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RESEARCH ARTICLE

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The PI3K/AKT signaling pathway: Associations of miRNAs with dysregulated gene expression in colorectal cancer

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The PI3K/AKT-signaling pathway is one of the most frequently activated signal-transduction pathways in cancer. We examined how dysregulated gene expression is associated with miRNA expression in this pathway in colorectal cancer (CRC). We used data from 217 CRC cases to evaluate differential pathway gene expression between paired carcinoma and normal mucosa and identify miRNAs that are associated with these genes. Gene expression data from RNA-Seq and miRNA expression data from Agilent Human miRNA Microarray V19.0 were analyzed. We focused on genes most associated with CRC (fold change (FC) of >1.5 or <0.67) that were statistically significant after adjustment for multiple comparisons. Of the 304 genes evaluated, 76 had a FC of <0.67, and 57 had a FC of >1.50; 47 of these genes were associated with miRNA differential expression. There were 145 mRNA:miRNA seed-region matches of which 26 were inversely associated suggesting a greater likelihood of a direct association. Most miRNA:mRNA associations were with factors that stimulated the pathway. For instance, both *IL6R* and *PDGFRA* had inverse seed-region matches with seven miRNAs, suggesting that these miRNAs have a direct effect on these genes and may be key elements in activation of the pathway. Other miRNA:mRNA associations with similar impact on the pathway were miR-203a with ITGA4, miR-6071 with *ITGAV*, and miR-375 with THBS2, all genes involved in extracellular matrix function that activate PI3Ks. Gene expression in the PI3K/Akt-signaling pathway is dysregulated in CRC. MiRNAs were associated with many of these dysregulated genes either directly or in an indirect manner.

KEYWORDS
colorectal cancer, miRNA, mRNA, PI3K/AKT signaling

1 | INTRODUCTION

Phosphoinositide 3-kinases (PI3K) are a family of enzymes, first discovered in 1985 that have been classified into three groups.[1] Class I PI3Ks are the best characterized and have been implicated in cancer given their role in various cellular processes including cell differentiation, metabolism, inflammation, cell motility, and cancer progression.[2] The PI3Ks can be activated by an array of stimuli, including growth factors, cytokines, and hormones through various membrane receptors such as receptor tyrosine kinases (RTKs) that include

EGFR (epidermal growth factor receptor) and *PDGFR* (platelet-derived growth factor receptor).[3] Downstream of PI3K, *AKT* (alias protein kinase B or PKB) is activated by PI3K. *AKT*, initially identified as a component of insulin intracellular signaling, is involved in the regulation of key factors related to cell growth, survival, and proliferation, including activation of mammalian target of rapamycin complex 1 (mTORC made up of *mTOR*, Raptor *mLST8*, and *PRAS40*)[4]; ribosomal S6 kinases; pro-apoptotic FOXO proteins; IKK which induces NFκB transcriptional activity; *CREB* (cAMP response element-binding protein) a cellular transcription factor that influences cell survival; and *BAD* (Bcl-2-associated death promoter) involved in the initiation of apoptosis.[2,5,6] *PTEN* (phosphatase and tensin homolog), a tumor suppressor gene, can inactivate this pathway and has been associated with multiple cancers and alterations in cell cycle regulation and apoptosis.[7] We have shown that genetic variation in *PIK3CA* and *AKT1* is associated with a strong increased risk of colon cancer.[8] Akt has been shown to be a key regulator in cell survival and proliferation.[9] *PIK3CA*, the human gene encoding PI3K, also was importantly related to survival after diagnosis with colon cancer, suggesting that genetic variation in the PI3K/Akt pathway influences survival.[10]

The PI3K/AKT signaling-pathway is recognized as being one of the most frequently activated signal transduction pathways in cancer.[2] MiRNAs can directly influence gene expression by binding to the 3'un-translated region (UTR) of the target mRNA and causing mRNA transcript degradation or repression of the target gene translation.[11,12] There is evidence that through these mechanisms miRNAs may influence genes that are central to the PI3K/Akt-signaling pathway. Among the miRNAs reported to influence this pathway are miR-133a, let-7, miR-21, miR-205, miR-200, miR-130b, miR-155, and miR-106b.[13,14]

In this paper we ascertained 342 genes in the PI3K/Akt-signaling pathway using Kyoto Encyclopedia of Genes and Genomes (KEGG) and evaluated 304 for which we have adequate expression data in our CRC samples. We identified genes in this pathway that are dysregulated in that the colorectal tumor mRNA expression levels are significantly different than mRNA expression in normal colorectal mucosa. For those genes that were dysregulated, we further evaluated their associations with expression levels of miRNAs. We hypothesized that genes in the PI3K/Akt-signaling pathway are dysregulated in in colorectal cancer (CRC) and that miRNAs are linked to this dysregulation.

2 | METHODS

2.1 | Study participants

Study participants come from two population-based case-control studies that included all incident colon and rectal cancer between 30 and 79 years of age in Utah or were members of Kaiser Permanente in Northern California (KPNC). Participants were non-Hispanic white, Hispanic, or black for the colon cancer study; the rectal cancer study also included people of Asian race.[15,16] Case diagnosis was verified by tumor registry data as a first primary adenocarcinoma of the colon or rectum and were diagnosed between October 1991 and September 1994 (colon study) and between May 1997 and May 2001 (rectal study).[17] The Institutional Review Boards at the University of Utah and at KPNC approved the study.

2.2 | RNA processing

Formalin-fixed paraffin embedded tissue from the initial biopsy or surgery was used to extract RNA. RNA was extracted, isolated, and purified from carcinoma tissue and adjacent normal mucosa as previously described.[18] We observed no differences in RNA quality based on age of the tissue. The same RNA preparation was used to generate both the mRNA and miRNA data.

2.3 | mRNA: RNA-Seq sequencing library preparation and data processing

Total RNA from 245 colorectal carcinoma and normal mucosa pairs was chosen for sequencing based on availability of RNA and high quality miRNA data; 217 pairs passed quality control (QC) and were used in these analyses. RNA library construction was done with the Illumina TruSeq Stranded Total RNA Sample Preparation Kit with Ribo-Zero. The samples were then fragmented and primed for cDNA synthesis, adapters were then ligated onto the cDNA, and the resulting samples were then amplified using PCR; the amplified library was then purified using Agencount AMPure XP beads. A more detailed description of the methods can be found in our previous work.[19] Illumina TruSeq v3 single read flow cell and a 50 cycle single-read sequence run was performed on an Illumina HiSeq instrument. Reads were aligned to a sequence database containing the human genome (build GRCh37/hg19, February 2009 from genome.ucsc.edu) and alignment was performed using novoalign v2.08.01. Total gene counts were calculated for each exon and UTR of the genes using a list of gene coordinates obtained from http://genome.ucsc.edu. We disregarded genes that were not expressed in our RNA-Seq data or for which the expression was missing for the majority of samples.[19]

2.4 | miRNA

The Agilent Human miRNA Microarray V19.0 was used. Data were required to pass stringent QC parameters established by Agilent that included tests for excessive background fluorescence, excessive variation among probe sequence replicates on the array, and measures of the total gene signal on the array to assess low signal. Samples failing to meet quality standards were re-labeled, hybridized to arrays, and re-scanned. If a sample failed QC assessment a second time, the sample was excluded from analysis. The repeatability associated with this microarray was extremely high ($r = 0.98$)[17]; comparison of miRNA expression levels obtained from the Agilent microarray to those obtained from qPCR had an agreement of 100% in terms of directionality of findings and the FCs were almost identical.[20] To normalize differences in miRNA expression that could be attributed to the array, amount of RNA, location on array, or factors that could erroneously influence miRNA expression levels, total gene signal was

normalized by multiplying each sample by a scaling factor which was the median of the 75th percentiles of all the samples divided by the individual 75th percentile of each sample.[21]

2.5 | *PI3K/Akt*-signaling genes

The Kyoto Encyclopedia of Genes and Genomes (KEGG) (www.genome.jp/kegg-bin/show_pathway?hsa04151) pathway map for PI3K-AKT-signaling was used to identify genes associated with this pathway. Using this map, we identified 342 genes, 304 of which had sufficient expression in CRC tissue for inclusion in the study (Supplemental Table S1).

2.6 | Statistical methods

We utilized a negative binomial mixed effects model in SAS (accounting for carcinoma/normal status as well as for subject effect) to determine genes in the PI3K/Akt-signaling pathway that had a significant difference in expression between individually paired colorectal carcinoma and normal mucosa (ie, differentially expressed) and their related fold changes (FC). In this test we offset the overall exposure as the log expression of all identified protein-coding genes (n = 17,461). The Benjamini and Hochberg[22] procedure was used to control the false discovery rate (FDR) using a value of 0.05 or less. A FC greater than one indicates a positive differential expression (ie, up-regulated in carcinoma), while a FC between zero and one indicates a negative differential expression (ie, down-regulated in carcinoma). We calculated level of expression of each gene by dividing the total expression for that gene in an individual by the total expression of all protein-coding genes per million transcripts (RPMPCG or reads per million protein-coding genes). We focused on those genes with FC of >1.50 or <0.67 for analysis with differential miRNA expression as these levels of FC may have a greater biological significance than smaller FCs. There were 814 miRNAs expressed in greater than 20% of normal colorectal mucosa that were analyzed; differential expression was calculated using subject-level paired data as the expression in the carcinoma tissue minus the expression in the normal mucosa. In these analyses, we fit a least squares linear regression model to the RPMPCG differential expression levels and miRNA differential expression levels. P-values were generated using the bootstrap method by creating a distribution of 10 000 F statistics derived by resampling the residuals from the null hypothesis model of no association between gene expression and miRNA expression using the boot package in R. Linear models were adjusted for age and sex. Multiplicity adjustments for gene/miRNA associations were made at the gene level using the FDR by Benjamini and Hochberg.[22]

2.7 | Bioinformatics analysis

We analyzed miRNAs and targeted mRNAs for seed region matches. The mRNA 3′ UTR FASTA as well as the seed region sequence of the associated miRNA were analyzed to determine seed region pairings between miRNA and mRNA. MiRNA seed regions were calculated as described in our previous work[23]; we calculated and included seeds of six, seven, and eight nucleotides in length. A seed match between the miRNA and the mRNA would increase the likelihood that identified genes associated with a specific miRNA were more likely to have a direct association, especially if there was an inverse association, given a higher propensity for binding and thus mRNA degradation. As miRTarBase[24] uses findings from many different investigations spanning across years and alignments, we used FASTA sequences generated from both GRCh37 and GRCh38 Homo sapiens alignments, using UCSC Table Browser (https://genome.ucsc.edu/cgi-bin/hgTables).[25] We downloaded FASTA sequences that matched our Ensembl IDs and had a consensus coding sequences (CCDS) available. Analysis was done using scripts in R 3.2.3 and in perl 5.018002.

