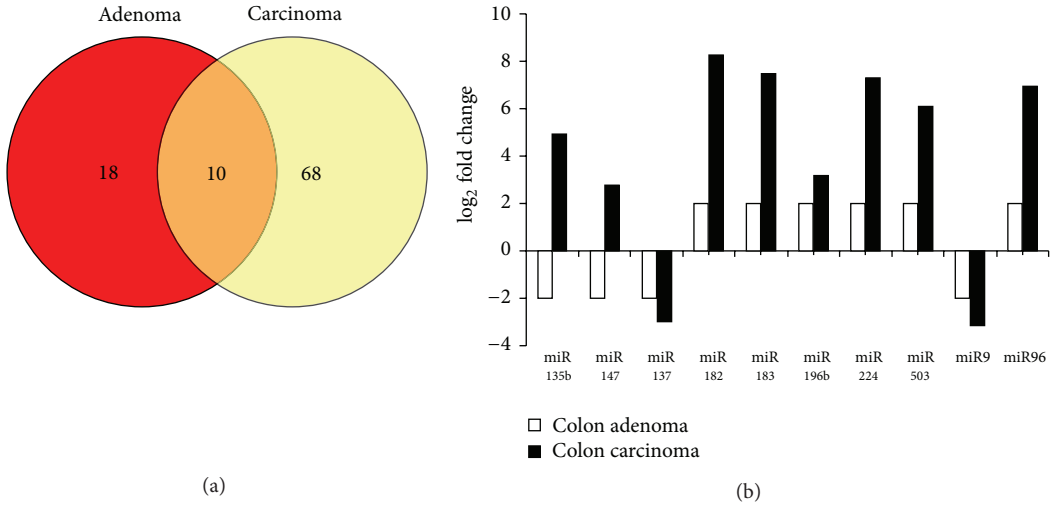



Figure 1: (a) The Venn diagram shows 10 unique miRNAs that were found to be commonly expressed in both adenoma and carcinoma of the colon. (b) Among these miRs, *miR-135b* and *miR-147* showed differential expression patterns. Conversely, the other miRNAs are either up in adenoma (*miR-182, miR-183, miR-196b, miR-224, miR-503,* and *miR-96*) or down in carcinoma (*miR-137* and miR-9).

variance (ANOVA) was applied on the whole data set, and differentially expressed gene list was then generated using an FDR (Benjamini Hochberg) of 0.05 with 2-fold change cutoff.

## 4. Functional and Pathway Analyses

To define biological networks among the differentially regulated genes in adenoma and carcinoma, pathway analyses were performed using Ingenuity Pathways Analysis software (IPA) (Ingenuity Systems, Redwood City, CA, USA). Differentially expressed mRNAs and miRNAs that were specific for colorectal adenoma and carcinoma was imported into IPA. The association analysis between miRNAs and gene signatures from the list of imported molecules were deduced using IPA knowledgebase. Expression values were plotted against the respective miRNAs and genes to identify the expression patterns, and only those miRNAs and genes that were differentially expressed between adenoma and carcinoma were considered for further IPA analysis. The functional analysis of IPA identified the biological functions and/or diseases that were most significantly altered for the differentially expressed gene set. The canonical pathway analysis identified the pathways that were most significantly induced in adenoma and carcinoma of the colon. The significance of the association between the data set and the canonical pathways was calculated by ratio and/or Fisher's exact test. The genes or gene products are represented as nodes, and the biological relationship between two nodes is represented by a line. Those molecules that are not linked were removed from the IPA analysis.

## 5. Results

The expression analysis of miRNAs associated with colorectal adenoma and carcinoma in the present study led to the identification of several *miRNAs* that were differentially expressed in colorectal adenoma (see Table S1A in Supplementary Material available online at http://dx.doi.org/10.1155/2014/526075) and carcinoma (Table S1B). These *miRNAs* consisted of both tumor suppressors and oncogenes. The following oncomirs, namely, *miR-21, miR-20a, miR-224,* and *miR-18a,* were upregulated in colorectal carcinoma, whereas the following tumor suppressors, namely, *miR-29a* and *miR-29c, miR-145, miR-35,* and *miR-26a,* were downregulated. In contrast, only one of the oncomirs, the oncogenic *miR*-18a, was upregulated, and there were no known tumor suppressors that were downregulated in colorectal adenoma.

