

Supplementary Files

Novel blood-based microRNA biomarker panel for early diagnosis of chronic pancreatitis

Lei Xin^{1*}, M.D., Jun Gao^{1*}, Ph.D., Dan Wang^{1*}, M.D., Jin-Huan Lin¹, M.D., Zhuan Liao¹, M.D., Jun-Tao Ji¹, M.D., Ting-Ting Du¹, M.D., Fei Jiang¹, M.D., Liang-Hao Hu¹, M.D., Zhao-Shen Li¹, M.D.

¹Department of Gastroenterology, Changhai Hospital, the Second Military Medical University, Shanghai, China

* Dr. Xin, Dr. Gao and Dr. Wang contributed equally to this work.

Supplementary Tables

GOID	Ontology	Term	P-value
GO:0003835	MF	beta-galactoside alpha-2,6-sialyltransferase activity	2.69E-15
GO:0019778	MF	APG12 activating enzyme activity	1.64E-06
GO:0008234	MF	cysteine-type peptidase activity	3.17E-06
GO:0004839	MF	ubiquitin activating enzyme activity	3.18E-06
GO:0034596	MF	phosphatidylinositol phosphate 4-phosphatase activity	4.94E-06
GO:0003906	MF	DNA-(apurinic or apyrimidinic site) lyase activity	1.12E-05
GO:0008373	MF	sialyltransferase activity	2.94E-05
GO:0044020	MF	histone methyltransferase activity (H4-R3 specific)	8.37E-05
GO:0016765	MF	transferase activity, transferring alkyl or aryl (other than methyl) groups	0.000152
GO:0016316	MF	phosphatidylinositol-3,4-bisphosphate 4-phosphatase activity	0.000358
GO:0034593	MF	phosphatidylinositol bisphosphate phosphatase activity	0.0007102
GO:0019104	MF	DNA N-glycosylase activity	0.0009819
GO:0008281	MF	sulfonylurea receptor activity	0.0009956
GO:0042611	CC	MHC protein complex	3.17E-06
GO:0030870	CC	Mre11 complex	4.94E-06
GO:0030427	CC	site of polarized growth	5.90E-06
GO:0044421	CC	extracellular region part	9.66E-06
GO:0005576	CC	extracellular region	1.12E-05
GO:0030426	CC	growth cone	1.15E-05
GO:0044459	CC	plasma membrane part	8.11E-05
GO:0044295	CC	axonal growth cone	9.83E-05
GO:0030016	CC	myofibril	0.000288
GO:0043292	CC	contractile fiber	0.0002918
GO:0005615	CC	extracellular space	0.000358
GO:0005744	CC	mitochondrial inner membrane presequence translocase complex	0.000358
GO:0032501	BP	multicellular organismal process	1.97E-06
GO:0006305	BP	DNA alkylation	3.17E-06
GO:0006306	BP	DNA methylation	3.17E-06

GO:0060561	BP	apoptotic process involved in morphogenesis	9.66E-06
GO:0016486	BP	peptide hormone processing	1.15E-05
GO:0043067	BP	regulation of programmed cell death	1.47E-05
GO:0042981	BP	regulation of apoptotic process	1.56E-05
GO:0072023	BP	thick ascending limb development	2.94E-05
GO:0072233	BP	metanephric thick ascending limb development	2.94E-05
GO:0050896	BP	response to stimulus	2.99E-05
GO:0010623	BP	developmental programmed cell death	3.27E-05
GO:0044728	BP	DNA methylation or demethylation	3.51E-05
GO:0007588	BP	excretion	5.45E-05
GO:0044707	BP	single-multicellular organism process	6.82E-05
GO:0010941	BP	regulation of cell death	7.68E-05
GO:0006914	BP	autophagy	0.0001079
GO:0021859	BP	pyramidal neuron differentiation	0.0001379
GO:0021860	BP	pyramidal neuron development	0.0001379
GO:0072021	BP	ascending thin limb development	0.0001977
GO:0072218	BP	metanephric ascending thin limb development	0.0001977
GO:0003407	BP	neural retina development	0.0002895
GO:0006304	BP	DNA modification	0.0003002
GO:0003285	BP	septum secundum development	0.000358
GO:0050808	BP	synapse organization	0.000425
GO:0051491	BP	positive regulation of filopodium assembly	0.000425
GO:0051023	BP	regulation of immunoglobulin secretion	0.0005942
GO:0000244	BP	assembly of spliceosomal tri-snRNP	0.000743
GO:0072236	BP	metanephric loop of Henle development	0.000743
GO:0051489	BP	regulation of filopodium assembly	0.0007666

*BP: biological_process; CC: cellular_component; MF: molecular_function

Supplementary Table S1. GO functional enrichment for the targets of DEmiRs in early CP

