

MircoRNA-33a inhibits epithelial-to-mesenchymal transition and metastasis and could be a prognostic marker in non-small cell lung cancer

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Supplementary Table 1. Association of miR-33a levels and clinicopathological factors in 53 NSCLC patients

Factors		No. of cases	miR-33a level		<i>P</i> value
			Hight (n / %)	Low (n / %)	
Age	≤ 50 y	22	13 / 59.09	9 / 40.91	0.43
	> 50 y	31	17 / 54.83	14 / 45.17	
Gender	Male	27	16 / 59.25	11 / 40.75	0.77
	Female	26	14 / 53.85	12 / 46.15	
WHO grade	I-II	28	11 / 39.29	17 / 60.71	0.002
	III-IV	25	19 / 76.00	6 / 24.00	
Tumor size	≤ 5 cm	29	16 / 55.17	13 / 44.83	0.07
	> 5 cm	24	14 / 58.33	10 / 41.67	

Supplementary Table 2. Univariate and multivariate analyses for overall survival by Cox regression model

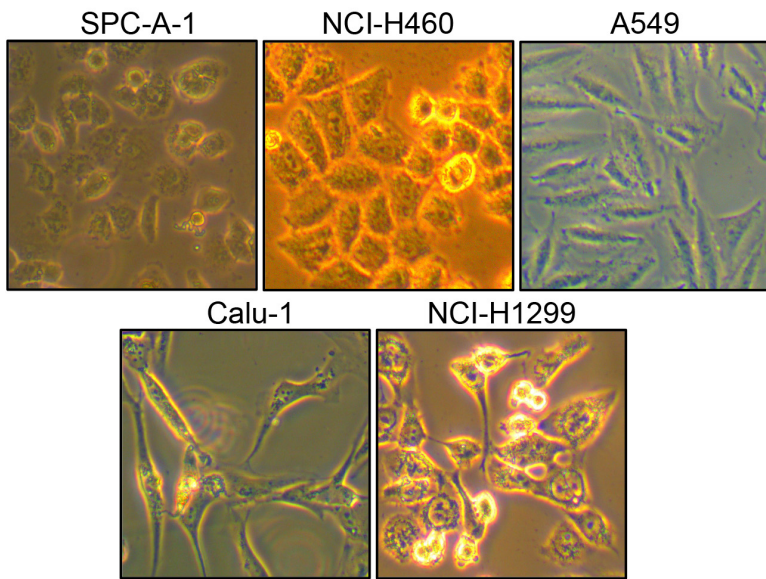
Factors	Univariate analysis		Multivariate analysis	
	Hazard ration	<i>P</i> value	Hazard ration	<i>P</i> value
Age	1.21	0.41	1.18	0.36
Gender	0.91	0.72	0.94	0.68
WHO grade	3.12	0.002	2.97	0.004
Tumor size	2.32	0.08	2.39	0.07
miR-33a level	2.13	0.01	2.35	0.008

Supplementary Table 3. RNA oligonucleotide sequences

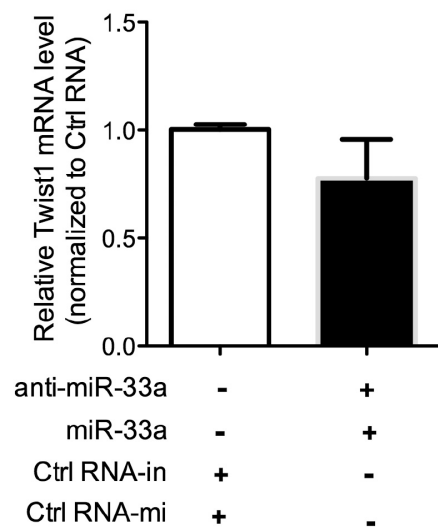
RNA oligos		Sequences (5' to 3')
miR-33a mimics	sense	gugcauuguaguugcauugca
	antisense	caaugcaacuacaauugcacuu
mimics NC	sense	uucuccgaacgugucacgutt
	antisense	acgugacacguucggagaatt
miR-33a inhibitor		ugcaaugcaacuacaauugcac
inhibitor NC		caguacuuuuguguaguacaa
Twist1 siRNA	sense	gguacaucgacuuccucuatt
	antisense	uagaggaagucgauguacctt

