PROTEOMICS :
A Practical Approach
for Cholangiocarcinoma Markers

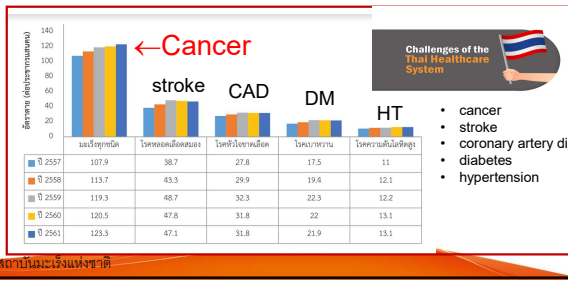
Siriporn Proungvitaya, PhD.

New chapter :
Biomarkers for prognosis of meningioma patients:
A systematic review and meta-analysis

1

"NCD Countdown 2030: worldwide trends in non-communicable disease mortality and progress towards Sustainable Development Goal target 3.4"

Bennett, James E et al.
The Lancet, Volume 392, Issue 10152, 1072 - 1088

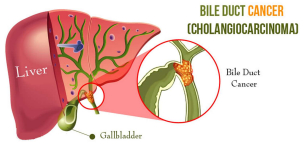


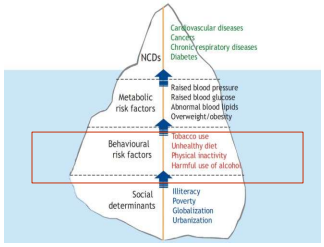
	มะเร็งทุกชนิด	โรคหลอดเลือดสมอง	โรคหัวใจขาดเลือด	โรคเบาหวาน	โรคความดันโลหิตสูง
ปี 2557	107.9	38.7	27.8	17.5	11
ปี 2558	113.7	43.3	29.9	19.4	12.1
ปี 2559	119.3	48.7	32.3	22.3	12.2
ปี 2560	120.5	47.8	31.8	22	13.1
ปี 2561	123.3	47.1	31.8	21.9	13.1

สถาบันมะเร็งแห่งชาติ

2

Cholangiocarcinama (CCA)





"Interaction between chemical carcinogens, especially nitrosamines, and OV infestation may play a role in the development of cholangiocarcinoma in Thailand."

Int J Cancer
. 1991 Jul 30;48(6):821-5. doi: 10.1002/ijc.2910480606.

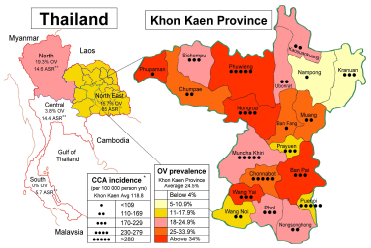
https://tinyurl.com/4wp43f9n

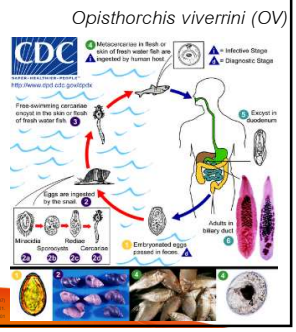
https://bsahely.com/2015/10/16/article-version-of-presentation-the-secret-to-a-healthy-nation/

3

Cholangiocarcinama and *OV prevalence*

Opisthorchis viverrini (OV)





https://tinyurl.com/yck9ny7y

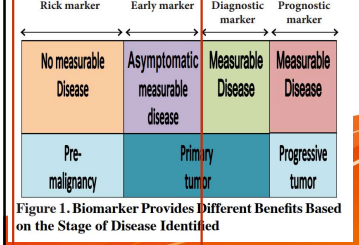
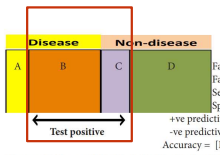


Figure 1. Biomarker Provides Different Benefits Based on the Stage of Disease Identified



False +ve = C
False -ve = A
Sensitivity = B/A+B
Specificity = D/C+D
+ve predictive value = B/B+C
-ve predictive value = D/A+D
Accuracy = [B+D]/[A+B+C+D]

Figure 2. Several Indices Indicate the Diagnostic Value of Each Test

Wongkham S, Silsirivanit A. State of serum markers for detection of cholangiocarcinoma. Asian Pac J Cancer Prev. 2012;13 Suppl:17-27. PMID: 23480761.

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Sensitivity, specificity,
positive predictive value (PPV) and negative predictive value (NPV)

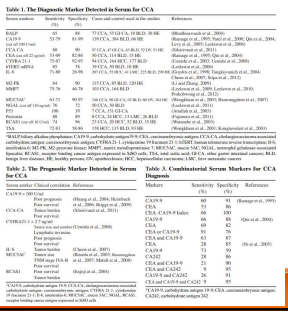


Table 1. The Diagnostic Marker Detected in Serum for CCA

Table 2. The Prognostic Marker Detected in Serum for CCA

Table 3. Combinatorial Serum Markers for CCA Diagnosis

Cutoff

	Disease present	Disease absent
Positive test	True positive	False positive
Negative test	False negative	True negative

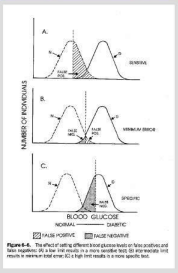
GEEKYMEDICS.COM

https://geekymedics.com/sensitivity-specificity-ppv-and-npv/?_escaped_fragment_=

Wongkham S, Silsirivanit A. State of serum markers for detection of cholangiocarcinoma. Asian Pac J Cancer Prev. 2012;13 Suppl:17-27. PMID: 23480761.

