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A preliminary study on the effects of human microRNAs on gut microbiota

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ABSTRACT

MicroRNAs (miRNAs) play a key role in disease manifestation, metabolic processes and immune regulation and modulation of the host. MiRNAs can be found in abundance in fecal samples of humans and are present in intracellular, extracellular fluid and extracellular vesicles. Abundance of miRNAs is found to be in ileal lumen as compared to the colon, whereas gut microbiota is more in colon, not in ileum. Still the effect of miRNA is found where the gut microbiota is more, which is by co-localizing with the nucleic acid of the bacteria. Some human miRNAs can regulate the non-coding RNAs in bacterial species like Escherichia coli and Fusobacterium nucleatum by entering into the bacterial cell in the intestine. By repressing the activity of the noncoding RNAs of intestinal bacterial species, human miRNAs bring about the increase or decrease in population of gut microbiota. With the change in the population of the certain microbiota species in the intestine, the host becomes more susceptible to the dysbiosis and becomes vulnerable to intestinal localization of pathogenic bacteria causing diseases like inflammatory bowel disease. With the usage of bioinformatics tool, this study has deepened into interaction of human miRNA and E. coli non-coding RNA, thereby opening up a detailed analysis into interaction of host with its gut microbiome and how the interaction is responsible for diseases in host.

Keywords: *miRNA,* Gut Microbiome, Inflammatory Bowel Disease, Inflammation.

INTRODUCTION

MicroRNAs (miRNAs) are the small non-coding ribonucleic acid (RNA) molecules with a single stranded structure spanning a length of about 18 to 25 nucleotides. These have a major role in modifying gene expression by an effective binding to their RNA targets. MiRNAs can inhibit messenger RNAs (mRNA) which encode the information in genetic materials into proteins. Along with this, they also have effect on other RNA molecules. These miRNAs can silence various RNA expressions via repression of translation of the corresponding messenger RNA (mRNA), and can have an invasion into mitochondria and modulate mitochondrial RNAs [1]. Moreover, it has been seen from the evolutionary point of view that mitochondria had originated from bacteria [2]. These two findings together lay the foundation for the possibility the entrance of human miRNAs into the gut bacteria. Then, to practically prove the entrance and colocalization of human miRNA with bacterial nucleic acid, Cy3-conjugated miRNAs were taken with Escherichia coli (*E. coli)* expressing green fluorescent protein (GFP), and was observed under confocal microscope [3]. It was found that the Cy3-conjugated miRNAs entered *E. coli* and co-localized with *E. coli* nucleic acids. Thus, the theoretical and practical evidence suggest that human miRNAs enter the gut bacteria. After the entrance into the bacteria, these miRNAs target bacterial RNAs [3].

MATERIALS AND METHODS

The effect of human miRNAs on *E. coli* has been analyzed from the cellular level and has been extensively studied deep into the effectiveness on microbes. The initial transcription of the gene

coding for miRNA is taken over by the enzyme RNA polymerase II, and the first transcript is termed as pri-miRNA. This pri-miRNA is long and contains a hairpin structure and sequences flanking that hairpin structure. This hairpin structure of pri-miRNA is cleaved by RNA polymerase III. After the cleavage, it undergoes another processing step by DROSHA and DGCR8 to form precursor miRNA. DICER enzyme converts precursor miRNA to mature miRNA in cytoplasm. The miRNA then forms miRNA induced silencing complex (miRISC) with Argonaut and other proteins and the complex acts on the target RNA. The miRNA can be collected from feces and introduced into bacteria to study the effect of human miRNA inside bacterial system. From the findings of Liu, et al., 2016, it is observed that wild type variant fecal miRNA has given rise to normal gut microbiota, but, DICER knockout variant has resulted in dysbiosis. The common fecal miRNA of mouse and human have been selected and their interaction with *E. coli* has been studied. Mice fecal miRNA collection sample showed the abundance of miR-1224, miR-2146, miR-2134, miR-2141 and miR-34c. Furthermore, miR-1246, miR-601, miR-630, miR-2116-5p, miR-320e, miR-1224-5p, miR-155-5p and miR-194-5p have been found to be in majority in human fecal miRNA collection sample. After all, it has been found that, human and mice fecal miRNA have 17 miRNAs in common.

