Hsa-miR-3120/miR-214 Mirror miRNAs Alteration in Colorectal Cancer

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Research Article

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Abstract

Colorectal cancer is one of the most common cancers, and various studies have shown that many genes, including miRNAs, play important roles in the development of this cancer. Here, we investigated the molecular and cellular effect of miR-3120 and its mirror miRNA, miR-214, in colorectal cancer using bioinformatics and experimental techniques. Microarray data analysis showed that miR-3120/miR-214 expression are deregulated in colorectal cancer tumors and RT-qPCR analysis of these miRNAs showed a negative expression correlation in different colorectal cancer-originated cell lines. Also, RT-qPCR result indicated that miR-3120 and miR-214 affects each other expression. Overexpression of miR-3120 in HCT-116 cell line followed by qRT-PCR showed increased expression of SMAD3, SMAD4 and AKT2 genes, whereas overexpression of miR-214 inversed this effect. In addition, the expression of specific target genes of each microRNA showed a pattern of co-expression with its microRNAs. Also, investigating the effect of miR-3120/miR-214 on the cell cycle, showed their promoting effect on the progression of the cell cycle. Overall, these data suggest that miR-3120/miR-214 may be involved in regulating the molecular pathways of colorectal cancer, and part of this regulation could be related to the interaction of these genes with each other.

Introduction

Despite significant advances in the treatment of colorectal cancer, metastatic death and recurrence of the disease still exist(Aran, Victorino et al. 2016). Studies show that CRC is a heterogeneous disease that develops through both the accumulation of genetic mutations and epigenetic changes (Aran, Victorino et al. 2016, Punt, Koopman et al. 2017). In this regard, understanding the molecular mechanisms involved in cancer can be useful for finding new solutions(Mollaei, Safaralizadeh et al. 2019, Takahashi, Prieto-Vila et al. 2019).

MicroRNAs are a class of small non-coding RNAs that control gene expression by controlling the translation process by affecting mRNA expression or stability(Oliveto, Mancino et al. 2017, O'Brien, Hayder et al. 2018). Various studies have shown that miRNAs are involved in the important cellular processes, including cell proliferation, apoptosis, differentiation, and so on(Gurtan and Sharp 2013, Gebert and MacRae 2019). Therefore, depending on what genes they target and regulate, their expression may change during tumorigenesis, and they may play the role of oncogenes or suppressor tumors(Zhang, Pan et al. 2007, Svoronos, Engelman et al. 2016).

miR-3120 was first discovered in 2010 by data mining in data related to melanoma(Stark, Tyagi et al. 2010), and then during another study in 2012, this microRNA was introduced as the first mirror microRNA for miR-214(Scott, Howarth et al. 2012). During the research that has been done so far, the targeting of the Axin2 gene as well as the HSP70 gene by miR-3120 has been investigated(Scott, Howarth et al. 2012, Hongdan, Feng et al. 2018). MiR-3120 is located in the cytogenic band 1q24.3 within the 14th intron of dynamin 13 gene. In addition to being involved in neuronal communication, antigen delivery, etc., the gene that hosts the miR-3120 also plays an important role in the development of cancer(Meng 2017).

While the miR-3120 is transcribed and processed from the DNM3 gene, miR-214, along with its host gene, which is a long noncoding RNA called DNM3OS, is transcribed in the anti-sense and complementary gene(Watanabe, Sato et al. 2008). Perhaps such microRNAs play important roles in regulating each other by regulating each other or hosting genes that remain unknown.

The aim of this study was to investigate the molecular and cellular effects of miR-3120 and its mirror miRNA (miR-214) on colorectal cancer using bioinformatics and experimental methods. The results of this study show that these two miRNAs can play a role in colorectal cancer by affecting different target genes' expression and part of this regulation could be related to the interaction of these miRNAs with each other.

Methods And Material

Bioinformatics

GSE126093 experiment were download from the NCBI GEO database. Data were normalized and expression of genes and microRNAs was analyzed with DESeq2 package. TargetScan (http://www.targetscan.org/vert_72/), Diana-microT-CDS (http://diana.imis.athena-innovation.gr/DianaTools/index.php?r=microT_CDS/index) and MiRDB (http://mirdb.org/) prediction tools were used for miRNA-target prediction.

