Supplementary Information

MALDI-TOF MS-based total serum protein fingerprinting for liver cancer diagnosis

Han-Gyu Park[1,+], Kyoung-Soon Jang[2,+], Hae-Min Park[3], Won-Suk Song[4], Yoon-Yi Jeong[1], Da-Hee Ahn[1], Seong-Min Kim[1], Yung-Hun Yang[5] and Yun-Gon Kim*[,1]

[1]Department of Chemical Engineering, Soongsil University, Seoul 06978, Korea

[2]Biomedical Omics Center, Korea Basic Science Institute, Cheongju 28119, Korea

[3]Departments of Chemistry and Molecular Biosciences, Northwestern University, Evanston, Illinois 60208, United States

[4]School of Chemical and Biological Engineering, Seoul National University, Seoul 08826, Korea

[5]Department of Biological Engineering, Konkuk University, Seoul 05029, Korea

*Corresponding author contact information:

Yun-Gon Kim, Prof.

Department of Chemical Engineering, Soongsil University

369 Sangdo-Ro, Seoul, Korea

E-mail: ygkim@ssu.ac.kr

Phone: +82-2-828-7099

+These authors contributed equally to this work

Table S1. Information of serum from patients with liver cancer used our MALDI-TOF MS-based serum protein fingerprinting platform for liver cancer diagnosis

	MSP / RTC	UICC stage	TNM Classification	Hepatitis type	Gender	Age	Pathology report
1	Liver cancer MSP	Stage Ⅰ	T1N0M0	C	M	55	HCC, trabecular type backgroud of chronic hepatitis, HCV related grade1 and stage1
2		Stage Ⅰ	T1N0M0	C	M	66	HCC, NOS
3		Stage Ⅰ	T1N0M0	C	M	68	HCC, trabecular type backgroud of chronic hepatitis, HBV related grade 1 and stage 0
4		Stage Ⅰ	T1N0M0	C	M	72	Liver cell carcinoma
5		Stage Ⅰ	T1N0M0	C	M	81	HCC, trabecular type backgroud of fatty liver
6		Stage Ⅰ	T1N0M0	C	M	77	HCC, trabecular type. background of macro and microvesicular steatosis
7		Stage Ⅰ	T1N0M0	C	M	47	Not available
8		Stage Ⅰ	T1N0M0	B	M	65	Not available
9		Stage Ⅰ	T1N0M0	B	M	66	Not available
10		Stage Ⅰ	T1N0M0	B	M	60	Not available
11		Stage Ⅰ	T1N0M0	B	M	48	Not available
12		Stage Ⅱ	T2N0M0	C	F	80	HCC, trabecular type. background of cirrhosis
13		Stage Ⅱ	T2N0M0	B	M	32	Not available
14		Stage Ⅱ	T2N0M0	B	M	57	Not available
15		Stage Ⅱ	T2N0M0	B	M	77	Not available
16		Stage Ⅲ	T3N0M0	C	M	64	HCC, trabecular type, background of macro and micronodular cirrhosis, unknown etiology
17		Stage Ⅲ	T3N0M0	C	M	70	HCC, trabecular type with clear resection margin
18		Stage Ⅲ	T3N0M0	C	M	46	HCC, trabecular type, angiolymphatic invasion identified. (portal vein invasion) background of macro and micro steatosis
19		Stage Ⅳ	T2NXM1	C	M	60	adenocarcinoma, NOS

