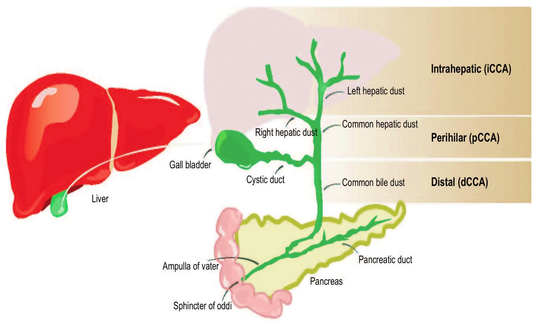
Identification of protein A, B, C, D and E as biomarkers to distinguish between hepatocellular carcinoma and cholangiocarcinoma in 3D culture by using Label-free quantitative proteomics

Penchatr Diskul-Na-Ayudthaya[1], Meghna Phanichkrivalkosil[1], Daranee Chokchaichamnankit[1], Churat Weeraphan[1],Jisnuson Svasti[1,2] and Chantragan Srisomsap[1]

1.Laboratory of Biochemistry, Chulabhorn Research Institute, Bangkok 10210, Thailand
2.Applied Biological Sciences Program, Chulabhorn Graduate Institute, Bangkok 10210, Thailand

Introduction:

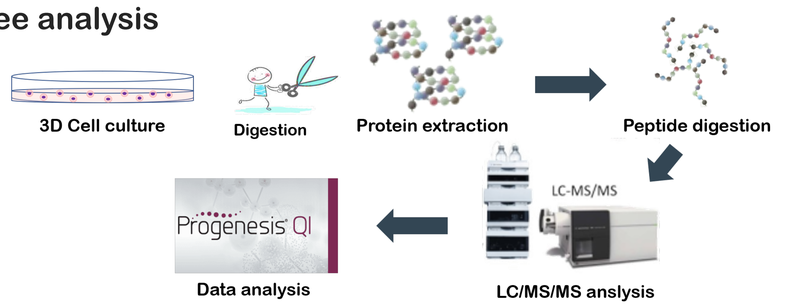


Cholangiocacinoma (CCA) is a type of cancer that form in the slender tubes or bile ducts as shown in the picture. Bile ducts are branched tubes, which connect to liver. CCA is classified by its location in relation to the liver. The first one called “Intrahepatic cholangiocarcinoma (iCCA)”, which begins in the small bile ducts within liver. And the second one called “Extrahepatic cholangiocarcinoma”, which occur outside the liver. This type type divides into 2 types, Perihilar cholangiocacinoma (pCCA) which begins in an area called the hilum, where the major bile ducts join and leave the liver. And Distal cholangiochacinoma (dCCA), which begins in bile ducts outside liver because CCA is located nearby liver[1]. The difficullty to diagnose the cancer between them is still the problem.

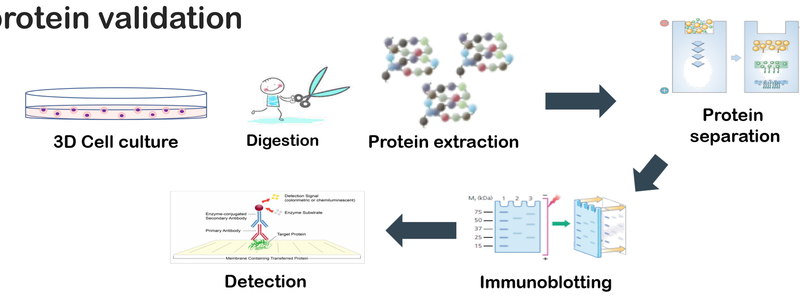
Liver cancer is the 3rd leading cause of death worldwide in 2020. But the highest rates are observed in Eastern Asia, South-East Asia (including Thailand) [2-3]. Most people who develop hepatocarcinoma (HCC) and CCA are older than 65. Because these cancers are often not discovered until they have already spread [4]. Most of the patiens have no symtoms in the early stages. It becomes a challenging to treat effetively. So, biomarker(s) for early detection becomes an important tool to diagnose and differentiate between HCC and CCA.

Methods:

- Label-free analysis



- Target protein validation



Results:

Table 1. Proteins increased in CCA compared to HCC cells

Description	Max fold change	Highest	Lowest
Protein A	7.44	M213	S102
Cornifin-A	4.97	M213	S102
Desmoplakin	2.95	M213	S102
Tumor protein D54	2.67	M213	S102
Glutathione S-transferase P	2.50	M213	S102
Cystatin-B	2.47	M213	S102
Ezrin	1.92	M213	S102
Protein B	1.86	HuCCA1	S102
Protein C	1.84	M213	S102
Peroxiredoxin-5, mitochondrial	1.83	HuCCA1	S102
Fascin	1.81	M213	S102
Cofilin-1	1.72	M213	S102
Plectin	1.70	M213	S102
Protein D	1.59	HuCCA1	S102
Cathepsin D	1.59	M213	S102
Vitronectin	1.58	M213	S102
Tropomodulin-3	1.55	M213	S102

Table 2. Proteins increased in HCC compared to CCA cells

Description	Max fold change	Highest	Lowest
Protein E	5.03	S102	HuCCA1
Delta(14)-sterol reductase	4.26	S102	M213
Aldo-keto reductase family 1 member C1	3.71	S102	HuCCA1
Aldo-keto reductase family 1 member C3	3.71	S102	HuCCA1
UDP-glucuronosyltransferase 1-9	3.69	S102	HuCCA1
Aldo-keto reductase family 1 member C2	3.26	S102	HuCCA1
Aldo-keto reductase family 1 member B10	3.14	S102	M213
60S ribosomal protein L37a	2.74	S102	M213
UDP-glucose 6-dehydrogenase	2.71	S102	HuCCA1
Glutathione S-transferase A2	2.61	S102	HuCCA1
60S ribosomal protein L15	2.59	S102	M213
Thioredoxin domain-containing protein 5	2.56	S102	M213
Tubulin beta chain	2.45	S102	M213
Growth factor receptor-bound protein 7	2.45	S102	HuCCA1
Heat shock protein beta-1	2.08	S102	M213

