Bio-Medical Materials and Engineering 26 (2015) S1985–S1991
DOI 10.3233/BME-151502
IOS Press

Integrative network analysis of rifampin-regulated miRNAs and their functions in human hepatocytes

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Abstract. Rifampin is an important drug used in the treatment of tuberculosis, and it increases the drug metabolism in human hepatocytes. Previous studies have shown that rifampin can indirectly influence drug deposition through the regulation of molecular interactions of miRNA, PXR and other genes. The potential functions of miRNAs associated with rifampin-induced drug disposition are poorly understood. In this study, significantly differentially expressed miRNAs (SDEM) were extracted and used to predict the miRNA-regulated co-expression target genes (MCeTG). Additionally, a miRNA-regulated co-expressed protein interaction network (MCePIN) was constructed for SDEM by extending from the protein interaction network (PIN). The functioning of the miRNAs were analyzed using GO analysis and KEGG pathway enrichment analysis. A total of 20 miRNAs belonging to SDEM were identified, and 632 miRNA-regulated genes were predicted. The MCePIN was constructed by extending from PIN, and 10 miRNAs and 33 genes that are relevant to 7 functions, including response to wounding, wound healing, response to drug, defense response, inflammatory response, liver development and drug metabolism, were discerned. The results provided by this study offer valuable insights into the effect of rifampin on miRNAs, genes and protein levels.

Keywords: Rifampin, miRNA, gene, hepatocyte, PPI network, P450

1. Introduction

Rifampin is an effective antimicrobial drug that is usually used to treat tuberculosis and other infections [1]. Rifampin works by inducing the drug-metabolizing enzymes and transporters [2]. Rifampin, a typical ligand of the Pregnane X receptor (PXR), has been shown to strongly stimulate

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drugs by activating the PXR [3-5], and is also an inducer of many cytochrome P450 (p450) enzymes [2]. And RXR regulator mediates the gene expression including some genes of P450 [6].

MicroRNAs (miRNAs) are a class of 22nt non-coding RNAs that typically regulate protein-coding genes at the post-transcription level [7, 8], and are predicted to regulate more than one-third of the protein-genes in the human genome [9]. Rifampin-induced miRNA expression changes are correlated with rifampin treatment changes in PXR and P450 [6, 10-12]. MiRNA can indirectly influence rifampin-induced drug deposition through gene regulation [11, 13-15]. Proteins are synthesized from mRNA templates through a highly conserved process, many studies have reported that the functions of miRNAs can be understood by analyzing miRNAs-regulated mRNA-related PIN [16-18].

In this study, miRNA and mRNA expression profiles were combined to better understand the functions of rifampin-regulated miRNAs and their potential molecular mechanisms within human hepatocytes. Following this initial analysis, the target genes of each differentially expressed miRNA were predicted. Furthermore, MCeTG were extracted, and rifampin-induced miRNAs-regulated co-expression PIN was constructed by integrating the PPI network. In addition, the functioning of the different miRNAs was analyzed by assessing the underlying MCePIN of each miRNA using functional enrichment analysis. The results provided by this study offer new insights into the miRNA and miRNA function when interacting with rifampin.

2. Materials and methods

2.1. Data source

The mRNA expression dataset (n=12780) and miRNA expression dataset (n=334) were obtained as in Ramanoorthy, et al [9]. Briefly, seven hepatocyte samples from seven different human were treated with rifampin, and the RNA was isolated from tye samples using the miRNease kit. The expressions of 754 miRNAs were measured using the Taqman OpenArray Human miRNA Panel. The mRNA expressions were obtained using EZBead preparation, Next-Gene sequencing, read quality assessment, sequence alignment and RNA-Seq differential expression analysis.

2.2. Statistical analysis

Benjamini-Hochberg correction was applied to control for the false discovery rate (FDR) [19]. A p-value was calculated for each miRNA and each gene of two groups (normal and rifampin-treated samples) with an unpaired, two-tailed Student's t test.

