Novel prognostic biomarkers for hepatocellular carcinoma

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Factors determining the prognosis of HCC

- Tumor burden (size)
- Tumor number
- Presence of vascular invasion (biologic invasiveness)
- Presence of extrahepatic metastasis
- Tumor pathology (differentiation, satellite nodule)

- On-treatment response (imaging)

- **AFP or PIVKA-II (DCP) levels at diagnosis**

Biomarkers in HCC

- Usually molecules directly produced by cancer cells or as an effect of the tumor on healthy tissue

- Serum proteins, oncofetal antigens, hormones, metabolites, receptors, enzymes, genetic materials

- Ideal cancer biomarkers
  - increase in small cancer (high sensitivity)
  - no increase in non-cancer (high specificity)
  - correlate or increase with tumor progression
  - correlate with other poor prognostic indicators
  - predict the prognosis

AFP and PIVKA-II

- α-fetoprotein (AFP)
  - A representative tumor marker of HCC for more than 40 years
  - Elevated in 50~80% of HCC patients, but not specific to HCC
  - Predicts recurrence

- Des-γ-carboxy prothrombin (DCP) = PIVKA-II
  - Liebman et al. first reported, in 1984
  - Another useful diagnostic tumor marker less sensitive more specific
  - Relation to PVI, tumor size, intrahepatic metastasis, recurrence

Liebman, et al. NEJM 1984; Imamura, et al. Br J Surg 1999; Kim DY, et al. Oncology 2007

- AFP and DCP
  - Are produced independently by HCC
  - May serve complementarily in the diagnosis of HCC

Fujiyama S et al. Dig Dis Sci 1991; Aoyagi Y et al. Cancer 1996

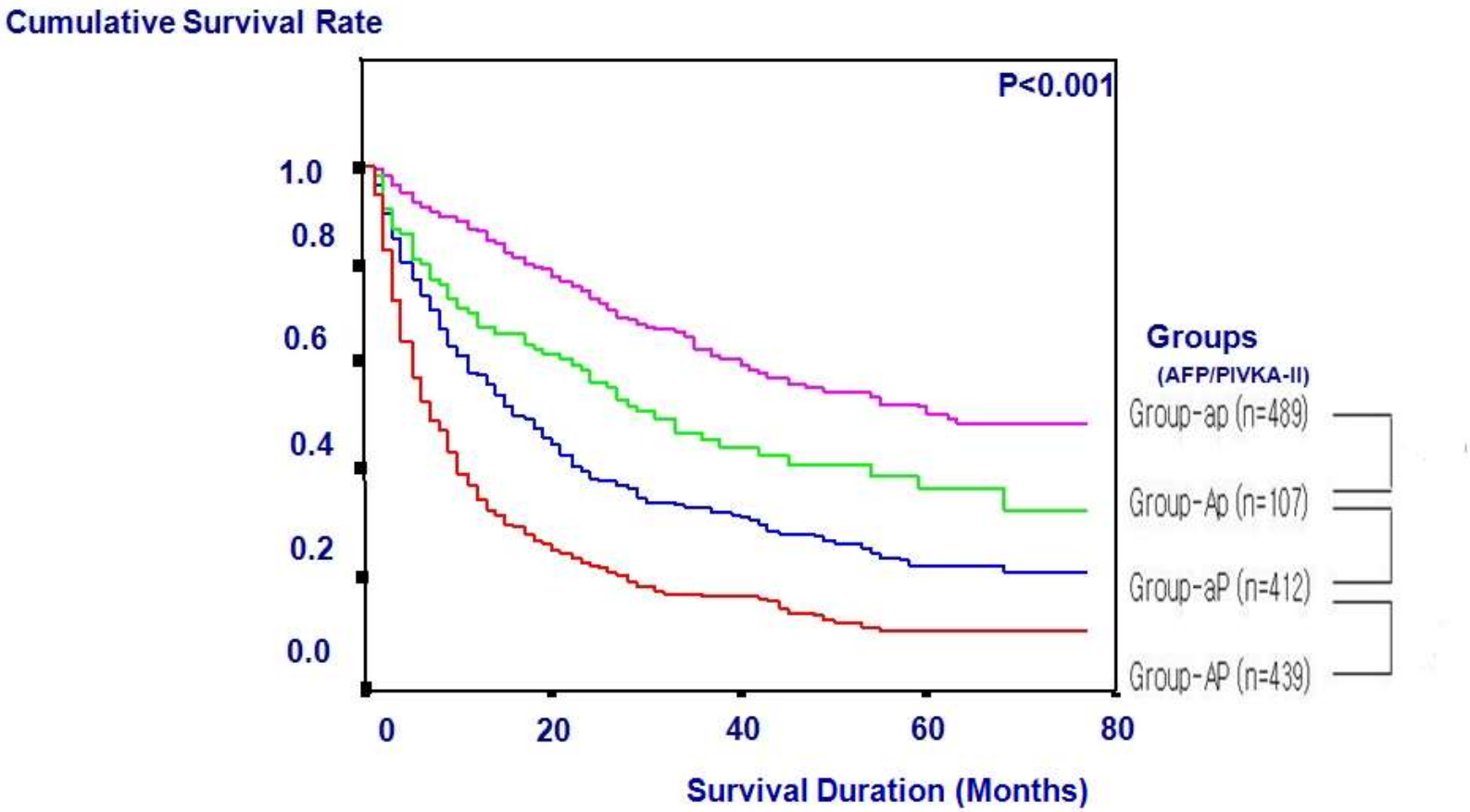
- Combined measurement of AFP and PIVKA-II appears to be useful in predicting the prognosis of HCC

Prognosis of HCC with different sets of serum AFP and PIVKA-II levels

- Retrospective cohort between Jan. 2003 and Dec. 2007 in YUMC
- 1,447 patients with HCC (EASL guideline[1])
- Underlying liver disease
  - ✓ HBV (n=1048, 72.4%)
  - ✓ HCV (n=151, 10.4%)
  - ✓ others (n=248,17.2%)
- Four groups
- The cutoff value
  - ✓ AFP > 400ng/ml[2]
  - ✓ PIVKA-II > 100mAU/ml[3]