Ten unique miRNAs were commonly expressed in both adenoma and carcinoma (Figure 1(a)). Amongst these, both *miR-135b* and *miR-147* showed differential expression patterns, being downregulated in colorectal adenoma and upregulated in carcinoma. The following *miRNAs,* namely, *miR-182, miR-183, miR-196b, miR-224, miR-503,* and *miR-96,* were upregulated while *miR-137* and *miR-9* were downregulated in both adenoma and carcinoma, respectively. However, both upregulation and downregulation of the individual miRNAs were relatively higher in colorectal carcinoma compared to adenoma (Figure 1(b)).

Among the genes regulated by these 10 commonly expressed *miRNAs*, the zinc-finger BED domain containing 3 (*ZBED3*), and solute carrier family 10 (sodium/bile acid cotransporter family), member 3 (*SLC10A3*) genes showed differential expression patterns, being downregulated in colorectal adenoma and upregulated in carcinoma (Figure 2(a)). In addition, *FOXQ1* gene expression was 10-fold higher in colorectal carcinoma compared to colorectal adenoma (Figure 2(b)).

Comparison of 2406 genes in adenoma and 1403 genes in carcinoma revealed unique predictive markers as well as the miRNAs and their regulatory targets associated with adenoma-carcinoma sequel (Figure 3(a)). Screening for the regulatory genes in both adenoma and carcinoma that

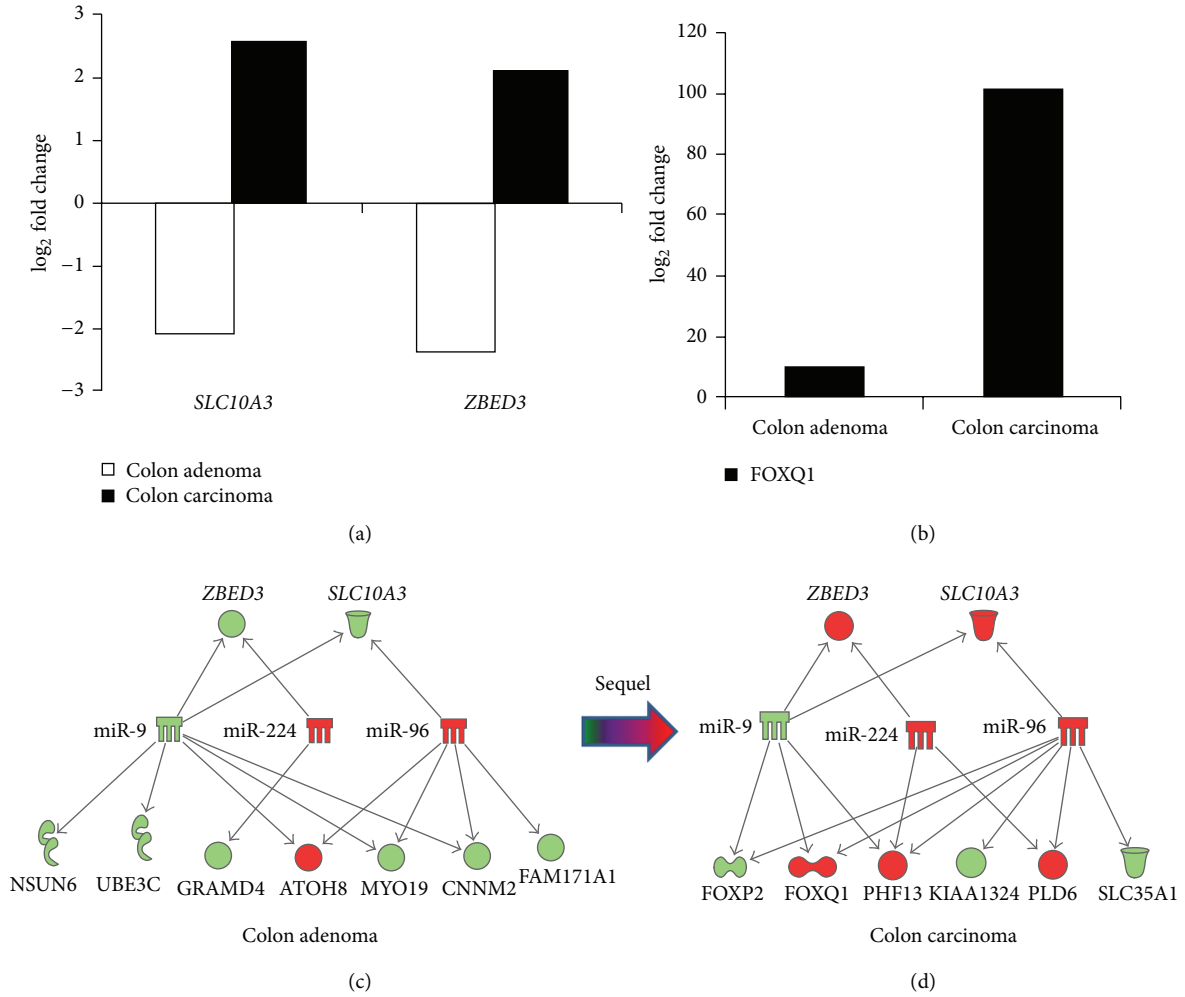
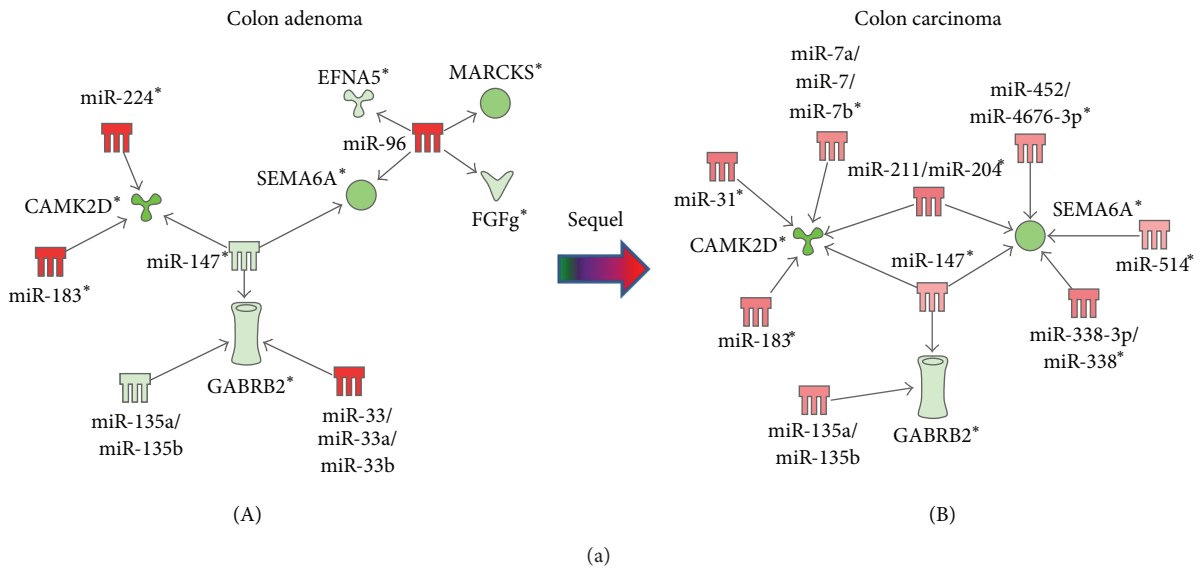



