

uPE1 Study Examples by Chr 13 Group

2018.6.16.

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Types of Approaches for Functional Annotation

1. Wet Experiments: Direct experimental evidence of function

(1) **Pathway Analysis (Method 1) (hypothesis & in vitro)**

(2) **Cellular Approach (Method 2) (in vitro)**

(3) **Genetic Approach: Functional complementation (Method 3)(in vivo)**

(4) Enzyme assays and other biochemical assays (binding, mutation, RNAi, 2-hybrid, proteotypic peptide (for uPE1) etc. and more.....

2. In silico Experiment: Indirect evidence of function

(1) Expression analysis

(2) Structure analysis

(3) Sequence analysis

Combined Approaches: To make close coordination between computational and experimental and theoretical researchers. (utilizing hypothesis generation from large data sets, targeted data gathering).

Selection of Target uPE1

① Total uPE1 protein : **1,232** (neXtProt 2017-08-01 released)

② Chromosome 13's uPE1 : **25**

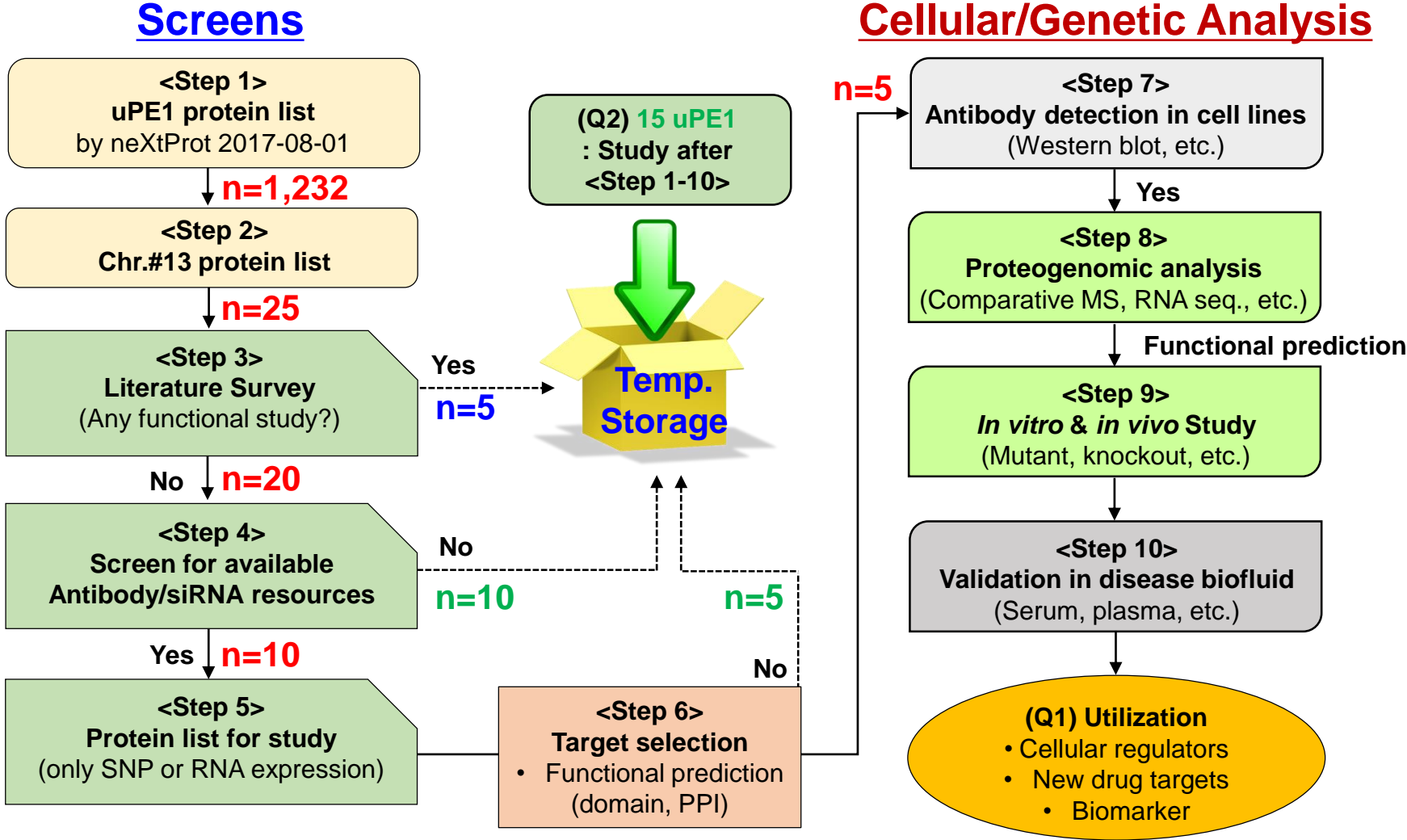
③ Published cellular function in RNA/protein level : **5**
(Paper research, e.g. Pubmed)