Results:

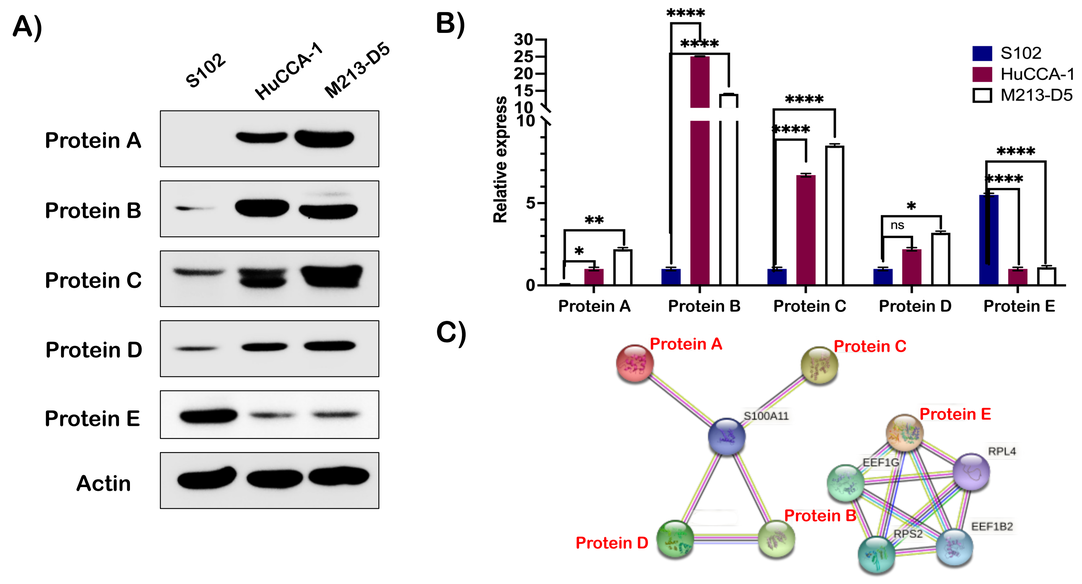


Figure 1. Validation of candidates proteins. (A-B) Immunoblotting and band intensities of 5 candidate proteins, (C)STRING network analysis of 5 significantly protein candidates.

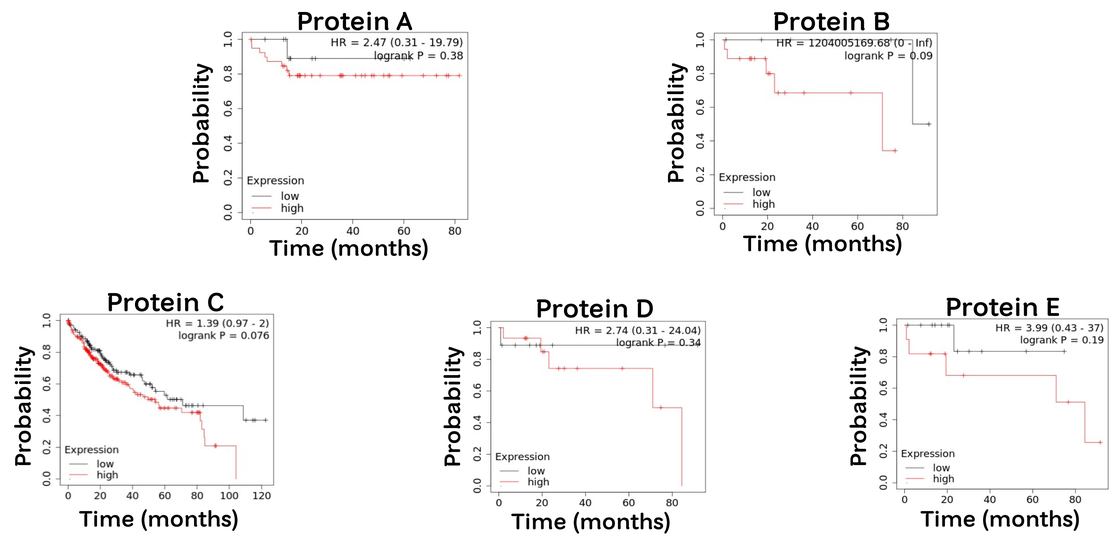


Figure 2. Survival rate of Liver cancer patients by Kaplan-Meier. (A-E) Survival curves of the mRNA expression levels of Protein A, B, C, D and E for all liver cancer patients (n= 2,779). Red line showed patients who have high level of target gene and black showed the patients who have low level of target gene.

Conclusion:

According to this experiment, we found 5 potential biomarkers to distinguish between hepatocellular carcinoma (HCC) and cholangiocarcinoma (CCA). We obtained more than 500 proteins which involved in protein binding, catalytic activity, structural molecule activity, transport regulator activity and transporter activity. Among these proteins, 4 interesting proteins named protein A, B, C and D were highly expressed in CCA compared to HCC cells. While protein E was highly expressed only in HCC compared to CCA cells. However, tissue samples from patients will be needed for further study. Taken together, our results might provide 5 candidate biomarkers to differentiate the difference between hepatocellular carcinoma and cholangiocarcinoma, which may help for cancer diagnosis and treatment in the future.

Acknowledgement:

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