20		Stage Ⅳ	T2NXM1	C	F	68	HCC, lung metastasis
21		Stage Ⅳ	T4NXM1	B	M	59	Not available
22		Unknown	T2NXM0	C	M	29	HCC, trabecular type backgroud of cirrhosis
23		Unknown	T3NXM0	C	M	55	Not available
24		Unknown	T2NXM0	C	F	58	HCC, Edmondson's grade 2
25		Unknown	T2NXM0	C	M	59	Not available
26		Unknown	T3NXM0	C	M	63	HCC, trabecular type
27		Unknown	T1NXM0	C	M	67	Not available
28		Unknown	T2NXM0	C	M	55	Not available
29		Unknown	T1NXM0	C	M	59	Not available
30		Unknown	T3NXM0	B	M	68	Not available
31		Unknown	T3NXM0	B	F	43	Not available
32		Unknown	T3NXM0	B	M	70	Not available
33		Unknown	T3NXM0	B	M	62	Not available
34		Unknown	T2NXM0	B	M	59	Not available
35		Unknown		C	F	65	HCC, bile duct invasion
36				C	M	57	Cholangiocarcinoma, forming(MF) type
37				C	F	58	HCC, trabecular type. background of liver cirrhosis
38				C	M	35	High grade dysplastic nodule, background of HBV-associated liver cirrhosis
39				C	M	61	HCC, trabecular and psedoglandular type, background of macro and microvesicular steatosis
40				C	M	80	HCC, trabecular and psedoglandular type, background of chronic hepatitis, with periportal fibrosis
41	Blind test	Stage Ⅰ	T1N0M0	C	M	74	Liver cell carcinoma, liver cirrhosis
42		Stage Ⅰ	T1N0M0	C	F	71	Not available

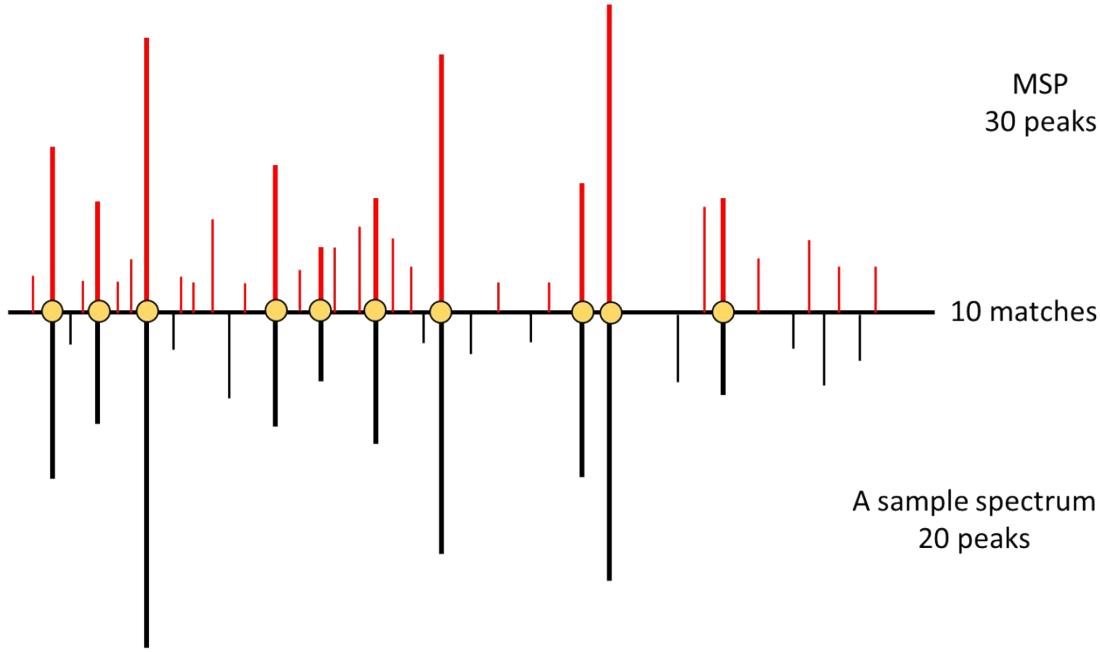
43		Stage Ⅰ	T1N0M0	B	M	65	Not available
44		Stage Ⅱ	T2N0M0	C	M	81	Not available
45		Stage Ⅲ	T3N0M0	B	F	71	Not available
46		Stage Ⅳ	T1NXM1	C	M	74	Adenocarcinoma, poorly differentiated, possibly metastatic
47		Stage Ⅳ	T4NXM1	B	F	71	Not available
48		Unknown	T1NXM0	C	M	75	Not available
49		Unknown	T3NXM0	B	F	69	Not available
50		Unknown	T2NXM0	B	M	76	Not available
51		Unknown	T3NXM0	B	M	51	Not available
52		Unknown		C	M	65	HCC, pseudoglandular type backgroud of cirrhosis alcoholic related
53				C	F	55	HNF1a-mutated hepatocellular adenoma