④ Usable antibody, siRNA (or cDNA) : **10/20**
(Abcam, Santa-cruz. Co.)

⑤ Prediction of function (domain, PPI)

⑥ Interesting target selection : **5**

Process for Chr.#13 uPE1 Study



<Step 3> Literature Survey

for those target genes in cellular/disease function

uPE1 name	Paper	Study contents
1. EPSTI1	Hastie E, <i>Oncotarget</i> . 2016	Drug-resistance marker in pancreatic cancer.
	Tan YY, <i>Am J Cancer Res</i> . 2016	Its miRNA dysregulates breast cancer.
	Meng X, <i>Mediators Inflamm</i> . 2015	EPSTI1-mediated anti-HCV activity by PKR.
	Capdevila-Busquets E, <i>PLoS One</i> . 2015	Apoptotic activity in breast cancer.
2. SLAIN1	<i>Mol Psychiatry</i> . 2017	Loss-of-function mutation in autosomal recessive.
	<i>Ann Hum Genet</i> . 2016	Obesity variant from whole exome sequence data.
	<i>Prostate</i> . 2015	SLAIN1-targeted miRNA promotes Caspase-8 apoptotic pathway in prostate cancer.
	<i>PLoS One</i> . 2014	Invasive-related SLAIN1 by proteomics in melanoma patients.
	<i>Int J Dev Biol</i> . 2010	Stem cell-development gene in nervous system.
3. N4BP2L1	Sasahira T, <i>Virchows Arch</i> . 2016	Proliferative and invasive activity, oral cancer biomarker.
4. C1QTNF9B (CTRP9B)	Peterson JM, <i>BBRC</i> . 2009	Adiponectin-associated secreted multimer form by CTRP9B+CTRP1+CTRP9+CTRP10+CRF. (PPI study)
5. ZAR1L	Hu J, <i>Dev Dyn</i> . 2010	Proliferation activity in mouse.

Proteins with Unknown Functions at the Protein Levels (only with SNP or RNA expression Level)

