

Supplementary tables

Table S1. Features of plasma samples that were used for discovery and verification

Disease		Gender ¹	Age	Disease		Gender	Age
HD	1	M	32	CC	1	M	71
	2	M	34		2	M	73
	3	M	26		3	F	50
	4	M	35		4	M	49
	5	F	27		5	F	32
	6	F	37		6	M	61
	7	M	41		7	M	59
	8	M	34		8	F	62
	9	F	30		9	M	67
	10	M	29		10	M	69
	11	M	33		11	M	55
	12	M	26		12	M	75
	13	F	33		13	M	81
CP	1	F	52	GC	1	M	64
	2	M	37		2	M	60
	3	F	37		3	M	37
	4	M	47		4	M	42
	5	M	41		5	M	62
	6	M	63		6	F	66
	7	M	68		7	M	69
	8	M	53		8	M	78
	9	M	47		9	M	70
	10	M	71		10	M	54
	11	M	30		11	M	72
	12	M	21		12	M	60
PC	1	F	47		13	M	63
	2	M	56	HCC	1	M	57
	3	M	72		2	M	43
	4	M	36		3	M	52
	5	M	57		4	M	56
	6	F	70		5	M	60
	7	M	60		6	M	49
	8	M	69		7	M	58
	9	F	69		8	M	71
	10	F	70		9	M	40
	11	M	61		10	M	55
	12	F	42		11	M	70
	13	M	62		12	M	36
					13	F	46

¹gender: M, male; F, female

Abbreviations: CA19-9, carbohydrate antigen 19-9; CFB, complement factor b; PC, pancreatic cancer; HD, healthy donor; CP, chronic pancreatitis; HCC, hepatocellular carcinoma; CC, cholangiocarcinoma; GC, gastric cancer.

Table S2. Features of plasma samples that were used for independent verification

Disease		Gender ¹	Age	Disease		Gender	Age	Disease		Gender	Age	
HD	1	M	32	HD	40	M	49	PC	23	M	53	
	2	M	34		41	M	57		24	F	52	
	3	M	26		42	M	55		25	M	66	
	4	M	35		43	M	59		26	M	50	
	5	F	27		44	F	46		27	F	57	
	6	M	41	CP	1	F	52		28	M	60	
	7	M	34		2	M	37		29	M	49	
	8	M	29		3	F	37		30	F	50	
	9	M	33		4	M	47		31	M	64	
	10	M	26		5	M	41		32	M	75	
	11	F	33		6	M	63		33	M	63	
	12	F	50		7	M	68		34	F	75	
	13	F	70		8	M	53		35	F	53	
	14	M	42		9	M	47		36	M	73	
	15	M	69		10	M	71		37	F	55	
	16	M	70		11	M	30		38	M	61	
	17	M	56		12	M	21		39	M	65	
	18	M	65	PC	1	M	52		40	M	46	
	19	F	57		2	M	55		41	F	74	
	20	M	61		3	M	58		CC	1	M	71
	21	M	72		4	M	73			2	M	73
	22	M	62		5	F	47	3		F	50	
	23	F	69		6	M	56	4		M	49	
	24	M	62		7	M	72	5		F	32	
	25	F	66		8	M	36	6		M	61	
	26	F	55		9	M	57	7		M	59	
	27	M	54		10	F	70	8		F	62	
	28	M	64		11	M	60	9		M	67	
	29	F	44		12	M	69	10		F	71	
	30	M	62		13	F	69	11		M	69	
	31	M	69		14	F	70	12		M	55	
	32	F	49	15	M	61	13	M		75		
	33	M	50	16	F	42	14	M		81		
	34	F	58	17	F	56	15	F	62			
	35	F	61	18	M	31	16	M	69			
	36	M	62	19	M	62	17	M	71			
	37	M	49	20	F	74	18	M	60			
	38	M	79	21	F	52	19	M	55			
	39	M	41	22	M	70	20	M	62			