Table S2. The score obtained by comparing integrated MSPs with healthy controls serum (n=18) of blind test set for validation

Name	Healthy control MSP	Liver cancer MSP
Healthy control 1	2.595±0.099	1.736±0.130
Healthy control 2	2.399±0.078	1.702±0.105
Healthy control 3	2.390±0.019	1.797±0.048
Healthy control 4	2.456±0.056	1.834±0.170
Healthy control 5	2.427±0.050	1.934±0.011
Healthy control 6	2.608±0.026	1.885±0.039
Healthy control 7	2.573±0.026	1.703±0.095
Healthy control 8	2.507±0.044	0.913±0.108
Healthy control 9	2.640±0.073	1.810±0.052
Healthy control 10	2.608±0.045	1.682±0.085
Healthy control 11	2.639±0.063	1.598±0.059
Healthy control 12	2.639±0.047	1.514±0.021
Healthy control 13	2.249±0.054	0.919±0.135
Healthy control 14	2.675±0.029	1.608±0.045
Healthy control 15	2.611±0.023	1.671±0.121
Healthy control 16	2.701±0.082	1.646±0.185
Healthy control 17	2.772±0.015	1.796±0.033
Healthy control 18	2.626±0.074	1.393±0.012

Suppl Table S3. The score obtained by comparing integrated MSPs with liver cancer serum (n=13) of blind test set for validation

Name	Healthy control MSP	Liver cancer MSP	Hepatitis type	UICC stage
Liver cancer 1	1.401±0.035	2.363±0.057	C	Ⅰ
Liver cancer 2	1.557±0.162	2.409±0.078	C	Ⅳ
Liver cancer 3	1.115±0.011	2.268±0.043	C	Ⅱ
Liver cancer 4	1.733±0.037	2.347±0.016	B	Ⅲ
Liver cancer 5	1.528±0.111	2.433±0.057	B	Ⅳ
Liver cancer 6	1.470±0.079	2.181±0.068	B	Ⅰ
Liver cancer 7	1.202±0.023	2.045±0.040	C	Ⅰ
Liver cancer 8	1.288±0.029	2.120±0.020	C	Unknown
Liver cancer 9	1.633±0.046	2.446±0.038	C	Unknown
Liver cancer 10	1.126±0.069	2.171±0.013	B	Unknown
Liver cancer 11	1.410±0.078	2.198±0.026	C	Unknown
Liver cancer 12	1.489±0.089	2.342±0.057	B	Unknown
Liver cancer 13	0.999±0.053	2.205±0.041	B	Unknown



Three factors for score value

- The number of matched peaks/the number of a sample spectrum peaks (10/20)
- The number of matched peaks/the number of MSP peaks (10/30)
- Comparison of intensity of matched peaks