Cell culture and transfection

The SW480, HCT116, and HT29 colorectal cancer cell lines and HEK293 cell line were purchased from the Pasteur institute, IRAN. The cells were cultured in DMEM supplemented with 10% (v/v) fetal bovine serum (FBS, Hyclone) in a humidified incubator at 37 °C. MiR-3120, miR-214 and control vectors were transfected with Turbofect according to the manufacturer's instructions.

Quantitative real-time PCR

Total RNA samples were extracted using RiboEx according to the manufacturer's instructions. M-MLV reverse transcriptase (Fermentas) was used to generate cDNA, which was later used as a PCR template. Quantitative real-time PCR (qRT-PCR) was performed in triplicate. The expression of U48 and B2M was used as internal controls.

Flow cytometry

36 hours after transfection, the cells were dissociated into a single cell suspension by trypsin digestion. After being washed with PBS, the cells were incubated with PI stain for 10 min before flow cytometric analysis. Flowjo software was used for data analysis.

Statistical analysis

Statistical analysis was performed using Graphpad prism sofware. Data are expressed as the mean ± standard error of the mean (SEM) of three independent experiments. T-test was used to evaluate statistical difference. P<0.05 was considered to indicate a statistically significant result.

Result

MiR-3120 is differentially expressed in colorectal cancer tumors

The GSE126093 results was first downloaded from the NCBI GEO database. In this experiment, the sequences of 10 colorectal tumor specimens were compared with 10 control specimens from the surrounding tissue. Data were normalized and expression of the genes and miRNAs was analyzed with DESeq2 package and 41 miRNAs with LogFC> 1 and P-Value <0.05 were obtained. Among the differentially expressed miRNAs (with unknown function in colorectal cancer), miR-3120-5p/3p showed the highest expression difference, so it was chosen for further investigation. In addition, miR-214-5p/3p expression in colorectal cancer was evaluated through the aforementioned experiment and the results showed a decreased expression level of this miRNA (especially miR-214-3p) in the most of cancer samples compared to the normal ones (Fig. 1).

MiR-3120-5p and miR-214-3p expressions are inversely correlated

MiR-3120-5p and miR-214-3p exist as mirror sequences in the genome, so they are likely to act as regulators of each other and also different target genes. In order to investigate the possible relationship between these two miRNAs in colorectal cancer, the expression of miR-3120-5p and miR-214-3p was evaluated in 3 different cell colorectal cancer cell lines (HT29, HCT116, SW480). As shown in the Fig. 2, miR-3120-5p shows the highest expression in SW480 and the lowest expression in HT29, while miR-214-3p shows the highest expression in HT29 and the lowest expression in SW480. In other words, these two genes represent the opposite pattern of expression.

Effect of miR-3120/miR-214 overexpression on each other and target genes expression

To investigate the effect of this microRNA on target genes, HCT-116 cells were transfected with miR-3120/miR-214-expressing and mock vectors. RT-qPCR of the expression of the miRNAs indicated that transfection was successful. MiR-3120 expression was respectively up-regulated and down-regulated in miR-3120 and miR-214 transfected cells. However, unlike the miR-3120 expression, miR-214 expression

was down-regulated and up-regulated in miR-3120 and miR-214 transfected cells, respectively (Fig. 3). The result indicated that miR-3120 and miR-214 affects each other expression.

Next, to find regulatory mechanism of miR-3120, TargetScan, Diana-microT-CDS, and miRDB software tools were used to investigate the probable target genes of miR-3120-5p/3p and miR-214-5p-3p. Some of these genes studied were specific to the miR-3120, some were common, and some were specific to the miR-214. These software tools predicted SMAD3, SMAD4 and AKT2 as a potential common target genes of miR-3120 and miR-214. MicroRNA-target prediction results are presented in the Fig. 4A.

