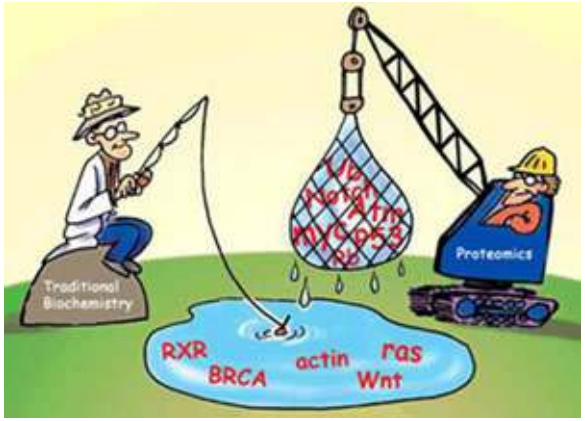
Gate to Omics

- General concept of proteOmics and more!

병리학교실
조 남훈



Contents

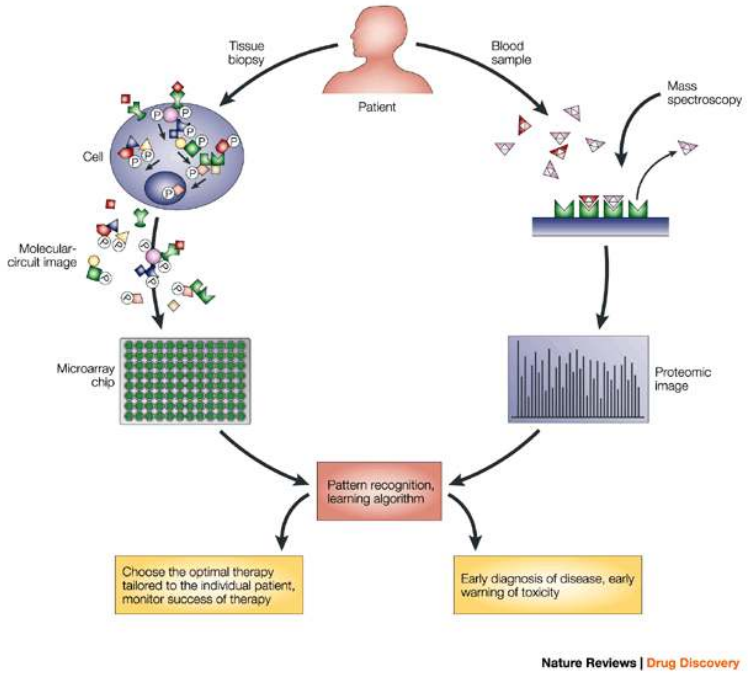
1. Design of study
2. Phenomics
3. Proteomics principle
4. Clinical application of display proteomics
4. Tissue MALDI-imaging MS and application
5. Rho family and cancer
6. Conclusion and message

Project Overview [I]

- With What
  - In vitro: cell line으로 실험관 배양
  - Ex vivo: primary culture 후 동물에 재이식
  - In vivo: mouse injection으로 채내실험
  - Human: 인체 시료 조직등 이용한 실험
- By What
  - DNA: regulation, mutation
  - RNA: transcription, regulation, expression
  - Protein: interaction, function, expression
  - Epigenetic: posttranslational modification

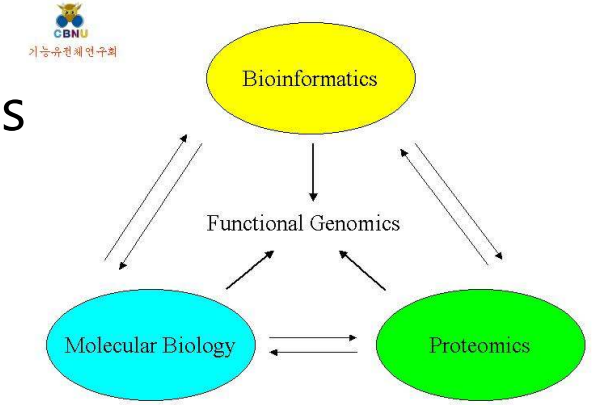
Project Overview [II]

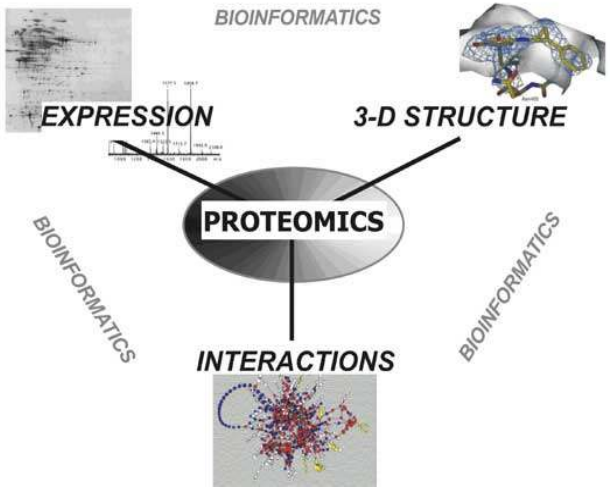
- How
  - Screening
    - DNA array/chip/array CGH
    - cDNA array/RT-PCR-based array
    - Proteomics/protein chip
    - Phosphoproteomics/Methylation chip
  - Targeting
    - PCR-based blot/ ISH
    - RT-PCR-based blot/RT-ISH
    - Blotting/immuno-based IF/IHC
  - Functional
    - Kinetic assay
    - Interaction Affinity assay: Chip/FRET/IP/co-IP/yeast two hybrid assay
    - Invasion/migration/motility assay



Project Overview [III]

- Screening high-throughput technology; Omics
- Omics: Large scale, high-throughput process-BIT
  - Genomics
  - Post genomics/Functional genomics
    - pharmacogenomics
    - Transcriptomics
    - Proteomics
    - Metabolomics
    - Kinomics
    - Physiomics
    - Nutrinomics
    - Epigenomics
  - Clinomics : Application
  - ***Phenomics (?) : validation***





Phenomics

- Phenomics define to find and correlate the whole morphologic spectrum (from the normal to abnormal spatial concept) with 'Omics data with preservation of integral parts of cell and tissue structure

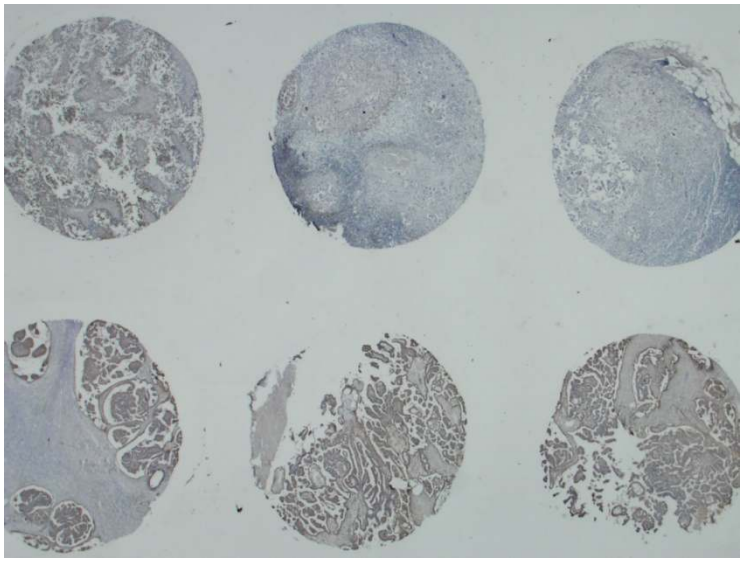
- In the narrow technical concept of phenomics, tissue microarray (TMA) is regarded as high throughput technique.
  - Limitation to overall configuration
  - Accurate knowledge of tissue morphology
  - Good clinical repository data-based well-trained TMA set
  - Standardization of interpretation algorithm

- Normal vs. abnormal (dysplasia-neoplasia)
  - Normal: phenotypically normal, but unknown gnotypic alteration
  - Diverse spectrum in normal
  - Incipient or cryptogenic or subtle or subclinical alteration
  - Gradual or abrupt transition of morphologic spectrum
  - Tissue specificity a/w microenvironment

- Finish line of Omics and New Start line of Fused Omics

Examples of Phenomics

- Screening purpose
  - From brain to toe
  - For a new molecule specificity/sensitivity evaluation
- Organotypic cancer TMA
  - according to pT/pN/pM
  - according to cell/histologic type
  - m/c used
- Specific zone TMA
  - Collection of intraepithelial neoplasia
  - Sorting to atypia zone
  - Capsule/margin zone
  - Vigorous necrosis d/t iatrogenic regression



- Limitation
  - **Tissue ischemia times** (intra– and postsurgical)
    Following tumor resection, approx. 25-30% of proteins and 20-25% of genes are differentially expressed within the first 30 minutes.
  - Spatial heterogeneity (periphery vs. epicenter)
  - Angiogeneis model
  - Average index model (Ki67….)
  - EMT model (focal patch expression)
  - TME model (surrounding stroma)

Websites useful

- http://www.genecards.org (무료)
  - Overall survey for specific gene
- http://www.ingenuity.com/index.html (유료)
  - Gene map networks
- http://cmbi.bjmu.edu.cn/transmir (무료)
  - miRNA-TF network

Short-cut to a thesis [III]
-Order of writing-

1. Goal (introduction)	Think always before
2. Materials	Note at the bench
3. Methods	Note at the bench
4. Results	Note at the bench
5. Discussion	Never touch before
6. References	With discussion
7. Introduction	After discussion
8. Abstract	Summary of Final message
9. Title	Shorter Precis

Proteomics 원리 및 응용

조 남훈

연세의대 병리학교실

Proteome Effect

- 1970-1990 Human Genome project
- 1990년 이후 postgenome era
- 1995년 proteome 탄생

- Functional genomics
  - Posttranslational modificaiton
  - One gene-multiple proteins

- Subcellular profiling
  - Shuttling or movement

- Integrated dogma
  - Approach to final conclusion

Proteome 구분

- Expression (display) proteomics
  - Disease-specific expression
    - Biomarker discovery
  - Drug action of mechanism
    - Drug candidate
- Cell-map proteomics
  - Molecular pathophysiology
  - Proteomics in situ MS

mRNA-protein관계

- Genome-mRNA 전사단계에서 coding sequence선택 변수
- mRNA-protein 번역단계에서 변형과정
- 번역후 변형과정 (post-translational modification)
  - (de)phospholy, (de)glycosyl, (de)acetylation

- 60 cell lines glutathion S-transferase mRNA-protein expression 비교 (k=0.43)
  Tew, *et al., Mol Pharmacol 50:149, 1996*

Step for proteomics

1. Protein preparation
2. Protein separation
3. Image analysis
4. Protein excision
5. Mass spectrophotometry
6. Protein identificaiton
7. Protein validation

Protein Preparation

Rule of thumb: minimize freeze-thaw cycle

1. Fresh serum

   aliquot to 4 tubes, each 25 μL, centrifuge, supernatant into 0.5 μL alliquot, and frozen

2. Body fluids

   avoid pink or red sample for hemolysis

3. Biopsy tissues

   avoid formalin, if not, rinsing with saline, mincing with a clean razor blade into pieces (less than 0.5 cm or 50 mg), and immediately frozen

4. Urine: low concentration and salt/urea

   10mL tube alliquot- 10mL Acetone precipitaiton or ultracentrifugation (200,000g for 120min at 4℃)

Protein Separation

- 1st EP: pI 분류 (net charge)
- 2nd EP: MW분류 (SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis)
- 2D-GE: cornerstone of proteome study
- Limitation of 2D-GE:
  - Membrane proteins
  - Too large or small proteins
  - Highly basic or acidic proteins
- Strategies for shortcoming
  - Fractionation by ion-exchange chromatography
  - Affinity chromatography
  - Subcellular fractionation
  - 1D and subsequent high performance liquid chromatography (HPLC)
- 염색

  Coomassie brilliant blue staining (detection limit > 100ng)
  Silver staining (detection limit > 1~2ng)
  SYPRO RUBY

2-D PAGE

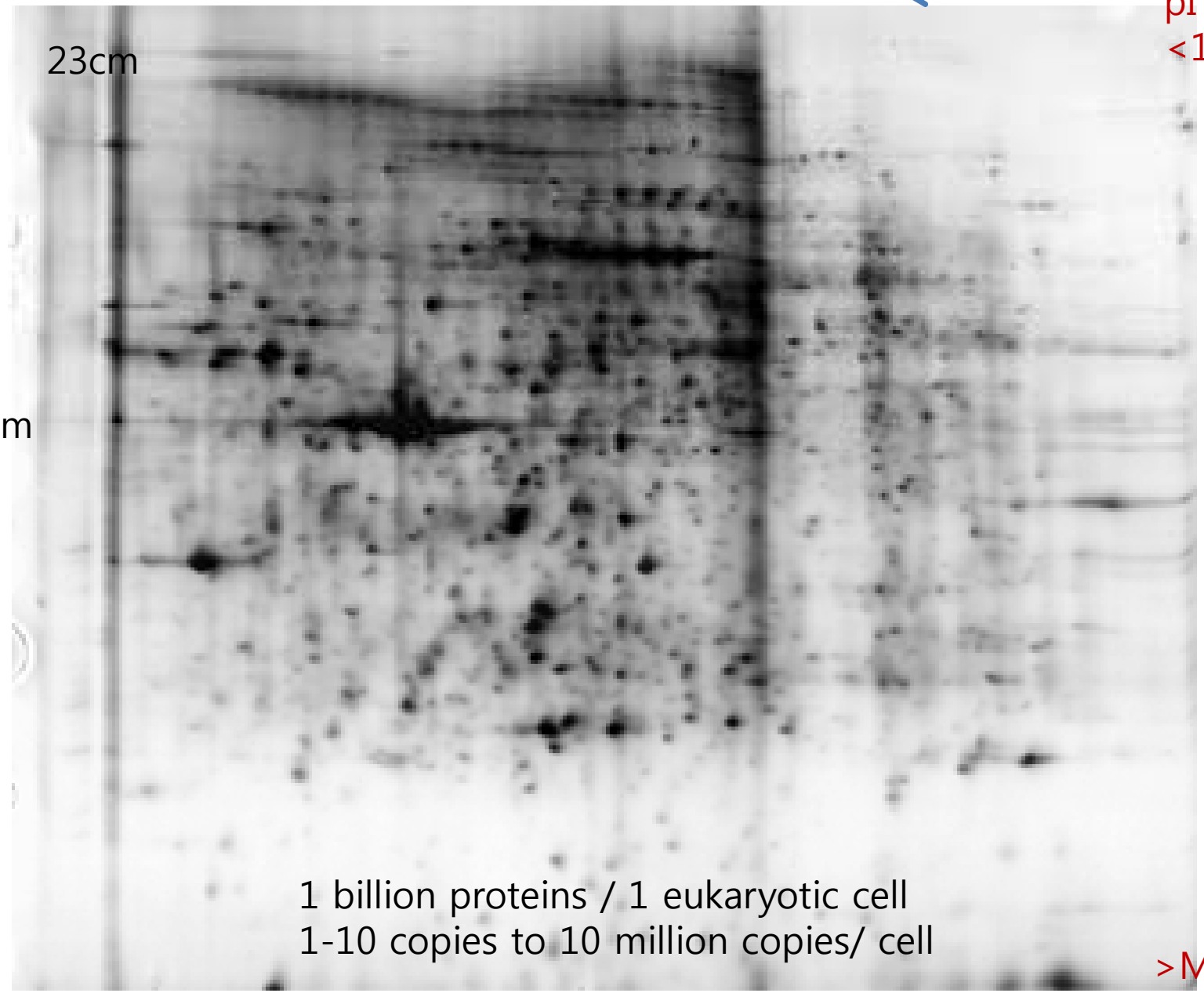
Upto 3,300 proteins
~10 fmol (10^{-15} mol)detection

>pI 4.0

IEF (isoelectric focusing)

pI <10.0
<150 MW

MW



1 billion proteins / 1 eukaryotic cell
1-10 copies to 10 million copies/ cell

>MW 15

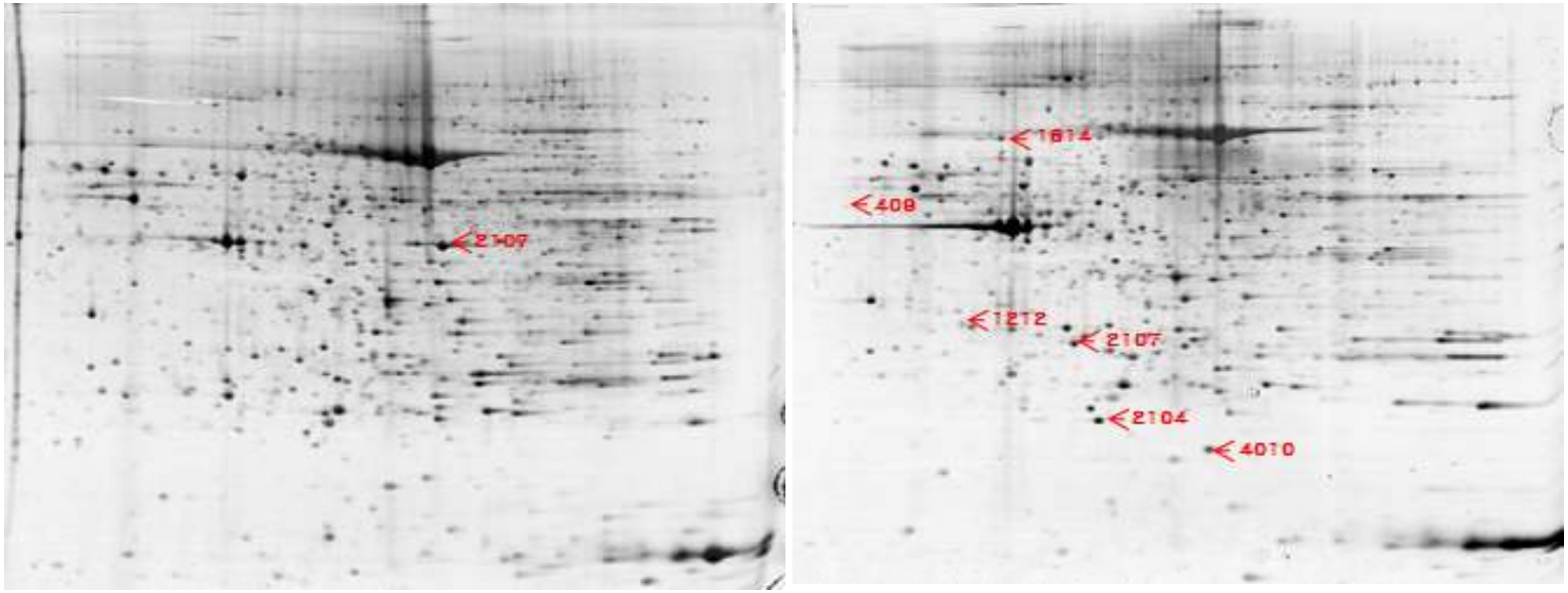
Image analysis

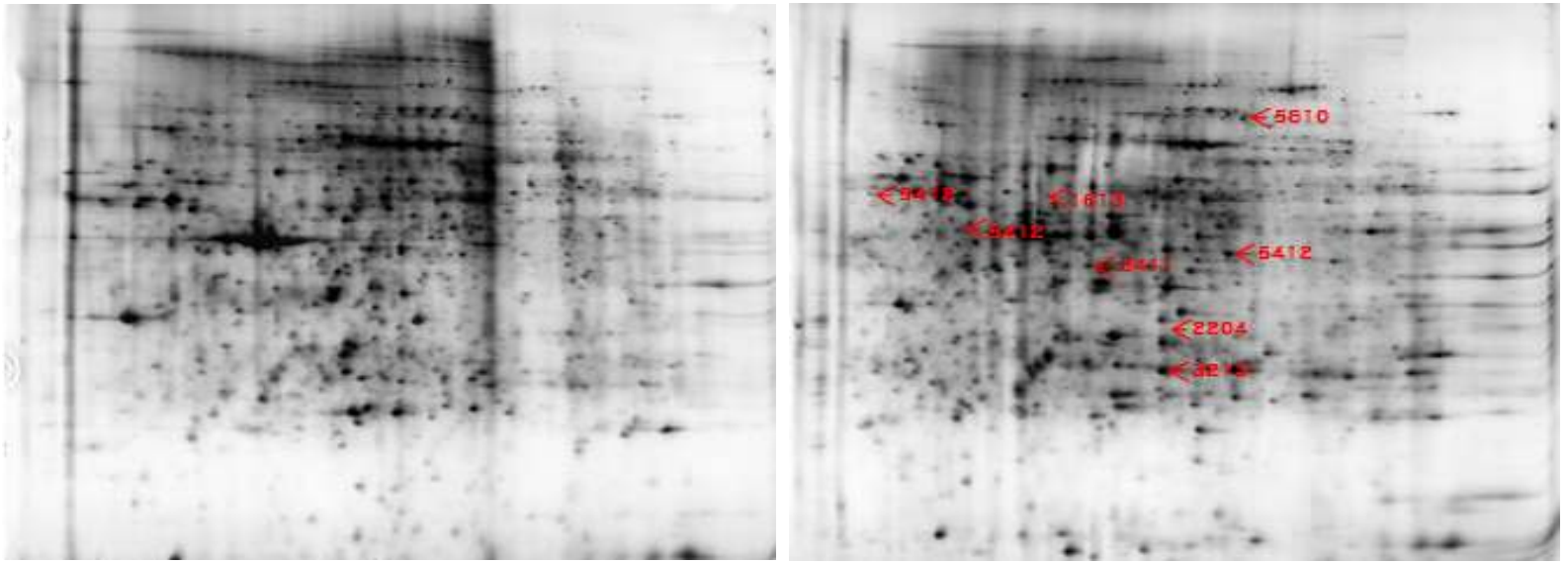
- Imaging system
  - Densitometry analysis
- Software analysis
  - PDQuest (100 gel image동시 분석)
  - MELANIE, IMAGE MASTER, HERMes, GELLABE
- Protein DB comparative analysis
  - SWISS-2D db
  - Molecular/medical/scientific knowledge support