It is also important to find out that whether the human miRNA can enter gut bacteria to influence it. It was verified by the findings of Zhang, et al. (2014), which depicts the proof that miRNA found in the muscle cells of human enter the mitochondria of the cell and affect the gene expression in the mitochondria. In the view of evolution, it has been observed that mitochondria has been derived from Bacteria [2]. These two major findings make the basis of the possibility of entrance of human miRNA into gut bacteria. Further, to prove the entrance and colocalization of human miRNA with bacterial nucleic acid practically, Cy3-conjugated miRNAs had been taken with *E. coli* expressing GFP, and confocal microscope was used to observe and analyze it [3]. Here, Cy3-conjugated miRNAs entered *E. coli* and had a co-localization with *E. coli* nucleic acids. Therefore, the entrance of human miRNA into *E. coli* and possibly other gut bacteria has been both theoretically and experimentally proved.

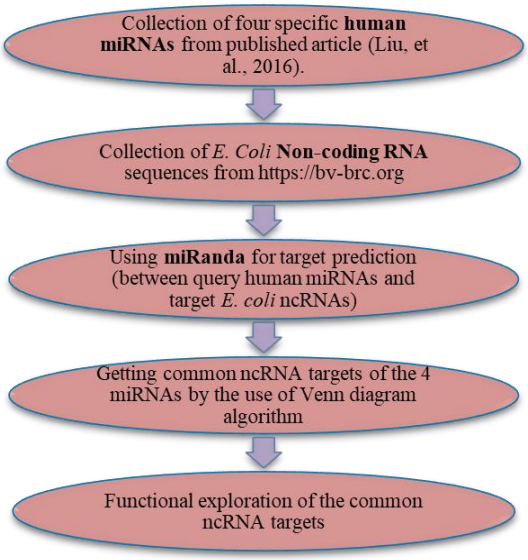
To check out whether the host miRNA can specifically affect gene expression, *E. coli* was cultured in the presence of miR-1226-5p, miR-4747-3p, miR-1224-5p for 4 hours. Then RNA transcripts were isolated and using qPCR, transcript levels of yegH mRNA, rutA mRNA, RNase P coding RNA and fucO mRNA were quantified. It has been found that with the administration of miR-1226-5P, the yegH mRNA showed an increase, ribonuclease P (RNase P) got increased by the effect of miR-4747-3p, rutA mRNA quantity declined by the effect of miR-1224-5p and fucO mRNA decreased by miR-623.

As the study of the effects of human miRNAs on *E. coli* mRNAs has already been done in the reference of Liu et al. (2016), we have focused on the target of ncRNAs, to target another prospective of those human miRNAs [3].

Sequence of steps in finding the targets of human miRNAs

The materials used for the study of the interaction between human miRNA and the transcriptome of *E. coli* are all derived from the available data from online bioinformatics resource portals. We have got four specific miRNAs of human that affect *E. coli* nucleic acid, which are hsa-miR-4747-3p, hsa-miR-1224-5p, hsa-miR-1226-5p and hsa-miR-623, which have been collected from the findings of Liu et al. (2016) evidently [3]. The specimen used for the experiment is the MG1655 substrain of the *E. coli* K-12 strain. The ncRNA data of this variant of *E. coli* is derived from the website - "*Escherichia coli* str. K-12 substr. MG1655: Genome Overview" (bv-brc.org/view/Genome/511145.12). After collecting both the host miRNAs and non-coding RNAs of *E. coli*, we used miRanda bioinformatics tool for target prediction. Using this tool, the targets of four selected human miRNAs were found in the non-coding RNA sequences of *E. coli*. The whole process of target prediction and functional analysis can be summarized in the form of a flow chart diagram as depicted here in the Figure 1.

Figure 1: Flow chart proposing the overall method of target prediction and functional analysis of ncRNA targets in *E. coli*