6

Validity: Sensitivity & Specificity



Low cutoff → High sensitivity
→ Low specificity
→ False positives

Moderate cutoff → balance

High cutoff → Low sensitivity
→ High specificity
→ False negatives

Courtesy, S. Fleming, 2011

https://www.dreamstime.com/vector-cartoon-stick-figure-drawing-conceptual-illustration-man-businessman-shooting-arrow-target-bow-missing-image162946700

https://www.slideshare.net/slideshow/diagnotic-and-screening-tests/13223632

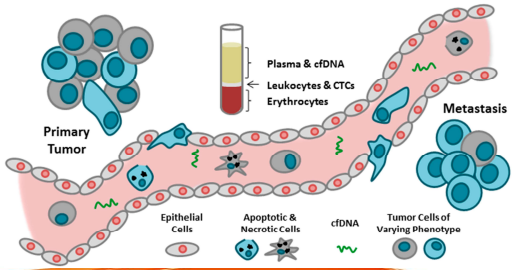
7

Discovered by Proteomics

- Bioinformatics (bioinfo.)
  - Human Plasma Proteome Project
  - The Human Protein Atlas project
  - GEPIA (Gene Expression Profiling Interactive Analysis)
- Evaluation test
  - Elisa
  - Western blot
  - Dot blot
  - HPLC
  - Common reagent
- Sample (praparation) types
- Mass spectrometry
  - Review
  - Bioinfo.
  - AI
  - etc.

8

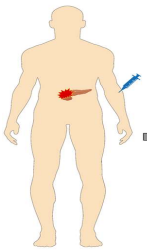
"Serum DNA methylation as a biomarker for differentiating cholangiocarcinoma from other biliary diseases"

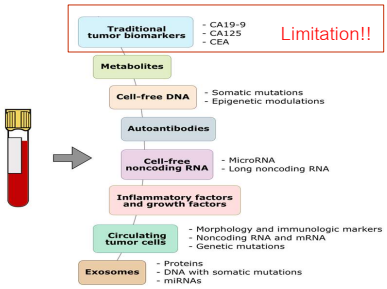


https://tinyurl.com/3n3kt6v9

9

Medical Technology





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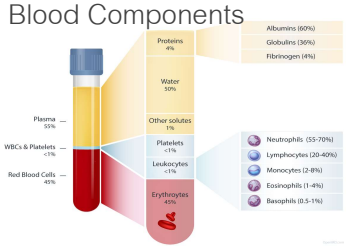
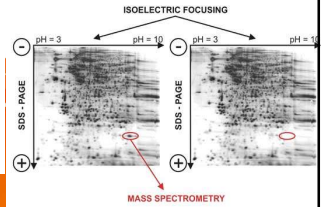
10

Blood Components



https://openmd.com/guide/blood-components

HUPO Human Proteome Organisation



2D protein electrophoresis

https://myendoconsult.com/learn/plasma-proteins-a-comprehensive-review/

11

Mass spectrometry

Dr. Sittiruk Roytrakul

12

Proteomics for cholangiocacinoma markers

- Chapter 1 Secretome
- Chapter 2 Bioinformatics for marker selections
- Chapter 3 Protein-protein interactions
- Chapter 4 Mitochondrial markers
- Chapter 5 Metabolomics of bile acids
- Summary for Future

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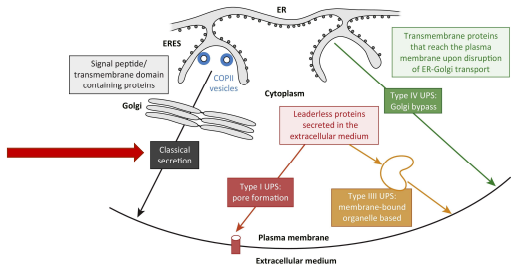
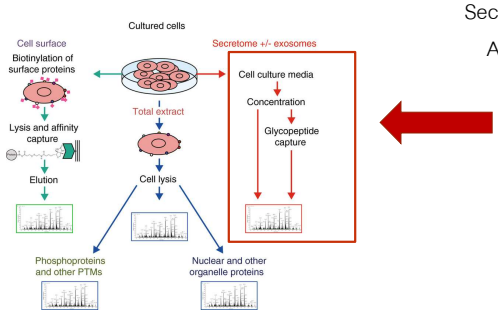
#1 Secretome



Trends Cell Biol
2017 Mar;27(3):230-240. doi: 10.1016/j.tcb.2016.11.007

14

Secretome Analysis



https://www.researchgate.net/figure/Analysis-of-the-proteome-of-cell-populations-by-sub-compartment-Proteomics-is_fig2_261514808

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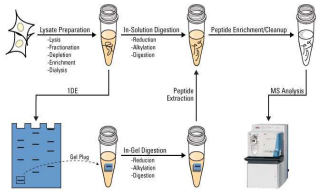
Work Flow



https://tinyurl.com/bdzhzw7h

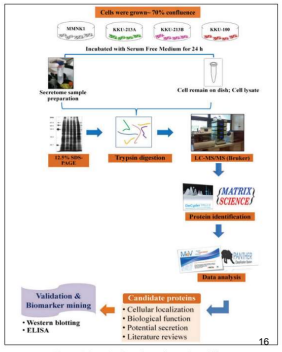


Figure 1-3 Outline of experimental workflow

Asian Pac J Cancer Prev
2012;13 Suppl:107-14.

16

Expression protein profile pattern

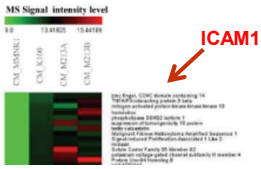
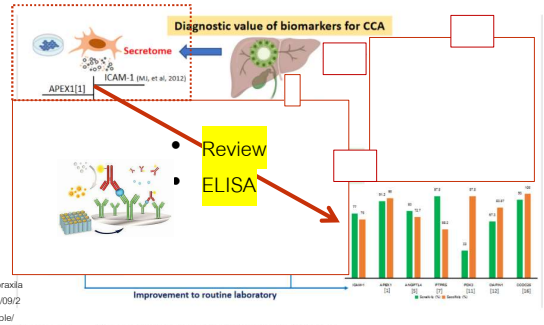


Figure 1-4 Expression profile pattern of total 90 significantly aberrant proteins. Heat map of the quantitative differential expressed of three CCA secretomes and control, MMNK1. Each row represents an individual protein, and each column represents MMNK1, KKU-100, KKU-M213A and KKU-M213B secretome, respectively (bar chart represents the signal intensity level as shown in corresponding color).

