

miR-193a-5p targeting SRSF6 promotes pancreatic cancer cell metastasis

Table S1. miR-193a-5p expression and clinicopathological features in 40 patients with pancreatic ductal carcinoma (PADC)

Characteristics	Expression of miR-193a-5p		p value*
	low	high	
Sex			0.076
male	17	12	
female	3	8	
Age			0.705
≤60	15	16	
>60	5	4	
Tumor Differentiation			0.569
Well-differentiated	7	4	
Moderately differentiated	9	11	
Poorly differentiated	4	5	
T Classification			0.584
T1	2	3	
T2	8	5	
T3	10	12	
N Classification			0.009*
N0	12	4	
N1	8	16	
TNM stage			0.038*
I/II	9	3	
III/IV	11	17	

Median expression level was used as a cutoff to divide the 40 patients into miR-193a-5p low group (n = 20) and miR-193a-5p high group (n = 20). Two-sided χ^2 test. *P < 0.05.

Table S2. Sequences of mimics, inhibitors and siRNAs, and of primers used for qRT-PCR, AS validation, RT-PCR and plasmid construction

RNA/Primer names	Sequences
pre-miR-193a-5p	5'-UGGGUCUUUGCGGGCGAGAUGA-3' (sense) 5'-AUCUCGCCCCGAAAGACCCAUU-3' (antisense)
pre-miR-NC	5'-UUCUCCGAACGUGUCACGUTT-3' (sense) 5'-ACGUGACACGUUCGGAGAATT-3' (antisense)
anti-miR-193a-3p	5'-UCAUCUCGCCCCGAAAGACCCA-3'
anti-miR-NC	5'-CAGUACUUUUGUGUAGUACAA-3'
SRSF6-siRNA-1	5'-GGAUACAGCAGUCGGAGAATT-3' (sense) 5'-UUCUCCGACUGCUGUAUCCTT-3' (antisense)
SRSF6-siRNA-2	5'-GCAGUUGGCAAGAUUUAATT-3' (sense) 5'-UUUAAAUCUUGCCAACUGCTT-3' (antisense)
SRSF6-siRNA-3	5'-GCAGGAAAUCUAGAUCAAATT-3' (sense) 5'-UUUGAUCUAGAUUUCUGCTT-3' (antisense)
OGDHL ex3+ si-1	5'-GGACAGCUUCUUCAGGAATT-3' (sense) 5'-UUCUCCUGAAGAAGCUGUCCTT-3' (antisense)
OGDHL ex3+ si-2	5'-GGACCAAGACCAGCAAUUTT-3' (sense) 5'-AAUUUGCUGGUCUUGGUCCTT-3' (antisense)
Sequences of primers used for qRT-PCR	
hsa-miR-193a-5p forward	5'-ACGCTGGGTCCTTGCGG-3'
hsa-miR-193a-5p reverse	5'-TATGTTGTTCCAGACTCCTTCAC-3'
U6 forward	5'-ATTGGAACGATACAGAGAAGATT-3'
U6 reverse	5'-GGAACGCTTCACGAATTG-3'

miR-193a-5p targeting SRSF6 promotes pancreatic cancer cell metastasis

SRSF6 forward	5'-ACATAGGACGCCTGAGCTACA-3'
SRSF6 reverse	5'-GCCGTACCCATTTTGGAGGTCTA-3'
GAPDH forward	5'-GAGTCAACGGATTGGTCTG-3'
GAPDH reverse	5'-TTGATTTGGAGGGATCTCG-3'
Sequences of primers used for AS validation	
pGint-OGDHL ex3+ forward	5'-TGTTGGAAAACCCCCAGAG-3'
pGint-OGDHL ex3+ reverse	5'-GAGGGCACAAAGGAGTCCAG-3'
pGint-ECM1 ex4- forward	5'-ACCCTGACTCCTCTCAGCAT-3'
pGint-ECM1 ex4- reverse	5'-TTGGGCAGGTAGCAGCTTTT-3'
Sequences of primers used for RT-PCR	
OGDHL forward	5'-CATCGACAAATCCAGCGAGAT-3'
OGDHL reverse	5'-ATCCTCTCATGGTACATGCC-3'
ECM1 forward	5'-GACAGAGTCAAGTGACGCC-3'
ECM1 reverse	5'-CTTCTGTTTCATTGGGGTGCTG-3'
Sequences of primers used for plasmid construction	
pcDNA3.1-SRSF6 forward	5'-CTAGCTAGCGCCACCATGCCGCGCTCTACATAG-3'
pcDNA3.1-SRSF6 reverse	5'-CCGGAATTCTTAATCTCTGGAACTCGACCTGG-3'
pcDNA3.1-ECM1 forward	5'-CTAGCTAGCGCCACCATGGGGACCACAGCCAGAGC-3'
pcDNA3.1-ECM1 reverse	5'-CCGGAATTCTCATTCTTCTTGGGCTCAG-3'
SRSF6-WT forward	5'-TCGAGGGCTCTAAGGAAATGGTGGCATGAAGACCTCTCCCTTCTTTGTAGAATTAAGT-3'
SRSF6-WT reverse	5'-CTAGACTTAATTCTACAAAGAAGGGAGAGGGTCTTCATGCCACCATTTCTTAGAGCCC-3'
SRSF6-MUT forward	5'-TCGAGGGCTCTAAGGAAATGGTGGCATGCCTCAAATCTCCCTTCTTTGTAGAATTAAGT-3'
SRSF6-MUT reverse	5'-CTAGACTTAATTCTACAAAGAAGGGAGATTGAGGCATGCCACCATTTCTTAGAGCCC-3'