2) Collection of required data from sources

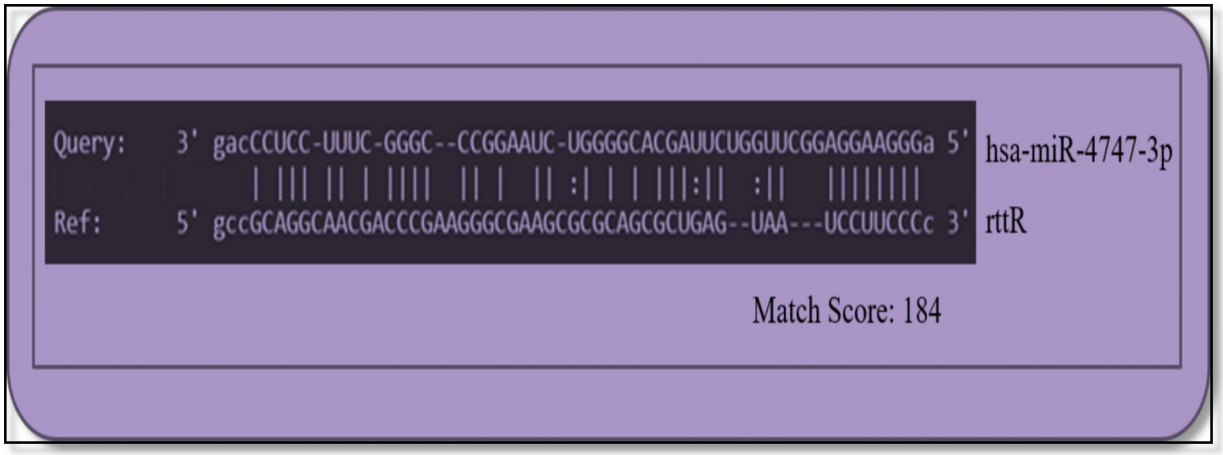
With reference to the publication of Liu et al. (2016), the miRNAs targeting the RNAs of *E. coli* were picked out [3]. Specifically, hsa-miR-4747-3p, hsa-miR-1224-5p, hsa-miR-1226-5p and hsa-miR-623 have been found to target *E. coli* nucleic acid sequences. We collected the sequences of these miRNAs from https://rnacentral.org/. The sequences of 60 ncRNAs of *E. coli* str. K-

12 substr. MG1655 were derived from (bv-brc.org/view/Genome /511145.12), under Refseq source.

miRNA Target Prediction

The bioinformatics tool – miRanda has been used in this analysis which helps to identify potential target sites for miRNA. The developers have used C programming language to write the algorithm of miRanda and has been rendered as sharable and changeable under General Public License. It has been installed in an Ubuntu operating system, and the procedure for installation has been taken from anaconda.org/bioconda/miranda. The four mentioned human miRNA sequences have been used as the query sequence in this program and the sixty non-coding RNA sequences collected from (bv-brc.org/view/Genome/511145.12) have been taken as the target sequences for each of the four human miRNAs. Each individual query miRNA sequence was aligned against 60 non-coding RNAs of *E. coli* via miRanda command (Figure 2). To get the most feasible thermodynamic binding interactions and to avoid mismatching, the energy threshold has been taken as -20 Kcal/mol and the score threshold has been taken 140 in miRanda algorithm. The gap open penalty has been considered -9 and the gap extend penalty to be -4, with a scaling parameter of 4.

Figure 2: Source data with the miRanda algorithm which has the activity of searching for complementarity matches between query miRNAs and reference RNAs (target RNAs). Here, as an example, the possible complimentary match between human miRNA hsa-miR-4747-3p and rttR ncRNA has been shown.



RESULTS AND DISCUSSION

The list of miRNAs known to affect intestinal microbes is mentioned in Table 1.

Table 1: List of human microRNAs targeting bacterial nucleic acid with function (the dash symbols shows that there is no existing data for respective headings).

