KALC 2022 Korean Association for Lung Cancer International Conference
November 10-11, 2022 | Lotte Hotel World, Seoul, Korea

Identification of potential secretome biomarkers for early stage lung adenocarcinoma in Filipino patients

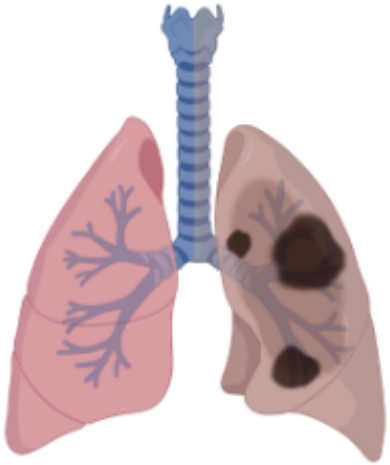
Dave Laurence A. Juntilla[1] , Ben Joshua O. Porras[1] , Lorenzo M. Zarate[1] , Venus B. Pondevida[2] , Ferdinand D. Mira[2] , Jayson L. Arce[2] ,

Efreihm Jovi T. De Guzman[2] , Herdee Gloriane C. Luna[3] , Baby Rorielyn T. Dimayacyac-Esleta[2] , and Eloise I. Prieto[1]

[1] National Institute of Molecular Biology and Biotechnology, University of the Philippines Diliman, Metro Manila 1101 Philippines
[2] Institute of Chemistry, University of the Philippines Diliman, Metro Manila 1101 Philippines
[3] Lung Center of the Philippines, Metro Manila 1100 Philippines

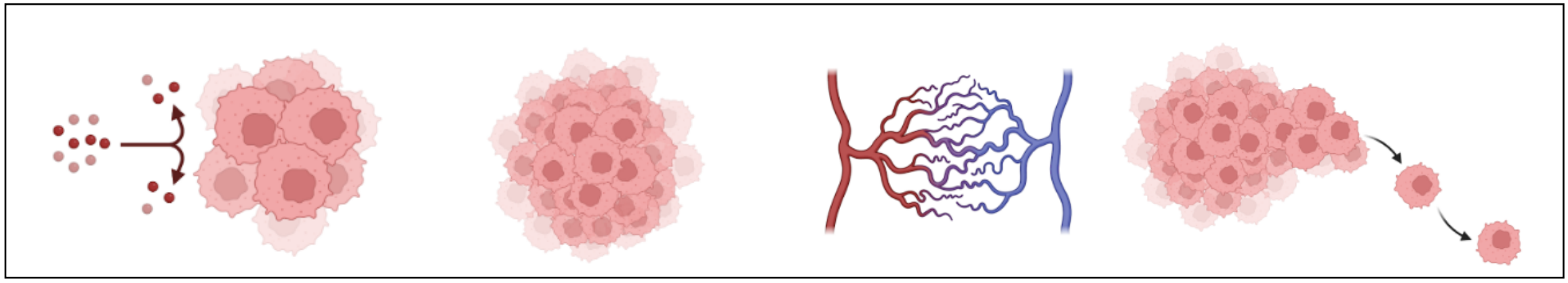
Background and Aim

- Lung cancer is the leading cause of cancer-related mortality worldwide, with non-small-cell lung carcinoma (NSCLC) accounting for 80-85% of all casualties.
  - Less than 15% overall 5-year survival rate
  - Most patients develop metastasis and systemic disease
- Multiple key pathways are dysregulated in NSCLC (e.g. cell growth, proliferation, angiogenesis, and apoptosis)



Non-small cell lung cancer (85%)
Adenocarcinoma (40%)
Squamous cell carcinoma (30%)
Large cell carcinoma (15%)

Small cell lung cancer (15%)

