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REVIEW

The functional role of miRNAs in colorectal cancer: insights from a large population-based study

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ABSTRACT Identification of causal microRNAs (miRNAs) in colorectal cancer (CRC) is elusive, due to our lack of understanding of how specific miRNAs affect biological pathways and outcomes. An miRNA can regulate many mRNAs and an mRNA can be associated with many miRNAs; appreciation of these complex networks in which miRNAs operate is necessary to transition from identifying dysregulated miRNAs to identifying individual miRNAs or groups of miRNAs that are suitable for therapeutic purposes. The aim of the paper is to compile results from a population-based study (n = 1,954 cases with matched carcinoma/normal tissue) of miRNAs in CRC. The information gained allows for cohesive and comprehensive insight into miRNAs and CRC in terms of function and impact. Comparison of miRNA expression with mRNA expression from nine signaling pathways in carcinogenic processes allowed us to identify miRNA targets within a biological context. MiRNAs that directly influence mRNA expression may be effective biomarkers or therapeutic targets.

KEYWORDS mRNA; miRNA; pathways; colorectal cancer

Introduction

MicroRNAs (miRNAs) are small, noncoding RNAs that post-transcriptionally regulate mRNA expression by binding to the 3'UTR of protein-coding genes. As such, miRNAs are thought to be key regulators in carcinogenesis. MiRNAs were first reported as being associated with colorectal cancer (CRC) in 2003[1]. Since then, research focusing on miRNAs has expanded rapidly, in the number of studies, the miRNAs examined, and the types of studies conducted. Studies that focus on few miRNAs and those that have undertaken large-scale discovery have identified hundreds of dysregulated miRNAs in CRC. However, findings are not unanimous as to which miRNAs are dysregulated in CRC nor is there a universal point at which miRNAs are considered dysregulated. Statistically significant associations have unknown biological significance when a fold change (FC) difference between carcinoma and normal mucosa is minimal (for instance a FC of 1.1). Correspondingly, the level of expression change between carcinoma and normal mucosa that is needed to have a biological significance is unknown, however setting some threshold for meaningful differences, such as a 50% or twofold change, when considering important miRNAs for determining functionality, could help avoid considering small changes that stem from noise in the data. Determining important miRNAs can be difficult, since results can vary by type of study, which range from population-based epidemiologic approaches to those using cell lines to evaluate the influence of specific miRNAs. Small studies of targeted miRNAs can identifiy "statistically significant" miRNAs that would not hold the same level of significance after adjustment for multiple comparisons of all miRNAs conducted in large discovery studies. We have previously addressed some of the issues surrounding studying miRNAs in terms of measurement of miRNA expression, standardizing miRNA expression, and using existing databases to determine target genes[2,3].

The utilization of individually paired-carcinoma and normal mucosa is important when determining differential expression as well as the impact of miRNA expression on disease and prognosis[4]. As we have previously reported, repeat sampling of study data shows considerable variation in results when looking only at absolute miRNA expression in carcinomas or if using non-paired normal mucosa expression data, while much greater consistency in findings occurrs when differential expression is calculated from individually paired carcinomas and normal mucosa samples[4,5]. We have shown that diet, alcohol, and smoking are associated with

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miRNA expression, as are genetic variants[6-10]. MiR-17-5p, miR-106b-5p, miR-19b-3p, and miR-20b-5p were downregulated in smokers; miR-106b-5p, miR-145-5p, and miR-17-5p were downregualted in subjects who consumed wine; and miR-145-5p was positively associated with whole grain intake. Individually paired tissue samples control for confounding factors, either genetic or environmental, that may influence miRNA expression. Using paired data also can help overcome variance in differential miRNA expression derived from tissue processing, since an individual's carcinoma and normal mucosa tissues are processed in the same manner. However, given the additional cost and availability of samples, individually paired analysis is often not undertaken, contributing to greater difficulty in determining important miRNAs.

The biggest obstacle in advancing miRNA into translational research is determining how miRNAs impact biological pathways and disease processes. It is well known that miRNAs regulate many genes and many genes are regulated by multiple miRNAs. These co-regulatory networks of genes and miRNAs complicate our understanding of functionality and bring into question the importance of pursuing a single miRNA as a therapeutic target without understanding the broader complex in which it belongs. Our goal in this paper is to summarize key findings from our large epidemiological study of miRNAs in CRC. This study was conducted both as a means of discovering new potentially important miRNAs as well as replicating previously reported miRNAs that were associated with CRC; we believe this summary can help focus further research. Additionally, consolidating our study findings may highlight which miRNAs will serve as the best therapeutic targets, either individually, or in groups. We believe that information gained from our large study of 1,954 individuals diagnosed with CRC, who have paired carcinoma and normal mucosa miRNA expression data on over 2,000 miRNAs (Agilent Human miRNA Microarray V19.0) allows for cohesive and comprehensive insight into miRNAs as they relate to CRC. Of the 1,954 individuals included in this study, 1,855 of these individuals had information on survival and 217 of the 1,954 individuals had paired carcinoma and normal gene expression data obtained from RNAseq data.

Differentially expressed miRNAs in CRC

In order for an miRNA to be functionally important in CRC, it is assumed that they have to be dysregulated in CRC tumors compared to normal mucosa. Of the 2,006 miRNAs analyzed in our study, 63.7% were expressed in carcinoma tissue and 63.26% were expressed in normal mucosa[3]. Of those miRNAs expressed in over 80% of the population (598 miRNAs), 86% of these miRNAs were statistically significantly (adjusted P value < 0.05) differentially expressed between carcinoma and normal mucosa. However, of those significantly and differentially expressed, 45 miRNAs (8.7%) had a FC of > 1.50 or < 0.68[3]. The level of FC that is biologically important is not clear and it should be recognized that utilization of FC as a tool to identify important miRNAs also has limitations, one being that if there are low levels of miRNA expression a large FC can be detected. Furthermore, calculation of FC is difficult when the miRNA is not expressed in either the carcinoma or normal mucosa. Identification of miRNAs that may have the greatest functional significance when most miRNAs are "dysregulated" can be challenging. In our study, we utilized a random forest technique that allowed us to identify a subset of these miRNAs that best distinguished differences in miRNA expression between carcinoma and normal mucosa[11]. Using this statistical method we identified 16 miRNAs important in colon cancer and 17 miRNAs important in rectal cancer (**Table 1**); four miRNAs were uniquely associated with colon cancer (miR-663a, miR-4538, miR-215, and miR-192-5p) and five miRNAs were uniquely associated with rectal cancer (miR-4323, miR-150-5p, miR-4749-3p, miR-424-3p, and miR-6073)[11]. Three of the miRNAs identified in colon cancer had a FC of > 0.67 (miR-4538 FC 0.73, miR-378a-3p FC 0.75, and miR-378i FC 0.76) and two of the miRNAs identified in rectal cancer had a FC of > 0.67 (miR-378i FC 0.76 and miR-378a-3p FC 0.74); these miRNAs may not have been considered important if a strict FC cutpoint had been applied. These miRNAs (shown in **Table 1** in numerical order) are one set of miRNAs that appear to predict differential expression when considered together. However, given the number of dysregulated miRNAs in CRC, it is highly probable that other miRNAs also have functional significance.

While at the population level miRNAs are up-regulated, down-regulated, or not dysregulated when comparing carcinoma tissue to normal mucosa; it should be kept in mind that not all individuals in the population have the up-regulated or down-regulated miRNA in their carcinoma tissue. However, our data suggest that miRNAs appear to be more stable than mRNAs when considering the percentage of the population with a dysregulated miRNA compared to a dysregulated mRNA[12]. Additionally, those miRNAs with a higher FC appear to have a greater percentage of the population with a dysregulated miRNA. The set of miRNAs identified by our random forest analysis were, for the most

Table 1 Summary of miRNAs identified as being important in CRC based on random forest assessment

Down-regulated miRNAs	MiR-145-5p, miR-150-5p, miR-192-5p, miR-215, miR-378a-3p, miR-378i, miR-4323, miR-4538, miR-4539, miR-4749-3p, miR-6073
Up-regulated miRNAs	MiR-17-59, miR-20a-5p, miR-21-5p, miR-3651, miR-424-3p, miR-4506, miR-663a, miR-663b, miR-92a-3p, miR-93-5p

part, highly dysregulated, making them targets that have an application at the population level. Knowing that dysregulated miRNAs with smaller FCs may be dysregulated in a smaller subset of the population also suggests the importance of focusing on higher FC in miRNA expression when determining which miRNAs to target for further functionality studies.

Infrequently expressed miRNAs

In our data, 38.79% of miRNAs were expressed in less than 20% of colorectal carcinomas (498 miRNAs) and 36.11% of miRNAs (457 miRNAs) were infrequently expressed in normal mucosa[3]. A reasonable question is, are these infrequently expressed miRNAs of biological importance or are they merely noise in the data? Given our large sample size we were able to examine in more detail infrequently expressed miRNA. Our approach to this question was to focus on those miRNAs that were infrequently expressed, but when expressed had higher levels of expression; what we hoped was beyond "noise" in the data.

