

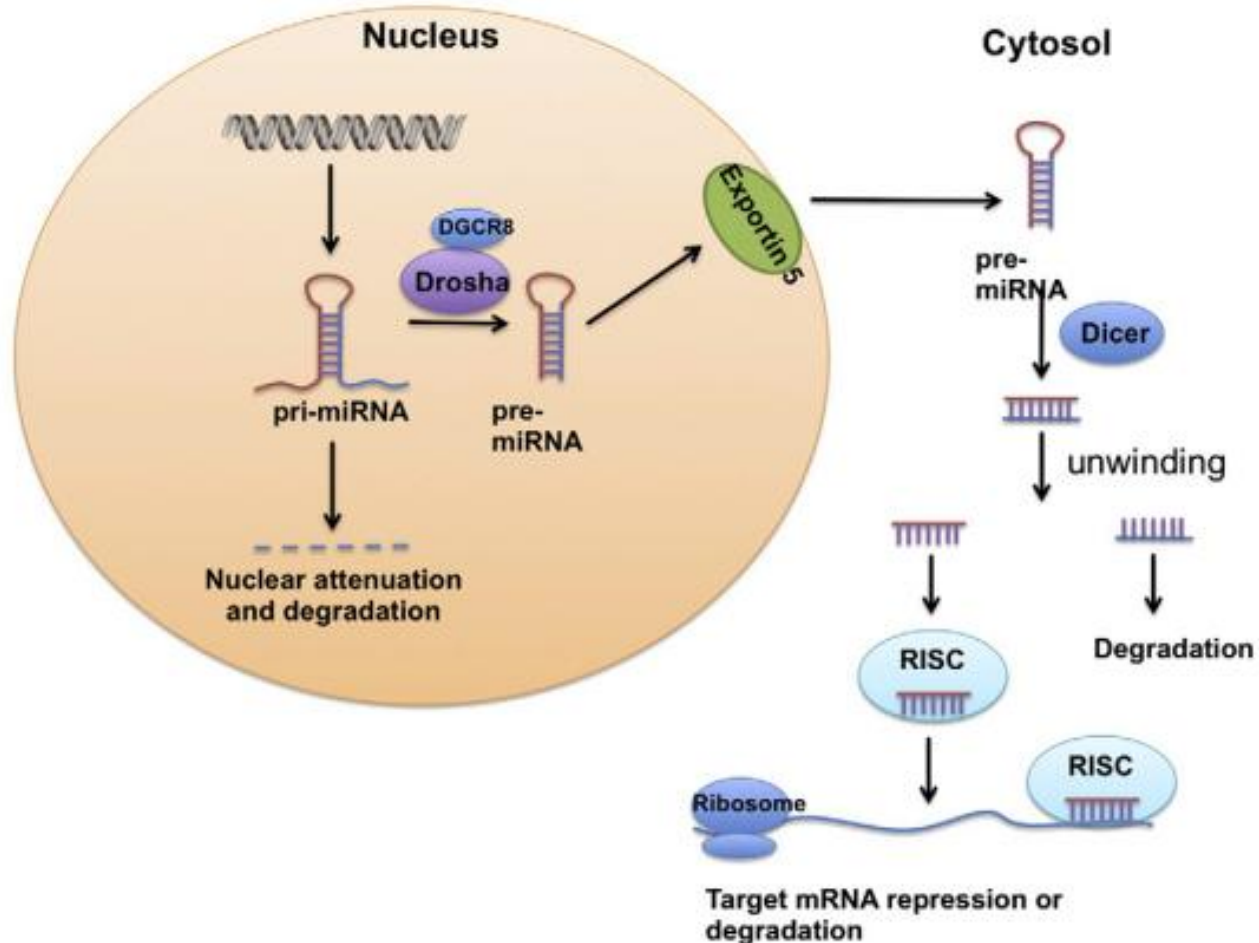
Integrate bioinformatics analysis in identifying a novel microRNA regulates HCV replication

Ju-Chien Cheng, Ph.D.