The effect of miR-3120/miR-214 transfection on different types of predicted genes related to different signaling pathways was evaluated. The results of RT-qPCR of the genes expression showed that in most cases these two genes have distinctive effects on the target genes. The results showed that miR-3120 overexpression ended in up-regulated SMAD4, AKT2 expression and down-regulated TGFBR2, Klf4, Erbb2 expression. While, miR-214 overexpression decreased all of the predicted genes expression (Fig. 4B).

MiR-3120 and miR-214 promotes cell cycle progression

To examine the effect of miR-3120 and miR-214 on the cell cycle progression, cell cycle assay was performed. 36 hours after transfection of HCT-116 cells with vectors containing miR-3120, miR-214 or control vectors, cell cycle assay was performed by PI-staining flow cytometry. The results showed that overexpression of miR-3120 and miR-214, decreased the number of cells in phase G1 and G2 and increased the number of cells in phase S. In other word, these miRNAs promote the cell cycle progression in HCT116 cells (Fig. 5).

Discussion

Colorectal cancer is one of the most common and deadly cancers that is caused by many genetic and environmental factors(Aran, Victorino et al. 2016, Rawla, Sunkara et al. 2019). Therefore, identifying genetic factors can help us understand the gene network that is involved in cancer formation. MiRNA as one of the regulatory factors for gene expression can play important roles in regulating this gene network(Plaisier, Pan et al. 2012).

Others have shown that miR-3120 regulates Wnt signaling pathway through direct targeting of Axin2 gene (Hongdan, Feng et al. 2018). It also upregulates the expression of Sox2, Nanog and Oct4 genes stemness markers and increases the invasion of tumor cells and is considered as an oncomiR(Hongdan, Feng et al. 2018). Interestingly, this microRNA is a mirror microRNA for hsa-miR-214 (Scott, Howarth et al. 2012). No such mirror genes were reported before, and the presence of these two microRNAs with nearly identical 3p and 5p sequences due to the palindrome sequence of this region of genome suggests that the expression of these two microRNAs and their target genes may be influenced by each other's effects. In the present study, using bioinformatics and experimental approaches, we tried to investigate the molecular and cellular effects of miR-3120 and its mirror miRNA (miR-214) on colorectal cancer.

Microarray data analysis showed that miR-3120 is up-regulated in colorectal cancer tumors compared with normal groups. However, in contrary to miR-3120 expression, miR-214 expression is down-regulated in colorectal tumors. Consistently, expression analysis of these two miRNAs in three CRC cell lines (HT29, HCT116 and SW480), showed opposite expression pattern for miR-3120 and miR-214. Also, RT-qPCR result indicated that miR-3120 and miR-214 affects each other's expression. This data is in consistent with previous data, which shows that the antisense gene can affect the expression of the sense gene(Su, Xiong et al. 2010, Balbin, Malik et al. 2015).

Different bioinformatics databases were used to find the molecular pathways regulated by miR-3120 and miR-214. Some of the predicted target genes were specific to the miR-3120, some were specific to miR-214, and some were common between the two miRNAs. To evaluate the effect of miR-3120/miR-214 on the predicted target genes expression, overexpression strategy was applied. The results of RT-qPCR of the genes expression showed that in most cases, these two miRNAs have distinctive effects on the target genes expression. Overexpression of miR-3120 in HCT-116 cell line followed by qRT-PCR indicated increased expression of SMAD3, SMAD4 and AKT2 genes, whereas overexpression of miR-214 inversed this effect. Previous studies have shown that miR-3120(Scott, Howarth et al. 2012, Hongdan, Feng et al. 2018) and miR-214(Penna, Orso et al. 2015) displays specific and even contrasting functions in different tumor types by differential mRNA targeting. Thus, these mirror miRNAs may play different roles according to co-expression with its own direct or indirect target genes such as SMAD3, SMAD4 and AKT2. To investigate cellular effect of miR-3120/miR-214 colorectal cancer, cell cycle analysis was performed and the result showed that forced expression of miR-3120 and miR-214, decreased the number of cells in phase G1 and increased the number of cells in phase S. In other word, these miRNAs promote cell cycle progression in HCT116 cells.