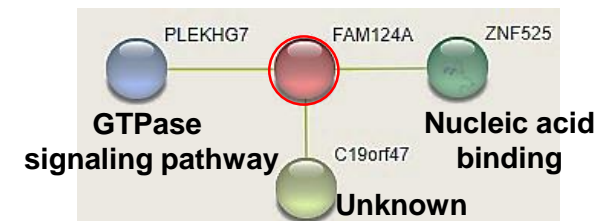
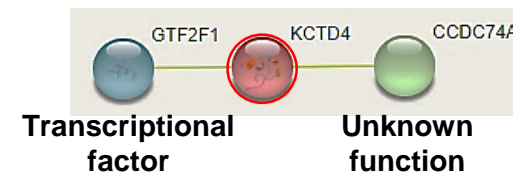
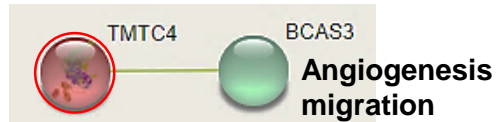
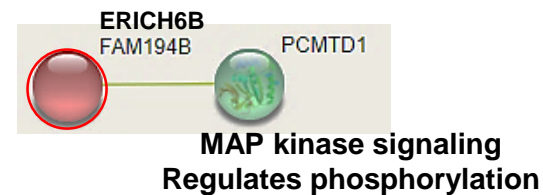
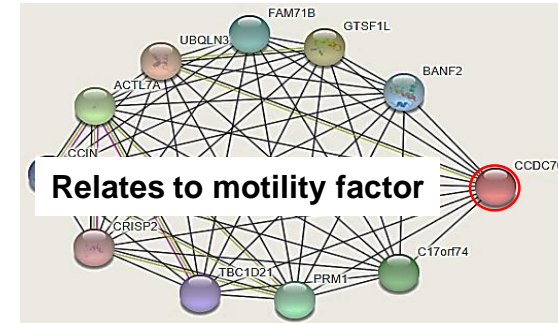
Search date: 2017.10.

uPE1 name	Paper	Study contents
1. SPRYD7	<i>PLoS Genet.</i> 2017	Genome-wide association study (GWAS).
	<i>Hum Mol Genet.</i> 2014	Genome-wide association study (GWAS).
2. UBL3	<i>Med Oncol.</i> 2012	Cervical cancer recurrence-signature gene by microarray.
	<i>OMICS.</i> 2015	Decreased and hypermethylated in esophageal cancer by genome-wide methylation analysis.
3. FAM124A	<i>Yi Chuan.</i> 2015	Body weight-related SNP from Genome-wide association study (GWAS).
	<i>Hum Genomics.</i> 2017	Increased in pneumonia (폐렴) from RNA sequencing.
4. CCDC70	<i>Zhonghua Nan Ke Xue.</i> 2016	Expression in the mouse testis during spermatogenesis.
5. CCDC122	<i>Int J Dermatol.</i> 2016	Chinese leprosy (나병)-related SNP gene from Genome-wide association study (GWAS).
	<i>Hum Genet.</i> 2014	Brazilian leprosy (나병)-related SNP gene from Genome-wide association study (GWAS).
6. KCTD4	No paper	
7. ERICH6B	No paper	
8. GPALPP1	No paper	
9. PROSER1	No paper	
10. TMTC4	No paper	

Functional Prediction: Combination

Reference of predicted function
(domain, interaction, expression site)

- ① PubMed
- ② Inferred from Interaction (e.g., STRING)
 - I. Physical interaction
 - II. Gene neighborhood
 - III. Gene fusion
 - IV. Gene co-occurrence
 - V. Gene co-expression
 - VI. Text mining (literature co-occurrence)
- ③ Genecard : GO annotation
- ④ UniProt : alternative name, AA sequence
- ⑤ ProteinAtlas : cell and tissue expression
- ⑥ SMART : domain function
- ⑦ Wikipedia
- ⑧ Google, etc.



Selection of 10 uPE1s in Chr.#13

	Gene Name	Alternative Name	Usable Antibody	Domain ^a and PPI ^b (for prediction)	Exp. in tissue ^c	Exp. in cell ^c
1.	KCTD4		Santa. sc-100998	BTB/POZ domain (interacts with histone deacetylase)	Brain	Microtubule
2.	SPRYD7	C13orf1, CLLD6	Santa. sc-81861	SPRY domain (regulate ATP production)	Brain, lung, testis	Vesicle
3.	TMTC4	TMTC4	AbCam. Ab83730	TPR domain in C-term. (protein folding, protein translocation)	All tissues	Vesicle
4.	GPALPP1	KIAA1704, LSR7	AbCam. Ab105795	DUF3752 domain (unknown function)	All tissues	Nucleus
5.	ERICH6B	FAM194B	Santa. sc-515452	Interact with PCMTD1 (MAP kinase signaling)	Testis	Nucleus, Cytosol
6.	CCDC70	CC70	AbCam. Ab87103	Coiled-coil domain (Interacts with spermatogenesis factor)	Testis	Spermatocytes, Adipocyte, Membrane, Nucleus, Mitochondria
7.	PROSER1	C13orf23, KIAA2032	AbCam. Ab173591	DUF4476 domain (unknown function)	All tissues	Nucleus, Cytosol
8.	FAM124A	F124A	AbCam. Ab179758	Interact with PLEKHG7 (GTPase activator)	Brain, Muscle, Breast, Intestine	Nucleus, Cytosol
9.	UBL3		Santa. sc-514190	Membrane-anchored ubiquitin (regulate proteolysis)	Intestine	Extracellular, membrane, Cytosol
10.	CCDC122	CC122	Thermo. PA5-24342	Coiled-coil domain (Interacts to immune response factor)	All tissues	Nucleus, Cytosol