Supplementary Table 4. Primer sequences for RT-PCR

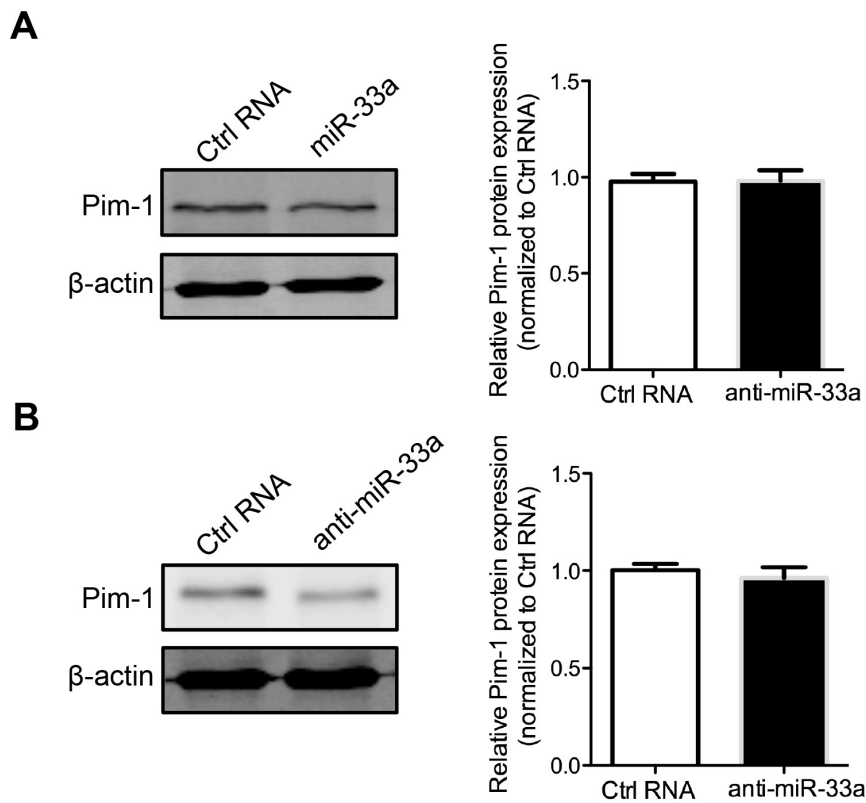
Gene	Forward primer (5' to 3')	Reverse primer (5' to 3')
CDH1	gcattggcacatacactctctct	tcggttaccgtgatcaaaatctc
Vimentin	aggaatggctcgtcaccttcgtgaata	ggagtgtcggttgtaagaactagagct
Twist1	catgtccgcgtcccaactag	tgtccattttctccttctctcg
β -actin	cagaaggagattactgctctggct	tactctgcttgctgatccacatc
U6 snRNA	ctcggttcggcagcaca	aacgcttcacgaatttgcgt
miR-33a	cctcataagcgggtgcattgta	tatgctgttctcgtctctgtgtc
Twist1 3'UTR	ctagtctagacattctcaagaggtcgtgccaa	ctagtctagatagacaccggatctatttgcattttac
Twist1 3'UTR MUT	ctagtctagacattctcaagaggtcgtgccaa	ctagtctagatagacaccggatctattttaccatgg
Twist1 gene	gcgatccatgatgcaggacgt	gcgaattcctagtgggacgcgga



Supplementary Figure 1. The morphology of different NSCLC cells was observed under a phase contrast microscope (100 ×).



Supplementary Figure 2. Endogenous transcript level of Twist1 in response to miR-33a is rescued when miR-33a is cotransfected with the miR-33a inhibitor. Ctrl RNA-in, control RNA inhibitor; Ctrl RNA-mi, control RNA mimic.



Supplementary Figure 3. miR-33a does not regulates Pim-1 expression in lung cancer cells. Western blotting and semi-quantitative analyses of Pim-1 (another target of miR-33a, *Oncogene* 2012, 31:918) protein expressions in NCI-H1299 and SPC-A-1 cells were performed after transfections of miR-33a (A) and anti-miR-33a (B), respectively.