Figure S1. Example of three factors considered in computation of score value

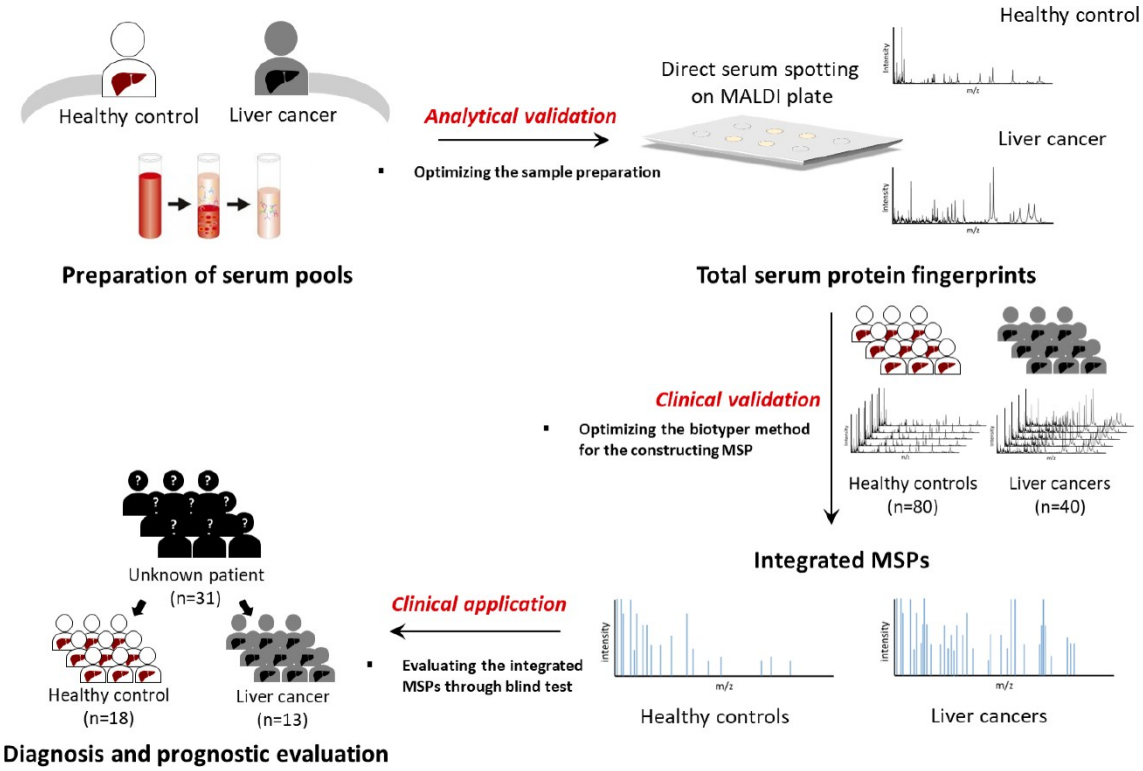


Figure S2. Overall workflow of the liver cancer diagnostic method *via* MALDI-based total serum fingerprinting