a. Domain function : SMART (<http://smart.embl-heidelberg.de/>)

b. Protein-protein interaction : STRING (<http://string-db.org/>)

c. Cell and tissue expression : ProteinAtlas (<https://www.proteinatlas.org/>) 8

Cancer-related **5** uPE1 Candidates

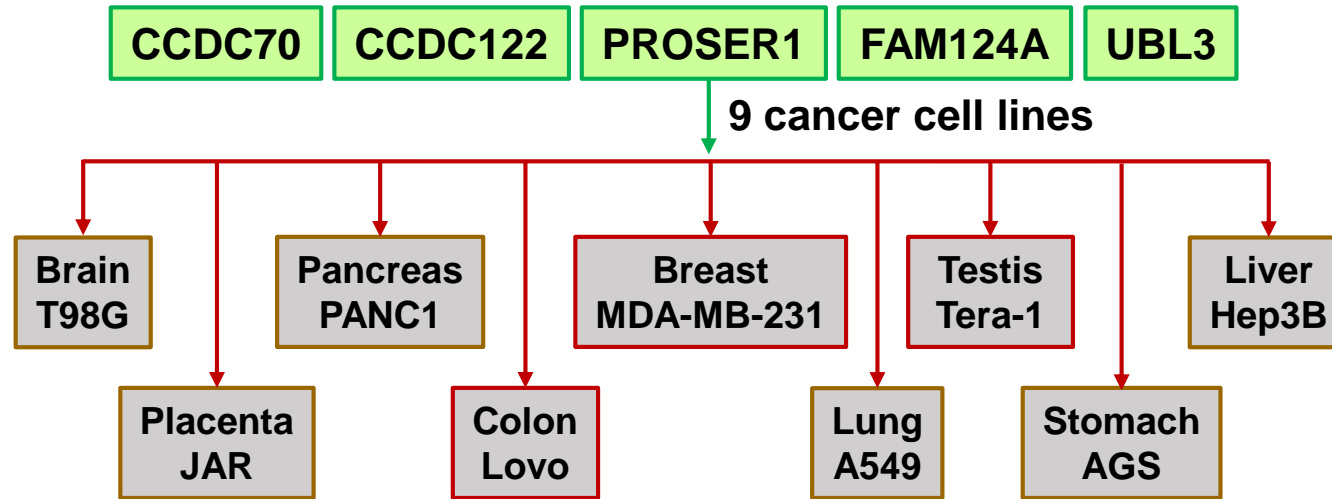
(from Domain function, PPI)