Table S3. Antibodies used for western blotting (WB), RNA-binding protein immunoprecipitation (RIP)

Protein	Applications	Antibody	Origin	dilution	Molecular weight
GAPDH	WB	D16H11, Cell Signaling Technology	Rabbit	1:1000	36 KD
E-cadherin	WB	3195, Cell Signaling Technology	Rabbit	1:1000	135 KD
ZEB-1	WB	3396, Cell Signaling Technology	Rabbit	1:1000	200 KD
MMP-9	WB	3852, Cell Signaling Technology	Rabbit	1:1000	92 KD
MMP-2	WB	4022, Cell Signaling Technology	Rabbit	1:1000	72 KD
Snail	WB	3879, Cell Signaling Technology	Rabbit	1:1000	29 KD
Vimentin	WB	ab92547, Abcam	Rabbit	1:1000	57 KD
Ago2	RIP	03-110, Merck Millipore	Mouse	1:10	100 KD
IgG	RIP	ab18413, Abcam	Mouse	1:10	150 kD
SRSF6	WB, IHC	ab140623, Abcam	Rabbit	1:1000, 1:50	40 KD
OGDHL	WB, IHC	17110-1-AP, Proteintech	Rabbit	1:1000, 1:50	115 KD
ECM1	WB, IHC	11521-1-AP, Proteintech	Rabbit	1:1000, 1:50	61 KD

Table S4. Screening of 93 predicted targets of miR-193a-5p

No.	Name	Cancer Related	Reported in Pancreatic Cancer	Expression in pancreatic tumor	Good survival related	P-Value	Migration and invasion related	Reported miR-193a-5p target	ΔG (kcal/mol)
1	SRSF6	√	×	Lower	√	0.0015	√	×	-22.6
2	KIAA0100	√	×	Lower	√	0.037	√	×	-22.6
3	USP6	√	×	Lower	√	0.00078	√	×	-23.8
4	CNTFR	√	×	Lower	√	0.0051	×		
5	KIAA0895L	√	×	Lower	√	0.00024	×		
6	PIP4K2B	√	×	Lower	√	0.0038	×		
7	MBD6	√	×	Lower	√	0.018	×		

miR-193a-5p targeting SRSF6 promotes pancreatic cancer cell metastasis

8	PCDHA13	√	×	Lower	√	0.025	×
9	PRPS1	√	×	Lower	√	0.031	×
10	ZNF70	√	×	Lower	√	0.033	×
11	FADS1	√	×	Lower	×		
12	BMF	√	×	Lower	×		
13	SMARCD1	√	×	Lower	×		
14	MLLT10	√	×	Lower	×		
15	CHERP	√	×	Lower	×		
16	EBF3	√	×	Lower	×		
17	GATAD2B	√	×	Lower	×		
18	ANKS1A	√	×	Lower	×		
19	RBBP6	√	×	Lower	×		
20	HECTD3	√	×	Lower	×		
21	FBXL3	√	×	Lower	×		
22	FANCD2	√	×	Lower	×		
23	PHACTR4	√	×	Lower	×		
24	EEF1A1	√	×	Lower	×		
25	MYO9B	√	×	Lower	×		
26	TCF3	√	×	Lower	×		
27	ADAMTS2	√	×	Lower	×		
28	PCDHA5	√	×	Higher			
29	PCDHA7	√	×	Higher			
30	PCDHA8	√	×	Higher			
31	PCDHA2	√	×	Higher			
32	PCDHA4	√	×	Higher			
33	PCDHA1	√	×	Higher			
34	PCDHAC2	√	×	Higher			
35	GANAB	√	×	Higher			
36	UBE2D2	√	×	Higher			
37	CEP57	√	×	Higher			
38	CRYBG3	√	×	Higher			
39	TSC22D2	√	×	Higher			
40	DDA1	√	×	Higher			
41	GBA2	√	×	Higher			
42	EIF4EBP2	√	×	Higher			
43	OTUD7B	√	×	Higher			
44	GPR39	√	×	Higher			
45	MYO1D	√	×	Higher			
46	CSNK2A1	√	×	Higher			
47	PCDHA11	√	√				
48	ACVR1	√	√				
49	BTG1	√	√				
50	GNAQ	√	√				
51	NETO2	√	√				
52	RAP2A	√	√				
53	SPOCK1	√	√				
54	PCDH10	√	√				
55	COL1A1	√	√				
56	CDK14	√	√				
57	MMP16	√	√				