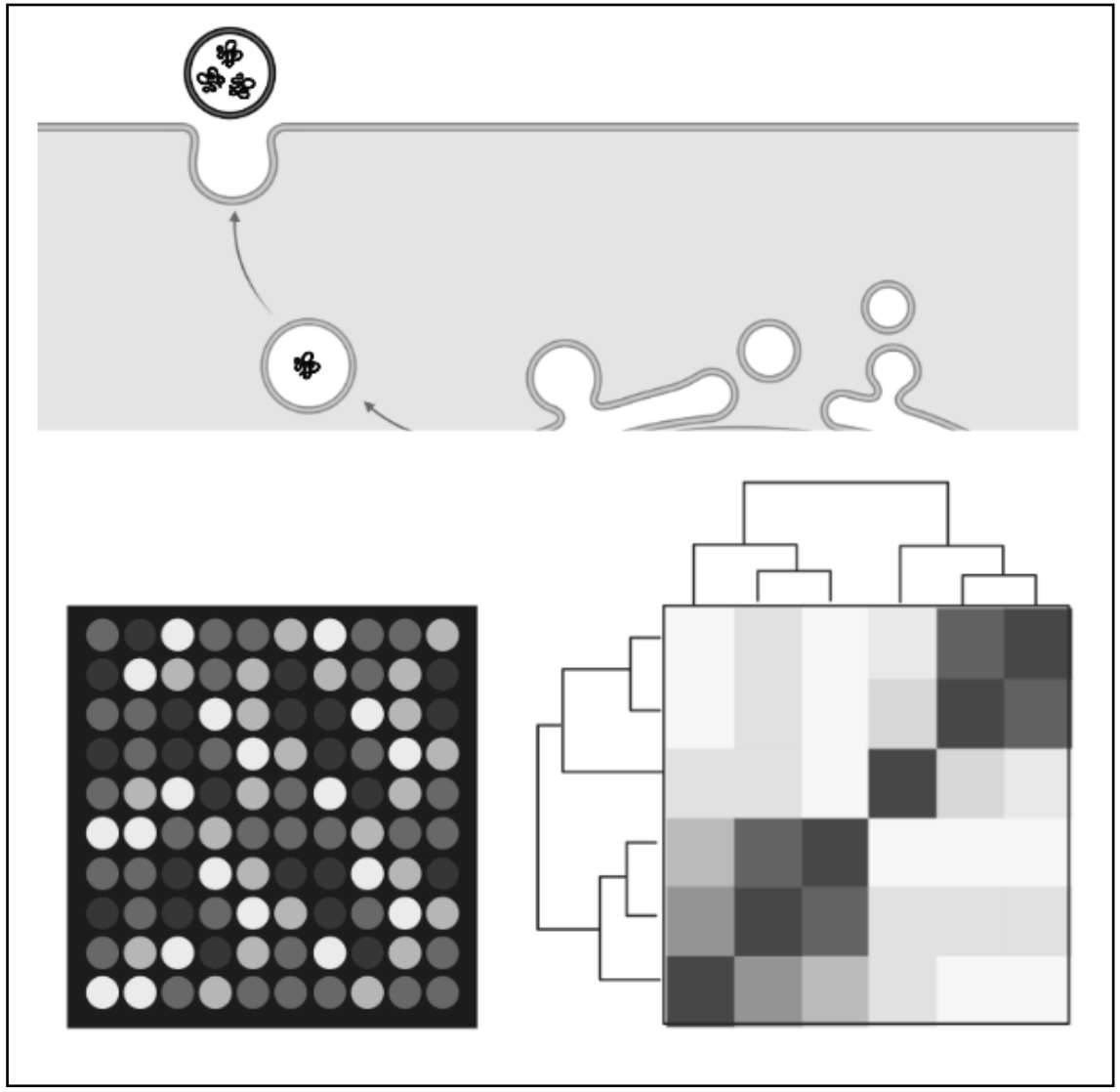


Background and Aim

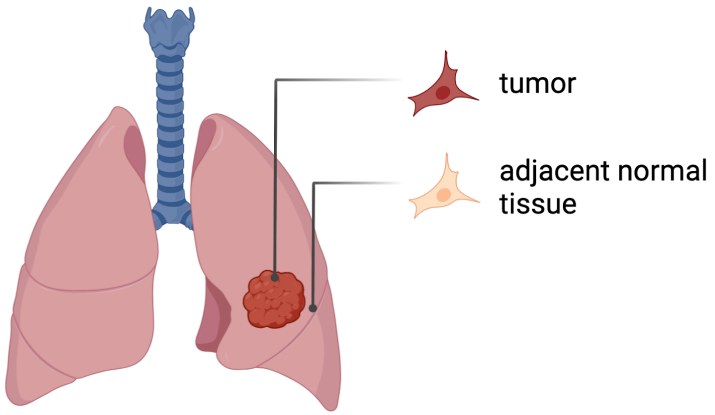
- **Cancer secretome:** proteins and other soluble factors shed or secreted by cancer cells
- The cancer secretome is a promising source of biomarker candidates as it plays a role in various tumor-promoting processes
- A major advantage of the cancer secretome is its accessibility, as secreted proteins are more likely to reach the blood and other bodily fluids
- Secretome biomarkers may be applied in the detection, diagnosis, prognosis, and treatment response selection & prediction

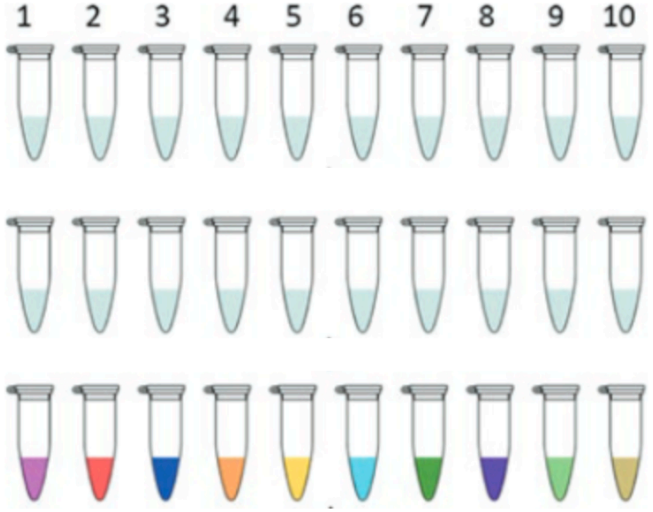
Aim of the Study

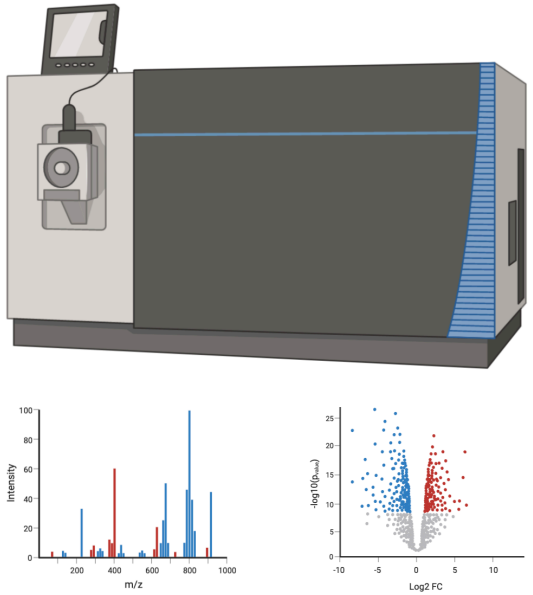
- This study aims to identify a panel of potential secretome biomarkers for NSCLC through Orbitrap LC-MS/MS proteomics and evaluate the prognostic value of these biomarkers



Methods







Harvesting of tumor & adjacent normal tissue from 7 Filipino early-stage NSCLC patients

Tryptic digestion, desalting, and TMT 10-plex labeling

Orbitrap LC-MS/MS, data processing, and identification of differentially expressed proteins

Methods

DTU

SecretomeP - 2.0
Prediction of non-classical protein secretion

SignalP - 6.0
Signal peptides and their cleavage sites in all domains of life

TargetP - 2.0
Subcellular location of proteins: mitochondrial, chloroplastic, secretory pathway, or other

