

## ORIGINAL ARTICLE

# Analysis of Differentially Expressed Proteins Involved in miR-449a for Hepatocellular Carcinoma by iTRAQ Proteomics

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### SUMMARY

**Background:** To investigate the relationship between down-regulation of miR-449a and prognosis of hepatocellular carcinoma (HCC) and to elucidate the potential target proteins of miR-449a.

**Material and Methods:** The expression of miR-449a in 142 HCC tissues was detected by RT-PCR. The correlation between down-regulation of miR-449a and prognosis of HCC was statistically analyzed during clinical follow-up. The Bel-7042 HCC cell line in miR-449a-mimic and miR-449a-inhibitor model was used, and the potential target protein of miR-449a was screened by isobaric tags for relative and absolute quantitation (iTRAQ) technology.

**Results:** miR-449a was significantly down-regulated in HCC tissues, which was significantly associated with post-operative metastasis ( $p < 0.0001$ ) and recurrence ( $p < 0.0001$ ). The median overall survival time in the low-expression group of miR-449a was significantly lower than that of the high-expression group (19 months vs. 37 months,  $p = 0.001$ ). In addition, the tumor-free survival time of the low-expression group was significantly lower than that of the high-expression group (14 months vs. 24 months,  $p = 0.001$ ). iTRAQ analysis screened out 137 differential proteins, among which 88 were up-regulated and 49 were down-regulated. GO clustering, KEGG pathway, and STRING analysis were performed, suggesting that these differential proteins have complicated functions, such as ATP binding, metal ion binding, RNA binding, human papillomavirus infection, and Epstein-Barr virus infection.

**Conclusions:** miR-449a was negatively correlated with HCC prognosis. The differential proteins screened by iTRAQ can provide the basis for studying the target proteins regulated by miR-449a and understanding the pathogenesis of HCC.

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## Supplementary Tables and Figures

Table S1. Differentially expressed proteins between miR-449a-mimic/miR-449a-inhibitor.

No.	Protein ID	Protein_name	Cover percent (100%)	MW (Da)	PI	miR-449a-mimic/ inhibitor ratio	p-value
1	<a href="#">P09914</a>	IFIT1	12.08	5,1713	8.55	1.906	2.79269E
2	<a href="#">P20592</a>	MX2 Interferon-induced GTP-binding protein	3.22	82,088	8.91	1.746	7.20231E
3	<a href="#">Q8NGA1</a>	OR1M1	2.22	35,151	9.18	1.604	1.44911E
4	<a href="#">Q9BTM9</a>	URM1	11.11	7,143	5.24	1.593	5.51826E
5	<a href="#">P49137</a>	MAPKAPK2	5.75	45,567	8.87	2.369	2.97089E
6	<a href="#">Q32P41</a>	TRMT5 tRNA (guanine-N(1)-methyltransferase	3.73	58,246	8.78	1.801	6.58833E
7	<a href="#">Q9UII2</a>	ATPIF1 Isoform 1 of ATPase inhibitor	16.98	12,249	9.34	1.610	1.43456E
8	<a href="#">P23258</a>	MZT2B	5.74	12,395	10.49	2.550	1.53677E
9	<a href="#">P05161</a>	ISG15 Ubiquitin-like protein	30.91	17,887	6.83	2.095	4.80534E
10	P13984	General transcription factor IIF subunit 2	5.22	28,380	9.24	2.904	1.15713E
11	Q14696	MESDC2 LDLR chaperone MESD	5.56	26,076	7.6	2.573	3.84254E
12	Q9BY32	ITPA Inosine triphosphate pyrophosphatase	28.35	21,445	5.5	2.263	5.75748E
13	Q9NUQ7	UFSP2	8.27	44,162	8.6	2.654	9.48566E
14	O15258	RER1	5.14	24,817	9.66	2.788	1.37883E
15	Q9UDY8	Mucosa-associated lymphoid tissue lymphoma translocation protein 1	1.46	92,271	5.47	2.875	2.92552E
16	O14879	IFIT3 Interferon-induced protein with tetratricopeptide repeats 3	6.94	55,984	5.12	1.848	9.80503E
17	P01889	HLA class I histocompatibility antigen, B-73 alpha chain	17.36	40,435	5.84	2.451	1.69941E
18	Q5T6V5	C9orf64 UPF0553 protein	10.26	39,028	5.61	2.377	3.80674E
19	P19971	Homo sapiens endothelial cell growth factor 1	5.95	50,404	5.36	2.153	1.25503E
20	Q9ULK4	MED23	1.82	157,112	7.3	1.706	2.6312E
21	O95613	PCNT Isoform 1 of Pericentrin	0.93	378,033	5.4	1.922	2.73215E
22	Q5GLZ8	HERC4 Isoform 1 of Probable E3 ubiquitin-protein ligase	3.31	118,562	5.8	2.662	2.8368E
23	Q9NPJ6	MED4	6.85	28,045	5.16	1.662	4.11836E
24	Q15165	Serum paraoxonase/arylesterase 2	2.4	41,528	5.35	2.195	4.78893E
25	P42224	Signal transducer and activator of transcription 1-alpha/beta	22	87,334	5.74	2.820	4.96811E
26	P29317	EPHA2 Ephrin type-A receptor 2	4	108,265	5.86	2.788	5.11947E
27	P04439	HLA class I histocompatibility antigen, B-55 alpha chain	18.23	40,496	5.66	2.099	5.38156E
28	P05062	ALDOB Fructose-bisphosphate aldolase	7.28	34,724	8.4	2.215	6.12455E
29	Q9BYX2	TBC1D2 Isoform 6 of TBC1 domain family member 2A	1.28	54,418	6.7	2.327	6.87408E
30	P49902	NT5C2 Cytosolic purine 5'-nucleotidase	7.31	64,969	5.75	2.520	7.31691E

