

An iTRAQ Based Quantitative Proteomic Strategy to Explore Novel Secreted Proteins in Metastatic Hepatocellular Carcinoma Cell Lines

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Table S1. Secreted proteins identified in the MHCC97L and HCCLM3 cell lines.

Protein Name	Gene	97L	Pvalue	LM3	Pvalue	LM3:97L	Pvalue	Sig- nalp	SecretomeP	GO-CC
		CM:L		CM:L		(CM)				
Fibronectin	FN1	1.58	3.52E-11	1.87	7.33E-15	1.18	6.89E-09	√	unknown	extracellular region
Filamin-A	FLNA	2.58	3.89E-02	2.21	3.76E-02	0.73	9.65E-01	-	no secret	extracellular region
Pyruvate kinase isozymes M1/M2	PKM2	0.76	7.07E-01	3.25	5.08E-05	3.91	8.85E-04	-	no secret	cytoplasm/Nucleus
Retinal dehydrogenase 1	ALDH1A1	2.23	2.88E-03	3.80	4.57E-04	1.10	3.57E-01	-	no secret	cytoplasm
Phosphoglycerate kinase 1	PGK1	5.45	1.11E-04	1.75	1.08E-03	1.54	1.17E-02	-	no secret	cytoplasm
Serum albumin	ALB	48.31	5.16E-04	43.65	1.38E-03	0.94	1.48E-01	√	unknown	extracellular region
Heat shock cognate 71 kDa protein	HSPA8	1.87	1.90E-02	1.96	6.28E-03	1.16	4.65E-01	-	no secret	plasma membrane
Alpha-enolase	ENO1	1.82	1.71E-02	3.31	4.64E-03	1.91	1.27E-01	-	no secret	plasma membrane
Glyceraldehyde-3-phosphate dehydrogenase	GAPDH	0.63	5.09E-01	1.54	1.85E-02	2.63	6.08E-03	-	no secret	cytoplasm
Hepatocyte growth factor receptor	MET	51.05	1.09E-03	56.49	3.17E-03	1.34	2.48E-02	√	unknown	plasma membrane
78 kDa glucose-regulated protein	HSPA5	0.29	1.61E-03	2.61	1.31E-01	1.34	8.01E-01	√	classic	plasma membrane
Fructose-bisphosphate aldolase A	ALDOA	2.03	5.50E-02	1.56	9.06E-02	1.29	2.61E-01	-	no secret	extracellular region
Ubiquitin-like modifier-activating enzyme 1	UBA1	1.74	3.75E-02	1.45	1.57E-01	1.18	5.35E-01	-	nonclassic	Unknown
Clusterin	CLU	41.69	5.77E-05	59.16	1.04E-02	1.39	2.04E-01	√	unknown	extracellular region
Peroxisomal bifunctional enzyme	EHHADH	49.66	1.52E-03	66.07	7.21E-04	1.58	3.37E-01	-	no secret	mitochondrion
Nicotinamide phosphoribosyltransferase	NAMPT	1.06	7.45E-01	2.54	4.46E-02	1.24	1.97E-01	-	no secret	extracellular space
Heat shock 70 kDa protein 1	HSPA1A	1.46	2.27E-01	2.17	8.78E-02	1.54	3.08E-01	-	no secret	endoplasmic reticulum
Fatty acid synthase	FASN	1.26	5.29E-01	1.56	2.01E-01	1.29	2.65E-01	-	no secret	Golgi apparatus
Peroxisiredoxin-1	PRDX1	4.25	3.11E-02	2.96	2.89E-02	1.33	3.23E-01	-	nonclassic	cytoplasm/Nucleus
Galectin-3-binding protein	LGALS3BP	27.04	3.78E-02	48.31	8.05E-07	1.66	1.61E-02	√	classic	extracellular region
Transketolase	TKT	4.17	3.24E-03	2.03	7.71E-03	1.00	7.93E-01	-	no secret	cytoplasm
Proactivator polypeptide	PSAP	22.91	1.94E-01	25.35	2.42E-03	1.07	9.21E-01	√	unknown	extracellular region
Plasminogen activator inhibitor 1	SERPINE1	40.18	9.13E-03	46.56	4.12E-04	1.38	1.62E-01	√	unknown	extracellular region
Liver carboxylesterase 1	CES1	2.44	1.75E-02	1.94	4.72E-02	0.63	1.33E-01	√	unknown	endoplasmic reticulum
Peptidyl-prolyl cis-trans isomerase B	PPIB	0.82	5.40E-01	2.63	2.29E-02	1.58	1.68E-01	√	unknown	endoplasmic reticulum
Serotransferrin	TF	39.81	8.83E-06	52.00	6.97E-03	0.79	1.88E-01	√	unknown	extracellular region
Polypyrimidine tract-binding protein 1	PTBP1	1.79	4.00E-01	2.65	1.48E-01	1.24	9.20E-01	-	no secret	nucleus
Complement factor H	CFH	33.73	2.06E-03	47.42	7.51E-02	1.50	1.10E-01	√	unknown	extracellular region
Aldehyde dehydrogenase, dimeric NADP-preferring	ALDH3A1	0.45	1.83E-01	2.33	1.84E-01	1.19	8.43E-01	-	nonclassic	endoplasmic reticulum