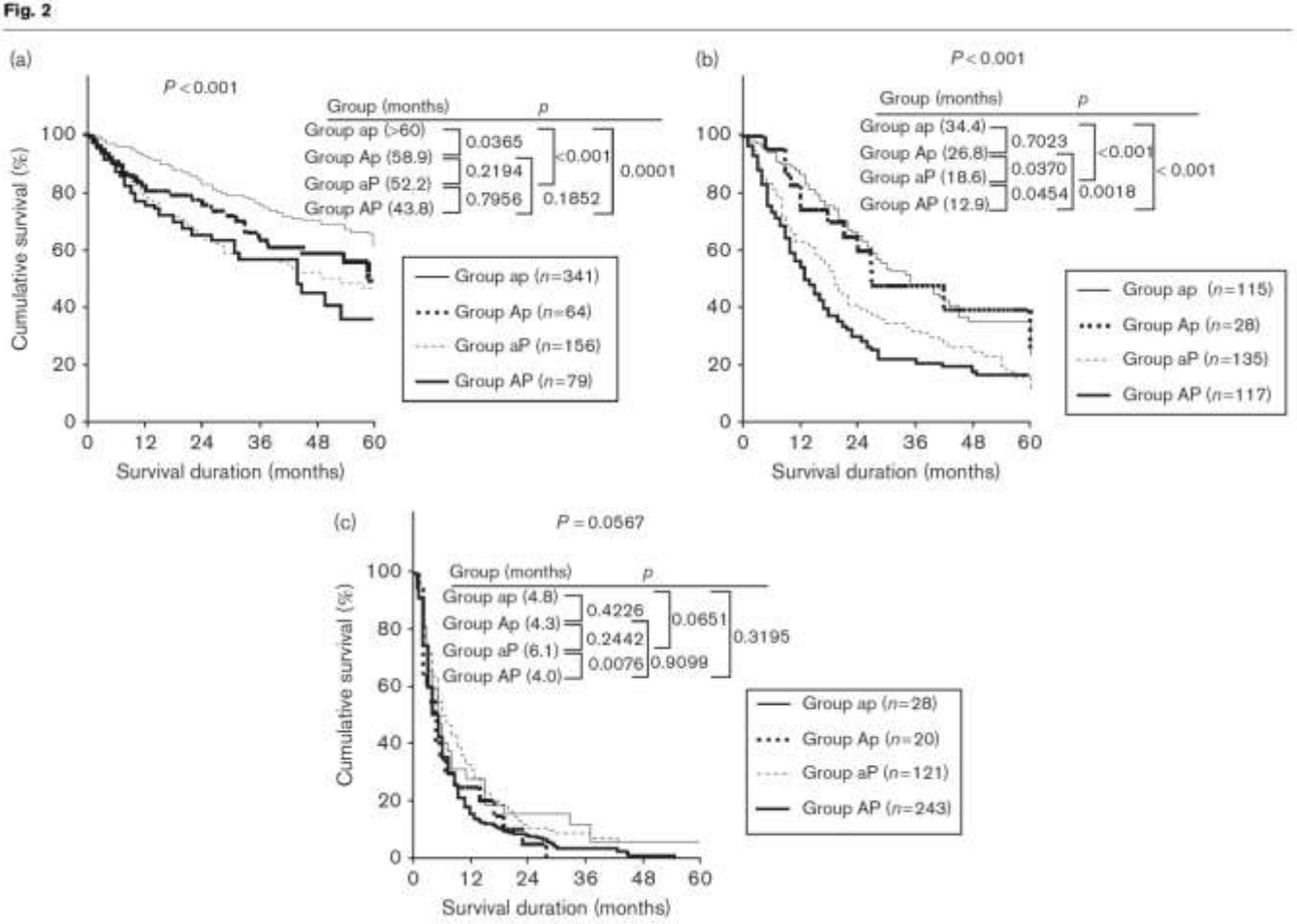
	AFP	PIVKA-II
Group ap	↓	↓
Group Ap	↑	↓
Group aP	↓	↑
Group AP	↑	↑

