

Supplemental Table 1: Characteristics and clinical features of serum samples. ALT, alanine aminotransferase; AST, aspartate aminotransferase.

Group* (Case number)	Age \pm SD (Year)	Gender	ALT \pm SD (IU/L)	AST \pm SD(IU/L)
Group A: Normal control (160)	37.52 \pm 12.09 range:20-75	female: 75 male: 85	19.15 \pm 7.53 range:5.1-37.6	21.81 \pm 6.19 range:11.6-33.6
Group B: Asymptomatic HBV carrier (55)	35.80 \pm 11.40 range:21-67	female: 22 male: 33	24.76 \pm 7.43 range:10.7-39.0	20.88 \pm 4.45 range:11.8-38.3
Group C: Chronic hepatitis B (80)	36.22 \pm 12.18 range:20-86	female: 25 male: 55	261.68 \pm 209.89 range:81.2-1602.3	205.10 \pm 142.47 range:82.3-877.5
Group D: HBV-positive HCC (65)	53.43 \pm 9.10 range:40-83	female: 6 male: 59	—	—
Group E: Chronic hepatitis C (48)	35.96 \pm 7.15 range:24-58	female: 9 male: 39	—	—
Group F: HBV-positive HCC (55)	52.83 \pm 7.85 range:42-70	female: 9 male: 46	—	—
Group G: HCC-free control (50)	47.72 \pm 12.09 range: 36-72	female: 8 male: 42	27.3 \pm 11.29 range:6.6-34.6	26.72 \pm 6.57 range:10.2-39.1

***Group inclusion criteria:**

Group A: anti-HBs, HBsAg and HBc negative; anti-HCV and/or HCV RNA negative; No other systematic disease.

Group B: HBsAg and anti-HBc positive; anti-HCV and/or HCV RNA negative; No clinical symptoms of hepatitis; No clinical evidence of liver cirrhosis; No other systematic disease.

Group C: HBsAg and anti-HBc positive for at least 6 months; anti-HCV and/or HCV RNA negative; No clinical evidence of liver cirrhosis; No other systematic disease.

Group D and F: HBsAg and anti-HBc positive; anti-HCV and/or HCV RNA negative; HCC confirmed by biopsy; No clinical evidence of liver cirrhosis.

Group E: anti-HCV and/or HCV RNA positive; HBsAg negative; No clinical evidence of liver cirrhosis; No other systematic disease.

Group G: anti-HBs, HBsAg and HBc negative; anti-HCV and/or HCV RNA negative; No HCC confirmed by biopsy; No clinical evidence of liver cirrhosis.

Supplemental Table 2. Solexa sequencing results for a pooled serum sample from 30 normal controls or 30 pooled chronic hepatitis B patients. The results are normalized against spiked-in plant miRNA-168.