3 | RESULTS

Of the 217 study participants, 169 had been diagnosed with colon cancer, 118 were male, and 161 were non-Hispanic white (Table 1). The mean age of the study population was 64.8 years. The majority of

TABLE 1 Description of Study Population

	N	%
Site		
Colon	169	77.9
Rectal	48	22.1
Sex		
Male	118	54.4
Female	99	45.6
Age		
Mean (SD)	64.8	10.1
Race		
Non-Hispanic White	161	74.2
Hispanic	14	6.5
Non-Hispanic Black	8	3.7
Unknown	34	15.7
AJCC stage		
1	58	27.1
2	61	28.5
3	72	33.6
4	23	10.8
Tumor phenotype		
TP53 mutated	103	47.5
KRAS mutated	69	31.8
BRAF mutated	21	10.1
CIMP high	45	20.7
MSI	29	13.4
Vital status		
Dead	92	42.6
Alive	124	57.4

TABLE 2 Statistically significant differentially expressed PI3K/AKT pathway genes whose fold change is <0.67 or >1.5

Gene	Tumor mean	Normal mean	Fold change	*P*-value	Adjusted *P*-value
TCL1A	0.19	3.21	0.06	1.46E-18	5.40E-18
PCK1	42.44	305.32	0.14	1.11E-46	1.98E-45
GNG7	3.66	22.76	0.16	2.58E-49	5.59E-48
TNXB	23.48	134.34	0.17	1.24E-63	1.26E-61
FGF9	1.66	9.08	0.18	4.27E-30	3.17E-29
COL4A6	3.80	16.61	0.23	1.94E-37	2.10E-36
COL6A5	0.97	3.97	0.24	1.34E-13	3.85E-13
CREB3L3	0.69	2.76	0.25	1.88E-10	4.22E-10
IL6R	25.93	101.24	0.26	2.77E-58	1.40E-56
ITGA8	9.84	37.30	0.26	2.42E-46	4.09E-45
COL4A5	10.70	40.45	0.26	4.50E-25	2.21E-24
NGFR	2.10	7.67	0.27	9.76E-26	5.30E-25
LPAR1	12.91	46.29	0.28	6.42E-52	1.63E-50
COL4A4	13.58	47.42	0.29	6.88E-33	5.98E-32
PDGFD	5.90	19.58	0.30	8.17E-28	5.10E-27
TNR	0.53	1.75	0.30	4.45E-11	1.06E-10
FIGF	0.36	1.14	0.31	4.89E-08	9.23E-08
CHRM1	1.10	3.43	0.32	4.01E-12	1.03E-11
GHR	6.68	20.21	0.33	3.79E-30	2.88E-29
PIK3R6	3.89	11.43	0.34	1.35E-26	8.02E-26
G6PC	0.25	0.74	0.34	8.92E-04	1.27E-03
COL2A1	1.47	4.17	0.35	6.37E-11	1.49E-10
G6PC2	0.33	0.92	0.36	1.63E-04	2.52E-04
IGF1	13.85	38.80	0.36	5.43E-28	3.59E-27
RELN	5.68	15.85	0.36	8.90E-18	3.15E-17
SGK1	50.97	137.98	0.37	4.36E-38	5.10E-37
EIF4E1B	0.16	0.42	0.38	7.63E-03	9.81E-03
FGFR2	26.51	68.82	0.39	2.32E-21	9.93E-21
COL4A3	12.26	31.40	0.39	1.96E-23	8.77E-23
BCL2	24.42	62.11	0.39	3.73E-39	4.72E-38
GYS2	0.23	0.58	0.40	5.32E-04	7.82E-04
PIK3CG	13.99	35.04	0.40	4.52E-28	3.06E-27
CHAD	8.65	21.44	0.40	2.76E-25	1.42E-24
PRKAA2	8.33	20.32	0.41	1.41E-08	2.84E-08
EFNA5	10.41	25.33	0.41	2.50E-15	7.92E-15
FGF10	1.70	4.06	0.42	1.85E-10	4.21E-10
KIT	14.58	33.84	0.43	6.08E-17	2.10E-16
LAMC3	4.06	9.43	0.43	2.41E-14	7.17E-14
IL7R	35.03	79.98	0.44	8.00E-29	5.65E-28
EGF	2.83	6.34	0.45	1.71E-05	2.87E-05
PPP2R3A	28.10	62.55	0.45	3.67E-37	3.84E-36
PPP2R2B	1.72	3.78	0.46	9.70E-10	2.12E-09
GNG2	13.93	29.33	0.47	4.15E-24	1.91E-23
GNGT2	0.71	1.46	0.49	1.10E-05	1.87E-05
CREB3L1	96.13	195.06	0.49	1.51E-16	5.15E-16

(Continues)

TABLE 2 (Continued)

Gene	Tumor mean	Normal mean	Fold change	*P*-value	Adjusted *P*-value
CD19	3.78	7.59	0.50	2.65E-08	5.17E-08
ITGA4	36.27	71.10	0.51	1.71E-25	8.99E-25
FGFR3	36.15	70.78	0.51	1.13E-18	4.28E-18
ITGB7	11.13	21.51	0.52	6.05E-18	2.17E-17
GNGT1	0.48	0.91	0.53	2.33E-03	3.15E-03
PDGFRA	88.47	166.61	0.53	5.08E-26	2.81E-25
COL9A2	26.53	49.91	0.53	4.06E-12	1.03E-11
LAMB4	0.79	1.47	0.54	2.64E-04	4.05E-04
CHRM2	2.32	4.20	0.55	1.89E-03	2.61E-03
PHLPP2	79.32	139.85	0.57	1.77E-28	1.22E-27
FGF2	10.04	17.66	0.57	2.79E-13	7.86E-13
TEK	8.92	15.47	0.58	5.93E-12	1.49E-11
LPAR4	0.40	0.68	0.58	2.33E-02	2.87E-02
FGF13	2.42	4.18	0.58	2.84E-05	4.69E-05
PIK3CD	27.33	46.48	0.59	9.96E-22	4.33E-21
CSF1	28.49	47.90	0.59	3.95E-21	1.67E-20
FGF7	8.17	13.70	0.60	1.49E-08	2.97E-08
ITGA7	21.33	35.69	0.60	1.74E-16	5.89E-16
LPAR3	1.36	2.26	0.60	1.41E-03	1.98E-03
IL3RA	2.21	3.59	0.62	2.82E-03	3.80E-03
ITGA2B	0.54	0.86	0.62	9.90E-03	1.26E-02
CSF3	1.03	1.66	0.62	8.63E-03	1.10E-02
EPOR	6.81	10.87	0.63	2.99E-08	5.72E-08
NR4A1	201.17	319.66	0.63	5.51E-11	1.31E-10
EFNA2	5.04	8.00	0.63	1.35E-06	2.42E-06
FASLG	1.07	1.70	0.63	4.96E-03	6.48E-03
BCL2L11	56.19	88.47	0.64	1.23E-23	5.58E-23
LPAR5	25.37	39.84	0.64	5.44E-20	2.15E-19
SGK2	42.18	65.06	0.65	1.12E-09	2.42E-09
PHLPP1	42.56	65.01	0.65	1.03E-20	4.16E-20
THBS1	585.51	881.12	0.66	3.69E-18	1.34E-17
NOS3	23.67	15.66	1.51	1.27E-09	2.72E-09
PDGFC	28.55	18.83	1.52	1.45E-09	3.08E-09
YWHAE	214.66	141.14	1.52	8.22E-28	5.10E-27
ITGB8	74.17	47.90	1.55	1.88E-15	6.02E-15
YWHAH	102.59	66.00	1.55	1.54E-25	8.22E-25
EIF4B	299.92	192.20	1.56	1.47E-39	1.94E-38
LAMA5	242.34	155.24	1.56	3.52E-14	1.03E-13
ITGB1	328.08	209.70	1.56	2.06E-34	1.90E-33
EFNA4	15.36	9.82	1.57	1.17E-14	3.55E-14
GNG10	6.37	4.00	1.59	2.92E-08	5.62E-08
CCND2	773.45	483.06	1.60	3.23E-16	1.08E-15
ITGB5	110.37	68.02	1.62	4.02E-23	1.77E-22
COL6A3	893.58	541.26	1.65	2.92E-18	1.07E-17
VEGFA	532.25	316.77	1.68	5.74E-32	4.71E-31

(Continues)

TABLE 2 (Continued)

