

Long Non-coding RNA *H19* Inhibits Adipocyte Differentiation of Bone Marrow Mesenchymal Stem Cells through Epigenetic Modulation of Histone Deacetylases

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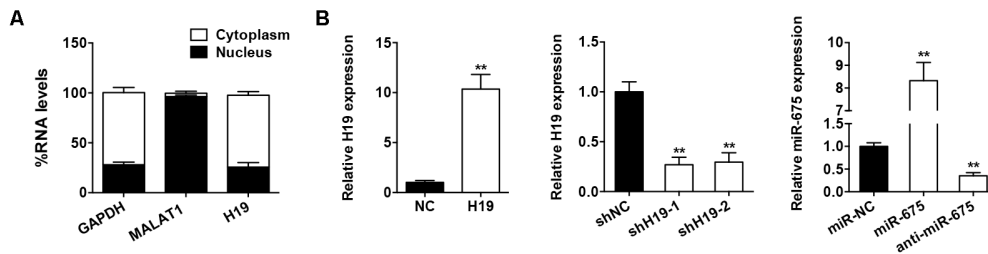
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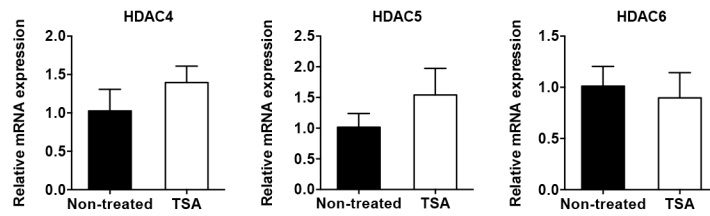
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Supplementary Information

Supplementary Figure S1. *H19* and *miR-675* expression in BMSCs transfected with lentiviruses. (A) Percentage of nuclear and cytoplasmic RNA levels of *H19*, *MALAT1*, and *GAPDH* measured by qRT-PCR after subcellular fractionation in BMSCs. (B) Left: relative expression of *H19*, as determined by qRT-PCR analysis, in BMSCs transfected with *H19* and the vacant vector (NC). Middle: relative expression of *H19* in BMSCs transfected with sh*H19* and the non-targeting vector (shNC). Right: relative expression of *miR-675* in BMSCs transfected with *miR-675*, anti-*miR-675*, or the scrambled vector (miR-NC). Data are shown as mean \pm SD (** $P < 0.01$).



Supplementary Figure S2. Relative mRNA expression of *HDACs 4-6* in BMSCs treated with or without TSA (400 nM). Results are presented as mean \pm SD.



Supplementary Table S1. The miR-675 potential target sites in HDAC4 transcript according to the RNA22 software

miRNA	leftmost position of predicted targeted site	cDNA region	folding energy (in -Kcal/mol)	predicted target site	base pairs in putative heteroduplex	span of target	p-value
has-miR-675	4183	3'UTR	-22.4	CACGGGAAGCCTTTCTGCCGCCCA	19	24	0.116
has-miR-675	6166	3'UTR	-16.8	CCACTTGGGTTTTTCCACTGCAGCCA	16	26	0.0919
has-miR-675	6231	3'UTR	-17.9	AACCCAGGGACCCTTGTTGCCTG	14	23	0.186
has-miR-675	6602	3'UTR	-12.4	GGTCTTGGGACTTGTTTGACTT	14	22	0.18
has-miR-675	6671	3'UTR	-14.7	AGGAGCTGGTCCATTCCCCATTC	13	23	0.0978
has-miR-675	6766	3'UTR	-17.9	CTCTGGGGATCCTGCCACCT	16	20	0.124
has-miR-675	8170	3'UTR	-18.5	CGCCGCCCGCCCTCACTGGCCT	16	22	0.147
has-miR-675	7360	3'UTR	-23.4	CGCTGGGACTGGCCGCCCC	15	19	0.261