miRNA	Control	HBV	miRNA	Control	HBV
hsa-miR-122a	1	69501	hsa-miR-151	40	16
hsa-miR-423	40	5741	hsa-miR-92b	0	16
hsa-miR-92a	68	3356	hsa-miR-142	110	14
hsa-miR-451	11660	2194	hsa-miR-486-3p	0	14
hsa-let-7c	3	1073	hsa-miR-455-3p	0	11
hsa-miR-23a	3	530	hsa-miR-101	502	9
hsa-miR-16	1361	377	hsa-miR-30a	0	9
hsa-let-7f	402	250	hsa-miR-197	0	9
hsa-let-7g	412	205	hsa-miR-574-3p	0	9
hsa-miR-23b	1	202	hsa-miR-532	6	7
hsa-miR-223	8	168	hsa-miR-923	6	7
hsa-miR-342-3p	1	152	hsa-miR-365	0	7
hsa-miR-375	0	148	hsa-miR-7	95	5
hsa-miR-99a	0	127	hsa-miR-106a	32	5
hsa-miR-150	2	120	hsa-miR-30e	16	5
hsa-miR-21	187	102	hsa-miR-652	9	5
hsa-miR-103	201	91	hsa-miR-148b	7	5
hsa-miR-191	127	91	hsa-miR-106b	280	2
hsa-miR-221	1	89	hsa-miR-186	68	2
hsa-miR-125b	1	89	hsa-miR-142-3p	65	2
hsa-let-7i	215	86	hsa-miR-363	62	2
hsa-miR-93	102	70	hsa-miR-130a	23	2
hsa-miR-185	370	61	hsa-miR-29c	21	2
hsa-miR-378	34	61	hsa-miR-20b	20	2
hsa-miR-629	1	59	hsa-miR-130b	12	2
hsa-miR-222	3	57	hsa-miR-146b	9	2
hsa-miR-10a	1	52	hsa-miR-374a	8	2
hsa-miR-885	0	48	hsa-miR-660	6	2
hsa-miR-99b	0	48	hsa-miR-424	5	2
hsa-miR-193b	0	43	hsa-miR-532-3p	1	2
hsa-miR-27b	2	41	hsa-miR-193a	0	2
hsa-miR-15a	93	39	hsa-miR-20a	297	0
hsa-miR-26a	110	36	hsa-miR-19b	97	0
hsa-miR-107	109	34	hsa-miR-144	64	0
hsa-miR-27a	0	34	hsa-miR-18a	24	0
hsa-miR-128a	2	25	hsa-miR-340	22	0
hsa-miR-483-3p	0	25	hsa-miR-182	16	0
hsa-miR-483	0	25	hsa-miR-98	13	0
hsa-miR-139	0	23	hsa-miR-183	7	0
hsa-miR-17	111	20	hsa-miR-126	7	0
hsa-miR-128b	2	20	hsa-miR-210	6	0
hsa-miR-1	1	20	hsa-miR-362	5	0
hsa-miR-146a	22	18	hsa-miR-96	5	0
hsa-miR-26b	57	16	hsa-miR-454	4	0

Supplemental Table 3. Fold changes of 13 miRNAs between persistent asymptomatic HBV carriers and chronic hepatitis B cases.

miRNA	Sample/No	Mean Fold \pm S.D.	P
miR-375	Asymptomatic HBV carriers /55	1.00 \pm 1.00	Ref.
	Chronic hepatitis B /80	0.89 \pm 0.56	0.36
miR-92a	Asymptomatic HBV carriers /55	1.00 \pm 0.98	Ref.
	Chronic hepatitis B /80	0.96 \pm 0.53	0.72
miR-10a	Asymptomatic HBV carriers /55	1.00 \pm 0.64	Ref.
	Chronic hepatitis B/80	0.90 \pm 0.55	0.28
miR-223	Asymptomatic HBV carriers /55	1.00 \pm 0.43	Ref.
	Chronic hepatitis B /80	0.97 \pm 0.30	0.56
miR-423	Asymptomatic HBV carriers /55	1.00 \pm 2.51	Ref.
	Chronic hepatitis B/80	0.34 \pm 0.52	0.06
miR-23b	Asymptomatic HBV carriers /55	1.00 \pm 1.38	Ref.
	Chronic hepatitis B/80	0.91 \pm 0.90	0.59
miR-23a	Asymptomatic HBV carriers /55	1.00 \pm 3.90	Ref.
	Chronic hepatitis B/80	0.56 \pm 1.05	0.32
miR-342-3p	Asymptomatic HBV carriers /55	1.00 \pm 0.87	Ref.
	Chronic hepatitis B /80	1.15 \pm 0.69	0.22
miR-99a	Asymptomatic HBV carriers /55	1.00 \pm 0.85	Ref.
	Chronic hepatitis B /80	0.92 \pm 0.45	0.42
miR-122a	Asymptomatic HBV carriers /55	1.00 \pm 0.96	Ref.
	Chronic hepatitis B /80	0.98 \pm 0.70	0.85
miR-125b	Asymptomatic HBV carriers /55	1.00 \pm 1.39	Ref.
	Chronic hepatitis B /80	1.02 \pm 0.72	0.93
miR-150	Asymptomatic HBV carriers /55	1.00 \pm 3.66	Ref.
	Chronic hepatitis B /80	0.63 \pm 0.83	0.36
Let-7c	Asymptomatic HBV carriers /55	1.00 \pm 0.74	Ref.
	Chronic hepatitis B/80	0.71 \pm 0.47	0.05

