Electronic Supplementary Material

miR-20a regulates expression of the iron exporter ferroportin in lung cancer

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SUPPLEMENTARY MATERIAL:

Name	5' Sequence 3'					
hs-ACTB_F	TTCCGCTGCCCTGAGGCACTCT					
hs-ACTB_R	TCTGCTGGAAGGTGGACAGCGA					
hs-pre-ACTB_F	CTATTCTCGCAGCTCACCATG					
hs-pre-ACTB_R	CAGCTCCCCTACCTGGTG					
hs-FPN_F	TGCTGTTTGCAGGCGTCATT					
hs-FPN_R	TTGCAGCAACTGTGTCACAGTT					
hs-pre-FPN_F	TACCCATTGGGAAGGGGAAT					
hs-pre-FPN_R	CCAGGGGTTTTGGCTCAGTA					
hs-HIF1A_F	CCCCAGATTCAGGATCAGACA					
hs-HIF1A_R	GGGACTATTAGGCTCAGGTGAACTT					
hs-RPL19_F	TCGCCTCTAGTGTCCTCCG					
hs-RPL19_R	GCGGGCCAAGGTGTTTTTC					
hs-RNU6	GTGCTCGCTTCGGCAGCACATATAC					
hs-miR-20a-5p	TAAAGTGCTTATAGTGCAGGTAGG					
hs-miR-21-5p	TAGCTTATCAGACTGATGTTGAGG					

Table S1: Primers used for qPCR analysis of mRNA and miRNA transcripts

Name	5' Sequence 3'
h-FPN-F	AAAGCTAGCATGACCAGGGCGGGAG
h-FPN-R	AAACTCGAGAACAACAGATGTATTTGCTTGATTT
Flag_F	[Phos]AATTCTAGACTACAAGGACGACGATGACAAGTGAGC
Flag_R	[Phos]GGCCGCTCACTTGTCATCGTCGTCCTTGTAGTCTAG

Table S2: Primers used to generate FPN expression constructs. Sequences with5'phosphate represented as [Phos] were annealed using oligo annealing buffer followed bydirect ligating into the vector.

Name	5' Sequence 3'
hs-FPN_F	GAGAGCTCGGAAGGAAAATCAAGCAAATACA
hs-FPN_R	GAGCTAGCTTGGGGAACAGAATTCAGGA
hs-HIF1A_F	GAGAGCTCACTCAGAGCTTTGGATCAAGTT
hs-HIF1A_R	GAGCTAGCGCCTGGTCCACAGAAGATGT
hs-RPL19_F	[Phos]CAACCTCCCACTTTGTCTGTACATACTGGCCTCTGTGATTACA
	TAGATCAGCCATTAAAATAAAACAAGCCTTAATCTGCG
hs-RPL19_R	[Phos]CTAGCGCAGATTAAGGCTTGTTTTATTTTAATGGCTGATCTAT
	GTAATCACAGAGGCCAGTATGTACAGACAAAGTGGGAGGTTGAGC
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hs-miR-20a-5p(+)_F	[Phos]CCTACCTGCACTATAAGCACTTTAG
hs-miR-20a-5p(+)_R	[Phos]CTAGCTAAAGTGCTTATAGTGCAGGTAGGAGCT
hs-miR-20a-5p(-)_F	[Phos]CTAAAGTGCTTATAGTGCAGGTAGG
hs-miR-20a-5p(-)_R	[Phos]CTAGCCTACCTGCACTATAAGCACTTTAGAGCT

Table S3: Primers used for the amplification of 3'-UTR sequences. Sequences with 5'phosphate represented as [Phos] were annealed using oligo annealing buffer followed by direct ligating into the vector.

