PLASMA PROTEOMIC PROFILING IDENTIFIES NOVEL BIOMARKERS FOR CHOLANGIOCARCINOMA

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Introduction

Cholangiocarcinoma (CCA) is one of the highly aggressive malignant tumors that arise from the cholangiocytes lining biliary trees. This type of cancer represents 10–25% of primary liver cancers worldwide and is highly prevalent in Asian countries including Thailand. CCA is highly lethal because most are locally advanced at patient presentation where therapies have limited benefit. Currently available tumor markers, e.g., CA19-9 and CEA, are not specific to CCA, thereby novel CCA biomarker is an unmet need. This study explored the feasibility of a translational proteomic approach on CCA biomarkers discovery. The list of possible candidates were obtained from our previous studies, together with the label-free quantitation. Proteins of interest were selected and translated into clinically compatible ELISA immunoassay for CCA biomarker investigation.

Methods

Plasma samples (CCAs, Normal and disease control)

- High-abundant proteins depletion using MARS14 column (Agilent, Santa Clara, CA, USA.)
  - Collected flow-through fraction
    - Digested peptides by in-solution tryptic digestion
      - Peptides were identified using nano-LC/MS/MS and Progenesis software
- 1D SDS-PAGE
  - Western Blot Analysis
- ELISA (n=63; 26 CCA vs. 37 non-CCA)
  - Data Analysis

Results

Table 1 Differentially expressed plasma proteins in cholangiocarcinoma patients identified by nano-LC-MS/MS compared with normal control subjects.

Accession	Protein Name	MW	Score	Fold change	Function
Up-regulated					
S10A8_HUMAN	Protein S100-A8	10.89	261.89	2.16	Inflammatory response
Protein A	Protein A	13.29	326.65	2.14	Inflammatory response
APOE_HUMAN	Apolipoprotein E	36.25	821.02	2.11	Metabolism
CO3_HUMAN	Complement C3	188.57	156.54	1.75	Cellular process/ complement activation/ proteolysis
IC1_HUMAN	Plasma protease C1 inhibitor	55.35	585.7	1.67	Blood coagulation
B2MG_HUMAN	Beta-2-microglobulin	13.82	158.71	1.67	FNA, function not assigned
Protein D	Protein D	39.89	814.23	1.53	Metabolism
ACTB_HUMAN	Actin, cytoplasmic 1	42.05	223.21	1.38	Cell motility and contraction
ITIH3_HUMAN	Inter-alpha-trypsin inhibitor heavy chain H3	100.07	504.52	1.37	FNA, function not assigned
ALDOA_HUMAN	Fructose-bisphosphate aldolase A	39.85	44.38	1.33	Glycolysis and gluconeogenesis
ITIH4_HUMAN	Inter-alpha-trypsin inhibitor heavy chain H4	103.52	2297.37	1.32	Metabolism
TRIPB_HUMAN	Thyroid receptor-interacting protein 11	228.13	42.94	1.31	Protein glycosylation
A1BG_HUMAN	Alpha-1B-glycoprotein	54.79	1680.61	1.27	FNA, function not assigned
CD14_HUMAN	Monocyte differentiation antigen CD14	40.68	169.51	1.26	Protein binding
CFAB_HUMAN	Complement factor B	86.85	1965.83	1.25	Complement activation
PROF1_HUMAN	Profilin-1	15.22	50.91	1.25	FNA, function not assigned
Down-regulated					
GELS_HUMAN	Gelsolin	86.04	1212.38	0.78	Ciliogenesis
ITIH1_HUMAN	Inter-alpha-trypsin inhibitor heavy chain H1	101.78	1312.09	0.77	hyaluronan metabolic process
PP1A_HUMAN	Serine/threonine-protein phosphatase PP1-alpha catalytic subunit	38.23	46.89	0.76	FNA, function not assigned
PLMN_HUMAN	Plasminogen	93.25	2104.49	0.70	Proteolysis
APOC2_HUMAN	Apolipoprotein C-II	11.28	71.84	0.64	Lipid metabolic process
CHLE_HUMAN	Cholinesterase	68.94	88.39	0.62	FNA, function not assigned
APOC3_HUMAN	Apolipoprotein C-III	10.85	202.08	0.38	Enzyme activator activity/ homeostatic process

Conclusions

- Translational proteomic approach is feasible for CCA biomarker investigation. It also provides multiple potential biomarkers for clinical diagnosis.
- ROC showed that protein A had higher performance with the area under the curve (AUC) of 0.835 (80.8% sensitivity, 83.8% specificity) as compared to other proteins.
- The combination of A, B and D proteins hold good promise as a potential multiplexing biomarker of CCA.
- With this investigation, further studies on these proteins should be validated in an independent cohort or multicenter study.