miR-193a-5p targeting SRSF6 promotes pancreatic cancer cell metastasis

58	NUAK1	√	√
59	VCL	√	√
60	MEIS1	√	√
61	AJUBA	√	√
62	SENP1	√	√
63	PBX1	√	√
64	PCDHA12	x	
65	PCDHA6	x	
66	PCDHA10	x	
67	PCDHA9	x	
68	PCDHA3	x	
69	PCDHAC1	x	
70	NLN	x	
71	SLC30A5	x	
72	ITSN1	x	
73	IPPK	x	
74	OLA1	x	
75	ZMYM4	x	
76	WIZ	x	
77	IFFO2	x	
78	ZNF827	x	
79	XK	x	
80	ANGEL1	x	
81	CHST14	x	
82	GOLGA8B	x	
83	GOLGA8A	x	
84	GDE1	x	
85	C18orf25	x	
86	SYT12	x	
87	TMTC3	x	
88	SEC61A1	x	
89	TAOK1	x	
90	SLC7A1	x	
91	NUP210	x	
92	CLPB	x	
93	SLC7A6	x	

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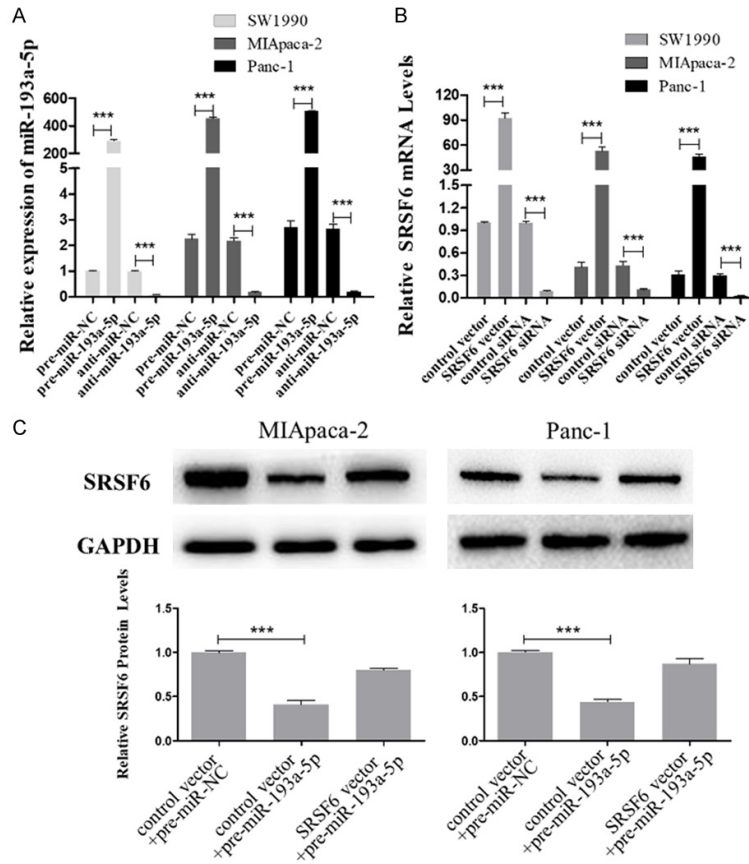