Table S1. Differentially expressed proteins between miR-449a-mimic/miR-449a-inhibitor (continued).

No.	Protein ID	Protein name	Cover percent (100%)	MW (Da)	PI	miR-449a-mimic/ inhibitor ratio	p-value
31	P10321	HLA class I histocompatibility antigen, B-2 alpha chain	18.23	40,521	5.85	2.007	9.84389E
32	Q03013	GSTM2P1	9.24	21,321	5.95	2.475	9.94003E
33	O43808	SLC25A17	4	25,721	10.33	2.671	1.36203E
34	Q9Y3Z3	SAM domain and HD domain-containing protein 1	4.47	72,200	6.69	2.899	1.64807E
35	Q15185	Prostaglandin E synthase 3	16.13	14,573	4.13	2.439	2.52295E
36	Q08AF3	SLFN5 cDNA FLJ52101	5.4	43,892	8.4	2.418	2.92606E
37	Q29983	HLA class I histocompatibility antigen, Cw-12 alpha chain	20.77	40,885	5.91	1.831	3.16924E
38	Q8WY22	BRI3-binding protein	6.77	27,835	9.48	2.637	6.62116E
39	Q8IXT5	RBM12B	2.5	102,704	7.09	2.147	7.31777E
40	O60443	Non-syndromic hearing impairment protein 5	1.81	54,554	5.03	2.676	0.00011
41	O95999	BCL10 B-cell lymphoma/leukemia 10	4.29	26,251	5.57	2.655	0.00012
42	Q8NAV1	PRPF38A Isoform 1 of Pre-mRNA-splicing factor 38A	2.56	37,476	9.97	1.697	0.00015
43	Q06323	PSME1 Proteasome activator complex subunit 1	45.38	28,723	5.78	2.583	0.00016
44	P06241	FYN Isoform 1 of Tyrosine-protein kinase Fyn	1.49	60,761	6.23	2.515	0.00018
45	P61769	B2M Beta-2-microglobulin	8.4	13,714	6.06	1.895	0.00021
46	Q15154	PCM1	2.4	84,066	4.93	1.994	0.00021
47	Q5VZF2	Muscleblind-like protein 2	5.36	40,517	8.77	1.767	0.00022
48	Q658Y4	FAM91A1	2.74	93,908	5.95	2.824	0.00029
49	P0C0L4	C4A	2.06	192,843	6.66	2.544	0.00032
50	Q86VM9	sapiens conserved nuclear protein NHN1	0.92	108,976	8.52	1.945	0.00035
51	Q96ST3	Paired amphipathic helix protein	4.08	145,173	6.82	2.211	0.00035
52	Q9UKV8	Isoform 2 of Protein argonaute-2	3.88	93,619	9.38	2.207	0.00039
53	O95218	Zinc finger Ran-binding domain-containing protein 2	5	36,318	9.79	1.654	0.00042
54	P30405	PPIF Peptidyl-prolyl cis-trans isomerase F	29.47	22,040	9.49	1.717	0.00044
55	Q08380	Galectin-3-binding protein	9.77	64,096	5.29	1.893	0.00047
56	Q9UBB4	ATXN10 ataxin-10 isoform 2	11.44	46,286	4.97	2.356	0.00051
57	Q53H82	LACTB2 Beta-lactamase-like protein 2	9.03	32,805	6.32	1.577	0.00055
58	P00734	F2 Prothrombin	2.41	70,036	5.64	1.846	0.00057
59	P20585	MSH3 DNA mismatch repair protein	2.29	127,410	8.2	2.269	0.00070
60	P15169	CPN1	7.35	22,879	5.16	2.769	0.00079
61	Q96ST2	IWS1 Isoform 3	2.12	69,108	4.81	2.414	0.00081
62	P84090	ERH Enhancer of rudimentary homolog	17.31	12,259	5.62	2.278	0.00086
63	Q9UL46	PSME2	22.05	29,126	6.27	1.595	0.00086
64	P68871	HBB Hemoglobin subunit beta	74.15	15,998	6.74	1.892	0.00089