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Name	5' Sequence 3'
hs-FPN-MT1_F	TCAGTTTGCAACATGTCTGTACCAAGATGGTAGAATGCCTTAACCG TTTATA
hs-FPN-MT1_R	TATAAACGGTTAAGGCATTCTACCATCTTGGTACAGACATGTTGCA AACTGA
hs-FPN-MT2_F	GATGGTAGAATGCCTTAACCGTTTATATGCAGAATCATGGAGACTG CAATAC
hs-FPN-MT2_R	GTATTGCAGTCTCCATGATTCTGCATATAAACGGTTAAGGCATTCT ACCATC
hs-FPN-MT3_F	GGAGACTGCAATACGTTGCTATGAGCAGAATCTTTATCCTTGGAGT TTAAT
hs-FPN-MT3_R	ATTAAACTCCAAGGATAAAGATTCTGCTCATAGCAACGTATTGCAG TCTCC
hs-HIF1A-MT1_F	TGGTATTTAAACCATTGCATTGCAGTAGCATCAGAATAAAAAATGC ACCTTTTTATTTATTTATTTTGG
hs-HIF1A-MT1_R	CCAAAAATAAATAAATAAAAAGGTGCATTTTTTATTCTGATGCTAC TGCAATGCAA
hs-HIF1A-MT2_F	TACTGCTGGCAAAGCATTATTATTATGTATTCTGTGAAAAAAAA
hs-HIF1A-MT2_R	AGGCAGTTGAAAAATTTTTACACCTTTTTTTTCACAGAATACATAA ATAATAATGCTTTGCCAGCAGTA
hs-HIF1A-MT3_F	TTTTCTTTTGAGCTGGCATTCTGACTATAGAAACATCAGATGATTTC TCTGAATTG
hs-HIF1A-MT3_R	CAATTCAGAGAAATCATCTGATGTTTCTATAGTCAGAATGCCAGCT CAAAAGAAAA
hs-HIF1A-MT4_F	AGTATGTTAATTTGCTCAAAATACAATGTTTGATTTTATGCAGAAT GTCGCTATTAACATCCTTTTTTT
hs-HIF1A-MT4_R	AAAAAAGGATGTTAATAGCGACATTCTGCATAAAATCAAACATT GTATTTTGAGCAAATTAACATACT

 Table S4: Primers used to mutagenize the miR-20a-5p seed sequences

Figure S1



Figure S1. Putative miR-20a binding sites in the 3'UTRs of human (A) HIF1A and (B) FPN. The nucleotides that are mutated in the wild type 3'UTRs are indicated in bold and lower case.



Figure S2. Boxplots indicating expression levels of the indicated member of the miR-17 seed family in the tumor and matching healthy tissue within TCGA datasets of (A) lung adenocarcinoma (LUAD) and (B) lung squamous cell carcinoma (LUSC) patients. 2-tailed Student's t test was used to calculate statistical significance.

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Figure S3. Scatter plots indicating correlations of FPN mRNA levels to the indicated member of the miR-17 seed family within TCGA datasets of (A) lung adenocarcinoma (LUAD) and (B) lung squamous cell carcinoma (LUSC) patients. Pearson's product moment correlation was used to calculate statistical significance.

Figure S4



Figure S4. Scatter plots indicating correlation of FPN mRNA levels to the indicated member of the miR-17 seed family within TCGA datasets of (A) hepatocellular carcinoma (LIHC) and (B) breast cancer (BRCA) patients. Pearson's product moment correlation was used to calculate statistical significance.



Figure S5. Chromatograms illustrating the transfection efficiency of the FPN expression construct (pcDNA FPN-EGFP) into H1299 cells. Green fluorescence intensity was analyzed by flow cytometry following transient transfection of pcDNA FPN-EGFP into H1299 cells after (A) 24 hrs (B) 48 hrs and (C) 72 hrs.

Lung squamous cell carcinoma (LUSC)

Figure S6. TCGA RNA-sequencing datasets available from Lung adenocarcinoma (LUAD) and Lung squamous cell carcinoma (LUSC) patients were downloaded from the UCSC cancer genomic browser (https://genome-cancer.ucsc.edu). Correlations between miR-485 and FPN mRNA expression levels are represented as scatter plots for (A) LUAD (n = 524, r = -0.127, P = 0.003) and (B) LUSC (n = 513, r = -0.16, P = 0.0002). Pearson's product moment correlation was used to calculate stastistical significance. Boxplots illustrate that in patients with LUAD (C) miR-485 expression is not significantly elevated (n = 46, P = 0.0567) and that (D) miR-485 expression. In patients with LUSC (E) miR-485 is significantly increased compared to normal (n = 45, P = 0.0058) and (F) miR-485 expression is significantly lower (n = 513, P < 0.0001) compare to miR-20a levels. 2-tailed Student's t test was used to calculate statistical significance. RPKM = Reads Per Kilobaseper Million mapped reads.

Figure S7. H1650 cells were transfected with 50 nM of (A) miR-20a mimic or (B) FPN siRNA1. After transfection TFR1 mRNA levels were analyzed by quantitative RT-PCR at an interval of 24 hrs for up to 4 days. Experiments were performed in triplicates and repeated at least three times. Data were normalized to ACTB. Data are represented as mean \pm SEM, and the values from negative control (NC) mimic were set to 1, ***P* < 0.001, ****P* < 0.001, 2-tailed Student's t test was used to calculate statistical significance.

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	Ē	S-CLL8 SL-BCBL1- DLBCL-DLB	old IL2						1			
	/ E	AML3-d0 B-ALL5-d2	9						1			
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		Jerebeilum Dvary Jterus							E			
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Figure S8. Heatmap illustrating comparative expression levels of miR-20a and miR-485-3p in various human tissues and cell lines.