Gene name	Protein name	Predicted Function (Domain, PPI)	Exp. in cell	Exp. in tissue	<i>C.elegans</i> Homolog
PROSER1	Proline and serine-rich protein 1	Domain unknown function. Increase in colon and stomach cancer as RNA level (no PPI in STRING)	Nucleus, cytosol	All tissues	-
FAM124A	Protein FAM124A	Domain unknown function. Interact with PLEKHG7 (Rho-Family GTPase signaling pathway: cell motility & tumorigenesis)	Nucleus, cytosol	Brain, muscle, breast, intestine	-
UBL3	Ubiquitin-like protein 3	Regulate proteolysis, DNA repair and transcription. Gene phenotype: γ -H2AX phosphorylation (DNA damage response marker).	Extracellular, membrane, cytosol	Intestine	C46F11.6 (mutant order by S.H.)
CCDC70	Coiled-coil domain-containing protein 70	Domain unknown function. Interacts with motility factor (PRM1, CCIN, GTSF1L, ACTL7A)	Adipocyte, membrane, nucleus, mitochondria	Testis	-
CCDC122	Coiled-coil domain-containing protein 122	Domain unknown function. SNP mutation in Leprosy disease. Related to cytokine response factors (RIPK2, LACC1, TNFSF15, LTA4H, SLA11A1, CCL18)	Nucleus, cytosol	All tissues	-

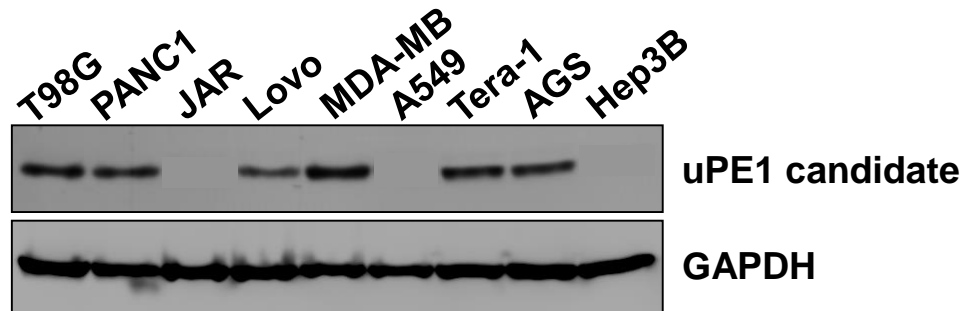
Plan 1

uPE1-expressed Cell and MS Confirmation

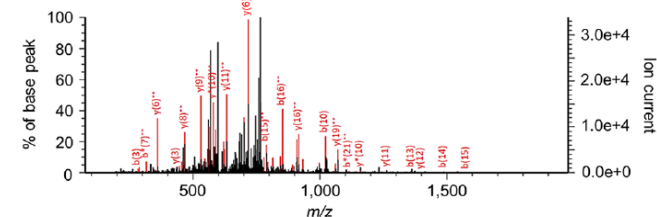
Five uPE1 Candidates



Protein analysis (Predicted data)



MS analysis (Predicted data)



Plan 2-1

Cellular Functional Validation of uPE1

Basic Cell Study

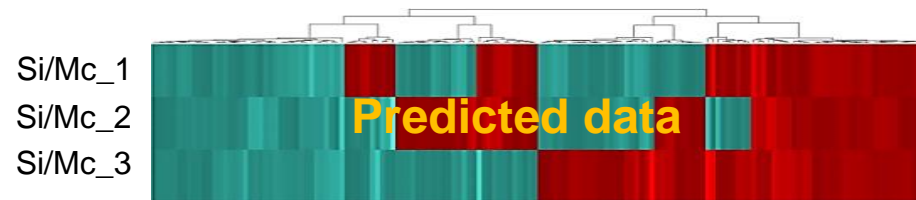
uPE1-containing
Cancer cell line

uPE1
siRNA, cDNA

Cell effect

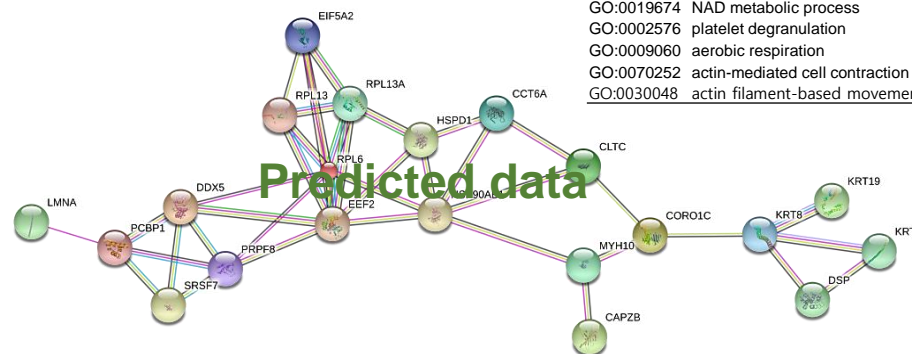
- Proliferation
- Migration
- Specific marker
- etc.