Normal

Tumor

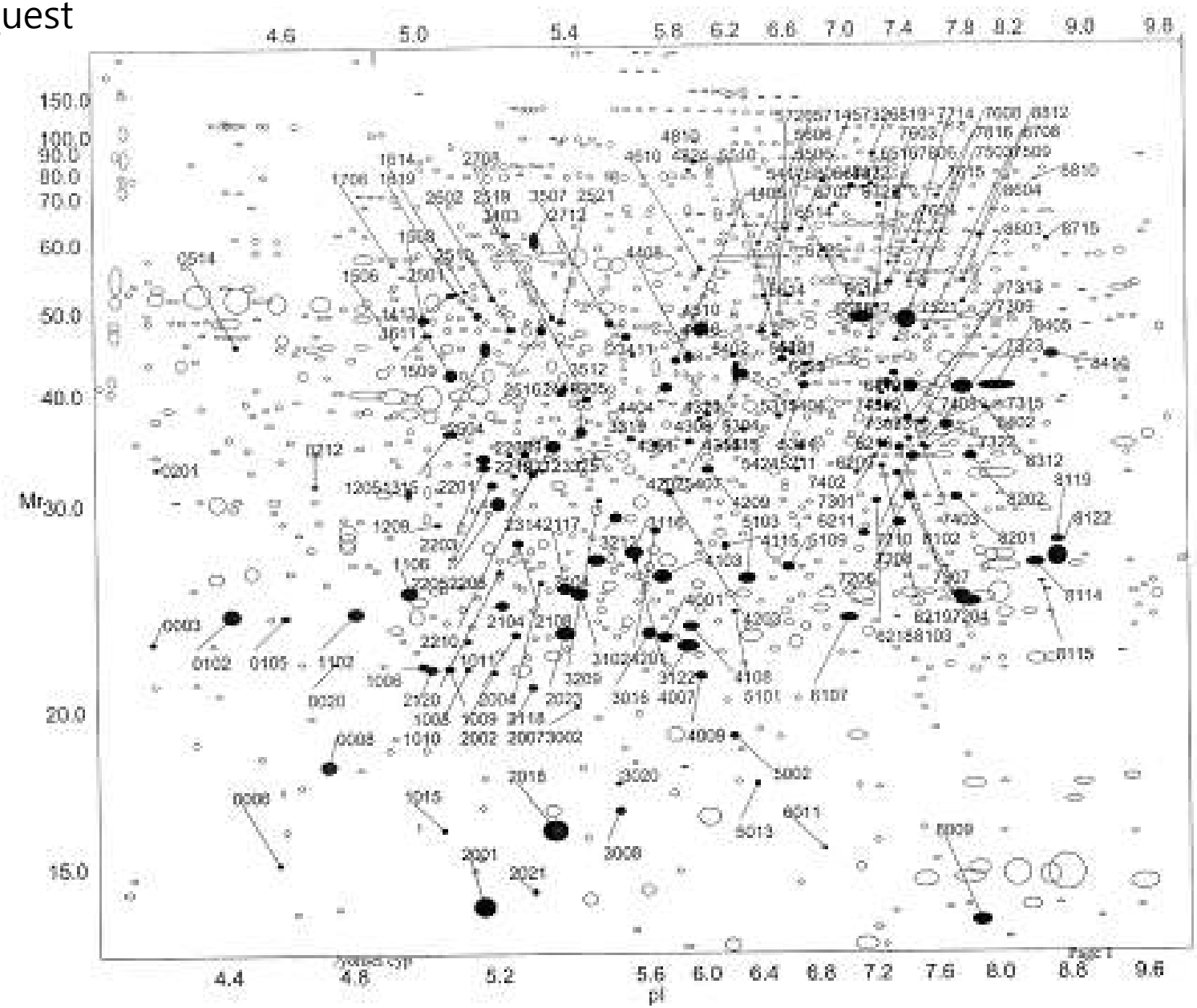
A



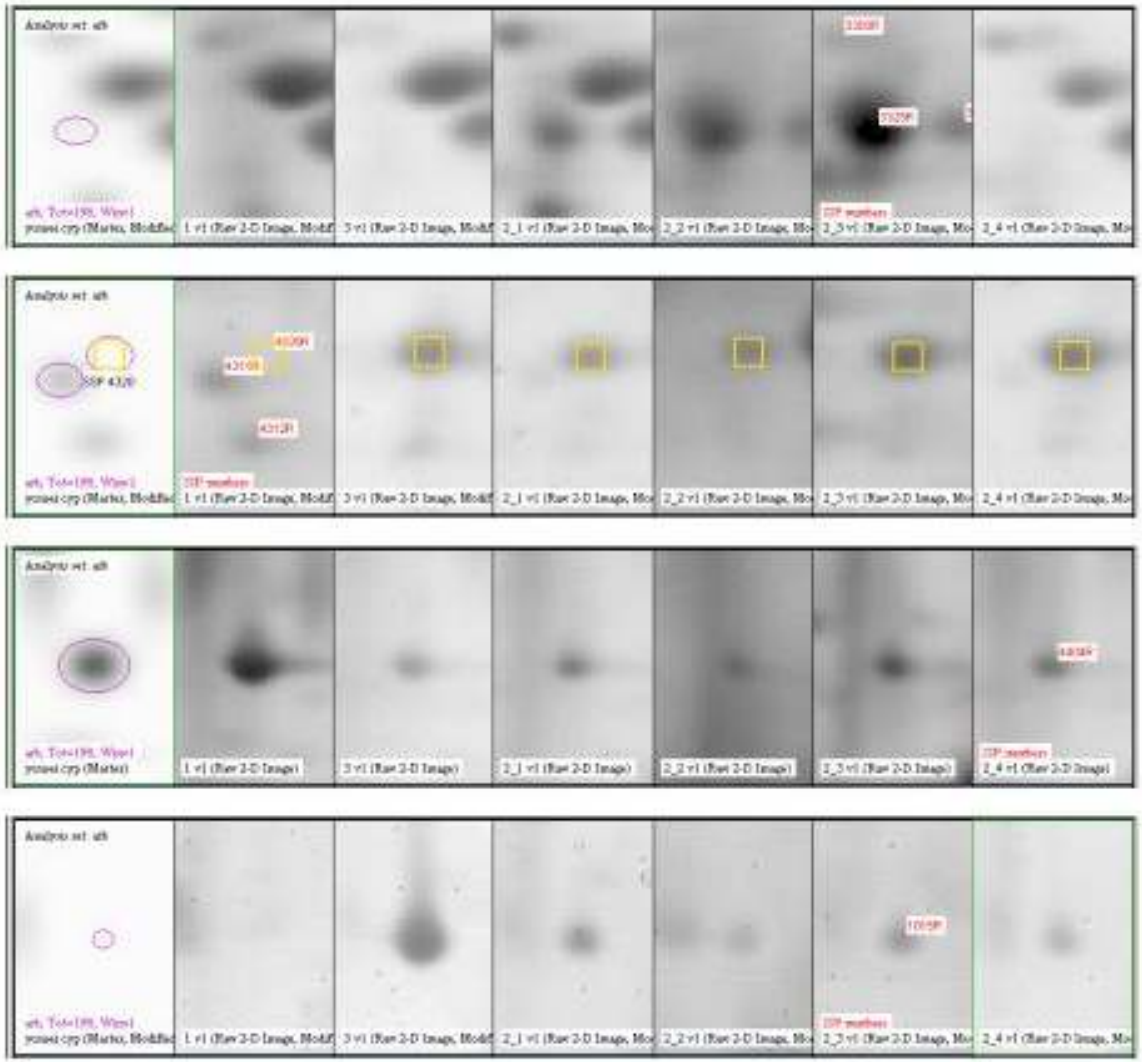


B

PDQuest


4.6
5.0
5.4
5.8
6.2
6.6
7.0
7.4
7.8
8.2
9.0
9.8
150.0
100.0
90.0
80.0
70.0
60.0
50.0
40.0
Mr
30.0
20.0
15.0
4.4
4.8
5.2
5.6
6.0
6.4
6.8
7.2
7.6
8.0
8.6
9.6
pI

Protein Excision



Mass Definitions

- Molecular masses are measured in Daltons (Da) or mass units (u). Dalton is defined as 1/12 of the mass of a ^{12}C atom. Relative molecular mass (Mr) is unitless because it is a ratio. Mass-to-charge ratio (m/z) is often used as the abscissa in a mass spectrum because a mass spectrometer separates ions on this basis.

- **"Average mass"** is calculated by summing the weighted average masses ("atomic weights") of the constituent atoms. The result is a weighted average over all of the naturally occurring isotopes present in the compound. This is the common chemical molecular weight that is used for stoichiometric calculations (H =1.0080, C = 12.011, O= 15.994, etc.). The mass cannot be calculated as accurately as the monoisotopic mass because of variations in natural isotopic abundances.

- **"Monoisotopic mass"**is calculated by summing the exact masses of the most abundant isotope of each element present, i.e., ^{1}H = 1.007825, ^{12}C = 12.000000 (by definition), ^{16}O = 15.994915, etc. This is the most accurately defined molecular mass and is preferred if a measurement can be made with sufficient resolution.

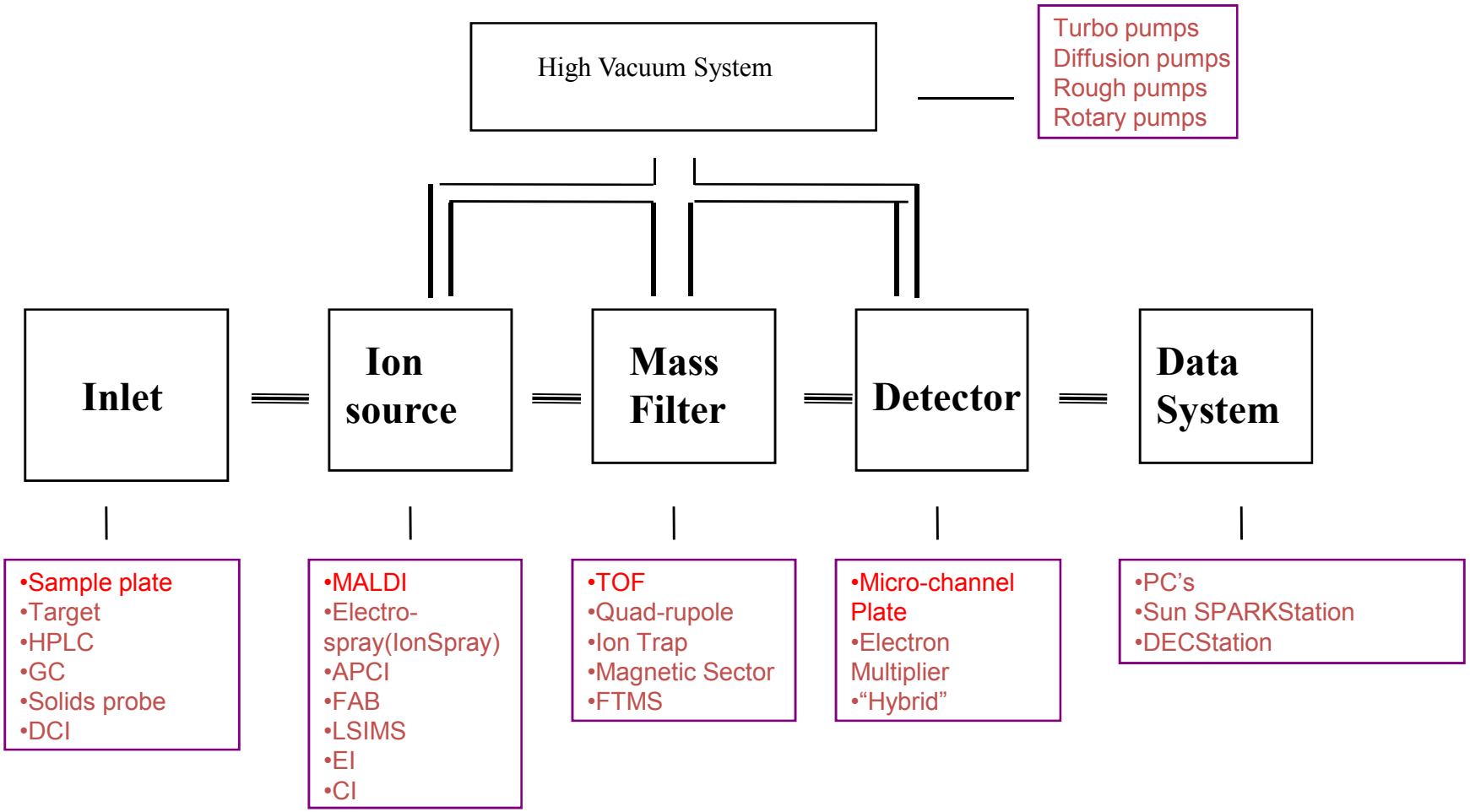
MALDI-TOF 질량분석기

- Matrix Assisted Laser Desorption Ionization
- Time Of Flight Mass Spectrometry

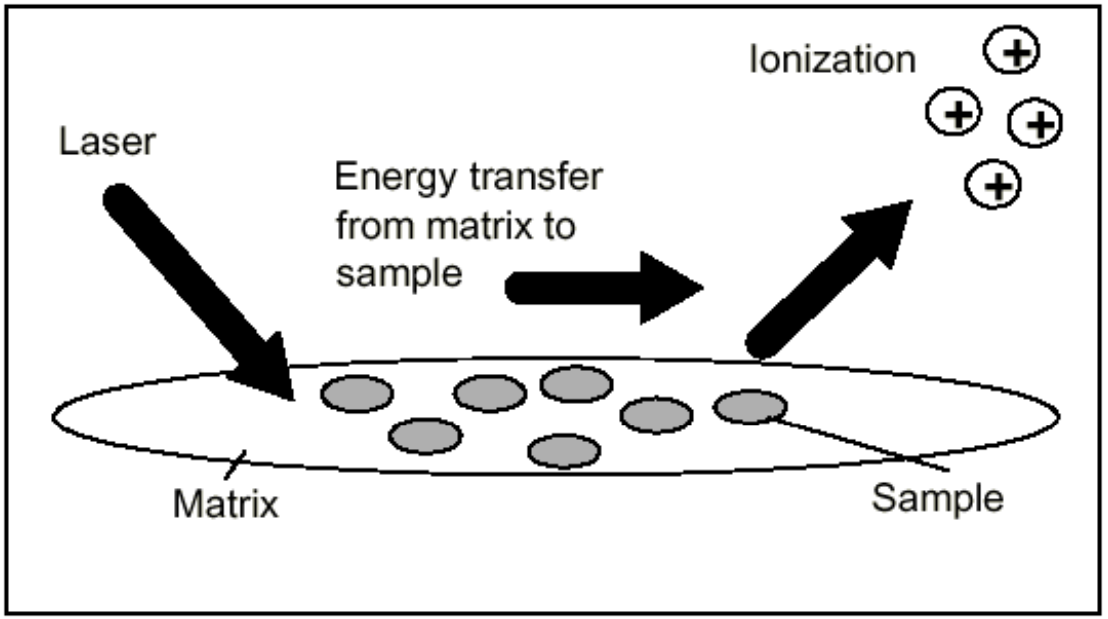
Voyager DE-STR

Voyager DE-Pro

질량분석기의 구성

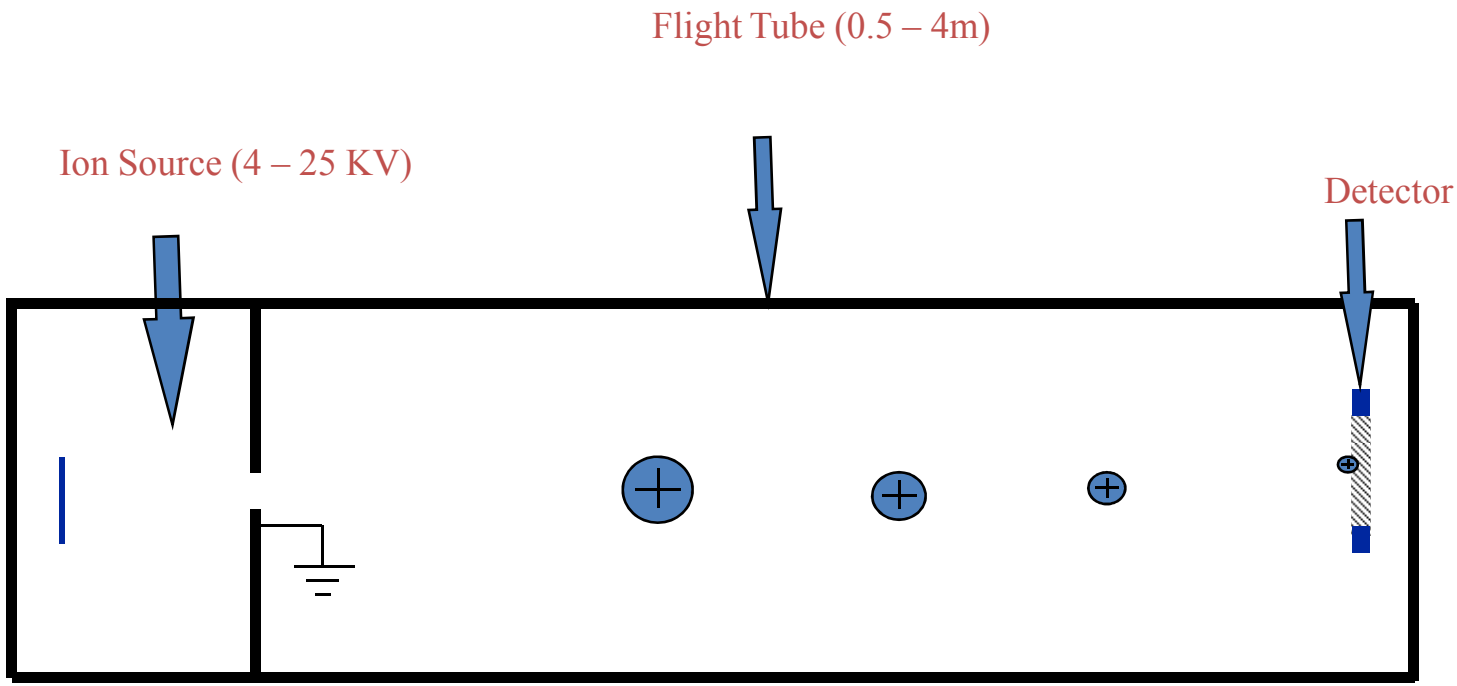

High Vacuum System
Turbo pumps
Diffusion pumps
Rough pumps
Rotary pumps
Inlet
Ion source
Mass Filter
Detector
Data System
•Sample plate
•Target
•HPLC
•GC
•Solids probe
•DCI
•MALDI
•Electro-spray(IonSpray)
•APCI
•FAB
•LSIMS
•EI
•CI
•TOF
•Quad-rupole
•Ion Trap
•Magnetic Sector
•FTMS
•Micro-channel Plate
•Electron Multiplier
•"Hybrid"
•PC's
•Sun SPARKStation
•DECStation

Matrix-Assisted Laser Desorption Ionization (MALDI)



In Matrix-Assisted Laser Desortion Ionization (MALDI), sample is embedded in a low molecular weight, UV-adsorbing matrix that enhances intact desorption and ionization of the sample. The matrix is present in vast excess of sample, and therefore isolates individual sample molecules.

