

Weighted miRNA co-expression networks analysis identifies circulating miRNA predicting overall survival in hepatocellular carcinoma patients

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Supplementary materials and methods

Enrichment Analysis

Mirna targets were searched in miRTarBase (<http://mirtarbase.mbc.nctu.edu.tw/php/index.php>)[1] release 7.0. Predicted Targets were searched in miRDB database(<http://mirdb.org>)[2]. Target enrichment analysis was performed pooling the results from miRTarBase and miRDB search by running the GeneCodis online tool (<https://genecodis.genyo.es/>)[3] on KEGG Pathway database with default settings.

Mir-Target enrichment analysis

In miRDB 272 and 925 transcripts were predicted as possible miR-4507 and miR-3185 targets, respectively (File S1). While 66 and 166 transcripts were associated to miR-4507 and miR-3185, respectively, in miRTarBase. None of the two miRNA has strongly validated targets, the only data available in miRTarBase, consists of weekly associated targets. The target enrichment analysis assigned a role in cancer for both miRNAs (Fig. S3 and Fig. S4). MiR-3185 targets were enriched in 36 pathways, with the cancer-related pathways being the top scoring ones (Fig. S3), while mir-4507 targets were significantly enriched in 7 pathways, 5 of them related to cancer (Fig. S4)

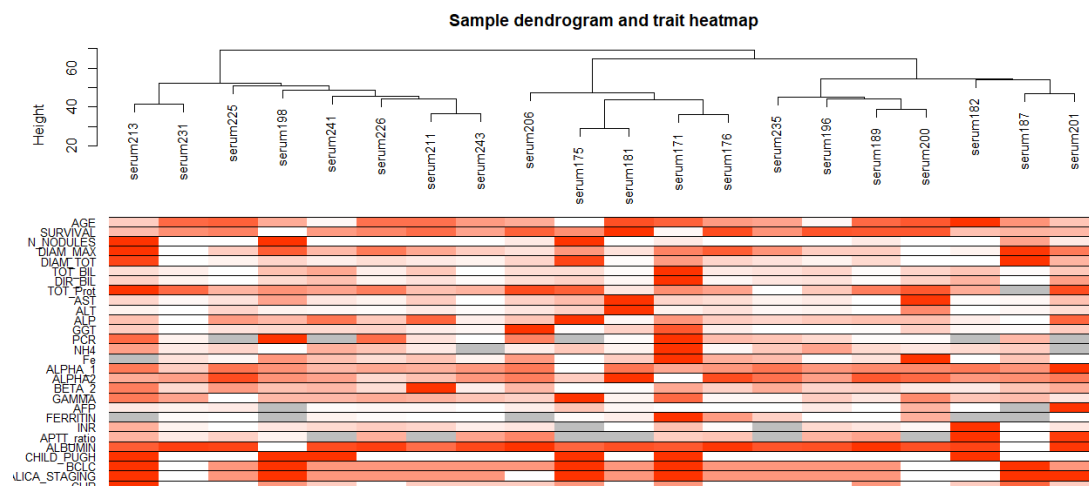


Figure S1. Clustering dendrogram of samples based on their Euclidean distance and heatmap of the clinical variables associated with each sample

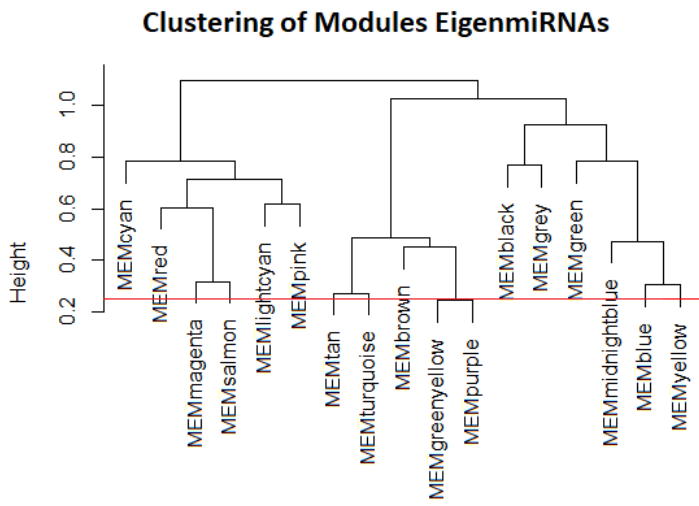


Figure S2. Clustering of the Modules EigenmiRNAs. The cluster merge height was set at 0.25



Figure S3. Bars-Chart of the 36 enriched pathways related to miR-3185 targets. The enrichment analysis was performed by using the Fisher's exact test with a Hypergeometric distribution corrected for multiple testing by using the False Discovery Rate (FDR) correction