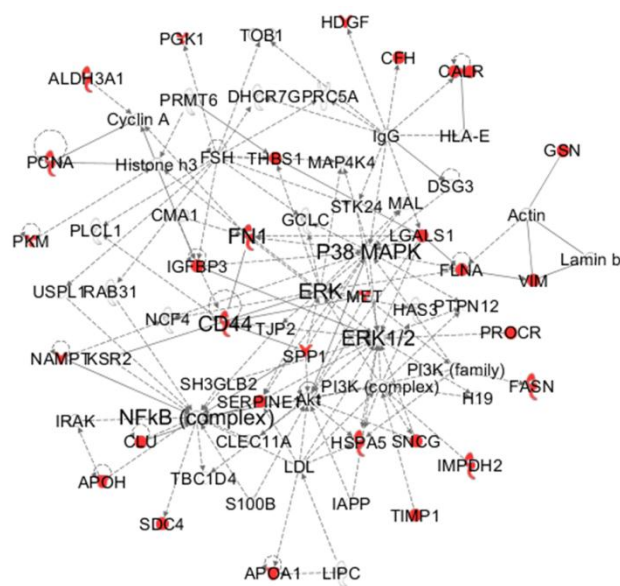
Alpha-2-HS-glycoprotein	AHSG	59.70	3.25E-02	66.68	1.01E-01	0.98	6.20E-01	√	classic	extracellular region
Acidic leucine-rich nuclear phosphoprotein 32 family member B	ANP32B	2.05	9.37E-01	2.49	3.79E-01	1.56	3.41E-01	-	no secret	nucleus
Interleukin enhancer-binding factor 3	ILF3	1.94	5.17E-01	1.43	8.62E-01	0.37	3.74E-01	-	no secret	cytoplasm/Nucleus
Insulin-like growth factor-binding protein 2	IGFBP2	34.04	6.58E-03	44.06	6.23E-03	1.61	2.41E-01	√	unknown	extracellular region
Collagen alpha-1(VI) chain	COL6A1	38.02	8.21E-02	51.52	7.12E-02	1.77	4.00E-01	√	classic	extracellular region
L-lactate dehydrogenase A chain	LDHA	2.17	1.03E-01	1.22	4.70E-01	1.28	9.91E-01	-	nonclassic	Cytoplasm
Galectin-1	LGALS1	3.44	8.89E-02	2.61	2.45E-01	0.85	3.89E-01	-	no secret	extracellular region
Nucleolin	NCL	2.03	4.73E-01	1.50	2.42E-01	1.04	5.05E-01	-	no secret	cytoplasm/Nucleus
Galectin-4	LGALS4	4.53	9.44E-02	4.41	1.37E-01	0.58	4.34E-01	-	no secret	plasma membrane
Cathepsin D	CTSD	6.85	4.60E-02	3.19	7.67E-02	1.18	6.38E-01	√	unknown	extracellular region
Osteopontin	SPP1	42.85	4.66E-03	46.13	3.87E-03	1.72	2.20E-01	√	unknown	extracellular region
Nucleobindin-1	NUCB1	23.55	1.05E-02	23.12	1.55E-02	1.04	9.47E-01	√	classic	extracellular region
Sulfhydryl oxidase 1	QSOX1	20.32	8.78E-02	60.81	6.22E-02	0.95	8.60E-01	√	membrain	extracellular region
Cystatin-C	CST3	46.56	1.31E-03	53.46	1.18E-03	1.43	1.83E-01	√	unknown	extracellular region
14-3-3 protein beta/alpha	YWHAB	1.84	3.61E-01	3.31	1.99E-01	1.57	4.48E-01	-	no secret	Cytoplasm
Thrombospondin-1	THBS1	27.54	1.12E-01	38.02	1.01E-01	1.42	5.78E-01	√	unknown	extracellular region
Heterogeneous nuclear ribonucleoprotein Q	SYNCRIP	3.16	2.07E-01	2.88	2.24E-01	0.88	8.06E-01	-	no secret	endoplasmic reticulum
Interleukin enhancer-binding factor 2	ILF2	1.58	4.39E-01	1.60	4.33E-01	0.98	9.85E-01	-	no secret	cytoplasm/Nucleus
Ceruloplasmin	CP	35.65	1.43E-01	40.55	1.21E-01	1.64	5.25E-01	√	unknown	extracellular region
Nascent polypeptide-associated complex subunit alpha	NACA	2.54	2.53E-01	2.91	2.22E-01	1.74	3.87E-01	-	no secret	cytoplasm/Nucleus