GOID	Ontology	Term	Level	MIN-p-value	MAX-p-value	AVG-p-value
GO:0042611	CC	MHC protein complex	6	1.6066E-33	3.167E-06	1.583E-06
GO:0044421	CC	extracellular region part	2	5.22874E-85	9.662E-06	4.831E-06
GO:0005576	CC	extracellular region	1	1.59705E-94	1.121E-05	5.604E-06
GO:0044459	CC	plasma membrane part	5	8.06328E-69	8.113E-05	4.056E-05
GO:0030016	CC	myofibril	8	1.85948E-31	0.000288	0.000144
GO:0043292	CC	contractile fiber	7	1.24842E-28	0.0002918	0.0001459
GO:0005615	CC	extracellular space	3	7.89242E-53	0.000358	0.000179
GO:0032501	BP	multicellular organismal process	1	3.81019E-74	1.967E-06	9.834E-07
GO:0050896	BP	response to stimulus	1	7.80417E-63	2.99E-05	1.495E-05
GO:0044707	BP	single-multicellular organism process	2	4.25127E-69	6.816E-05	3.408E-05
GO:0007588	BP	excretion	4	2.14248E-05	5.45E-05	3.796E-05
GO:0003407	BP	neural retina development	10	2.32321E-05	0.0002895	0.0001563
GO:0006305	BP	DNA alkylation	8	3.16666E-06	0.0003543	0.0001787
GO:0006306	BP	DNA methylation	9	3.16666E-06	0.0003543	0.0001787
GO:0003835	MF	beta-galactoside alpha-2,6-sialyltransferase activity	5	2.6925E-15	2.002E-07	1.001E-07

*BP: biological_process; CC; CC: cellular_component; MF: molecular_function

Supplementary Table S2. Common GO terms regulated by DE miRNAs in late CP and early CP

pathwayId	pathwayName
path:00071	Fatty acid metabolism
path:04010	MAPK signaling pathway
path:04012	ErbB signaling pathway
path:04020	Calcium signaling pathway
path:04120	Ubiquitin mediated proteolysis
path:04141	Protein processing in endoplasmic reticulum
path:04142	Lysosome
path:04144	Endocytosis
path:04150	mTOR signaling pathway
path:04210	Apoptosis
path:04310	Wnt signaling pathway
path:04360	Axon guidance
path:04510	Focal adhesion
path:04666	Fc gamma R-mediated phagocytosis
path:04722	Neurotrophin signaling pathway
path:04810	Regulation of actin cytoskeleton
path:04910	Insulin signaling pathway
path:04916	Melanogenesis
path:05100	Bacterial invasion of epithelial cells
path:05120	Epithelial cell signaling in Helicobacter pylori infection
path:05200	Pathways in cancer
path:05212	Pancreatic cancer
path:05214	Glioma
path:05215	Prostate cancer
path:05218	Melanoma
path:05220	Chronic myeloid leukemia
path:05222	Small cell lung cancer
path:05223	Non-small cell lung cancer

Supplementary Table S3. Common pathways regulated by DEmiRs of late CP and early CP.

miRNAs	Primers	Sequences
Control	P1-F	TGAGAAGTCCGTCACACACCA
	P1-R	TAAGTCGTCTGTCAGCAGCAC
hsa-miR-130a	h-P130a-F	TGAGAAGTCCGTCACACACCA
	h-P130a-R	GGCGACAGTGCAATGTTAA
hsa-miR-221	h-P221-3p-F	AGTCACATCTACATTGTCTGC
	h-P221-3p-R	TGAGAAGTCCGTCACACACCA
hsa-miR-320a/b	h-P320a/b-F	CACAAAAGCTGGGTTGAGAGG
	h-P320-R	TGAGAAGTCCGTCACACACCA
hsa-miR-320c/d	h-P320c/d-F	CAGTAGGAAAAGCTGGGTTGAG
	h-P320-R	TGAGAAGTCCGTCACACACCA

Supplementary Table S4. List of miRNA-specific primer sequences

Appendix 1. M-ANNHEIM clinical staging of chronic pancreatitis.

Asymptomatic chronic pancreatitis

Stage 0 of subclinical chronic pancreatitis

- a. Period without symptoms (determination by chance, eg, autopsy)
- b. Acute pancreatitisVsingle episode (possible onset of chronic pancreatitis)
- c. Acute pancreatitis with severe complications

Symptomatic chronic pancreatitis

Stage 1 without pancreatic insufficiency

- a. (Recurrent) acute pancreatitis (no pain between episodes of acute pancreatitis)
- b. Recurrent or chronic abdominal pain (including pain between episodes of acute pancreatitis)
- c. 1a/b with severe complications

Stage 2 of partial pancreatic insufficiency

- a. Isolated exocrine (or endocrine) pancreatic insufficiency (without pain)
- b. Isolated exocrine (or endocrine) pancreatic insufficiency (with pain)
- c. 2a/b with severe complications

Stage 3 of painful complete pancreatic insufficiency

- a. Exocrine and endocrine insufficiency (with pain, e.g. requiring pain medication)
- b. 3a with severe complications

Stage 4 of secondary painless disease (burnout)

- a. Exocrine and endocrine insufficiency without pain and without severe complications
- b. Exocrine and endocrine insufficiency without pain and with severe complications

Supplementary Figures Legends

Supplementary Figure S1. GO functional enrichment for the targets of DEmiRs in early CP

Supplementary Figure S2. The enriched GO terms of DEmiRs in early CP and late CP and their hierarchical relationships in molecular function

Supplementary Figure S3. The enriched GO terms of DEmiRs in early CP and late CP and their hierarchical relationships in molecular function