Human miRNAs	Associated bacteria	Target RNA	Effects inside the Bacteria	Author
miR-101	*Fusobacterium nucleatum*	-	-	Liu, et al., 2016 [3]
hsa-miR-515-5p	*Fusobacterium nucleatum*	16s rRNA	Promotes 16s rRNA activity	Liu et al., 2016 [3]
miR-876-5p	*Fusobacterium nucleatum*	-	-	Liu et al., 2016 [3]
hsa-miR-325	*Fusobacterium nucleatum*	16s rRNA	Promotes 16s rRNA activity	Liu et al., 2016 [3]
hsa-miR-1253	*Fusobacterium nucleatum*	-	Promotes *F. nucleatum* growth	Liu et al., 2016 [3]
hsa-miR-194-5p	*Salmonella Typhimurium*	-	Promotes the growth of *S. typhimurium*	Herrera-Uribe et al., 2022[4]
hsa-miR-148-3p	Enterobacteriaceae	-	Promotes the growth of microbe causing inflammation	Bi et al., 2020 [5]
hsa-miR-27b-3p	Enterobacteriaceae	-	Promotes the growth of microbe causing inflammation	Bi et al., 2020 [5]
hsa-miR-4747-3p	*E. coli*	RNase P coding RNA	Activity of RNse P increased	Liu et al., 2016 [3]
hsa-miR-1224-5p	*E. coli*	rutA mRNA	Activity of rutA decreased	Liu et al., 2016 [3]
hsa-miR-1226-5p	*E. coli*	yegH mRNA	Activity of yegH mRNA increased	Liu et al., 2016 [3]
hsa-miR-623	*E. coli*	fucO mRNA	Activity of fucO decreased	Liu et al., 2016 [3]
hsa-miR-144	*Bacteroidetes*	-	Promotes growth of *Bacteroidetes*	Rojas-Feria et al., 2018
hsa-miR-519	*Bacteroidetes*	-	Promotes growth of *Bacteroidetes*	Rojas-Feria et al., 2018 [6]
hsa-miR-211	*Bacteroidetes*	-	Promotes growth of *Bacteroidetes*	Rojas-Feria et al., 2018 [6]
hsa-miR-30d	*Akkermansia muciniphila*	-	Increases the abundance of *A. muciniphila* in the gut	Liu, et al., 2019 [7]
hsa-miR-30d	*Helicobacter pylori*	-	Increases *H. pylori* intracellular survival	Yang, et al., 2016 [8]

Analysis of the effect of human miRNAs on E. coli

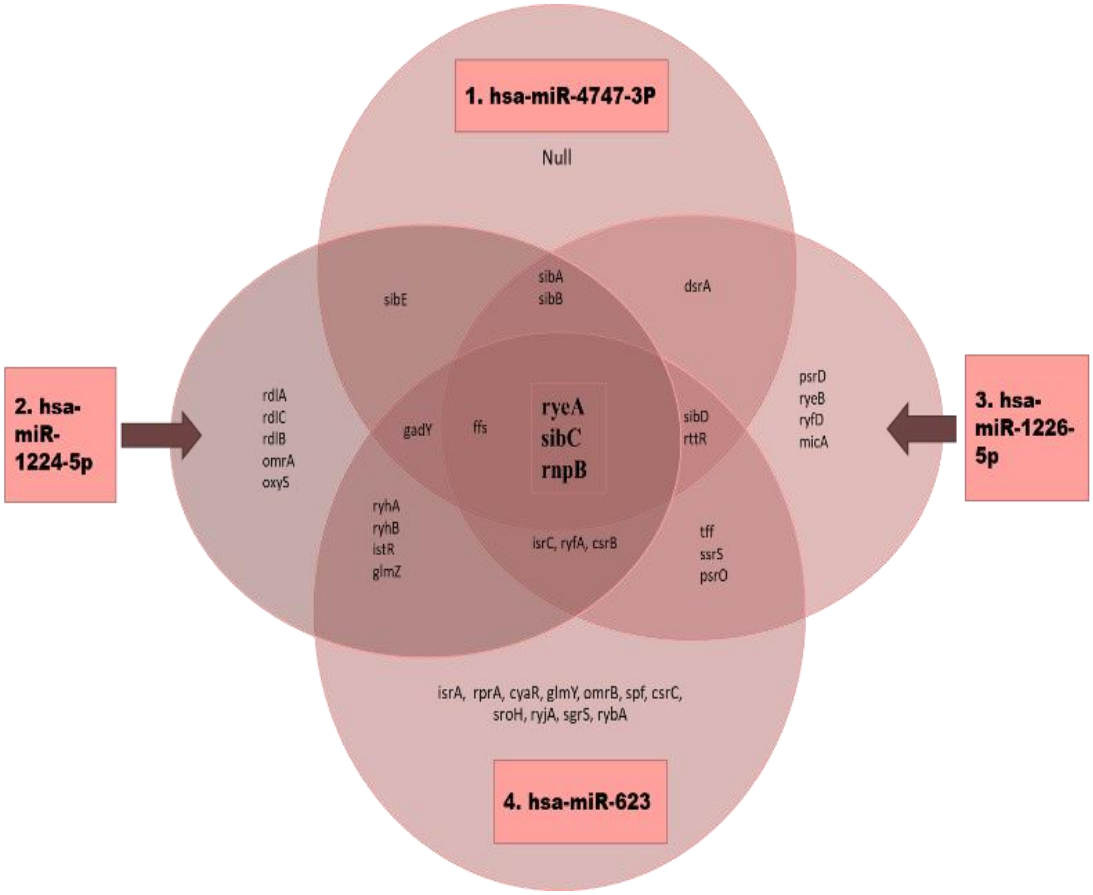
Observations and results found in the target prediction are as follows. The use of miRanda dynamic programming alignment has led us to find out that hsa-miR-4747-3p has 12 ncRNA targets; hsa-miR-1224-5p has 20 ncRNA targets; hsa-miR-1226-5p has 18 ncRNA targets, and lastly, there are 29 ncRNA targets for hsa-miR-623 (Table 2).