A. Comparative MS analysis



B. Functional prediction

GO ID	GO Term	P value
GO:0033275	actin-myosin filament sliding	0.00018
GO:0030049	muscle filament sliding	0.00018
GO:0019674	NAD metabolic process	0.00110
GO:0002576	platelet degranulation	0.00017
GO:0009060	aerobic respiration	0.00100
GO:0070252	actin-mediated cell contraction	0.00016
GO:0030048	actin filament-based movement	0.00002



C. Validation through cell study

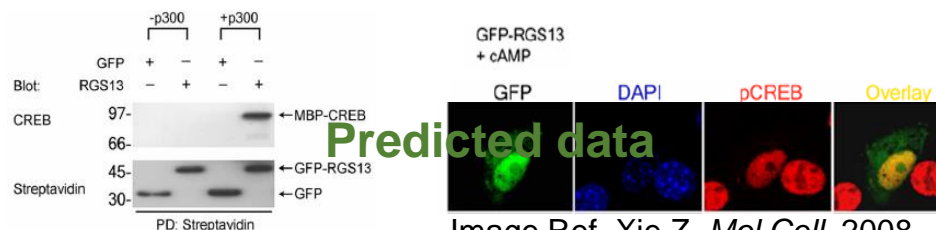
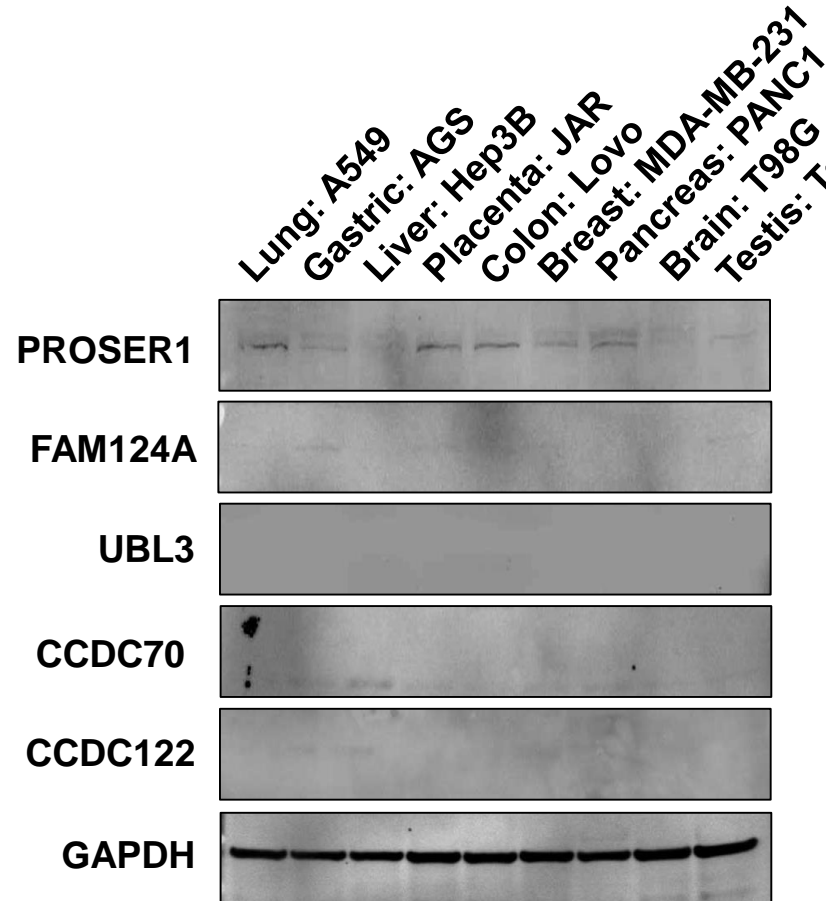


Image Ref. Xie Z, Mol Cell. 2008.