Gene	Tumor mean	Normal mean	Fold change	*P*-value	Adjusted *P*-value
ITGAV	191.03	113.43	1.68	2.91E-30	2.27E-29
TNC	156.39	92.44	1.69	7.51E-11	1.73E-10
FGF1	4.77	2.79	1.71	9.30E-06	1.59E-05
CDK6	289.15	166.12	1.74	1.71E-35	1.63E-34
YWHAB	389.26	223.04	1.75	1.32E-37	1.49E-36
TP53	105.07	59.63	1.76	3.25E-24	1.54E-23
RHEB	46.51	26.01	1.79	1.22E-30	9.79E-30
PPP2R3B	4.34	2.42	1.79	1.09E-08	2.20E-08
YWHAQ	130.47	72.74	1.79	8.52E-37	8.63E-36
CCNE1	8.70	4.80	1.81	2.93E-10	6.49E-10
CDK2	45.70	24.96	1.83	8.37E-33	7.07E-32
PDGFRB	166.24	87.76	1.89	8.03E-28	5.10E-27
RPS6	850.00	447.37	1.90	5.64E-45	9.02E-44
LAMC2	138.11	70.95	1.95	3.56E-24	1.67E-23
DDIT4	66.29	32.77	2.02	3.01E-25	1.53E-24
YWHAG	241.75	119.30	2.03	1.69E-52	4.68E-51
F2R	51.19	25.16	2.03	2.84E-26	1.63E-25
COL4A1	509.38	243.49	2.09	1.17E-43	1.78E-42
FGF18	1.90	0.85	2.22	1.27E-05	2.14E-05
BCL2L1	144.28	64.05	2.25	1.06E-56	4.02E-55
COL9A1	10.41	4.59	2.27	3.34E-09	6.90E-09
EIF4EBP1	20.83	8.84	2.36	3.54E-25	1.76E-24
COL9A3	17.74	7.51	2.36	1.74E-12	4.68E-12
BRCA1	87.46	35.35	2.47	2.77E-41	3.83E-40
CDK4	66.65	26.90	2.48	9.86E-58	4.28E-56
ITGA2	206.67	82.69	2.50	1.27E-48	2.41E-47
HSP90AA1	627.50	246.84	2.54	7.12E-59	4.33E-57
CCND1	317.79	122.64	2.59	1.53E-59	1.16E-57
FN1	1461.37	554.51	2.64	8.52E-34	7.62E-33
HSP90AB1	593.22	213.90	2.77	6.27E-75	1.91E-72
EFNA3	24.98	8.69	2.88	5.14E-29	3.72E-28
OSM	6.68	2.31	2.90	4.35E-16	1.44E-15
COL1A2	1838.76	588.95	3.12	9.45E-49	1.91E-47
ITGA11	64.23	19.89	3.23	1.31E-36	1.29E-35
MET	302.60	84.88	3.56	1.25E-68	1.89E-66
MYC	181.11	49.00	3.70	5.76E-55	1.95E-53
COL1A1	3190.86	754.73	4.23	1.19E-54	3.61E-53
FGF20	0.72	0.14	5.29	2.29E-05	3.83E-05
THBS2	212.12	32.95	6.44	1.67E-51	3.91E-50
COMP	9.53	1.45	6.57	8.75E-20	3.41E-19
GNG4	35.42	4.39	8.07	2.36E-38	2.87E-37
FGF19	1.39	0.15	9.42	6.08E-13	1.68E-12
SPP1	38.03	3.28	11.60	5.67E-42	8.21E-41

study participants (55.6%) were diagnosed at either AJCC Stage 1 or 2. *TP53* mutations were the most common tumor molecular phenotype of cases (47.5%) and *BRAF*-mutated tumors were the least common tumor molecular phenotype observed (10.1%).

Of the 304 genes evaluated, 254 (83.6%) were statistically significantly dysregulated after adjustment for multiple comparisons. Of the 254 significantly dysregulated genes, 76 had a FC of <0.67, and 57 had a FC of >1.50 (Table 2) (Supplemental Table S2 lists expression results for all genes in the pathway). Among the most dysregulated genes (both up and down-regulated) were genes involved in extracellular matrix (ECM) functions including collagen type genes (such as *COL4A6*, *COL4A4*, *COL2A1*, *COL4A3*, and *COL6A5*), tenascin genes (*TNC* and *TNXB*), thrombospondin genes (*THBS2*), and integrin subunit alpha and subunit beta genes. The cytokine-related genes, *IL3RA*, *IL6R*, and *IL7R* were significantly down-regulated. Several growth factor genes, including *IGF1*, *FGFR2*, *FGF9*, *FGF10*, *EGF*, and *PDGFD* were down-regulated in CRC tumor tissue. *PIK3CG* and *PIK3CD* also were significantly down-regulated (FC 0.40 and 0.59 respectively), which could influence down-stream genes including *BRCA1*, *CCND1*, *MYC*, *BCL2*, and *CREB*. Figure 1 highlights those genes that are down-regulated (yellow) and up-regulated (green) in the PI3K/Akt-signaling pathway.

Differential mRNA expression of 47 of the 133 genes with a FC of <0.67 or >1.50 were associated with differential miRNA expression. Of these 47 genes, 36 genes had seed-region matches with their associated miRNAs (Table 3). Of the 147 mRNA:miRNA associations with seed-region matches, 26 were inversely associated meaning that mRNA was down-regulated when the miRNA was up-regulated or the mRNA was up-regulated when the miRNA was down-regulated, suggesting a greater likelihood of a direct effect. While some genes only had a seed-region match with one miRNA, such as *IGF1*, *PHLPP2*, *SGK2*, *ITGA4*, *CDK4*, *THBS1*, *ITGA11*, *CREB3L1*, and *GNG2*, most had seed-region matches with multiple miRNAs. *IL6R* had a seed-region match with 11 miRNAs with the majority being inversely associated, *ITGA2* had a seed-region match with 14 miRNAs, and *YWHAB* had a seed-region match with 19 miRNAs. Several miRNAs had seed-region matches with multiple genes: miR-1246, miR-21-3p, miR-21-5p, miR-23a-3p, miR-375, and miR-934 with two genes; let-7i-5p, miR193b-3p, miR-196-5p, miR-19b-3p, miR-221-3p, and miR-24-3p with three genes; miR-106b-5p, miR199a-5p, miR-199b-5p, miR-29b-3p, miR-3651, and miR-501-3p with four genes; miR-145-5p and miR-93-5p with five genes; miR-199a-3p, miR-203a, and miR-27a-3p with six genes; miR-150-5p, miR-214-3p and miR-650 with seven genes; miR-17-5p, miR-20a-5p, and miR-20b-5p with eight genes. Expression levels of all of the genes associated with miR-203a expression were inversely associated, suggesting direct associations. Figure 1 highlights the miRNAs with seed-region matches associated with various components of the PI3K/Akt-signaling pathway.

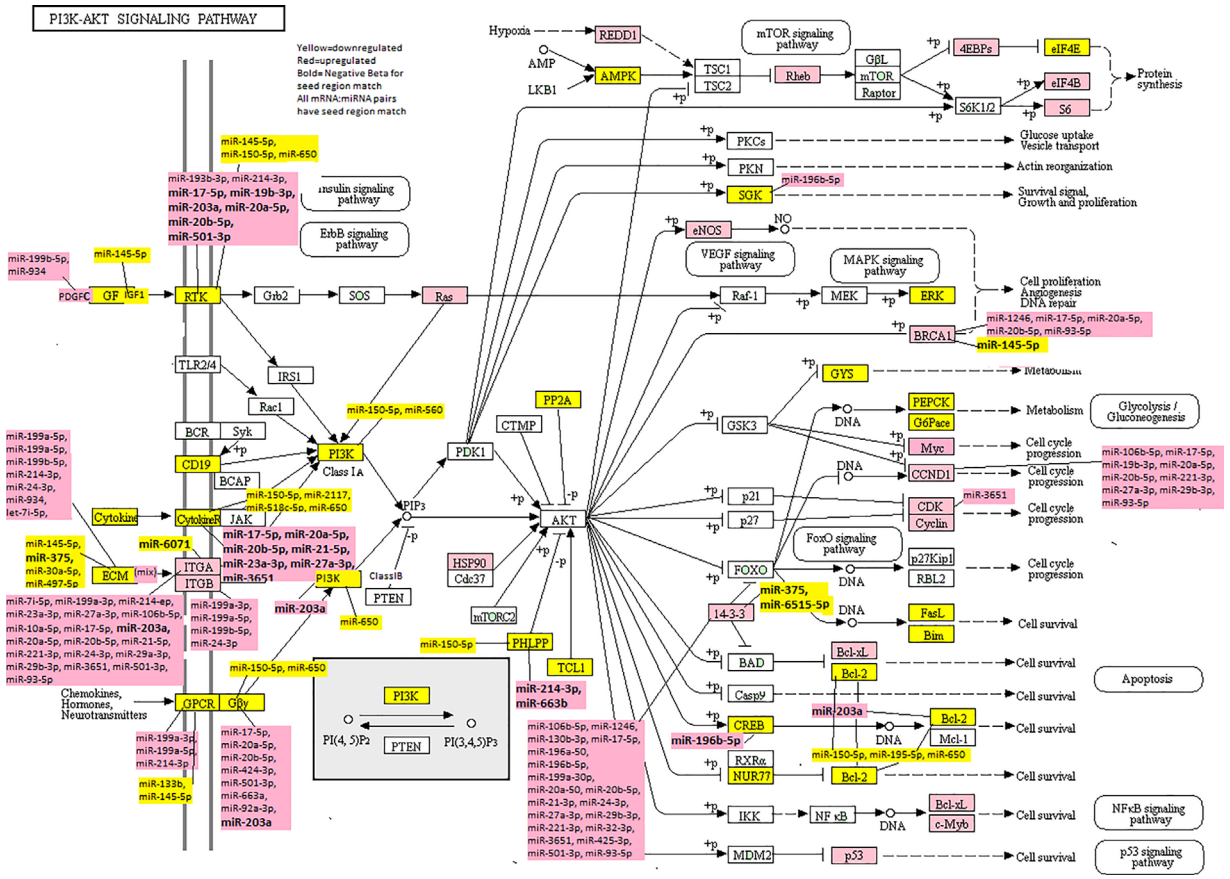

FIGURE 1 Kegg PI3K/Akt-Signaling pathway with highlighted dysregulated mRNA and their associated seed-region matched miRNA. Bold items depict negative beta coefficient between miRNA and mRNA

TABLE 3 Associations between PI3K/AKT pathway genes with miRNAs that have a seed match