According to the evidence obtained in this study, with respect to miR-3120 target genes and its increased expression in colon cancer compared to normal, this miRNA seems to have an oncomeric role. These data suggest that miR-3120 and its mirror miRNA, miR-214, may be involved in regulating the molecular pathways of colorectal cancer, and part of this regulation could be related to the interaction of these miRNAs with each other. Further studies are needed to investigate the exact molecular mechanism of these two mirror miRNAs in cancer.

Declarations

Funding

Not applicable

Conflicts of interest

The authors declare no conflict of interest.

Author contributions

SB and BMS conceived and designed research. SB conducted experiments. SB and MJ analyzed data. MJ wrote the manuscript. BMS revised the manuscript. All authors read and approved the manuscript.

Ethics approval

This article does not contain any studies with human participants or animals performed by any of the authors.

Consent to participate

This article does not contain any studies with human participants.

Data availability

Data available on request

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Figures

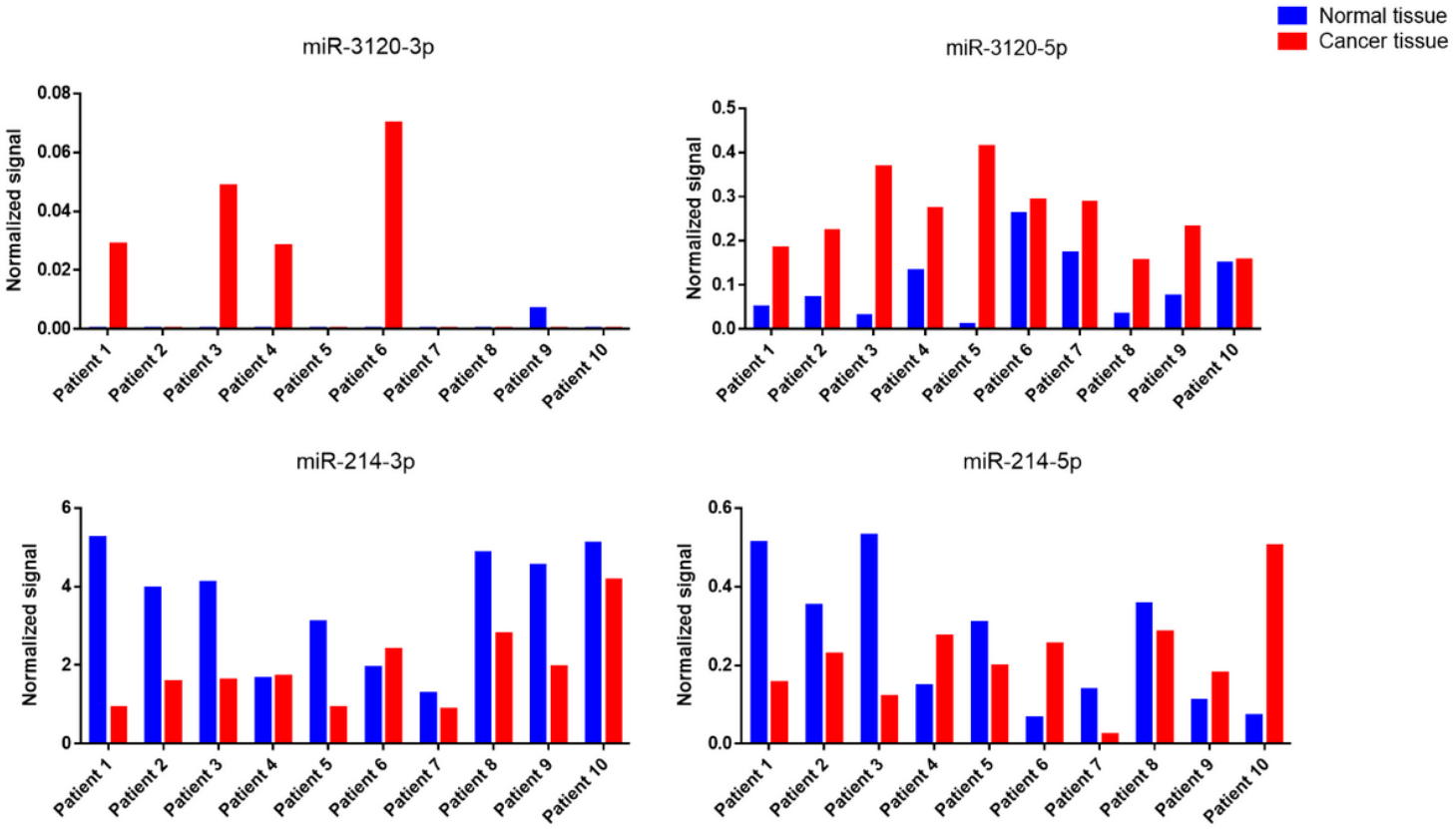

Figure 1

miR-3120 / miR-214 are differentially expressed in colorectal cancer. GSE126093 microarray data analysis (normalized data) showed that miR-3120-5p/3p has higher expression level in colorectal cancer samples, compared to normal ones. On contrary, miR-214 expression in colorectal cancer showed a decreased expression level in the most of colorectal cancer samples, compared to the normal ones.

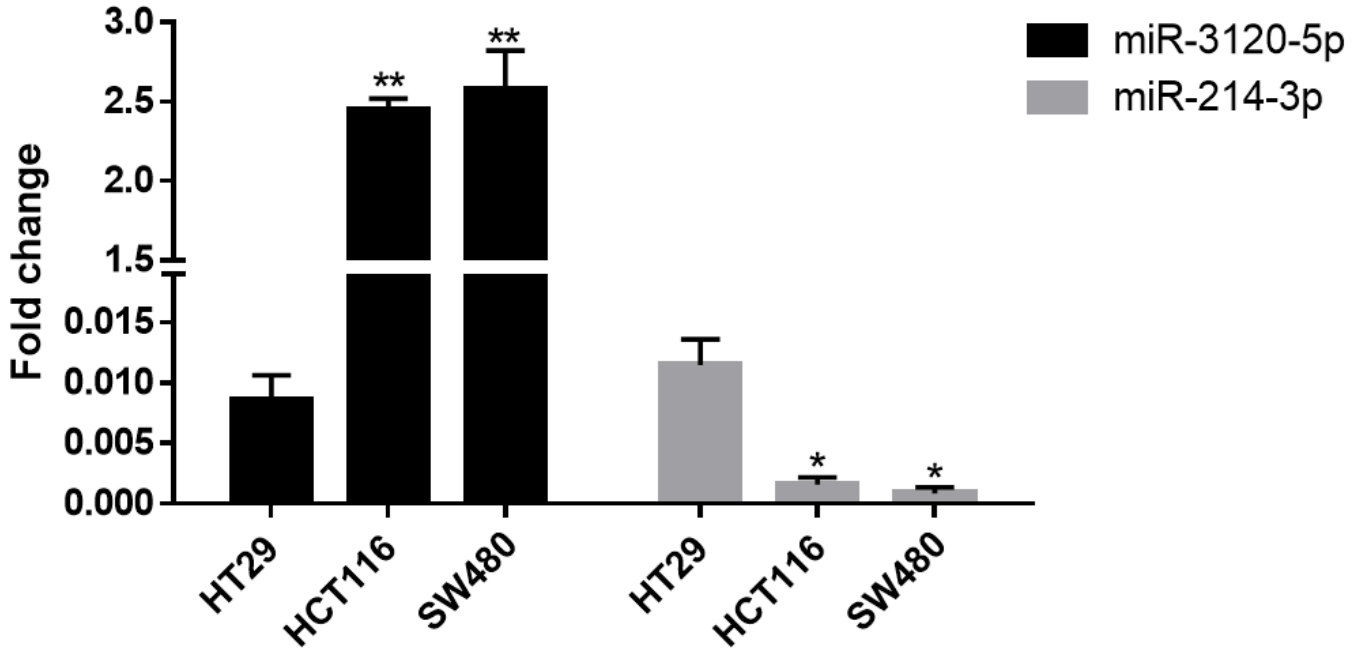

Figure 2

Inverse expression correlation of miR-3120-5p and miR-214-3p in colorectal cancer cells. RT-qPCR data show that miR-3120-5p has the highest expression in SW480 and the lowest expression in HT29, while miR-214-3p shows the highest expression level in HT29 and the lowest expression in SW480 cells. Error bar indicates SEM ; *$p < 0.05$;**$p < 0.01$.