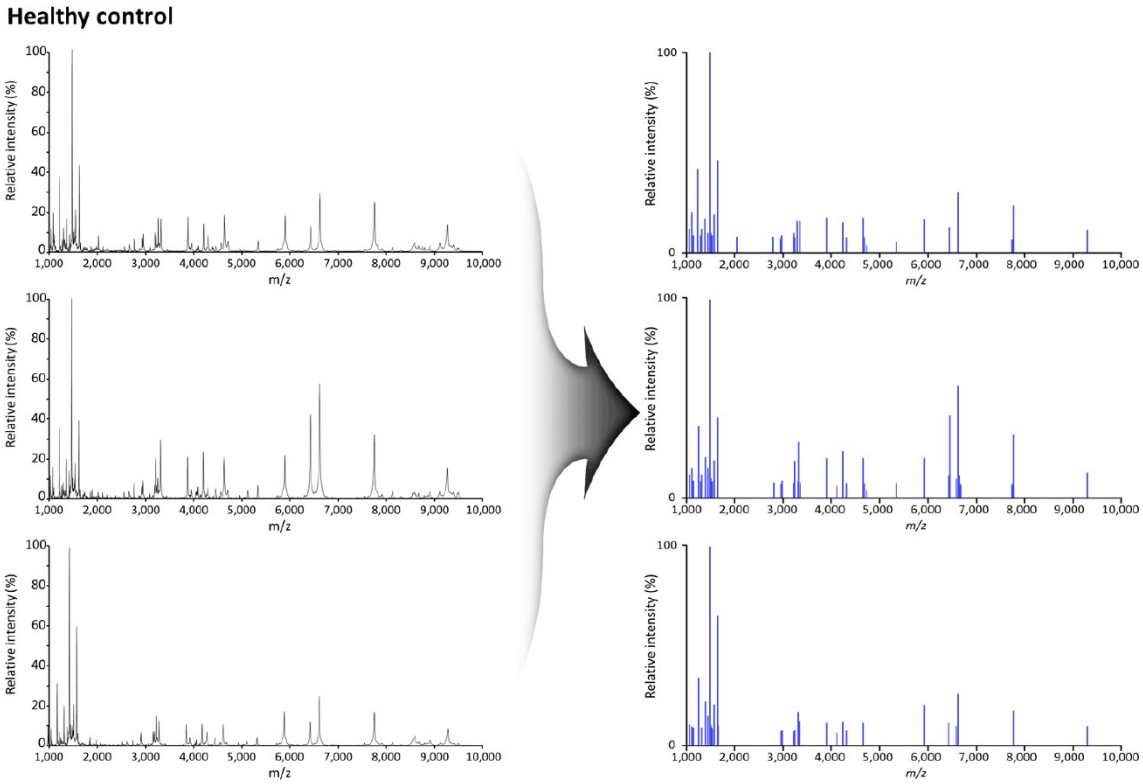


Figure S3. One of five healthy control serum samples that were repeated analyzed three times. The results show no significant differences among repeatedly analyzed MS spectra