Asian Pac J Cancer Prev
2012;13 Suppl:107-14.

17



https://blog.praxilabs.com/2021/09/20/elisa-principle/

Figure S1 Diagnostic value of biomarkers for CCA
https://pubmed.ncbi.nlm.nih.gov/?term=proungvitaya%20s&sort=date

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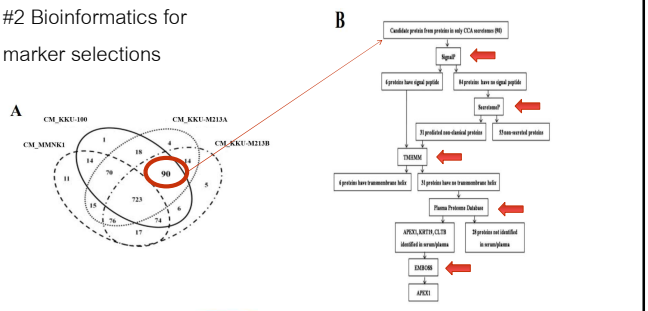
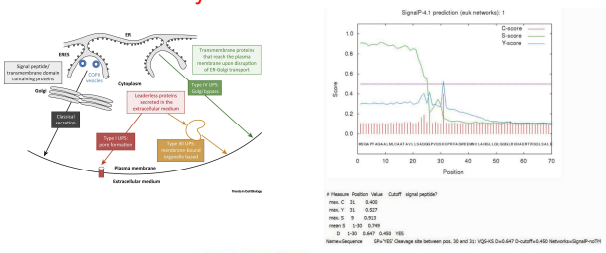
#2 Bioinformatics for marker selections



Biomolecules 2019 Aug 26;9(9):413. doi: 10.3390/biom9090413.

Figure 2-6 Data analysis from mass spectrometry and selected candidate protein

Prediction of signal peptides using the SignalP 4.1 server: classically secreted proteins



Trends Cell Biol 2017 Mar;27(3):230-240. doi: 10.1016/j.tcb.2016.11.007

Figure 2-1 The output result of the SignalP 4.1

20

Search for non-classically secreted proteins using SecretomeP 2.0 server

Protocol

1. https://services.healthtech.dtu.dk/service.php?SecretomeP-2.0
2. Sequence submission: paste the sequence(s) and/or upload a local file
3. For example, "APEX1" from https://www.uniprot.org/uniprot/P27695
4. Check the NN-score in the output result (Figure 2-2).

EXAMPLE OUTPUT

```
# Name          NN-score  Odds   Weighted  Warning
#                         by prior
# ================================================================================
FGF1_HUMAN      0.847     4.267  0.009     -
FGF4_HUMAN      0.945     6.804  0.014     signal peptide predicted by SignalP
ATRX_HUMAN      0.093     0.205  0.000     -
```

In the example above the first protein, known to enter the non-classical secretory pathway, FGF1_HUMAN, is correctly predicted as secretory. The second classical secreted protein, FGF4_HUMAN, is correctly predicted as secretory, but a warning that a signal peptide is predicted is reported. The third protein, ATRX_HUMAN, a known nuclear protein, receives a low score, thus is correctly classified.

Figure 2-2 The output result of the SecretomeP 2.0

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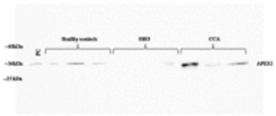
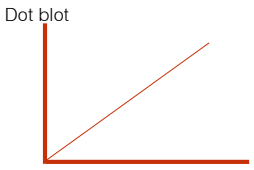
3 of 90 markers

- TMHMM
- Plasma proteome database



- APEX1
- Protein 2
- Protein 3



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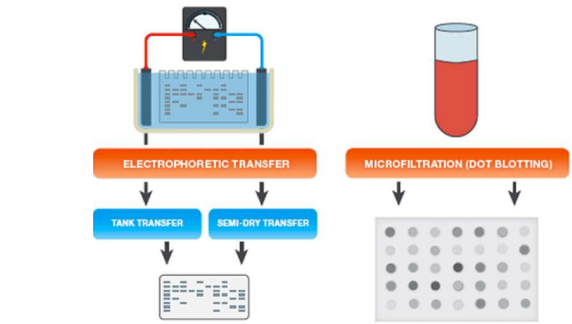

https://polizshoes.weebly.com/blog/biorad-western-blot-protocol

23

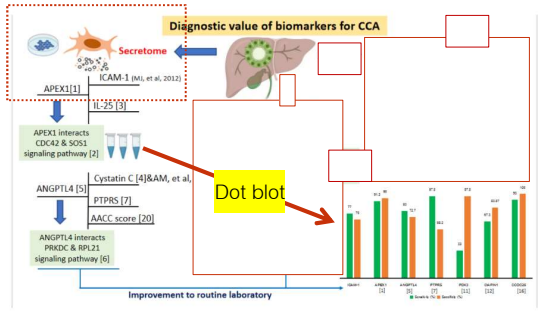

Figure S1 Diagnostic value of biomarkers for CCA

https://pubmed.ncbi.nlm.nih.gov/?term=proungvitaya%20s&sort=date

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#3 Protein-protein Interactions