Gene name	Tumor mean	Normal mean	Fold change	miRNA	Tumor mean	Normal mean	Fold change	Beta	Raw *P*-value	FDR *P*-value
BRCA1[a]	87.46	35.35	2.47	hsa-miR-1246	629.21	412.81	1.52	0.25	0.0008	0.0383
				hsa-miR-145-5p	132.97	223.14	0.60	−0.28	0.0004	0.025
				hsa-miR-17-5p	61.04	16.38	3.73	0.28	<0.0001	0.0116
				hsa-miR-20a-5p	70.78	17.61	4.02	0.25	0.0006	0.0383
				hsa-miR-20b-5p	17.65	3.30	5.35	0.25	0.0004	0.0326
				hsa-miR-93-5p	41.72	15.20	2.74	0.30	<0.0001	0.0116
IGF1	13.85	38.80	0.36	hsa-miR-145-5p	132.97	223.14	0.60	0.28	<0.0001	0.0407
PHLPP2	79.32	139.85	0.57	hsa-miR-150-5p	14.90	39.17	0.38	0.29	<0.0001	0.0271
TNC	156.39	92.44	1.69	hsa-miR-199a-5p	20.18	9.28	2.17	0.25	0.0002	0.0163
				hsa-miR-199b-5p	4.69	1.53	3.07	0.27	0.0002	0.0203
PHLPP1	42.56	65.01	0.65	**hsa-miR-214-3p**	13.24	6.13	2.16	−0.26	0.0002	0.0488
				hsa-miR-663b	65.50	32.21	2.03	−0.26	<0.0001	0.0488
ITGB5	110.37	68.02	1.62	hsa-miR-199a-3p	44.83	22.53	1.99	0.32	<0.0001	0.0163
				hsa-miR-199a-5p	20.18	9.28	2.17	0.36	<0.0001	0.0163
				hsa-miR-199b-5p	4.69	1.53	3.07	0.28	0.0002	0.0233
				hsa-miR-24-3p	106.75	62.39	1.71	0.25	0.0006	0.0444
SGK2	42.18	65.06	0.65	hsa-miR-196b-5p	17.89	5.53	3.24	0.37	<0.0001	0.0271
PIK3CG	13.99	35.04	0.40	**hsa-miR-203a**	12.52	3.70	3.38	−0.34	<0.0001	0.0203
				hsa-miR-650	4.51	16.60	0.27	0.36	<0.0001	0.0203
COL1A1	3190.86	754.73	4.23	hsa-miR-199a-5p	20.18	9.28	2.17	0.29	<0.0001	0.0163
				hsa-miR-199b-5p	4.69	1.53	3.07	0.30	<0.0001	0.0163
				hsa-miR-214-3p	13.24	6.13	2.16	0.34	<0.0001	0.0163
				hsa-miR-934	4.36	0.94	4.66	0.58	<0.0001	0.0163
CCND1	317.79	122.64	2.59	hsa-miR-106b-5p	15.90	5.19	3.06	0.25	0.0006	0.0349
				hsa-miR-17-5p	61.04	16.38	3.73	0.30	<0.0001	0.0244
				hsa-miR-19b-3p	29.80	10.42	2.86	0.28	0.0003	0.0244
				hsa-miR-20a-5p	70.78	17.61	4.02	0.28	<0.0001	0.0244
				hsa-miR-20b-5p	17.65	3.30	5.35	0.29	<0.0001	0.0244
				hsa-miR-221-3p	13.53	4.12	3.28	0.26	0.0003	0.0349
				hsa-miR-27a-3p	56.26	23.29	2.42	0.27	0.0002	0.0244
				hsa-miR-29b-3p	24.31	9.83	2.47	0.27	0.0003	0.0244
				hsa-miR-93-5p	41.72	15.20	2.74	0.26	0.0002	0.0244
PDGFRB	166.24	87.76	1.89	hsa-miR-193b-3p	9.12	5.42	1.68	0.27	0.0002	0.0181
				hsa-miR-214-3p	13.24	6.13	2.16	0.38	<0.0001	0.0102
ITGA4	36.27	71.10	0.51	**hsa-miR-203a**	12.52	3.70	3.38	−0.30	<0.0001	0.0116
YWHAQ	130.47	72.74	1.79	hsa-miR-106b-5p	15.90	5.19	3.06	0.29	<0.0001	0.0116
				hsa-miR-17-5p	61.04	16.38	3.73	0.30	<0.0001	0.0116
				hsa-miR-20a-5p	70.78	17.61	4.02	0.30	<0.0001	0.0116
				hsa-miR-20b-5p	17.65	3.30	5.35	0.31	<0.0001	0.0116
				hsa-miR-27a-3p	56.26	23.29	2.42	0.24	0.001	0.0471
				hsa-miR-425-5p	11.76	6.97	1.69	0.23	0.0011	0.0471
				hsa-miR-93-5p	41.72	15.20	2.74	0.33	<0.0001	0.0116
PDGFRA	88.47	166.61	0.53	hsa-miR-145-5p	132.97	223.14	0.60	0.26	0.0002	0.0163
				hsa-miR-150-5p	14.90	39.17	0.38	0.24	0.0004	0.0233
				hsa-miR-17-5p	61.04	16.38	3.73	−0.28	<0.0001	0.0136

(Continues)

TABLE 3 (Continued)

Gene name	Tumor mean	Normal mean	Fold change	miRNA	Tumor mean	Normal mean	Fold change	Beta	Raw *P*-value	FDR *P*-value
				hsa-miR-19b-3p	29.80	10.42	2.86	−0.29	<0.0001	0.0136
				hsa-miR-203a	12.52	3.70	3.38	−0.30	<0.0001	0.0136
				hsa-miR-20a-5p	70.78	17.61	4.02	−0.28	0.0002	0.0163
				hsa-miR-20b-5p	17.65	3.30	5.35	−0.30	<0.0001	0.0136
				hsa-miR-29b-3p	24.31	9.83	2.47	−0.27	0.0003	0.0222
				hsa-miR-501-3p	7.07	2.95	2.39	−0.25	0.0004	0.0233
				hsa-miR-650	4.51	16.60	0.27	0.32	<0.0001	0.0136
CDK4	66.65	26.90	2.48	hsa-miR-3651	58.66	25.92	2.26	0.26	0.0003	0.0488
THBS1	585.51	881.12	0.66	hsa-miR-145-5p	132.97	223.14	0.60	0.29	<0.0001	0.0407
ITGA11	64.23	19.89	3.23	hsa-miR-193b-3p	9.12	5.42	1.68	0.28	<0.0001	0.0102
ITGAV	191.03	113.43	1.68	hsa-let-7i-5p	62.16	39.97	1.56	0.31	0.0004	0.0271
				hsa-miR-199a-3p	44.83	22.53	1.99	0.30	<0.0001	0.0136
				hsa-miR-214-3p	13.24	6.13	2.16	0.29	<0.0001	0.0136
				hsa-miR-23a-3p	174.68	87.53	2.00	0.27	0.0003	0.0244
				hsa-miR-27a-3p	56.26	23.29	2.42	0.25	0.0012	0.0425
				hsa-miR-6071	0.97	1.70	0.57	−0.23	0.0011	0.0425
PDGFC	28.55	18.83	1.52	hsa-miR-199b-5p	4.69	1.53	3.07	0.28	0.0002	0.0488
				hsa-miR-934	4.36	0.94	4.66	0.27	<0.0001	0.0407
CREB3L1	96.13	195.06	0.49	**hsa-miR-196b-5p**	17.89	5.53	3.24	−0.28	0.0002	0.0203
IL6R	25.93	101.24	0.26	hsa-miR-150-5p	14.90	39.17	0.38	0.30	<0.0001	0.0136
				hsa-miR-17-5p	61.04	16.38	3.73	−0.24	0.0008	0.0362
				hsa-miR-20a-5p	70.78	17.61	4.02	−0.24	0.0003	0.0163
				hsa-miR-20b-5p	17.65	3.30	5.35	−0.29	0.0002	0.0136
				hsa-miR-2117	1.50	4.09	0.37	0.22	0.001	0.0388
				hsa-miR-21-5p	463.11	167.37	2.77	−0.29	<0.0001	0.0136
				hsa-miR-23a-3p	174.68	87.53	2.00	−0.25	0.0007	0.0335
				hsa-miR-27a-3p	56.26	23.29	2.42	−0.28	<0.0001	0.0136
				hsa-miR-3651	58.66	25.92	2.26	−0.25	0.0003	0.0163
				hsa-miR-518c-5p	1.76	2.90	0.61	0.22	0.0014	0.0475
				hsa-miR-650	4.51	16.60	0.27	0.29	0.0002	0.0136
COL6A3	893.58	541.26	1.65	hsa-let-7i-5p	62.16	39.97	1.56	0.26	<0.0001	0.0081
				hsa-miR-214-3p	13.24	6.13	2.16	0.43	<0.0001	0.0081
ITGA2	206.67	82.69	2.50	hsa-miR-106b-5p	15.90	5.19	3.06	0.23	0.001	0.0339
				hsa-miR-10a-5p	44.64	26.74	1.67	0.25	0.0006	0.0257
				hsa-miR-17-5p	61.04	16.38	3.73	0.28	<0.0001	0.0081
				hsa-miR-203a	12.52	3.70	3.38	0.34	<0.0001	0.0081
				hsa-miR-20a-5p	70.78	17.61	4.02	0.27	<0.0001	0.0081
				hsa-miR-20b-5p	17.65	3.30	5.35	0.33	<0.0001	0.0081
				hsa-miR-21-5p	463.11	167.37	2.77	0.28	0.0002	0.0116
				hsa-miR-221-3p	13.53	4.12	3.28	0.37	<0.0001	0.0081
				hsa-miR-24-3p	106.75	62.39	1.71	0.23	0.0016	0.0432
				hsa-miR-29a-3p	110.29	51.04	2.16	0.24	0.0006	0.0257
				hsa-miR-29b-3p	24.31	9.83	2.47	0.22	0.0022	0.0497
				hsa-miR-3651	58.66	25.92	2.26	0.22	0.002	0.0479
				hsa-miR-501-3p	7.07	2.95	2.39	0.26	0.0003	0.0163

(Continues)

TABLE 3 (Continued)