Disease		Gender	Age
CC	21	F	67
	22	F	62
GC	1	M	71
	2	F	36
	3	M	64
	4	M	60
	5	F	46
	6	M	37
	7	M	42
	8	M	62
	9	F	66
	10	M	69
	11	M	78
	12	F	49
	13	M	70
	14	F	69
	15	F	53
	16	M	54
	17	F	74
	18	M	72
	19	M	60
	20	F	69
	21	M	63
	22	M	49
	23	M	34
	24	M	66
	25	M	52
	26	M	72
	27	M	48
	35	F	66
	28	M	72
	29	M	57
	30	M	72
	31	M	55
	32	M	61
	33	M	46
	34	M	68
35	M	58	

Disease		Gender	Age
HCC	1	M	49
	2	M	40
	3	M	55
	4	M	70
	5	M	36
	6	F	46
	7	M	44
	8	M	62
	9	F	67
	10	M	52
	11	M	34
	12	M	67
	13	M	67
	14	M	50
	15	F	67
	16	F	52
	17	M	55
	18	M	49
	19	M	43
	20	M	52
	21	M	54
	22	M	47
	23	M	54
	24	M	43
	25	M	64
	26	M	57
	27	M	57
	35	F	70
	28	F	48
	29	F	57
	30	M	52
31	M	61	

¹gender: M, male; F, female

Abbreviations: CA19-9, carbohydrate antigen 19-9; CFB, complement factor b; PC, pancreatic cancer; HD, healthy donor; CP, chronic pancreatitis; HCC, hepatocellular carcinoma; CC, cholangiocarcinoma; GC, gastric cancer.

Table S3. LC condition for 4.6 × 100 mm column

	Time (min)	%B	Flow Rate (mL/min)	Max. Pressure (bar)
1	0.00	0.00	0.125	60
2	18.00	0.00	0.125	60
3	18.01	0.00	1.000	60
4	20.00	0.00	1.000	60
5	20.01	100.00	1.000	60
6	27.00	100.00	1.000	60
7	27.01	0.00	1.000	60
8	38.00	0.00	1.000	60

Supplementary Table 4. Identification of differentially expressed proteins by 2-D DIGE analysis with MALDI-TOF or LC-MS/MS*.

(a) MALDI-TOF

Spot No.	Accession No.	Protein Name	Seq. Coverage (%)	Score	MW (kDa)	pI	Mass values searched	Mass values matched	Mass values unmatched	expect values	peptide
3	gi223170	Fibrinogen gamma	34	96	51.5	5.4	70	16	54	6.10E-05	K.TSEVKQLIK.A K.AIQLTYNPDESSKPNMIDAATLK.S K.AIQLTYNPDESSKPNMIDAATLK.S + Oxidation (M) K.AIQLTYNPDESSKPNMIDAATLKS.R.K K.AIQLTYNPDESSKPNMIDAATLKS.R.K + Oxidation (M) R.YLQEIYNSNQK.I K.QSGLYFIKPLK.A K.EGFGHLSPTGTTEFWLGNEK.I R.VELEDWNGRTSTADYAMFK.V + Oxidation (M) K.VGPEADKYR.L K.TRWYSMK.K K.TRWYSMK.K + Oxidation (M) R.WYSMKK.T + Oxidation (M) K.TTMKIIPFNR.L + Oxidation (M) R.LTIGEGQQHHLGGAK.Q R.LTIGEGQQHHLGGAKQAGDV.-
4	gi3421149	E6-AP ubiquitin-protein ligase fragment	80	67	100.7	5.1	57	6	51	0.055	R.YYHQLTEGCGNEACTNEFCASCPTFLR.M R.MDNNAAAIKALELYK.I K.INAKLCDPHPSK.K K.KGASSAYLENSK.G K.GASSAYLENSKGAPNNSCSEIK.M K.GAPNNSCSEIKMNK.K
5	gi4557325	Apolipoprotein E Precursor	42	70	36.1	5.7	70	13	57	0.027	R.WVQTLSEVQEELLSSQVTQELR.A K.AYKSELEEQLTPVAEETR.A R.LSKELQAAQAR.L