Table S1. Differentially expressed proteins between miR-449a-mimic/miR-449a-inhibitor (continued).

No.	Protein ID	Protein_name	Cover percent (100%)	MW (Da)	PI	miR-449a-mimic/inhibitor ratio	p-value
65	Q9BQC6	MRP63 Ribosomal protein 63	12.75	12,266	11.45	2.190	0.00102
66	P17096	High mobility group protein HMG	4.88	34,301	10.39	2.147	0.00107
67	Q9HAS0	C17orf75 Protein Njmu-R1	2.78	44,621	4.98	2.399	0.00107
68	Q7Z4W1	DCXR L-xylulose reductase isoform 2	4.13	25,743	8.33	2.318	0.00136
69	Q13823	GNL2 Nucleolar GTP-binding protein 2	3.56	83,654	9.27	2.474	0.00137
70	Q6NUK1	Calcium-binding mitochondrial carrier protein	7.13	53,354	6	2.728	0.00142
71	Q15392	DHCR24 Delta(24)-sterol reductase	10.85	60,101	8.42	2.179	0.00167
72	Q99595	TIMM17A Mitochondrial import inner membrane translocase subunit	12.87	18,023	7.72	2.576	0.00177
73	P04179	SOD2 superoxide dismutase [Mn], mitochondrial isoform A precursor	17.12	24,750	8.35	2.330	0.00189
74	Q9H477	RBKS Ribokinase	2.48	34,143	4.94	2.217	0.00219
75	Q9UL25	Ras-related protein Rab-21	12	24,347	8.11	1.854	0.00222
76	P28065	PSMB9 Isoform LMP2.L of Proteasome subunit beta type-9	13.7	23,264	4.93	1.725	0.00229
77	P82673	MRPS35 Isoform 1 of 28S ribosomal protein S35	18.89	36,844	8.41	1.878	0.00233
78	O43293	DAPK3 Death-associated protein kinase 3	4.63	52,535	6.44	2.294	0.00243
79	Q92536	SLC7A6 Y+L amino acid transporter 2	4.08	56,827	5.63	2.273	0.00250
80	Q96CP7	TLCD1 TLC domain-containing protein 1	8.5	28,548	9.54	2.233	0.00255
81	P48735	IDH2 Isocitrate dehydrogenase	4	45,179	7.63	1.977	0.00257
82	P55265	ADAR Isoform 5 of Double-stranded RNA-specific adenosine deaminase	11.71	103,641	8.89	2.685	0.00263
83	Q9H0M0	WWP1 Isoform 1 of NEDD4-like E3 ubiquitin-protein ligase	2.39	105,201	5.64	1.734	0.00322
84	Q9UGT4	SUSD2 Sushi domain-containing protein 2	3.16	90,207	5.84	2.533	0.00355
85	Q4U2R6	MRPL51 39S ribosomal protein L51	8.59	15,095	11.27	2.272	0.00359
86	P11717	IGF2R Cation-independent mannose-6-phosphate receptor	0.84	274,372	5.64	1.931	0.00384
87	Q15274	QPRT Nicotinate-nucleotide pyrophosphorylase	4.04	30,845	5.81	2.737	0.00397
88	Q13501	SQSTM1 Isoform 1 of Sequestosome-1	15.45	47,687	5.1	1.767	0.00397
89	Q9NWX4	C4orf27 UPF0609 protein	4.05	39,436	6.34	-1.938	0.00381
90	P01009	SERPINA1 Isoform 3 of Alpha-1-antitrypsin	4.9	34,755	5.04	-1.678	0.00377
91	P08123	COL1A2 Collagen alpha-2(I) chain	3.22	129,312	9.08	-2.501	0.00358