Our data suggest that infrequently expressed miRNAs may be important in defining tumor phenotype[13] and survival after a diagnosis with colorectal cancer[14]. **Table 2** summarizes those miRNAs that may have a functional signficiance because they influence survival, mainly when up-regulated in the tumor. In most instances, having these miRNAs up-regulated in tumors resulted in worse survival. While we do not understand how these miRNAs function, their association with survival implies that they may have a functional significance in the carcinogenic process.

Determining functionality

MiRNAs are key regulators of gene expression, hence they are important to the carcinogenic process. An initial step in determining biological impact is to identify which genes are targeted by various miRNAs. Methods such as Western blot and reporter assays measure protein expression levels, an important consideration given that miRNAs are thought to work post-transcriptionally. However, most databases such as miRTarBase, have incorporated miRNA target gene data that are based on less strong evidence of associations such as microarray, RNASeq, and Northern blot[15]. While these methods don't validate miRNA targets using protein expression and instead measure gene expression, several studies have shown that, despite miRNAs having their impact post-transcriptionally, they usually also alter gene expression[16,17]. Given that databases are restricted to existing literature and that the literature does not uniformly represent all miRNAs[2], we utilized RNASeq gene expression data to gain insight into miRNAs associated with gene expression. By examining how change in miRNA expression was associated with change in mRNA expression we gained insight into potentially important miRNAs and their targeted genes. **Figure 1** provides an example of how these differences are correlated (beta coefficient -0.30).

Seed-region matches between the miRNA and the 3'UTR of the mRNA suggest a greater propensity for binding and therefore an increased likelihood that the miRNA directly influences mRNA expression; previously we investigated miRNA and mRNA FASTA sequences for matches of 6, 7, and 8 contiguous nucleotides to elucidate direct interactions, in which mRNA expression is reduced[18]. Thus, looking at seed region matches between miRNA and mRNA is one method to identify potentially important target genes for an miRNA. However, it has been suggested that other factors, such as binding at energy-based sites, may be important when identifying target genes. Ding and colleagues[19] used energy-based sites as a second set of criteria when identifying

Table 2 Infrequently expressed miRNAs that may influence survival in CRC

	Improved survival	Worse survival
Up-regulated in carcinoma tissue	MiR-347a-5p, miR-590-5p, miR-362-5p, miR-632	MiR-124-3p, miR-143-5p, miR-145-3p, miR-31-5p, miR-99b-5p, miR-1, miR-124-3p, miR-133a, miR-143-5p, miR-3622b-3p, miR-378g, miR-548aw, miR-371-3p
Down-regulated in carcinoma tissue		MiR-645

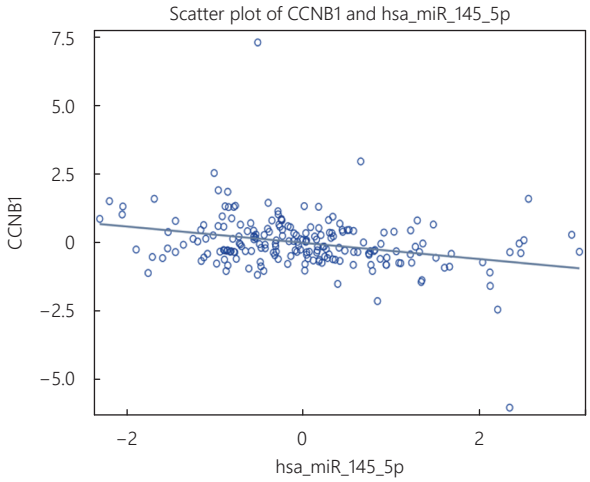

Figure 1 Scatter plot of differentially expressed *CCNB1* (cyclin B1) with differentially expressed miR-145-5p.

candidate target sites; seed-region matching remained the first line of candidate site identification. It has been suggested that as much as 16% of miRNA:mRNA interactions may not involve contacts within seed regions and are considered seedless interactions[20]. In our studies, we used seed-region matching to provide additional information on our miRNA-mRNA associations, which, taken together with a negative beta coefficient between the two, provides additional support for their direct binding. A negative beta coefficient combined with the identification of a seed region match suggests that, as the miRNA binds to the mRNA, it decreases the mRNA expression. Because we only looked at seed matching, we did not consider seedless interactions. We believe that looking at seed-region matches between miRNA and mRNA provides support that miRNAs are targeting specific genes that may be important in CRC.

While direct binding between an miRNA and an mRNA shows support for that gene being a target of the miRNA, indirect associations between miRNAs and mRNAs also have potential biological importance. Indirect associations are seen in our studies when the differential expression of an miRNA is statistically significantly associated with the differential expression of an mRNA, but as one increases the other increases, or they are inversely associated but without a seed-region match. Indirect effects most likely occurr in feed-forward loops[21-23]. In feed-forward loops, regulators such as miRNAs can have either the same effect (repression of expression) or opposite effects on each other[22]. In feed-forward loops, a transcription factor (TF) such as *TP53* can regulate the miRNA and the target gene (TG), which in turn is regulated by the miRNA. 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. Studies suggest that regulatory pathways involving miRNAs are prevalent mechanisms of altering gene expression[22].

Tumor suppressor genes (TSG) and oncogenes (OG) play important roles in the carcinogenic process by controlling cell growth and inhibiting tumor formation. MiRNAs are thought to play similar roles in the carcinogenic process and it has been suggested that they work with OGs and TSGs[24]. Determining the association between miRNAs and TSGs and OGs is another avenue in which to pursue insight into miRNA functionality. In our data, we observed that miRNAs most likely have both direct and indirect effects on TSGs and OGs[25], suggesting that they work as intermediary regulators between OGs and TSGs, and help balance up and down regulation of genes that in turn influence cell proliferation and apoptosis. Looking at the associated miRNAs, TSGs and OGs suggests that miRNA dysregulation in key signaling pathways is important to CRC[25]. Increased inflammation, angiogenesis, and decreased immune response are hallmarks of many of the major pathways in which dysregulated TSGs and OGs operate with miRNAs. A comprehensive evaluation of all genes within these pathways, in conjunction with all miRNAs expressed in CRC, provided further insight into functionality of miRNAs and possible target sites for intervention.

Key pathways assessed with miRNAs were: apoptosis[26], cell cycle[27], JAK-STAT signaling[28], MAPK signaling[29], NFκB signaling[30], PI3K/AKT[31], TGFβ signaling[12] , p53 signaling[32], and Wnt signaling pathways[33]. Focusing on the those miRNAs (triangle shape) identified through random forrest analysis with an miRNA: mRNA seed region match (mRNAs designated by square shape), one can see how the same miRNA is associated with multiple genes in multiple pathways, and the same is true for mRNAs (**Figure 2**); **Supplementary Table S2** shows mRNAs and miRNAs in more detail. Likewise, mRNAs are associated with multiple miRNAs in different pathways.

Further evaluation of the miRNA:mRNA associations within these nine pathways shows that 88 of the 814 miRNAs (10.8%) expressed in either carcinoma or normal mucosa in over 20% of the population were associated with one or more of the nine pathways. As is shown in **Figure 3** (**Supplementary Table S2** shows mRNAs and miRNAs within pathways in more detail), the majority of associations were indirect, implying that many effects of the miRNAs come from their involvement in feedback loops, rather than directly binding

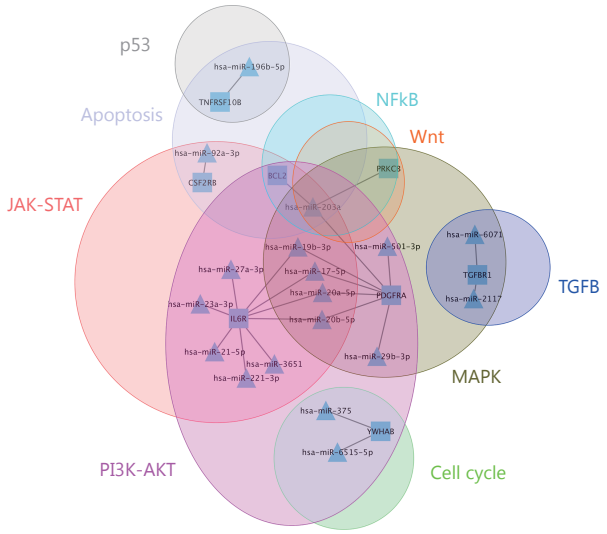

Figure 2 Overlap of key miRNAs and genes in various pathways in CRC.