Gene name	Tumor mean	Normal mean	Fold change	miRNA	Tumor mean	Normal mean	Fold change	Beta	Raw *P*-value	FDR *P*-value
				hsa-miR-93-5p	41.72	15.20	2.74	0.26	0.0005	0.0239
COL1A2	1838.76	588.95	3.12	hsa-let-7i-5p	62.16	39.97	1.56	0.24	0.0002	0.0239
				hsa-miR-193b-3p	9.12	5.42	1.68	0.29	<0.0001	0.0148
				hsa-miR-199a-3p	44.83	22.53	1.99	0.36	<0.0001	0.0116
YWHAB	389.26	223.04	1.75	hsa-miR-106b-5p	15.90	5.19	3.06	0.30	<0.0001	0.0029
				hsa-miR-1246	629.21	412.81	1.52	0.24	0.0008	0.0099
				hsa-miR-130b-3p	8.74	4.89	1.79	0.18	0.012	0.0485
				hsa-miR-17-5p	61.04	16.38	3.73	0.44	<0.0001	0.0029
				hsa-miR-196a-5p	6.70	4.21	1.59	0.23	0.0007	0.0234
				hsa-miR-196b-5p	17.89	5.53	3.24	0.39	<0.0001	0.0095
				hsa-miR-199a-3p	44.83	22.53	1.99	0.22	0.001	0.0029
				hsa-miR-19b-3p	29.80	10.42	2.86	0.37	<0.0001	0.0029
				hsa-miR-20a-5p	70.78	17.61	4.02	0.45	<0.0001	0.0029
				hsa-miR-20b-5p	17.65	3.30	5.35	0.42	<0.0001	0.0029
				hsa-miR-21-3p	22.68	9.89	2.29	0.33	<0.0001	0.0029
				hsa-miR-24-3p	106.75	62.39	1.71	0.20	0.0067	0.035
				hsa-miR-27a-3p	56.26	23.29	2.42	0.28	<0.0001	0.0029
				hsa-miR-32-3p	4.74	2.81	1.68	0.18	0.0115	0.0468
				hsa-miR-3651	58.66	25.92	2.26	0.29	<0.0001	0.0029
				hsa-miR-375	20.50	54.53	0.38	−0.26	0.0002	0.0048
				hsa-miR-501-3p	7.07	2.95	2.39	0.19	0.0078	0.0376
				hsa-miR-6515-5p	1.20	4.41	0.27	−0.25	0.0005	0.0083
				hsa-miR-93-5p	41.72	15.20	2.74	0.30	<0.0001	0.0029
GNG4	35.42	4.39	8.07	hsa-miR-17-5p	61.04	16.38	3.73	0.23	0.001	0.0388
				hsa-miR-20a-5p	70.78	17.61	4.02	0.24	0.0004	0.025
				hsa-miR-20b-5p	17.65	3.30	5.35	0.22	0.0014	0.0407
				hsa-miR-424-3p	39.81	25.37	1.57	0.35	<0.0001	0.009
				hsa-miR-501-3p	7.07	2.95	2.39	0.23	0.0008	0.0326
				hsa-miR-663a	374.83	234.91	1.60	0.25	0.0008	0.0326
				hsa-miR-92a-3p	121.60	41.18	2.95	0.34	<0.0001	0.009
TNXB	23.48	134.34	0.17	hsa-miR-30a-5p	2.38	4.61	0.52	0.25	0.0004	0.0444
				hsa-miR-497-5p	1.77	7.12	0.25	0.30	<0.0001	0.0271
IL7R	35.03	79.98	0.44	hsa-miR-150-5p	14.90	39.17	0.38	0.32	<0.0001	0.0271
				hsa-miR-650	4.51	16.60	0.27	0.26	<0.0001	0.0271
YWHAG	241.75	119.30	2.03	hsa-miR-21-3p	22.68	9.89	2.29	0.27	<0.0001	0.0116
				hsa-miR-221-3p	13.53	4.12	3.28	0.29	<0.0001	0.0116
				hsa-miR-27a-3p	56.26	23.29	2.42	0.26	<0.0001	0.0116
				hsa-miR-29b-3p	24.31	9.83	2.47	0.24	0.0007	0.0438
PIK3CD	27.33	46.48	0.59	hsa-miR-150-5p	14.90	39.17	0.38	0.35	<0.0001	0.0271
				hsa-miR-650	4.51	16.60	0.27	0.31	<0.0001	0.0271
BCL2	24.42	62.11	0.39	hsa-miR-150-5p	14.90	39.17	0.38	0.34	<0.0001	0.0203
				hsa-miR-195-5p	3.59	12.18	0.29	0.24	0.0003	0.0488
				hsa-miR-203a	12.52	3.70	3.38	−0.27	<0.0001	0.0203
				hsa-miR-650	4.51	16.60	0.27	0.38	<0.0001	0.0203
GNG7	3.66	22.76	0.16	hsa-miR-150-5p	14.90	39.17	0.38	0.28	<0.0001	0.0407

(Continues)

TABLE 3 (Continued)

Gene name	Tumor mean	Normal mean	Fold change	miRNA	Tumor mean	Normal mean	Fold change	Beta	Raw *P*-value	FDR *P*-value
				hsa-miR-650	4.51	16.60	0.27	0.30	<0.0001	0.0407
CHRM2	2.32	4.20	0.55	hsa-miR-133b	1.71	6.94	0.25	0.36	<0.0001	0.0271
				hsa-miR-145-5p	132.97	223.14	0.60	0.35	<0.0001	0.0271
F2R	51.19	25.16	2.03	hsa-miR-199a-3p	44.83	22.53	1.99	0.28	0.0002	0.0326
				hsa-miR-199a-5p	20.18	9.28	2.17	0.27	0.0004	0.0407
				hsa-miR-214-3p	13.24	6.13	2.16	0.30	<0.0001	0.0271
THBS2	212.12	32.95	6.44	hsa-miR-199a-3p	44.83	22.53	1.99	0.35	<0.0001	0.0081
				hsa-miR-214-3p	13.24	6.13	2.16	0.42	<0.0001	0.0081
				hsa-miR-375	20.50	54.53	0.38	−0.24	0.0005	0.0271
GNG2	13.93	29.33	0.47	**hsa-miR-203a**	12.52	3.70	3.38	−0.28	<0.0001	0.0271
				hsa-miR-650	4.51	16.60	0.27	0.34	<0.0001	0.0271

[a]Bold text highlights negative beta coefficient between differential expression of miRNA and mRNA.

Table 4 shows associations between mRNA and miRNAs without seed-region match, suggesting an indirect association between the miRNA and the mRNA, in that while miRNA and mRNA are associated, it is through the binding with other mRNA that influence the signaling pathway. In total there were 165 associations without seed-region matches. Like those miRNA and mRNA with seed-region matches, differential expression of most genes were associated with multiple miRNAs and miRNAs were associated with multiple genes. Several of the miRNAs that had seed-region matches to the mRNA, such as let-7i-5p, were also associated with other genes, but without seed-region matches. A summary of the miRNA and gene expression associations by seed-region match can be seen in Table 5. Most miRNAs have both direct associations, supported by seed-region matches, and indirect associations with pathway genes.

4 | DISCUSSION

The PI3K/Akt-signaling pathway is frequently activated in cancer. Of the 304 genes evaluated 133 (43.8%) had FC differences that were <0.67 or >1.50, which is slightly higher than the rate of dysregulated genes for all protein-coding genes (37.5%). Forty-seven of the 133 dysregulated genes were associated with miRNA expression. For those mRNA and miRNAs with a seed region match, 26 were inversely associated suggesting a greater likelihood of a direct association. Several genes within this signaling pathway have become therapeutic targets, thus a better understanding of the PI3K/Akt-signaling pathway can provide insight into the underlying biology associated with these targets.

During cellular stress, as in oncogenesis, the PI3K/Akt-signaling pathway is a key regulator of cell survival.[26] PI3K can be divided into two major subclasses that have been associated with cancer.[4] Subclass IA is activated by receptors with a protein tyrosine kinase (RPTK) activity; hormones, cytokines, growth factors, insulin receptors activate the subclass IA. The IB subclass of PI3Ks is activated by receptors coupled with G proteins (GPCR). In this signaling pathway AKTs are key intermediates. There are three AKTs, amplification of AKT2 has been detected in colorectal tumors and has been positively associated with tumor aggressiveness.[2,27] In our data AKT2 was significantly up-regulated, although the FC was modest (FC = 1.15). Amplification of AKTs, alters several downstream molecules including mTOR, MYC, eukaryotic initiation factor of translation (eIF4E), IKK, cyclin D1, CREB, CASP9, and BAD. Both BAD and CASP9 were down-regulated in our data (FC of 0.71 and 0.74, respectively) and MYC and cyclin D1 were up-regulated (FC 3.70 and 2.59, respectively) as could be expected when AKT2 would be up-regulated.[28] PTEN is a negative regulator of the PI3K/Akt-signaling pathway and was significantly down-regulated, although the FC was modest (FC = 0.83). Many of the expression levels that we observed are consistent with an activated PI3K/Akt-signaling pathway that would promote tumorigenesis.

Several parts of the PI3K/Akt-signaling pathway appear to have dysregulated genes. Most major junctures in the pathway had both up and down-regulated genes. One example are the genes and receptors associated with extracellular matrix integrity including collagen genes *COL1A1*, *COL1A2*, and *COL4A1* that were significantly up-regulated with strong FCs (FC 4.32, 3.12, and 2.09, respectively) while other collagen genes were down-regulated (Supplemental Table S2 list all genes analyzed and FCs). Tenascin genes involved in cell to matrix interaction and extracellular matrix formation were down-regulated (*TNN* FC 0.67, *TNXB* FC 0.17, and *TNC* FC 1.69) as were the integrin genes, which are extracellular matrix receptors for collagens and related proteins (*ITGA11* FC 3.23, *ITGA2* FC 2.50, *ITGA2B* FC 0.62, *ITGA4* FC 0.51, *ITGA8* FC 0.26, and *ITGAV* FC 1.68). The PI3K genes also showed both up and down-regulation, such as *PIK3CA* with a FC of 1.22, *PIK3CD* FC 0.59, and *PIK3CG* FC 0.40. The ultimate impact of these genes on tumorigenesis, may depend on the balance of up- and down-regulation and how it enables cells to survive and grow.