Protein Detection (by WB)



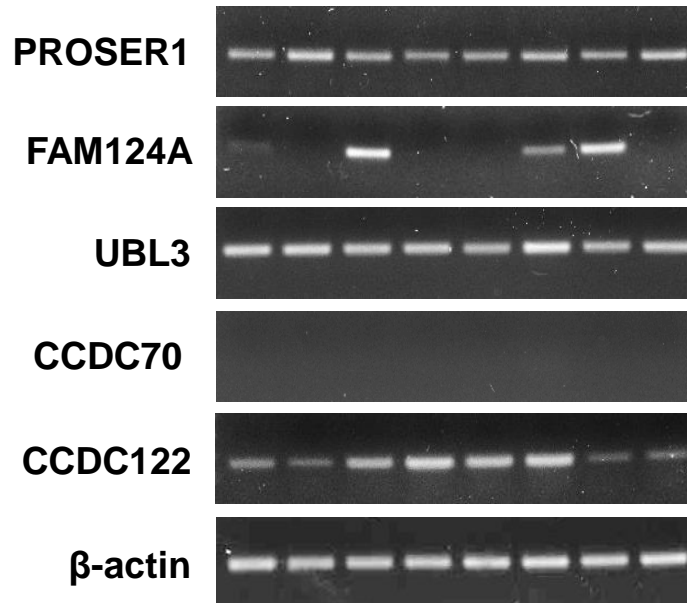
- Cell lysates: 30 μ g
- Antibody (100:1, App. WB): PROSER1 (Abcam, ab173591), FAM124A (Thermo, PA5-44353), UBL3 (Santa-cruz, sc-514190), CCDC70 (Thermo, PA5-31679), CCDC122 (Thermo, PA5-24342)
- Antibody assay does not show the bands. MS analysis is required.
- **Problems: Signals of 4 uPE1s were very low and thus they will be subjected to either OE or KD assay.**

Molecular Detection in 8 Different Cell Lines

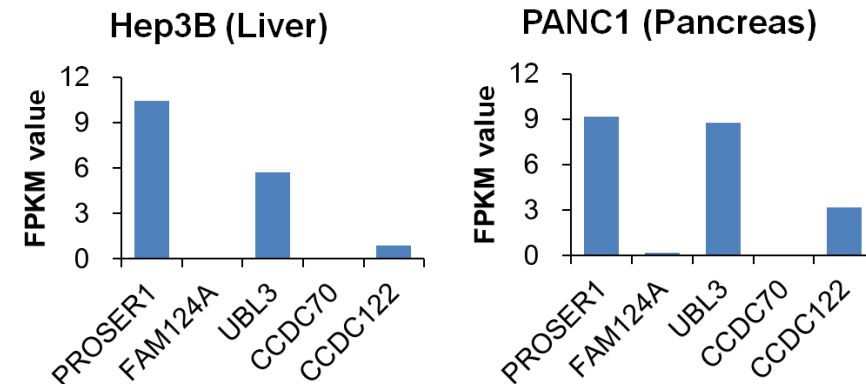
※ PCR Analysis with NT primers

- ① PROSER1: 4 primers
- ② FAM124A : 4 primers
- ③ UBL3 : 4 primers
- ④ CCDC70 : 5 primers
- ⑤ CCDC122 : 3 primers

Lung: A549
Gastric: AGS
Colon: Lovo
Breast: MDA-MB-231
Pancreas: PANC1
Liver: SK-Hep7
Testis: Tera1
Brain: T98G

