Differential regulation of microRNAs in nonalcoholic fatty liver disease

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Mechanism of miRNA

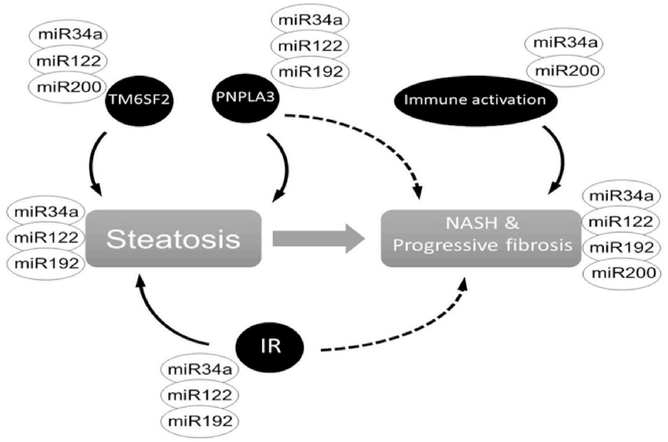

Figure 1. An illustration demonstrating differential associations of circulating miRNA with pathogenic factors in NAFLD.

Patient Characteristics

Total (n)	217
Age (mean, IQR)	50.9 (42.9-60.5)
Female (%)	40.7
Hispanic ethnicity (%)	13.7
BMI (mean, IQR)	34.0 (29.6-36.7)
Diabetes (%)	25.4
Hypertension (%)	38.7
Fasting lipids (mean, IQR)	
Triglycerides	196.3 (107-259)
LDL-C	109.8 (83-132)
HDL-C	45.9 (37-53)
Fibrosis stage (%)	
0- 1	53.3
2	33.0
3-4	13.7
NAS score (mean, IQR)	4.6 (4-6)
PNPLA3 genotype (%)	
CC	37.3
CG	41.2
GG	21.5
TM6SF2 genotype (%)	
CC	79.7
CT or TT	20.3
eLP-IR score (mean, IQR)	66.3 (54.4-83.0)
ADA2 (mean, IQR)	6.8 (4.1-8.3)
CK18 (mean, IQR)	377.3 (135.8-518.0)

Abstract

Background & Aims:

Nonalcoholic fatty liver disease (NAFLD) is a heterogeneous disease driven by both genetic and environmental factors. Several microRNAs (miRNA) have been implicated in the pathogenesis of NAFLD. We aimed to examine the differential relationship between a panel of miRNAs (miR-34a, miR-122, miR-191, miR-192, and miR-200a) and NAFLD disease phenotype as well as genetic and metabolic factors related to NAFLD pathogenesis.

Methods:

A total of 170 subjects with biopsy proven NAFLD from a prospective NAFLD registry were included in the study. The levels of target miRNAs and housekeeping controls were measured by qPCR using plasma samples from patients in the biorepository. We studied the relationship between the miRNA levels and histological features of NAFLD, NAFLD-related genotypes, insulin resistance measured by the enhanced Lipoprotein Insulin Resistance Index (eLP-IR), and the activity of adenosine deaminase 2 (ADA2), a marker of macrophage activation.

Results:

Four of the miRNAs: miR-34a, miR-122, miR-192 and miR-200a, were strongly correlated with the stage of liver fibrosis with each stage of fibrosis associated with 0.26 to 0.44 standard deviation (SD) increases in miRNA concentration in the plasma (all p values < 0.001). While miR-34a, miR-122, miR-192 and miR-200a were associated with ballooning degeneration, , only miR-34a, miR-122 and miR-192 were associated with hepatic steatosis and lobular inflammation. These observations were mirrored by a robust association between eLP-IR, a measure of insulin resistance, and miR-34a, miR-122 and miR-192 (p values <0.001 – 0.002). The GC and GG variants at rs738409 of PNPLA3 were associated with the higher levels of miR-34a, miR-122, and miR-192, but not miR-200a, whereas the CT and TT variants at rs58542926 of TM6SF2 were associated with the higher levels of miR-34a, miR-122, and miR-200a, but not miR-192. Finally, miR-34a and miR-200 were associated with an elevated activity of ADA2. In contrast, miR-191 was not found to be associated with NAFLD histological phenotype, insulin resistance, the PNPLA3 and TM6SF2 genotypes or inflammation as measured by ADA2 and CK-18.