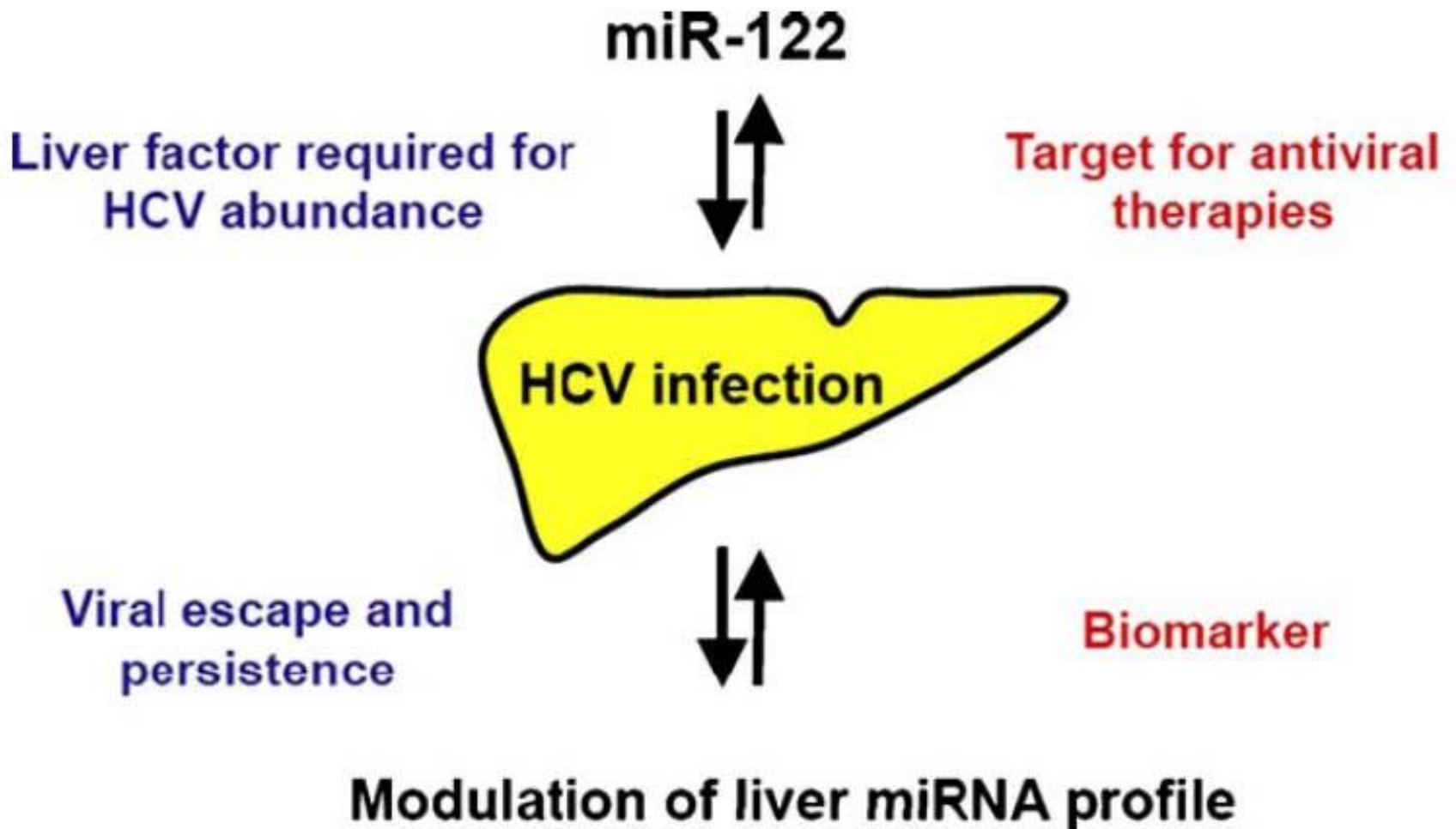
鄭如茜 副教授

醫學檢驗生物技術學系/研究所

Outline of miRNA biogenesis



Clinical impact of HCV-miRNA interaction



Virus associated with miRNA

Host microRNA	Actions of cellular miRNAs in viral infections
hsa-miR-32	Restrict the replication of primate foamy virus type 1 (PFV-1) <i>(Science, 2005)</i>
hsa-miR-122	Stimulate translation of hepatitis C virus RNA (HCV) <i>(Science, 2005)</i>
hsa-miR-199a*	Regulation of the hepatitis C virus genome replication <i>(Journal of Hepatology, 2009)</i>
hsa-miR-29a	Interfere with viral nef protein expression and HIV-1 replication <i>(Retrovirology, 2008)</i>
hsa-miR-155	EBV induced miR-155 attenuates NF-kB signaling and stabilizes latent virus persistence <i>(Journal of Virology, 2008)</i>