Baseline AFP and PIVKA-II level might be helpful for predicting prognosis



Kim DY, et al. Eur J Gastroenterol Hepatol 2012

Low baseline AFP/DCP levels predicts better survival in each TNM stage

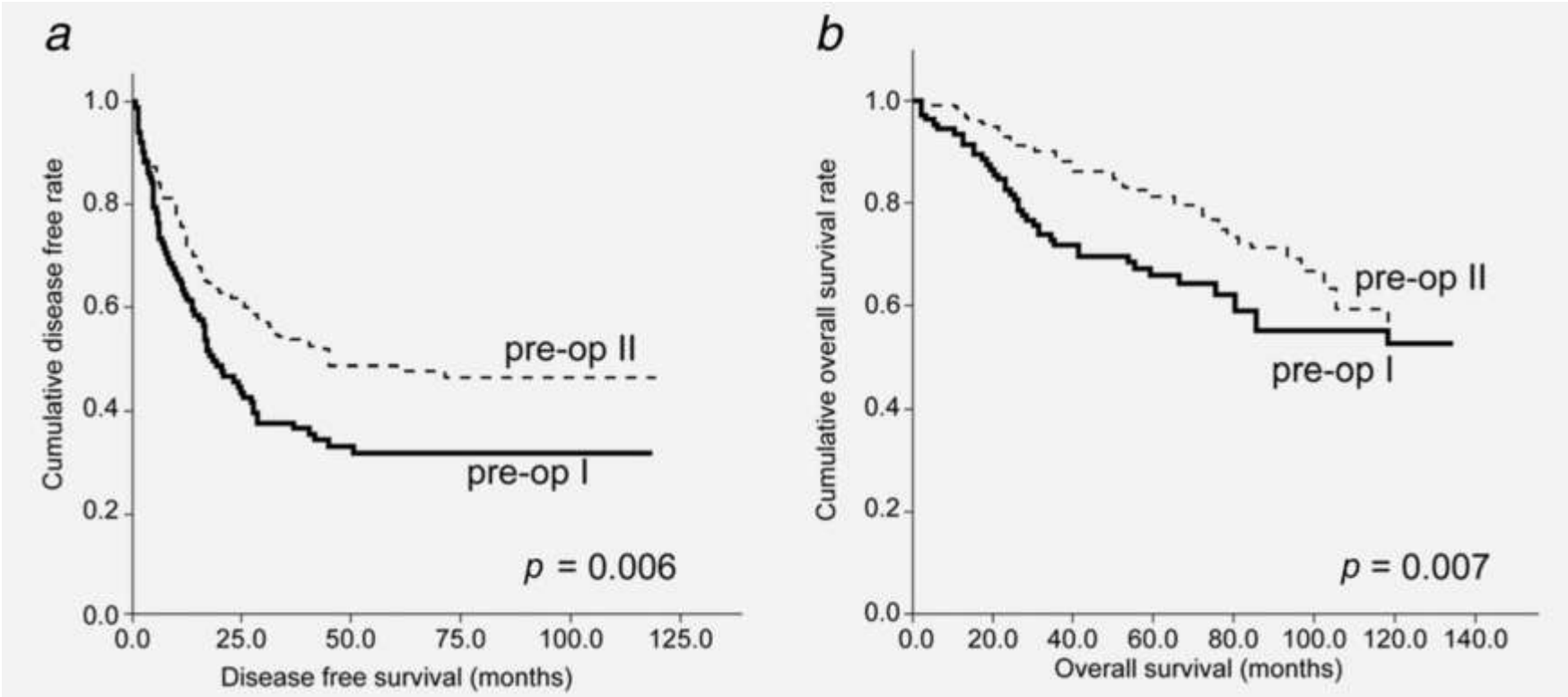


Survival of the four groups according to the TNM by LCSGJ. (a) TNM stages I and II; (b) TNM stage III; and (c) TNM stage IV. LCSGJ, Liver Cancer Study Group of Japan; TNM, tumor-node-metastasis.

Kim DY, et al. Eur J Gastroenterol Hepatol 2012

Simultaneous measurement of pre-operative AFP and PIVKA-II predicts recurrence after curative resection in HBV-related HCC

- Between Jan. 2001 and Dec. 2007 at YUMC (*n* = 267)



Cut-off: AFP, 20 ng/ml, DCP 40 mAU/ml

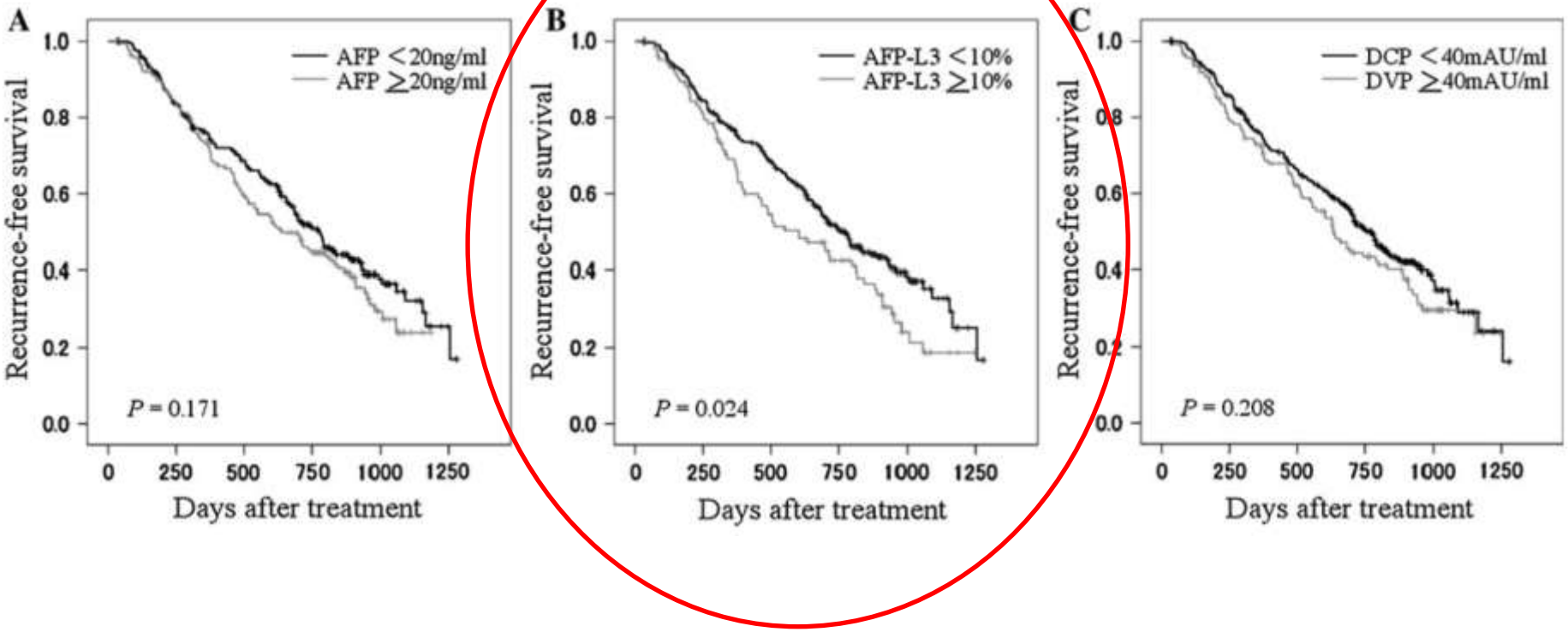
Chon YE, et al. Int J Cancer 2012

AFP-L3 as a novel biomarker for HCC

- Lens culinaris agglutinin-reactive fraction of alpha-fetoprotein
  - Very specific marker for HCC
  - Early diagnosis and prediction of prognosis and malignant potential
  - Described as percentage of AFP (%)
  - Affected by total AFP level