to an mRNA. We determined that 40 miRNAs had a direct effect on gene expression because of a high likelihood of binding to the mRNA. Those miRNAs involved in regulating gene expression in various pathways are: miR-106b-5p (JAK-STAT-signaling), miR-1243 (Wnt-signaling), miR-1271-5p (NFκB-signaling), miR-145-5p (cell cycle control, apoptosis, and PI3K-AKT-signaling), miR-150-5p (cell cycle control, p53-signaling, apoptosis), miR-17-5p (JAK-STAT-signaling, NFκB-signaling, MAPK-signaling, PI3K-AKT-signaling), miR-193b-3p (MAPK-signaling), miR-195-5p (JAK-STAT-signaling, cell cycle control, apoptosis), miR-196b-5p (p53-signaling, apoptosis, NFκB-signaling, PI3K-AKT-signaling), miR-199a-5p (NFκB-signaling), miR-19b-3p (JAK-STAT-signaling, MAPK-signaling, PI3K-AKT-signaling), miR-203a (JAK-STAT-signaling, apoptosis, NFκB-signaling, MAPK-signaling, PI3K-AKT-signaling, Wnt-signaling), miR-204-3p (Wnt-signaling), miR-20a-5p (JAK-STAT-signaling, MAPK-signaling, PI3K-AKT-signaling), miR-20b-5p (JAK-STAT-signaling, apoptosis, NFκB-signaling, MAPK-signaling, PI3K-AKT-signaling), miR-2117 (MAPK-signaling), miR-214-3p (NFκB-signaling, PI3K-AKT-signaling), miR-215 (NFκB-signaling), miR-21-5p (JAK-STAT-signaling, PI3K-AKT-signaling), miR-221-3p (JAK-STAT-signaling, MAPK-signaling, PI3K-AKT-signaling), miR-23a-3p (JAK-STAT-signaling, PI3K-AKT-signaling), miR-27a-3p (JAK-STAT-signaling, PI3K-AKT-signaling), miR-29b-3p (apoptosis, MAPK-signaling, PI3K-AKT-signaling), miR-324-5p (NFκB-signaling), miR-3591-3p (Wnt-signaling), miR-365a-3p (NFκB-signaling), miR-375 (cell cycle control, TGFβ-signaling, NFκB-signaling, PI3K-AKT-signaling), miR-424-3p (cell cycle control), miR-429 (JAK-STAT-signaling, NFκB-signaling, MAPK-signaling), miR-4749-3p (TGFβ-signaling), miR-501-3p (apoptosis, MAPK-signaling, PI3K-AKT-signaling), miR-590-5p (NFκB-signaling), miR-6071 (MAPK-signaling, PI3K-AKT-signaling), miR-650 (JAK-STAT-signaling, cell cycle control, p53-signaling, apoptosis, PI3K-AKT-signaling), miR-6515-5p (cell cycle control, PI3K-AKT-signaling), miR-663b (NFκB-signaling, PI3K-AKT-signaling), miR-92a-3p (JAK-STAT-signaling, apoptosis), miR-934 (NFκB-signaling), miR-93-5p (JAK-STAT-signaling). These miRNAs also were involved indirectly in several pathways (**Supplementary Table S2**).

Many of the miRNAs have previously been reported associated with genes or biological responses that could impact CRC. For instance, miR-590-5p, miR-106b, and miR-93 have been associated with *PTEN* in the PI3K/AKT pathway[34,35]; miR-145 has been associated with cell-cycle related factors such as *CDK4* and cyclin E2[36]; miR-150 has been associated with immune response[37] and reducing inflammatory cytokine production[38]; miR-17-5p has been shown to inhibit proliferation and trigger apoptosis[39]; miR-199 has previously been associated with *IKKB*[40] and with *ITGA3*[41]; miR-203 as being associated with *BIRC5* which encodes survivin[42]; miR-20 has previously been associated with cyclin D1[43]; miR-221 has been reported as being associated with TNFα[40]; miR-650 has been associated with *BCL2* and *AKT2* and has been shown to promote cell proliferation and invasion[44-46]. However, our findings add to the functional information we have for these miRNAs in that we observed their associations with genes not previously reported and with specific disease pathways. Additionally, our findings show associations for other miRNAs that are directly related to signaling pathways and to genes that are dysregulated in CRC.

To further understand how miRNAs work in complex networks, we have assessed the interactions between TFs and miRNAs, both of which play important roles in regulating gene expression. We found that both miRNAs and TFs influence mRNA expression, and effect of one regulator influences the impact the other has on mRNA expression[47,48]. In normal colonic mucosa we identified significant feed back loop (FBL) interactions involving miR-1258, miR-145-5p, miR-150-5p, 193b-3p, miR-330-3p, and miR-4469. In differential expression we identified FBL interactions involving miR-23a-3p and miR-4469. MiR-330-3p and miR-4469 were associated with one mRNA each in a few pathways, and no significant findings were identified with miR-1258. MiR-150-5p had numerous indirect associations in seven of the pathways (apoptosis, cell cycle, JAK-STAT-

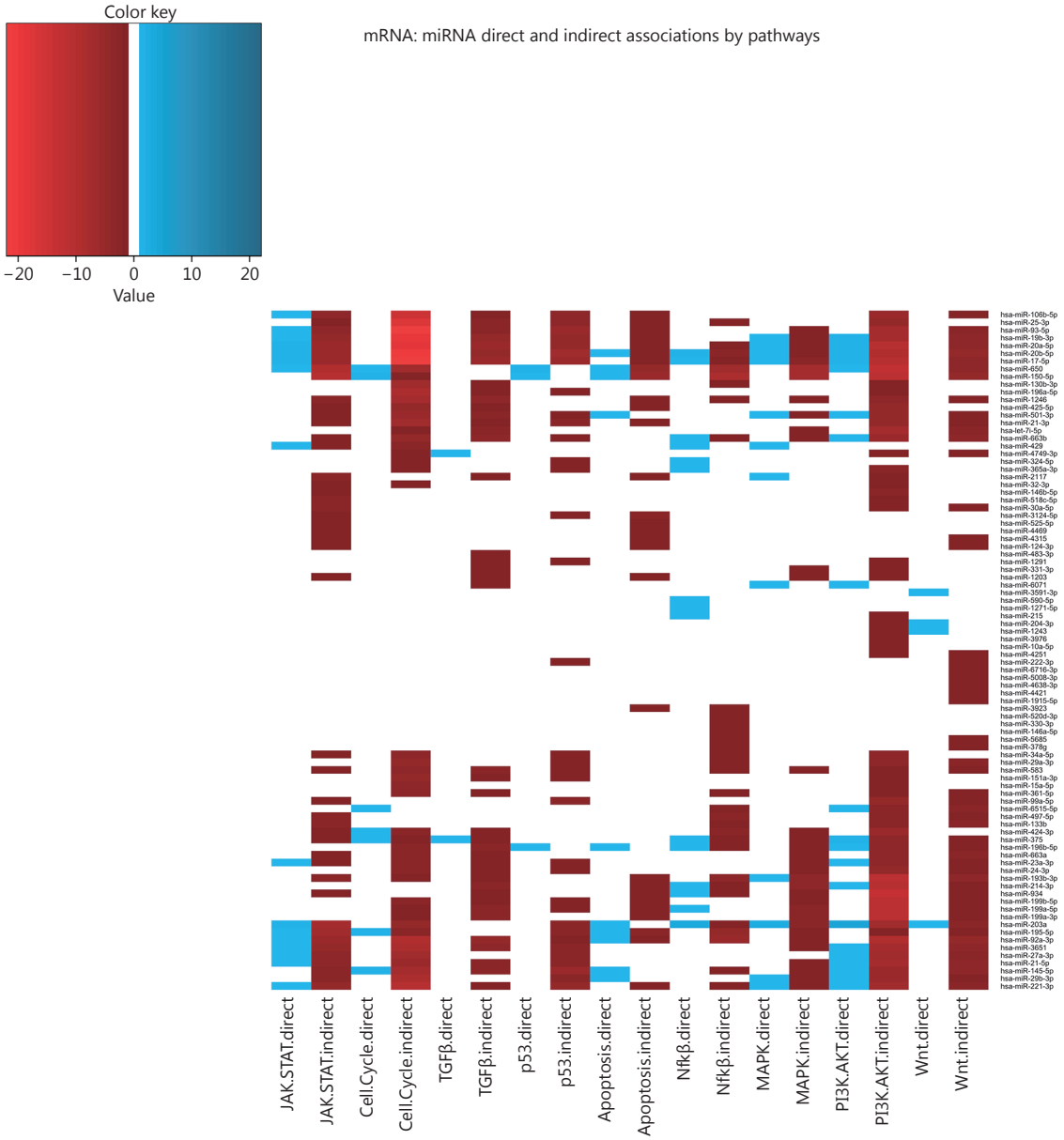

Figure 3 Heatmap of direct and indirect associations of miRNAs within nine pathways.

signaling, MAPK-signaling, NFκB-signaling, PI3K-AKT-signaling, and Wnt-signaling), and had direct associations in apoptosis, cell cycle, and p53-signaling. MiR-145-5p had numerous indirect associations in all pathways except apoptosis, and was involved in direct associations in apoptosis, cell cycle, and PI3K-AKT-signaling. MiR-23a-3p had a direct association with *IL6R* in JAK-STAT-signaling and PI3K-AKT-signaling, and indirect associations with seven mRNAs in the cell cycle, MAPK-signaling, PI3K-AKT-signaling, TGFβ-signaling, p53-signaling, and Wnt-signaling pathways. While some mRNAs were consistent across these studies, such as *MYC*, which has roles in numerous pathways and is a TF, many of the mRNAs associated with these miRNAs varied between our pathway analysis and FBL analysis, highlighting the variance that occurs in these types of investigations.