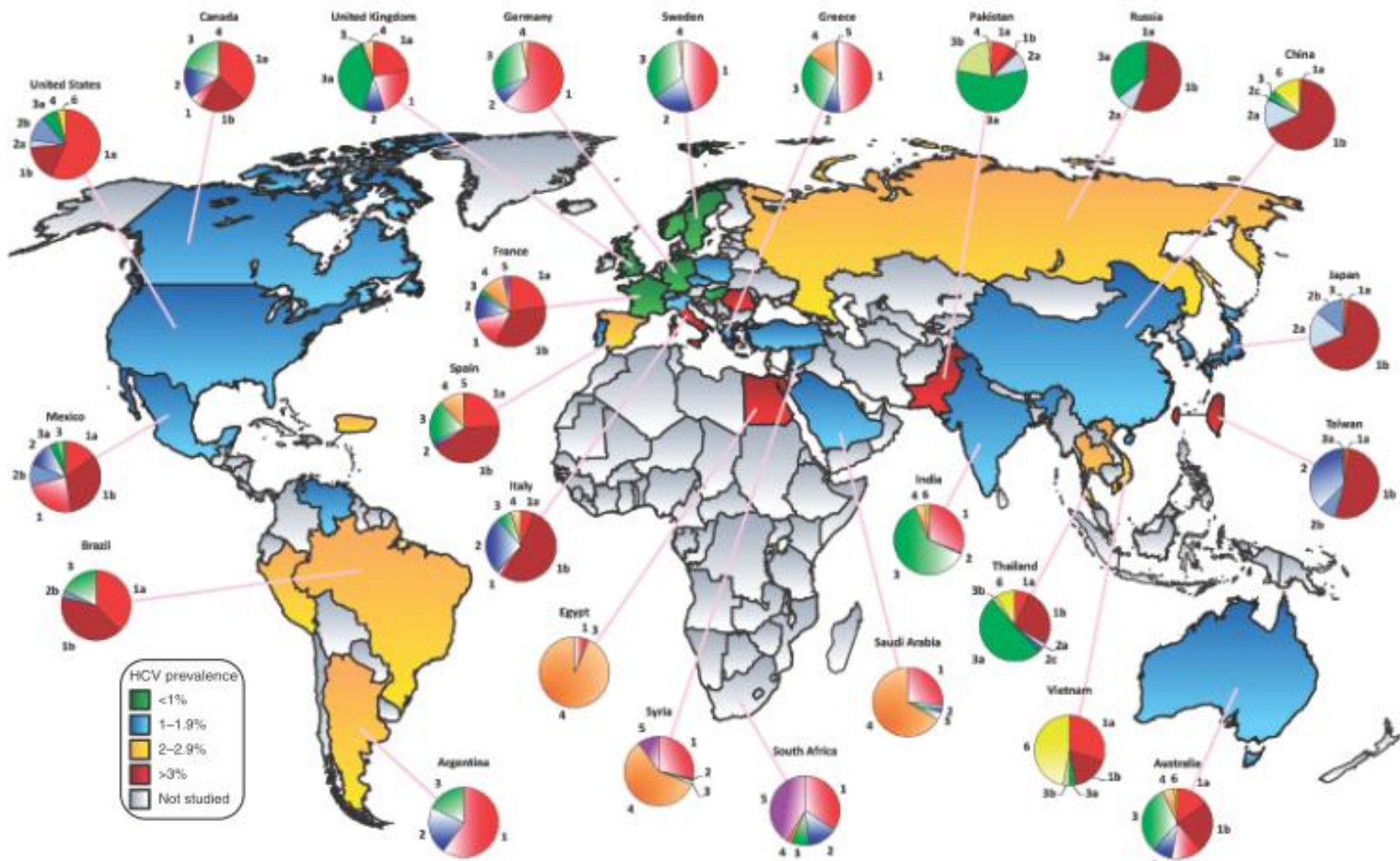


Fig. 1. Hepatitis C virus prevalence among adults and genotype distribution.

The overall prevalence of viral hepatitis worldwide is estimated to be 3-5% with over 170 million people infected with HCV

Bioinformatic strategy for identifying liver miRNAs with target sites on HCV genome

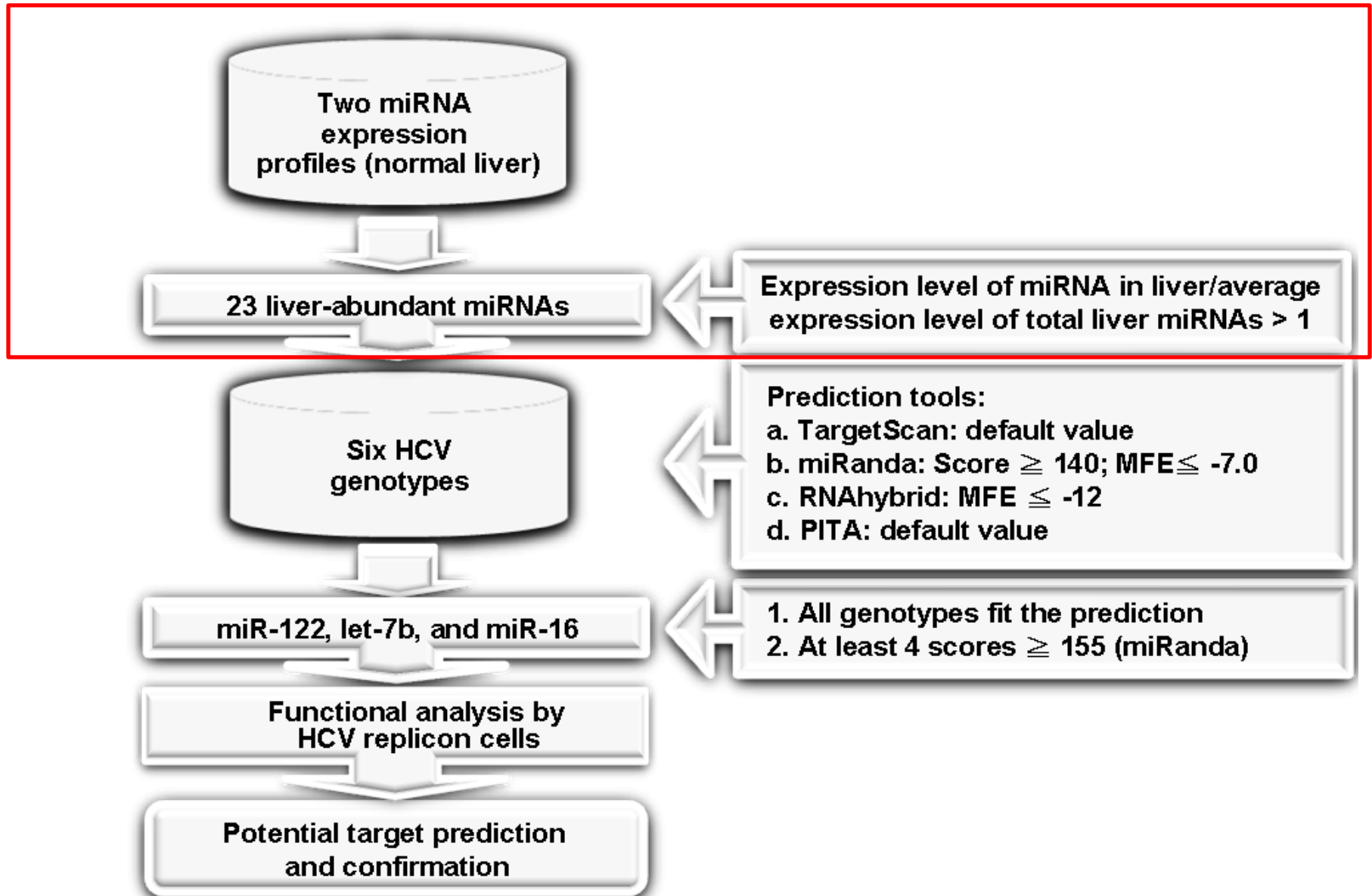


Table S1. The miRNAs that are abundantly expressed in human liver.