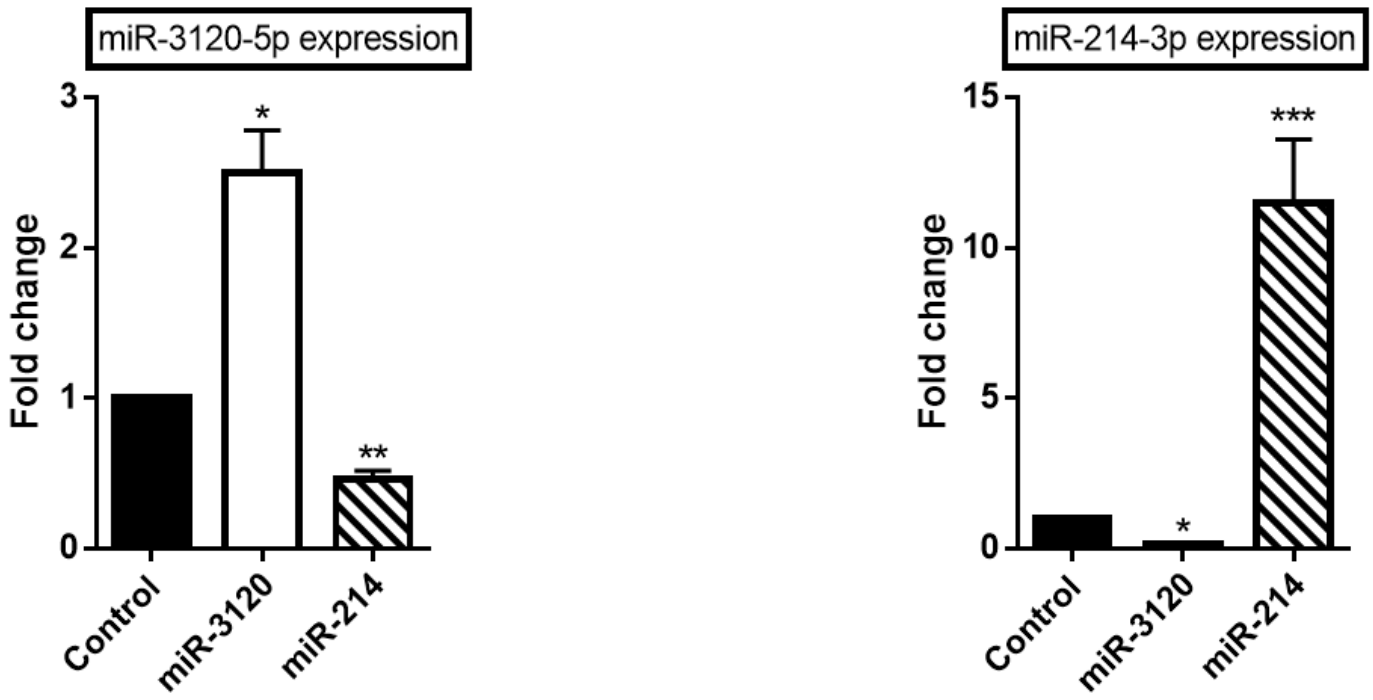

Figure 3

Hsa-miR-3120 and miR-214 affects each other expression. RT-qPCR results indicated that miR-3120 expression level was up-regulated and down-regulated following the overexpression of miR-3120 and miR-214 cassettes in the cells, respectively. However, miR-214 expression was down-regulated and up-regulated following the overexpression of miR-3120 and miR-214, respectively. Error bar indicates SEM ; *p < 0.05;**p < 0.01; ;***p < 0.001.