Keratin, type II cytoskeletal 1	KRT1	36.31	9.86E-02	15.14	1.52E-01	0.28	2.17E-01	-	no secret	plasma membrane
Hepatoma-derived growth factor	HDGF	2.36	2.70E-01	3.22	2.04E-01	1.58	4.43E-01	-	no secret	extracellular region
Insulin-like growth factor-binding protein 3	IGFBP3	12.25	1.23E-01	15.14	1.18E-01	1.54	5.01E-01	√	unknown	extracellular region
Glia-derived nexin	SERPINE2	31.92	1.04E-02	36.98	6.17E-03	0.94	5.09E-01	√	unknown	extracellular region
Apolipoprotein A-1	APOA1	71.12	1.87E-02	3.84	1.80E-01	0.19	1.50E-01	√	classic	extracellular region
Superoxide dismutase [Cu-Zn]	SOD1	1.85	1.16E-01	1.69	1.55E-01	1.05	8.36E-01	-	nonclassic	extracellular region
Laminin subunit beta-1	LAMB1	13.06	9.59E-02	7.52	1.21E-01	0.86	7.65E-01	√	classic	extracellular region
Agrin	AGRN	5.35	1.29E-01	7.45	1.11E-01	1.56	4.43E-01	√	classic	extracellular region
CD44 antigen	CD44	30.48	3.33E-02	7.73	1.33E-01	1.18	7.36E-01	√	unknown	extracellular region
Endothelial protein C receptor	PROCR	8.24	1.54E-01	11.59	1.30E-01	1.50	5.08E-01	√	unknown	plasma membrane
Cystatin-B	CSTB	1.96	3.32E-01	1.41	5.35E-01	0.47	3.05E-01	-	no secret	cytoplasm/Nucleus
Putative heterogeneous nuclear ribonucleoprotein A1-like protein 3	HNRPA1L3	1.60	4.37E-01	1.34	6.43E-01	0.74	6.33E-01	-	no secret	Cytoplasm
Inter-alpha-trypsin inhibitor heavy chain H2	ITIH2	10.19	2.14E-01	28.31	1.05E-01	1.98	3.70E-01	√	classic	extracellular region
Glutathione reductase, mitochondrial	GSR	1.60	4.49E-01	2.17	2.83E-01	1.31	5.57E-01	-	nonclassic	plasma membrane
Metalloproteinase inhibitor 1	TIMP1	31.33	1.38E-01	35.32	1.07E-01	1.47	4.83E-01	√	classic	extracellular region
Protein AMBP	AMBP	10.47	1.65E-01	12.13	1.18E-01	1.43	5.22E-01	√	unknown	extracellular region
Carbonic anhydrase 12	CA12	2.42	1.34E-01	2.47	1.47E-01	1.19	6.12E-01	√	membrain	plasma membrane
Golgi membrane protein 1	GOLM1	3.31	2.00E-01	8.32	1.16E-01	1.54	4.63E-01	√	membrain	Golgi apparatus
Alpha-2-macroglobulin	A2M	8.02	1.17E-01	13.18	9.46E-02	1.16	7.54E-01	√	unknown	extracellular region
Myristoylated alanine-rich C-kinase substrate	MARCKS	1.84	3.59E-01	1.69	4.03E-01	2.27	2.82E-01	-	no secret	plasma membrane
Vimentin	VIM	1.80	2.90E-01	0.04	6.22E-02	0.03	5.36E-02	-	nonclassic	plasma membrane