Table S1. Differentially expressed proteins between miR-449a-mimic/miR-449a-inhibitor (continued).

No.	Protein ID	Protein_name	Cover percent (100%)	MW (Da)	PI	miR-449a-mimic/ inhibitor ratio	p-value
92	O15260	SURF4	19.35	21,127	6.07	-1.934	0.00293
93	Q9UBM7	DHCR7	8.53	29,189	9.21	-2.345	0.00287
94	Q13206	Probable ATP-dependent RNA helicase DDX10	7.04	90,315	8.92	-1.627	0.00280
95	Q9H204	MED28 Mediator of RNA polymerase II transcription subunit 28	7.87	19,520	5.39	-2.063	0.00270
96	P00338	LDHA Isoform 1 of L-lactate dehydrogenase A chain	40.06	36,688	8.44	-2.002	0.00253
97	Q9UII2	ATP6V1H 52 kDa protein	7.22	51,570	5.86	-1.915	0.00242
98	Q92600	Rattus norvegicus rcd1 homolog 1	8.53	14,653	9.1	-2.055	0.00228
99	Q5T280	C9orf114	5.15	44,003	9.9	-2.283	0.00224
100	P49427	CDC34 Ubiquitin-conjugating enzyme E2 R1	2.97	26,736	4.41	-2.226	0.00211
101	P10412	HIST1H1E Histone H1.4	33.79	21,865	11.03	-1.968	0.00209
102	P55327	TPD52	23.38	31,170	5.86	-2.484	0.00209
103	P20962	PTMS Parathymosin	22.55	11,530	4.14	-2.115	0.00183
104	Q15291	Isoform 2 of Retinoblastoma-binding protein 5	8.2	55,071	4.82	-1.897	0.00180
105	O43670	Isoform 1 of Zinc finger protein 207	7.11	50,750	9.19	-1.931	0.00177
106	P55327	TPD52 tumor protein D52 isoform 2	39.13	22,477	5.3	-2.685	0.00163
107	O15460	Isoform IIa of Prolyl 4-hydroxylase subunit alpha-2	6.75	60,632	5.5	-1.841	0.00153
108	Q5TC12	Homo sapiens ATP synthase mitochondrial F1 complex assembly factor 1	13.93	14,036	9.24	-2.219	0.00147
109	P16403	HIST1H1C Histone H1.2	30.52	21,364	10.94	-2.411	0.00121
110	Q9UMY4	SNX12 Isoform 1 of Sorting nexin-12	19.77	19,730	7.71	-2.066	0.00116
111	Q9UJ83	HACL1	6.57	14,544	5.22	-2.300	0.00115
112	P47914	RPL29;RPL29P4 Ribosomal protein L29	14.29	17,951	11.67	-2.195	0.00090
113	P35900	KRT20 Keratin, type I cytoskeletal 20	5.42	48,486	5.52	-2.012	0.00063
114	Q15796	SMAD2 Isoform Short of Mothers against decapentaplegic homolog 2	4.35	48,955	6.67	-2.293	0.00052
115	Q9H6S0	YTHDC2	3.11	82,988	7.02	-2.026	0.00050
116	Q13017	ARHGAP5 Isoform 1 of Rho GTPase-activating protein 5	1.6	172,458	6.18	-2.310	0.00039
117	O75426	FBXO24 Isoform 2 of F-box only protein 24	3.77	36,353	9.52	-1.662	0.00031
118	Q8IUE6	HIST2H2AB Histone H2A type 2-B	22.31	13,995	10.88	-2.412	0.00026
119	Q6P1M3	LLGL2 Isoform C of Lethal giant larvae protein homolog 2	1.27	113,447	7.22	-2.551	0.00020
120	Q07020	RPL18 60S ribosomal protein L18	25	21,634	11.73	-1.701	0.00014
121	Q96BN8	FAM105B Protein FAM105B	7.39	40,262	5.35	-1.940	0.00010