The miRNAs and target genes we identified were limited to the nine pathways we determined as being important in CRC, given the number of TSGs, OGs, and TFs in these pathways that were associated with miRNAs. We undoubtedly missed associations utilizing only our gene expression data, however given the nature of the study, it was impossible to obtain

information on proteins. It is probable that important associations were not identified given the post-transcriptional role of miRNAs. However, it is also likely that the associations identified are real, and therefore merit validation in other similarily designed studies is needed.

Ongoing challenges and conclusions

While advancements are being made in understanding how miRNAs function in the carcinogenic process, there are many challenges when transitioning miRNAs from the discovery stage to their entering the realm of possible therapeutic agents. One of the biggest challenges stems from the fact that miRNAs target multiple genes and genes are targeted by multiple miRNAs. It is unclear if altering a single miRNA is sufficient to achieve a desired response, or if multiple miRNAs have to be considered as a complex that work within a given system to attain the outcome. Likewise, when investigating a specific miRNA that is associated with multiple genes, it is unclear whether regulation of a particular mRNA produces a certain outcome, or if this effect is the result of the regulation of a subgroup of the associated mRNAs or all of the mRNAs. Most functional studies have looked at single miRNAs when determining their functionality. Also of consideration is the fact that using a target miRNA may have unwanted effects, given that an miRNA can target many genes. It is unclear if a simple approach within the complex human system is sufficient to meaningfully pinpoint miRNAs for theraupeutic purposes. While we know that miRNAs are a part of a complex network, pinpointing their functionality within that framework remains a challenge.

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Conflict of interest statement

No potential conflicts of interest are disclosed.

References

1. Michael MZ, O'Connor SM, Van Holst Pellekaan NG, Young GP, James RJ. Reduced accumulation of specific microRNAs in colorectal neoplasia. Mol Cancer Res. 2003; 1: 882-91.
2. Slattery ML, Herrick JS, Stevens JR, Wolff RK, Mullany LE. An assessment of database-validated microRNA target genes in normal colonic mucosa: implications for pathway analysis. Cancer Inform. 2017; 16: 1176935117716405.
3. Slattery ML, Herrick JS, Pellatt DF, Stevens JR, Mullany LE, Wolff E, et al. MicroRNA profiles in colorectal carcinomas, adenomas and normal colonic mucosa: variations in miRNA expression and disease progression. Carcinogenesis. 2016; 37: 245-61.
4. Stevens JR, Herrick JS, Wolff RK, Slattery ML. Power in pairs: assessing the statistical value of paired samples in tests for differential expression. BMC Genomics. 2018; 19: 953.
5. Slattery ML, Herrick JS, Pellatt DF, Mullany LE, Stevens JR, Wolff E, et al. Site-specific associations between miRNA expression and survival in colorectal cancer cases. Oncotarget. 2016; 7: 60193-205.
6. Slattery ML, Herrick JS, Mullany LE, Stevens JR, Wolff RK. Diet and lifestyle factors associated with miRNA expression in colorectal tissue. Pharmgenomics Pers Med. 2017; 10: 1-16.
7. Slattery ML, Trivellas A, Pellatt AJ, Mullany LE, Stevens JR, Wolff RK, et al. Genetic variants in the TGFβ-signaling pathway influence expression of miRNAs in colon and rectal normal mucosa and tumor tissue. Oncotarget. 2017; 8: 16765-83.
8. Mullany LE, Herrick JS, Wolff RK, Stevens JR, Slattery ML. Association of cigarette smoking and microRNA expression in rectal cancer: insight into tumor phenotype. Cancer Epidemiol. 2016; 45: 98-107.
9. Mullany LE, Herrick JS, Wolff RK, Stevens JR, Slattery ML. Alterations in microRNA expression associated with alcohol consumption in rectal cancer subjects. Cancer Causes Control. 2017; 28: 545-55.
10. Stevens JR, Herrick JS, Wolff RK, Slattery ML. Identifying factors associated with the direction and significance of microRNA tumor-normal expression differences in colorectal cancer. BMC Cancer. 2017; 17: 707.
11. Pellatt DF, Stevens JR, Wolff RK, Mullany LE, Herrick JS, Samowitz W, et al. Expression Profiles of miRNA Subsets Distinguish Human Colorectal Carcinoma and Normal Colonic Mucosa. Clin Transl Gastroenterol. 2016; 7: e152.
12. Pellatt AJ, Mullany LE, Herrick JS, Sakoda LC, Wolff RK, Samowitz WS, et al. The TGFβ-signaling pathway and colorectal cancer: associations between dysregulated genes and miRNAs. J Transl Med. 2018; 16: 191.
13. Slattery ML, Lee FY, Pellatt AJ, Mullany LE, Stevens JR, Samowitz WS, et al. Infrequently expressed miRNAs in colorectal cancer tissue and tumor molecular phenotype. Mod Pathol. 2017; 30: 1152-69.
14. Slattery ML, Pellatt AJ, Lee FY, Herrick JS, Samowitz WS, Stevens JR, et al. Infrequently expressed miRNAs influence survival after

diagnosis with colorectal cancer. Oncotarget. 2017; 8: 83845-59.
15. Chou CH, Chang NW, Shrestha S, Hsu SD, Lin YL, Lee WH, et al. miRTarBase 2016: updates to the experimentally validated miRNA-target interactions database. Nucleic Acids Res. 2016; 44: D239-47.
16. Guo HL, Ingolia NT, Weissman JS, Bartel DP. Mammalian microRNAs predominantly act to decrease target mRNA levels. Nature. 2010; 466: 835-40.
17. Baek D, Villén J, Shin C, Camargo FD, Gygi SP, Bartel DP. The impact of microRNAs on protein output. Nature. 2008; 455: 64-71.
18. Mullany LE, Herrick JS, Wolff RK, Slattery ML. MicroRNA seed region length impact on target messenger RNA expression and survival in colorectal cancer. PLoS One. 2016; 11: e0154177.
19. Ding J, Li XM, Hu HY. TarPmiR: a new approach for microRNA target site prediction. Bioinformatics. 2016; 32: 2768-75.
20. Helwak A, Kudla G, Dudnakova T, Tollervey D. Mapping the human miRNA interactome by CLASH reveals frequent noncanonical binding. Cell. 2013; 153: 654-65.
21. Lin Y, Zhang Q, Zhang HM, Liu W, Liu CJ, Li QB, et al. Transcription factor and miRNA co-regulatory network reveals shared and specific regulators in the development of B cell and T cell. Sci Rep. 2015; 5: 15215.
22. Martinez NJ, Walhout AJM. The interplay between transcription factors and microRNAs in genome-scale regulatory networks. Bioessays. 2009; 31: 435-45.
23. Mangan S, Alon U. Structure and function of the feed-forward loop network motif. Proc Natl Acad Sci U S A. 2003; 100: 11980-5.
24. Kent OA, Mendell JT. A small piece in the cancer puzzle: microRNAs as tumor suppressors and oncogenes. Oncogene. 2006; 25: 6188-96.
25. Slattery ML, Herrick JS, Mullany LE, Samowitz WS, Sevens JR, Sakoda L, et al. The co-regulatory networks of tumor suppressor genes, oncogenes, and miRNAs in colorectal cancer. Genes Chromosomes Cancer. 2017; 56: 769-87.
26. Slattery ML, Mullany LE, Sakoda LC, Wolff RK, Samowitz WS, Herrick JS. Dysregulated genes and miRNAs in the apoptosis pathway in colorectal cancer patients. Apoptosis. 2018; 23: 237-50.
27. Mullany LE, Herrick JS, Sakoda LC, Samowitz W, Stevens JR, Wolff RK, et al. miRNA involvement in cell cycle regulation in colorectal cancer cases. Genes Cancer. 2018; 9: 53-65.
28. Mullany LE, Herrick JS, Sakoda LC, Samowitz W, Stevens JR, Wolff RK, et al. MicroRNA-messenger RNA interactions involving JAK-STAT signaling genes in colorectal cancer. Genes Cancer. 2018; 9: 232-46.
29. Slattery ML, Mullany LE, Sakoda LC, Wolff RK, Samowitz WS, Herrick JS. The MAPK-signaling pathway in colorectal cancer: dysregulated genes and their association with microRNAs. Cancer Inform. 2018; 17: 1176935118766522.
30. Slattery ML, Mullany LE, Sakoda L, Samowitz WS, Wolff RK, Stevens JR, et al. The NF-κB signalling pathway in colorectal cancer: associations between dysregulated gene and miRNA expression. J Cancer Res Clin Oncol. 2018; 144: 269-83.
31. Slattery ML, Mullany LE, Sakoda LC, Wolff RK, Stevens JR, Samowitz WS, et al. The PI3K/AKT signaling pathway: associations of miRNAs with dysregulated gene expression in colorectal cancer. Mol Carcinog. 2018; 57: 243-61.
32. Slattery ML, Mullany LE, Wolff RK, Sakoda LC, Samowitz WS, Herrick JS. The p53-signaling pathway and colorectal cancer: Interactions between downstream p53 target genes and miRNAs. Genomics. 2018. (in press)
33. Slattery ML, Mullany LE, Sakoda LC, Samowitz WS, Wolff RK, Stevens JR, et al. Expression of Wnt-signaling pathway genes and their associations with miRNAs in colorectal cancer. Oncotarget. 2018; 9: 6075-85.
34. Zhang GJ, San YZ, Zhang HQ, Zhang JF, Yang Z, Yu ZF, et al. MiR-590-5p as potential oncogenic microRNA of human colorectal cancer cells by targeting PTEN. Int J Clin Exp Pathol. 2017; 10: 1322-30.
35. Li NN, Miao Y, Shan YJ, Liu B, Li Y, Zhao LF, et al. MiR-106b and miR-93 regulate cell progression by suppression of PTEN via PI3K/Akt pathway in breast cancer. Cell Death Dis. 2017; 8: e2796.
36. Hermeking H. MicroRNAs in the p53 network: micromanagement of tumour suppression. Nat Rev Cancer. 2012; 12: 613-26.
37. Tsitsiou E, Lindsay MA. microRNAs and the immune response. Curr Opin Pharmacol. 2009; 9: 514-20.
38. Sang W, Wang Y, Zhang C, Zhang DZ, Sun C, Niu MS, et al. MiR-150 impairs inflammatory cytokine production by targeting ARRB-2 after blocking CD28/B7 costimulatory pathway. Immunol Lett. 2016; 172: 1-10.
39. Li H, Zhou H, Luo JS, Huang J. MicroRNA-17-5p inhibits proliferation and triggers apoptosis in non-small cell lung cancer by targeting transforming growth factor β receptor 2. Exp Ther Med. 2017; 13: 2715-22.
40. Contreras J, Rao DS. MicroRNAs in inflammation and immune responses. Leukemia. 2012; 26: 404-13.
41. Koshizuka K, Hanazawa T, Kikkawa N, Arai T, Okato A, Kurozumi A, et al. Regulation of *ITGA3* by the anti-tumor *miR-199* family inhibits cancer cell migration and invasion in head and neck cancer. Cancer Sci. 2017; 108: 1681-92.
42. Bian K, Fan J, Zhang X, Yang XW, Zhu HY, Wang L, et al. MicroRNA-203 leads to G1 phase cell cycle arrest in laryngeal carcinoma cells by directly targeting survivin. FEBS Lett. 2012; 586: 804-9.
43. Karimkhanloo H, Mohammadi-Yeganeh S, Ahsani Z, Paryan M. Bioinformatics prediction and experimental validation of microRNA-20a targeting Cyclin D1 in hepatocellular carcinoma. Tumour Biol. 2017; 39: 1010428317698361.
44. Ye Y, Zhuang JH, Wang GY, He SF, Ni J, Xia W, et al. microRNA-605 promotes cell proliferation, migration and invasion in non-