Several miRNAs have previously been associated with genes in the PI3K/Akt-signaling pathway.[13] In the study by Josse and colleagues, the PI3K/Akt-signaling pathway was one of the top pathways associated with dysregulated miRNA expression levels.[13] MiRNAs identified as being important to this signaling pathway were miR-143, miR-145, miR-133a, and miR-223. We only identified miR-145-5p as

TABLE 4 Associations between PI3K/AKT pathway genes with miRNAs that do not have a seed region match

Gene name	Tumor mean	Normal mean	Fold change	miRNA	Tumor mean	Normal mean	Fold change	Raw *P*-value	FDR *P*-value
BRCA	87.46	35.35	2.47	hsa-miR-19b-3p	29.80	10.42	2.86	0.0008	0.0383
				hsa-miR-25-3p	30.05	12.78	2.35	0.0002	0.0163
				hsa-miR-425-5p	11.76	6.97	1.69	0.001	0.0452
				hsa-miR-650	4.51	16.60	0.27	0.0002	0.0163
				hsa-miR-92a-3p	121.60	41.18	2.95	0.0005	0.0291
PHLPP2	79.32	139.85	0.57	hsa-miR-196b-5p	17.89	5.53	3.24	<0.0001	0.0271
TNC	156.39	92.44	1.69	hsa-let-7i-5p	62.16	39.97	1.56	<0.0001	0.0163
				hsa-miR-146b-5p	4.46	2.67	1.67	0.0004	0.0296
				hsa-miR-199a-3p	44.83	22.53	1.99	<0.0001	0.0163
				hsa-miR-214-3p	13.24	6.13	2.16	<0.0001	0.0163
				hsa-miR-21-5p	463.11	167.37	2.77	0.0004	0.0296
				hsa-miR-934	4.36	0.94	4.66	<0.0001	0.0163
HSP90AA1	627.50	246.84	2.54	hsa-miR-203a	12.52	3.70	3.38	<0.0001	0.0271
ITGB5	110.37	68.02	1.62	hsa-miR-193b-3p	9.12	5.42	1.68	<0.0001	0.0163
				hsa-miR-214-3p	13.24	6.13	2.16	<0.0001	0.0163
				hsa-miR-365a-3p	8.43	4.33	1.94	0.0007	0.0475
				hsa-miR-934	4.36	0.94	4.66	<0.0001	0.0163
				hsa-miR-99a-5p	6.30	3.70	1.71	0.0006	0.0444
HSP90AB1	593.22	213.90	2.77	hsa-miR-203a	12.52	3.70	3.38	<0.0001	0.0271
				hsa-miR-20a-5p	70.78	17.61	4.02	0.0002	0.0326
				hsa-miR-221-3p	13.53	4.12	3.28	0.0002	0.0326
				hsa-miR-6515-5p	1.20	4.41	0.27	<0.0001	0.0271
				hsa-miR-663b	65.50	32.21	2.03	<0.0001	0.0271
OSM	6.68	2.31	2.90	hsa-miR-424-3p	39.81	25.37	1.57	<0.0001	0.0407
				hsa-miR-934	4.36	0.94	4.66	<0.0001	0.0407
COMP	9.53	1.45	6.57	hsa-miR-425-5p	11.76	6.97	1.69	<0.0001	0.0407
				hsa-miR-934	4.36	0.94	4.66	<0.0001	0.0407
PIK3CG	13.99	35.04	0.40	hsa-miR-150-5p	14.90	39.17	0.38	<0.0001	0.0203
COL1A1	3190.86	754.73	4.23	hsa-miR-193b-3p	9.12	5.42	1.68	0.0002	0.0233
				hsa-miR-199a-3p	44.83	22.53	1.99	0.0002	0.0233
				hsa-miR-215	49.53	77.27	0.64	0.0007	0.0438
				hsa-miR-375	20.50	54.53	0.38	0.0007	0.0438
YWHAE	214.66	141.14	1.52	hsa-miR-424-3p	39.81	25.37	1.57	0.0002	0.0407
CCND1	317.79	122.64	2.59	hsa-miR-203a	12.52	3.70	3.38	0.0002	0.0244
				hsa-miR-21-5p	463.11	167.37	2.77	0.0006	0.0349
PDGFRB	166.24	87.76	1.89	hsa-miR-199a-3p	44.83	22.53	1.99	<0.0001	0.0102
				hsa-miR-199a-5p	20.18	9.28	2.17	<0.0001	0.0102
				hsa-miR-199b-5p	4.69	1.53	3.07	<0.0001	0.0102
				hsa-miR-934	4.36	0.94	4.66	<0.0001	0.0102
ITGA4	36.27	71.10	0.51	hsa-miR-150-5p	14.90	39.17	0.38	<0.0001	0.0116
				hsa-miR-650	4.51	16.60	0.27	<0.0001	0.0116
YWHAH	102.59	66.00	1.55	hsa-miR-650	4.51	16.60	0.27	<0.0001	0.0163
LAMA5	242.34	155.24	1.56	hsa-miR-17-5p	61.04	16.38	3.73	<0.0001	0.0203
				hsa-miR-193b-3p	9.12	5.42	1.68	<0.0001	0.0203
				hsa-miR-331-3p	14.64	9.30	1.57	0.0002	0.0271

(Continues)

TABLE 4 (Continued)

Gene name	Tumor mean	Normal mean	Fold change	miRNA	Tumor mean	Normal mean	Fold change	Raw *P*-value	FDR *P*-value
YWHAQ	130.47	72.74	1.79	hsa-miR-19b-3p	29.80	10.42	2.86	0.0002	0.0181
				hsa-miR-21-3p	22.68	9.89	2.29	0.0009	0.0471
				hsa-miR-221-3p	13.53	4.12	3.28	0.0002	0.0181
				hsa-miR-25-3p	30.05	12.78	2.35	<0.0001	0.0116
				hsa-miR-29a-3p	110.29	51.04	2.16	0.0011	0.0471
				hsa-miR-29b-3p	24.31	9.83	2.47	0.001	0.0471
				hsa-miR-3651	58.66	25.92	2.26	0.0006	0.0444
				hsa-miR-92a-3p	121.60	41.18	2.95	0.0011	0.0471
CDK4	66.65	26.90	2.48	hsa-miR-17-5p	61.04	16.38	3.73	<0.0001	0.0271
				hsa-miR-20a-5p	70.78	17.61	4.02	<0.0001	0.0271
				hsa-miR-20b-5p	17.65	3.30	5.35	0.0002	0.0407
				hsa-miR-93-5p	41.72	15.20	2.74	<0.0001	0.0271
MYC	181.11	49.00	3.70	hsa-miR-1246	629.21	412.81	1.52	0.0002	0.0163
				hsa-miR-17-5p	61.04	16.38	3.73	<0.0001	0.0136
				hsa-miR-19b-3p	29.80	10.42	2.86	0.0002	0.0163
				hsa-miR-20a-5p	70.78	17.61	4.02	<0.0001	0.0136
				hsa-miR-20b-5p	17.65	3.30	5.35	0.0002	0.0163
				hsa-miR-3651	58.66	25.92	2.26	0.0003	0.0188
				hsa-miR-375	20.50	54.53	0.38	<0.0001	0.0136
				hsa-miR-501-3p	7.07	2.95	2.39	0.0003	0.0188
				hsa-miR-583	6.61	3.22	2.05	0.0004	0.0233
				hsa-miR-663a	374.83	234.91	1.60	0.0003	0.0188
				hsa-miR-663b	65.50	32.21	2.03	<0.0001	0.0136
				hsa-miR-92a-3p	121.60	41.18	2.95	<0.0001	0.0136
ITGA11	64.23	19.89	3.23	hsa-miR-199a-3p	44.83	22.53	1.99	<0.0001	0.0102
				hsa-miR-199a-5p	20.18	9.28	2.17	<0.0001	0.0102
				hsa-miR-199b-5p	4.69	1.53	3.07	<0.0001	0.0102
				hsa-miR-214-3p	13.24	6.13	2.16	<0.0001	0.0102
				hsa-miR-934	4.36	0.94	4.66	<0.0001	0.0102
				hsa-miR-99a-5p	6.30	3.70	1.71	0.0006	0.0136
ITGAV	191.03	113.43	1.68	hsa-miR-1203	1.76	2.83	0.62	<0.0001	0.0136
				hsa-miR-1243	1.48	3.20	0.46	0.0005	0.0291
				hsa-miR-199a-5p	20.18	9.28	2.17	0.0003	0.0244
				hsa-miR-204-3p	0.92	2.37	0.39	0.0011	0.0244
				hsa-miR-21-3p	22.68	9.89	2.29	0.0009	0.0425
				hsa-miR-21-5p	463.11	167.37	2.77	0.0002	0.0203
				hsa-miR-24-3p	106.75	62.39	1.71	0.001	0.0425
				hsa-miR-34a-5p	25.15	12.32	2.04	0.0007	0.038
				hsa-miR-4251	3.31	1.77	1.86	0.001	0.0425
				hsa-miR-934	4.36	0.94	4.66	<0.0001	0.0136
PDGFC	28.55	18.83	1.52	hsa-miR-99a-5p	6.30	3.70	1.71	<0.0001	0.0407
CREB3L1	96.13	195.06	0.49	hsa-miR-193b-3p	9.12	5.42	1.68	<0.0001	0.0136
				hsa-miR-375	20.50	54.53	0.38	<0.0001	0.0136
				hsa-miR-424-3p	39.81	25.37	1.57	0.0005	0.0407
				hsa-miR-6515-5p	1.20	4.41	0.27	0.0002	0.0203

(Continues)

TABLE 4 (Continued)