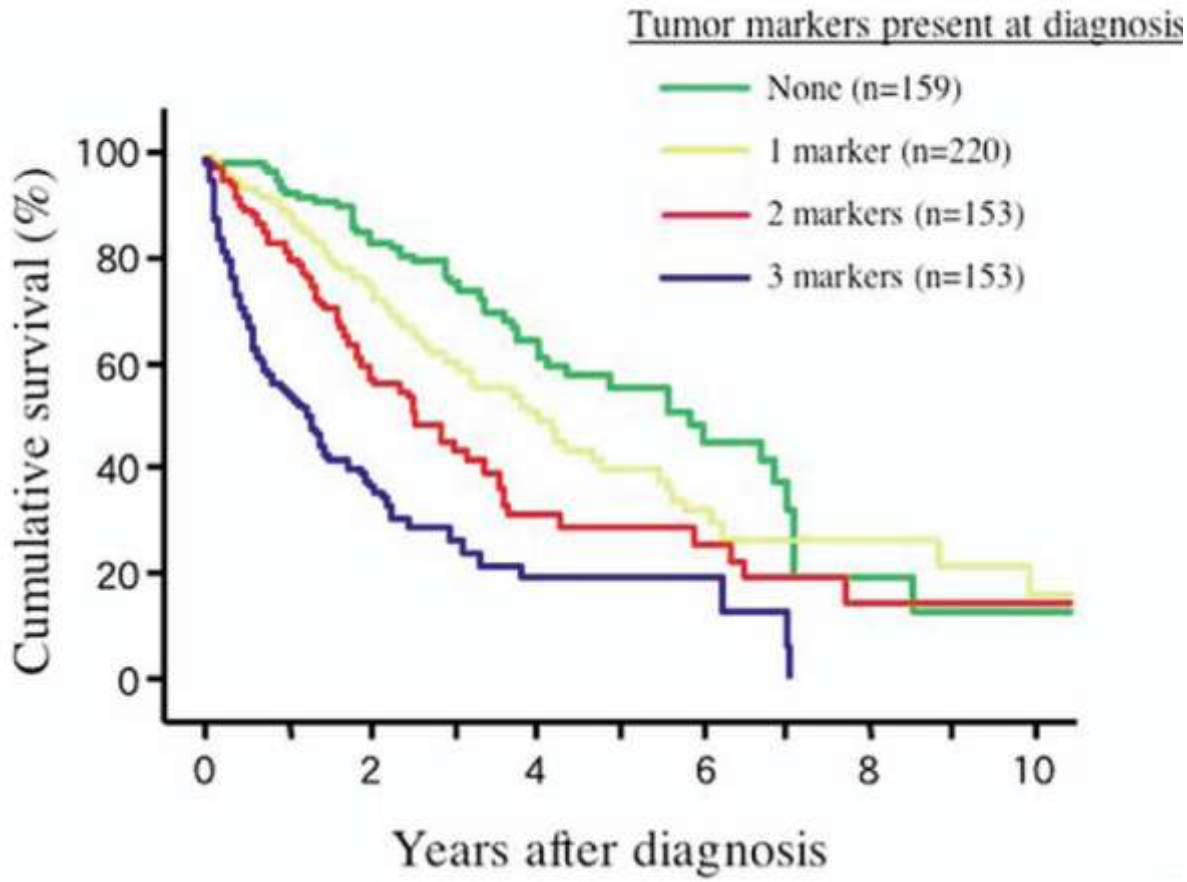
- Highly sensitive AFP-L3
  - Micro-total analysis system (μTAS)
  - Detected in very low total AFP level

Prediction of recurrent HCC after resection using hsAFP-L3



Tamura Y, et al. Dig Dis Sci 2013

Combined measurement of AFP, DCP, AFP-L3 for the prediction of prognosis



Cut-off: AFP, 20 ng/ml, DCP 40 mAU/ml, AFP-L3, 10%

Toyota H, et al.
Clin Gastroenterol Hepatol 2006

Proteomics, Huge input → Small output

The candidates-11 to validate

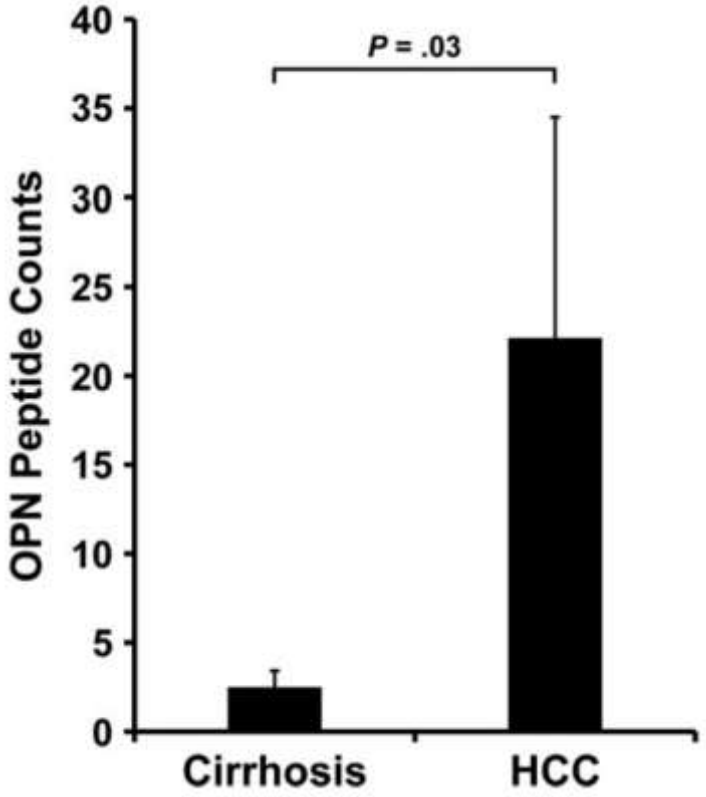
- FBXO38
- MUC19
- FGG
- DSC2
- TKT
- CAT
- ANXA1
- ANXA3
- MMP2
- FTH1
- PTPRG
- COL5A3
- MEGF8
- NENF
- ESAM
- HOXA4
- COL6A3
- COL6A1
- COL4A3
- MAN2A1
- MAN2A2
- ASXL3
- CACNA2D1
- SVEP1
- CSTA
- ARMC2
- HMBS
- CFHR3
- F11
- CDH1
- S100A6
- PCDH12
- PDGFD
- PDGFB
- HGF
- Calreticulum
- CD14

Exciting novel biomarkers for early HCC detection?

Biomarker	Clinical use
AFP	**Early diagnosis, prediction of prognosis**
AFP-L3	**Early diagnosis, prediction of prognosis**
DCP	**Early diagnosis, prediction of prognosis**
Gamm-glutamyl transferase	Early diagnosis
Alpha-1-fucosidase	Early diagnosis
Glypican-3	Early diagnosis
Human carbonyl reductase 2	**Prediction of prognosis?**
Golgi phosphoprotein 2	**Tumor aggressiveness**
Transforming growth factor beta	**Tumor invasiveness**
Hepatocyte growth factor (HGF)	**Prognosis and recurrence**
Tumor specific growth factor	Diagnosis
Epidermal growth factor receptor family	**Recurrence**

Osteopontin

- An acidic, highly phosphorylated and glycosylated calcium-binding secretory protein, is expressed widely and has a diverse range of functions. These include cell adhesion and migration, immune and inflammatory response, antiapoptosis, suppression of nitric oxide synthase and bone calcification.