Conclusions:

Four miRNAs demonstrated differential associations with various pathogenic factors in NAFLD, suggesting their distinctive pathways of activation. Our study suggests that circulating miRNAs are potential biomarkers to characterize subtypes of NAFLD patients. .

Conclusions

- ❖ NAFLD is a heterogeneous disease with influence from genetics, insulin resistance and immune dysregulation
- ❖ MiRNAs correlate differentially with risk factors of NAFLD, disease activity, and progression
- ❖ MiRNAs can potentially be used as tools to help predict NAFLD phenotypes that influence disease severity

Table 1

	miR-34a β[1], p value	miR-122 β, p value	miR-192 β, p value	miR-200a β, p value	miR-191 (control) β, p value
eLP-IR score	0.012 <0.001	0.011 0.001	0.011 0.002	0.002 0.7	0.001 0.7
BMI	0.009 0.5	0.002 0.9	-0.012 0.3	-0.010 0.5	-0.012 0.3
Diabetes	0.728 <0.001	0.154 0.4	0.354 0.04	0.464 0.01	-0.009 1.0
Dyslipidemia					
TG	0.001 0.07	0.002 0.03	0.002 0.002	0.001 0.2	-0.0001 0.9
LDL-C	-0.002 0.3	-0.001 0.7	-0.0003 0.9	-0.002 0.5	0.003 0.2
HDL-C	-0.014 0.03	-0.015 0.02	-0.014 0.02	0.004 0.6	-0.009 0.1
Hypertension	0.496 0.001	0.033 0.8	0.097 0.523	0.374 0.03	-0.088 0.6
ADA2	0.104 <0.001	0.064 0.02	0.062 0.02	0.101 0.001	0.016 0.6
CK18	0.002 <0.001	0.001 <0.001	0.002 <0.001	0.0004 0.09	-0.0002 0.3

Univariate associations between miRNA and non-genetic pathogenic factors in NAFLD.

Table 2

	miR-34a β[1], p value	miR-122 β, p value	miR-192 β, p value	miR-200a β, p value	miR-191 (control) β, p value
eLP-IR score	0.010 0.035	0.009 0.050	0.012 0.011	0.007 0.2	0.003 0.5
PNPLA3					
CC	Ref	Ref	Ref	Ref	Ref
CG or GG	0.689 0.001	0.788 <0.001	0.770 <0.001	0.397 0.1	0.204 0.4
TM6SF2					
CC	Ref	Ref	Ref	Ref	Ref
CT or TT	0.545 0.025	0.204 0.4	0.359 0.1	0.344 0.2	-0.294 0.3
ADA2	.087 0.001	0.057 0.027	0.050 0.049	0.100 0.001	0.022 0.4

Multivariate analysis between miRNA and key pathogenic factors in NAFLD

Table 3

	miR-34a β, p value	miR-122 β, p value	miR-192 β, p value	miR-200a β, p value	miR-191 (control) β, p value
Fibrosis	0.445 <0.001	0.264 <0.001	0.315 <0.001	0.271 <0.001	-0.019 0.8
Steatosis	0.363 0.001	0.415 <0.001	0.367 0.001	0.116 0.3	-0.018 0.9
Lobular inflammation	0.480 <0.001	0.402 0.003	0.364 0.007	0.151 0.3	-0.018 0.9
Ballooning	0.487 <0.001	0.404 <0.000	0.325 0.002	0.330 0.004	-0.187 0.07

Univariate associations between microRNA and histological features of NAFLD