Figure 2: (a) Zinc-finger *BED* domain-containing 3 (*ZBED3*) and the solute carrier family 10 (sodium/bile acid cotransporter family) and me*MB*er 3 (*SLC10A3*) genes that were regulated by the 10 common miRNAs showed differential expression patterns between adenoma and carcinoma, respectively. (b) Similarly, *FOXQ1* gene expression was 10-fold higher in colon carcinoma when compared to the adenoma of the colon. (c) The upregulation of miR-224 and miR-96 in colon adenoma could cause the downregulation of both *ZBED3* and *SLC10A3*. (d) Though both miRNAs were upregulated in colon carcinoma, the expression of *ZBED3* and *SLC10A3* was not influenced by these miRNAs.

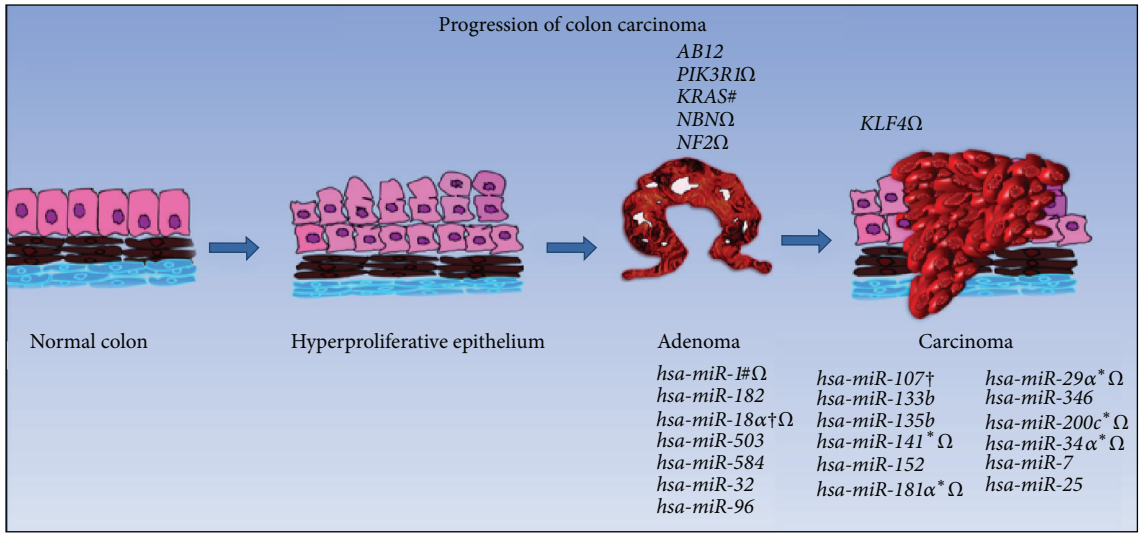
are controlled by the two differentially expressed miRNAs (*miR-135b* and *miR-147*) led to the identification of *MiR-33* being uniquely expressed in colon adenoma (Figure 3(A)). In addition, the *Myoglobin* (*MB*) gene was commonly expressed in both colorectal adenoma and carcinoma that also showed differential expression pattern being upregulated in colorectal adenoma and downregulated in carcinoma (Figure 4(a)). Furthermore, the various signalling mechanisms/interaction networks that are associated with the MB gene based on mRNA-miRNA integration analysis were identified (Figure 4(b)). These include the tumor suppressors (TP53), the angiogenic factors (VEGFA), cell proliferation, adhesion, and inflammatory molecules (EGF, VACM1, and IFN$\gamma$).

The network and pathway analyses led to the identification of the cellular and molecular functions associated with miRNAs in both adenoma and carcinoma. The identified miRNAs were primarily associated with various cellular functions including cell proliferation, cell motility, cell cycle, and cell death. The miRNAs that were specific to cellular proliferation were found to be more associated with colon carcinoma (Table S2A), while those miRNAs associated with cell morphology, cell signaling, and interaction were unique to colon adenoma (Table S2B). In general, most of the miRNAs identified in the current study were associated with diseases and disorders that have been previously reported (Table S2C).

The IPA network analysis identified genes such as insulin 1 (*Ins1*), *miR-375*, pyruvate dehydrogenase kinase-1(*PDK1*), and 3-phosphoinositide dependent protein kinase-1(*PDPK1*) were more represented in colon adenoma (Table S3A), whereas bruton agammaglobulinemia tyrosine kinase (*BTK*), CASP8 and FADD-like apoptosis regulator (*CFLAR*), EGF containing fibulin-like extracellular matrix protein 2 (*EFEMP2*), interleukin 18 (*IL18*), and *miR-346* were the more represented gene networks in colon carcinoma (Table S3B). As




(a)




(b)

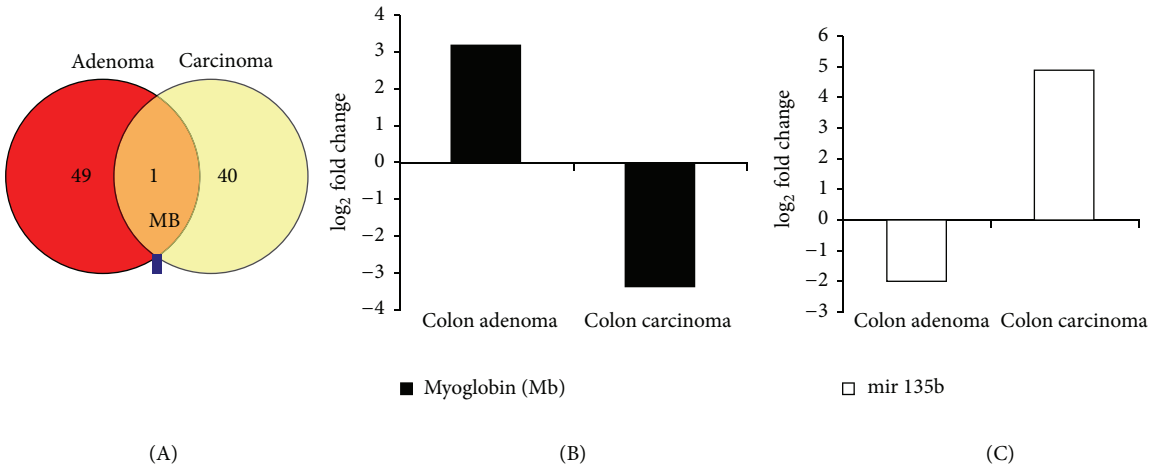
Figure 3: (a) We compared 2406 genes in adenoma and 1403 genes in carcinoma to find the regulatory genes controlled by miRNAs that potentially mediate the carcinogenesis in adenoma-carcinoma sequel. (A) miR-33 is unique to colon adenoma stage and is responsible for the suppression of gamma-aminobutyric acid receptor beta-subunit gene (*GABRB2*). (B) The other miRNA which was differentially expressed was miR-147. We identified the matching partners for *miR-147* by comparing the same gene sets used for miR-135b. Interestingly, both *miR-147* and miR-135a/*miR-135b* were not expressed in colon adenoma; however, they are both upregulated in colon carcinoma. (b) Graphical representation of adenoma-carcinoma sequel starting from normal colon and specific miRNAs and gene (oncogenes and tumor suppressors) associated with the sequel. $^{\Omega}$Tumor suppressor, $^{\#}$oncogene, $^{\dagger}$oncomirs, and $^{*}$mature miRNA sequence from the opposite arm of the precursor.