References

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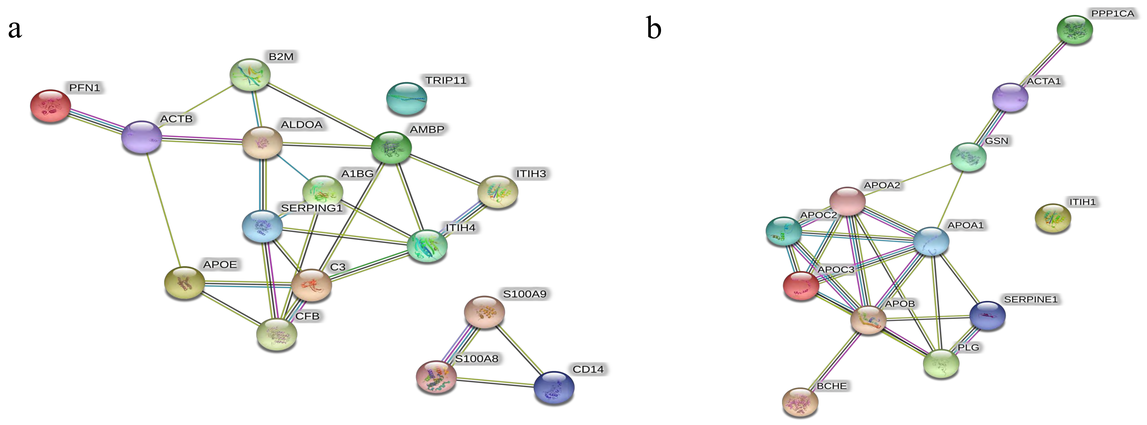

Figure 1. Protein-protein interactions of proteins with 1.25 fold expression (a: more than 1.25 fold, b: less than 1.25 fold) were predicted by STRING. Colors: Green gene neighborhood; red gene fusion; blue co-occurrence; black co-expression; purple experiments; cyan databases; yellow text mining; and grey homology.

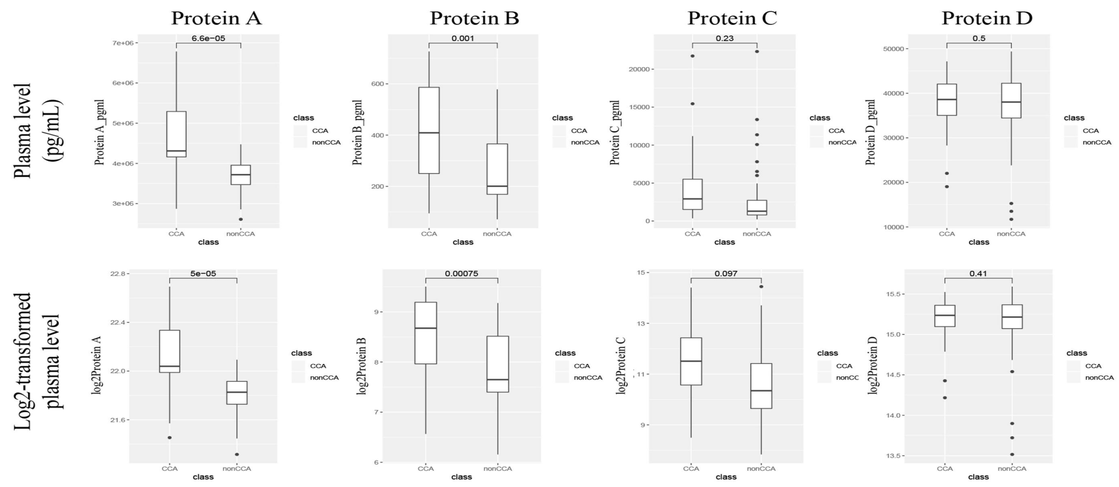

Figure 2. Candidate biomarkers in CCA (n = 26) vs. non-CCA (n = 37; 17 disease controls, 20 healthy individuals) as measured by ELISA. a, plasma levels (pg/mL) b, log2 transformation was performed to adjust normal data distribution.