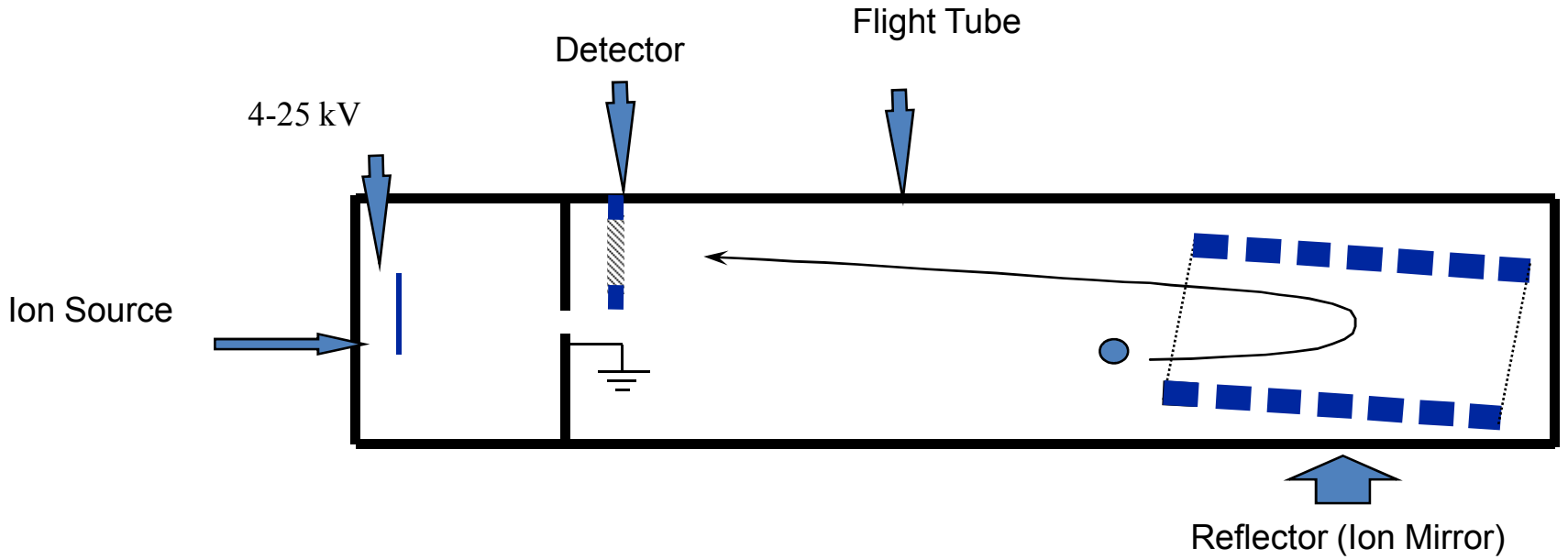
Linear TOF에서 분자 이온들의 분리



가벼운 이온들은 무거운 이온들보다 먼저 검출기에 도착한다.

이 비행시간(time of flight)은 질량으로 다음 식에 의해 변환된다 ($KE=1/2mv^2$).

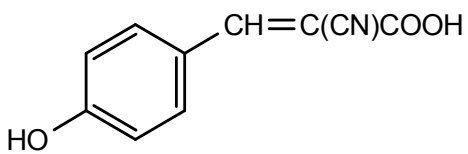
Reflector TOF내 분자이온의 비행



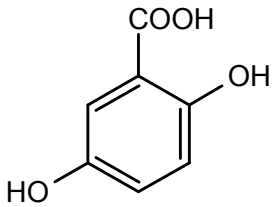
- 주어진 진공상태의 비행관 (tube) 내에서 이온이 비행할 수 있는 거리를 길게 해주어 분리능(resolution)을 향상 되는 디자인
- Peptide등의 정확한 질량 분석이 필요한 Proteomics 응용에 적합함
- Reflector (ion mirror)를 비행관의 끝에 설치하여 이온 비행 방향을 바꾸어 주고 또 하나의 검출기(detector) 를 반대편에 설치함.
- 이 디자인의 장비로는 응용에 따라 Linear 또는 Reflector 모드로 사용함

Matrix 종류

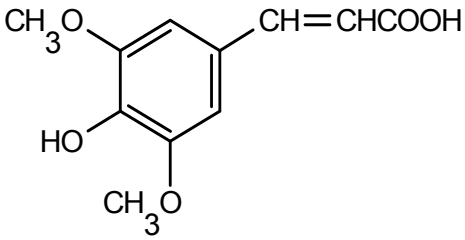
Matrix 종류	응용
Sinapinic Acid	Proteins >10kDa
α -Cyano-4-hydroxy-cinnamic acid (**CHCA**)	Peptides<10kDa
2,5-Dihydroxybenzoic acid (DHB)	Neutral Carbohydrates, synthetic polymers
Super DHB	Proteins, Glycosylated proteins
3-Hydroxypicolinic acid	Oligonucleotides
HABA	Proteins, Oligosaccharides



α-cyano-4-hydroxycinnamic acid



2,5-dihydroxybenzoic acid (2,5-DHB)



Sinapinic acid (3,5-Dimethoxy-4-hydroxy cinnamic acid)

Mass spectrophotometry (MS)

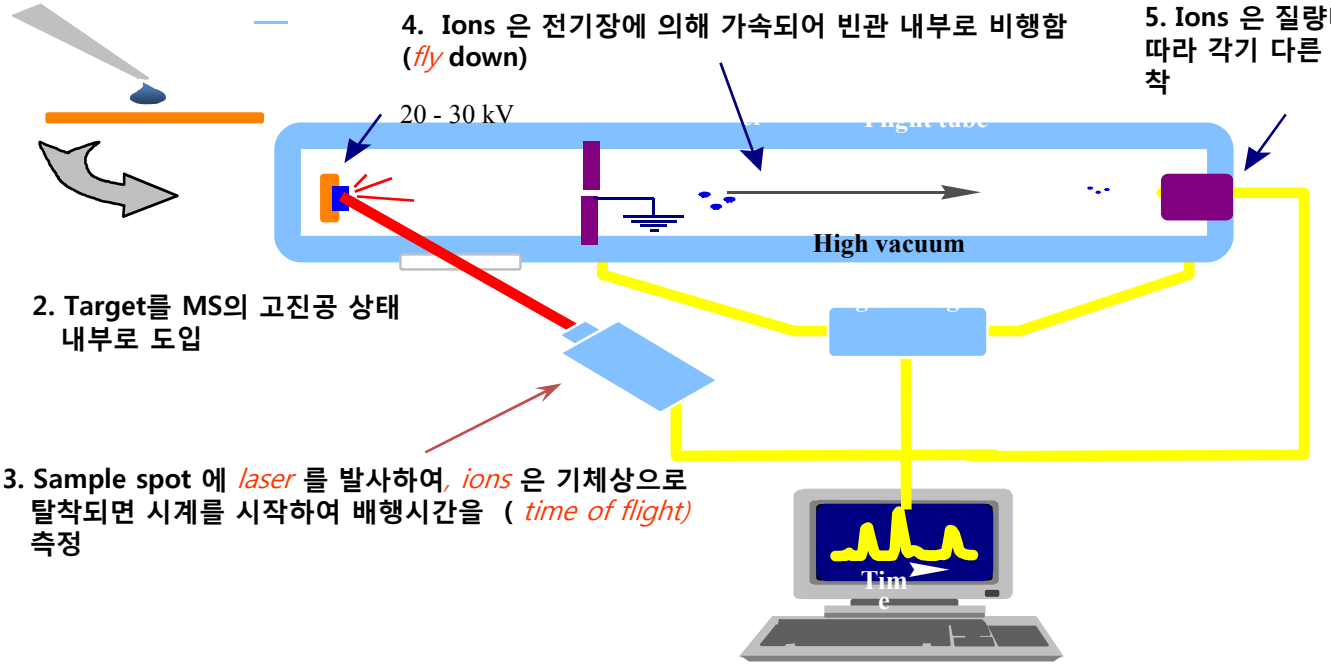
- Peptide mass fingerprinting
  - Gel상에서 원하는 단백질 spot을 찾은 다음, trypsin과 같은proteolytic enzyme을 이용하여 작은 크기의 peptide로 분해
  - 이 peptide의 질량을 MALDI-TOF 질량분석계를 이용하여 측정
  - 여기서 나온 결과 (m/z value)를 가지고 기존의database를 탐색하여 이들과 일치하는 peptide를 찾는방법
- Peak detected
  - average MW 아닌 monoisotopic MW
  - Signal vs. noise구분 가능
- MS/MS: tandem mass spectrometer
  - 분리된 단백질들의 정체확인을 위한 다른 방법은 위에서 얻은 tryptic peptide의 아미노산 배열을 MS/MS를 이용하여 알고 일치하는 sequence를 포함하는 단백질을 db에서 찾아보는 방법

System Components

1. 시료는 *matrix* 와 혼합하여 target에 건조함

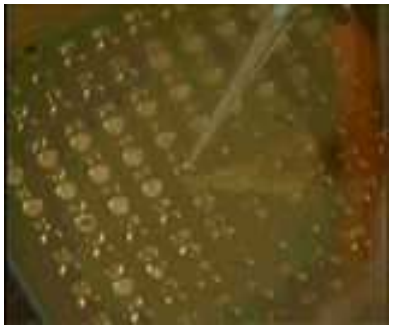
4. Ions 은 전기장에 의해 가속되어 빈관 내부로 비행함 (*fly* down)

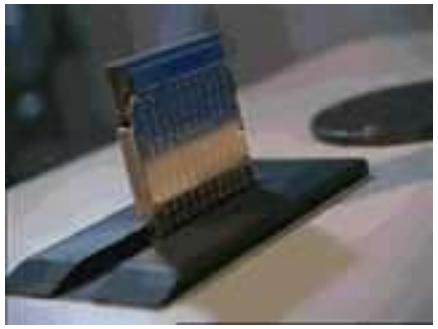
5. Ions 은 질량대 전하(mass to charge) 에 따라 각기 다른 시간(*times)* 에 검출기에 도착

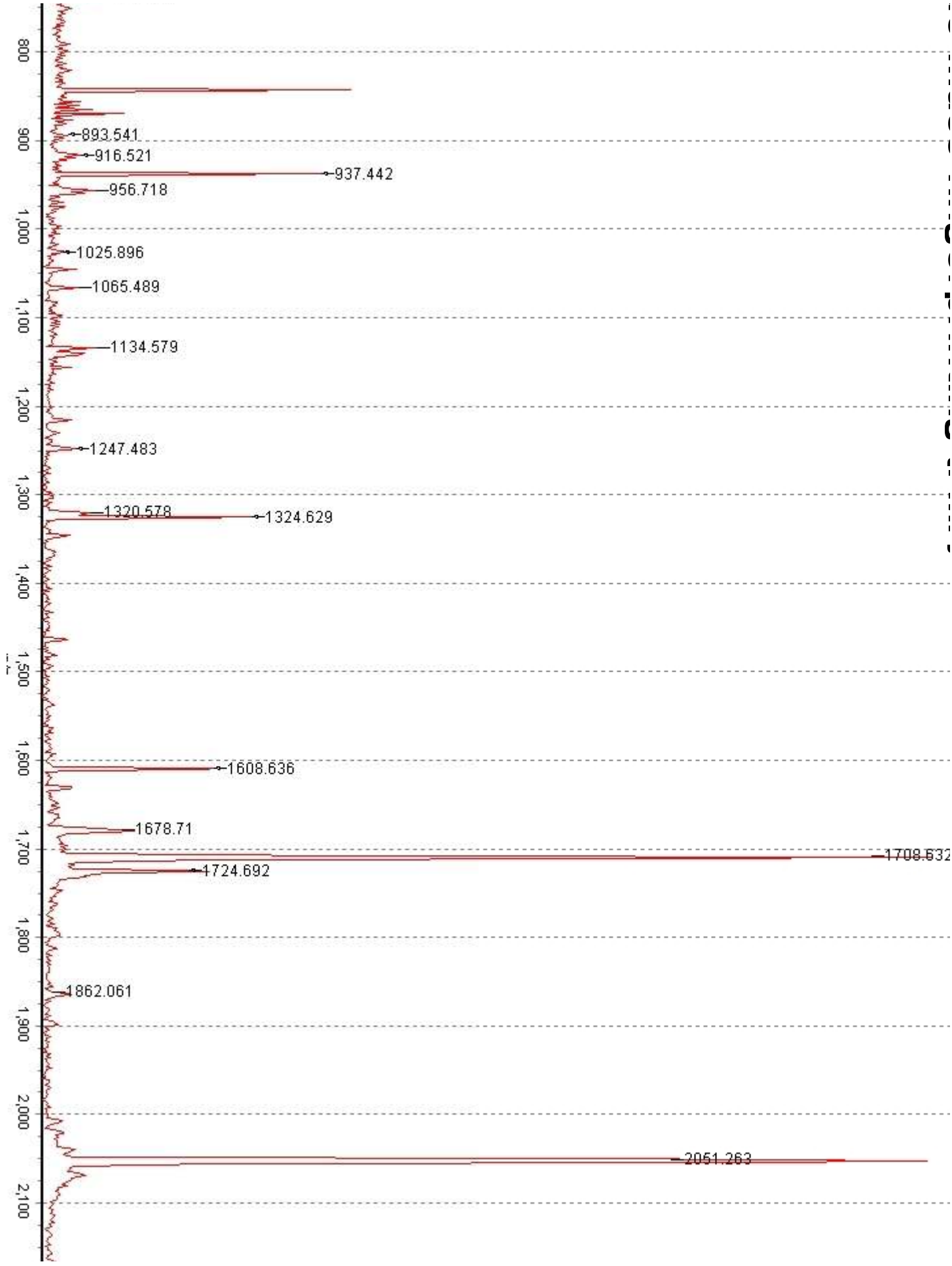


2. Target를 MS의 고진공 상태 내부로 도입

3. Sample spot 에 *laser* 를 발사하여, *ions* 은 기체상으로 탈착되면 시계를 시작하여 배행시간을 (*time of flight)* 측정






800
900
1,000
1,100
1,200
1,300
1,400
1,500
1,600
1,700
1,800
1,900
2,000
2,100
893.541
916.521
937.442
956.718
1025.896
1065.489
1134.579
1247.483
1320.578
1324.629
1608.636
1678.71
1708.632
1724.692
1862.061
2051.263

Protein Identification

Probability of Matching index with theoretical peptide database

Acc. #: P30304 **Species:** HUMAN **Name:** M-phase inducer phosphatase 1 (Dual specificity phosphatase Cdc25A)
Index: 129733 **MW:** 58797 Da **pI:** 6.3

m/z Submitted	MH^+ Matched	Delta ppm	Modifications		Start	End	Missed Cleavages	Database Sequence
972.5135	972.5552	-43			111	119	0	(K) LLGCSPALK (R)
1065.4968	1065.5403	-41			378	385	0	(K) EFVIIDCR (Y)
1091.5472	1091.5485	-1.2			448	456	1	(R) DRLGNEYPK (L)
1092.5343	1092.5842	-46			343	352	0	(K) GYLFHTVAGK (H)
1106.5668	1106.5345	29			467	475	1	(K) GGYKEFFMK (C)
1521.7929	1521.7987	-3.8			358	371	0	(K) YISPEIMASVLNGK (F)
1534.8035	1534.7284	49			175	188	0	(R) QNSAQLGMLSSNER (D)
1537.7725	1537.7936	-14	1	Met-ox	358	371	0	(K)YISPEIMASVLNGK(F)
1705.8585	1705.8873	-17			307	322	0	(K) AHETLHQSLSLASSPK (G)
1778.8241	1778.9012	-43			423	436	1	(K) RVIVVFHCEFSSER (G)
2224.1046	2224.2052	-45	1	Met-ox	358	377	1	(K)YISPEIMASVLNGKFANLIK(E)
2232.2191	2232.1148	47			323	342	1	(K) GTIENILDNDPRDLIGDFSK (G)

Protein Identification

Probability of Matching index with theoretical peptide database

Spot Number	Candidate	MOWSE Score	Cov(%)	Protein MW(Da/pI)	Acession #
1123	ZW10 interactor (ZW10 interacting protein-1)	5300	29	31195/5.0	O95229
6123	DNA-repair protein XRCC3	1.78E+04	35	37850/8.8	O43542
6213	Mitogen-activated protein kinase 3	688	24	43136/6.3	P27361
5417	Caspase-2 precursor (CASP-2) (ICH-1 protea se)	1.59E+07	43	54040/6.2	Q16877

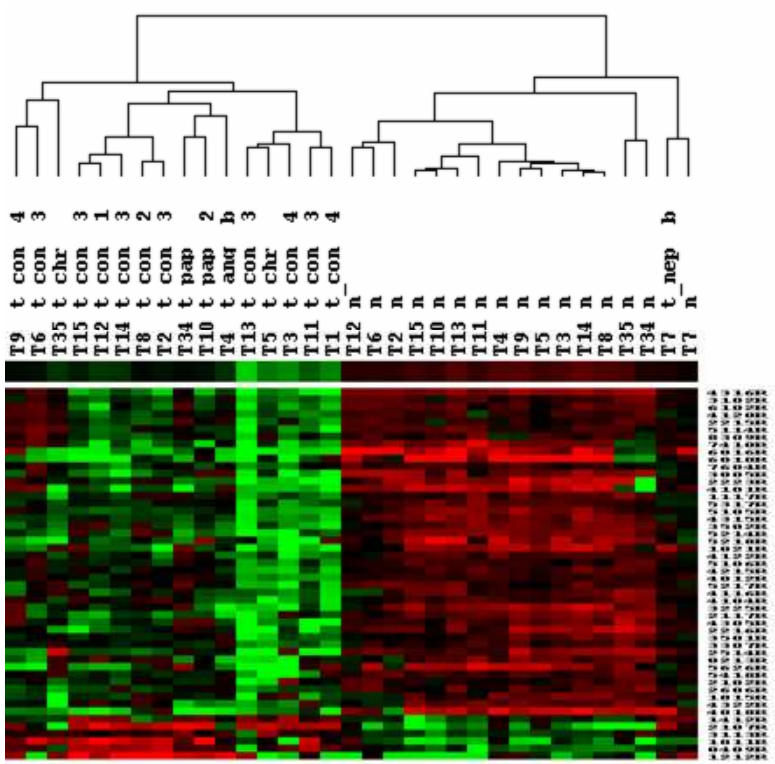
Validation of peptide detected

- Clustergram
  - Reverse clustergram
  - Heat map algorithm
- Distance map tree
  - Phylgenetic algorithm
- Tissue confirmation
  - WB
  - IF
  - IHC