TMHMM - 2.0
Prediction of transmembrane helices in proteins

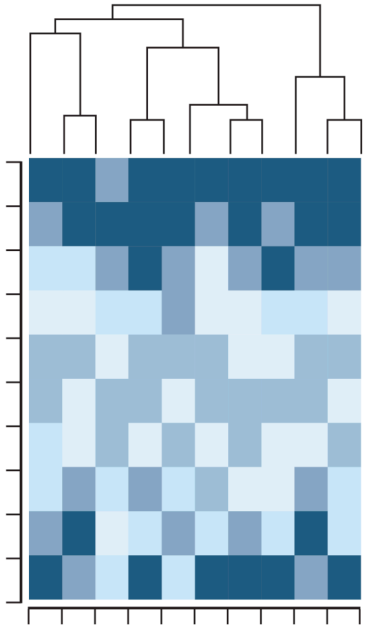
Vesiclepedia

ExoCarta

GEPIA2
Gene Expression Profiling Interactive Analysis

TCGA

GTEx



Prediction of secreted proteins using bioinformatics servers and databases

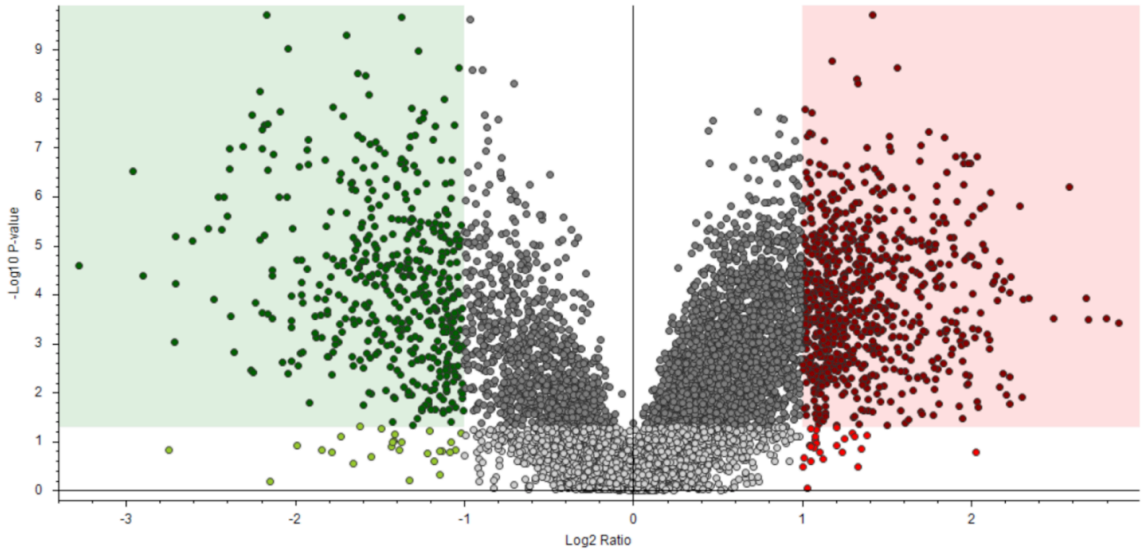
Validation of differentially expressed secretome genes using lung adenocarcinoma TCGA and GTEx databasets

Pathway analysis & cluster analysis of secretome gene expression profiles across 8 common cancer types

Results

Orbitrap LC-MS/MS analysis identified differentially expressed proteins

- TMT quantification identified 7,799 total proteins with false discovery rate < 0.01
- two-sample t-test identified a total of 816 significantly upregulated and 462 significantly downregulated proteins in tumor tissues (log2 fold change > 1, p > 0.05)



