miR-96 Regulates Liver Tumor-Initiating Cells Expansion by targeting TP53INP1 and Predicts Sorafenib Resistance

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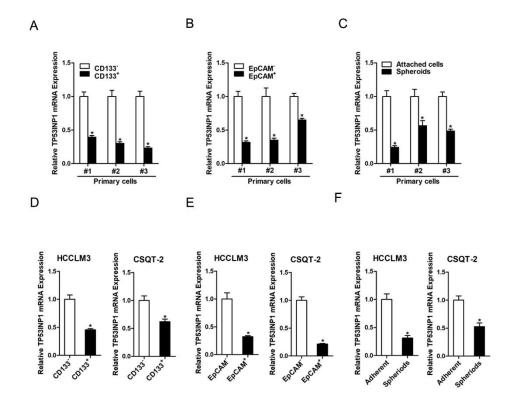
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Supplementary Figure 1

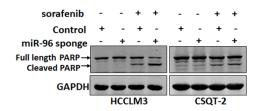


Supplementary Figure 1.

- A. The expression of TP53INP1 in CD133⁺ and CD133⁻ primary HCC cells was checked by real-time PCR assay. (n=3)
- B. The expression of TP53INP1 in EpCAM⁺ and EpCAM⁻ primary HCC cells was checked by real-time PCR assay. (n=3)
- C. Realtime–PCR analysis of TP53INP1 in primary HCC adherent and spheroids cells. (n=3)
- D. The expression of TP53INP1 in CD133⁺ and CD133⁻ HCC cell lines was checked by real-time PCR assay. (n=3)
- E. The expression of TP53INP1 in EpCAM⁺ and EpCAM⁻ HCC cell lines was checked by real-time PCR assay. (n=3)
- F. Realtime–PCR analysis of TP53INP1 in human HCC adherent and spheroids cells. (n=3) (Data are represented as mean±s.d.; *P<0.05; two-tailed Student's t-test.)

Supplementary Figure 2

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Supplementary Figure 1.

A. miR-96 knockdown and control hepatoma cells were treated with 10 μ M sorafenib as indicated for 48 hours. The protein of cleaved-PARP was determined by western blot.

Supplementary Table 1. Clinicopathologic Features of 91 HCC Specimens

Characteristics		miR-96 low	miR-96 high	n voluo	
Characteristics		(n=46)	(n=45)	p value	
Age(year)	≤50	24	25	>0.05	
	>50	22	21		
Gender	Male	38	37	>0.05	
	Female	8	8		
LID a A a	Positive	29	42	< 0.05	
HBsAg	Negative	17	3		
AED(u.~/L)	≤400	26	27	>0.05	
$AFP(\mu g/L)$	>400	20	18		
Tumor size(cm)	≤5	21	19	>0.05	
	>5	25	26		
Tumor number	Single	38	27	< 0.05	
i umor number	Multiple	8	18		
Portal vein tumor thrombus	Yes	6	4	>0.05	
	No	40	41		
Pathological satellite	Yes	21	32	< 0.05	
	No	25	13		
	A	32	13		
BCLC stage	B or C	14	32	< 0.05	
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HBsAg: hepatitis B virus surface antigen; AFP: α -fetoprotein; BCLC: Barcelona Clinic Liver Cancer Staging.

Supplementary Table 2. Primer List.

Gene	Forward primer	Reverse primer (5'-3')
β-actin(Human)	Forward (5'- 3')	GGCCCAGAATGCAGTTCGCCTT
	Reverse (5'- 3')	AATGGCACCCTGCTCACGCA
SOX2(Human)	Forward (5'- 3')	TGGAGAAGGAATGGTCCACTTC
	Reverse (5'- 3')	GGATAAGTACACGCTGCCCG
OCT4(Human)	Forward (5'- 3')	ATGTGCGCGTAACTGTCCAT
	Reverse (5'- 3')	CTGCAGTGTGGGTTTCGGGCA
SOX6(Human)	Forward (5'- 3')	CCTCTACCTCACCACATAAGC
	Reverse (5'- 3')	TCCACCACATCGGCAAGA
FOXO1(Human)	Forward (5'- 3')	GACAGCCCTGGATCACAGTTT
	Reverse (5'- 3')	CGGTCATAATGGGTGAGAGTCT
FOXOa3(Human)	Forward (5'- 3')	TTCCGTAAGCAAGCCGTGTA
	Reverse (5'- 3')	CGAGTCCGAAGTGAGCAGGT

TP53INP1 (Human)	Forward (5'- 3')	CCCCACCCCCATGTTTTACT	
	Reverse (5'- 3')	TTTCCTGGCCCTGGGACTAC	
miR-96 (Human)	Forward (5'- 3')	AGCAAAAATGTGCTAGTGCCAAA	
	Reverse (5'- 3')	AGCAAAAATGTGCTAGTGCCAAA	
U6 (Human)	Forward (5'- 3')	CTCGCTTCGGCAGCACA	
	Reverse (5'- 3')	AACGCTTCACGAATTTGCGT	

Supplementary Table 3. Antibody List.

Antigens	Manufacturer	Application
TP53INP1	Abcam, Cambridge, MA	1:500 for WB
PARP	Cell Signaling Technology, Beverly, MA	1:500 for WB
GAPDH	Cell Signaling Technology, Beverly, MA	1:5000 for WB
SOX-2	Cell Signaling Technology, Beverly, MA	1:500 for WB
OCT4	Cell Signaling Technology, Beverly, MA	1:500 for WB