Peroxioredoxin-2	PRDX2	1.09	8.65E-01	1.74	3.72E-01	1.24	6.52E-01	-	nonclassic	Cytoplasm
Histone H1.2	HIST1H1C	2.86	2.26E-01	4.97	1.51E-01	0.75	6.12E-01	-	no secret	Nucleus
Calcyclin-binding protein	CACYBP	1.34	5.79E-01	1.67	4.09E-01	1.26	6.47E-01	-	nonclassic	cytoplasm/Nucleus
Metalloproteinase inhibitor 2	TIMP2	50.12	6.47E-02	39.08	3.05E-02	0.62	4.44E-01	✓	classic	extracellular region
Exportin-2	CSE1L	0.79	6.68E-01	2.81	2.28E-01	1.15	7.73E-01	-	no secret	cytoplasm/Nucleus
Heterogeneous nuclear ribonucleoprotein D0	HNRNPD	1.51	4.74E-01	1.15	7.70E-01	0.96	9.60E-01	-	no secret	cytoplasm/Nucleus
Calreticulin	CALR	1.39	5.42E-01	1.61	4.31E-01	0.95	9.45E-01	✓	unknown	extracellular region
Threonyl-tRNA synthetase, cytoplasmic	TARS	0.36	2.34E-01	1.58	4.39E-01	3.10	2.09E-01	-	no secret	Cytoplasm
Laminin subunit alpha-5	LAMA5	7.45	1.85E-01	13.80	1.56E-01	1.49	5.91E-01	✓	classic	extracellular region
Cathepsin L1	CTSL1	7.66	1.20E-01	3.94	1.76E-01	1.13	8.00E-01	✓	classic	extracellular region
Hsc70-interacting protein	ST13	1.79	3.73E-01	2.00	3.25E-01	1.37	5.57E-01	-	nonclassic	cytoplasm
Angiopoietin-related protein 4	ANGPTL4	18.88	1.21E-01	28.84	1.07E-01	2.13	3.44E-01	✓	classic	extracellular region
Lumican	LUM	9.20	1.11E-01	8.39	1.15E-01	1.34	5.75E-01	✓	unknown	extracellular region
Proliferating cell nuclear antigen	PCNA	1.57	3.16E-01	1.11	8.23E-01	0.87	4.67E-01	-	nonclassic	nucleus
Protein-arginine deiminase type-1	PADI1	2.27	2.81E-01	1.75	3.85E-01	0.92	8.81E-01	-	no secret	cytoplasm
Glucosylceramidase	GBA	42.46	8.95E-02	21.48	1.02E-01	1.38	5.70E-01	✓	unknown	Lysosome
Sorbitol dehydrogenase	SORD	4.06	1.72E-01	1.26	6.48E-01	0.38	2.49E-01	-	nonclassic	extracellular region
Gamma-synuclein	SNCG	0.44	2.82E-01	1.58	4.41E-01	1.58	4.40E-01	-	no secret	cytoplasm
Syndecan-4	SDC4	3.10	2.07E-01	10.96	1.06E-01	2.96	2.34E-01	✓	unknown	Golgi apparatus
Beta-2-glycoprotein 1	APOH	14.06	9.34E-02	54.45	6.12E-02	1.22	6.89E-01	✓	unknown	extracellular region
Gelsolin	GSN	1.45	5.09E-01	1.60	4.34E-01	2.15	2.99E-01	✓	unknown	extracellular region
Heterogeneous nuclear ribonucleoprotein H3	HNRNPH3	0.45	2.94E-01	2.47	2.58E-01	3.28	2.01E-01	-	no secret	nucleus
Testis-specific protein 10-interacting protein	TSGA10IP	13.68	9.57E-02	12.94	9.50E-02	0.91	8.64E-01	-	nonclassic	Unknown
Inosine-5'-monophosphate dehydrogenase 2	IMPDH2	1.92	3.40E-01	0.90	8.49E-01	0.62	4.41E-01	-	no secret	cytoplasm