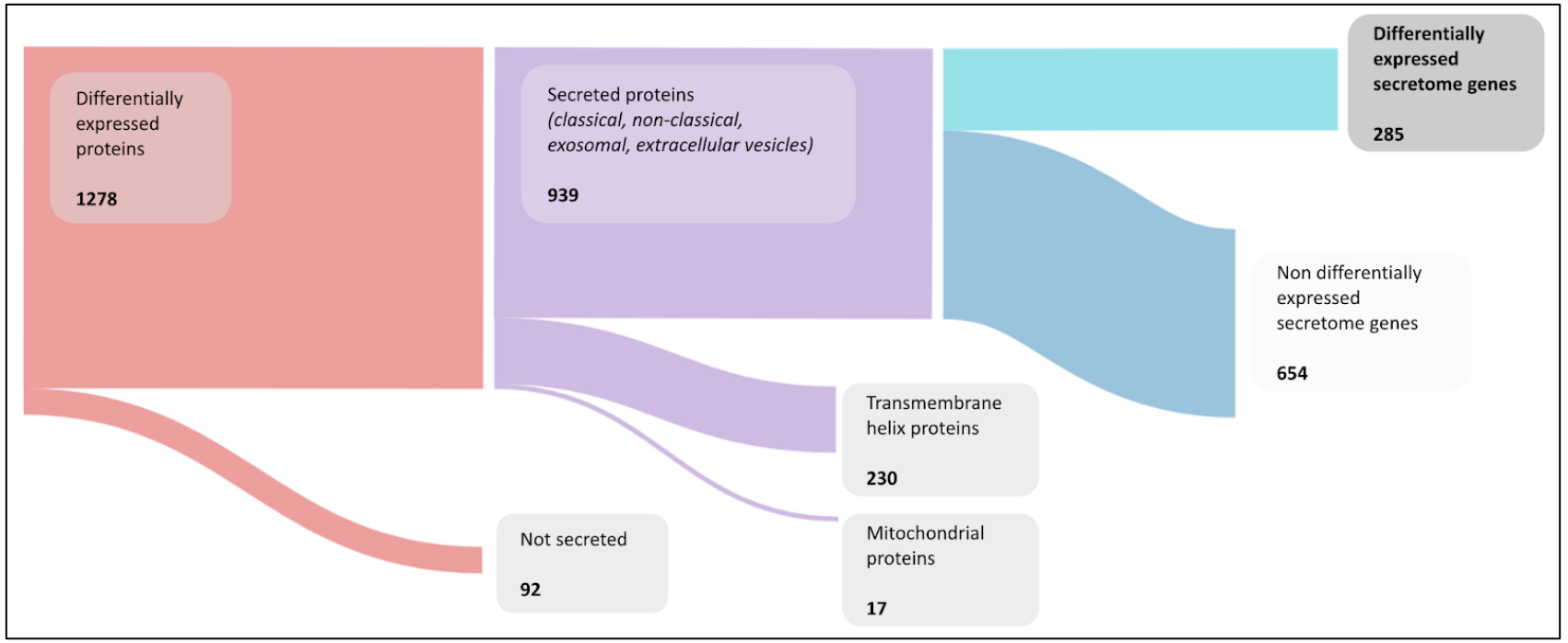
7,799 total proteins with false discovery rate < 0.01

- 816 upregulated proteins (log2FC > 1, p > 0.05)
- 462 downregulated proteins (log2FC > 1, p > 0.05)

Results

Bioinformatic analysis identified differentially expressed secreted proteins in NSCLC

- Secreted proteins were predicted using various in silico algorithms and databases
- The GEPIA 2 tool was used to identify genes that are differentially expressed in lung adenocarcinoma (LUAD) in the TCGA and GTEx databases



Results

Pathway and gene ontology analysis reveals cellular localization and biological functions of secretome genes

- Gene ontology analysis revealed involvement of predicted secretome proteins in metabolic pathways and processes related to nucleotide synthesis, replication initiation, and apoptosis
- Search Tool for Retrieval of Interacting Genes (STRING) analysis revealed that secretome proteins are located mainly in inflammasome complexes and other complexes that play a role in the progression of malignant human cancers

Cellular component

GO term	Description	Strength	False Discovery Rate
GO:0072557	IPAF inflammasome complex	1.42	0.0035
GO:0097169	AIM2 inflammasome complex	1.3	0.0374
GO:0005854	Nascent polypeptide-associated complex	1.3	0.0374
GO:0042555	MCM complex	1.25	0.00039
GO:0072559	NLRP3 inflammasome complex	1.22	0.0022

Biological processes (gene ontology)