Shang S, et al. Hepatology 2012

Characteristics of 55 HCC patients

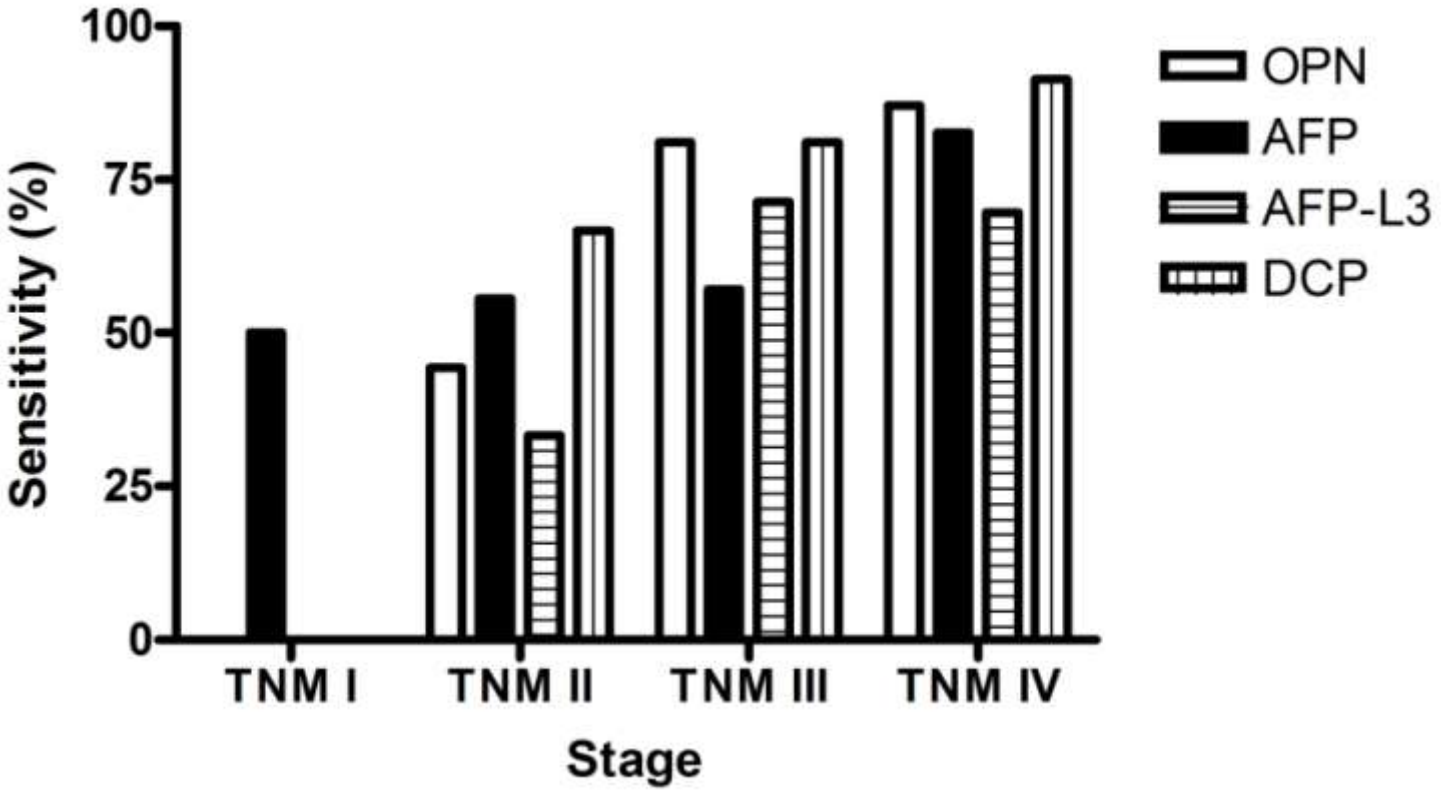
Variable	Value	Variable	Value
Patients number	55	Type of HCC (%)	
M:F (%)	42:13 (76.4:23.6)	Uninodular	11 (20.0)
Age, (mean±SD)	57.3±11.2	Multinodular	23 (41.8)
Median (range)	58 (35~84)	Massive	13 (23.6)
Etiology (%)		Infiltrative	8 (14.5)
HBV	49 (89.0)	**BCLC stage (%)**	
HCV	3 (5.5)	**A**	**9 (16.4)**
others	3 (5.5)	**B**	**18 (32.7)**
Child-Pugh class (%)		**C**	**26 (47.3)**
A	41 (74.5)	**D**	**2 (3.6)**
B	13 (23.6)	**TNM stage (%)**	
C	1 (1.8)	**I**	**2 (3.6)**
DM (%)	6 (10.9)	**II**	**9 (16.4)**
Histologic Dx (%)	3 (5.5)	**III/IV**	**21 (38.2)/23 (41.8)**

Sensitivity of each marker according to stage

		Sensitivity			
Stage	No. pt.	**OPN**	**AFP**	**AFP-L3**	**DCP**
TNM Ⅰ	2	**0**	**50%** (1/2)	**0**	**0**
TNM Ⅱ	9	**44.4%** (4/9)	**55.6%** (5/9)	**33.3%** (3/9)	**66.7%** (6/9)
TNM Ⅲ	21	**81.0%** (17/21)	**57.1%** (12/21)	**71.4%** (15/21)	**81.0%** (17/21)
TNM Ⅳ	23	**87.0%** (20/23)	**82.6%** (19/23)	**69.6%** (16/23)	**91.3%** (21/23)
BCLC-A	9	**33.3%** (3/9)	**66.7%** (6/9)	**44.4%** (4/9)	**77.8%** (7/9)
BCLC-B	18	**72.2%** (13/18)	**55.6%** (10/18)	**33.3%** (6/18)	**61.1%** (11/18)
BCLC-C	26	**92.3%** (24/26)	**76.9%** (20/26)	**73.1%** (19/26)	**92.3%** (24/26)
BCLC-D	2	**50%** (1/2)	**50%** (1/2)	**50%** (1/2)	**100%** (2/2)