Gene name	Tumor mean	Normal mean	Fold change	miRNA	Tumor mean	Normal mean	Fold change	Raw *P*-value	FDR *P*-value
				hsa-miR-92a-3p	121.60	41.18	2.95	<0.0001	0.0136
IL6R	25.93	101.24	0.26	hsa-miR-19b-3p	29.80	10.42	2.86	0.0002	0.0136
				hsa-miR-203a	12.52	3.70	3.38	0.0007	0.0335
				hsa-miR-221-3p	13.53	4.12	3.28	0.0015	0.0488
				hsa-miR-92a-3p	121.60	41.18	2.95	<0.0001	0.0136
COL6A3	893.58	541.26	1.65	hsa-miR-193b-3p	9.12	5.42	1.68	<0.0001	0.0081
				hsa-miR-199a-3p	44.83	22.53	1.99	<0.0001	0.0081
				hsa-miR-199a-5p	20.18	9.28	2.17	<0.0001	0.0081
				hsa-miR-199b-5p	4.69	1.53	3.07	<0.0001	0.0081
				hsa-miR-934	4.36	0.94	4.66	<0.0001	0.0081
ITGA2	206.67	82.69	2.50	hsa-miR-1291	5.52	3.67	1.51	0.0009	0.0318
				hsa-miR-19b-3p	29.80	10.42	2.86	<0.0001	0.0081
				hsa-miR-23a-3p	174.68	87.53	2.00	0.0002	0.0116
				hsa-miR-27a-3p	56.26	23.29	2.42	<0.0001	0.0081
				hsa-miR-34a-5p	25.15	12.32	2.04	<0.0001	0.0081
				hsa-miR-650	4.51	16.60	0.27	0.0002	0.0116
				hsa-miR-663b	65.50	32.21	2.03	0.0019	0.0116
				hsa-miR-92a-3p	121.60	41.18	2.95	0.0004	0.0116
COL1A2	1838.76	588.95	3.12	hsa-miR-199a-5p	20.18	9.28	2.17	<0.0001	0.0116
				hsa-miR-199b-5p	4.69	1.53	3.07	<0.0001	0.0116
				hsa-miR-214-3p	13.24	6.13	2.16	<0.0001	0.0116
				hsa-miR-934	4.36	0.94	4.66	<0.0001	0.0116
YWHAB	389.26	223.04	1.75	hsa-let-7i-5p	62.16	39.97	1.56	0.0022	0.0176
				hsa-miR-151a-3p	5.15	1.56	3.31	<0.0001	0.0029
				hsa-miR-15a-5p	7.69	5.07	1.52	0.0002	0.0048
				hsa-miR-193b-3p	9.12	5.42	1.68	0.0036	0.0234
				hsa-miR-199a-5p	20.18	9.28	2.17	0.0057	0.0113
				hsa-miR-199b-5p	4.69	1.53	3.07	0.0023	0.0311
				hsa-miR-21-5p	463.11	167.37	2.77	0.0002	0.0048
				hsa-miR-221-3p	13.53	4.12	3.28	<0.0001	0.0029
				hsa-miR-23a-3p	174.68	87.53	2.00	0.0005	0.0083
				hsa-miR-25-3p	30.05	12.78	2.35	<0.0001	0.0029
				hsa-miR-29a-3p	110.29	51.04	2.16	<0.0001	0.0029
				hsa-miR-29b-3p	24.31	9.83	2.47	<0.0001	0.0029
				hsa-miR-34a-5p	25.15	12.32	2.04	0.0005	0.0083
				hsa-miR-361-5p	11.62	6.20	1.87	0.001	0.0113
				hsa-miR-424-3p	39.81	25.37	1.57	<0.0001	0.0029
				hsa-miR-425-5p	11.76	6.97	1.69	0.0007	0.0095
				hsa-miR-4749-3p	8.01	12.04	0.67	0.0027	0.0191
				hsa-miR-663a	374.83	234.91	1.60	0.0056	0.0311
				hsa-miR-663b	65.50	32.21	2.03	<0.0001	0.0029
				hsa-miR-92a-3p	121.60	41.18	2.95	<0.0001	0.0029
GNG4	35.42	4.39	8.07	hsa-miR-3651	58.66	25.92	2.26	0.0008	0.0326
				hsa-miR-663b	65.50	32.21	2.03	<0.0001	0.009
TNXB	23.48	134.34	0.17	hsa-miR-133b	1.71	6.94	0.25	<0.0001	0.0271

(Continues)

TABLE 4 (Continued)

Gene name	Tumor mean	Normal mean	Fold change	miRNA	Tumor mean	Normal mean	Fold change	Raw *P*-value	FDR *P*-value
				hsa-miR-145-5p	132.97	223.14	0.60	<0.0001	0.0271
				hsa-miR-365a-3p	8.43	4.33	1.94	0.0008	0.0458
				hsa-miR-3976	2.97	1.24	2.39	0.0006	0.0444
				hsa-miR-99a-5p	6.30	3.70	1.71	0.0009	0.0458
IL7R	35.03	79.98	0.44	hsa-miR-203a	12.52	3.70	3.38	<0.0001	0.0271
YWHAG	241.75	119.30	2.03	hsa-miR-106b-5p	15.90	5.19	3.06	<0.0001	0.0116
				hsa-miR-17-5p	61.04	16.38	3.73	<0.0001	0.0116
				hsa-miR-19b-3p	29.80	10.42	2.86	0.0004	0.0362
				hsa-miR-20a-5p	70.78	17.61	4.02	0.0002	0.0203
				hsa-miR-20b-5p	17.65	3.30	5.35	0.0008	0.0465
				hsa-miR-21-5p	463.11	167.37	2.77	0.0006	0.0116
				hsa-miR-25-3p	30.05	12.78	2.35	<0.0001	0.0438
				hsa-miR-3651	58.66	25.92	2.26	0.0006	0.0438
				hsa-miR-650	4.51	16.60	0.27	0.0007	0.0438
				hsa-miR-93-5p	41.72	15.20	2.74	<0.0001	0.0116
BCL2L1	144.28	64.05	2.25	hsa-miR-92a-3p	121.60	41.18	2.95	<0.0001	0.0407
CD19	3.78	7.59	0.50	hsa-miR-150-5p	14.90	39.17	0.38	<0.0001	0.0407
F2R	51.19	25.16	2.03	hsa-let-7i-5p	62.16	39.97	1.56	0.0003	0.0407
				hsa-miR-146b-5p	4.46	2.67	1.67	0.0004	0.0407
				hsa-miR-193b-3p	9.12	5.42	1.68	0.0002	0.0326
				hsa-miR-199b-5p	4.69	1.53	3.07	<0.0001	0.0271
				hsa-miR-934	4.36	0.94	4.66	<0.0001	0.0271
THBS2	212.12	32.95	6.44	hsa-let-7i-5p	62.16	39.97	1.56	0.0003	0.0203
				hsa-miR-193b-3p	9.12	5.42	1.68	<0.0001	0.0081
				hsa-miR-199a-5p	20.18	9.28	2.17	<0.0001	0.0081
				hsa-miR-199b-5p	4.69	1.53	3.07	<0.0001	0.0081
				hsa-miR-934	4.36	0.94	4.66	<0.0001	0.0081
GNG2	13.93	29.33	0.47	hsa-miR-150-5p	14.90	39.17	0.38	<0.0001	0.0271
LPAR1	12.91	46.29	0.28	hsa-miR-21-5p	463.11	167.37	2.77	<0.0001	0.0407
				hsa-miR-497-5p	1.77	7.12	0.25	<0.0001	0.0407

being associated with *BRCA1*, *IGF1*, *PDGFRA*, *THBS1*, *CHRM2*, and *TNXB*. Other miRNAs associated previously with genes in PI3K/Akt-signaling pathway include miR-590-5p, 106b, and miR-93 with *PTEN*,[29,30] miR-497 with IGF1R,[31] miR-451 with *LKB1*, *AKT*, *PI3K*, and *BCL2*,[32] and miR-126 with *AKT*.[33] We observed associations between miR-497 and miR-106b-5p with various genes in the pathway. Differences between our study and the literature in detection of associations result from our focusing on those genes with higher FCs. Thus we did not assess genes with lower FC, such as *AKT* and *PTEN*, with miRNAs.

We utilized seed-region matches to identify miRNAs that were more likely to have a direct effect on the mRNA, leading to mRNA degradation from the miRNA binding with the 3′UTR of the mRNA. Those matches that were most likely to have a direct effect were the 26 matches where the expression of the miRNA was opposite that of the mRNA expression. Indirect effects are most likely operating feedback and feed-forward loops.[34–36] In feedback loops, regulators such as miRNAs and transcription factors (TFs) can have either the same effect (repression of expression) or opposite effects where the TF enhances the mRNA on each other.[35] In feed-forward loops, TF regulates the miRNA as well as a target gene (TG), which is in turn also regulated by the miRNA. In this instance, the miRNA may regulate the TG directly, through seed region binding leading to mRNA degradation or translational repression, or indirectly, through repression of the TF that is influencing transcription of the same TG. It has been suggested that regulatory paths involving miRNAs are prevalent mechanisms of gene expression.[35] Studies have shown that genes can share a regulatory network with miR-150-5p via feed forward loops.[34] As this miRNA was only associated with mRNAs with a positive beta coefficient, even when seed-region matches were identified, it is possible that this miRNA is involved in feed-forward loops in CRC.

TABLE 5 Associations between miRNAs and mRNA expression based on seed region matches