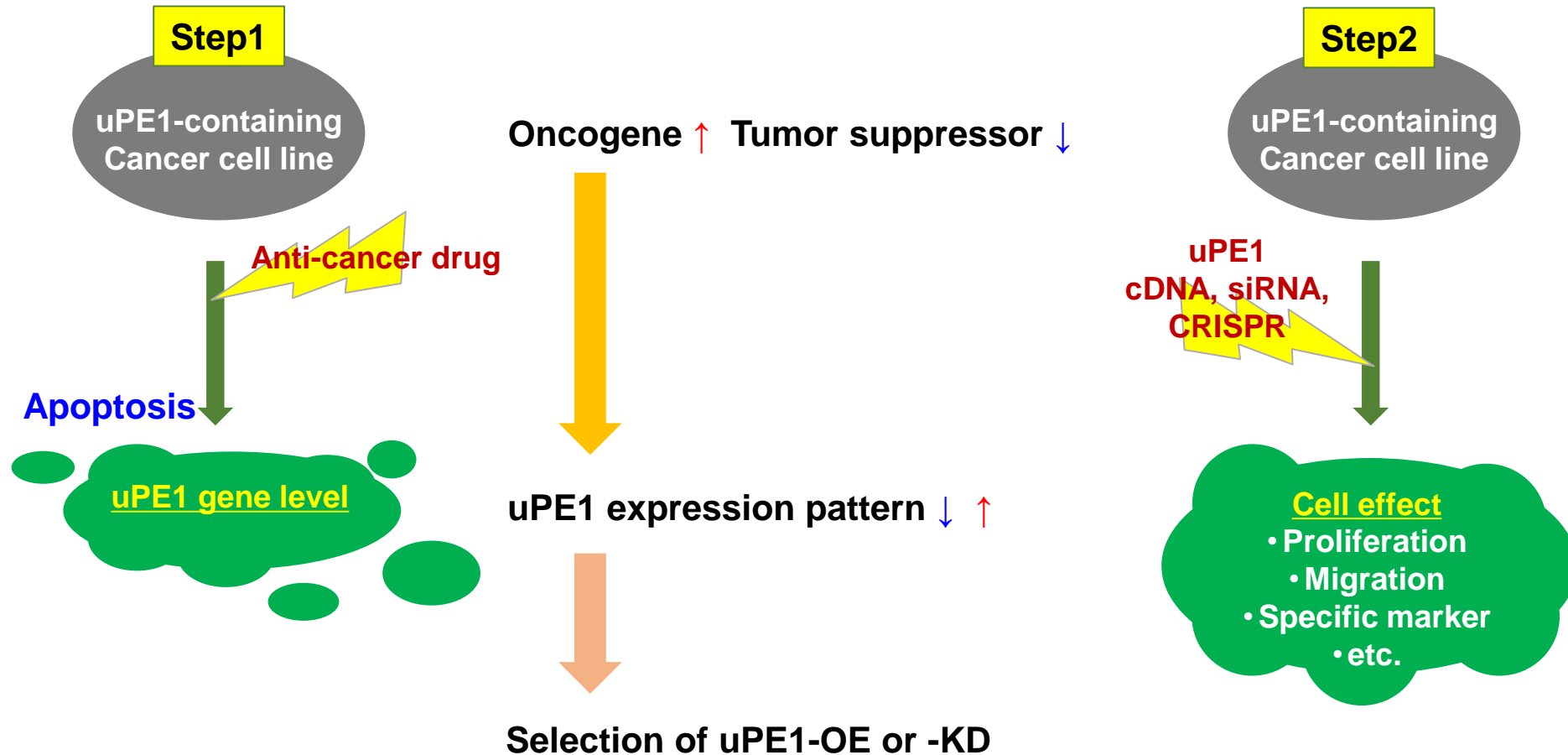


RNA seq. data (YPRC)



※ CCDC70 was tentatively excluded due to their absence by RNA Seq of Liver cancer, pancreatic cancer cells.

uPE1-OE or KD ?



Process for Chr.#13 uPE1 Study