expected, the 5 gene networks identified in colon adenoma and the genes that interact with each network were primarily associated with cancer, gastrointestinal disease, carbohydrate metabolism, growth, and cellular proliferation.

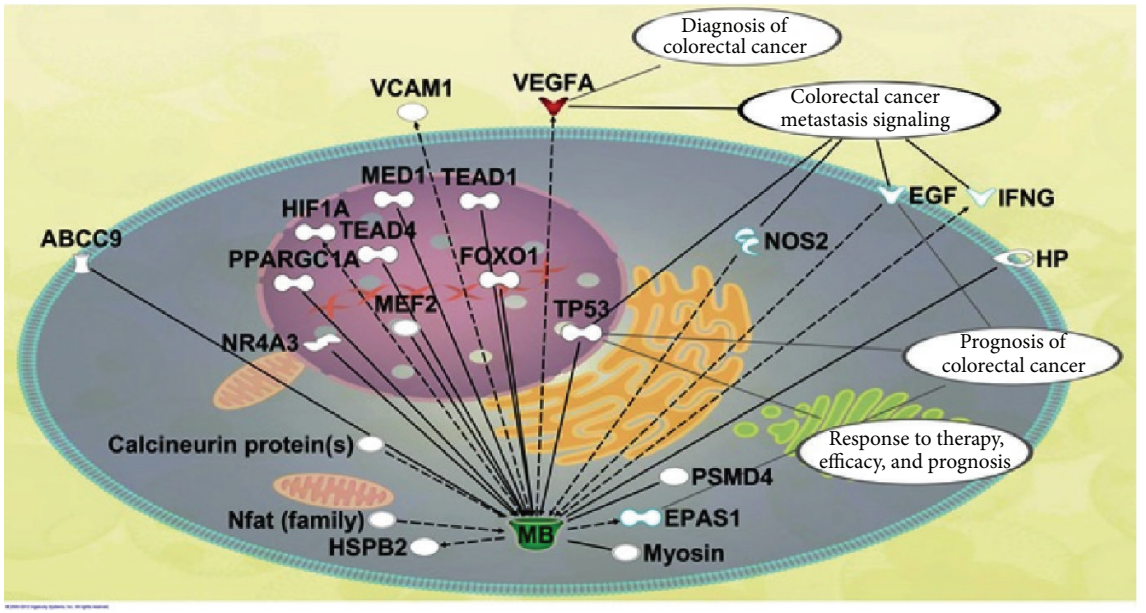
In addition, the *miRNA-mRNAs* association studies revealed distinct gene regulatory signatures in both adenoma and carcinoma. The following tumor suppressors genes, namely, phosphoinositide-3-kinase regulatory subunit 1-alpha (*PIK3R1*), nibrin (*NBN*), and neurofibromin 2 (*NF2*), were downregulated by miRNAs in adenomas (Table 2). Kruppel-like factor 4 (*KLF4*), tumor necrosis factor, alpha-induced protein 3 (*TNFAIP3*) and sterile alpha motif, and leucine zipper containing kinase AZK (*ZAK*) are some of the tumor suppressors that were downregulated in colon carcinoma. The list of miRNAs associated with these tumor suppressors in colon carcinoma is given in Table 3.