miRNA ID	miR-x /average miRNA expressed in liver	
	Liang <i>et al.</i> , 2007 [1]	Hsu SD <i>et al.</i> , 2008 [2]
hsa-let-7a	2.27	23.13
hsa-let-7b	1.43	23.13
hsa-let-7c	5.97	23.13
hsa-let-7f	2.74	1.02
hsa-miR-122	16.54	13.58
hsa-miR-125a	1.50	1.50
hsa-miR-125b	8.29	5.77
hsa-miR-126	8.14	3.82
hsa-miR-16	6.31	5.35
hsa-miR-192	10.19	1.88
hsa-miR-194	6.55	1.63
hsa-miR-19b	8.40	4.46
hsa-miR-20	7.23	2.01
hsa-miR-21	7.88	2.07
hsa-miR-23b	3.01	1.04
hsa-miR-26a	25.05	8.14
hsa-miR-26b	10.65	5.02
hsa-miR-27a	1.01	1.12
hsa-miR-27b	2.86	1.12
hsa-miR-30b	2.77	27.11
hsa-miR-30c	10.30	27.11
hsa-miR-92	11.44	9.95
hsa-miR-93	0.99	1.09

liver-abundant miRNA

miRNA in liver level
 total liver miRNA average level > 1

Two miRNA
expression
profiles (normal liver)

23 liver-abundant miRNAs

Expression level of miRNA in liver/average
expression level of total liver miRNAs > 1

Six HCV
genotypes

Prediction tools:
a. TargetScan: default value
b. miRanda: Score ≥ 140 ; MFE ≤ -7.0
c. RNAhybrid: MFE ≤ -12
d. PITA: default value

miR-122, let-7b, and miR-16

1. All genotypes fit the prediction
2. At least 4 scores ≥ 155 (miRanda)

Functional analysis by
HCV replicon cells

Potential target prediction
and confirmation

Table S2. The HCV genotypes that are subject to analysis in this study.

Genotype	Name	Accession no.	References
1a	H77C	AF011751	[3,4]
1b	HCV-N	AF139594	[3]
1b	Con1	AJ238799	[4]
2a	JFH-1	AB047639	[3,4]
3a	NZL1	D17763	[3]
4a	ED43	Y11604	[4]
5a	SA13	AF064490	[4]
6a	6a33	AY859526	[4]
6b	TH580	D84262	[5]

