

Molecular Pharmacology

MicroRNA hsa-miR-1301-3p regulates human *ADH6*, *ALDH5A1* and *ALDH8A1* in the ethanol-acetaldehyde-acetate metabolic pathway

Xubing Wang, Yanjie Zhao, Jiao Luo, Lin Xu, Xinmei Li, Yuan Jin,

Chuanhai Li, Meiyao Feng, Ying Wang, Jing Chen, Yufei Hou, Qianwen

Zhao, Jinquan Zhao, Baitang Ning, Yuxin zheng, and Dianke Yu

Supplemental Table 1. Sequences of oligonucleotides and primers

Name	Sequence (5'—3')
<i>For qRT-PCR</i>	
ADH6-F	CCTCCTGCAATGAGAGCTATGG
ADH6-R	GGATGTGCTGTCTGCTCTTCCA
ALDH5A1-F	AGGCTGGGATTCCTTCAGGTGT
ALDH5A1-R	ACAGAGTTTGCTGCGTGGTGCA
ALDH8A1-F	ATCCACTGGTGAGCATAGGTGC
ALDH8A1-R	GAGGCTCAACTTATCCACTCCC
GAPDH-F	GAAATCCCATCACCATCTTCCAGG
GAPDH-R	GAGCCCCAGCCTTCTCCATG
hsa-miR-1301-3p-F	TTGCAGCTGCCTGGGAGTG
hsa-miR-1301-3p-R	CAGTGCGTGTCGTGGAGT
U6-F	CTCGCTTCGGCAGCAC
U6-R	AACGCTTCACGAATTTGCGT
<i>For FREMSA</i>	
dye-ADH6	AGGGUCGUCCCUGCUGCAGAAGCUGUAU
dye-ALDH5A1	UGCCACGUGCCUGUGGCUGCAG
dye-ALDH8A1	GCCCAGGCGGUUGAGGCUGCAG
dye-miR-1301-3p	UUGCAGCUGCCUGGGAGUGACUUC
dye-ALDH5A1-2008	CUACAGAACAUGGGCCCAGAGC
dye-miR-330-5p	UCUCUGGGCCUGUGUCUUAGGC
miRNA negative control oligo	AACGCTTCACGAATTTGCGT

Supplemental Materials

Oligonucleotides for FRMESA

The RNA oligonucleotide for hsa-miR-330-5p was 5'-modified using DyLight 800 dye and named as dye-miR-330-5p, and the oligonucleotide corresponding to the MRE of hsa-miR-330-5p resident in *ALDH5A1* 3'-UTR was 5'-modified using Cy5.5 dye and designated as dye-ALDH5A1-2008.

Luciferase reporter gene construct

The core sequence containing the MRE of hsa-miR330-5p in *ALDH5A1* 3'-UTR was synthesized chemically and subcloned into the pMirGlo system, and designated as ALDH5A1-WT-2008. The resultant construct was sequenced to confirm authenticity.

Supplemental Figure Legends

Supplemental Figure 1. Interactions among dye-miR-1301-3p

oligonucleotides. (a) Free energy analyses of miRNA:miRNA duplex formed by hsa-miR-1301-3p. **(b)** Time-dependent formation of the dimeric dye-miR-1301-3p. *Lanes* 1-4 indicated the mobility of monomeric and dimeric dye-miR-1301-3p with the incubation time of 0, 5, 10, and 20 min, respectively.

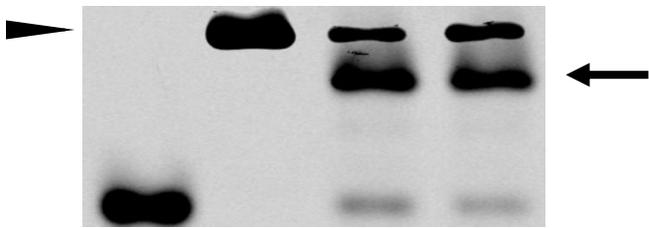
Supplemental Figure 2. The hsa-miR-330-5p interacted with *ALDH5A1*

3'-UTR. (a) The hsa-miR-330-5p interacted with *ALDH5A1* mRNA *in vitro*. *Lanes* 1 and 2 indicated the mobility of dye-miR-330-5p and dye-*ALDH5A1*-2008, respectively; *lane* 3 indicated the mobility status of the miRNA:mRNA complex formed by dye-miR-330-5p with dye-*ALDH5A1*-2008 oligonucleotides; *lane* 4 revealed the mobility shift status of miRNA:mRNA complex in the presence of excess unlabeled nonspecific competitors. NC, nonspecific competitor. The arrow, miRNA:mRNA complex; solid triangle, dimeric dye-*ALDH5A1*-2008. **(b)** hsa-miR-330-5p suppressed luciferase activity produced by *ALDH5A1* 3'-UTR. Construct containing the core sequence of *ALDH5A1* 3'-UTR was transiently transfected into 293T cells, together with 50 nmol/L hsa-miR-330-5p mimic or miRNA negative control. Cells were harvested at 24h after transfection. Three independent experiments were conducted in triplicate, and data were shown as mean \pm SD. **P* < 0.05 versus miRNA negative control.

Supplemental Figure 2

a

dye-miR-330-5p	+	-	+	+
dye-ALDH5A1-2008	-	+	+	+
Cold-NC (50x)	-	-	-	+



b