## 6. Discussion

*In silico* based high-throughput expression analyses are being widely used to decipher the changes at the gene, mRNA, and miRNA levels, and such analyses enable us to understand their molecular interactions and networks in both normal and diseased states. Change in the miRNA expression profile




(a)




(b)

Figure 4: (a)—(A) We identified *MB* gene as the differentially expressed target in colon adenoma and carcinoma that was under the control of miR-135b. (B) *MB* was upregulated in adenoma and downregulated in carcinoma, respectively. (C) The expression of *MB* was correlated with the inverse expression pattern of *miR-135b* in adenoma and carcinoma. (b) *Myoglobin* interaction network in colon carcinoma. The interaction partner of *Myoglobin* is represented along with the functional role they represent in colorectal cancer.

is one of the common features observed in the development of cancer [3, 21]. In the present study, genome-wide transcriptomic and miRNA analysis led to the identification of ten unique mRNAs that were commonly expressed in colorectal adenoma and carcinoma. In contrast to the other *miRNAs* that were expressed and mostly upregulated in both adenoma and carcinoma, two *miRNAs,* namely, *miR9* and *miR137,* were found to be downregulated in both colorectal adenoma and carcinoma. Decreased expression of *miR-137* which was primarily due to abnormal hypermethylation was identified to be one of the early events in colon carcinogenesis, and transfection of colon cancer cell lines with *miR-137* resulted in inhibition of cell proliferation [22]. Similarly, decreased expression of *miR-137* was also reported with oral cancer and glioblastoma [23] indicating that *miR-137* has tumor suppressor properties.

Two of the ten miRNAs, namely, *miRNA135b* and *miRNA147,* were differentially expressed, being downregulated in colorectal adenoma and upregulated in carcinoma. Our findings are in line with earlier studies, where *miR-147* was downregulated in colon adenoma [24] and *miR-135b* was upregulated in colon carcinoma [25]. Similarly, downregulation of *miR-135b* was found to be associated with the progression of oral carcinoma [23]. Moreover, *miR-135* is also reported to target the 39 untranslated regions of adenomatous polyposis coli (APC) gene, a key multifunctional tumor suppressor in sporadic and hereditary colon carcinoma, suppresses its expression [26, 27], and induces

Table 2: Differential expression of key miRNAs and their specific gene targets in colon adenoma.

| miRNAs | miRNA expression | miRNA regulated genes | Gene expression |
|---|---|---|---|
| hsa-miR-1$^{\#\Omega}$ | Down | ABI2 | Up |
| **hsa-miR-182** | Up | **PIK3R1**$^{\Omega}$ | Down |
| hsa-miR-18a$^{\dagger\Omega}$ | Up | KRAS$^{\#}$ | Down |
| **hsa-miR-503** | Up | **PIK3R1**$^{\Omega}$ | Down |
| **hsa-miR-584** | Up | **NBN**$^{\Omega}$ | Down |
| **hsa-miR-32** | Up | **NF2**$^{\Omega}$ | Down |
| **hsa-miR-96** | Up | **PIK3R1**$^{\Omega}$ | Down |

$^{\Omega}$Tumor suppressor, $^{\#}$oncogene, $^{\dagger}$oncomirs, and $^{*}$mature miRNA sequence from the opposite arm of the precursor.

downstream Wnt signaling [25]. Moreover, *miR-135b* has been identified as a novel biomarker for pancreatic ductal adenocarcinoma by global microRNA expression profiling of microdissected tissues [28].

Our study showed that upregulation of *miR-147* and *miR-135b* in colon carcinoma was involved in the specific downregulation of gamma-aminobutyric acid receptor beta-subunit gene (*GABRB2*). MiR-33 is unique to colon adenoma stage and is responsible for the suppression of *GABRB2* gene. *GABRB2* which is well known to be involved in transport processes of chloride channel has been previously reported as a colorectal cancer subtype classificatory [29]. *GABRB2* was also identified as a discriminatory transcript involved in the CRC-Benign versus CRC-Crohn's disease [29]. The miR-135b, present in the chromosome position 1q32.1 [24], could also play a role in the downregulation of *GABRB2* gene.