Supplemental Table 4. C_T value and fold changes of the 13 miRNAs between the 4 serum groups.

miRNA	Sample/No	Mean CT±S.D.	Mean Fold±S.D.	P*	P*
miR-375	Control group/160	20.29±1.02	1.00±0.78	Ref.	
	HBV group/135	13.03±1.30	176±173	2.33×10 ⁻²²	6.14×10 ^{-22 a}
	HCV group/48	18.76±1.27	3.45±3.86	6.58×10 ⁻⁵	1.63×10 ^{-20 b}
miR-92a	HBV positive HCC/65	16.98±1.27	11.6±12.4	2.90×10 ⁻⁹	3.56×10 ^{-6 c}
	Control group/160	19.78±1.26	1.00±0.98	Ref.	
	HBV group/135	18.06±0.78	2.76±2.60	5.37×10 ⁻¹²	4.21×10 ^{-21 a}
miR-10a	HCV group/48	15.49±0.51	14.6±5.26	8.53×10 ⁻²³	1.21×10 ^{-4 b}
	HBV positive HCC/65	16.24±1.68	16.7±27.4	1.92×10 ⁻⁵	5.48×10 ^{-1 c}
	Control group/160	24.01±4.78	1.00±1.34	Ref.	
miR-223	HBV group/135	14.13±0.67	74.2±33.0	8.05×10 ⁻⁵⁴	1.96×10 ^{-54 a}
	HCV group/48	29.22±1.33	0.003±0.004	5.38×10 ⁻¹⁷	2.07×10 ^{-54 b}
	HBV positive HCC/65	25.87±1.65	0.04±0.09	5.33×10 ⁻¹⁶	1.53×10 ^{-3 c}
miR-423	Control group/160	25.59±1.06	1.00±0.70	Ref.	
	HBV group/135	22.82±0.56	5.74±2.13	2.55×10 ⁻⁵⁶	3.46×10 ^{-58 a}
	HCV group/48	25.78±0.81	0.78±0.36	4.30×10 ⁻³	1.43×10 ^{-18 b}
miR-23b	HBV positive HCC/65	23.93±0.93	2.97±1.67	7.96×10 ⁻¹⁴	1.02×10 ^{-15 c}
	Control group/160	24.62±1.26	1.00±0.97	Ref.	
	HBV group/135	20.22±2.2	63.9±206	5.36×10 ⁻⁴	6.45×10 ^{-1 a}
miR-23a	HCV group/48	19.78±1.75	51.8±134	1.16×10 ⁻²	3.10×10 ^{-1 b}
	HBV positive HCC/65	19.22±1.38	44.9±46.5	1.56×10 ⁻¹⁰	7.34×10 ^{-1 c}
	Control group/160	34.63±2.27	1.00±2.34	Ref.	
miR-342-3p	HBV group/135	29.89±1.64	12.7±16.5	1.73×10 ⁻¹³	1.13×10 ^{-2 a}
	HCV group/48	29.84±3.22	58.5±120	1.75×10 ⁻³	1.02×10 ^{-4 b}
	HBV positive HCC/65	26.34±2.56	335±627	6.06×10 ⁻⁵	8.93×10 ^{-4 c}
miR-99a	Control group/160	33.33±2.14	1.00±2.94	Ref.	
	HBV group/135	28.69±1.71	26.2±128	2.38×10 ⁻²	9.33×10 ^{-1 a}
	HCV group/48	30.24±3.34	25.0±63.3	1.16×10 ⁻²	1.81×10 ^{-2 b}
miR-122a	HBV positive HCC/65	26.26±2.78	262±779	8.83×10 ⁻³	1.73×10 ^{-2 c}
	Control group/160	19.85±0.99	1.00±0.69	Ref.	
	HBV group/135	14.51±1.38	46.3±39.1	1.16×10 ⁻²⁶	7.45×10 ^{-26 a}
miR-125b	HCV group/48	18.77±0.96	2.08±1.35	1.88×10 ⁻⁶	1.59×10 ^{-20 b}
	HBV positive HCC/65	16.87±1.19	8.60±7.83	6.63×10 ⁻¹¹	7.50×10 ^{-9 c}
	Control group/160	28.69±1.69	1.00±3.54	Ref.	
miR-150	HBV group/135	24.51±1.03	8.04±6.65	2.20×10 ⁻²²	1.6×10 ^{-8 a}
	HCV group/48	27.84±1.64	1.93±5.62	2.83×10 ⁻¹	1.74×10 ^{-1 b}
	HBV positive HCC/65	24.78±1.7	13.7±32.9	2.83×10 ⁻³	6.11×10 ^{-3 c}
Let7c	Control group/160	36.62±2.34	1.00±1.94	Ref.	
	HBV group/135	29.27±1.49	71.6±68.4	5.79×10 ⁻²³	2.42×10 ^{-23 a}
	HCV group/48	38.63±1.28	0.12±0.17	6.29×10 ⁻⁸	1.37×10 ^{-21 b}
miR-125b	HBV positive HCC/65	34.04±2.05	4.09±5.38	2.48×10 ⁻⁵	1.25×10 ^{-7 c}
	Control group/160	26.95±3.33	1.00±2.22	Ref.	
	HBV group/135	20.92±1.13	12.5±11.9	6.54×10 ⁻²¹	3.15×10 ^{-23 a}
miR-125b	HCV group/48	28.19±1.43	0.11±0.19	1.38×10 ⁻⁶	7.50×10 ^{-23 b}
	HBV positive HCC/65	27.03±1.51	0.26±0.46	8.74×10 ⁻⁵	1.99×10 ^{-2 c}
	Control group/160	32.02±2.09	1.00±2.24	Ref.	
miR-150	HBV group/135	29.13±2.24	12.4±53.8	1.52×10 ⁻²	5.52×10 ^{-1 a}
	HCV group/48	30.30±2.31	8.56±31.1	9.90×10 ⁻²	1.48×10 ^{-1 b}
	HBV positive HCC/65	27.57±2.16	22.3±40.2	6.61×10 ⁻⁵	4.29×10 ^{-2 c}
Let7c	Control group/160	19.63±1.85	1.00±1.20	Ref.	
	HBV group/135	17.02±1.20	4.13±3.34	1.37×10 ⁻¹⁹	1.06×10 ^{-21 a}
	HCV group/48	19.08±0.66	0.81±0.37	8.24×10 ⁻²	6.30×10 ^{-3 b}
	HBV positive HCC/65	16.58±1.43	5.99±4.84	9.83×10 ⁻¹²	2.57×10 ^{-12 c}