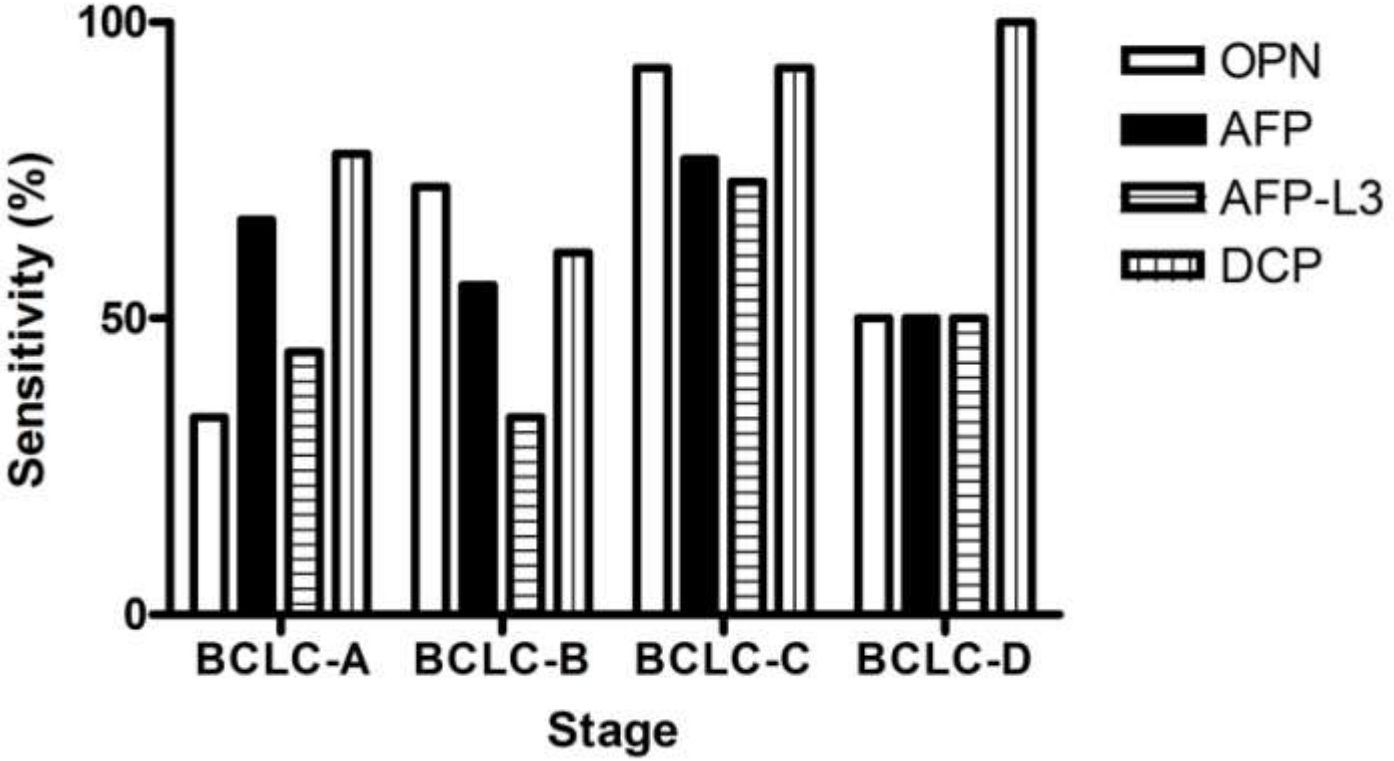
Best cut-off: OPN, 64.2ng/ml; AFP, 16.55ng/ml; AFP-L3, 10.35%; DCP, 0.295ng/ml.

Sensitivity of each marker according to TNM stage



Best cut-off: OPN, 64.2ng/ml; AFP, 16.55ng/ml; AFP-L3, 10.35%; DCP, 0.295ng/ml.

Sensitivity of each marker according to BCLC stage



Best cut-off: OPN, 64.2ng/ml; AFP, 16.55ng/ml; AFP-L3, 10.35%; DCP, 0.295ng/ml.

Overexpression of Osteopontin Is Associated with Intrahepatic Metastasis, Early Recurrence, and Poorer Prognosis of Surgically Resected Hepatocellular Carcinoma

Cancer 2003;98:119–27.

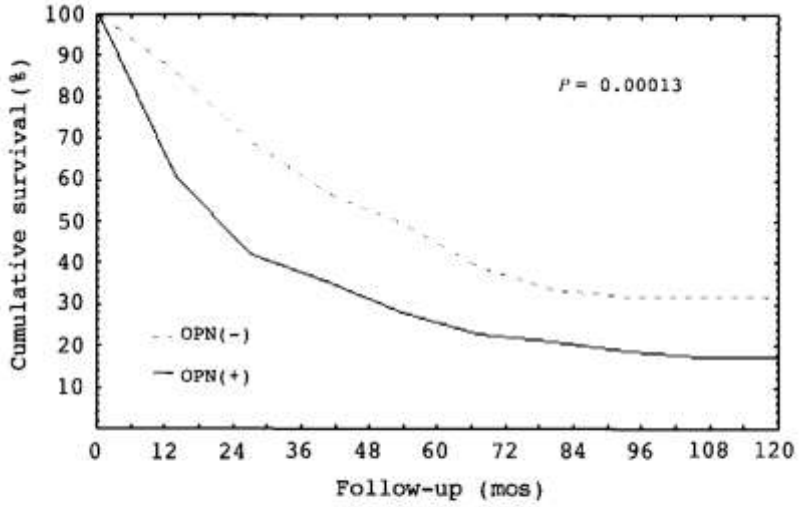
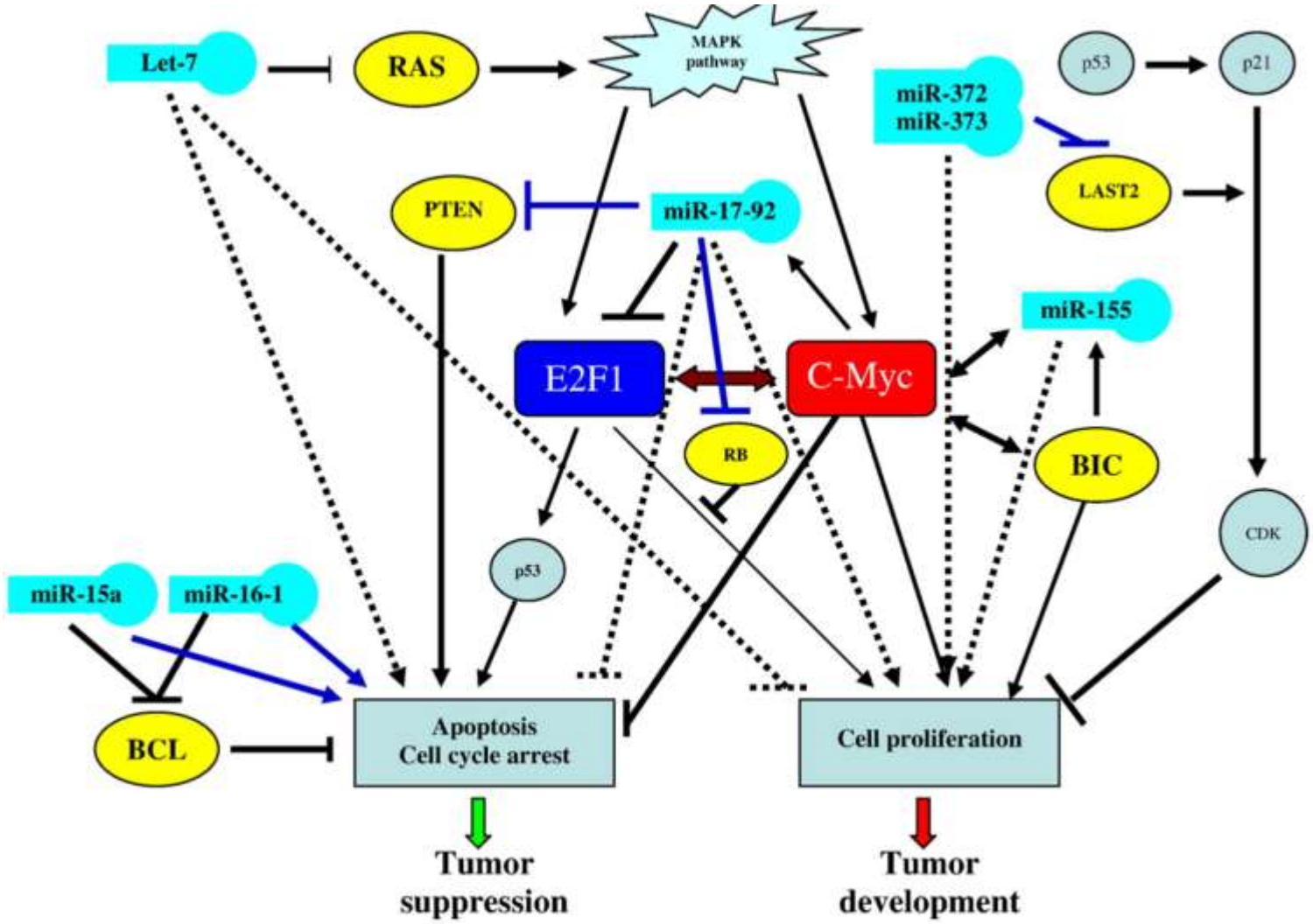