A

miRNA	Target gene	3'UTR length(bp)	MREs number prediction		
			Targetscan	Diana-microT-CDS	miRDB
miR-3120-5p	SMAD3	4680	3	3	3
	SMAD4	6592	3	6	2
	AKT1	1011	3	0	0
	AKT2	3572	1	0	0
	AKT3	5539	2	3	3
	ErbB3	1460	1	0	0
	Klf4	924	1	0	2
miR-3120-3p	YWHAE	907	1	1	1
	LRP6	5104	2	0	2
	TGFB1	3269	2	2	2
miR-214-3p	SMAD3	4680	2	9	0
	AKT2	3572	2	0	0
	ErbB2	982	2	12	0
miR-214-5p	SMAD4	4680	1	3	1

B

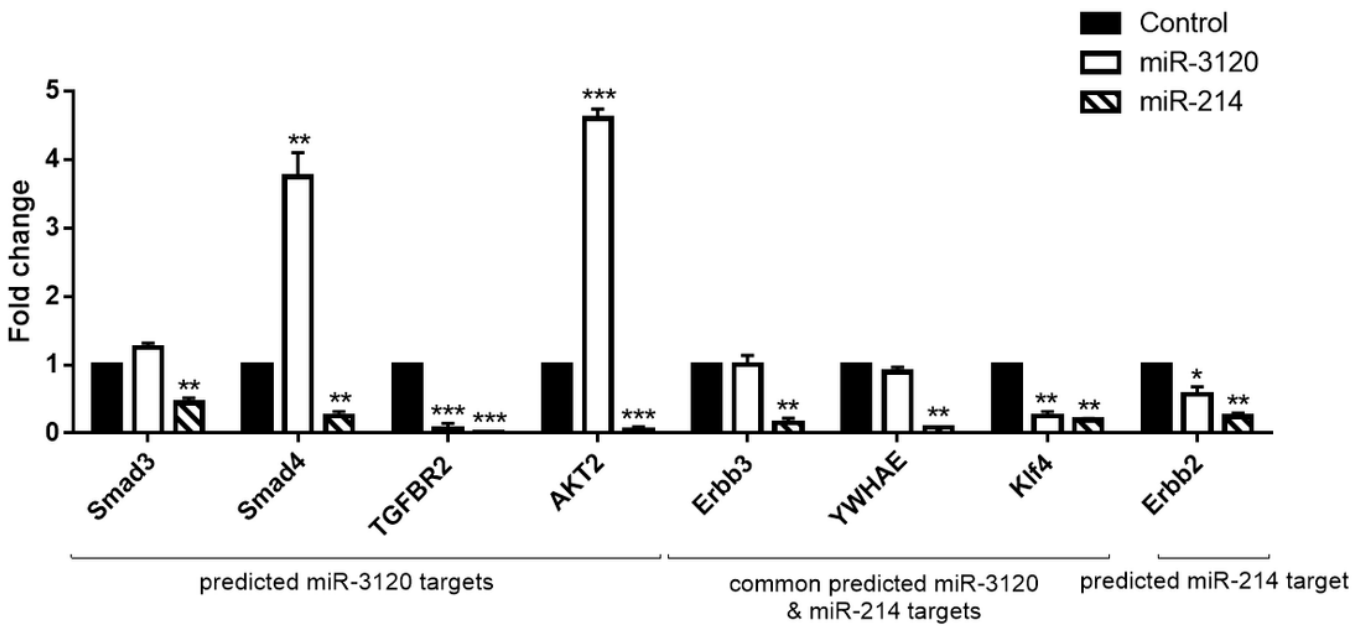

Figure 4

The effect of miR-3120/miR-214 expression on predicted target genes. A) TargetScan, Diana-microT-CDS, and miRDB software tools were used to investigate the probable target genes of miR-3120/miR-214. Smad3, Smad4 and AKT2 were predicted as common target genes of these miRNAs. B) The effect of miR-3120/miR-214 transfection on different types of genes that are related to different signaling pathways was evaluated. Some of these genes were specific to the miR-3120, some were common, and some were specific to the miR-214. RT-qPCR results indicated that miR-3120 overexpression resulted in SMAD4 and AKT2 upregulation while TGFBR2, Klf4, Erbb2 downregulation. However, miR-214 overexpression resulted in decreased expression level of all the tested genes. Error bar indicates SEM ; *p < 0.05;**p < 0.01; ;***p < 0.001.