Two miRNA
expression
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Expression level of miRNA in liver/average
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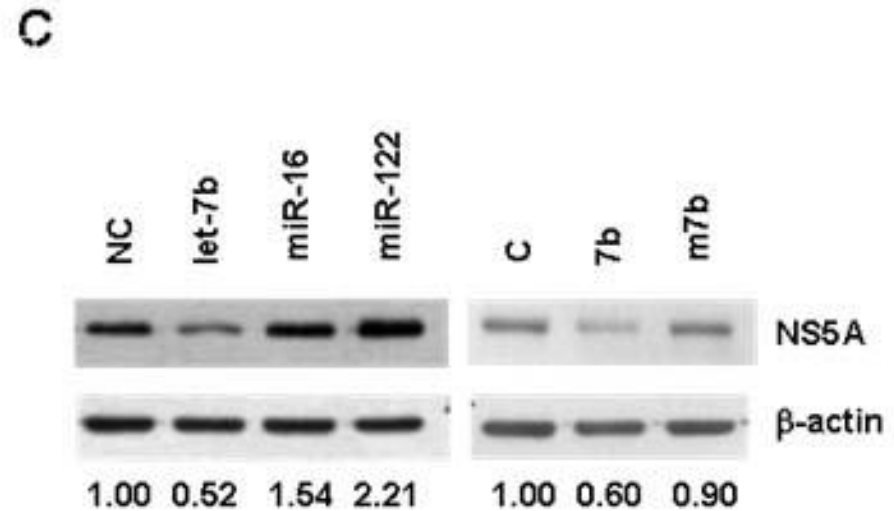
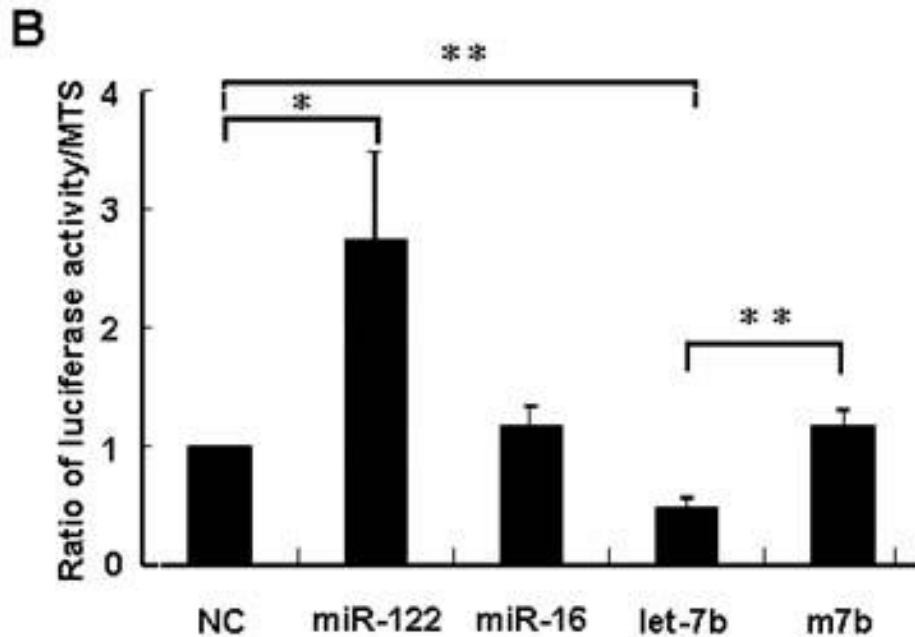
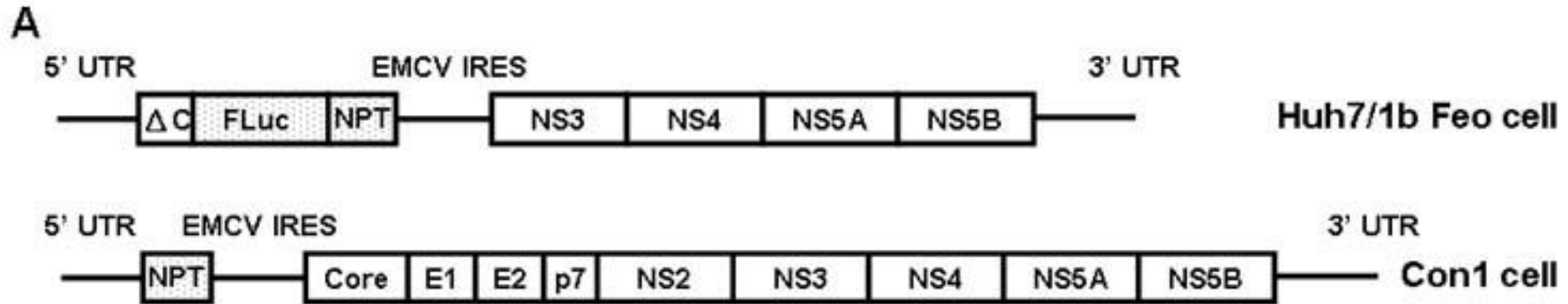
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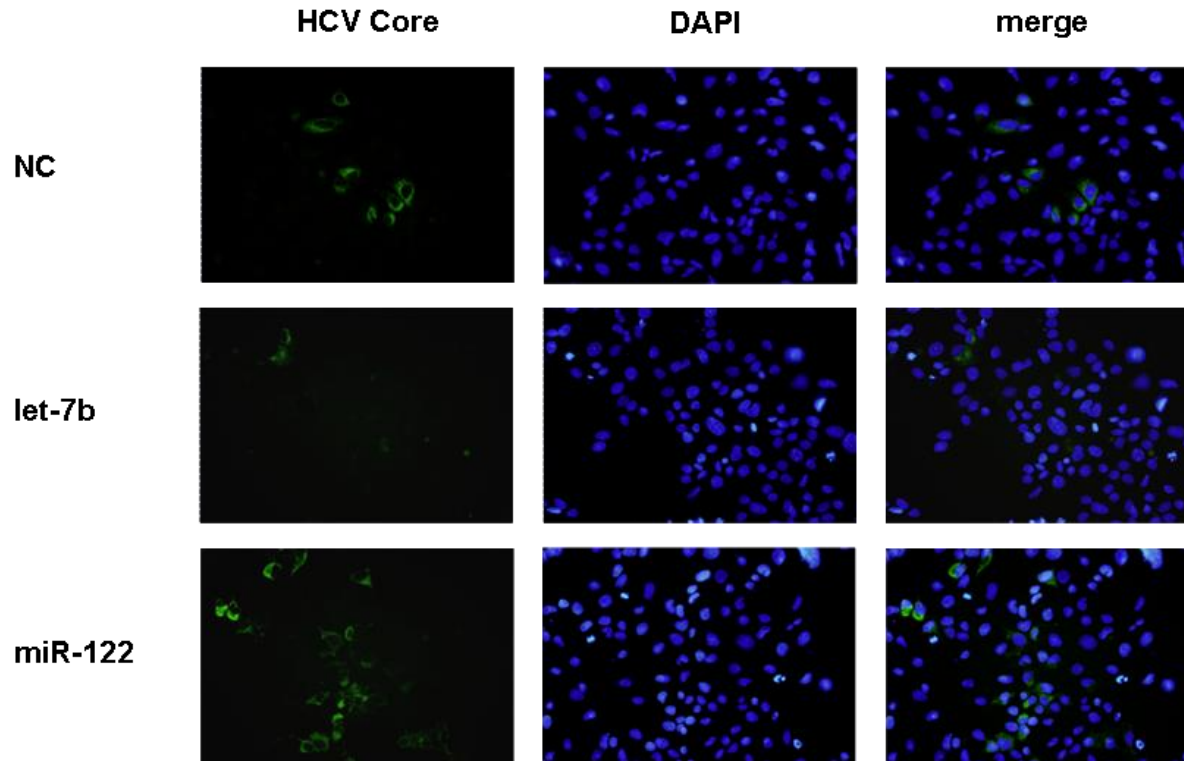
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Characterization of miRNAs with putative target sites on HCV genome