Table 2: List of *E. coli* ncRNA targets of human miRNAs obtained from the output of miRanda (Tick marks for a ncRNAs depict the proper complementary match to respective miRNA, found by the usage of miRanda).

Target E. coli ncRNAs	Human miRNAs			
	hsa-miR-4747-3p	hsa-miR-1224-5p	hsa-miR-1226-5p	hsa-miR-623
sibA	✓	✓	✓	
sibB	✓	✓	✓	
sibE	✓	✓		
dsrA	✓		✓	
ffs		✓	✓	
gadY	✓	✓		✓
sibD	✓			✓
rttR	✓			✓
isrC		✓	✓	✓
ryfA		✓	✓	✓
csrB		✓	✓	✓
ryhA		✓		✓
ryhB		✓		✓
istR		✓		✓
glmZ		✓		✓
tff			✓	✓
ssrS			✓	✓
psrO			✓	✓
psrD			✓	
ryeB			✓	
ryfD			✓	
micA			✓	
rdlA		✓		
rdlB		✓		
rdlC		✓		
omrA		✓		
oxyS		✓		
isrA				✓
rprA				✓
cyaR				✓
glmY				✓
omrB				✓
spf				✓
csrC				✓
sroH				✓
ryjA				✓
sgrS				✓
rybA				✓
ryeA	✓	✓	✓	✓
sibC	✓	✓	✓	✓
rnpB	✓	✓	✓	✓

ncRNA targets of all four miRNAs.

Figure 3: Venn Diagram showing the ncRNA targets of hsa-miR-4747-3p, hsa-miR-1224-5p, hsa-miR-1226-5P, hsa-miR-623. And, at the centre lies the common ncRNA targets of all four miRNAs.



Analysis on effect of human miRNAs on E. coli

Observations and results found in the target prediction are as follows. Use of miRanda dynamic programming alignment has led us to find out that hsa-miR-4747-3p has 12 ncRNA targets; hsa-miR-1224-5p has 20 ncRNA targets; hsa-miR-1226-5p has 18 ncRNA targets, and lastly, there are 29 ncRNA targets for hsa-miR-623 (Table 2).

From the raw aligned sequence data obtained by miRanda, the targets for all four human miRNAs were extracted separately and were listed in distinct text files for each four human miRNAs.

From those text files, the common non-coding RNAs of different sets of human miRNAs were extracted and mentioned in Venn diagram in Microsoft Excel software (Figure 3). Finally, By the help of miRanda program, the ncRNAs - ryeA, sibC and rnpB have been discovered to be the most common ncRNA targets of all the four human microRNAs named hsa-miR-4747-3p, hsa-miR-1224-5p, hsa-miR-1226-5P, hsa-miR-623.

Analysis on ryeA: RyeA is actually a small non-coding RNA found in E. coli with 234-249[9]. Another gene segment called sdsR can be observed opposite to the ryeA. The sdsR derived protein acts as a toxin to the cell, because it targets yhcB mRNA which codes for inner membrane protein involved in biogenesis of cell envelope maintenance, preventing proper cell development and cell shape maintenance [10]. RyeA suppresses the expression of sdsR [11]. The human miRNA affects the activity of ryeA which may cause changes to the normal activity of ryeA-sdsR activity in the cell.