Spot No.	Accession No.	Protein Name	Seq. Coverage (%)	Score	MW (kDa)	pI	Mass values searched	Mass values matched	Mass values unmatched	expect values	peptide
											R.LAVYQAGAR.E
											R.LAVYQAGAREGAER.G
											R.GLSAIRER.L
											R.LGPLVEQGR.V
											R.LGPLVEQGRV.R.A
											R.AATVGSLAGQPLQER.A
											R.AQAWGERL.R.A
											R.AKLEEQAQQIR.L
											R.LKSWFEPLVEDMQR.Q
											R.LKSWFEPLVEDMQR.Q + Oxidation (M)
6	gi4557325	Apolipoprotein E Precursor	41	103	36.1	5.7	71	17	54	1.20E-05	R.WVQTLSEVQEELLSSQVTQELR.A
											R.LSKELQAAQAR.L
											K.ELQAAQAR.L
											R.GEVQAMLGQSTEELRVR.L
											R.GEVQAMLGQSTEELRVR.L + Oxidation (M)
											K.RLAVYQAGAR.E
											R.LAVYQAGAR.E
											R.LAVYQAGAREGAER.G
											R.GLSAIRER.L
											R.LGPLVEQGRV.R.A
											R.VRAATVGSLAGQPLQER.A
											R.AATVGSLAGQPLQER.A
											R.AQAWGERL.R.A
											R.LQAEAFQAR.L
											R.LKSWFEPLVEDMQR.Q
											R.LKSWFEPLVEDMQR.Q + Oxidation (M)
											K.SWFEPLVEDMQR.Q
7	gi178835	Apolipoprotein E	46	97	36.1	5.7	70	16	54	5.20E-05	R.FWDYLR.W
											R.WVQTLSEVQEELLSSQVTQELR.A

Spot No.	Accession No.	Protein Name	Seq. Coverage (%)	Score	MW (kDa)	pI	Mass values searched	Mass values matched	Mass values unmatched	expect values	peptide
											K.AYKSELEEQLTPVAEETR.A
											R.LSKELQAAQAR.L
											R.GEVQAMLGQSTEELRVR.L
											R.GEVQAMLGQSTEELRVR.L + Oxidation (M)
											K.RLAVYQAGAR.E
											R.LAVYQAGAR.E
											R.LAVYQAGAREGAER.G
											R.GLSAIRER.L
											R.LGPLVEQGRVR.A
											R.VRAATVGS LAGQPLQER.A
											R.AATVGS LAGQPLQER.A
											R.AQAWGERLR.A
											R.LKSWFEPLVEDMQR.Q
											R.LKSWFEPLVEDMQR.Q + Oxidation (M)
8	gi178835	Apolipoprotein E	56	192	36.1	5.7	70	23	47	1.60E-14	R.QQTEWQSGQRWELALGR.F
											R.FWDYLR.W
											R.WVQTLSEVQEELLSSQVTQELR.A
											R.LSKELQAAQAR.L
											R.LVQYRGEVQAMLGQSTEELR.V
											R.GEVQAMLGQSTEELRVR.L
											R.VRLASHLR.K
											R.LASHLRK.L
											K.RLAVYQAGAR.E
											R.LAVYQAGAR.E
											R.LAVYQAGAREGAER.G
											R.GLSAIRER.L
											R.LGPLVEQGR.V
											R.LGPLVEQGRVR.A
											R.VRAATVGS LAGQPLQER.A