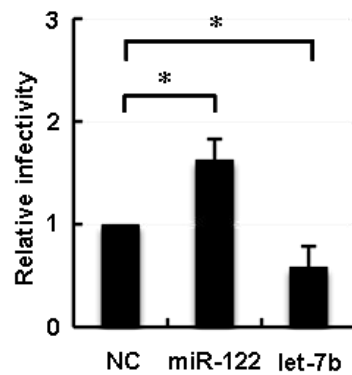


Let-7b reduces HCVcc infectivity

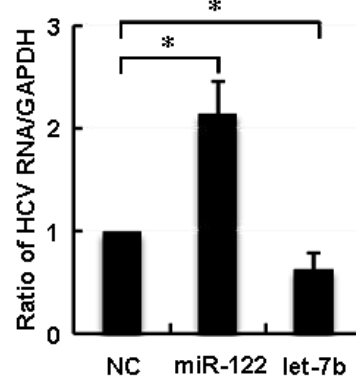
A



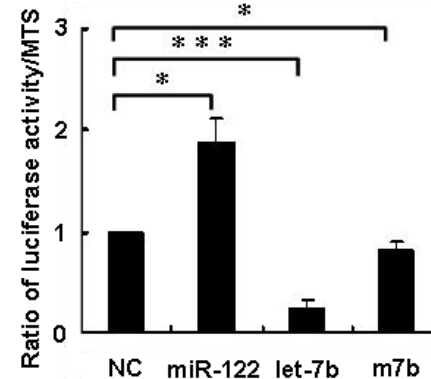
B



C



D



Let-7b physically interacts with HCV genome

miR-122 3'-UGUUUGUGGUAACAGUGUGAGGU-5'

S1-p34m

ACACAGCG

S1-p6m

ACUCUCCG

HCV

5'-ACCU GACACUCCGCCAUGAAU CACUCC CCUG... SL2

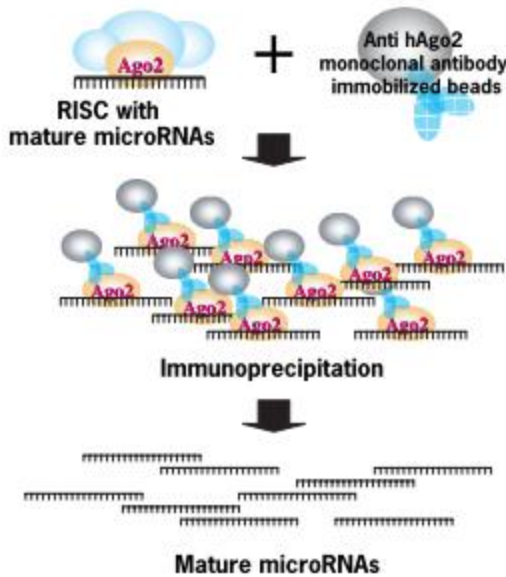
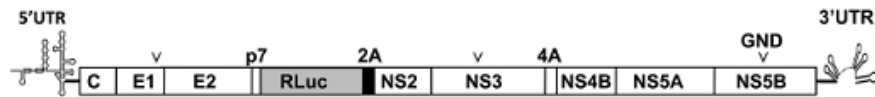
Site 1

Site 2

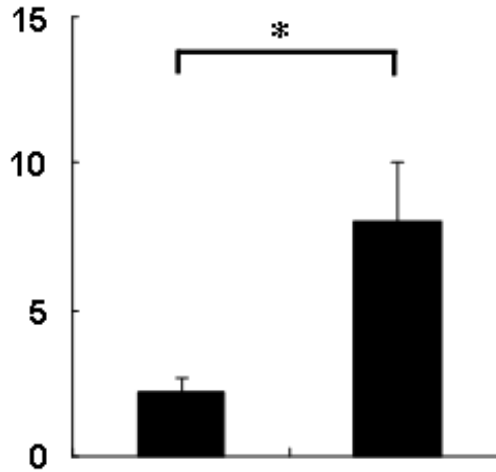
SL1

G C C C G G G
U A A U

A



Relative HCV RNA expression



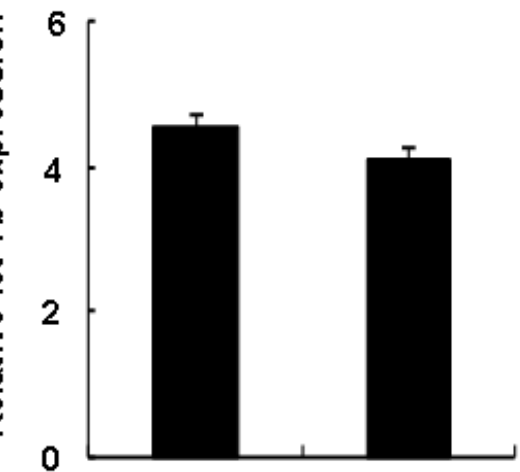
miR-122

wt

mut

(log₁₀)

Relative let-7b expression



miR-122

wt

mut

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expression
profiles (normal liver)