small cell lung cancer by directly targeting LATS2. Exp Ther Med. 2017; 14: 867-73.

45. Huang JY, Cui SY, Chen YT, Song HZ, Huang GC, Feng B, et al. MicroRNA-650 was a prognostic factor in human lung adenocarcinoma and confers the docetaxel chemoresistance of lung adenocarcinoma cells via regulating Bcl-2/Bax expression. PLoS One. 2013; 8: e72615.
46. Zhou CX, Cui FY, Li JL, Wang DY, Wei YZ, Wu Y, et al. MiR-650 represses high-risk non-metastatic colorectal cancer progression via inhibition of AKT2/GSK3β/E-cadherin pathway. Oncotarget. 2017; 8: 49534-47.
47. Mullany LE, Herrick JS, Wolff RK, Stevens JR, Samowitz W, Slattery ML. MicroRNA-transcription factor interactions and their combined effect on target gene expression in colon cancer cases. Genes Chromosomes Cancer. 2018; 57: 192-202.
48. Mullany LE, Herrick JS, Wolff RK, Stevens JR, Samowitz W, Slattery ML. Transcription factor-microRNA associations and their impact on colorectal cancer survival. Mol Carcinog. 2017; 56: 2512-26.

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Supplementary materials

Table S1 Associations between miRNAs identified in random forest assessment that are associated with genes with a seed region match and negative beta coefficient in CRC related pathways

MiRNA	Apoptosis	Cell cycle	JAK/STAT	MAPK	NFKB	PI3K/AKT	TP53	TGFB	WNT
MiR-106b-5p			*IL10RA*						
MiR-1243									*ROCK2*
MiR-1271-5p					*TRAF5*				
MiR-145-5p	*BIRC5*	*MAD2L1*, *SMC1A*				*BRCA1*			
MiR-150-5p	*BIRC5*	*CCNA2*, *PRKDC*			*PRKCB*		*RPM2*		
MiR-17-5p			*IL10RA*, *IL6R*	*PDGFRA*	*TNFRSF11A*	*PDGFRA*			
MiR-193b-3p				*DUSP4*					
MiR-195-5p	*BIRC5*	*CDC6*, *SMC1A*							
MiR-196b-5p						*CREB3L1*			
MiR-196b-5p	*TNFRSF10B*				*TNFRSF11A*		*TNFRSF10B*		
MiR-199a-5p					*TNFRSF11A*				
MiR-19b-3p			*IL6R*	*PDGFRA*		*PDGFRA*			
MiR-203a	*BLC2*		*LIFR*	*MEF2C*, *PDGFRA*, *PRKCB*	*BTK*, *BCL2*, *PLCG2*	*PIK3CG*; *ITGA4*; *PDGFRA*			
MiR-204-3p			*BCL2*						*ROCK2*
MiR-20a-5p			*1L10RA*, *IL6R*			*PDGFRA*; *IL6R*			
MiR-20b-5p	*CTSS*		*IL10RA*, *IL6R*		*BTK*	*PDGFRA*; *IL6R*, *BCL2*, *GNG2*			
MiR-2117				*TGFBR1*				*TGFBR1*	
MiR-214-3p					*TNFRSF11A*	*PHLPP2*			
MiR-215					*PLAU*				
MiR-21-5p			*IL6R*						
MiR-221-3p			*IL6R*						
MiR-23a-3p			*IL6R*						
MiR-27a-3p			*IL6R*						
MiR-29b-3p	*CASP7*					*PDGFRA*			
MiR-324					*CCL13*				
MiR-3591-3p									*SFRP5*
MiR-3651			*IL6R*						
MiR-365a-3p					*TNFRSF11A*				

Continued

Continued

MiRNA	Apoptosis	Cell cycle	JAK/STAT	MAPK	NFKB	PI3K/AKT	TP53	TGFB	WNT
MiR-375		*YWHAB*			*PLCG1*	*YWHAB*, *THBS2*		*TGIF2*	
MiR-424-3p		*CDC6*							
MiR-429			*IL10RA*	*RASGRP3*	*PLCG2*				
MiR-4749-3p								*TGIF2*	
MiR-501-3p	*CTSS*					*PDGFRA*			
MiR-6071				*TGFBR1*		*ITGAV*		*TGFBR1*	
MiR-650	*BIRC5*	*MAD2L1*	*PTPN11*				*PERP*; *RPM2*		
MiR-6515-5p		*YWHAB*				*YWHAB*			
MiR-663b						*PHLPP2*			
MiR-92a-3p	*CSF2RB*		*CSF2RB*		*TNFSF11A*				
MiR-934					*TNFRSF11A*				
MiR-93-5p			*IL10RA*						

Table S2 MiRNA and mRNA associations by pathway

MiRNA	Direction	Apoptosis	Cell cycle	JAK-STAT	MAPK	NFkB	PI3K-AKT	TGFB	p53	WNT
hsa-let-7i-5p	Indirect		*YWHAB*		*TGFBR1*		*COL1A2*, *COL6A3*, *F2R*, *ITGAV*, *THBS2*, *TNC*, *YWHAB*	*INHBA*, *TGFBR1*	*CCND1*, *CDK1*	*SFRP4*, *SFRP5*
hsa-miR-106b-5p	Direct			*IL10RA*						
hsa-miR-106b-5p	Indirect	*BIRC5*	*BUB3*, *CCNA2*, *CCND1*, *CDC25C*, *CDK1*, *MAD2L1*, *MCM3*, *MCM4*, *MCM6*, *PRKDC*, *RAD21*, *YWHAB*, *YWHAG*, *YWHAQ*	*CCND1*, *PTPN11*			*CCND1*, *ITGA2*, *YWHAB*, *YWHAG*, *YWHAQ*	*TGIF2*	*RRM2*	*CCND1*
hsa-miR-10a-5p	Indirect						*ITGA2*			
hsa-miR-1203	Indirect	*CSF2RB*		*CSF2RB*	*TGFBR1*		*ITGAV*	*TGFBR1*		
hsa-miR-124-3p	Indirect	*CSF2RB*		*CSF2RB*						*DAAM2*
hsa-miR-1243	Direct									*ROCK2*
hsa-miR-1243	Indirect						*ITGAV*			