Analysis on sibC

The gene for non-coding RNA sibC is encoded from the sibC gene and is found opposite to the gene forming toxic peptide called ibsC; where the sibC acts as antitoxin to the ibsC [12]. It has been found that ibsC accumulates in the inner membrane and creates depolarization of the membrane disturbing the integrity of the cell membrane. SibC prevents this cellular disturbance. And as the human miRNA targets the sibC ncRNA, hence, it may affect the vital activity of sibC in cell membrane integrity maintenance.

Analysis on rnpB

The ncRNA named rnpB is the RNA part of ribonuclease P (RNase P) enzyme. RnpB, being a ribozyme and catalytic subunit, helps RNase P in cleaving 5'-leader sequence from the precursor tRNA (pre-tRNA) [13, 14, 15]. So, the effect by human miRNA on rnpB may cause a serious load on the cell as it may cause the changes in protein synthesis machinery of E. coli.

Effects of human miRNAs on other gut microbes

Effects of human miRNAs on Fusobacterium nucleatum: Human microRNAs also have effects on F. nucleatum nucleic acids, affecting their vital processes including protein synthesis machinery. The human microRNAs named as MiR-

101, hsa-miR-515-5p, miR-876-5p, hsa-miR-1253, hsa-miR-325 use to target F. nucleatum nucleic acids [3]. From the same reference it has also been found that MiR-515-5p and hsa-miR-325 increases the 16s rRNA activity.

Effects of human miRNAs on Salmonella typhimurium: Hsa-miR-194 promotes the growth of S. typhimurium and hence it has been observed that there will be more invasion and adhesion of S. typhimurium if the expression of Hsa-miR-194 is more in the host cells, causing the infection in S. typhimurium infection cases [4]. When the expression of S. typhimurium is decreased, the growth of S. typhi also got inhibited and with decreased population there is lower invasion of the microbe. Both the hsa-miR-194a-5p and hsa-miR-194b have been found to show the same outcome of growth in the bacterial population which affects the S. typhimurium infection significantly.

Effects of human miRNAs on Enterobacteriaceae: Hsa-miR-148-3p and Hsa-miR-27b-3p supports the growth of microbes of Enterobacteriaceae, whose effects have been seen in Inflammatory Bowel Disease (IBD) patients [5]. These play a huge role in pathogenesis intestinal inflammation. These miRNAs also promote the growth of proteobacteria, which in turn decreases the intestinal mucus and compromises the intestinal barrier, also causing the decrease in immunity.

Effects of human miRNAs on Bacteroidetes: Hsa-miR-144, Hsa-miR-211, Hsa-miR-519 have been observed playing a major role in Crohn's disease (CD) [6]. They aid in increment in the population of Bacteroidetes in CD patients. In the pathogenesis of IBD, Bacteroidetes have been found to be harmful as compared to another major CD bacterial species Firmicutes which is tagged as beneficial during CD. The human miRNAs thus cause the severity of CD through increment Bacteroidetes population.

Effects of human miRNAs on Akkermansia muciniphila: The human miRNA hsa-miR-30d has been found abundantly in faeces of experimental autoimmune encephalomyelitis (EAE) patients. Hsa-miR-30d influences the lactase producing gene in Akkermansia muciniphila, which in turn acts in more production of lactase and the microbe is able to grow properly [7]. The increased population of A. muciniphila then increases regulatory T cells to suppress EAE symptoms.

Effects of human miRNAs on Helicobacter pylori: The infection caused by Helicobacter pylori in the intestine of humans results in induction of autophagy in gastric epithelial cells [8]. This autophagy in turn can stop the intracellular residence of H. pylori in epithelium. But, hsa-miR-30d downregulates the autophagy-related gene expression causing the inhibition of the autophagy process which leads to the increase in the survival of H. pylori inside the epithelial cells. Thus, the population of H. pylori is increased by human miRNA hsa-miR-30d.

CONCLUSION

In this paper we have studied the association between the non-coding sequence of the microbes, especially the E-coli with the human miRNAs. It appears that the miRNA shares some amount of complementary match and forms the basis of regulation. The study found that hsa-miR-4747-3p has 12 ncRNA targets; hsa-miR-1224-5p has 20 ncRNA targets; hsa-miR-1226-5p has 18 ncRNA targets, and 29 ncRNA targets for hsa-miR-623. Further we also found ncRNA targets of all four miRNAs which happens to suggest the presence of redundancy. However, further investigation is needed to understand the redundancy and the role of such ncRNA targets in the regulation.

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