SUPPLEMENTAL MATERIAL

MiR-103a-3p targets the 5´UTR of GPRC5A in pancreatic cells

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Supp. Table 1. Primers and sequences.

GPRC5A primer	**RT-PCR**	
	Forward:	GCTGCTCACAAAGCAACGAA
	Reverse:	ATAGAGCGTGTCCCCTGTCT
GPRC5A-5'UTR-CDS primer		
	Forward:	ATCAGCTAGCATTGTCGAGGCTTTAG
	Reverse:	TTTCGCGGCCGCATCCCTTTGAGCTCCC
GPRC5A-CDS primer		
	Forward:	CACGGATCCAACGCCTTGGCACTAGGGT
	Reverse:	TTTCGCGGCCGCATCCCTTTGAGCTCCC
GPRC5A-S11 sequence		
	S11WT	ACTGCTCGAGGCTGGCTTGTCCCGCTGGACTTGACGGCGGAGCTGCTGCAATCTCGCGGGCTGGGCCCTTGGCGGCCGCAGTC
	S11MT	ACTGCTCGAGGCTGGCTTGTCCCGCTGGACTTGACGGCGGATATTCTGCAATCTCGCGGGCTGGGCCCTTGGCGGCCGCAGTC
GPRC5A-S12 sequence		
	S12WT	ACTGCTCGAGGCACTAATGGTGCCTGTCTAGACTCTGACAGGTGGAGCTGCTGAGCTAACTACGGACCTGGTGGCGGCCGCAGTC
	S12MT	ACTGCTCGAGGCACTAATGGTGCCTGTCTAGACTCTGACAGGTGGATATTCTGAGCTAACTACGGACCTGGTGGCGGCCGCAGTC
GAPDH primer	**RT-PCR**	
	Forward:	GAAAGCCTGCCGGTGACTAA
	Reverse:	AGGAAAAGCATCACCCGGAG

SUPPLEMENTAL FIGURE CAPTIONS

Supp. Figure 1. MiR-103a-3p abundance affects both GPRC5A mRNA and protein levels. A: Rna22 predicts two miR-103a-3p binding sites, S11 and S12, in GPRC5A's 5´UTR. MiR-103a-3p expression level is changed after ectopic expression of pre-miR-103a-3p or Anti-miR-103a-3p. Taqman-for-microRNA assay was performed on cells transfected with pre-miR-103a-3p. Cells treated with pre-miR-scrambles were tested in parallel as control. Result shows that transfection leads to a nearly 1000x increase in the abundance of mature miR-103a-3p. B: Taqman-for-microRNA assay was performed on cells transfected with Anti-miR-103a-3p. Cells treated with Anti-miR-scramble were tested in parallel as control. Result shows that transfection leads to a nearly 10x decrease in the abundance of mature miR-103a-3p. All shown data are mean±sd. * P<0.05, ** P<0.01, *** P<0.001, n=3. U6, U6 snRNA. U6 is used as internal control.

Supp. Figure 2. GPRC5A is directly targeted by miR-103a-3p at its 5´UTR. A: The diagram (drawn to scale) shows the second predicted miR-103a-3p binding site (S12) within the 5´UTR of GPRC5A and the sequences of S12 wild type (S12WT, top) and mutant (S12MT, bottom) used in this study. B: Luciferase activity in MIA PaCa-2 cells upon co-transfection with indicated reporter constructs and miR-103a-3p. C. Luciferase activity in MIA PaCa-2 cells upon co-transfection with indicated reporter constructs and miR-103a-3p inhibitors. D. Luciferase activity in MIA PaCa-2 cells upon transfection of indicated reporter constructs and miR-103a-3p. E. Luciferase activity in MIA PaCa-2 cells upon transfection of indicated reporter constructs and miR-103a-3p inhibitors. Shown data are mean±sd. * P<0.05, ** P<0.01, *** P<0.001, n=3. S12WT, psiCHECK-2 vector containing miR-103a-3p binding site 2; S12MT, psiCHECK-2 vector containing mutant miR-103a-3p binding site 2.