25

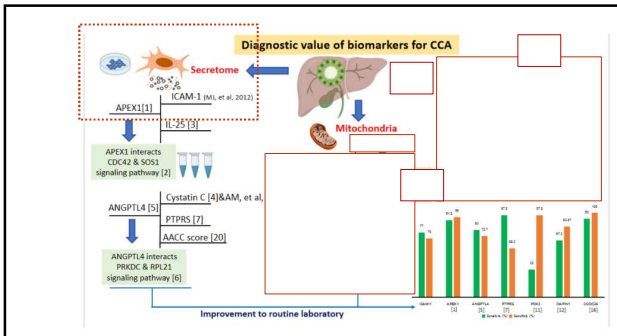


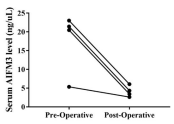
Figure S1 Diagnostic value of biomarkers for CCA

https://pubmed.ncbi.nlm.nih.gov/?term=proungvitaya%20s&sort=date

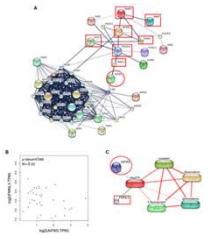
26

#4 Mitochondrial proteome analysis

Tumour Biol. 2016 Oct;37(10):13659-13667



Biomolecules. 2020 Jul 10;10(7):1021



- AIFM3
- PDK3
- CINPIN1

Cancer Genomics & Proteomics, January 2022, 19 (1) 25-49

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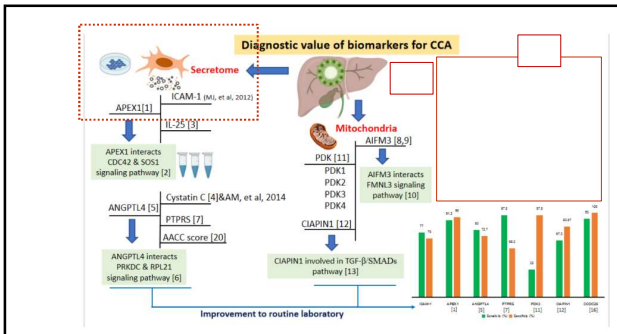

Figure S1 Diagnostic value of biomarkers for CCA

https://pubmed.ncbi.nlm.nih.gov/?term=proungvitaya%20s&sort=date

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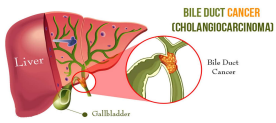
#5 Metabolomics of bile acids



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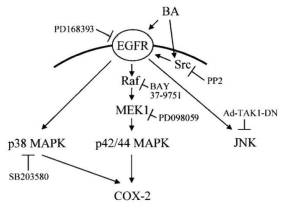

Figure 4-5 Proposed model of signaling pathways for bile acid-mediated COX-2 induction in human CCA cell lines [24]

1.Bile acids induce cyclooxygenase-2 expression via the epidermal growth factor receptor in a human cholangiocarcinoma cell line
Yoon, Jung-Hwan et al. Gastroenterology, Volume 122, Issue 4, 985 - 993

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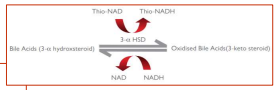
Bile acid determination

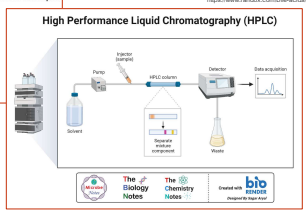


https://www.randox.com/bile-acids/30

Table 2

Spearman's correlation for the measurement of total bile acids using three commercial kits and the reference liquid chromatography-mass spectrometry (LC-MS) assay.

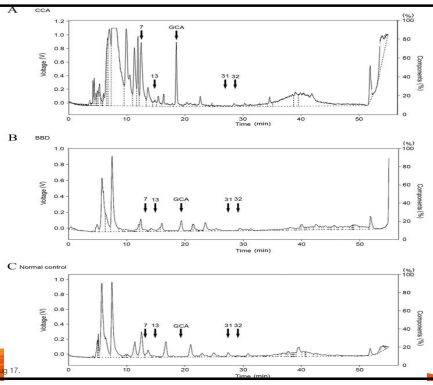
	LC-MS	Randox	Sentinel
Diazyme	r = 0.92 (p<0.001)	r = 0.92 (p<0.001)	r = 0.89 (p<0.001)
Randox	r = 0.99 (p<0.001)	-	r = 0.97 (p<0.001)
Sentinel	r = 0.97 (p<0.001)	-	-



https://microbenotes.com/high-performance-liquid-chromatography-hplc/

https://www.instruchemie.nl/bile-acids-reagent-set-enzymatic-method

HPLC



CCA

BBD

HE

Oncol Lett
. 2017 Oct;14(4):4943-4948.
doi: 10.3892/ol.2017.6763. Epub 2017 Aug 17.

CCDC25 in CCA tissue

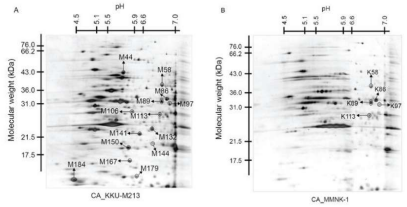


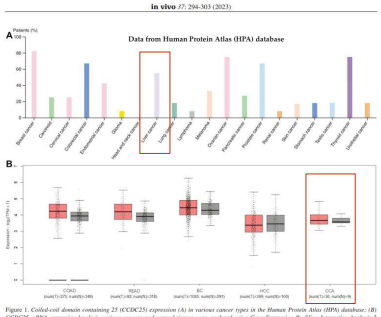
Figure 1. Two-dimensional electrophoresis patterns of protein spots of (A) CA-treated KKU-M213 cells, compared with (B) CA-treated MMNK-1 cells. The x-axis represents the isoelectric point values and the y-axis represents the molecular weight (kDa). CA, cholic acid.

CCA

Control

Oncol Lett
. 2017 Aug;14(2):2566-2572.
doi: 10.3892/ol.2017.6446. Epub 2017 Jun 21.