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Expression level of miRNA in liver/average
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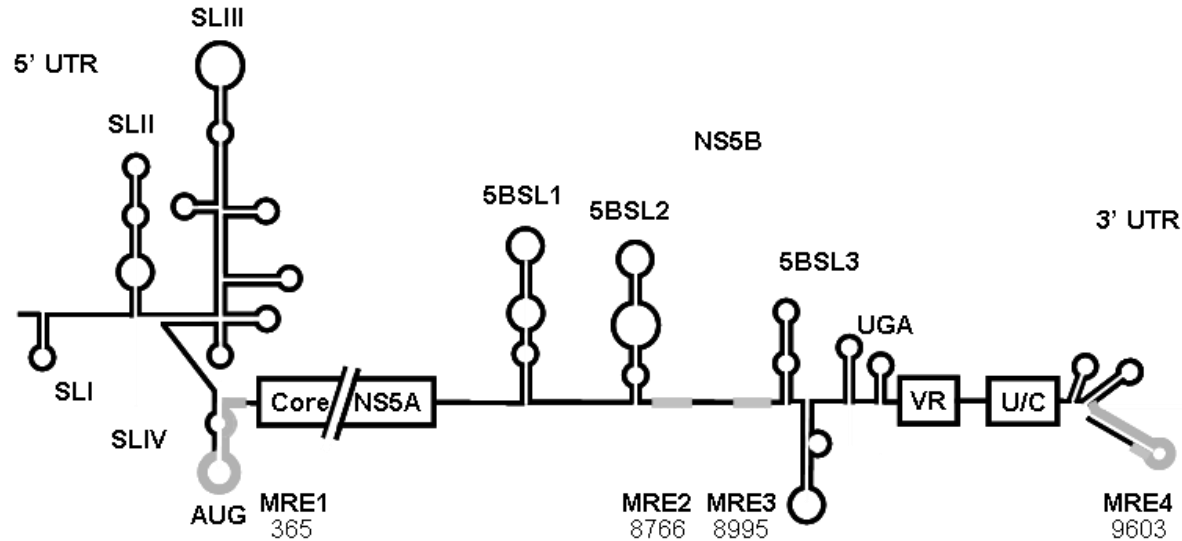
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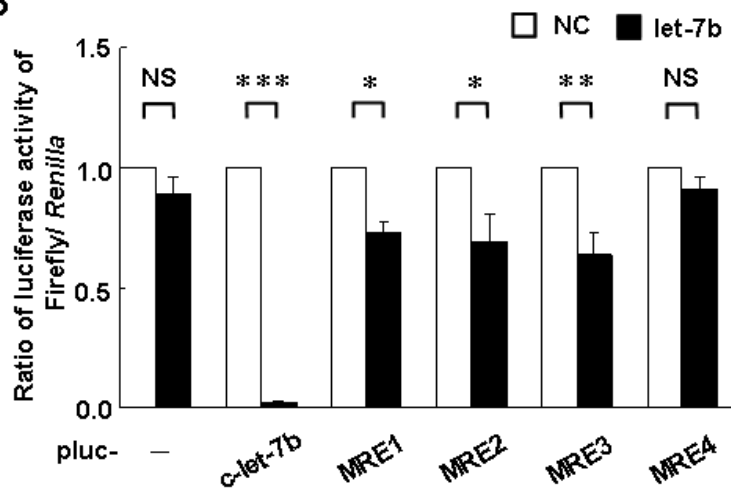
Potential target prediction
and confirmation

The MREs of let-7b are located at the NS5B coding sequences and 5'-UTR of HCV genome