GO term	Description	Strength	False Discovery Rate
GO:0019284	L-methionine salvage from S-adenosylmethionine	1.42	0.0367
GO:0006059	Hexitol metabolic process	1.42	0.0367
GO:0006267	Pre-replicative complex assembly involved in nuclear cell cycle dna replication	1.35	0.00046
GO:0019509	L-methionine salvage from methylthioadenosine	1.32	0.0118
GO:0006189	De novo imp biosynthetic process	1.25	0.0160

Molecular function (gene ontology)

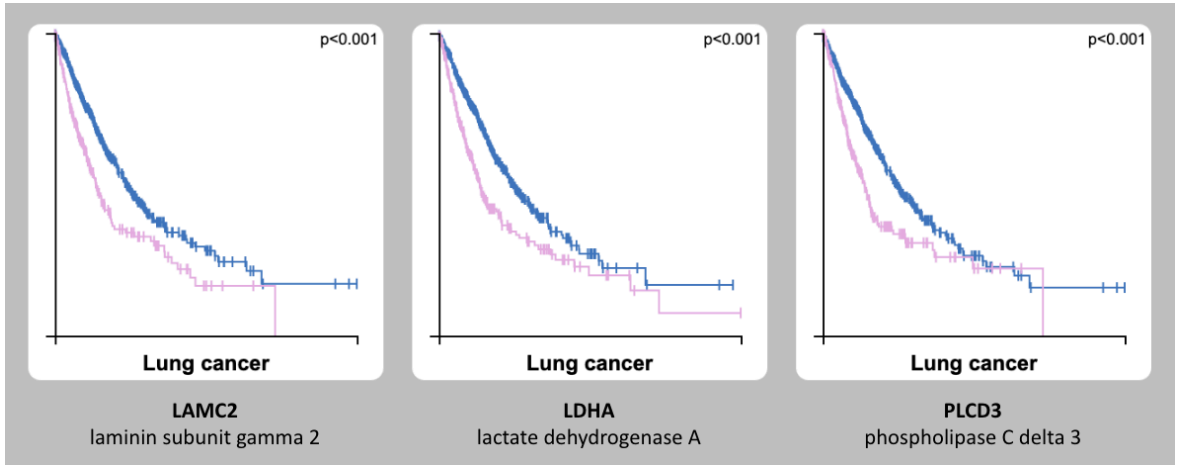
GO term	Description	Strength	False Discovery Rate
GO:0097200	Cysteine-type endopeptidase activity involved in execution phase of apoptosis	1.42	0.0055
GO:0097153	Cysteine-type endopeptidase activity involved in apoptotic process	1.21	3.85×10^{-5}
GO:0031821	G protein-coupled serotonin receptor binding	1.18	0.0219
GO:0016868	Intramolecular transferase activity, phosphotransferases	1.16	0.0013
GO:0097199	Cysteine-type endopeptidase activity involved in apoptotic signaling pathway	1.12	0.0301