Serum CCDC25 in cancers



Human protein atlas

GEPIA

Figure 1. Coiled-coil domain containing 25 (CCDC25) expression (A) in various cancer types in the Human Protein Atlas (HPA) database; (B) CCDC25 mRNA expression levels in various cancers and normal tissues were analyzed using Gene Expression Profiling Interactive Analysis 2 (GEPIA2) database. COAD: Colon adenocarcinoma; READ: rectum adenocarcinoma; BC: breast cancer; HCC: hepatocellular carcinoma; CCA: cholangiocarcinoma; T: tumor (red box); N: normal (grey box).

In Vivo
2023 Jan-Feb;37(1):294-303.
doi: 10.21873/invivo.13079.

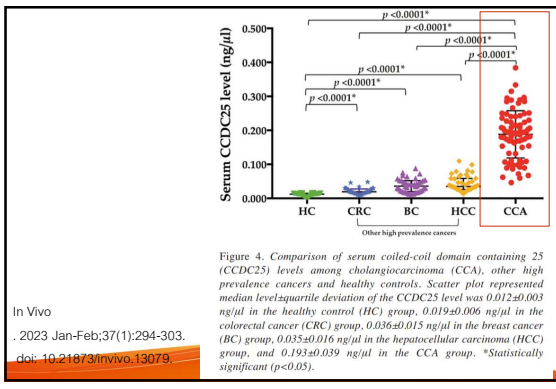


Figure 4. *Comparison of serum coiled-coil domain containing 25 (CCDC25) levels among cholangiocarcinoma (CCA), other high prevalence cancers and healthy controls. Scatter plot represented median level±quartile deviation of the CCDC25 level was 0.012±0.003 ng/μl in the healthy control (HC) group, 0.019±0.006 ng/μl in the colorectal cancer (CRC) group, 0.036±0.015 ng/μl in the breast cancer (BC) group, 0.035±0.016 ng/μl in the hepatocellular carcinoma (HCC) group, and 0.193±0.039 ng/μl in the CCA group. *Statistically significant (p<0.05).*

In Vivo
. 2023 Jan-Feb;37(1):294-303.
doi: 10.21873/invivo.13079.

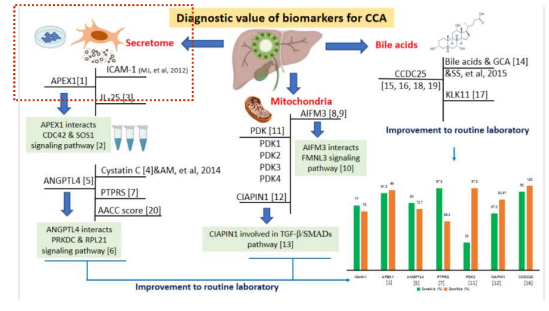


Figure S1 Diagnostic value of biomarkers for CCA

https://pubmed.ncbi.nlm.nih.gov/?term=proungvitaya%20s&sort=date

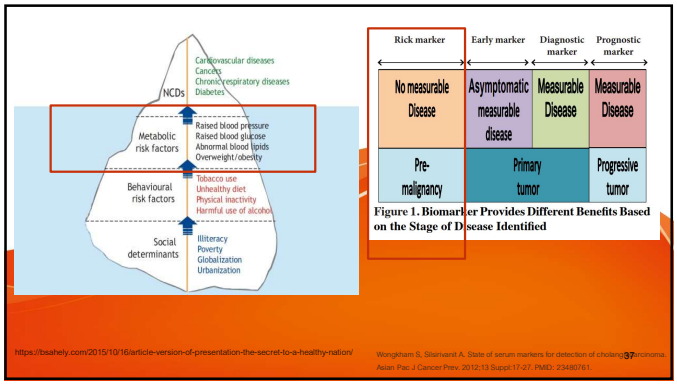
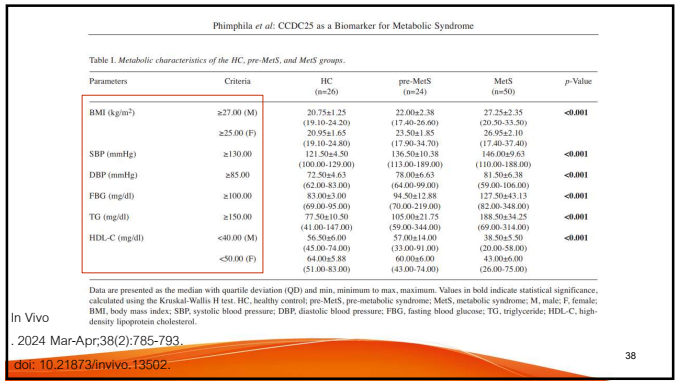


Figure 1. Biomarker Provides Different Benefits Based on the Stage of Disease Identified

https://bsahely.com/2015/10/16/article-version-of-presentation-the-secret-to-a-healthy-nation/

Wongkham S, Sirisrivanit A. State of serum markers for detection of cholangiocarcinoma. Asian Pac J Cancer Prev. 2012;13 Suppl:17-27. PMID: 23480761.



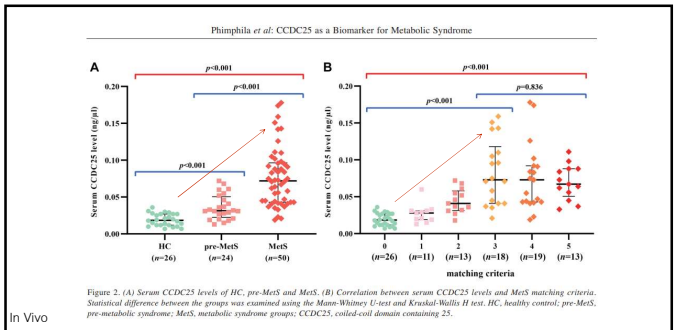
Phimphila *et al*: CCDC25 as a Biomarker for Metabolic Syndrome