Continued

Continued

MiRNA	Direction	Apoptosis	Cell cycle	JAK-STAT	MAPK	NFkB	PI3K-AKT	TGFB	p53	WNT
hsa-miR-1246	Indirect	*BIRC5*	*ESPL1, MCM6, MYC, PRKDC, RAD21, RBL1, YWHAB*	*MYC, PTPN11*	*MYC*	*PLCG1*	*BRCA1, MYC, YWHAB*	*MYC, RBL1, TGIF2*		*MYC*
hsa-miR-1271-5p	Direct					*TRAF5*				
hsa-miR-1291	Direct									
hsa-miR-1291	Indirect						*ITGA2*	*TGIF2*	*PERP*	
hsa-miR-130b-3p	Indirect		*CCNA2, ESPL1, MAD2L1, MCM4, PRKDC, YWHAB*			*PLCG1*	*YWHAB*	*TGIF2*		
hsa-miR-133b	Indirect			*FHL1, LIFR*		*CXCL12*	*CHRM2, TNXB*			*DAAM2, PRICKLE2*
hsa-miR-145-5p	Direct	*BIRC5*	*MAD2L1, SMC1A*				*BRCA1*			
hsa-miR-145-5p	Indirect		*BUB1B, CCNB1, CDC20, CDC25C, CDC6, MCM4, TFDP1*	*FHL1, LIFR*	*PDGFRA*	*CXCL12*	*CHRM2, IGF1, PDGFRA, THBS1, TNXB*	*TFDP1, THBS1*	*CCNB1, GTSE1, IGF1, RRM2, THBS1*	*DAAM2, PRICKLE2 , SFRP4*
hsa-miR-146a-5p	Indirect					*TRAF5*				
hsa-miR-146b-5p	Indirect			*STAT1*			*F2R, TNC*			
hsa-miR-150-5p	Direct	*BIRC5*	*CCNA2, PRKDC*						RRM2	
hsa-miR-150-5p	Indirect	*BCL2, CSF2RB, ITPR1, PIK3CD, TUBA1B*	*SMC1A*	*BCL2, CSF2RB, IL10RA, IL24, IL6R, IL6ST, IL7R, LIFR, PIK3CD, PTPN11*	*MAP4K1, MEF2C, PDGFRA, PRKCB, RAC2, RASGRP2, RASGRP3*	*BCL2, BTK, CCL21, CD40, CXCL12, PLCG2, PRKCB, ZAP70*	*BCL2, CD19, GNG2, GNG7, IL6R, IL7R, ITGA4, PDGFRA, PHLPP2, PIK3CD, PIK3CG*			*DAAM2, PLCB2, PRKCB, RAC2*
hsa-miR-151a-3p	Indirect		*CDC16, PRKDC, RAD21, YWHAB*				*YWHAB*	*TGIF2*	*PERP*	
hsa-miR-15a-5p	Indirect		*CDC16, RAD21, YWHAB*				*YWHAB*			
hsa-miR-17-5p	Direct			*IL10RA, IL6R*	*PDGFRA*	*TNFRSF11A*	*PDGFRA, IL6R*			

Continued

Continued

MiRNA	Direction	Apoptosis	Cell cycle	JAK-STAT	MAPK	NFkB	PI3K-AKT	TGFB	p53	WNT
hsa-miR-17-5p	Indirect	*BIRC5*	*ANAPC1*, *CCNA2*, *CCND1*, *CDC16*, *CDC25C*, *CDK1*, *CDK4*, *E2F5*, *ESPL1*, *MAD2L1*, *MCM3*, *MCM4*, *MCM6*, *MYC*, *PRKDC*, *RAD21*, *RBL1*, *TFDP1*, *YWHAB*, *YWHAG*, *YWHAQ*	*CCND1*, *MYC*, *PTPN11*	*HSPA8*, *MYC*	*PLCG1*	*BRCA1*, *CCND1*, *CDK4*, *GNG4*, *ITGA2*, *LAMA5*, *MYC*, *YWHAB*, *YWHAG*, *YWHAQ*	*E2F5*, *MYC*, *RBL1*, *TFDP1*, *TGIF2*	*CCND1*, *CDK1*, *CDK4*, *PERP*, *RRM2*	*CCND1*, *MYC*, *ROCK2*
hsa-miR-1915-5p	Indirect									*SFRP5*
hsa-miR-193b-3p	Direct				*DUSP4*					
hsa-miR-193b-3p	Indirect	*CTSK*	*YWHAB*	*FHL1*, *LIFR*	*IL1R1*, *MEF2C*, *PDGFRB*	*CXCL12*, *IL1R1*, *TNFRSF11A*	*COL1A1*, *COL1A2*, *COL6A3*, *CREB3L1*, *F2R*, *ITGA11*, *ITGB5*, *LAMA5*, *PDGFRB*, *THBS2*, *YWHAB*	*TGIF2*		*PRICKLE2*, *SFRP4*, *SFRP5*
hsa-miR-195-5p	Direct	*BIRC5*	*CDC6*, *SMC1A*	*PTPN11*						
hsa-miR-195-5p	Indirect	*BCL2*, *TUBA1B*	*CCNB1*, *MAD2L1*	*BCL2*, *FHL1*, *IL10RA*, *IL6ST*, *LIFR*	*MEF2C*	*BCL2*, *CCL21*, *CXCL12*, *PLCG2*	*BCL2*		*CCNB1*, *RRM2*	*DAAM2*
hsa-miR-196a-5p	Indirect		*ANAPC1*, *CCNA2*, *CDC25A*, *CDC6*, *MAD2L1*, *PRKDC*, *RAD21*, *YWHAB*				*YWHAB*	*TGIF2*	*GTSE1*, *STEAP3*	
hsa-miR-196b-5p	Direct	*TNFRSF10B*				*TNFRSF11A*	*CREB3L1*		*TNFRSF10B*	
hsa-miR-196b-5p	Indirect		*RBL1*, *YWHAB*		*DUSP4*	*PLCG1*	*PHLPP2*, *SGK2*, *YWHAB*	*RBL1*, *TGIF2*		*PLCB4*

Continued

Continued

MiRNA	Direction	Apoptosis	Cell cycle	JAK-STAT	MAPK	NFkB	PI3K-AKT	TGFB	p53	WNT
hsa-miR-199a-3p	Indirect	*TUBA1B*	*YWHAB*		*PDGFRB*, *TGFBR1*		*COL1A1*, *COL1A2*, *COL6A3*, *F2R*, *ITGA11*, *ITGAV*, *ITGB5*, *PDGFRB*, *THBS2*, *TNC*, *YWHAB*	*INHBA*, *TGFBR1*		*SFRP4*
hsa-miR-199a-5p	Direct					*TNFRSF11A*				
hsa-miR-199a-5p	Indirect	*TUBA1B*	*YWHAB*		*PDGFRB*, *TGFBR1*		*COL1A1*, *COL1A2*, *COL6A3*, *F2R*, *ITGA11*, *ITGAV*, *ITGB5*, *PDGFRB*, *THBS2*, *TNC*, *YWHAB*	*INHBA*, *TGFBR1*		*SFRP4*
hsa-miR-199b-5p	Indirect	*TUBA1B*	*CDC16*, *YWHAB*		*PDGFRB*		*COL1A1*, *COL1A2*, *COL6A3*, *F2R*, *ITGA11*, *ITGB5*, *PDGFC*, *PDGFRB*, *THBS2*, *TNC*, *YWHAB*	*INHBA*	*PERP*	*SFRP4*
hsa-miR-19b-3p	Direct			*IL6R*	*PDGFRA*		*IL6R*, *PDGFRA*			
hsa-miR-19b-3p	Indirect	*BIRC5*	*ANAPC1*, *BUB3*, *CCND1*, *CDC16*, *CDC25C*, *CDK1*, *ESPL1*, *MAD2L1*, *MCM3*, *MCM4*, *MCM6*, *MYC*, *PRKDC*, *RAD21*, *RBL1*, *TFDP1*, *YWHAB*, *YWHAG*, *YWHAQ*	*CCND1*, *MYC*, *PTPN11*	*MYC*		*BRCA1*, *CCND1*, *ITGA2*, *MYC*, *YWHAB*, *YWHAG*, *YWHAQ*	*MYC*, *RBL1*, *TFDP1*, *TGIF2*	*CCND1*, *CDK1*, *PERP*, *RRM2*	*CCND1*, *MYC*, *ROCK2*
hsa-miR-203a	Direct	*BCL2*		*BCL2*, *LIFR*	*MEF2C*, *PDGFRA*, *PRKCB*	*BCL2*, *BTK*, *PRKCB*, *PLCG2*	*BCL2*, *GNG2*, *ITGA4*, *PDGFRA*, *PIK3CG*			*PRKCB*