*ZBED3* gene is a novel axin-binding protein that was shown to be involved in Wnt/beta-catenin signaling modulation [30]. Stage specific expression of both *ZBED3* and *SLC10A3* was observed in our current study and has the potential to be used as a specific biomarker to differentiate adenoma and carcinoma of the colon [31, 32]. Forkhead box Q1 (FOXQ1) transcription factor has recently been reported to play an important role in the promotion of cancer through the upregulation of several genes that promote tumor growth through angiogenesis, antiapoptotic effects [33], and epithelial-mesenchymal transition (EMT) [34]. One of the reasons for the downregulation of *ZBED3* and *SLC10A3* could be due to the upregulation of *miR-224* and *miR-96* in adenoma; however, this needs experimental validation (Figure 2(a)). Probably the mechanism of upregulation of these two genes could either be a mutation or polymorphism in the mRNA coding region that could inhibit the binding of the miRNA to these genes [35]. In the presence of *miR-33, miR-135a/miR-135b* becomes inactive. MiRNA-miRNA synergistic network is least investigated though it has been postulated to be a key factor associated with complex diseases [36]. Our results indicate a potential involvement of both positive and negative synergetic roles for miRNAs in the progression sequel associated with colon adenoma to carcinoma.

We identified *MB* gene as the differentially expressed target in colon adenoma and carcinoma under the control of miR-135b. The gene was upregulated in adenoma and downregulated in carcinoma. The expression was correlated with the inverse expression pattern of *miR-135b* in adenoma and carcinoma. Although *MB* is expressed chiefly in cardiomyocytes and oxidative skeletal muscle fibres, recent studies identified low level of *MB* being expressed in various nonmuscle tissues [37]. Interestingly, *MB* gene has been widely implicated in epithelial cancers and given renewed importance in solid tumors [38, 39]. It was shown *in vitro* that *MB* was expressed in hypoxic and oxidative stress conditions associated with epithelial tumors [26]. *MB* is important in both oxygen transport and free radical scavenging, and its expression in human tumor cells promotes differentiation and inhibits metastasis [27]. Solid epithelial tumors such as colon carcinoma could take advantage of proteins such as *MB* to cope with hypoxic conditions and to control the metabolism of reactive oxygen and nitrogen species. Furthermore, our study showed for the first time, based on mRNA-miRNA integration analysis, the enhanced expression of *MB* in adenoma and drastic downregulation in carcinoma of the colon.

The downregulation of Kruppel-like family of transcription factors (*KLF4*) in colon cancer, and not in adenoma, was associated with the specific upregulation of known oncomirs such as *hsa-miR-107* as well as novel oncomirs identified in our study such as *hsa-miR-133b, hsa-miR135b, hsa-miR152, hsa-miR-7,* and *hsa-miR-25*. *KLF4* functions as a tumor suppressor in several tissues, including the colon, and its specific knockdown induces epithelial-mesenchymal transition which predisposes to the development of a subset of colorectal cancers involving Wnt/beta-catenin signalling mechanisms. [40]. *KLF4* also directly inhibits the expression of Bmi1 in colon cancer cells [41].

Phosphatidylinositol 3′-kinase p85alpha regulatory subunit 1 gene (*PIK3R1*) identified in the current study is also a tumor suppressor, which could potentially be downregulated through miRNA targets and requires validation. Studies on human tumor samples showed increased expression of a coordinately regulated module consisting of *PIK3R1* in advanced malignancy [42]. The PI3KR1 gene was also found to be an oncogene in human ovarian and colon tumors [43]. *PIK3R1* gene was also one of the genes upregulated by leptin, and this could be one of the causative factors in changing the response of colon epithelial cells possessing an *APC* mutation but not normal cells. Furthermore, the genes regulating the Wnt/beta-catenin-mediated pathway including *PIK3R1* were upregulated by leptin [44], which was consistent with the progression of colon carcinogenesis.

In addition, *Nibrin* (*NBN*) was found to be under the regulation of miRNAs and is associated with immortalization of colon carcinoma [45]. NBN was one of the nine genes showing altered expression in both low and high clinical stage colon carcinoma [45]. On the other hand, Neurofibromatosis 2 gene (NF2) is a candidate tumor suppressor gene in chromosome 22.q locus [46], and the colon cancers commonly have allelic losses of chromosome 22q. Consequently, NF2

Table 3: Differential expression of key miRNAs and their specific gene targets in colon carcinoma.