miRNA	mRNA associated with a seed match	mRNA associated without seed region match
hsa-let-7i-5p	*ITGAV*, *COL6A3*, *COL1A2*	*TNC*, *YWHAB*, *F2R*, *THBS2*
hsa-miR-10a-5p	*ITGA2*	
hsa-miR-106b-5p	*CCND1*, *YWHAQ*, *ITGA2*, *YWHAB*	*YWHAG*
hsa-miR-1203		*ITGAV*
hsa-miR-1243		*ITGAV*
hsa-miR-1246	*BRCA1*, *YWHAB*	*MYC*
hsa-miR-1291		*ITGA2*
hsa-miR-130b-3p	*YWHAB*	
hsa-miR-133b	*CHRM2*	*TNXB*
hsa-miR-145-5p	***BRCA1***[a] *IGF1*, *PDGFRA*, *THBS1*, *CHRM2*	*TNXB*
hsa-miR-146b-5p		*TNC*, *F2R*
hsa-miR-150-5p	*PHLPP2*, *PDGFRA*, *IL6R*, *IL7R*, *PIK3CD*, *BCL2*, *GNG7*	*PIK3CG*, *ITGA4*, *CD19*, *GNG2*
hsa-miR-151a-3p		*YWHAB*
hsa-miR-15a-5p		*YWHAB*
hsa-miR-17-5p	*BRCA1*, *CCND1*, *YWHAQ*, ***PDGFRA***, ***IL6R***, *ITGA2*, *YWHAB*,	*LAMA5*, *CDK4*, *MYC*, *YWHAG*
hsa-miR-193b-3p	*PDGFRB*, *ITGA11*, *COL1A2*	*ITGB5*, *COL1A1*, *LAMA5*, *CREB3L1*, *COL6A3*, *YWHAB*, *F2R*, *THBS2*
hsa-miR-195-5p	*BCL2*	
hsa-miR-196a-5p	*YWHAB*	
hsa-miR-196b-5p	***SGK2***, ***CREB3L1***, *YWHAB*	*PHLPP2*
hsa-miR-199a-3p	*ITGB5*, *ITGAV*, *COL1A2*, *YWHAB*, *F2R*, *THBS2*	*TNC*,*COL1A1*, *PDGFRB*, *ITGA11*, *COL6A3*
hsa-miR-199a-5p	*TNC*, *ITGB5*, *COL1A1*, *F2R*	*PDGFRB*, *ITGA11*, *ITGAV*, *COL6A3*, *COL1A2*, *YWHAB*, *THBS2*
hsa-miR-199b-5p	*TNC*, *ITGB5*, *COL1A1*, *PDGFC*	*PDGFRB*, *ITGA11*, *COL6A3*, *COL1A2*, *YWHAB*, *F2R*, *THBS2*
hsa-miR-19b-3p	*CCND1*, ***PDGFRA***, *YWHAB*	*BRCA1*, *YWHAQ*, *MYC*, *IL6R*, *ITGA2*, *YWHAG*
hsa-miR-203a	***PIK3CG***, ***ITGA4***, ***PDGFRA***, *ITGA2*, ***BCL2***, ***GNG2***	*HSP90AA1*, *HSP90AB1*, *CCND1*, *IL6R*, *IL7R*
hsa-miR-204-3p		*ITGAV*
hsa-miR-20a-5p	*BRCA1*, *CCND1*, *YWHAQ*, ***PDGFRA***, ***IL6R***, *ITGA2*, *YWHAB*, *GNG4*	*HSP90AB1*, *CDK4*, *MYC*, *YWHAG*
hsa-miR-20b-5p	*BRCA1*, *CCND1*, *YWHAQ*, ***PDGFRA***,***IL6R***, *ITGA2*, *YWHAB*, *GNG4*	*CDK4*, *MYC*, *YWHAG*
hsa-miR-2117	*IL6R*	
hsa-miR-21-3p	*YWHAB*, *YWHAG*	*YWHAQ*, *ITGAV*
hsa-miR-21-5p	***IL6R***, *ITGA2*	*TNC*, *CCND1*, *ITGAV*, *YWHAB*, *YWHAG*, *LPAR1'*
hsa-miR-214-3p	***PHLPP1***, *COL1A1*, *PDGFRB*, *ITGAV*, *COL6A3*, *F2R*, *THBS2*	*TNC*, *ITGB5*, *ITGA11*, *COL1A2*
hsa-miR-215		*COL1A1*
hsa-miR-221-3p	*CCND1*, *ITGA2*, *YWHAG*	*HSP90AB1*, *YWHAQ*, *IL6R*, *YWHAB*
hsa-miR-23a-3p	*ITGAV*, ***IL6R***	*ITGA2*, *YWHAB*
hsa-miR-24-3p	*ITGB5*, *ITGA2*, *YWHAB*	*ITGAV*
hsa-miR-25-3p		*BRCA1*, *YWHAQ*, *YWHAB*
hsa-miR-27a-3p	*CCND1*, *YWHAQ*, *ITGAV*, ***IL6R***, *YWHAB*, *YWHAG*	*ITGA2*
hsa-miR-29a-3p	*ITGA2*	*YWHAQ*, *YWHAB*
hsa-miR-29b-3p	*CCND1*, ***PDGFRA***, *ITGA2*, *YWHAG*	*YWHAQ*, *YWHAB*
hsa-miR-30a-5p	*TNXB*	
hsa-miR-32-3p	*YWHAB*	
hsa-miR-331-3p		*LAMA5*
hsa-miR-34a-5p		*ITGAV*, *ITGA2*, *YWHAB*
hsa-miR-361-5p		*YWHAB*

(Continues)

TABLE 5 (Continued)

miRNA	mRNA associated with a seed match	mRNA associated without seed region match
hsa-miR-365a-3p		*ITGB5, TNXB*
hsa-miR-3651	*CDK4*, ***IL6R***, *ITGA2, YWHAB*	*YWHAQ, MYC, GNG4, YWHAG*
hsa-miR-375	***YWHAB***, ***THBS2***	*COL1A1, MYC, CREB3L1*
hsa-miR-3976		*TNXB*
hsa-miR-424-3p	*GNG4*	*YWHAB,OSM, YWHAE, CREB3L1,*
hsa-miR-425-5p	*YWHAQ*	*BRCA1, YWHAB*
hsa-miR-4251		*ITGAV*
hsa-miR-4749-3p		*YWHAB*
hsa-miR-497-5p	*TNXB*	*LPAR1*
hsa-miR-501-3p	***PDGFRA***, *ITGA2, YWHAB, GNG4*	*MYC*
hsa-miR-518c-5p	*IL6R*	
hsa-miR-583		*MYC*
hsa-miR-6071	***ITGAV***	
hsa-miR-650	*PIK3CG, PDGFRA, IL6R, IL7R, PIK3CD, BCL2, GNG7, GNG2*	*BRCA1, ITGA4, YWHAH, ITGA2, YWHAG*
hsa-miR-6515-5p	***YWHAB***	*HSP90AB1, CREB3L1*
hsa-miR-663a	*GNG4*	*MYC, ITGA2, YWHAB*
hsa-miR-663b	***PHLPP1***	*HSP90AB1, MYC, YWHAB, GNG4*
hsa-miR-92a-3p	*GNG4*	*BRCA1, YWHAQ, MYC, CREB3L1, IL6R, ITGA2, YWHAB, BCL2L1*
hsa-miR-934	*COL1A1, PDGFC*	*TNC, OSM, PDGFRB, ITGA11, COL6A3, COL1A2, F2R, THBS2*
hsa-miR-93-5p	*BRCA1, CCND1,YWHAQ, ITGA2, YWHAB*	*ITGB5, CDK4, YWHAG*
hsa-miR-99a-5p		*ITGB5, ITGA11, PDGFC, TNXB*

[a]Bold text indicates an inverse direction of association.

Of the 26 mRNA:miRNA significant inverse association, both *IL6R* and *PDGFRA* were associated with seven miRNAs. Both of these genes stimulate Class IA PI3Ks that in turn alter AKT. Both of these genes were associated with multiple miRNAs in the miR-17 to miR-92 cluster, including miR-17-5p, miR-19b-3p, miR-20a-5p, miR-20b-5p, miR-29b, miR-27a-3p, and miR-23a-3p. Overexpression of miR-17-5p has been shown to inhibit proliferation and trigger apoptosis.[37] MiR-20 has previously been associated with cyclin D1[38] and has been shown to be associated with vascular endothelial growth factor, a cytokine, via activation of Akt signaling.[39] MiR-27a-3p has been shown to promote cell proliferation and invasion possibly through its association with the cytokine *TRAIL* (TNF-related apoptosis-inducing ligand)[40] or through its effects on insulin.[41] *IL6R* differential expression also was inversely associated with miR-21-5p suggesting a direct association between this mRNA and miRNA. MiR-21 has been previously linked to increased inflammation and has been shown to promote Akt signaling and exert an anti-apoptotic effect.[42] Other miRNA:miRNAs that had a greater potential for a direct effect given the inverse association between the miRNA and the mRNAs that stimulated Class IA PI3Ks were miR-203a, miR-375 and miR-607a through their inverse association with *ITGA4*, *ITGAV*, and *THBS2*. These direct associations also would impact the Class IA PI3Ks that could lead to indirect effects with other miRNA:mRNA associations. MiR-203a was inversely association with both *PIK3CG* and *GNG2* expression, again suggesting a direct association that would operate at the stimulate component of the pathway. Up-regulation of miR-203 has been shown to inhibit proliferation and metastasis in CRC.[43] These associations could serve as key targets for future research.

Several miRNAs may be important given the frequent number of associated genes both with and without seed-region matches although associations do not any miRNA:mRNAs with inverse associations. These include miR-150-5p, miR-199a-3p, miR-199a-5p, and miR-199b-5p with 11 mRNAs, miR-650 with 13 mRNAs, and miR-93-5p with 7 mRNAs. MiR-150 has been shown to impair inflammatory cytokine production[44]; ITGA3 has previously been associated with the anti-tumor miR-199 family. Over-expression of miR-93 has been shown to promote tumorigenesis and metastasis by activating the PI3K/Akt-signaling pathway.[45] *YWHAB* (14-3-3 proteinβ) was associated with 19 miRNA seed-region matches, two of which had inverse associations, miR-375 and miR-6515-5p.

Although our sample size is small, it is one of the largest available containing samples with paired carcinoma and normal mucosa data. While normal colonic mucosa may not be truly "normal," it is the closest normal mucosa that can be used for a matched-paired analysis. Normal colonic mucosa was taken from the same colonic site as the tumor to prevent differences in expression from being the result of tumor location. The same RNA prep was used to generate both miRNA and mRNA data, which could minimize differences in tissue samples that could contribute to associations. We focused only on those genes that were statistically significant and also had a FC of 1.5 or greater or 0.67 or

less. Using these criteria, we did not examine all statistically significant genes and miRNAs that were differentially expressed. Thus, genes like *PIK3CA*, which were statistically significantly dysregulated but the FC was 1.22 and *PTEN* with a FC of 0.83, were not examined further with miRNAs. A biologically important FC is not well defined, and by using set values for further follow-up we could have missed genes associated with miRNAs. Additionally, in our current analysis, we utilized a negative binomial model with a random subject effect. Other statistical methods based on fixed effect models such as DESEQ2 could have slightly different results. We exclusively used the KEGG pathway database to identify signaling pathway genes. Thus, other genes not identified in KEGG may importantly alter PI3K/Akt-signaling as well as influence miRNA expression. When evaluating miRNA with mRNAs, we could have further missed important associations since miRNAs have their impact post-transcriptionally. However, much of the current information on miRNA target genes comes from gene expression data and associations observed may have important biological meaning, but must be acknowledged as being incomplete.[24,46]

In conclusion, we find the PI3K/Akt-signaling pathway is dysregulated in CRC and that several of the dysregulated genes are associated with miRNAs. Several of these associations involve negative beta coefficients and seed-region matches between the miRNA and mRNA, suggesting that these miRNAs directly repress gene expression, and thus alter PI3K/Akt signaling; other associations we identified may be downstream effects resulting from the pathways disruption. These results need to be confirmed in other CRC studies, including laboratory-based setting. Given the prominence of the PI3K/Akt-signaling pathway in the carcinogenic process, it is important to obtain a better understanding of the underlying genomics of this pathway in an effort to help identify and refine therapeutic agents.

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AUTHORS' CONTRIBUTION

MS obtained funding, planned study, oversaw study data collection and analysis, and wrote the manuscript. JRS provided input into the statistical analysis. LEM conducted bioinformatics analysis and helped write manuscript. LCS provided data and input into the manuscript. RKW oversaw laboratory analysis and gave input into data interpretation. WS reviewed and edited the manuscript and did pathology overview for the study. JSH conducted statistical analysis and managed data. All authors approved final manuscript.

CONFLICTS OF INTEREST

All the authors declare that they have no conflict of interest.

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

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