FIGURE 5. Cumulative survival curve for 240 patients with primary unifocal hepatocellular carcinoma (HCC). HCC with osteopontin (OPN) mRNA overexpression, designated OPN (+), had a significantly worse 10-year survival rate than HCC without OPN mRNA overexpression ($n = 107$), designated OPN (−) ($P = 0.00013$; log rank test).

TABLE 3
Age-and-Gender-Adjusted Relative Risk values for Selected Predictors of Overall Survival at 120 Months after Resection of Hepatocellular Carcinoma

Predictor	RR	95% CI	*P* value
HBsAg			
Positive vs negative	1.08	0.78–1.49	0.647
AFP (ng/mL)			
≥ 400 vs. < 400	1.39	1.05–1.85	0.021
Child-Pugh grade			
B vs. A	1.34	0.77–2.32	0.298
Cirrhosis			
Yes vs. no	1.06	0.81–1.40	0.675
Tumor size (cm)			
3.1–5 vs. ≤ 3	1.79	1.21–2.65	0.004
> 5 vs. ≤ 3	2.03	1.45–2.82	< 0.001
Tumor grade			
II vs. I	1.51	1.1–2.32	0.012
III–IV vs. I	1.58	1.10–2.26	0.013
Tumor stage			
IIIA vs. I–II	1.36	0.97–1.92	0.078
IIIB–IV vs. I–II	2.82	2.06–3.86	< 0.001
Early recurrence[a]			
Yes vs. no	4.49	3.34–7.29	< 0.001
OPN overexpression			
Yes vs. no	1.32	1.01–1.72	0.039

MicroRNAs as oncogenes and tumor supressor genes



Zhang , et al. Dev Biol 2007;302:1-12

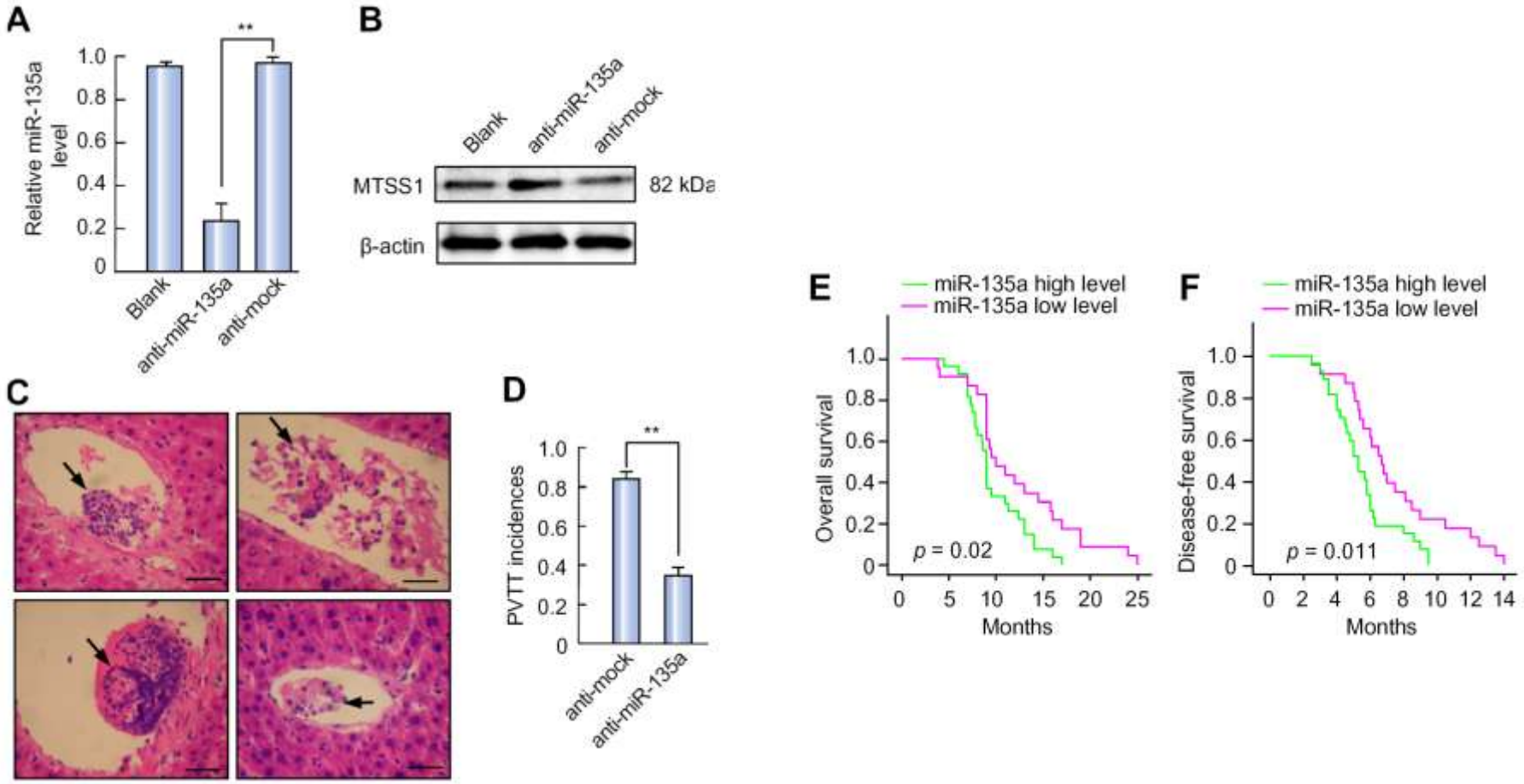
MicroRNA-related HCC aggressiveness (I)