* All P values are calculated by Student *t* test.

a “HBV group” vs “Chronic hepatitis C patients”

b “HBV group” vs “HBV positive HCC”

c “Chronic hepatitis C patients” vs “HBV positive HCC”

Supplemental Table 5. Fold changes of the six serum miRNAs between the HBV-positive HCC and the age-, gender-matched control groups.

miRNA	Sample/No.	Mean Fold ± S.D.	P*
miR-1	Control/50	1.00±1.37	Ref.
	HCC/55	3.52±2.66	1.37×10 ⁻¹¹
let-7f	Control/50	1.00±1.21	Ref.
	HCC/55	253.60±419.09	1.54×10 ⁻¹⁷
miR-25	Control/50	1.00±1.61	Ref.
	HCC/55	116.41±166.64	8.16×10 ⁻¹³
<u>miR-92a</u>	Control/50	1.00±1.83	Ref.
	HCC/55	4.97±7.91	1.60×10 ⁻¹⁰
miR-206	Control/50	1.00±2.40	Ref.
	HCC/55	2.98±3.94	8.36×10 ⁻⁸
<u>miR-375</u>	Control/50	1.00±0.17	Ref.
	HCC/55	3.97±0.96	1.16×10 ⁻¹⁷

* All *P* values are calculated by Wilcoxon rank-sum (Mann-Whitney) test.