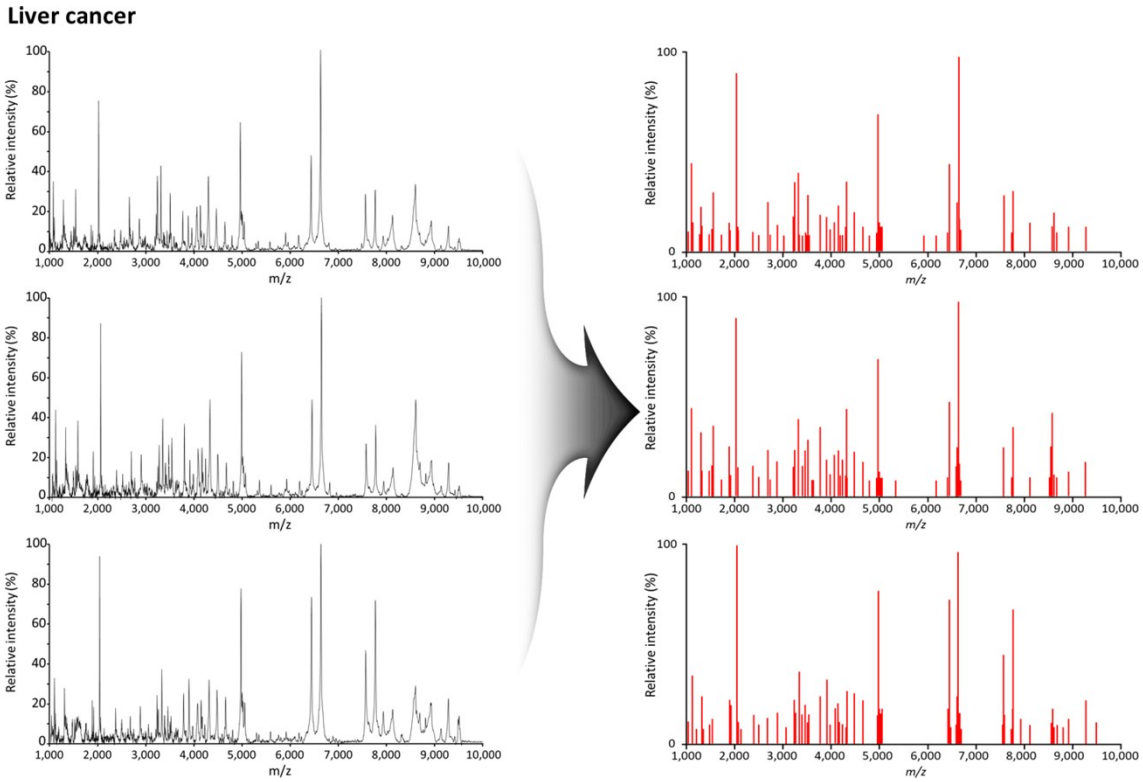


Figure S4. One of five liver cancer serum samples that were repeated analyzed three times. The results show no significant differences among repeatedly analyzed MS spectra

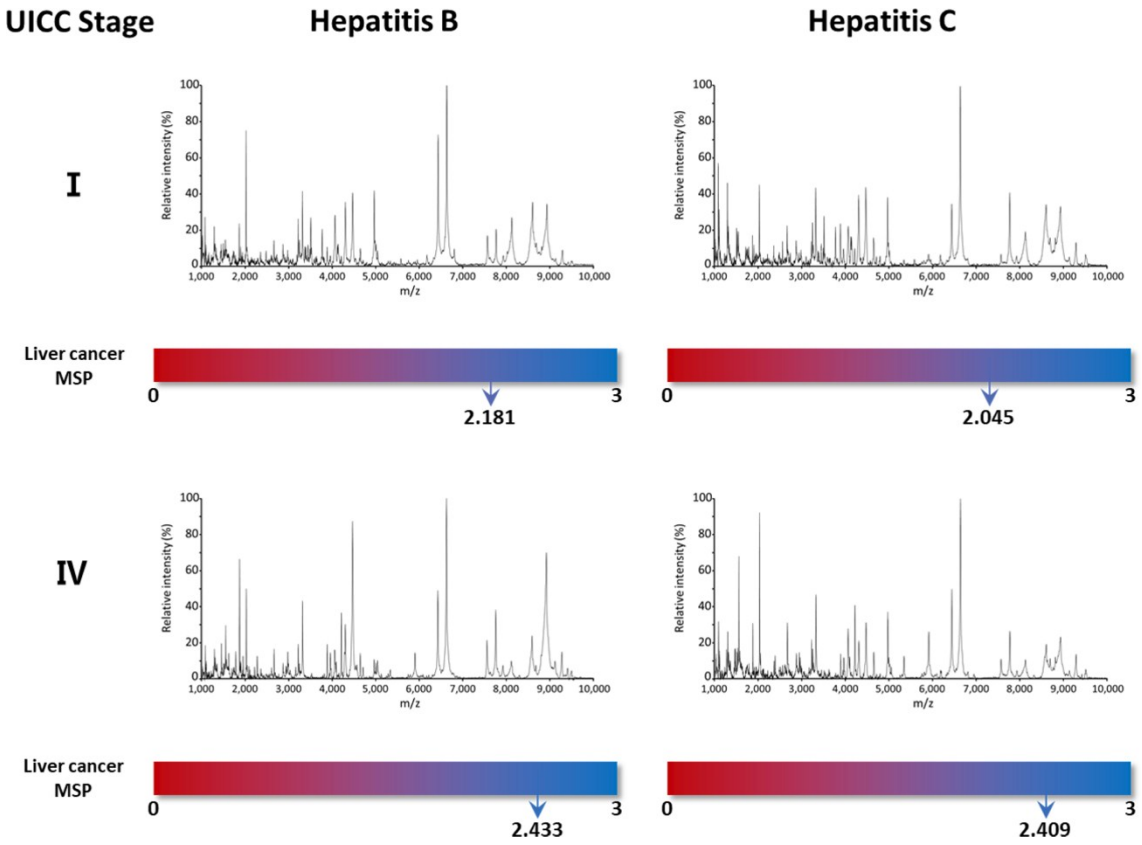


Figure S5. Comparison of MALDI-MS spectrum and score according to cancer stage.