Figure S1. Verification of miR-193a-5p, SRSF6 overexpression or knockdown efficiencies in breast cancer cells. A. miR-193a-5p levels in SW1990, MIAPaca-2 and Panc-1 cells transfected with pre-miR-NC, pre-miR-193a-5p, anti-miR-NC, or anti-miR-193a-5p. B. SRSF6 mRNA in SW1990, MIAPaca-2 and Panc-1 cells transfected with control vector, SRSF6 vector, control siRNA or SRSF6 siRNA. C. SRSF6 protein levels in SW1990 and Panc-1 cells transfected with pre-miR-NC plus control vector, pre-miR-193a-5p plus control vector, or pre-miR-193a-5p plus SRSF6 vector. All data are shown as the mean \pm SEM. *** $P < 0.001$.

miR-193a-5p targeting SRSF6 promotes pancreatic cancer cell metastasis

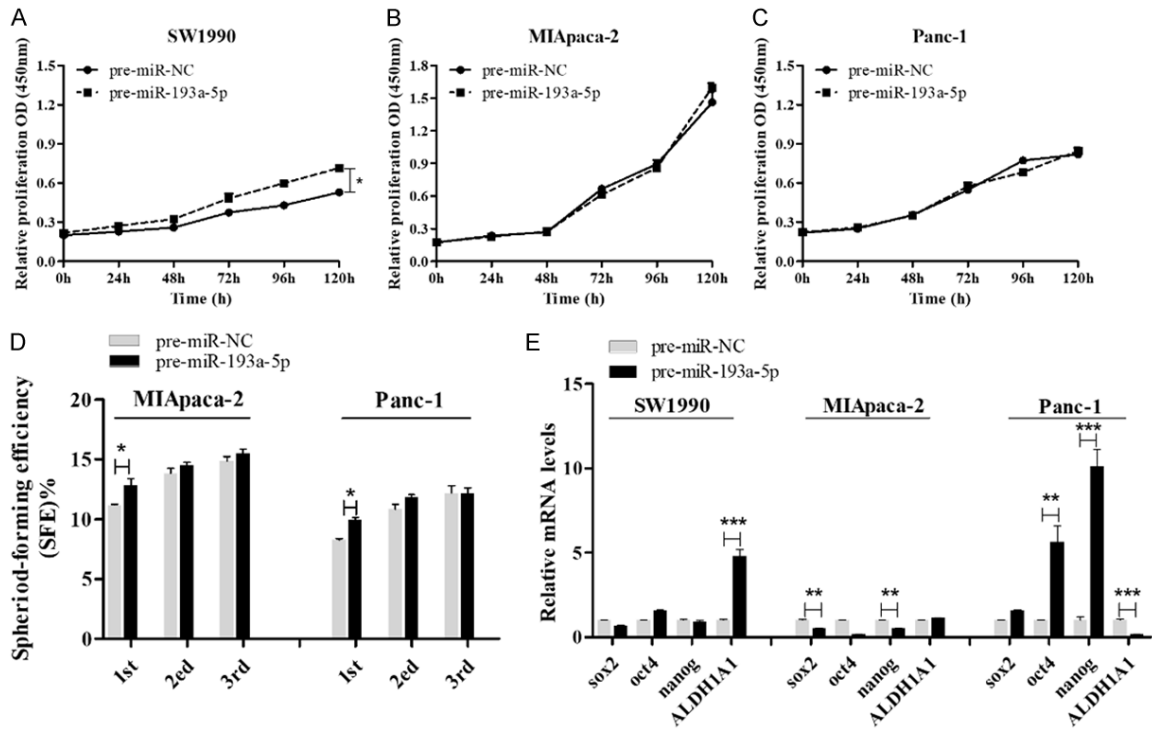


Figure S2. miR-193a-5p has no significant effects on pancreatic cancer cell proliferation, self-renewal, or drug resistance ability. (A-C) Proliferative abilities of SW1990 cells (A), MIApaca-2 cells (B) and Panc-1 cells (C) transfected with pre-miR-NC or pre-miR-193a-5p detected by CCK-8 assays. (D) The sphere-formation efficiencies (SFEs) of MIApaca-2 cells and Panc-1 cells transfected with pre-miR-NC or pre-miR-193a-5p. (E) mRNA levels of pluripotent transcription factors in SW1990 cells, MIApaca-2 cells and Panc-1 cells transfected with pre-miR-NC or pre-miR-193a-5p detected by qRT-pancreatic cancerR. All data are shown as the mean \pm SEM. * $P < 0.05$.