Continued

Continued

MiRNA	Direction	Apoptosis	Cell cycle	JAK-STAT	MAPK	NFkB	PI3K-AKT	TGFB	p53	WNT
hsa-miR-203a	Indirect		*CCND1*, *CDC25C*, *PRKDC*, *RAD21*	*CCND1*, *IL10RA*, *IL6R*, *IL7R*, *PTPN11*	*HSPA8*, *RAC2*	*CCL21*	*CCND1*, *HSP90AA1*, *HSP90AB1*, *IL6R*, *IL7R*, *ITGA2*		*CCND1*, *PERP*	*CCND1*, *RAC2*
hsa-miR-204-3p	Direct									*ROCK2*
hsa-miR-204-3p	Indirect						*ITGAV*			
hsa-miR-20a-5p	Direct			*IL6R*, *IL10RA*	*PDGFRA*		*IL6R*, *PDGFRA*			
hsa-miR-20a-5p	Indirect	*BIRC5*	*ANAPC1*, *CCND1*, *CDC16*, *CDK4*, *E2F5*, *ESPL1*, *MAD2L1*, *MCM3*, *MCM4*, *MCM6*, *MYC*, *PRKDC*, *RAD21*, *RBL1*, *TFDP1*, *YWHAB*, *YWHAG*, *YWHAQ*	*CCND1*, *MYC*, *PTPN11*	*MYC*	*PLCG1*	*BRCA1*, *CCND1*, *CDK4*, *GNG4*, *HSP90AB1*, *ITGA2*, *MYC*, *YWHAB*, *YWHAG*, *YWHAQ*	*E2F5*, *MYC*, *RBL1*, *TFDP1*, *TGIF2*	*CCND1*, *CDK4*, *PERP*, *RRM2*	*CCND1*, *MYC*, *ROCK2*
hsa-miR-20b-5p	Direct	*CTSS*		*IL6R*, *IL10RA*	*PDGFRA*	*BTK*, *TNFRSF11A*	*IL6R*, *PDGFRA*			
hsa-miR-20b-5p	Indirect	*BIRC5*	*ANAPC1*, *BUB3*, *CCNA2*, *CCND1*, *CDC16*, *CDC25C*, *CDK1*, *CDK4*, *MAD2L1*, *MCM3*, *MCM4*, *MCM6*, *MYC*, *PRKDC*, *RAD21*, *RBL1*, *TFDP1*, *YWHAB*, *YWHAG*, *YWHAQ*	*CCND1*, *MYC*, *PTPN11*	*MYC*	*CSNK2A2*, *PLCG1*	*BRCA1*, *CCND1*, *CDK4*, *GNG4*, *ITGA2*, *MYC*, *YWHAB*, *YWHAG*, *YWHAQ*	*MYC*, *RBL1*, *TFDP1*, *TGIF2*	*CCND1*, *CDK1*, *CDK4*, *PERP*, *RRM2*, *STEAP3*	*CCND1*, *CSNK2A2*, *MYC*, *ROCK2*
hsa-miR-21-3p	Indirect	*BIRC5*	*PRKDC*, *RAD21*, *TFDP1*, *YWHAB*, *YWHAG*, *YWHAQ*	*PTPN11*			*ITGAV*, *YWHAB*, *YWHAG*, *YWHAQ*	*TFDP1*	*PERP*, *RRM2*	*CTNNB1*
hsa-miR-21-5p	Direct			*IL6R*			*IL6R*			
hsa-miR-21-5p	Indirect		*CCND1*, *PRKDC*, *RAD21*, *YWHAB*, *YWHAG*	*CCND1*, *PTPN11*	*TGFBR1*		*CCND1*, *ITGA2*, *ITGAV*, *LPAR1*, *TNC*, *YWHAB*, *YWHAG*	*INHBA*, *TGFBR1*	*CCND1*, *PERP*	*CCND1*, *ROCK2*, *SFRP5*

Continued

Continued

MiRNA	Direction	Apoptosis	Cell cycle	JAK-STAT	MAPK	NFkB	PI3K-AKT	TGFB	p53	WNT
hsa-miR-2117	Direct				*TGFBR1*					
hsa-miR-2117	Indirect	*CSF2RB*		*CSF2RB*, *IL6R*			*IL6R*	*TGFBR1*		
hsa-miR-214-3p	Direct					*TNFRSF11A*	*PHLPP1*			
hsa-miR-214-3p	Indirect	*TUBA1B*			*IL1R1*, *PDGFRB*, *TGFBR1*	*IL1R1*	*COL1A1*, *COL1A2*, *COL6A3*, *F2R*, *ITGA11*, *ITGAV*, *ITGB5*, *PDGFRB*, *THBS2*, *TNC*	*INHBA*, *TGFBR1*		*SFRP4*
hsa-miR-215	Direct					*PLAU*				
hsa-miR-215	Indirect						*COL1A1*			
hsa-miR-221-3p	Direct			*IL6R*	*MEF2C*		*IL6R*			
hsa-miR-221-3p	Indirect	*CASP7*	*CCND1*, *CDK1*, *MCM3*, *MCM4*, *MCM6*, *PRKDC*, *RAD21*, *YWHAB*, *YWHAG*, *YWHAQ*	*CCND1*, *IL10RA*, *PTPN11*	*HSPA8*	*CCL13*	*CCND1*, *HSP90AB1*, *ITGA2*, *YWHAB*, *YWHAG*, *YWHAQ*	*TGIF2*	*CCND1*, *CDK1*, *RRM2*, *PERP*	*CCND1*, *CTNNB1*
hsa-miR-222-3p	Indirect								*PERP*	*SFRP5*
hsa-miR-23a-3p	Direct			*IL6R*			*IL6R*			
hsa-miR-23a-3p	Indirect		*PRKDC*, *YWHAB*		*TGFBR1*		*ITGA2*, *ITGAV*, *YWHAB*	*TGFBR1*	*PERP*	*SFRP5*
hsa-miR-24-3p	Indirect		*PRKDC*, *YWHAB*		*TGFBR1*		*ITGA2*, *ITGAV*, *ITGB5*, *YWHAB*	*TGFBR1*	*PERP*	*SFRP5*
hsa-miR-25-3p	Indirect	*BIRC5*	*BUB3*, *CCNA2*, *CCNB1*, *CDC25C*, *CDK1*, *ESPL1*, *MAD2L1*, *MCM3*, *MCM4*, *MCM6*, *PRKDC*, *RAD21*, *RBL1*, *SKP2*, *YWHAB*, *YWHAG*, *YWHAQ*	*PTPN11*		*PLCG1*	*BRCA1*, *YWHAB*, *YWHAG*, *YWHAQ*	*RBL1*, *TGIF2*	*CCNB1*, *CDK1*, *RRM2*	

Continued

Continued

MiRNA	Direction	Apoptosis	Cell cycle	JAK-STAT	MAPK	NFkB	PI3K-AKT	TGFB	p53	WNT
hsa-miR-27a-3p	Direct			*IL6R*			*IL6R*			
hsa-miR-27a-3p	Indirect		*CCND1*, *PRKDC*, *RAD21*, *YWHAB*, *YWHAG*, *YWHAQ*	*CCND1*, *PTPN11*			*CCND1*, *ITGA2*, *ITGAV*, *YWHAB*, *YWHAG*, *YWHAQ*		*CCND1*, *PERP*	*CCND1*, *CTNNB1*
hsa-miR-29a-3p	Indirect		*PRKDC*, *RAD21*, *YWHAB*, *YWHAQ*			TRAF5	*ITGA2*, *YWHAB*, *YWHAQ*		*PERP*	*ROCK2*, *SFRP5*
hsa-miR-29b-3p	direct	*CASP7*			*PDGFRA*		*PDGFRA*			
hsa-miR-29b-3p	Indirect		*CCND1*, *MCM3*, *MCM4*, *PRKDC*, *RAD21*, *YWHAB*, *YWHAG*, *YWHAQ*	*CCND1*, *PTPN11*	*HSPA8*		*CCND1*, *ITGA2*, *YWHAB*, *YWHAG*, *YWHAQ*		*CCND1*, *PERP*	*CCND1*
hsa-miR-30a-5p	Indirect			*FHL1*, *LIFR*			*TNXB*			*DAAM2*
hsa-miR-3124-5p	Indirect	*CSF2RB*, *TNFRSF10B*		*CSF2RB*					*TNFRSF10B*	
hsa-miR-32-3p	Indirect		*YWHAB*	*PTPN11*			*YWHAB*			
hsa-miR-324-5p	Direct					*CCL13*				
hsa-miR-324-5p	Indirect		*PRKDC*						*PERP*	
hsa-miR-330-3p	Indirect					*TNFRSF11A*				
hsa-miR-331-3p	Indirect				*TGFBR1*		*LAMA5*	*TGFBR1*		
hsa-miR-34a-5p	Indirect		*PRKDC*, *RAD21*, *YWHAB*	*PTPN11*		CCL13	*ITGA2*, *ITGAV*, *YWHAB*		*PERP*	
hsa-miR-3591-3p	Direct									*SFRP5*
hsa-miR-361-5p	Indirect		*CDC16*, *PRKDC*, *YWHAB*			*TNFRSF11A*	*YWHAB*	*TGIF2*		*SFRP5*
hsa-miR-3651	Direct			*IL6R*			*IL6R*			
hsa-miR-3651	Indirect		*CDK4*, *MYC*, *PRKDC*, *RAD21*, *RBL1*, *YWHAB*, *YWHAG*, *YWHAQ*	*IL10RA*, *MYC*, *PTPN11*	*MYC*		*CDK4*, *GNG4*, *ITGA2*, *MYC*, *YWHAB*, *YWHAG*, *YWHAQ*	*MYC*, *RBL1*, *TGIF2*	*CDK4*, *PERP*	*MYC*, *ROCK2*, *SFRP5*
hsa-miR-365a-3p	Direct					*TNFRSF11A*				
hsa-miR-365a-3p	Indirect		*PRKDC*				*ITGB5*, *TNXB*		*GTSE1*	
hsa-miR-375	Direct		*YWHAB*			*PLCG1*	*THBS2*, *YWHAB*	*TGIF2*		
hsa-miR-375	Indirect		*MYC*	*MYC*	*MYC*	*TNFRSF11A*	*COL1A1*, *CREB3L1*, *MYC*	*MYC*		*MYC*