Supplemental Table 6. Solexa sequencing results from serum samples pooled from 25 cirrhosis cases, 25 HBV-positive HCC cases or 25 health controls. Total copies sequenced in three groups: control, 946774; cirrhosis, 614273; HCC, 431633. The final copy number of each miRNA was normalized against total copy number.

miRNA No.	control	cirrhosis	HCC
miR-34c-5p	154	3059	79
miR-1301	0	464	0
miR-17	21	368	0
miR-204	0	211	0
miR-320c	0	570	0
miR-99a	864	5197	1625
miR-21	6	281	0
miR-22	0	277	0
miR-217	0	217	0
miR-449a	0	156	0
miR-455-5p	0	234	0
miR-199a-5p	0	165	0
miR-186	256	1071	0
miR-421	0	214	0
miR-411	0	208	0
miR-720	0	123	0
miR-135a	314	3254	870
miR-653	0	350	0
miR-514	0	538	0
miR-886-3p	0	126	0

Supplemental Table 7. Verified or predicted probable targets of the studied miRNAs that might be involved in the initiation and development of HBV-positive HCC.

miRNAs	Genes	Full name/Alternative titles	P value *
miR-375	JAK2	Janus Kinase 2	Krek et al, 2005
	C1QBP	Complement Component C1q-binding Protein	Krek et al, 2005
	TP53	Tumor Protein p53	4.06×10 ⁻⁴
miR-92a	FAN	Factor Associated with N-SMase Activation	2.46×10 ⁻¹¹
	DKK3	Dickkopf, <i>Xenopus</i> , Homolog of 3	3.36×10 ⁻⁹
	NOX4	NADPH Oxidase 4	1.40×10 ⁻⁶
	AURKA	Aurora Kinase A	1.42×10 ⁻⁵
miR-23a	CXCL12	Chemokine CXC Motif, Ligand 12	Lewis et al, 2003
miR-23b	NOTCH1	Notch, <i>Drosophila</i> , Homolog of, 1	Fukuda et al, 2005
	PTP4A2	Protein-Tyrosine Phosphatase, Type 4A, 2	1.42×10 ⁻⁶
miR-10a	HOXA1	Homeobox A1	Garzon et al, 2006
	GATA6	GATA-Binding Protein 6	7.01×10 ⁻⁶
	TBP	TATA Box-Binding Protein	2.03×10 ⁻⁵
mir-125b	Lin28	Lin28, <i>C. Elegans</i> , Homolog of	Wu et al, 2005
	BAK1	BCL2 Antagonist Killer 1	6.34×10 ⁻⁵
	TNF	Tumor Necrosis Factor	2.49×10 ⁻⁴
mir-122a	CCNG1	Cyclin G1	Gramantieri et al, 2007
	IGFBP1	Insulin-like Growth Factor-Binding Protein 1	1.66×10 ⁻⁵
	MET	MET Protooncogene	1.62×10 ⁻⁴
	WNT1	Wingless-Type MMTV Integration Site Family 1	1.93×10 ⁻⁴
	PLA2G7	Phospholipase A2, Group VII	2.33×10 ⁻⁴
miR-223	STMN1	Stathmin 1	Wong et al, 2008
miR-423	SHIP2	SH2-Containing Inositol Phosphatase 2	8.50×10 ⁻⁵
	IGFBP2	Insulin-like Growth Factor-Binding Protein 2	1.52×10 ⁻⁴
miR-342-3p	TENC1	Tensin-like C1 Domain-Containing Phosphatase	9.88×10 ⁻⁵
miR-99a	MTOR	Mammalian Target of Rapamycin	6.34×10 ⁻⁶
	RELB	V-Rel Avian Reticuloendotheliosis Viral Oncogene Homolog B	1.74×10 ⁻⁴
	FGF4	Fibroblast Growth Factor 4	2.32×10 ⁻⁴
Let7c	MYC	V-MYC Avian Myelocytomatosis Viral Oncogene Homolog	7.35×10 ⁻⁸
miR-150	IGFBP2	Insulin-like Growth Factor-Binding Protein 2	4.55×10 ⁻⁶

* The best *P* value of the selected miRNA for the transcript or reference.

Supplemental references:

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