2.3. Target gene prediction

The miRNAs were significantly different when the p-value < 0.01. The predicted miRNA target genes were predicted in Targetscan [20, 21], miRanda [22] and PicTar [23] using RmiR.hsa, which retrieved the targets or the miRNAs in miRNA target databases for given miRNAs or genes without any threshold. BioMart [24] was used to convert gene Entrez ID into the gene Symbol.

2.4. Constrction of MCePIN

The Human Protein Reference Database (HPRD) [25] includes 30047 proteins and 41327 protein-

protein interactions. The human liver protein interaction network (HLPN) is composed of 3484 interactions among 2582 proteins relevant to human liver. The MCePIN was created through the integration from the proteins and interactions of the HPRD and HLPN. However, duplicates were excluded [26]. The cytoscape [27], version 3.0.2 software was used to generate the network.

2.5. Enrichment analysis of modules

Enrichment analysis of the biological processes and pathways of the MCePIN was processed with the Database for Annotation, Visualization and Integrated Discovery (DAVID), which is a tool for biological interpretation of genes. Functional classification of important gene pathways was carried out using the Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG).

3. Results and discussions

3.1. MCePIN

MCePIN was constructed using the significantly different expressed miRNAs, their co-expressed significantly different expressed target genes and the interactions between them.

The differentially expressed miRNAs were retrieved based on a p-value ($p<0.05$). The upregutalted and downregulated miRNAs were identified using threshold (Fold Change>1) and threshold (Fold Change<1), respectively. There were 20 miRNAs, including 12 upregulated miRNAs and 8 downregulated miRNAs left (Table 1).

To extract target genes of 20 miRNAs in a liberal standard, Pictar, Targetscan and miRanda databases were used without setting any threshold. The target genes of the 20 miRNAs were the mixed dataset of the results from the three databases. In this analysis, our primary focus was on the genes in the gene expression profile. Therefore, 6211 genes were extracted, and the negatively regulated target genes of each miRNA were obtained using the fold change that represents a measure of expression changes. As a result, 4115 co-expression genes of the 20 miRNAs were retained. From this, there were 632 significantly differentially expressed genes, and 1187 co-expression miRNA-gene pairs were identified. The HPRD and HLPN were integrated by merging all the proteins and interactions, and then removing the duplicates. The human PIN included 42521 interactions among 10210 proteins.

Table 1

Differentially expressed miRNA dataset

miRNA	P-value	Fold Change	miRNA	p-value	Fold Change
upregulated			upregulated		
miR-886-3p	0.00017781	1.56454118	miR-660	0.02971142	1.26423062
miR-766	0.00746736	1.42202073	miR-638	0.0302057	1.67681216
miR-92a	0.01687698	1.13186208	miR-25	0.0337675	1.27581638
miR-107	0.01770697	2.18865184	miR-616	0.04464574	1.33365615
miR-30d#	0.01945112	1.15621932	miR-576-3p	0.04528093	2.19157158
miR-335	0.02415006	1.33006108	miR-218	0.04993545	1.9012223
Downregulated					
miR-186	0.00177975	0.83561977	miR-320	0.03756323	0.77870999
miR-361	0.01111667	0.7086572	miR-202	0.03960494	0.59880068
miR-95	0.02185477	0.63201986	miR-200b#	0.04256355	0.60539667
miR-345	0.0239214	0.81506025	let-7g	0.04347655	0.84016356

Based on the interactions in the human PIN, the relationships of the 632 proteins were established. Specifically, the miRNA-regulated PINs were extended by choosing the first level proteins nodes of genes. So, the MCePIN was constructed from 11219 interactions among 20 miRNAs and 632 proteins.

Among the 1187 co-expression miRNA-gene pairs, miR-660 negatively regulated the most genes (136), and miR-886 negatively regulated the least genes (12). In this analysis, the rifampin-associated genes were further to reveal potential miRNA-related PINs and the functions of miRNAs.