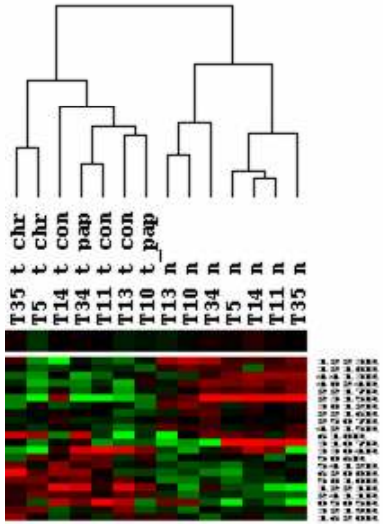
Expression profile and its similarity analysis

By clustering and profile distance calculation

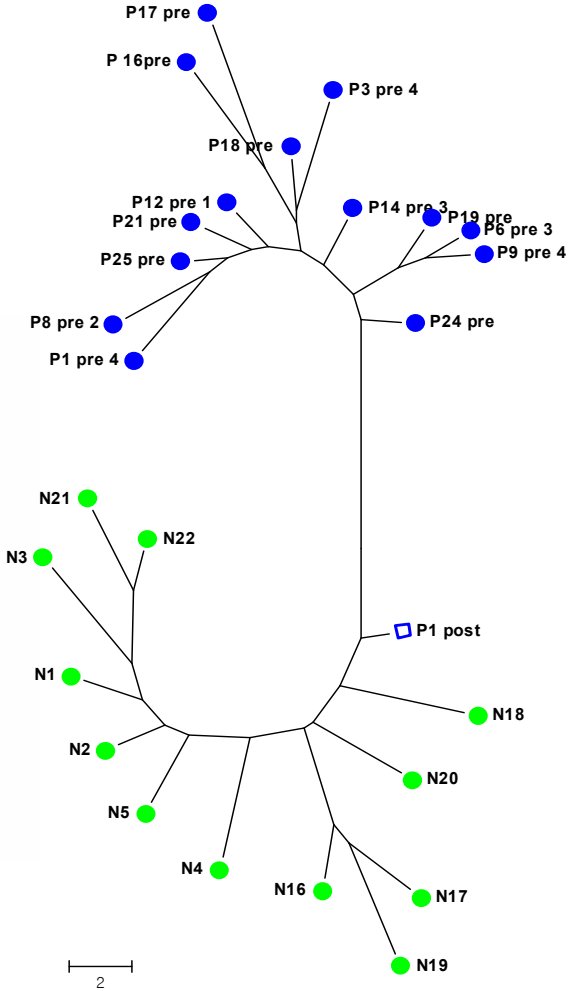
50 spots 0.05<P



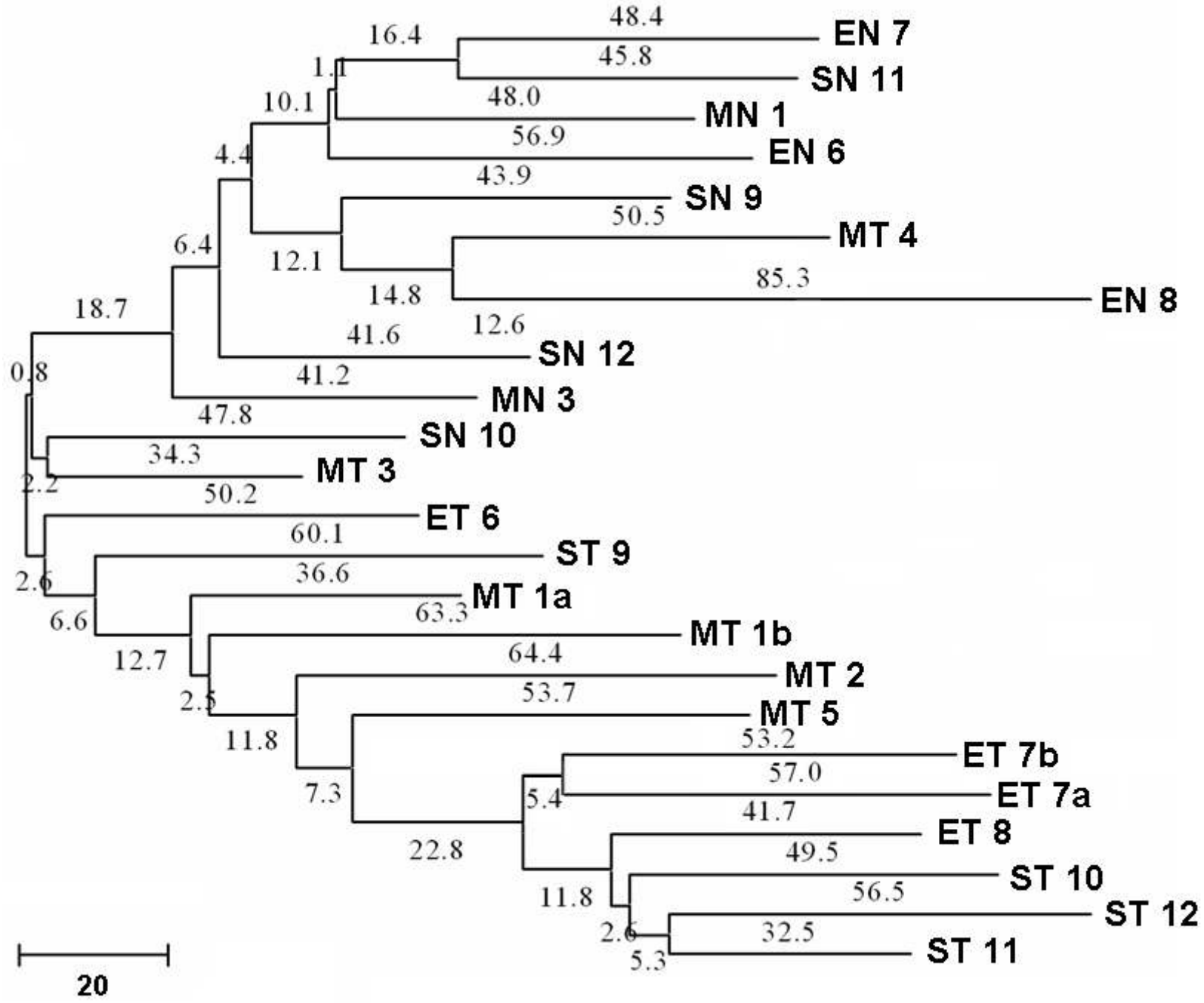
20 spots 0.05<P

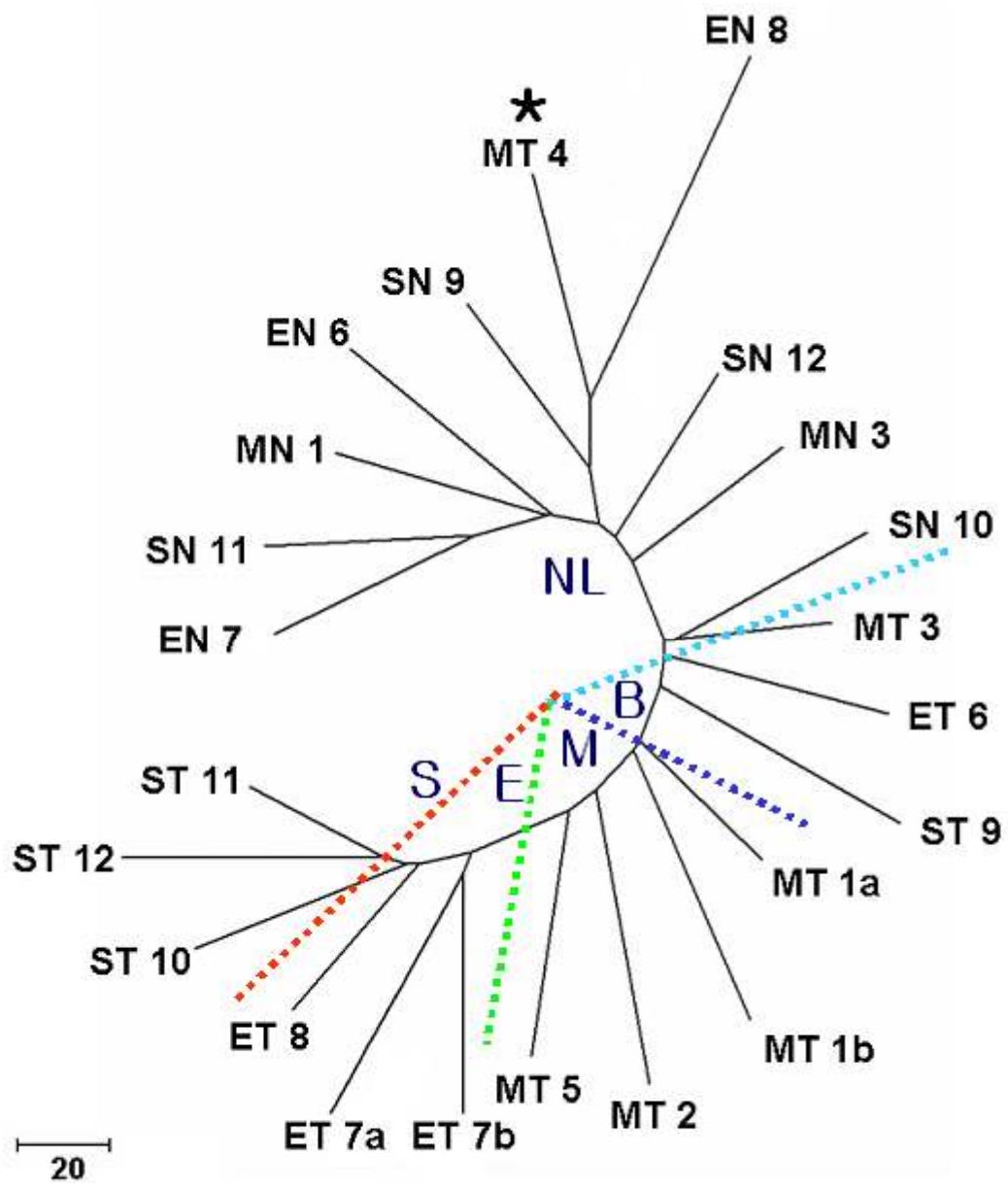


6 spots 0.05<P



Ovary ca proteomics classification



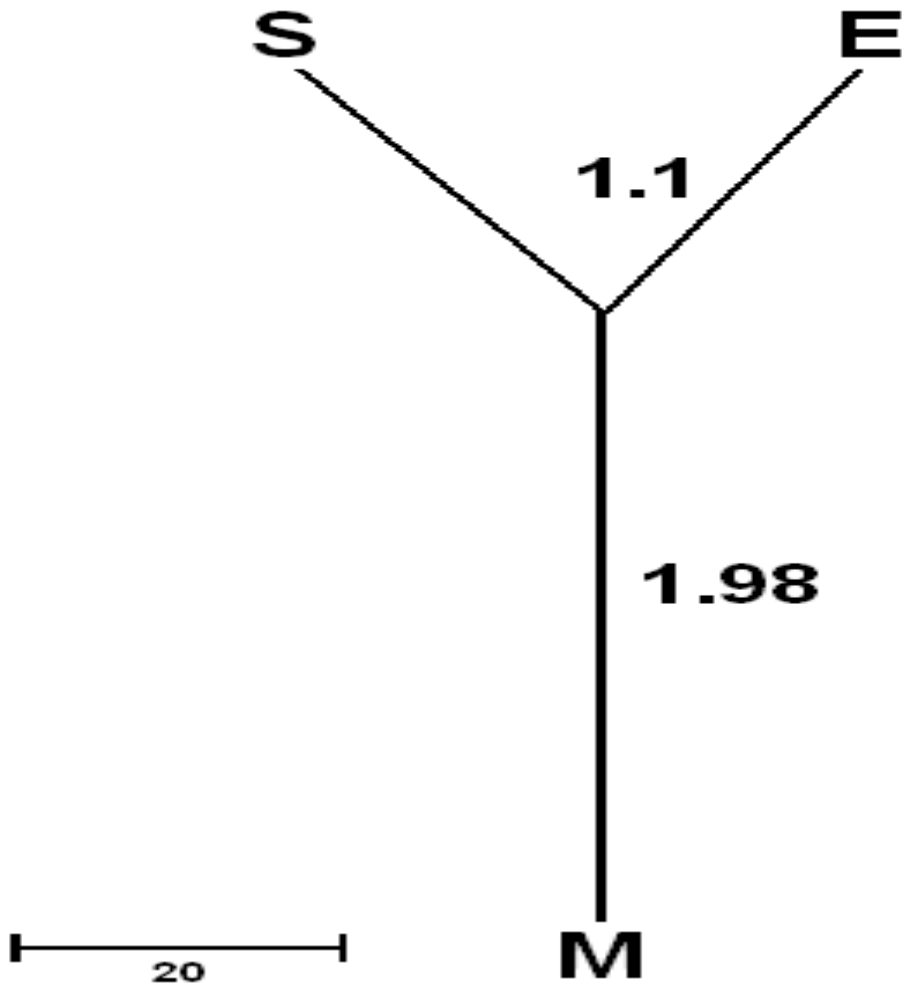


M
- T1: LMP mucinous
  - T1a: benign-looking
  - T1b: IECa
- T2: LMP mucinous with stromal microinvasion
- T3: IMBT
- T4: MMBT
- T5: Mucinous ca

E
- T6: Endometriotic cyst
- T7: Eca, sertoliform
  - T7a: sertoriform
  - T7b: conventional
- T8: Eca, well-diff

S
- T9: LMP Serous tumor
- T10:Serous ca, stage I
- T11:Serous ca,stage III
- T12: Serous ca,stage II

Serous and Endometrioid ca in ovary is similar in proteome

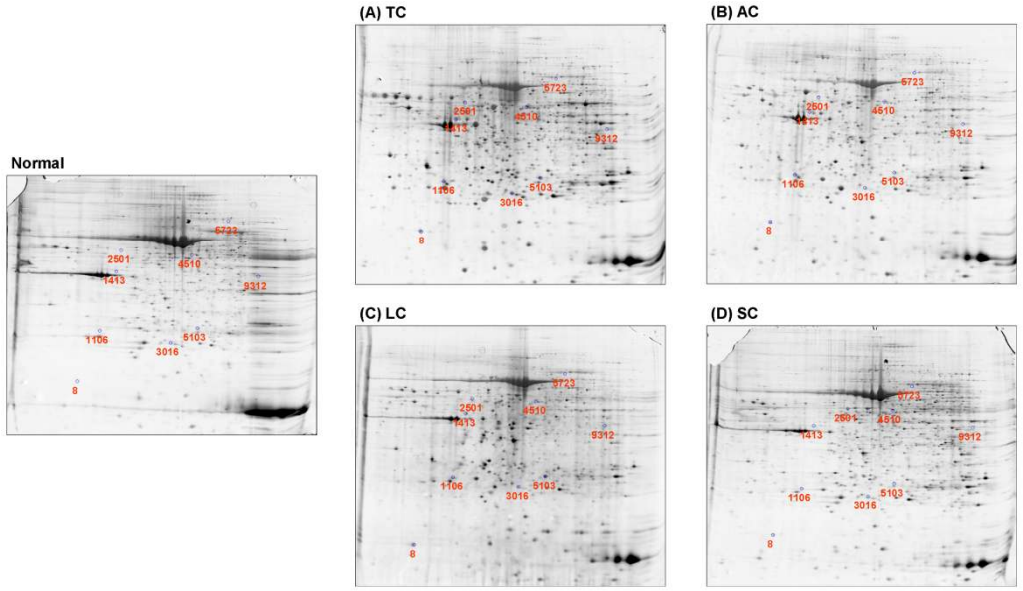


Ovary cancer proteomics classification

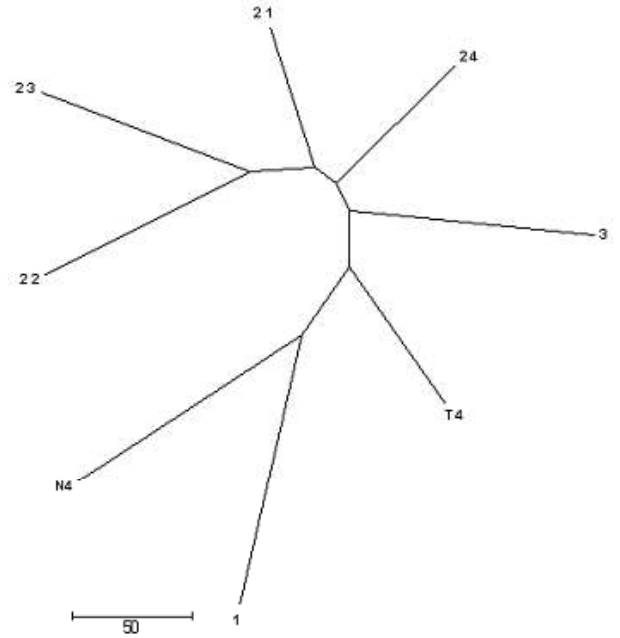
- ***J. Proteome Res.*, 2006, *5* (5), pp 1082–1090**

- **Comparative Proteomics of Ovarian Epithelial Tumors**
- Hee Jung An,[†] Dong Su Kim, Yong Kyu Park, Sei Kwang Kim, Yoon Pyo Choi,[§] Suki Kang,[‡] Boxiao Ding,[§] and **Nam Hoon Cho*[‡§]**
- We analyzed 12 ovarian epithelial tumors using 2D PAGE-based comparative proteomics to construct intra- and inter-tumoral distance map trees and to discover surrogate biomarkers indicative of an ovarian tumor. The analysis was performed after laser microdissection of 12 fresh-frozen tissue samples, including 4 serous, 5 mucinous, and 3 endometrioid tumors, with correlation with their histopathological characteristics. Ovarian epithelial tumors and normal tissues showed an apparent separation on the distance map tree. Mucinous carcinomas were closest to the normal group, whereas serous carcinomas were located furthest from the normal group. All mucinous tumors with aggressive histology were separated from the low malignant potential (LMP) group. The benign-looking cysts adjacent to the intraepithelial carcinoma (IEC) showed an expression pattern identical to that of the IEC area. The extent of change on the lineages leading to the mucinous and serous carcinoma was 1.98-fold different. The overall gene expression profiles of serous or endometrioid carcinomas appeared to be less affected by grade or stage than by histologic type. The potential candidate biomarkers screened in ovarian tumors and found to be significantly up-regulated in comparison to normal tissues were as follows: NM23, annexin-1, protein phosphatase-1, ferritin light chain, proteasome α-6, and NAGK (*N*-acetyl glucosamine kinase). In conclusion, ovarian mucinous tumors are distinct from other ovarian epithelial tumors. LMP mucinous tumors showing histologically aggressive features belong to mucinous carcinoma on the proteomic basis.