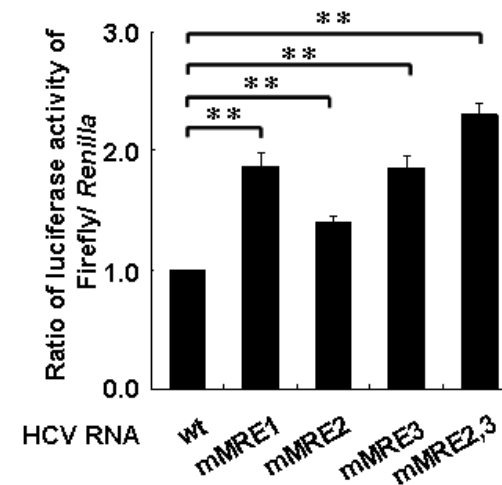
A



B



C

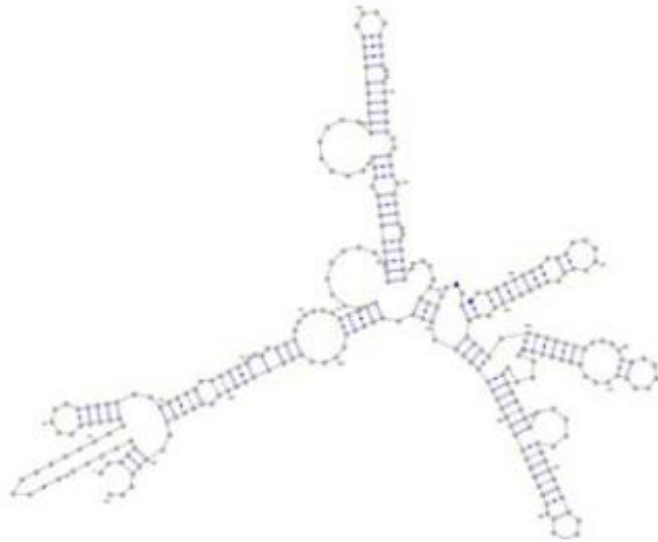


Consideration for generation of mMRE

- Silent mutations of coding region
- Secondary structure of HCV genome

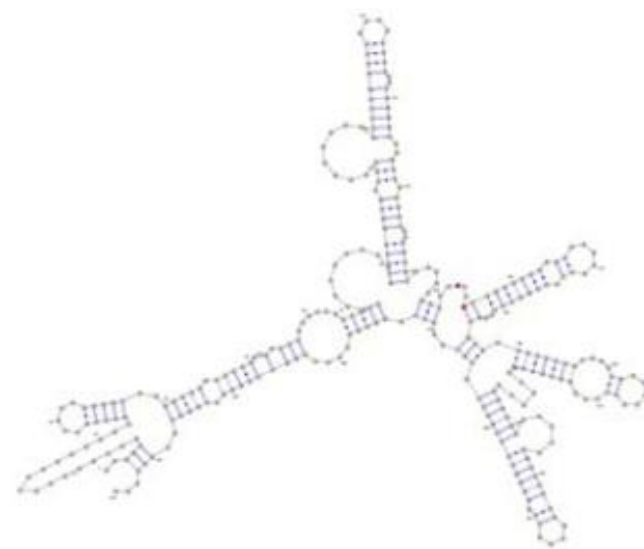
wild-type

miRNA 3' UUGGUGUGUUGGAUGAUGGAGU 5'
 | | | | : | | | | | | | | | |
Target 5' AGCCACUUGAC—CUACCUCA 3'



mMRE3

miRNA 3' UUGGUGUGUUGGAUGAUGGAGU 5'
 | | | | : | | | | | | | | | |
Target 5' AGCCACUUGAC—CUUCGCA 3'



RESEARCH ARTICLE

Let-7b is a novel regulator of hepatitis C virus replication

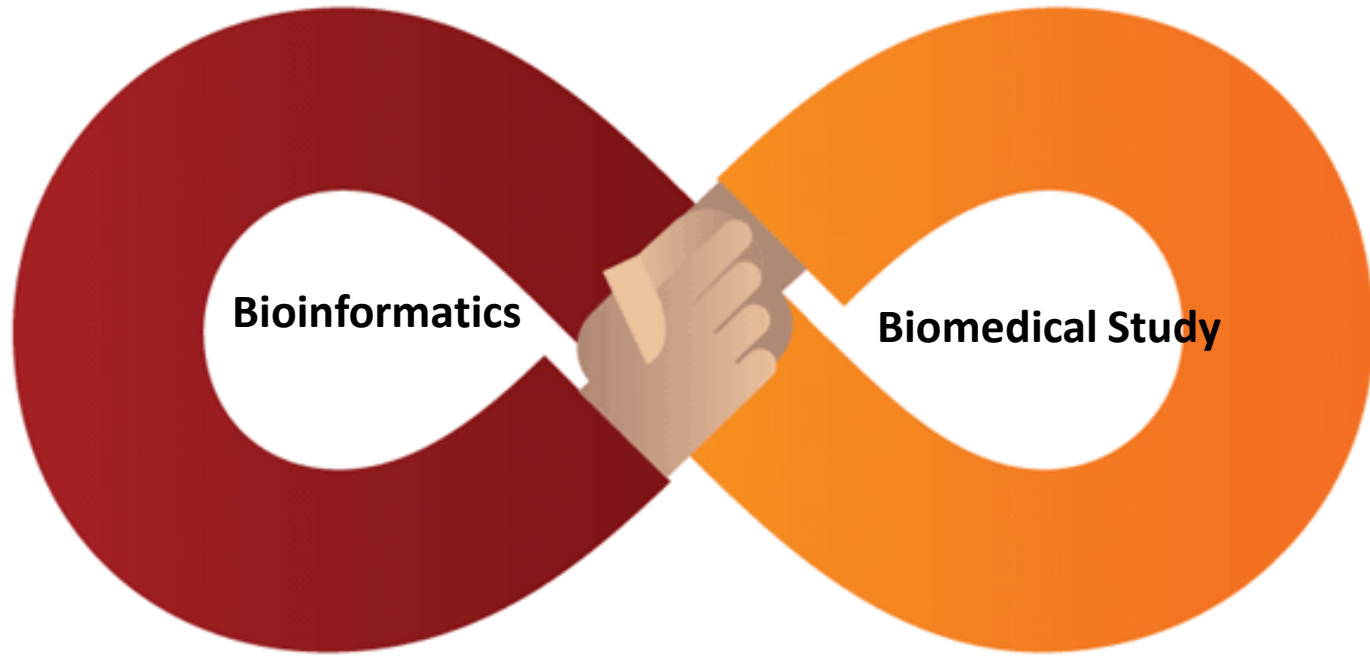
**Ju-Chien Cheng · Yung-Ju Yeh · Ching-Ping Tseng ·
Sheng-Da Hsu · Yu-Ling Chang · Naoya Sakamoto ·
Hsien-Da Huang**

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Summary

- Let-7b is a novel factor which plays a negative role in HCV expression.
- Let-7b binding sites at the coding sequences of NS5B and 5'-UTR of HCV genome that were conserved among various HCV genotypes.
- Let-7b-mediated suppression of HCV RNA accumulation was not dependent on inhibition of HCV translation.
- Let-7b and IFN α -2a also elicited a synergistic inhibitory effect on HCV infection.



Modified from OCLC Brad Gauder

Thank you for your attention!

Ju Laboratory