3.2. Enrichment analysis of miRNA functions

The biological classification tool DAVID, based on GO and KEGG, was used to describe the functional classification and signal pathways of the miRNAs and MCePIN genes. Based on the extension of network with PIN, GO terms and KEGG keys pathways associated with drug-induced were excluded. Functional annotation clustering was used to extract the concerned functions, and Go terms and KEGG pathways that contained "drug", "liver", "metabolic", "response", "wound" and "stimulus" were defined as drug-related functions. The results of the enriched biological processes for the genes and proteins related to the rifampin are listed in Table 2.

Table 2

Enrichment analysis of miRNA-regulated genes and proteins in MCePIN

DAVID	description	Gene	protein	p-value
GO:0009611, response to wounding	a stimulus indicating damage to the organism	22	186	6.0E-26
GO:0042060, wound healing	restore integrity to a damaged tissue	7	85	7.7E-18
GO:0042493, response to drug	a result of a drug stimulus	15	77	1.2E-13
GO:0006952, defense response	response to the presence of a foreign body or the occurrence of an injury	19	143	4.6E-5
GO:0006954, inflammatory response	The immediate defensive reaction to infection or injury	16	96	8.6E-4
GO:0001889, liver development	The process whose specific outcome is the progression of the liver over time	6	21	5.4E-6
KEGG_PATHWAY, Drug metabolism-P450	Drug metabolism	6	2	

Table 3

The rifampin-associated miRNAs and genes of MCePIN

function	miRNA	gene
response to wounding	miR-107, miR-186, miR-218, miR-576-3p, miR-886-3p, miR-335, miR-616, miR-766	AHSG, IL1RAP, IL1RN, THBS1, F8, IL10RB, IL20RB, SGMS1, P2RX7, CD55, CXCL13, HIF1A, RELA, IGF1, PLSCR4, SLC1A2, TPM1
wound healing	Let-7g, miR-107, miR-218, miR-576-3p, miR-766, miR-886-3p	HBEGF, SYT7, PLSCR4, TPM1, IGF1, F8
response to drug	miR-218, miR-766, miR-886-3p, miR-660, miR-576-3p	ABAT, CAV1, SLC1A2, BCHE, CAV2, HMGCS1, PLIN2, PPARG, P2RX7
defense response	miR-576-3p, miR-335, miR-186, miR-886-3p	CD55, CXCL13, HIF1A, RELA, AHSG, IL1RAP, IL1RN, THBS1, F8, IL10RB, IL20RB, SGMS1, PPARG, P2RX7
inflammatory response	miR-576-3p, miR-335, miR-186	CD55, CXCL13, HIF1A, RELA, AHSG, IL1RAP, IL1RN, THBS1, F8, IL10RB, IL20RB, SGMS1
liver development	miR-107, miR-186	ONECUT2, ARF6, SP3, TGFBR3, CEBPA, RELA
Drug metabolism-P450	miR-107, miR-335, miR-186	ADH1B, CYP3A5

Response to wounding, wound healing, response to drug, defense response, inflammatory response, liver development and drug metabolism are the seven different functions that were identified Table 2). The rifampin-associated miRNAs and genes are listed in Table 3.

A total of 33 different rifampin-associated genes were identified (Table 3). Among them, P450 and CYP3A5 have been reported to have important roles in drug metabolism [28-30], and SP3, ARF6, CEBPA, RELA are responsible for liver development [31-34]. In addition, CXCL13 and RELA have been previously reported as inflammatory markers [35, 36].

The ten rifampin-associated miRNAs that targeted the 33 genes are listed as follow: miR-107, miR-186, miR-218, miR-576-3p, miR-886-3p, miR-335, miR-616, miR-766, miR-218 and Let-7g. Previous studies have reported that rifampin induced expression change of genes and miRNAs [9, 10]. These results suggest that miR-107, miR-186, miR-218, miR-576-3p, miR-886-3p, miR-335, miR-616 and miR-766 were correlated with response to wounding via direct or indirect mechanisms through regulating genes. Additionally, let-7g, miR-107, miR-218, miR-576-3p, miR-799 and miR-886-3p were related to wound healing via induced 6 genes. Further, miR-218, miR-766, miR-886-3p, miR-660 and miR-576-3p enriched in response to drug, and miR-107 and miR-186 were candidates for participating in liver development. Similarly, miR-107, miR-335 and miR-186 were suggested to be related to drug metabolism through the regulation of ADH1B and CYP3A5 [37].