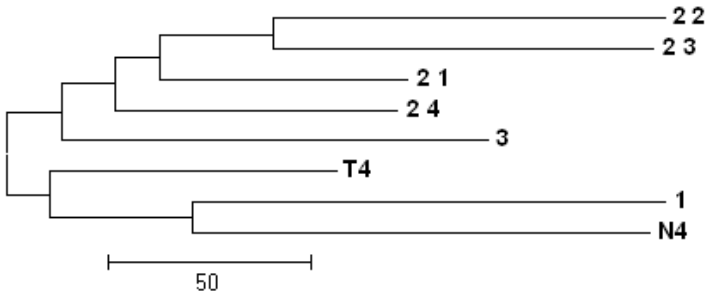
Lung NECa typing Recategorization



A



B



Comparative proteomics in Lung neuroendocrine cancer

J. Proteome Res., 2006, *5* (3), pp 643–650

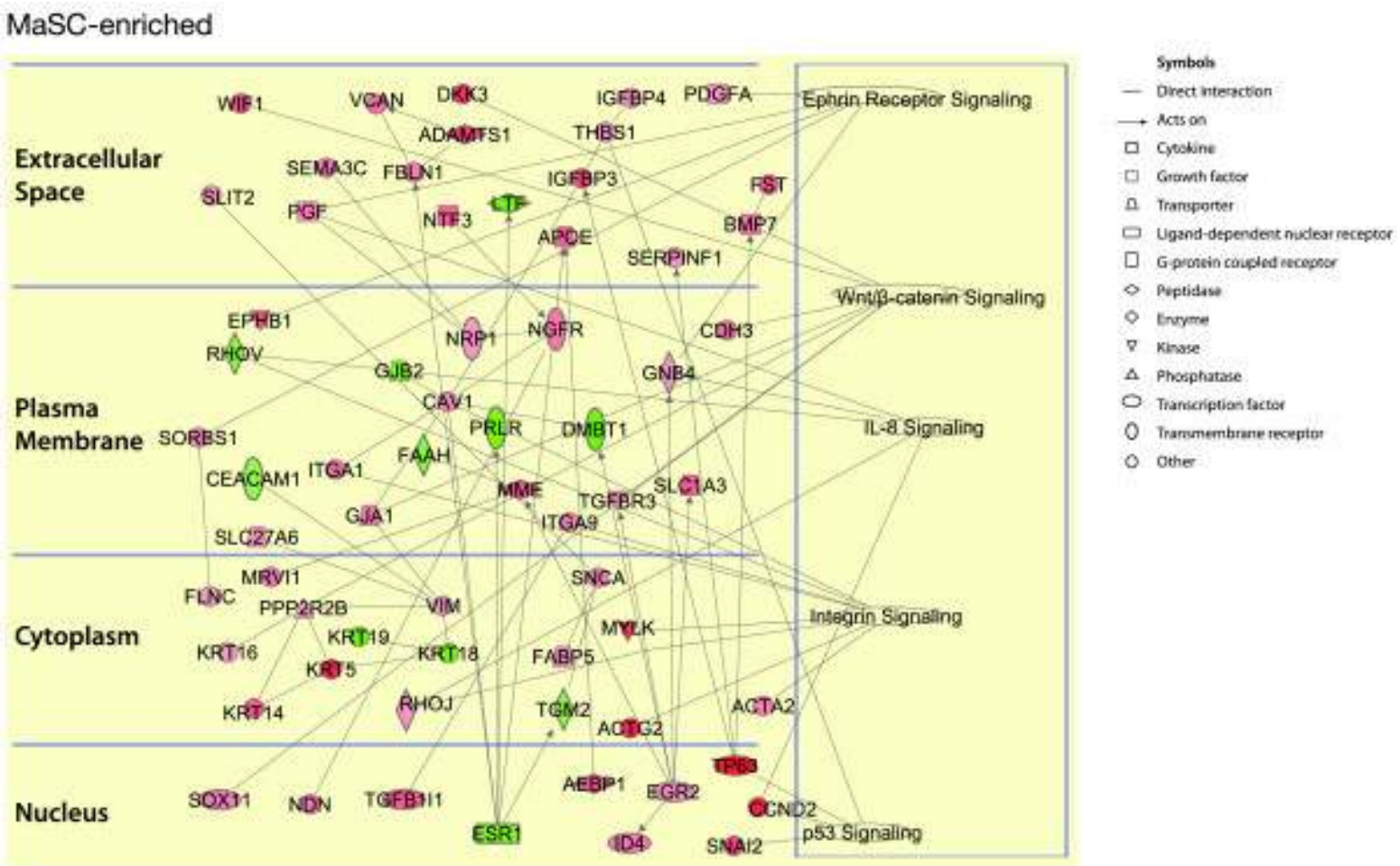
Comparative Proteomics of Pulmonary Tumors with Neuroendocrine Differentiation

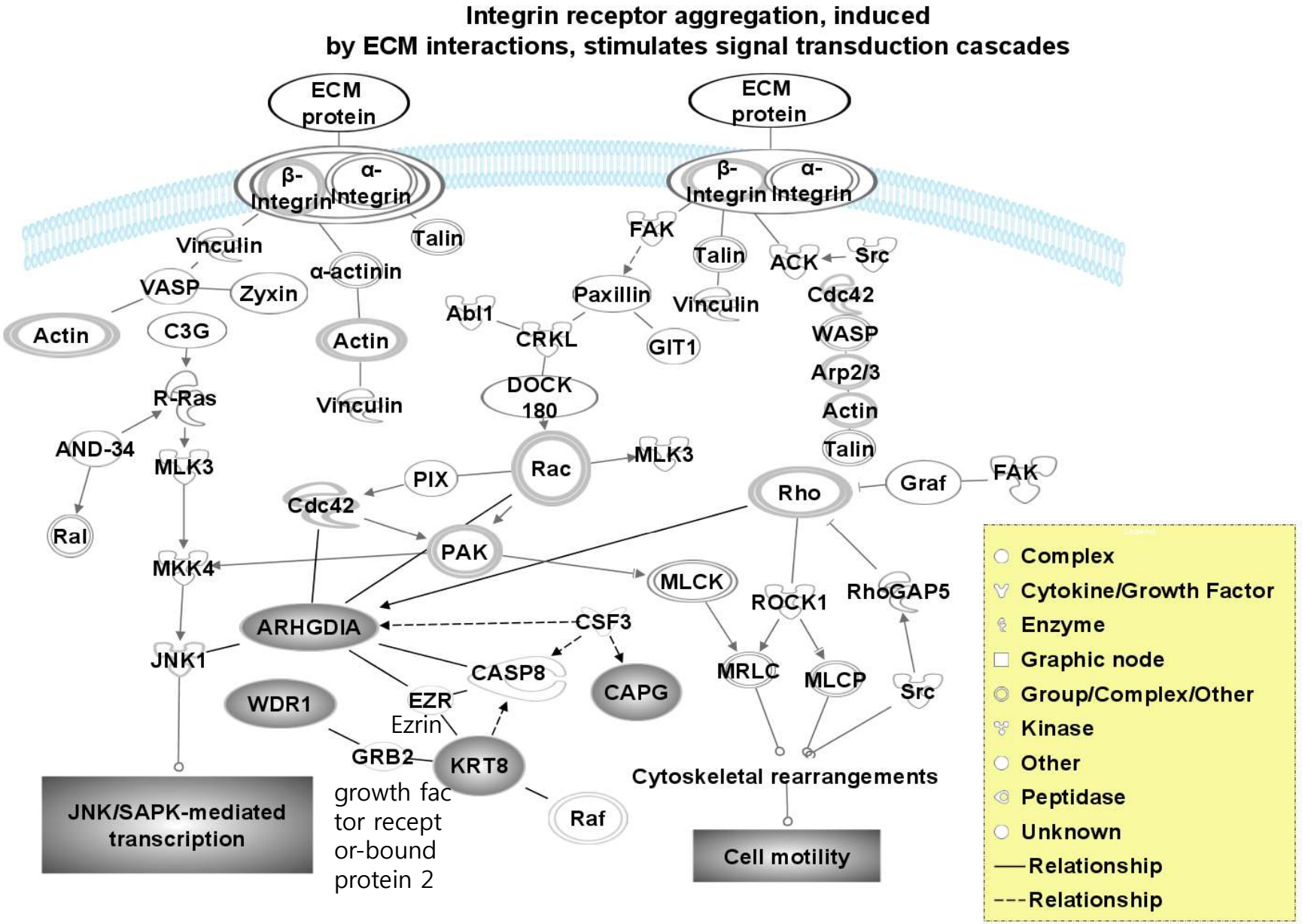
Nam Hoon Cho,*,†,§ Eun Suk Koh,‡ Dong Wha Lee,‡ Haeryoung Kim,† Youn Pyo Choi,†,§ Sang Ho Cho,† and Dong Su Kim|

We aimed to evaluate neuroendocrine pulmonary tumors (NEPT) by a novel method involving map tree construction by comparing all of the protein spots. We performed a proteomics analysis to assess the similarities in protein expression between neuroendocrine pulmonary tumors (NEPT), including typical carcinoids (TC), atypical carcinoids (AC), large cell neuroendocrine carcinomas (LCNEC) and small cell carcinomas (SCLC). Total protein lysates were obtained from seven histologically confirmed frozen NEPT tissues, including 1TC, 2 SCLC, and 4 cases ranging from AC to LCNEC. 2-DE demonstrated that TC was similar to normal lung. AC, LCNEC, and SCLC were similar to each other, forming a group separate from TC, however, SCLC at an early stage showed a similarity to TC. MALDI analysis detected 9 surrogate endpoint biomarkers, including eIF5A1, GST M3, cytokeratin 18 (CK 18), FK506-binding protein p59, p63, MAGE-D2, mitochondrial short-chain enoyl-coenzyme A hydratase 1, tranferrin and poly (rC) binding protein 1. Immunohistochemical staining revealed a gradual decrease in expression rate of p63 and CK 18 with poor differentiation of NEPT. Our results demonstrate that (1) the comparative proteomics of NEPT match the WHO classification except for AC and LCNEC; (2) SCLC show differences in their proteomics according to tumor stage; and (3) CK 18 and p63 may be useful as diagnostically and prognostically available markers.

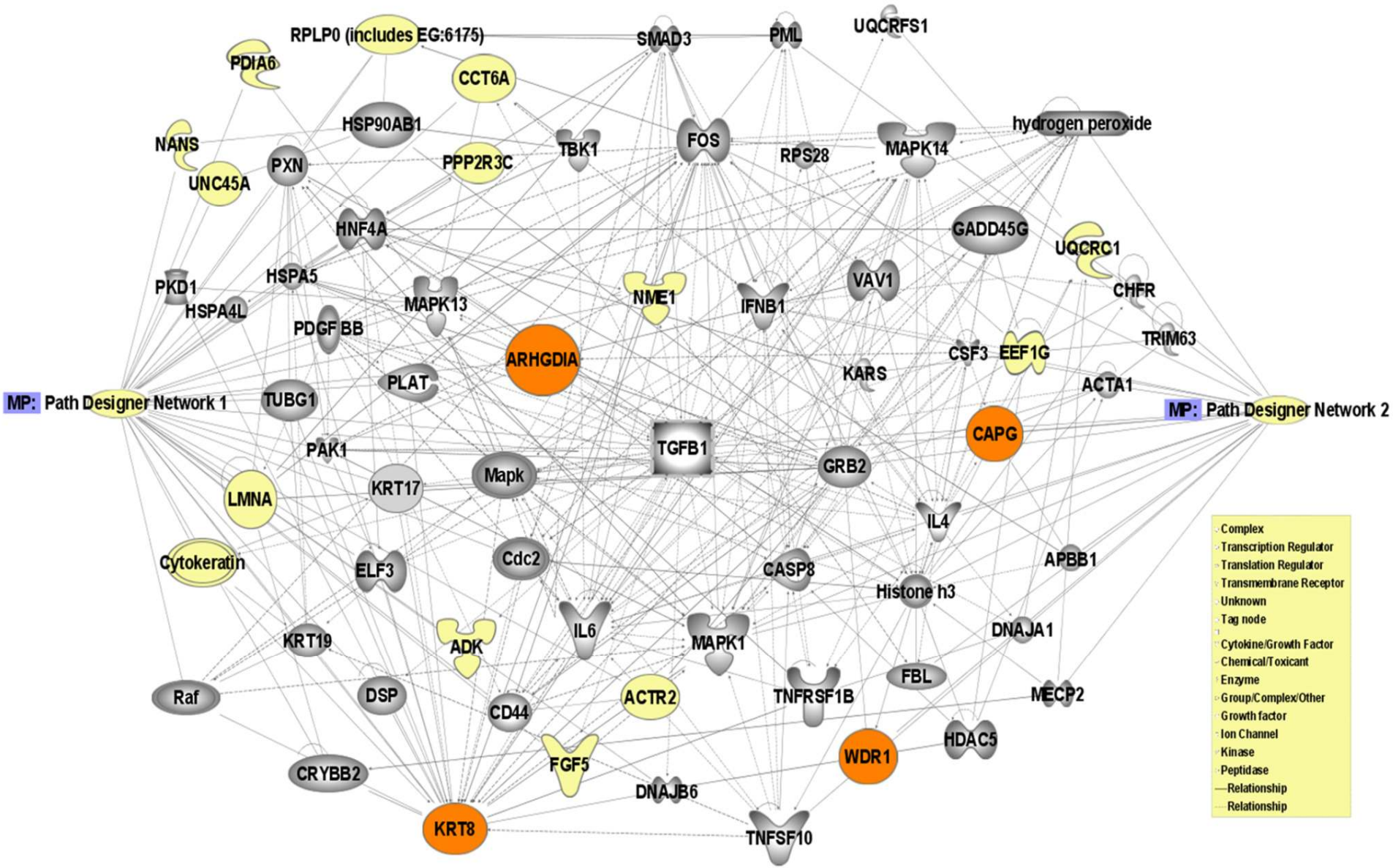
Example of narrow-down validation

IPA from proteomics or cDNA array in breast ca

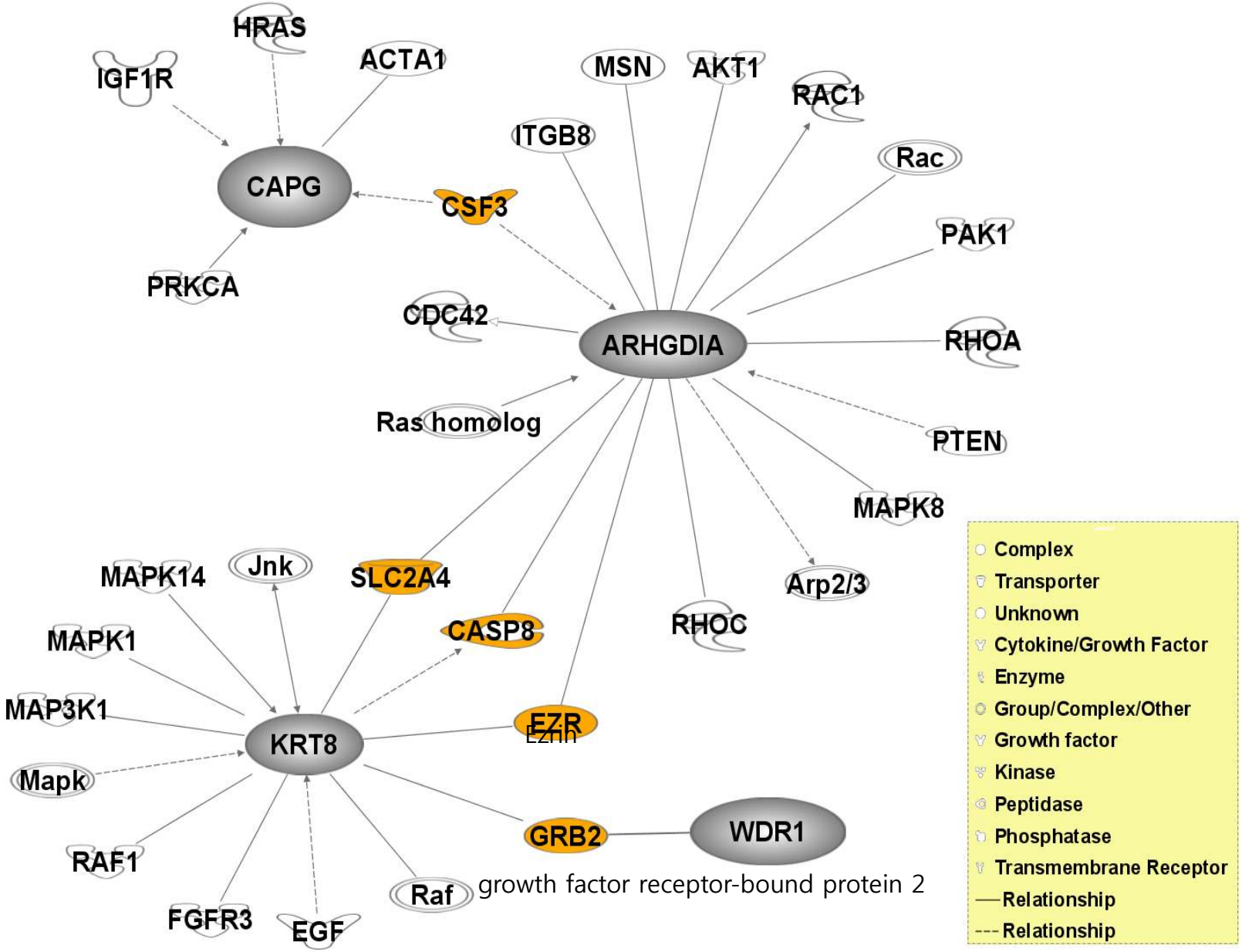


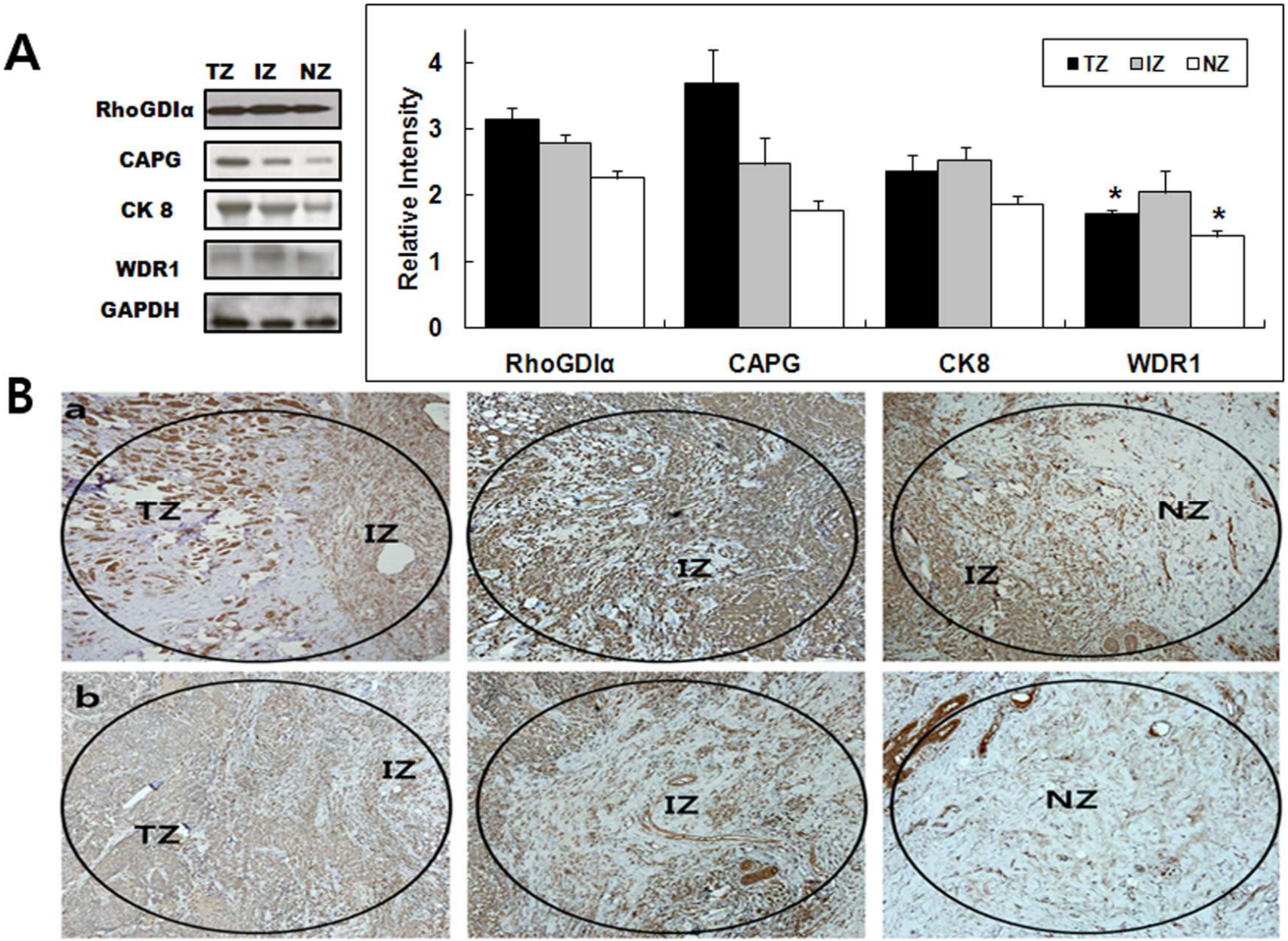


Protein network in regulation of actin-based motility by Rho. The diagram shows that a motility network including ARHGDA, WDR1, KRT8, and CAPG can be constructed through surrogate molecules in regulation of actin-based motility by Rho. These four molecules are closely linked to one another, but are not directly associated. Gray represents high upregulation and validation of protein expression of related genes by IPA analysis.