Spot No.	Accession No.	Protein Name	Seq. Coverage (%)	Score	MW (kDa)	pI	Mass values searched	Mass values matched	Mass values unmatched	expect values	peptide
											R.AATVGS LAGQPLQER.A
											R.AATVGS LAGQPLQERAQAWGER.L
											R.AQAWGERLR.A
											R.AKLEEQAQQIR.L
											K.LEEQAAQIRLQAEAFQAR.L
											R.LQAEAFQAR.L
											R.LKSWFEPLVEDMQR.Q
											R.LKSWFEPLVEDMQR.Q + Oxidation (M)
9	gi178835	Apolipoprotein E	42	116	36.1	5.7	70	17	53	6.30E-07	R.FWDYLR.W
											R.WVQTLSEVQEELLSSQVTQELR.A
											R.LSKELQAAQAR.L
											R.GEVQAMLGQSTEELRVR.L
											R.VRLASHLR.K
											R.LASHLRK.L
											K.RLAVYQAGAR.E
											R.LAVYQAGAR.E
											R.LAVYQAGAREGAER.G
											R.GLSAIRER.L
											R.LGPLVEQGRVR.A
											R.VRAATVGS LAGQPLQER.A
											R.AATVGS LAGQPLQER.A
											R.AATVGS LAGQPLQERAQAWGER.L
											R.AQAWGERLR.A
											R.LKSWFEPLVEDMQR.Q
											R.LKSWFEPLVEDMQR.Q + Oxidation (M)

(b) LC-MS/MS

Spot No.	Accession No.	Protein Name	Seq. Coverage (%)	Score	MW (kDa)	pI	Matched spectral	Unique sequence	Unique peptide	expect values	peptide
1	gi291922	Complement factor B	2	114	85.5	6.7	5	1	1	0.00012	K.VSEADSSNADWVTK.Q
										3.40E-05	K.VSEADSSNADWVTK.Q
										7.40E-06	K.VSEADSSNADWVTK.Q
										0.00011	K.VSEADSSNADWVTK.Q
										0.00096	K.VSEADSSNADWVTK.Q
2	gi291922	Complement factor B	2	67	85.5	6.7	3	1	1	0.00069	K.VSEADSSNADWVTK.Q
										0.00091	K.VSEADSSNADWVTK.Q
										0.00019	K.VSEADSSNADWVTK.Q
10	gi123508	Haptoglobin	6	140	45.2	6.1	3	2	2	0.02	R.TEGDGVYTLNDK.K
										0.0085	R.TEGDGVYTLNDK.K
										0.00097	R.TEGDGVYTLNDK.K

Protein scores showing greater than 66 for MALDI-TOF or individual ion scores showing greater than 42 for LC-MS/MS are regarded as significant ($p < 0.05$) where LC-MS/MS data have $FDR < 1\%$.

* These proteins were filtered according to three criteria: (1) protein with Student's t-test, $p < 0.05$ (2) proteins detected all in the duplicated testing; (3) spots of PC samples showed >1.4 -fold higher abundance compared with the control groups (HD and CP).

Table S5. Diagnosis of PC based on the optimal cut-off values of CFB determined by ROC analysis

	PC (n=41)		Others* (n=144)	
	CA 19-9 <37¹	CA 19-9 ≥37	CA 19-9 <37	CA 19-9 ≥37
CFB <78.4²	1 (2.4%)	10 (24.4%)	97 (67.3%)	43 (29.9%)
CFB ≥78.4	7 (17.1%)	23 (56.1%)	3 (2.1%)	1 (0.7%)
Only CA 19-9		33 (80.5%)		44 (30.5%)

¹The units of CA 19-9 level is U/ml.

²The units of CFB level is ng/ml.

Samples are considered as diagnostically positive (+) if CA 19-9 and/or CFB levels are higher or equal to each value.

Abbreviations: CA19-9, carbohydrate antigen 19-9; CFB, complement factor b; PC, pancreatic cancer; Others*: HD, healthy donor; CP, chronic pancreatitis; HCC, hepatocellular carcinoma; CC, cholangiocarcinoma; GC, gastric cancer.

Table S6. PC diagnosis based on optimal cut-off value of CA 19-9 and CFB in each group

	CFB ≥ 78.4ng/ml	CA 19-9 ≥ 37U/ml
HD	0%	0%
CP	8.30%	8.30%
PC	73.20%	80.50%
HCC	0%	61.30%
CC	0%	77.20%
GC	8.60%	17.10%

Abbreviations: CA19-9, carbohydrate antigen 19-9; CFB, complement factor b; PC, pancreatic cancer; HD, healthy donor; CP, chronic pancreatitis; HCC, hepatocellular carcinoma; CC, cholangiocarcinoma; GC, gastric cancer.