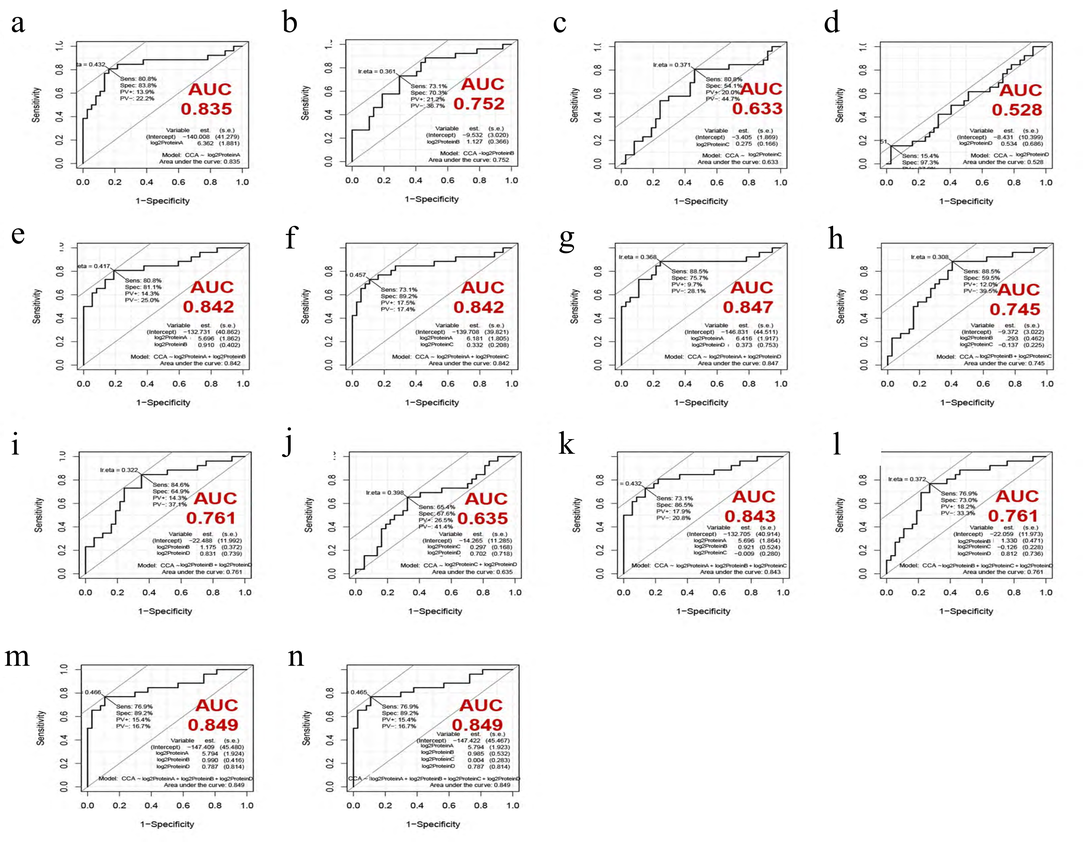

Figure 3. Diagnostic performance of candidate biomarkers in a study cohort. a, log2(Protein A); b, log2(Protein B); c, log2(Protein C); d, log2(Protein D); e, log2(Protein A)+log2(Protein B); f, log2(Protein A)+log2(Protein C); g, log2(Protein A)+log2(Protein D); h, log2(Protein B)+log2(Protein C); i, log2(Protein B)+log2(Protein D); j, log2(Protein C)+log2(Protein D); k, log2(Protein A)+log2(Protein B)+log2(Protein C); l, log2(Protein B)+log2(Protein C)+log2(Protein D), m, log2(Protein A)+log2(Protein B)+log2(Protein D); n, log2(Protein A)+log2(Protein B)+ log2(Protein C)+log2(Protein D). Abbreviation: AUC, area under the curve.

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