RPLP0 (includes EG:6175)
SMAD3
PML
UQCRFS1
PDIA6
CCT6A
HSP90AB1
hydrogen peroxide
NANS
PXN
PPP2R3C
TBK1
FOS
RPS28
MAPK14
UNC45A
HNF4A
GADD45G
UQCRC1
PKD1
HSPA5
MAPK13
NME1
IFNB1
VAV1
CHFR
HSPA4L
PDGF BB
TRIM63
ARHGDIA
CSF3
EEF1G
PLAT
KARS
ACTA1
MP: Path Designer Network 1
TUBG1
MP: Path Designer Network 2
CAPG
PAK1
TGFB1
GRB2
Mapk
KRT17
LMNA
IL4
Cytokeratin
ELF3
Cdc2
CASP8
APBB1
Histone h3
IL6
DNAJA1
KRT19
ADK
MAPK1
Raf
DSP
ACTR2
TNFRSF1B
FBL
MECP2
CD44
HDAC5
WDR1
FGF5
CRYBB2
DNAJB6
KRT8
TNFSF10
Complex
Transcription Regulator
Translation Regulator
Transmembrane Receptor
Unknown
Tag node
Cytokine/Growth Factor
Chemical/Toxicant
Enzyme
Group/Complex/Other
Growth factor
Ion Channel
Kinase
Peptidase
Relationship
Relationship

Gene 정보 찾기 :
http://www.ncbi.nlm.nih.gov/sites/entrez?Db=gene&Cmd=retrieve&dopt=full_report&list_uids=7430




A
TZ IZ NZ
RhoGDIα
CAPG
CK 8
WDR1
GAPDH
Relative Intensity
0
1
2
3
4
TZ IZ NZ
RhoGDIα
CAPG
CK8
WDR1
*
*
B
a
TZ
IZ
IZ
IZ
NZ
b
IZ
TZ
IZ
NZ

Comparative proteomics in breast cancer regarding invasion

- J Proteome Res. 2010 Nov 5;9(11):5638-45.
- **Proteomic molecular portrait of interface zone in breast cancer.**
- Kang S, Kim MJ, An H, Kim BG, Choi YP, Kang KS, Gao MQ, Park H, Na HJ, Kim HK, Yun HR, Kim DS, Cho NH.

  Department of Pathology, Yonsei University College of Medicine, Seoul, Korea.

Abstract

- Surgical tumor margins are intended to encompass residual tumor cells but may not always accurately delineate the boundary between tumor and normal tissue. Efforts to define tumor margins based on molecular analysis have achieved limited success. Furthermore, no clinical trials have addressed the scope of the tumor microenvironment. Here, we considered the tumor cell population and surrounding microenvironment in delineating tumor margins, classifying breast cancer into tumor and normal zones, and introducing the concept of an interface zone, the region between the invading tumor front and normal tissue, which develops during tumor invasion and metastasis through remodeling of the tumor microenvironment. Pathological signatures of invasion markers in tumor tissues are most dynamic within the invading tumor front. We compared protein profiles of tumor, normal, and interface zones using MALDI-MS. Proteins upregulated in the interface zone were identified by peptide mass fingerprinting and confirmed by database searching with chemically assisted MALDI-PSD spectra. Upregulation was confirmed for Rho GDIα, CAPG, WDR1, and CK8 by Western and immunohistochemical analyses. Our results demonstrate that the molecular profile of the interface zone is unique and suggest that upregulation of proteins here may be related to progression and metastasis of breast carcinomas.

comparative proteomics in Ut cervical cancer in a/w HPV

Proteomics 2005, 5, 1481–1493

REGULAR ARTICLE

Proteomic analysis of progressive factors in uterine cervical cancer

Yoon Pyo Choi2, Suki Kang1, Sunghee Hong1, Xianhe Xie2 and ***Nam Hoon Cho2***

Human papillomavirus (HPV) infections play a crucial role in the progress of cervical cancer. The high-risk HPV types are frequently associated with the development of malignant lesions. Some of the latest studies have demonstrated that the high-risk HPV 16 and 18 are predominantly detected in the more aggressive cancers. In the present study, we aimed to establish the proteomic

profiles and characterization of the tumor related proteins by using two-dimensional gel electrophoresis (2-DE) and matrix-assisted laser desorption/ionization-time of flight mass spectrometry (MALDI-TOF MS). For proteomic analysis, patients infected by HPV 16 or 18 were included in this study. We compared nuclear protein and cytoplasmic protein, separately by using the subcellular fraction. Differential protein spots between cervical cancer with high-risk HPV, HPV 16 or HPV 18, and HaCaT cell lines were characterized by 2-DE. Those proteins analyzed by peptide mass fingerprinting based on MALDI-TOF MS and database searching were

the products of oncogenes or proto-oncogenes, and the others were involved in the regulation of cell cycle, for general genomic stability, telomerase activation, and cell immortalization. However, there was no difference in protein characterization for cervical cancer between HPV 16 and HPV 18 infection. Nonetheless, these data are valuable for the mass identification of differentially

expressed proteins involved in human uterine cervical cancer. Moreover, the data has enormous value for establishing the human uterine cervical cancer proteome database that can be used in screening a molecular marker for the further study of human uterine cervical cancer, and also for studying any correlation among the cancers induced by HPV.

Keywords:

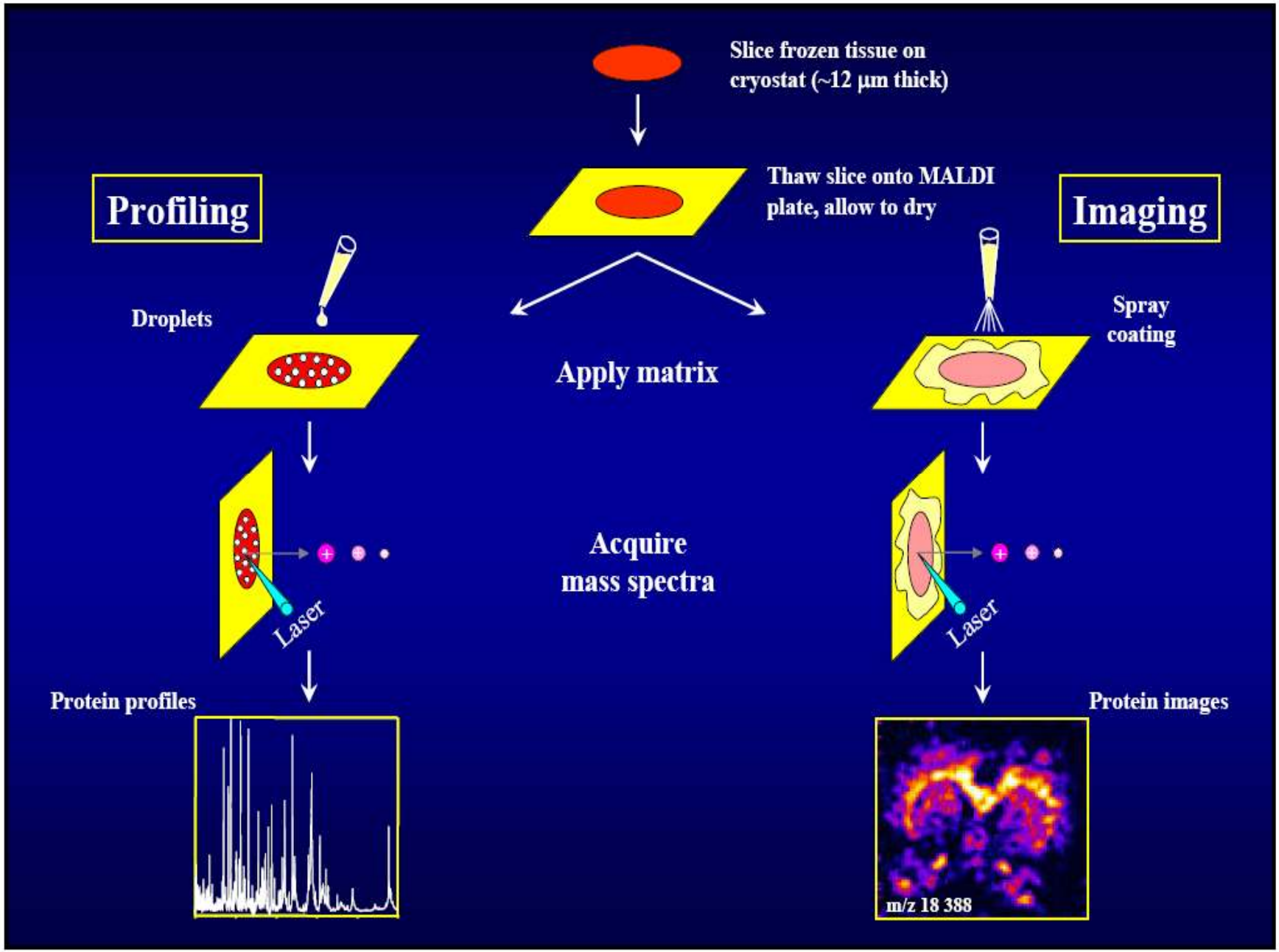
Differential expression protein / Human uterine cervical cancer / Matrix-assisted laser

desorption/ionization-time of flight mass spectrometry / Two-dimensional gel electrophoresis

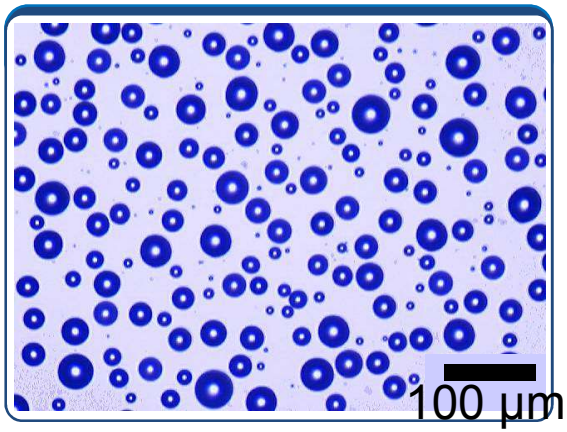
MALDI-Imaging MS

MALDI-MS

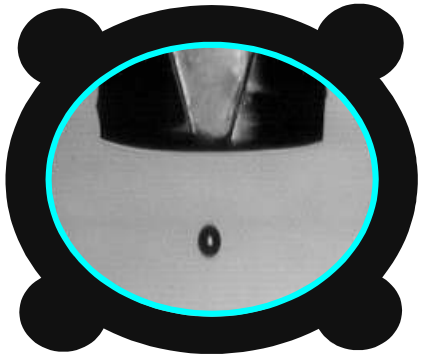
Image localization


Slice frozen tissue on cryostat (~12 µm thick)
Thaw slice onto MALDI plate, allow to dry
Profiling
Imaging
Droplets
Spray coating
Apply matrix
Acquire mass spectra
Laser
Laser
Protein profiles
Protein images
m/z 18 388

1. Bruker Imaging prep.



2. Shimadzu Chip 1000

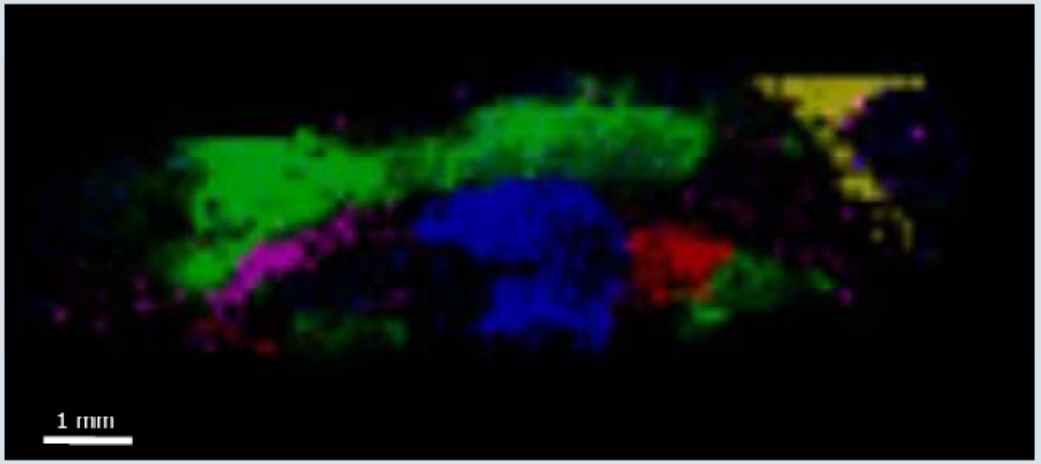


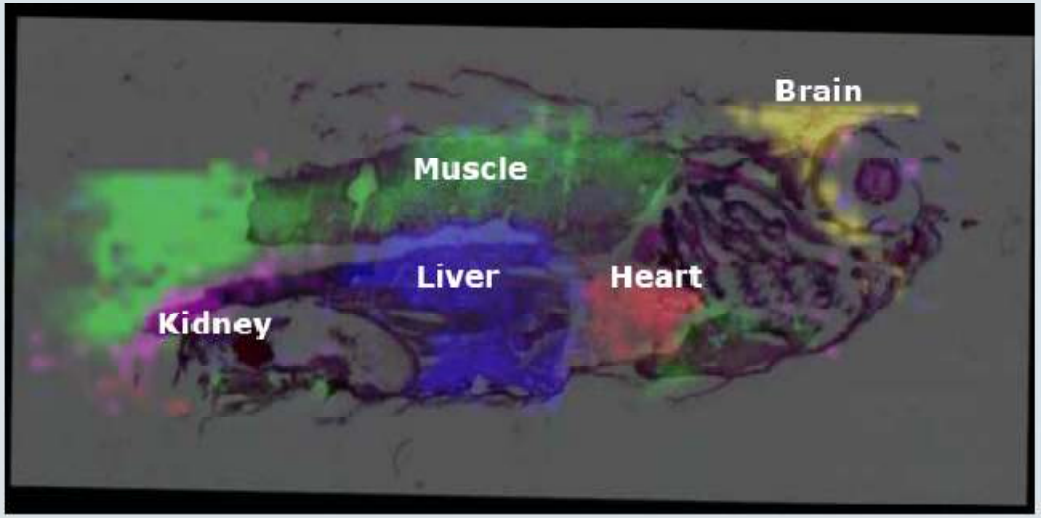
MALDI Imaging

Principle:

Acquisition of spatially resolved MALDI spectra

Intensity of specific signals is translated into colour intensity





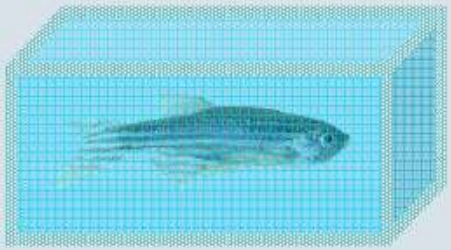
Example Zebrafish:

Resolution 200 µm

Matrix
Sinapinic acid

From Tissue to Image – the process

catch



freeze

take image
Video microscope/scanner

cryosection
-15 ° C

1. Apply MALDI matrix on tissue section
2. Align lab coordinates on slide and image in the software

ethanol wash
top. scanning

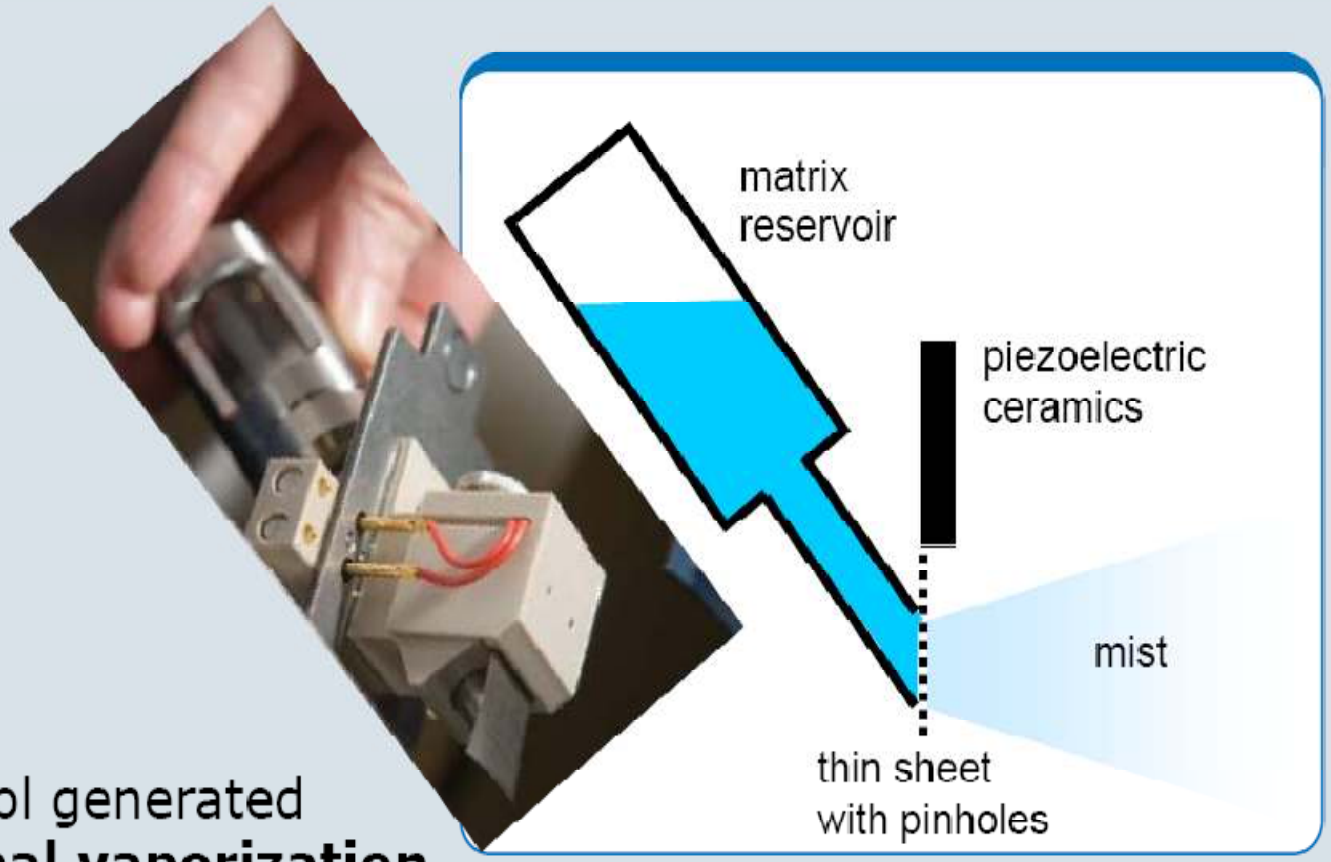
ImagePrep

Compact instrument
with integrated touchpanel
control

Teflon coated spray chamber

Spray head
with matrix reservoir

ImagePrep Droplet Generation



ImagePrep spray head

- Matrix aerosol generated by **vibrational vaporization**
- Soft, gravitational droplet deposition, **controlled atmosphere**