3.3. Analysis of rifampin-associated miRNAs and negatively regulated genes

To reveal the rifampin-associated miRNAs and genes, the miRNA-genes co-expression network was constructed (Figure 1).

There were 10 miRNAs that negatively regulated the 33 genes, and 62 miRNA-gene pairs in the network. To focus on the rifampin-associated miRNAs, miR-576-30, which controlled 11 genes, was designated as the most important in the network, and is suggested to participate in response to wounding, wound healing, response to drug, defense response and inflammatory response. The miR-186 negatively regulated 7 genes, including CYP3A5, and it is suggested that miR-186 associated with the drug metabolic process. Although miR-335 did not negatively regulate CYP3A5, which is relevant

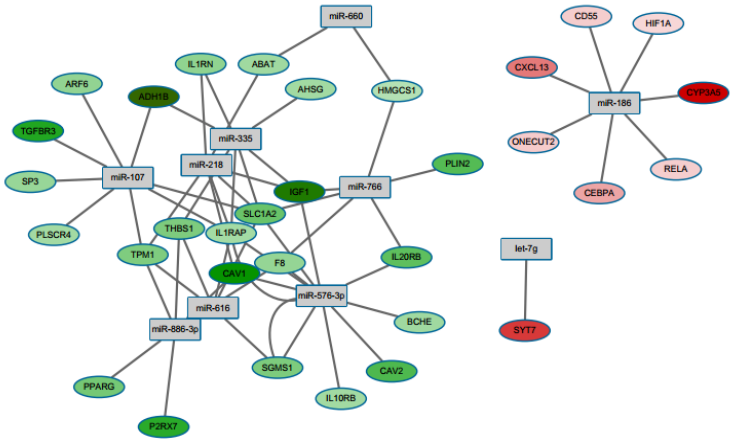

Fig. 1. Key miRNA-genes co-expression network. The yellow and grey rectangles represent the downregulated and upregulated miRNAs, respectively. The circles express the miRNA-regulated genes, and the regulatory relationships are denoted by colors, in which red and green indicate upregulated and downregulated genes, respectively. The depth of the color represents the size of the fold change.

to drug metabolism, it did negatively control ADH1B, which is also related to drug metabolism.

The 33 genes performed the functions directly or indirectly through proteins. ADH1B, IGF1, CAV1, and SGMS1 had the major quantity changes, and were regulated by multiple miRNAs. CYP3A5 is an important gene that takes part in drug metabolism and differentially expressed. These results suggest that these five genes have important functions in the rifampin-induced biological process.

4. Conclusion

To understand the rifampin-induced differentially expressed miRNAs and their functions, the miRNA and gene profile were integrated to identify significant differential miRNA and miRNAs negatively regulated genes, further to constructed MCePIN by PIN. Consequently, seven functions were extracted through GO analysis and KEGG pathway enrichment. Moreover, it is suggested that miRNAs and genes are associated with each function. As a whole, the results suggest that rifampin contributes to changes in the expression of multiple miRNAs. It provides evidence for an important regulatory function of miRNAs in the rifampin-induced biological process in human hepatocytes.

Acknowledgment

The authors would like to acknowledge the financial support of the National Key Scientific Instrument and Equipment Development Projects of China (2012YQ04014001 and 2012YQ04014010), National Natural Science Foundation of China (61071174), Fundamental Research Funds for the Central Universities (HEUCFT1102, HEUCFT1302 and HEUCFX41303), Science and technology Research Program of Heilongjiang Educational Committee (12541898).

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