Table 1. miRNAs aberrant expression in PVTT.

miRNA	Means ± SD	*p* value	Chromosome location	Potential targets
Increased expression >1.5-fold				
miR-135a	5.94 ± 0.10	<0.05	3p21.1, 12q23.1	*MTSS1, GAS7*
miR-302d	2.56 ± 0.34	<0.01	4q25	*RASSF2, RGL1*
miR-517b	2.35 ± 0.51	<0.05	19q13.42	*WNT4,TSC1*
miR-34a	2.06 ± 0.33	<0.05	1p36.22	*FKBP1B, FOXP1*
miR-424	1.92 ± 0.07	<0.01	Xq26.3	*MYB, PAPPA*
miR-130a	1.84 ± 0.06	<0.01	11q12.1	*DDX6, GJA1*
miR-195	1.66 ± 0.26	<0.05	17p13.1	*CD28, KIF23*
miR-624	1.63 ± 0.34	<0.05	14q12	*NBEA, NFIB*
miR-150	1.53 ± 0.11	<0.05	19q13.33	*RC3H1, PIK3R1*
miR-199b	1.52 ± 0.09	<0.05	9q34.11	*GARNL1, SULF1*
Decreased expression <0.6-fold				
miR-214	0.37 ± 0.07	<0.01	1q24.3	*RC3H1, ZFAND3*
miR-654	0.35 ± 0.14	<0.05	14q32.31	*MTSS1, KIF21B*
miR-675	0.33 ± 0.10	<0.01	11p15.5	*MARK4*
miR-503	0.32 ± 0.19	<0.05	Xq26.3	*ZNF423,TNRC6B*
miR-433	0.17 ± 0.18	<0.05	14q32.2	*NAV1, SORBS1*

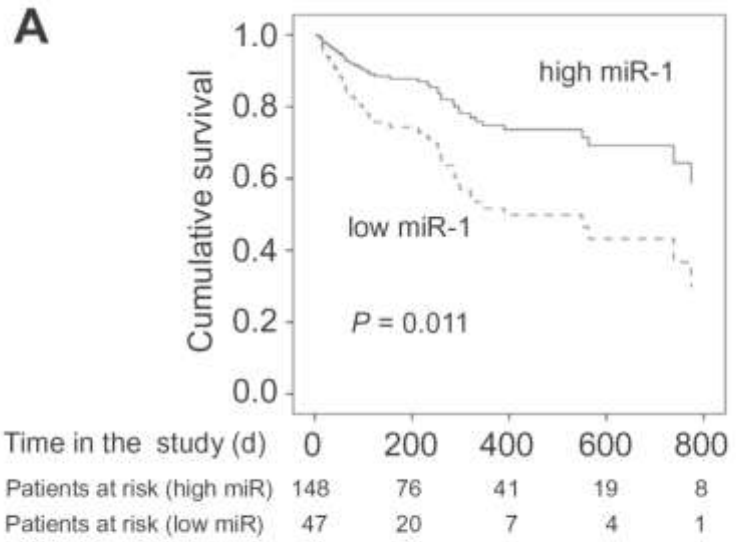
Liu , et al. J Hepatol 2012;56:389-396

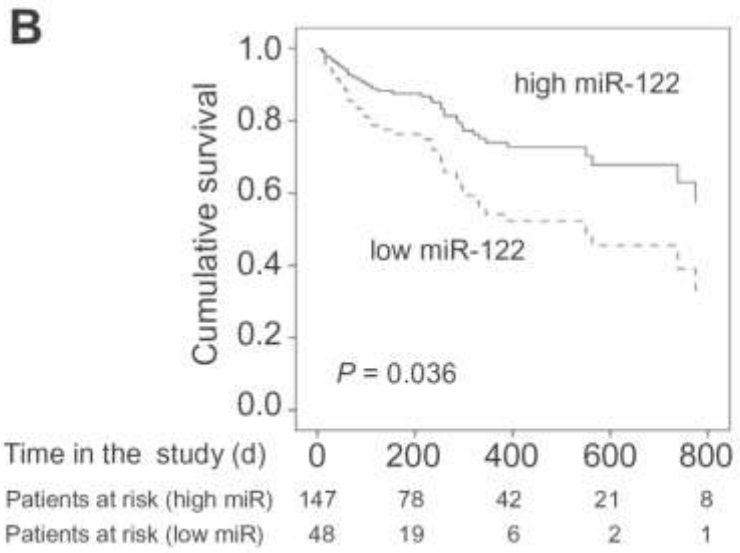
MicroRNA-related HCC aggressiveness (II)



Liu , et al. J Hepatol 2012;56:389-396

Serum microRNA as a prognostic biomarker





Koberle V, et al. Eur J Cancer 2013

Conclusions

- AFP and DCP: proven to predict prognosis of HCC
- AFP-L3: validation required
- other potential biomarkers including miRNA: still a long way