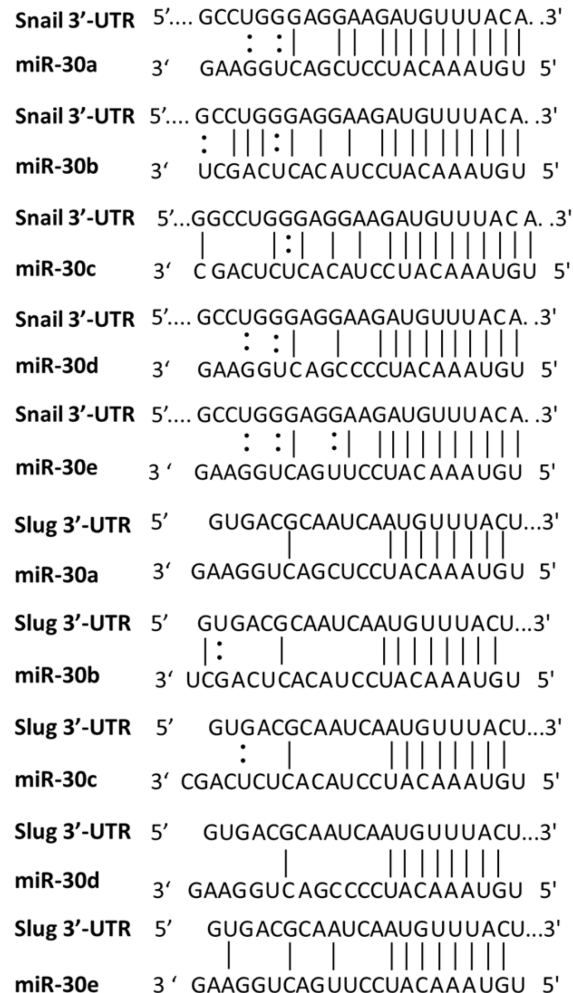
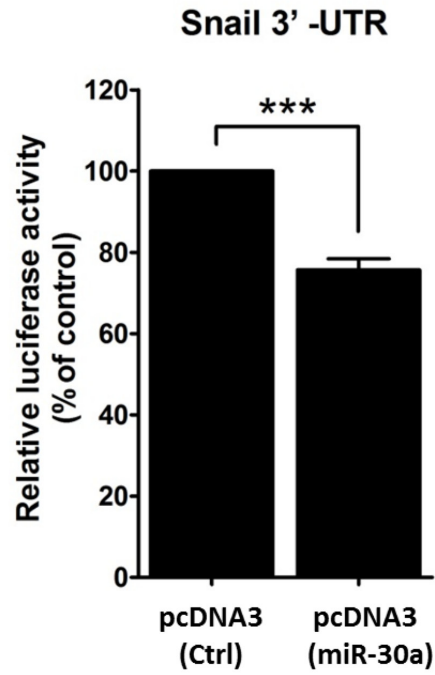
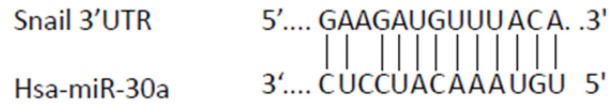


MicroRNA-30a increases tight junction protein expression to suppress the epithelial-mesenchymal transition and metastasis by targeting Slug in breast cancer

Supplementary Materials



Supplementary Figure S1: Prediction of the sequences within Snail mRNAs targeted by miR-30 family members. Computational algorithm results show that miR-30 family members share the same seed sequence, which targets the 3'-UTRs of *Snail* and *Slug*.



Supplementary Figure S2: *Snail* as a downstream target of miR-30a. Three algorithms, miRanda, miRWalk, and TargetScan, were used to identify putative interaction sites between *Snail* 3'-UTR and miR-30a. The consensus binding site in the *Snail* mRNA is shown. Expression of miR-30a reduced the activity of the luciferase gene fused to the *Snail* 3'-UTR by > 20%, supporting the notion that miR-30a downregulates Snail levels by targeting the 3'-UTR of the mRNA. The control (Ctrl) consisted of the *Snail* 3'-UTR construct along with pcDNA3.

Supplementary Table S1: Immunohistochemical analysis of slug, fascin, and claudins stratified by miR-30a expression levels in association with clinicopathological features of breast cancer

Cliniopathological features	miR-30a expression (T/N ratio) ^a	Slug	Fascin	CLDN-1	CLDN-2	CLDN-3
pT1, N1, Stage I	> 0.5	+/-	+	++	+++	+++
pT1, N0, Stage IIa	> 0.5	-	-	+++	++	+++
pT2, N1, Stage IIb	< 0.5	+++	++	-	+	-
pT2, N1, Stage IIb	< 0.5	+++	++	-	-	+/-
pT2, N2, Stage IIIa	< 0.5	+++	++	+	+/-	-
pT2, N2, Stage IIIa	< 0.1	+++	+++	+	-	+
pT2, N2, Stage IV	< 0.1	+++	+++	+	+/-	-
pT4, N2, Stage IIIb	< 0.1	++	++	+	-	+
pT4, N2, Stage IIIb	< 0.1	+++	+++	-	-	+/-
pT1, M1, Stage IV	< 0.1	+++	+++	-	-	-

^aDetailed quantitation for expression level of miR-30a in tumor tissues was described elsewhere [10]. The expression status of the miR-30a was defined by comparing miR-30a expression in tumor cells and adjacent non-tumor cells captured from the primary tumor site of the patient as described accordingly. Immunohistochemical staining and scoring system for the paraffin-embedded breast cancer tissue samples from patients were performed and assessed as described in Material and method. Tumor classification was referred to sixth edition of the AJCC Cancer Staging Manual [40].

Supplementary Table S2: Oligonucleotide primers used in this study

Gene	Sequence	
Plasmid construction		
plemiR-30a	F:	5'-CCGCTCGAGTCCATGAGCCTTCTTA 3'
	R:	5'-CGACGCGTTATTGCCCTACTACGC 3'
pcDNA3/miR-30a	F:	5'-CCAAGCTTCCATGAGCCTTCTTA 3'
	R:	5'-CGGAATTCTATTGCCCTACTACGC 3'
Slug 3'-UTR/wt	F:	5'-CCGCTCTAGATGGTTTGGTACTAATCATGAAG 3'
	R:	5'-TCCCAGTCTAGAACGCAATCAATGTTTACTCGAA 3'
Slug 3'-UTR/mut1	F:	5'-GTGACGCAATCA <u>AA</u> CAAAATGTCGAACAGAATGCATTTC 3'
	R:	5'-GAAATGCATTCTGTT <u>CGAC</u> ATTTGTTTGATTGCGTCAC 3'
Slug 3'-UTR/mut2	F:	5'-CAATGTAAATTT <u>GCGGTG</u> CTTTTATATGATTG 3'
	R:	5'-CAATCATATAAAAAGC <u>ACCG</u> CAAATTTACATTG 3'
Chromatin IP		
CLDN1 (+171/+189)	F:	5'-ATCCAGACTCCAGCGCCGCC 3'
	R:	5'-GACTCGCTCGGGCGCCCGCG 3'
CLDN2 (-803/-717)	F:	5'-TTCAAAGAGAGGCAGTGTA AAC 3'
	R:	5'-TGAGAACCTGTGGCTTCTCTACT 3'
CLDN3 (-462/-363)	F:	5'-ACCGCCGGACTCAGGCTGAGCC 3'
	R:	5'-GACACGGCTTCTCTCTCCTCACC 3'

The primer sequences with mutated nucleotides underlined are the sequences of the mismatch primers used to generate the different Slug 3'-UTR mutants.