Table 1. *Metabolic characteristics of the HC, pre-MetS, and MetS groups.*

Parameters	Criteria	HC (n=26)	pre-MetS (n=24)	MetS (n=50)	*p*-Value
BMI (kg/m²)	≥27.00 (M)	20.75±1.25 (19.10-24.20)	22.00±2.38 (17.40-26.60)	27.25±2.35 (20.50-33.50)	**<0.001**
	≥25.00 (F)	20.95±1.65 (19.10-24.80)	23.50±1.85 (17.90-34.70)	26.95±2.10 (17.40-37.40)	
SBP (mmHg)	≥130.00	121.50±4.50 (100.00-129.00)	136.50±10.38 (113.00-189.00)	146.00±9.63 (110.00-188.00)	**<0.001**
DBP (mmHg)	≥85.00	72.50±4.63 (62.00-83.00)	78.00±6.63 (64.00-99.00)	81.50±6.38 (59.00-106.00)	**<0.001**
FBG (mg/dl)	≥100.00	83.00±3.00 (69.00-95.00)	94.50±12.88 (70.00-219.00)	127.50±43.13 (82.00-348.00)	**<0.001**
TG (mg/dl)	≥150.00	77.50±10.50 (41.00-147.00)	105.00±21.75 (59.00-344.00)	188.50±34.25 (69.00-314.00)	**<0.001**
HDL-C (mg/dl)	<40.00 (M)	56.50±6.00 (45.00-74.00)	57.00±14.00 (33.00-91.00)	38.50±5.50 (20.00-58.00)	**<0.001**
	<50.00 (F)	64.00±5.88 (51.00-83.00)	60.00±6.00 (43.00-74.00)	43.00±6.00 (26.00-75.00)	

Data are presented as the median with quartile deviation (QD) and min, minimum to max, maximum. Values in bold indicate statistical significance, calculated using the Kruskal-Wallis H test. HC, healthy control; pre-MetS, pre-metabolic syndrome; MetS, metabolic syndrome; M, male; F, female; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; FBG, fasting blood glucose; TG, triglyceride; HDL-C, high-density lipoprotein cholesterol.

In Vivo
. 2024 Mar-Apr;38(2):785-793.
doi: 10.21873/invivo.13502.



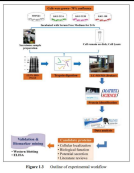
Phimphila *et al*: CCDC25 as a Biomarker for Metabolic Syndrome

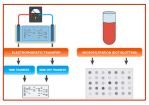
Figure 2. *(A) Serum CCDC25 levels of HC, pre-MetS and MetS. (B) Correlation between serum CCDC25 levels and MetS matching criteria. Statistical difference between the groups was examined using the Mann-Whitney U-test and Kruskal-Wallis H test. HC, healthy control; pre-MetS, pre-metabolic syndrome; MetS, metabolic syndrome groups; CCDC25, coiled-coil domain containing 25.*

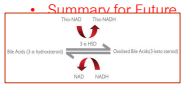
In Vivo
. 2024 Mar-Apr;38(2):785-793.
doi: 10.21873/invivo.13502.

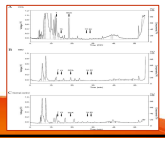
Marker routine detection

- Chapter 1 Secretome
- Chapter 2 Bioinformatics for marker selections
- Chapter 3 Protein-protein interactions
- Chapter 4 Mitochondrial markers
- Chapter 5 Metabolomics of bile acids
- Summary for Future









https://blog.praxilabs.com/2021/09/20/elisa-principle/

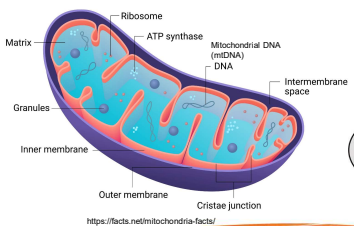
"Mitochondrial markers"

Daraporn Chua-on, Ph.D.
Postdoctoral researcher, KKU, Thailand

International Conference on Health Sciences
October 10, 2024

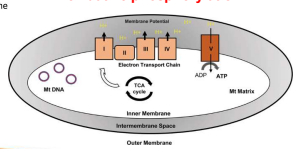
Mitochondria

MITOCHONDRIA



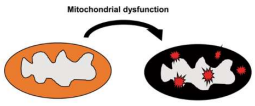
https://facts.net/mitochondria-facts/

ATP production by oxidative phosphorylation



Sreedhar et al., Cell Death & Disease. 2020 Jun;11:444

Mitochondria in physiological and pathological conditions



Mitochondrial metabolism	Dysfunctional mitochondria
Redox homeostasis	Oxidative stress
ATP production	Decreased ATP levels
Respiration	Dysfunctional OXPHOS
Biogenesis	Altered mitochondrial biogenesis
Calcium homeostasis	Calcium imbalance
Cellular growth	Cell death

Farhana et al., Cancers. 2023 Jul;15(5):3836

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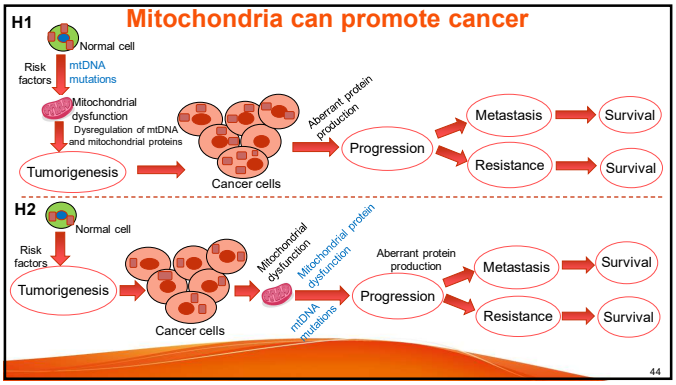
Mitochondria can promote cancer



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Mitochondrial marker projects in cholangiocarcinoma (CCA)