Note: " ✓ " means signal peptide predicted by SignalP

Table S2. identified secreted proteins related to HCC metastasis

Accession	Protein Name	Gene	LM3:97L (CM)	Pvalue
Up-regulated proteins with increasing potential of metastasis				
P14618	Pyruvate kinase isozymes M1/M2	PKM2	3.91	8.85E-04
P00558	Phosphoglycerate kinase 1	PGK1	1.54	1.17E-02
P06733	Alpha-enolase	ENO1	1.91	1.27E-01
P04406	Glyceraldehyde-3-phosphate dehydrogenase	GAPDH	2.63	6.08E-03
Q08426	Peroxisomal bifunctional enzyme	EHHADH	1.58	3.37E-01
P08107	Heat shock 70 kDa protein 1	HSPA1A	1.54	3.08E-01
Q08380	Galectin-3-binding protein	LGALS3BP	1.66	1.61E-02
P23284	Peptidyl-prolyl cis-trans isomerase B	PPIB	1.58	1.68E-01
Q92688	Acidic leucine-rich nuclear phosphoprotein 32 family member B	ANP32B	1.56	3.41E-01
P18065	Insulin-like growth factor-binding protein 2	IGFBP2	1.61	2.41E-01
P12109	Collagen alpha-1(VI) chain	COL6A1	1.77	4.00E-01
P10451	Osteopontin	SPP1	1.72	2.20E-01
P31946	14-3-3 protein beta/alpha	YWHAAB	1.57	4.48E-01
P00450	Ceruloplasmin	CP	1.64	5.25E-01
Q13765	Nascent polypeptide-associated complex subunit alpha	NACA	1.74	3.87E-01
P51858	Hepatoma-derived growth factor	HDGF	1.58	4.43E-01
P17936	Insulin-like growth factor-binding protein 3	IGFBP3	1.54	5.01E-01
O00468	Agrin	AGRN	1.56	4.43E-01
P19823	Inter-alpha-trypsin inhibitor heavy chain H2	ITIH2	1.98	3.70E-01
Q8NBJ4	Golgi membrane protein 1	GOLM1	1.54	4.63E-01
P29966	Myristoylated alanine-rich C-kinase substrate	MARCKS	2.27	2.82E-01
P26639	Threonyl-tRNA synthetase, cytoplasmic	TARS	3.10	2.09E-01
Q9BY76	Angiopoietin-related protein 4	ANGPTL4	2.13	3.44E-01
O76070	Gamma-synuclein	SNCG	1.58	4.40E-01
P31431	Syndecan-4	SDC4	2.96	2.34E-01
P06396	Gelsolin	GSN	2.15	2.99E-01
P31942	Heterogeneous nuclear ribonucleoprotein H3	HNRNPH3	3.28	2.01E-01
Down-regulated proteins with increasing potential of metastasis				
Q12906	Interleukin enhancer-binding factor 3	ILF3	0.37	3.74E-01
P04264	Keratin, type II cytoskeletal 1	KRT1	0.28	2.17E-01
P02647	Apolipoprotein A-I	APOA1	0.19	1.50E-01
P04080	Cystatin-B	CSTB	0.47	3.05E-01
P08670	Vimentin	VIM	0.03	5.36E-02
Q00796	Sorbitol dehydrogenase	SORD	0.38	2.49E-01



Supplementary Figure. Top network of the secreted proteins identified in metastatic HCC cell lines. Proteins in red were from our dataset,  represents cytokine/growth factor,  represents enzyme,  represents G protein coupled receptor,  represents kinase,  represents transcription regulator,  represents transporter, and  represents Group/Complex/Other.  means direct relationship and  means indirect relationship.