Table S1. Differentially expressed proteins between miR-449a-mimic/miR-449a-inhibitor (continued).

No.	Protein ID	Protein_name	Cover percent (100%)	MW (Da)	PI	miR-449a-mimic/inhibitor ratio	p-value
122	Q658P3	STEAP3 Isoform 1 of Metalloreductase	3.48	54,600	8.86	-2.650	2.0169E
123	O00479	HMGN4 High mobility group nucleosome-binding domain-containing protein 4	10	9,539	10.48	-2.797	1.48094E
124	P60983	GMFB Glia maturation factor, beta	14.94	18,110	5.21	-2.437	1.02915E
125	O60613	SEP15 15 kDa selenoprotein isoform 1 precursor	9.7	18,045	4.96	-1.653	5.70642E
126	P05204	HMGN2 Non-histone chromosomal protein HMG-17	13.33	9,393	10	-2.312	4.65183E
127	O14684	PTGES Prostaglandin E synthase	13.82	17,102	9.59	-2.649	4.22495E
128	Q01995	TAGLN Transgelin	9.95	22,611	8.87	-2.705	1.71068E
129	Q5SY16	NOL9 Polynucleotide 5'-hydroxyl-kinase	3.56	79,322	9.3	-2.252	2.75771E
130	Q9H6E5	TUT1 speckle targeted PIP5K1A-regulated poly(A) polymerase	2.52	98,434	6.02	-1.607	1.0265E
131	B1AJZ9	FHAD1 Isoform 3 of Forkhead-associated domain-containing protein 1	0.55	166,298	6.82	-1.677	3.37462E
132	P00326	ADH1C Alcohol dehydrogenase 1C	2.13	39,867	8.63	-1.709	7.62072E
133	Q9NP79	VTA1 Vacuolar protein sorting-associated protein	5.86	33,879	5.87	-1.713	6.27171E
134	P63313	TMSB10 Thymosin beta-10	31.82	5,026	5.31	-1.780	2.60148E
135	Q13547	HDAC1 Histone deacetylase 1	7.05	55,102	5.31	-1.932	0.08207
136	Q00534	CDK6 Cyclin-dependent kinase 6	11.66	36,938	6.02	-2.368	0.00860
137	P08581	MET Isoform 2 of Hepatocyte growth factor receptor	1.63	157,710	7.02	-1.749	0.00858