Assoc. Prof. Dr.Siriporn Proungvitaya

Dr. Daraporn Chua-on

Mitochondrial extraction from CCA tissues
↓
Proteome analysis

↓
Selection of candidate biomarkers
↓
Validation of clinical samples

Bioinformatics analysis

Serum AIFM3 in CCA sera (Biomolecules, 2020, Jul;10(7):1021

Dr. Daraporn Chua-on

Serum PDK3 in CCA sera (Oncol Lett, 2019, Jun;17(6): 5275–5282

Ms. Surangkana Sanmai

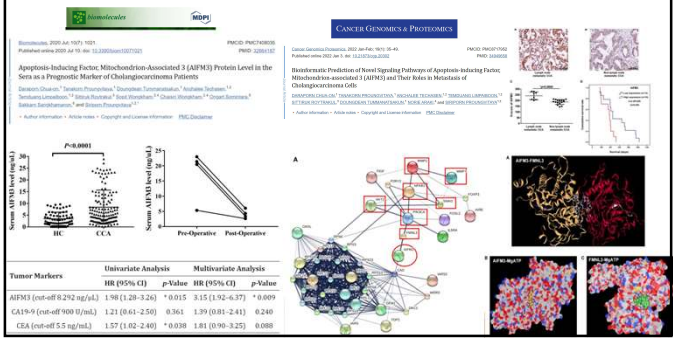
Serum CIAPIN1 in CCA sera (Diagnostics, 2021, Jun;11(6):1054

Dr. Son Dinh An Truong

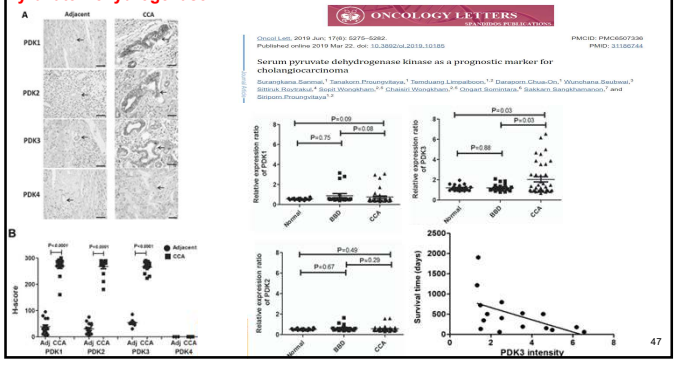
45

Apoptosis-Inducing Factor, Mitochondrion-Associated 3 (AIFM3)

46



Pyruvate Dehydrogenase Kinase 3 (PDK3)



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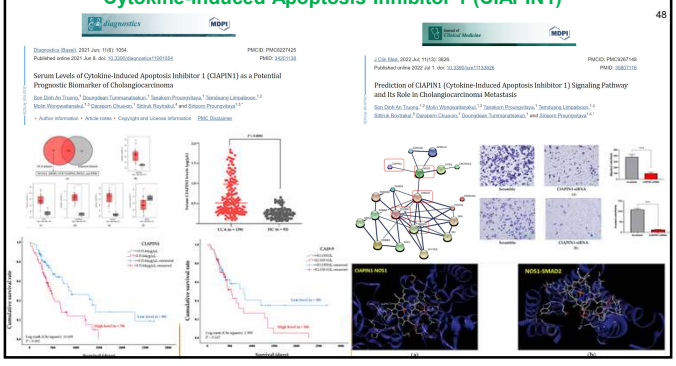
Cytokine-Induced Apoptosis Inhibitor 1 (CIAPIN1)

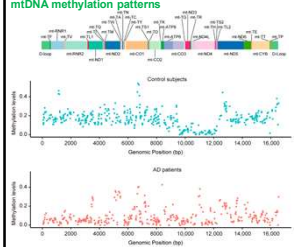
48



Current mitochondrial strategies for cancer diagnosis

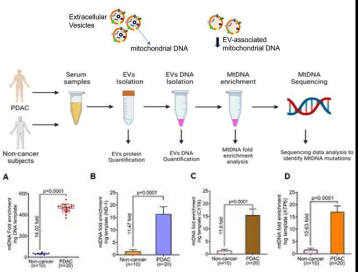
mtDNA mutation patterns
mtDNA mutation levels

mtDNA methylation patterns



Hypomethylation of mtDNA contributes to the pathogenesis of AD and potential application for the diagnosis of AD.

Ding et al., Diagnostics. 2023 Jul;13(14):2351



This study establishes, for the first time, that mtDNA mutations can be detected in circulating EVs and potentially serve as a tool for reliable PDAC diagnosis.

Vikramdeo et al., Scientific Reports. 2022 Jul;12:18455

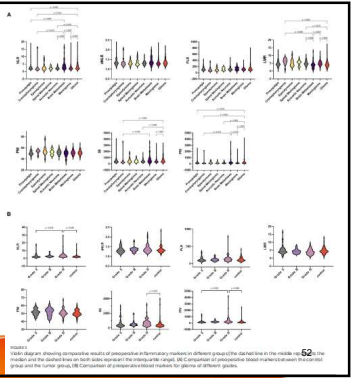
New chapter

	GRADE I ~85-95%	GRADE II ~5-10%	GRADE III ~1-5%
PREVALENCE			
DEMOGRAPHICS			
GENDER	Female > Male	Female ≥ Male	Female ≤ Male
DIAGNOSITIC CRITERIA	Mitoses < 4/10 hpf	Mitoses 4 - 19/10 hpf OR *3/5 of the following:* Necrosis High nuclear/cytoplastic ratio Prominent nucleoli Architectural sheeting Hypercellularity OR Clear cell/chordoid histology OR Brain Invasion	Mitoses ≥ 20/10 hpf OR Frank anaplasia OR Papillary/rhabdoid histology
HISTOLOGIC SUBTYPES	Meningothelial Fibrous (Fibroblastic) Transitional (Mixed) Psammomatous Angiomatous Microcystic Secretory Lymphoplasmacyte-rich Metaplastic	Atypical Chordoid Clear Cell	Anaplastic Papillary Rhabdoid
CLINICAL OUTCOMES AT 10 YEARS[†]			
OVERALL SURVIVAL	80-90%	50-79%	14-34%
PROGRESSION-FREE SURVIVAL	75-90%	23-78%	0%