Supplementary Figures

Figure S1. 2-D DIGE map of protein expressed in pancreatic cancer (PC) and chronic pancreatitis (CP) plasma.

A gel contained the same pooled internal standard sample minimal dye labeled with CyDye DIGE Fluor Cy2 (blue), whereas two protein samples were labeled with Cy3 (green) or Cy5 (red) minimal dyes.

Gel No.	Cy2	Cy3	Cy5
1	Pooled standard	Pancreatic Cancer	Chronic Pancreatitis

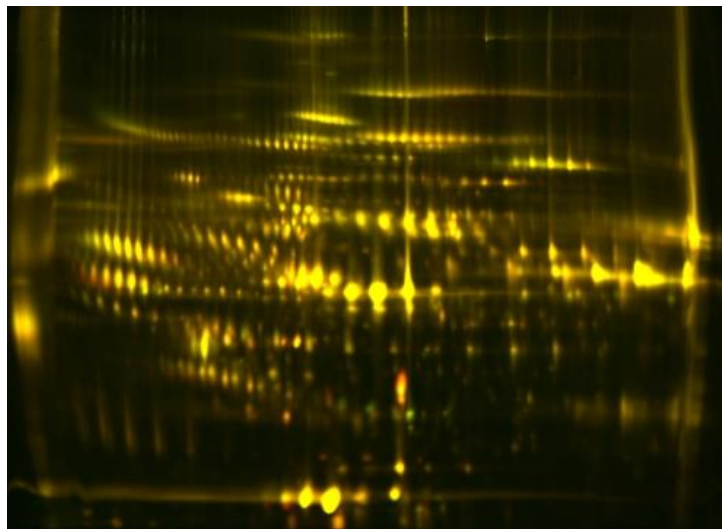
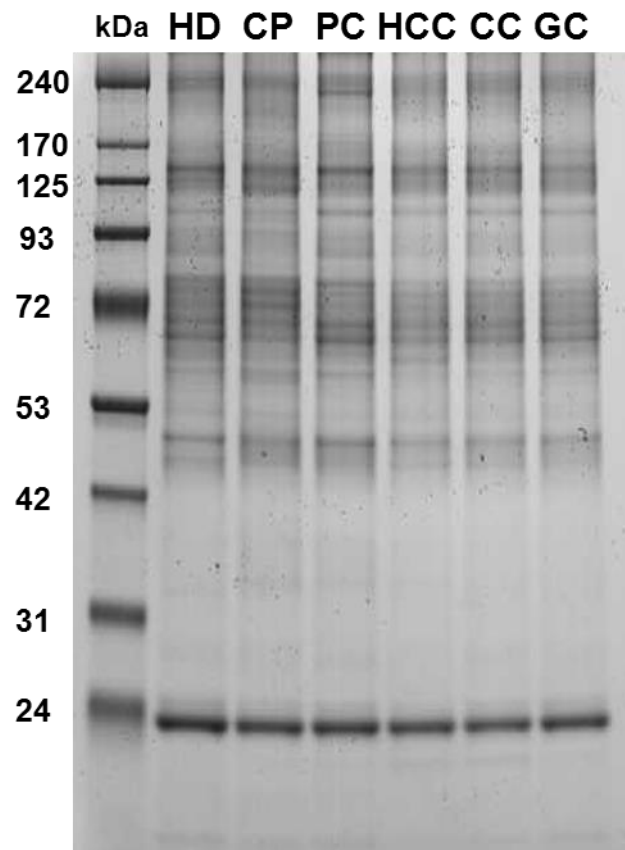


Figure S2. The overall protein profile of the pooled plasma samples.

The gel was stained with Coomassie brilliant blue G250 dye solution to ensure equal loading during western blot analysis.



* HD, healthy donor; CP, chronic pancreatitis; PC, pancreatic cancer; HCC, hepatocellular carcinoma; CC, cholangiocarcinoma; GC, gastric cancer.