Tissue MALDI imaging system

Main Unit

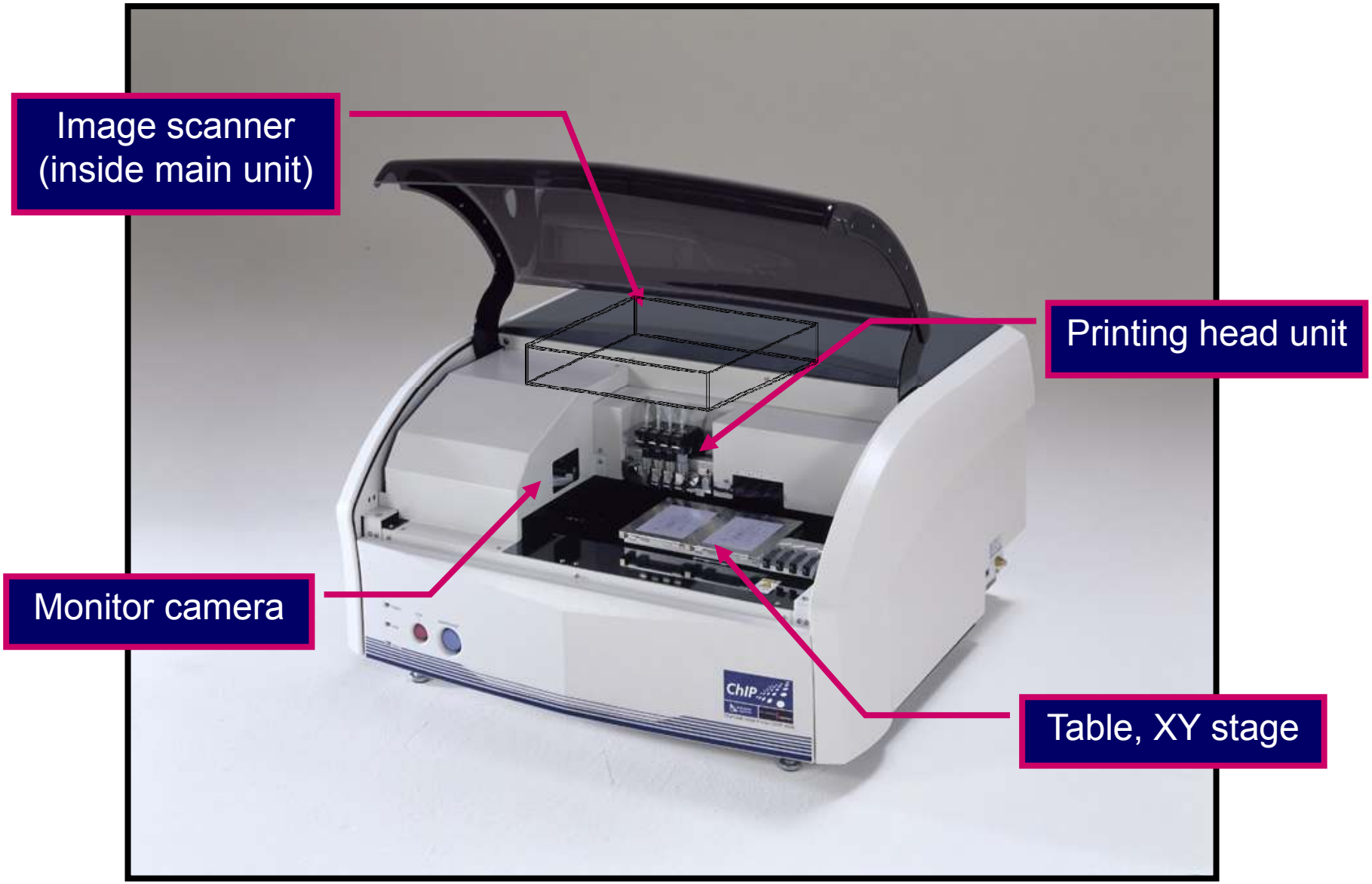
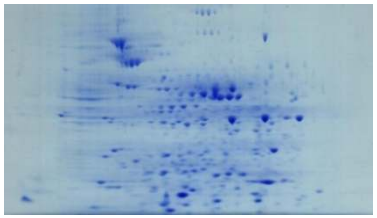


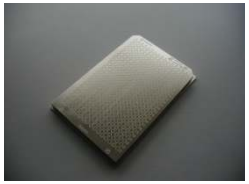
Image scanner
(inside main unit)
Printing head unit
Monitor camera
Table, XY stage

Microscale Proteome Analysis

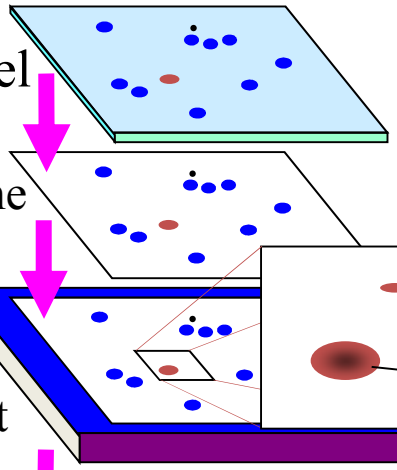


Gel

Processing excised gel piece

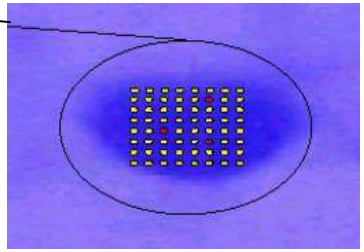


Target plate



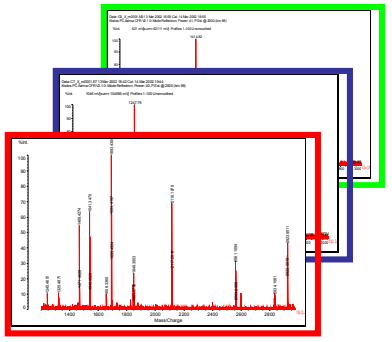
On-Membrane MALDI

AXIMA MALDI MS



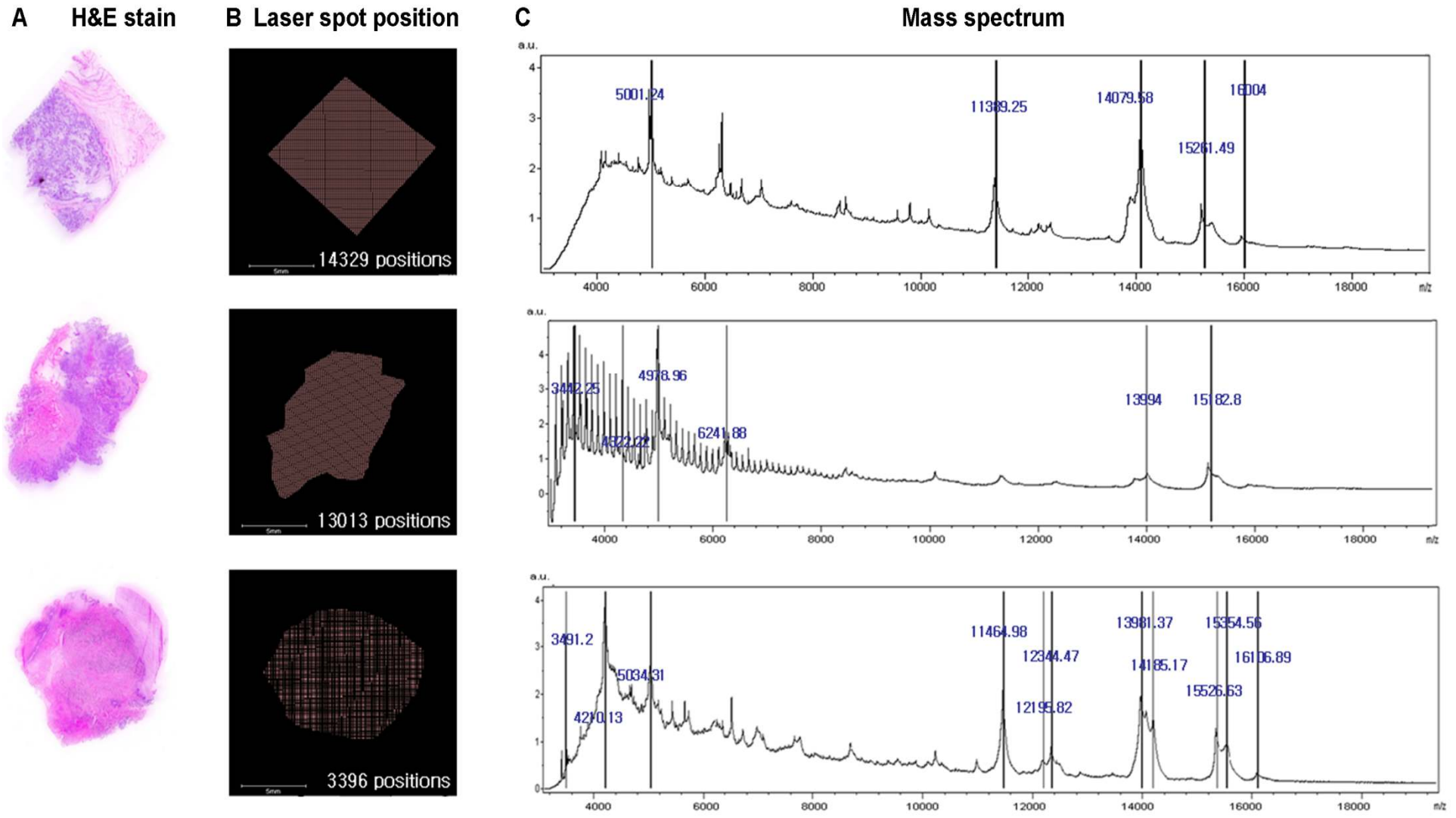
Processing on membrane

ChIP

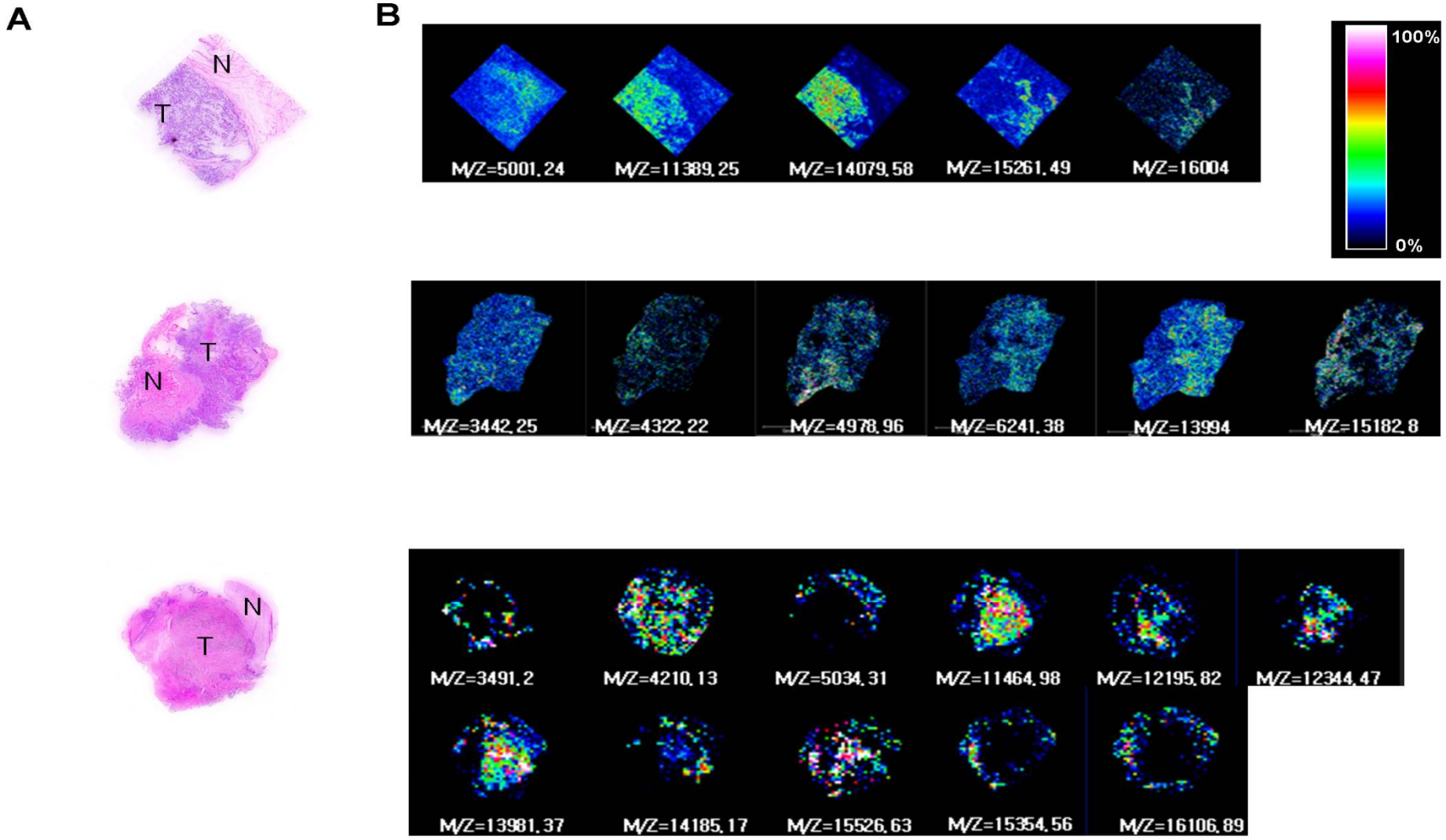


Peptide Mass Fingerprinting (PMF)