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MiRNA	Direction	Apoptosis	Cell cycle	JAK-STAT	MAPK	NFkB	PI3K-AKT	TGFB	p53	WNT
hsa-miR-378g	Indirect					*TRAF5*				*SFRP5*
hsa-miR-3923	Indirect	*CTSS*				*TRAF5*				
hsa-miR-3976	Indirect						*TNXB*			
hsa-miR-424-3p	Direct		*CDC6*							
hsa-miR-424-3p	Indirect		*YWHAB*, *YWHAE*	*OSM*	*DUSP4*	*PLCG1*, *TNFRSF11A*	*CREB3L1*, *GNG4*, *OSM*, *YWHAB*, *YWHAE*	*TGIF2*		
hsa-miR-425-5p	Indirect	*BIRC5*	*PRKDC*, *RAD21*, *YWHAB*, *YWHAQ*	*PTPN11*			*BRCA1*, *COMP*, *YWHAB*, *YWHAQ*	*TGIF2*		
hsa-miR-4251	Indirect						*ITGAV*			*SFRP5*
hsa-miR-429	Direct			*IL10RA*	*RASGRP3*	*PLCG2*				
hsa-miR-429	Indirect		*PRKDC*, *RAD21*							
hsa-miR-4315	Indirect	*CSF2RB*		*CSF2RB*						CTNNB1
hsa-miR-4421	*Indirect*									SFRP5
hsa-miR-4469	Indirect	*CSF2RB*		*CSF2RB*						
hsa-miR-4638-3p	Indirect									*SFRP5*
hsa-miR-4749-3p	Direct							*TGIF2*		
hsa-miR-4749-3p	Indirect		*YWHAB*				*YWHAB*			*ROCK2*
hsa-miR-483-3p	Indirect							*TGIF2*		
hsa-miR-497-5p	Indirect			*IL6ST*, *LIFR*		*CCL21*, *CXCL12*	*LPAR1*, *TNXB*			*DAAM2*
hsa-miR-5008-3p	Indirect									*SFRP5*
hsa-miR-501-3p	Direct	*CTSS*			*PDGFRA*		*PDGFRA*			
hsa-miR-501-3p	Indirect		*CDC25C*, *MAD2L1*, *MYC*, *PRKDC*, *YWHAB*	*MYC*	*MYC*		*GNG4*, *ITGA2*, *MYC*, *YWHAB*	*MYC*, *TGIF2*	*PERP*	*MYC*
hsa-miR-518c-5p	Indirect			*CNTF*, *IL6R*			*IL6R*			
hsa-miR-520d-3p	Indirect					*TRAF5*				
hsa-miR-525-5p	Indirect	*CSF2RB*		*CSF2RB*						
hsa-miR-5685	Indirect					*TRAF5*				*SFRP5*
hsa-miR-583	Indirect		*MAD2L1*, *MYC*, *SMC1A*	*MYC*	*MYC*	*PLCG1*	*MYC*	*MYC*, *TGIF2*	*STEAP3*	*MYC*
hsa-miR-590-5p	Direct					*TNFRSF11A*				
hsa-miR-6071	Direct				*TGFBR1*		*ITGAV*			
hsa-miR-6071	Indirect							*TGFBR1*		
hsa-miR-650	Direct	*BIRC5*	*MAD2L1*	*PTPN11*			*ITGA2*		*PERP*, *RRM2*	

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MiRNA	Direction	Apoptosis	Cell cycle	JAK-STAT	MAPK	NFkB	PI3K-AKT	TGFB	p53	WNT
hsa-miR-650	Indirect	*BCL2*, *CSF2RB*, *ITPR1*, *PIK3CD*	*CCNA2*, *MCM4*, *PRKDC*, *RAD21*, *YWHAG*, *YWHAH*	*BCL2*, *CSF2RB*, *IL10RA*, *IL6R*, *IL6ST*, *IL7R*, *LIFR*, *PIK3CD*	*MAP4K1*, *MEF2C*, *PDGFRA*, *PRKCB*, *RAC2*, *RASGRP2*, *RASGRP3*	*BCL2*, *BTK*, *CCL21*, *CD40*, *PLCG2*, *PRKCB*	*BCL2*, *BRCA1*, *GNG2*, *GNG7*, *IL6R*, *IL7R*, *ITGA4*, *PDGFRA*, *PIK3CD*, *PIK3CG*, *YWHAG*, *YWHAH*			*PLCB2*, *PRKCB*, *RAC2*
hsa-miR-6515-5p	Direct		*YWHAB*				*YWHAB*			
hsa-miR-6515-5p	Indirect					*CSNK2A2*	*CREB3L1*, *HSP90AB1*			*CSNK2A2*, *ROCK2*
hsa-miR-663a	Indirect		*MYC*, *YWHAB*	*MYC*	*MYC*		*GNG4*, *MYC*, *YWHAB*	*MYC*		*MYC*, *ROCK2*
hsa-miR-663b	Direct					*TNFRSF11A*	*PHLPP1*			
hsa-miR-663b	Indirect		*MYC*, *PRKDC*, *RBL1*, *YWHAB*	*MYC*	*MYC*	*PLCG1*	*GNG4*, *HSP90AB1*, *ITGA2*, *MYC*, *YWHAB*	*MYC*, *RBL1*, *TGIF2*	*PERP*	*MYC*, *PLCB4*, *ROCK2*
hsa-miR-6716-3p	Indirect									*SFRP5*
hsa-miR-92a-3p	Direct	*CSF2RB*		*CSF2RB*						
hsa-miR-92a-3p	Indirect	*BCL2L1*	*ANAPC1*, *CDC16*, *MYC*, *PRKDC*, *RAD21*, *RBL1*, *TFDP1*, *YWHAB*, *YWHAQ*	*BCL2L1*, *IL6R*, *MYC*, *PTPN11*	*MYC*	*BCL2L1*, *CSNK2A2*, *PLCG1*, *TNFRSF11A*, *TRAF5*	*BCL2L1*, *BRCA1*, *CREB3L1*, *GNG4*, *IL6R*, *ITGA2*, *MYC*, *YWHAB*, *YWHAQ*	*MYC*, *TFDP1*, *TGIF2*, *RBL1*	*PERP*	*CSNK2A2*, *MYC*, *PLCB4*, *ROCK2*
hsa-miR-93-5p	Direct			*IL10RA*						
hsa-miR-93-5p	Indirect	*BIRC5*	*ANAPC1*, *BUB1*, *BUB3*, *CCNA2*, *CCNB1*, *CCND1*, *CDC25C*, *CDC6*, *CDK1*, *CDK4*, *ESPL1*, *MAD2L1*, *MCM3*, *MCM4*, *MCM6*, *PRKDC*, *RAD21*, *RBL1*, *SKP2*, *YWHAB*, *YWHAG*, *YWHAQ*	*CCND1*, *PTPN11*	*HSPA8*		*BRCA1*, *CCND1*, *CDK4*, *ITGA2*, *YWHAB*, *YWHAG*, *YWHAQ*	*TGIF2*, *RBL1*	*CCNB1*, *CCND1*, *CDK1*, *CDK4*, *RRM2*	*CCND1*, *ROCK2*
hsa-miR-934	Direct					*TNFRSF11A*				

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MiRNA	Direction	Apoptosis	Cell cycle	JAK-STAT	MAPK	NFkB	PI3K-AKT	TGFB	p53	WNT
hsa-miR-934	Indirect	*TUBA1B*		*OSM*	*PDGFRB*	*PLAU*	*COL1A1*, *COL1A2*, *COL6A3*, *COMP*, *F2R*, *ITGA11*, *ITGAV*, *ITGB5*, *OSM*, *PDGFC*, *PDGFRB*, *THBS2*, *TNC*	*INHBA*		*SFRP4*
hsa-miR-99a-5p	Indirect		*LIFR*			*ITGA11*, *ITGB5*, *PDGFC*, *TNXB*		*GTSE1*, *RRM2*	*SFRP4*	