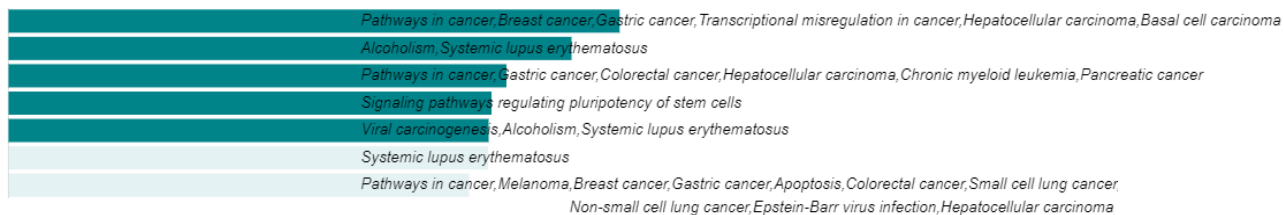


Figure S4. Bars-Chart of 7 enriched pathways related to miR-4507 targets. The enrichment analysis was performed by using the Fisher’s exact test with a Hypergeometric distribution corrected for multiple testing by using the False Discovery Rate (FDR) correction

Table S1. Demographic and clinical characteristics of the studied populations

	Training Cohort (n=20)	Validation Cohort (n=102)
Patients characteristics		
Age (mean, 95%CI)	68 (64-71)	70 (48-87)
Sex (M/F)	17/3	81/ 21
Aetiology		
Alcohol metabolic	16	56
Alcohol metabolic viral		10
Viral	4	36
Disease scores		
CTP A/B/C	14/6/0	73/22/3
BCLC 0/A/B/C-D	3/12/5/0	8 / 61 / 25 / 6
Alpha fetoprotein		
<20 ng/mL	15	54
20 - 400 ng/mL	5	14
>400 ng/mL	0	10

Table S2. Number of miRNAs included in each module.

Module	N° of miRNAs
Black	13
Blue	37
Brown	27
Cyan	9
Green	19
Greenyellow	10
Lightcyan	5
Magenta	11
Midnightblue	9
Pink	13
Purple	10
Red	16
Salmon	9
Tan	10
Turquoise	46
Yellow	23
Grey	7

Table S3. Clinical traits significantly associated to MEMs

MEM color	Significant positive correlation	Significant Negative correlation
Red	CRP, AFP	Survival, Albumin
Magenta	CRP, INR, APTT ratio	
Salmon	Alpha 1 , AFP, INR, APTT ratio	
Lightcyan	Alpha 1, AFP, ITALICA staging	
Pink	Alpha1	
Tan	ALT, AST	Beta 2
Turquoise	ALT	
Green	GGT, Ferritin	
Blue	Ferritin	
Grey	Clip score.	

Table S4. MiRNA included in MEM red ranked based on GS p value

miRNAs in MEM Red	GS.survival	p.GS.survival	MM.red	p.MM.red
miR-3185	0.81	1.69E-05	-0.52	0.018
miR-4507	0.70	0.001	-0.72	0.001
miR-125b-5p	0.65	0.002	-0.29	0.222
miR-30d-5p	0.65	0.002	-0.28	0.229
miR-450b-5p	-0.56	0.010	0.93	3.84E-09
miR-603	-0.49	0.028	0.88	3.77E-07
miR-3646	-0.48	0.034	0.61	0.004
miR-628-5p	-0.47	0.035	0.69	0.001
miR-570	-0.43	0.057	0.81	1.28E-05
miR-4423-3p	-0.39	0.093	0.89	1.19E-07
mir-548aa	-0.34	0.144	0.84	3.33E-06
miR-548aj-3p	-0.34	0.147	0.67	0.001
miR-4529-3p	-0.32	0.166	0.67	0.001
miR-19a-3p	-0.31	0.187	0.83	4.86E-06
miR-3128	-0.26	0.274	0.81	1.64E-05
miR-3201	-0.24	0.306	0.76	9.25E-05

Table S5. Circulating miRNA expression

miRNA	Survival time (mean 95%CI)			p value
	≤12 months	12 – 24 months	≥24 months	
miR-3185	18.61 (13.92-23.31)	29.88 (21.27-38.49)	35.68 (28.42-42.94)	<i>p</i> =0.03
miR-4507	0.71 (-0.67-2.09)	20.99 (9.50-32.46)	32.22 (20.47-43.96)	<i>p</i> =0.014
miR-450	0.21 (0.11-0.33)	0.15 (0.056-0.24)	0.17 (0.10-0.23)	<i>p</i> =0.6

References

- 1 Chou C-H, Shrestha S, Yang C-D *et al.* miRTarBase update 2018: a resource for experimentally validated microRNA-target interactions. *Nucleic Acids Res.* 46(D1), D296–D302 (2018).
- 2 Chen Y, Wang X. miRDB: an online database for prediction of functional microRNA targets. *Nucleic Acids Res.* 48(D1), D127–D131 (2020).
- 3 Tabas-Madrid D, Nogales-Cadenas R, Pascual-Montano A. GeneCodis3: a non-redundant and modular enrichment analysis tool for functional genomics. *Nucleic Acids Res.* 40(Web Server issue), W478-483 (2012).

File S1. List of the miR-4507 and miR-3185 predicted targets