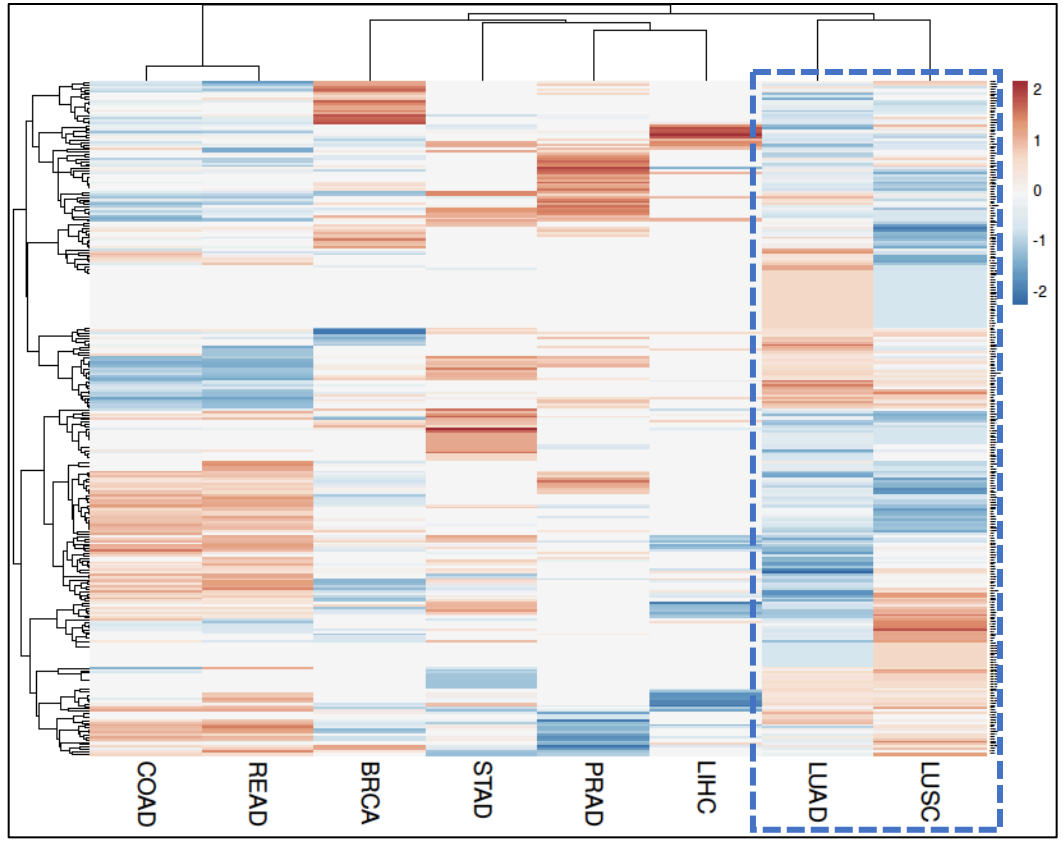
Results

Lung adenocarcinoma (LUAD) and lung squamous cell carcinoma (LUSC) exhibit expression profiles that are distinct from other prevalent cancer types.

- Euclidean cluster analysis was performed on the expression profiles of secretome genes across the 8 most prevalent cancer types

High relative expression of 3 genes (LAMC2, LDHA, PLCD3) is strongly associated with lower overall survival in lung cancer patients





(BRCA: breast invasive carcinoma; COAD: colon adenocarcinoma; LIHC: liver hepatocellular carcinoma; LUAD: lung adenocarcinoma; LUSC: lung squamous cell carcinoma; PRAD: prostate adenocarcinoma; READ: rectum adenocarcinoma; STAD: stomach adenocarcinoma)

Conclusions

- This analysis demonstrates a proteomics workflow coupled with a bioinformatics pipeline to identify a shortlist of secreted proteins that may be used as non-invasive biomarkers for NSCLC
- The panel of proteins exhibits an expression profile distinct from other cancer types
- High expression of 3 secretome genes (LAMC2, LDHA, PLCD3) is associated with poor overall survival in lung cancer patients, demonstrating their prognostic value

Selected References:

- Bray, F., Ferlay, J., Soerjomataram, I., Siegel, R., Torre, L., & Jemal, A. (2018). Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin, 68(6), 394-424. doi: 10.3322/caac.21492.
- de Oliveira, G., Freire, P.P., Cury, S.S., de Moraes, D., Oliveira, J.S., Dal-Pai-Silva, M., do Reis, P.P. & Carvalho, R.F. (2020). An integrated meta-analysis of secretome and proteome identify potential biomarkers of pancreatic ductal adenocarcinoma. Cancers (Basel), 12(3), 716. Doi: 10.3390/cancers12030716
- Schaaij-Visser, T. B., de Wit, M., Lam, S. W., & Jimenez, C. R. (2013). The cancer secretome, current status and opportunities in the lung, breast and colorectal cancer context. Biochimica et Biophysica Acta (BBA) - Proteins and Proteomics, 1834(11), 2242- 2258. doi: 10.1016/j.bbapap.2013.01.029.

Acknowledgements:

DEPARTMENT OF SCIENCE AND TECHNOLOGY
PCHRD
PHILIPPINE COUNCIL FOR HEALTH RESEARCH AND DEVELOPMENT

中央研究院
ACADEMIA SINICA