Supp. Figure 3. Scheme of GPRC5A over-expression vector. A: The diagram shows the GPRC5A cDNA (5´UTR, CDS and 3´UTR). B: The diagram shows the GPRC5A 5´UTR and CDS regions that are cloned into the pcDNA3.1 vector with NheI and NotI as the restriction sites. C: The diagram shows the GPRC5A CDS region that is cloned into the pcDNA3.1 vector with NheI and NotI as the restriction sites.

Supp. Figure 4. Scheme of GPRC5A 5´UTR binding site over-expression vector. A: The diagram shows the GPRC5A 5´UTR. The blue and red-brown segments represent the miR-103a-3p binding sites 1 and 2, respectively. B: The diagram shows our 'sponge' S11WTL that contains 10 tandem copies of binding site 1 (S11WT) cloned into the pcDNA3.1 vector with NheI and NotI as the restriction sites. C: The diagram shows our 'control-sponge' S11MTL that contains 10 tandem copies of the mutant binding site 1 (S11MT) cloned into the pcDNA3.1 vector with NheI and NotI as the restriction sites.

Supp. Figure 5. Over-expression of 5´UTR MRE decreases miR-103a-3p levels and increases GPRC5A mRNA levels. A: Increase in GPRC5A mRNA levels is promoted by over-expression of the wild type sponge compared to the control sponge in HPNE cells. B: Increase in GPRC5A protein levels is promoted by over-expression of the wild type sponge compared to the control sponge in HPNE cells. C. Quantification result of Supp. Figure 5B. D. Increase in GPRC5A mRNA levels is promoted by over-expression of a luciferase construct containing the single copy of wild type sponge (S12WT) compared to the control sponge (S12MT) in HPNE cells. All shown data are mean±sd. * P<0.05, ** P<0.01, *** P<0.001, n=3. GAPDH, glyceraldehyde-3-phosphate dehydrogenase; U6, U6 snRNA; GAPDH and U6 are used as internal controls. S11WTL, pCDNA vector containing 10 tandem copies of the wild type miR-103a-3p binding site S11; S11MTL, pCDNA vector containing 10 tandem copies of the mutant miR-103a-3p binding site S11. S12WT, psiCHECK-2 vector containing the miR-103a-3p binding site S12; S12MT, psiCHECK-2 vector containing the mutant miR-103a-3p binding site S12.

Supplemental Figure 6. GPRC5A's 5´UTR alone can function as a decoy for miR-103a-3p. A. GPRC5A mRNA levels were tested by RT-PCR in HPNE cells treated with Pre-miR-103a-3p in addition to co-transfecting with GPRC5A-S11WTL or GPRC5A-S11WTL. B. GPRC5A protein levels were tested by Western Blots in HPNE cells treated with Pre-miR-103a-3p in addition to co-transfecting with GPRC5A-S11WTL or GPRC5A-S11MTL. C. Quantification result of supp.Fig.6B. All shown data are mean±sd. * P<0.05, ** P<0.01, *** P<0.001, n=3. GAPDH, glyceraldehyde-3-phosphate dehydrogenase; GAPDH and Actin are internal controls. S11WTL, pcDNA vector containing 10 tandem copies of miR-103a-3p binding site S11; S11MTL, pcDNA vector containing 10 tandem copies of mutant miR-103a-3p binding site S11.

Supp. Figure 7. Over-expression of 5´UTR MRE increases GPRC5A mRNA levels in different cell lines. A: GPRC5A mRNA levels increased when we over-expressed the wild type 5´UTR MRE (S12WT) compared to the mutant site in Panc-1 cells. B: GPRC5A protein levels increased when we over-expressed wild type 5´UTR MREs (S11WTL and S12WT) compared to the mutant site in HEK-293T cells. C: Quantification result of Supp. Figure 6C. All shown data are mean±sd. * P<0.05, ** P<0.01, *** P<0.001, n=3. GAPDH, glyceraldehyde-3-phosphate dehydrogenase; GAPDH and Actin are used as internal controls. S12WT, psiCHECK-2 vector containing the miR-103a-3p binding site S12; S12MT, psiCHECK-2 vector containing the mutant miR-103a-3p binding site 2.