The bold item indicates the predicted target site of maximum likelihood

Supplementary Table S2. The miR-675 potential target sites in HDAC5 transcript according to the RNA22 software

miRNA	leftmost position of predicted targeted site	cDNA region	folding energy (in -Kcal/mol)	predicted target site	base pairs in putative heteroduplex	span of target	p-value
has-miR-675	4443	3'UTR	-19.1	AGAACCAGGTCTGCTCCCCACCT	13	23	0.261
has-miR-675	3925	3'UTR	-12.3	GTCCCTCCCCCATTTCCTGCATTC	13	26	0.023
has-miR-675	5208	3'UTR	-13.7	TCATGTAAACCCACTCCTTGCTT	14	23	0.221
has-miR-675	5162	3'UTR	-17.8	GGCTCCCGGCCCTTACCCACTC	16	23	0.127
has-miR-675	4210	3'UTR	-14.7	TTCCCTTCTTTCGTCCTCACCA	13	24	0.00691

The bold item indicates the predicted target site of maximum likelihood

Supplementary Table S3. The miR-675 potential target sites in HDAC6 transcript according to the RNA22 software

miRNA	leftmost position of predicted targeted site	cDNA region	folding energy (in -Kcal/mol)	predicted target site	base pairs in putative heteroduplex	span of target	p-value
has-miR-675	3832	3'UTR	-19.1	AGAATACGGTCCCTCTTCACCT	13	22	0.127
has-miR-675	3903	3'UTR	-18.7	GATGAGGGGTAGCCTCCCCTGCATCC	15	27	0.0179

The bold item indicates the predicted target site of maximum likelihood

Supplementary Table S4. Sequences of RNA and DNA Oligonucleotides

Name	Sense Strand/Sense Primer (5'-3')	Antisense Strand/Antisense Primer (5'-3')
Primers for qRT-PCR		
H19	GCACCTTGGACATCTGGAGT	TTCTTTCCAGCCCTAGCTCA
PPAR γ	GCTGTTATGGGTGAAACTCTG	ATAAGGTGGAGATGCAGGTTC
C/EBP α	GCAAGGCCAAGAAGTCGGTGGAC	TGCCCATGGCCTTGACCAAGGAG
FABP4	AGCACCATAACCTTAGATGGGG	CGTGGAAGTGACGCCTTTCA
HDAC1	CCGCATGACTCATAATTTGC	TGGAGCGCAAGAATTTAATG
HDAC2	AATTTGCGCATGTTACCTCA	TATCCGCTTGTCTGATGCTC
HDAC3	GATGGCATTGATGACCAGAG	GATCACAGCCCAGAGAGTCA
HDAC4	CGTCCCGGTACTTGTATGTG	ATTCCACGGAAACGATAGC
HDAC5	TTCTTTGGACCAGAGTTCCC	GTTGGGTTCAGAGGCTGTTT
HDAC6	CGGAGGGTCCTTATCGTAGA	GTAGCGGTGGATGGAGAAAT
GAPDH	GGTCACCAGGGCTGCTTTTA	GGATCTCGCTCCTGGAAGATG
miR-675 RT primer	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACCACTGTG	
miR-675	GTGCTGGTGCGGAGAGG	GTGCAGGGTCCGAGGT

U6	CTCGCTTCGGCAGCACA	AACGCTTCACGAATTTGCGT
Mimics		
miR-675 mimic	UGGUGCGGAGAGGGCCACAGUG	CUGUGGGCCCUCUCCGCACCAUU
mimic NC	UCACAACCUCCUAGAAAGAGUAGA	UACUCUUUCUAGGAGGUUGUGAUU
siRNAs		
siHDAC4	CGACAGGCCUCGUGUAUGATT	UCAUACACGAGGCCUGUCGTT
siHDAC5	GGACUUCUCUGCACAGCAUTT	AUGCUGUGCAGAGAAGUCCTT
siHDAC6	GAGGGUCCUUAUCGUAGAUTT	AUCUACGAUAAGGACCCUCTT
siNC	UUCUCCGAACGUGUCACGUTT	ACGUGACACGUUCGGAGAATT

Abbreviations: C/EBP α , CCAAT-enhancer-binding proteins- α ; FABP4, fatty acid binding protein 4; GAPDH, Glyceraldehyde 3-phosphate dehydrogenase; HDAC, histone deacetylases; PPAR γ , peroxisome proliferator-activated receptor- γ .