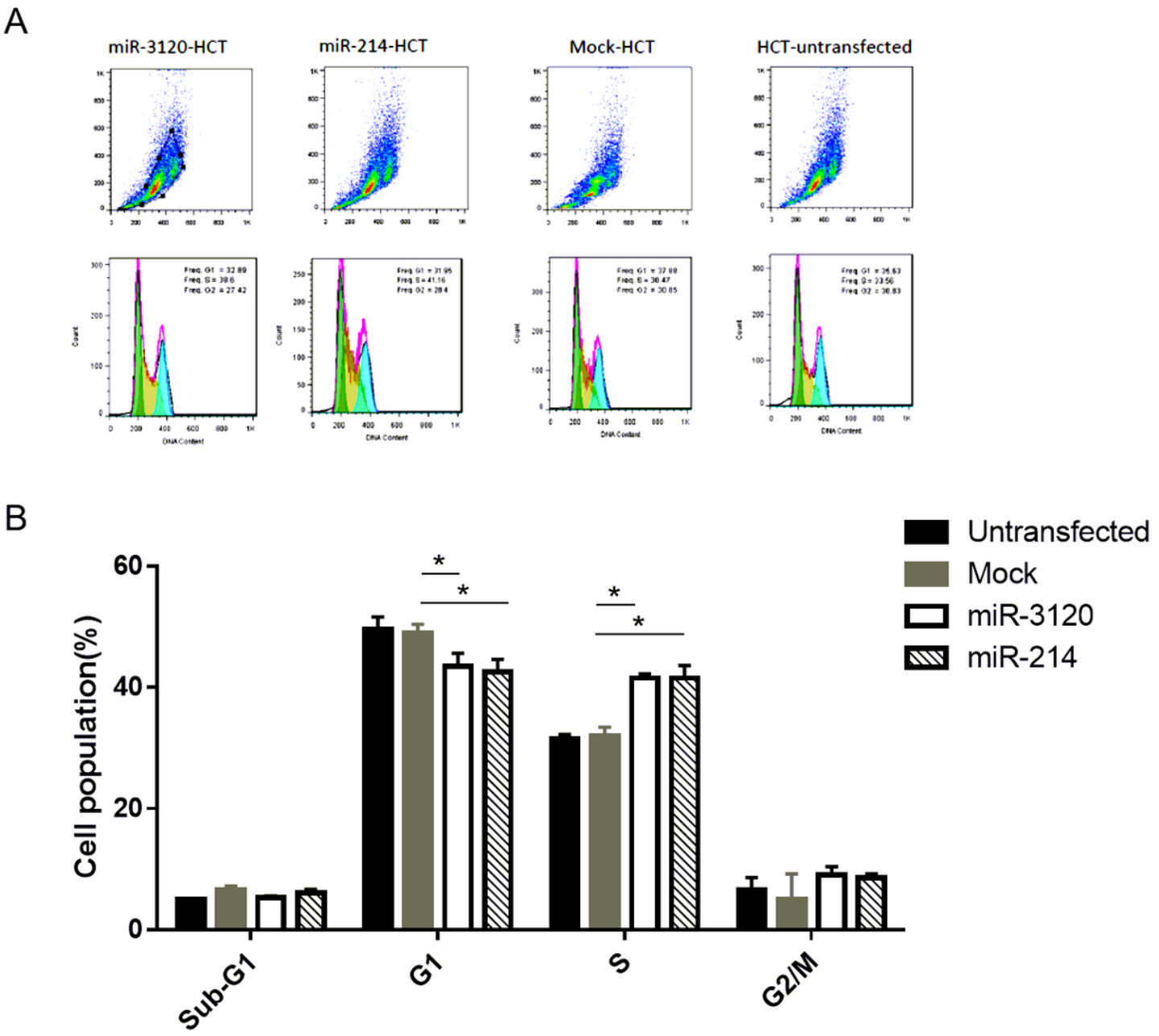

Figure 5

MiR-3120 and miR-214 promotes cell cycle. Histogram(A) and bar plots(B) of PI-staining flow cytometry results show that overexpression of miR-3120 and miR-214, decreased the number of cells in phase G1 and increased the number of cells in phase S. Error bar indicates SEM ; *p < 0.05.