| miRNAs | miRNA expression | miRNA regulated genes | Gene expression |
|---|---|---|---|
| hsa-let-7i$^{*\Omega}$ | Up | AKAP5, EPHA4, KLF9, and PPP1R12B | Down |
| **hsa-miR-107**$^{\dagger}$ | Up | **KLF4**$^{\Omega}$ | Down |
| **hsa-miR-133b** | Up | **KLF4**$^{\Omega}$ | Down |
| **hsa-miR-135b** | Up | **KLF4**$^{\Omega}$ | Down |
| hsa-miR-141$^{*\Omega}$ | Up | FOXA1, NR3C1, and PRKACB | Down |
| **hsa-miR-152** | Up | **KLF4**$^{\Omega}$ | Down |
| hsa-miR-181a$^{*\Omega}$ | Up | AKAP5, KITLG, MARCKS, NR3C1, PLCL2, and PPP1R12B | Down |
| **hsa-miR-29a**$^{*\Omega}$ | Up | AKAP5, **KLF4**$^{\Omega}$ | Down |
| **hsa-miR-346** | Up | **KLF4**$^{\Omega}$ | Down |
| **hsa-miR-200c**$^{*\Omega}$ | Up | **KLF4**$^{\Omega}$, KLF9, LPAR1, MARCKS, NR3C1, PPP1R12B, and PRKACB | Down |
| hsa-miR-34a$^{*\Omega}$ | Up | IL6R, KITLG | Down |
| **hsa-miR-7** | Up | **KLF4**$^{\Omega}$ | Down |
| **hsa-miR-25** | Up | **KLF4**$^{\Omega}$ | Down |
| hsa-miR-125a$^{*\Omega}$ | Down | TNFAIP3$^{\Omega}$ | Up |
| hsa-miR-137 | Down | ZAK$^{\Omega}$ | Up |
| hsa-miR-143$^{*\Omega}$ | Down | ITGA6 | Up |
| hsa-miR-145$^{*\Omega}$ | Down | ABCC1, ATRX, ETS2, INHBB, MYC$^{\#}$, and SCARB1 | Up |
| hsa-miR-195$^{*\Omega}$ | Down | AXIN2, CXCL10, GABRE, IRAK2, KSR1, PSME3, SERPINE2, TGFBR3, UBE2S, VEGFA, ZAK$^{\Omega}$, and ZYX | Up |
| hsa-miR-26a$^{*\Omega}$ | Down | CDK8, COL1A2, INHBB, PMAIP1, PPP1R3D, and STK4 | Up |
| hsa-miR-34b$^{*\Omega}$ | Down | ZAK$^{\Omega}$ | Up |
| hsa-miR-9$^{\Omega}$ | Down | DNAJB1, FOXO3$^{\#}$, INHBB, ITGA6, KSR1, NCOA3, NEDD4, and PPAT | Up |

$^{\Omega}$Tumour suppressor, $^{\#}$oncogene, $^{\dagger}$oncomirs, and $^{*}$mature miRNA sequence from the opposite arm of the precursor.

gene was found to be the target of potential miRNAs in colon carcinoma in our study.

## 7. Conclusions and Future Directions

In the present study, we have exploited the high-throughput expression analyses strategies to critically delineate the mRNA as well as miRNA expression profiles of adenoma and carcinoma of the colon. Besides, we have identified novel miRNA regulatory networks that regulate the transcription of mRNAs required for the adenoma and carcinoma sequel. It was significant to note that none of the miRNAs and their gene targets identified either in adenoma or carcinoma had overlapped in their expression patterns. This shows that specific miRNAs are expressed in a stage specific manner and regulate their candidate genes in colon adenoma and carcinoma, respectively. These transcriptional networks that regulate genes involved in the molecular functions associated with the differentiation of adenoma into carcinoma provide the predictive markers of colon adenoma-carcinoma sequel and together with RNAa/RNAi strategies to increase the expression of the tumor suppressor genes as well as silencing oncogenes would add tremendously to early detection and management of colon carcinoma.

## Conflict of Interests

The authors of the paper, do not have a direct financial relation with the commercial identities mentioned in the paper that might lead to a conflict of interests.

## Authors' Contribution

Kothandaraman Narasimhan and Jayapal Manikandan conceived the study, participated in its design and coordination, and helped to draft the paper. Kalamegam Gauthaman, Peter Natesan Pushparaj, Govindasamy Meenakumari, Adeel Gulzar Ahmed Chaudhary, Adel Abuzenadah, Mamdooh Abdullah Gari, and Mohammed Al Qahtani participated in the design of the study and performed the statistical analysis as well as helping to draft the paper.

## Acknowledgments

The authors wish to acknowledge the Centre for Excellence in Genomic Medicine Research (CEGMR), King Abdul Aziz University, for providing funds for the project (grant title: Identification of circulating nucleic acids as markers for colorectal cancer) as well as for providing space and computing infrastructure to carry out the work. This project

was supported by the NSTIP strategic technologies program in the Kingdom of Saudi Arabia, Award no. 11-MED1550-03, and the authors, therefore, acknowledge with thanks STU, KAU, for technical financial support.

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