miR-193a-5p targeting SRSF6 promotes pancreatic cancer cell metastasis

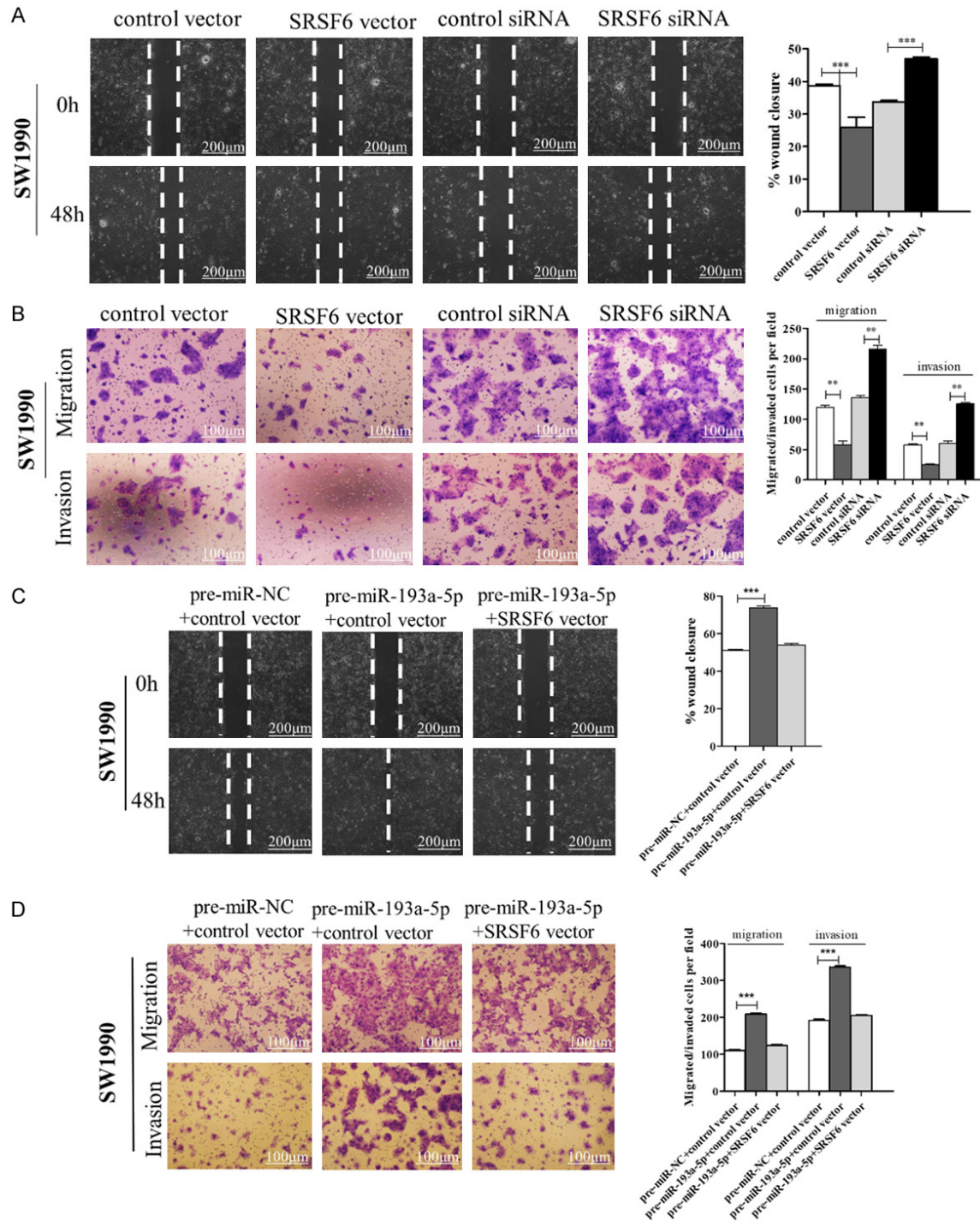


Figure S3. miR-193a-5p promotes pancreatic cancer cell migration and invasion via targeting SRSF6 in SW1990 cells. (A, B) Migration and invasion of SW1990 cells transfected with control vector, SRSF6 vector, control siRNA or SRSF6 siRNA detected by wound healing assay (A) or migration and invasion assay (B). Scale bar, 100 μ m. (C, D) Migration and invasion of SW1990 cells transfected with pre-miR-NC plus control vector, pre-miR-193a-5p plus control vector, or pre-miR-193a-5p plus SRSF6 vector detected by wound healing assay (C) or migration and invasion assay (D). Scale bar, 100 μ m. All data are shown as the mean \pm SEM. ** $P < 0.01$; *** $P < 0.001$.