Convenient and reliable peripheral blood markers

Cancer ☑ chronic inflammation

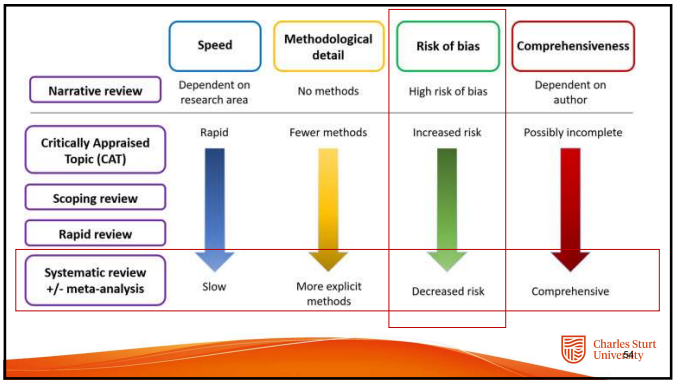
- neutrophil–lymphocyte ratio (NLR)
- platelet–lymphocyte ratio (PLR)
- lymphocyte–monocyte ratio (LMR)



Yang Y, Hu F, Wu S, Huang Z, Wei K, Ma Y and Ou-Yang Q (2023) Blood-based biomarkers: diagnostic value in brain tumors (focus on glioma). *Front. Neurol.* 14:1297835. doi: 10.3389/fneur.2023.1297835

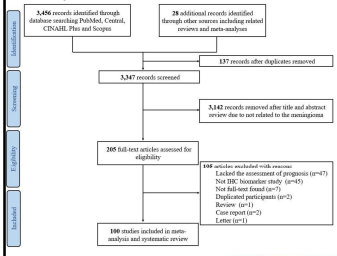
New chapter : Brain tumor

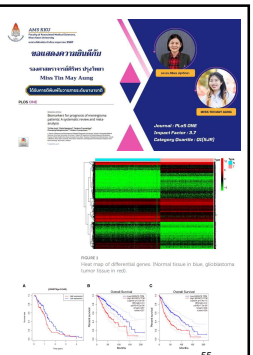
- Various biomarkers → the results are inconsistent
- In this study → a systematic review and meta-analysis to assess the prognostic biomarkers for patients with meningioma



Tools for brain cancer marker selection

Systematic review 2024 May 17;19(5):e0303337.





Luo YH, Wang K, Zhan L, Zeng F, Zheng J, Chen S, Duan X, and Ai D (2024) EXOC7 as a prognostic biomarker in glioblastoma and its association with glioblastoma immune infiltration, migration and invasion. Front. Oncol. 13:1214413. doi: 10.3389/fonc.2023.1214413

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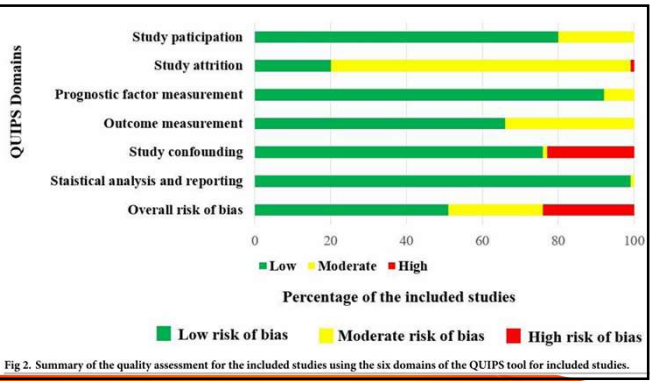

Fig 2. Summary of the quality assessment for the included studies using the six domains of the QUIPS tool for included studies.

Association of biomarkers and prognosis in the meta-analysis

To evaluate the prognostic value of biomarkers of meningioma patients, we performed a meta analysis of at least two or more studies of the association between 11 biomarkers (PR, cyclin A, TOP2A, p21, MCM6,H3k27me3, Bcl-2, p53, VEGF, PHH3, and Ki-67/MIB-1) and their respective prognostic outcomes using the univariate or multivariate HR and 95% CI by forest plot of random-effects model.

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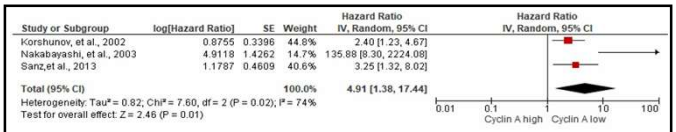

Fig 4. Association between cyclin A expression and RFS of meningioma patients by forest plot.

- Cyclin A is a member of cells cycle protein and implicated in the control of DNA replication.
- It is linked to both CDK 1 (cyclin dependent kinase) and CDK2, and has functions in, both S phase and mitosis stages of the cell cycle.
- High expression of cyclin A is observed in various human malignancies like astrocytoma, breast cancer, and liver cancer.
- Our meta-analysis revealed the association of the high expression of cyclin A with the high-risk of recurrence of meningioma patients.
- This marker could be used as predictors of recurrence for meningioma patients.
- Abnormal expression of cyclin A may linked to the irregular cell proliferation of meningioma patients.

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Discovered by Proteomics

- Bioinformatics(bioinfo.)
  - Human Plasma Proteome Project
  - The Human Protein Atlas project
  - GEPIA (Gene Expression Profiling Interactive Analysis)
- Evaluation
  - Elisa
  - Western blot
  - Dot blot
  - HPLC
  - Common reagent
- Sample praparation
- Mass spectrometry
- Review
- Bioinfo.
- AI
- etc.

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References

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https://pubmed.ncbi.nlm.nih.gov/?term=proungvitaya%20s&sort=date

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