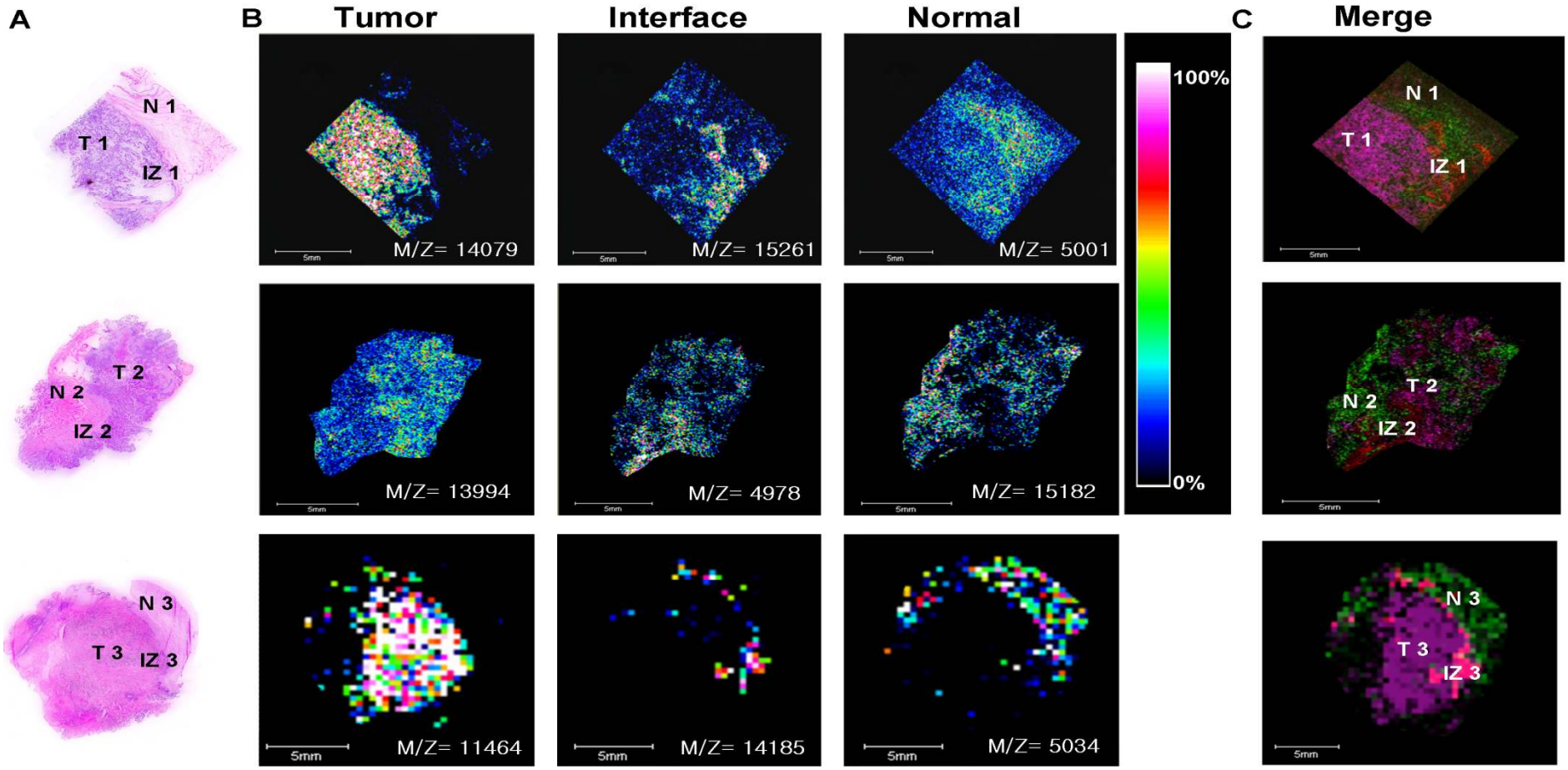
MALDI imaging-PMF using FFT



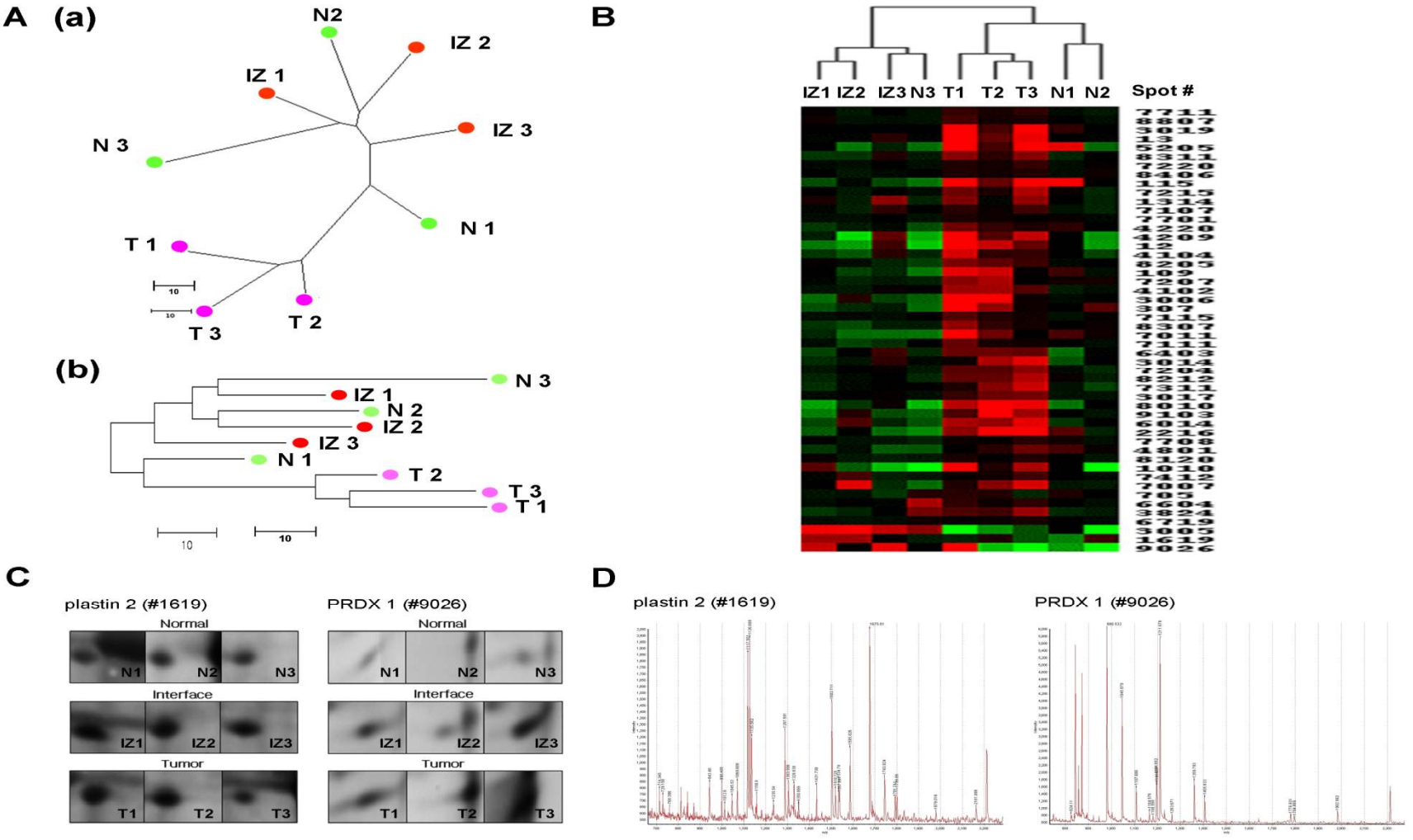
MALDI-tissue image correlation



Ovarian Cancer biomarker



Tissue MALDI–2D GE–PMF

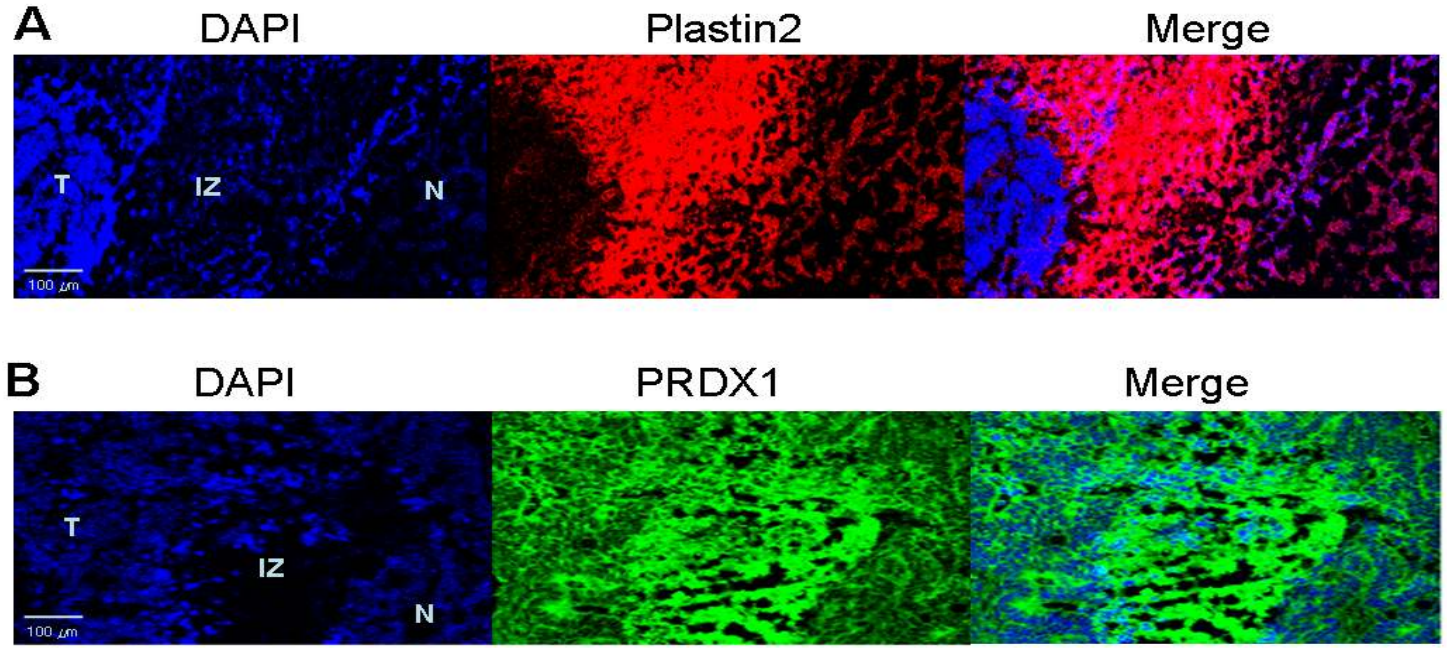


Marker identification and validation

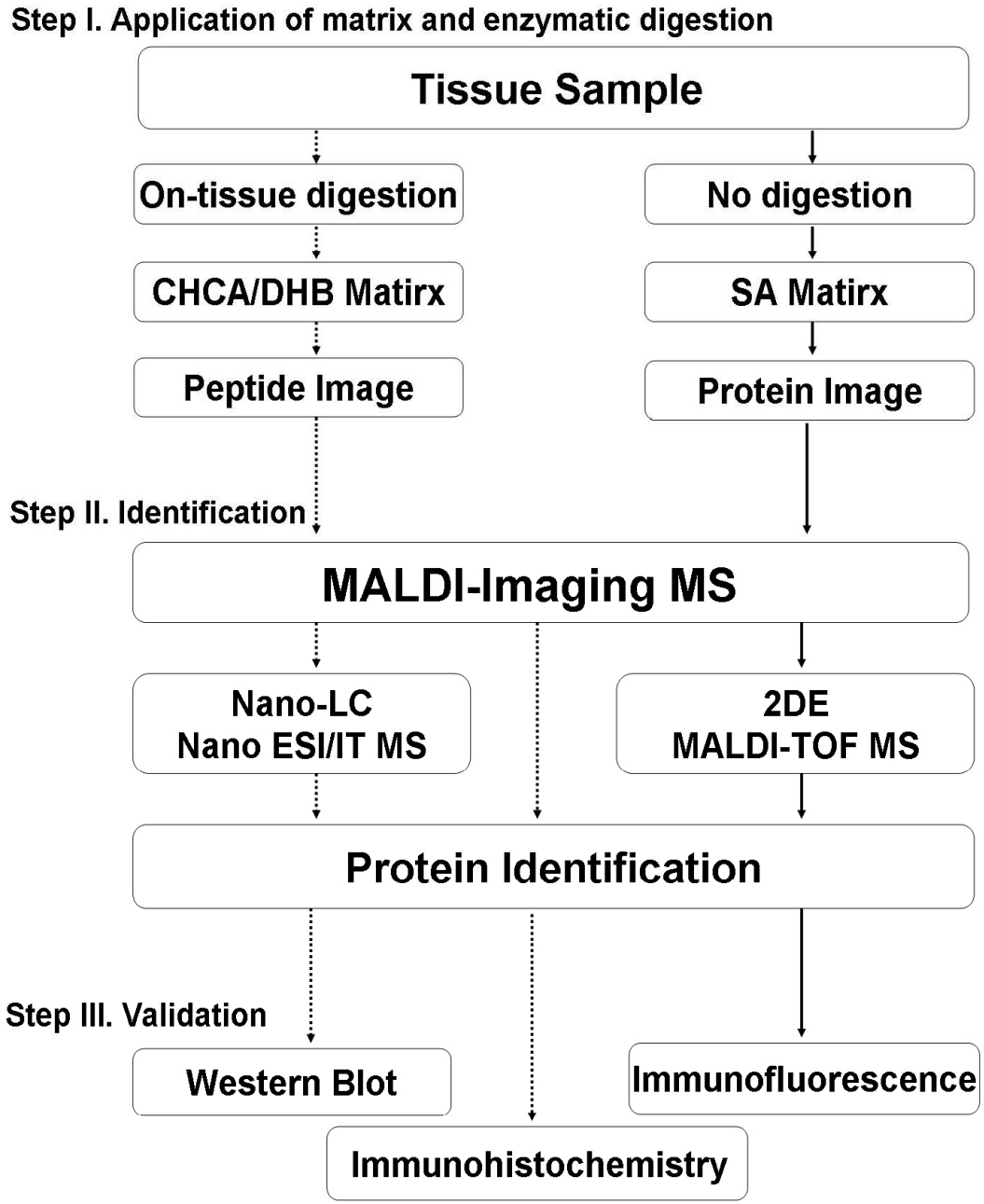
Table 1. Protein Identification of Interface Overexpression.

Spot No.	Protein name	Accession No.	PI a/ PI b	MW a/ MW b (K Da)	Sequence Coverage (%)	Est'Z Score	Fold IZ/N c	Fold IZ/T d
9026	PRDX 1	CAI13096	9.58/6.4	25.82/19.13	46	2.36	3.1	1.8
1619	Plastin 2	P13796	4.81/5.2	67.26/70.84	31	2.41	1.76	1.72

*The peptide profiles of the protein spots treated with trypsin were analyzed by MALDI-TOF MS. ProFound (http://129.85.19.192/profound_bin/WebProFound.exe) was used to search the protein database for protein identification using peptide mass fingerprinting (PMF). The mass and p*I* values specified are theoretically matched by a database search. a: observed, b: theoretically calculated, *c*: the rate of increase in intensity (average interface sample intensity/average normal sample intensity), d: the rate of increase in intensity (average interface sample intensity/average tumor sample intensity).



Schematic representation of the MALDI-IMS technology



1) Application of matrix and enzymatic digestion of proteins.
2) Identification of proteins using protein database matching.
3) Validation of protein identities. Recently, identification of proteins may be directly performed from the MALDI-IMS without any analytic process of extraction and proteolysis.

Solid line, performed in the present study;
Dotted line, not performed in the present study, but possible.
CHCA, α-Cyano-4-hydroxycinnamic acid;
DHB, 2,5-dihydroxybenzoic acid;
SA, sinapinic acid;
MS, mass spectrometry;

Journal of proteome research

Technical Note

Molecular Proteomics Imaging of Tumor Interfaces by Mass Spectrometry

SUKI KANG, Hyo Sup Shim, Jong Sik Lee, Dong Su Kim, Hak Yong Kim, Seong Hyun Hong, Pan Soo Kim, Joo Heon Youn, and ***Nam Hoon Cho***

J. Proteome Res., ***12 October 2009***

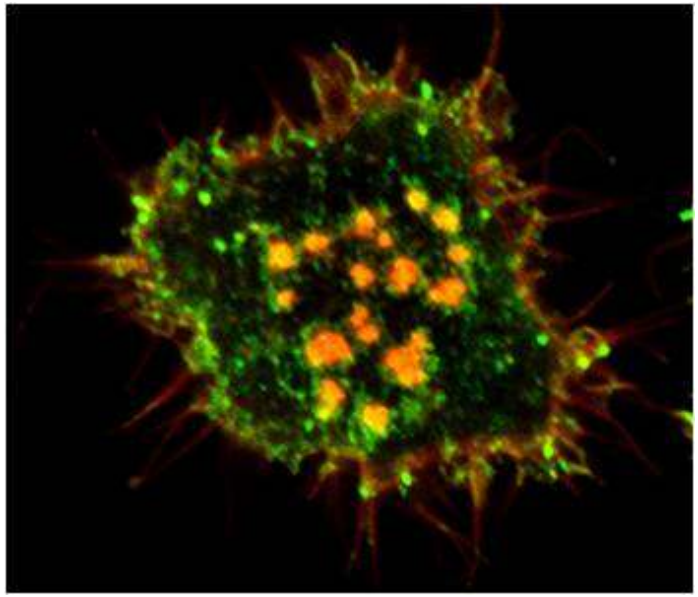
Abstract

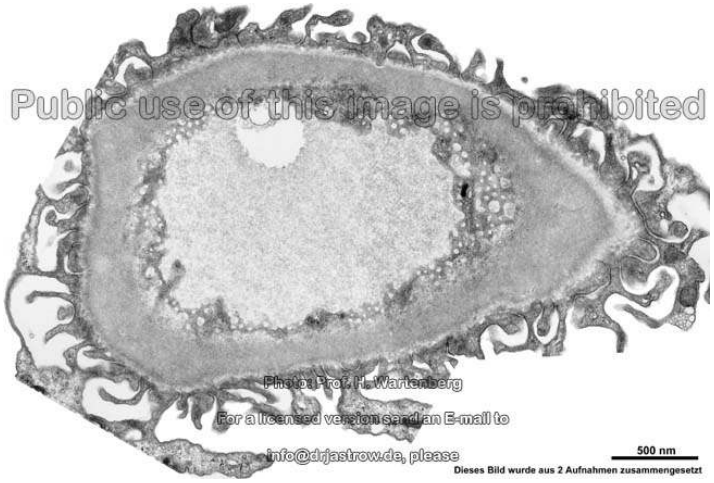
The specific molecular profiles of ovarian cancer interface zones (IZ), the region between tumors and normal tissues, were evaluated using a new method involving matrix-assisted laser desorption/ionization (MALDI)-imaging mass spectrometry (IMS). We analyzed three ovarian serous carcinomas using MALDI-IMS. Principal component analysis (PCA) was used to evaluate the quality of tissue spatial features based on MALDI-IMS, and for analysis of large datasets of MALDI-IMS. 2-dimensional gel electrophoresis and fluorescence microscopy were used to verify interface-specific proteins. Unique profiles were identified for the tumors, the normal zone, and the IZ. Through MALDI analysis, two interface-specific proteins, plastin 2 and peroxiredoxin 1 (PRDX 1) were identified as differentially regulated between zones. Fluorescence microscopy revealed high expression levels of plastin 2 and PRDX 1 along the IZ of ovarian tumors. This comparative proteomics study using tissue MALDI-IMS, suggested that the IZ is different from the adjacent tumor and normal zones, and that plastin 2 and PRDX 1 may be interface markers specific to ovarian tumors.

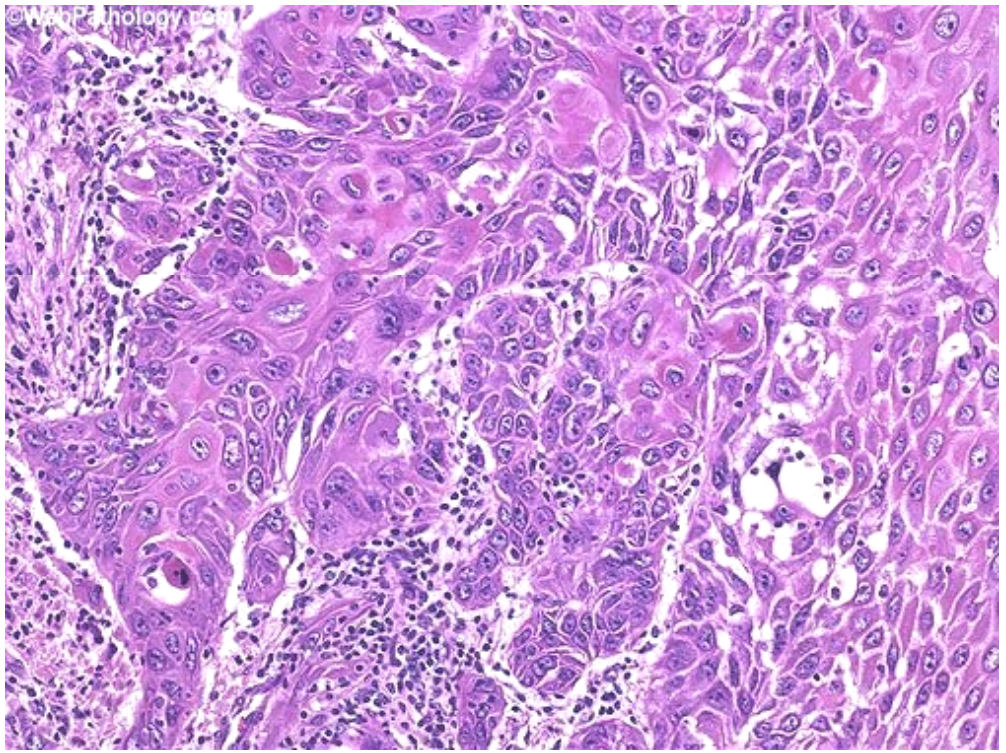
Rho family in breast ca as diagnostic & prognostic markers

- Cell Motility Factors screened by proteomics in breast ca
  - Rho family: GTPase
  - ER/Her2 status correlation
  - Triple negative breast ca biomarker
  - personalized targeted therapy

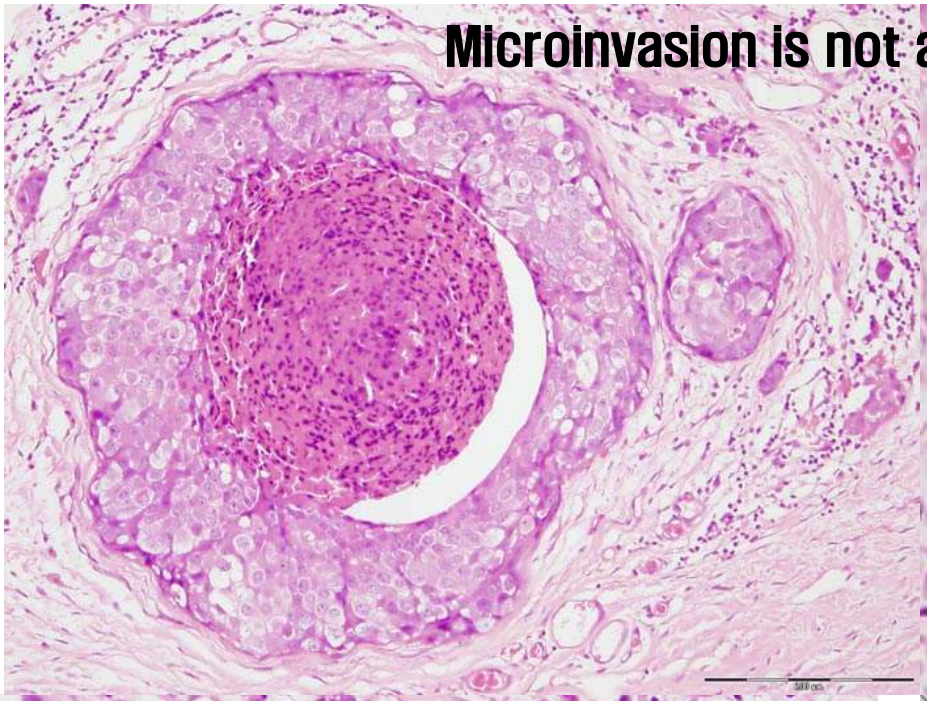
Cell invasion vs. Tumor invasior

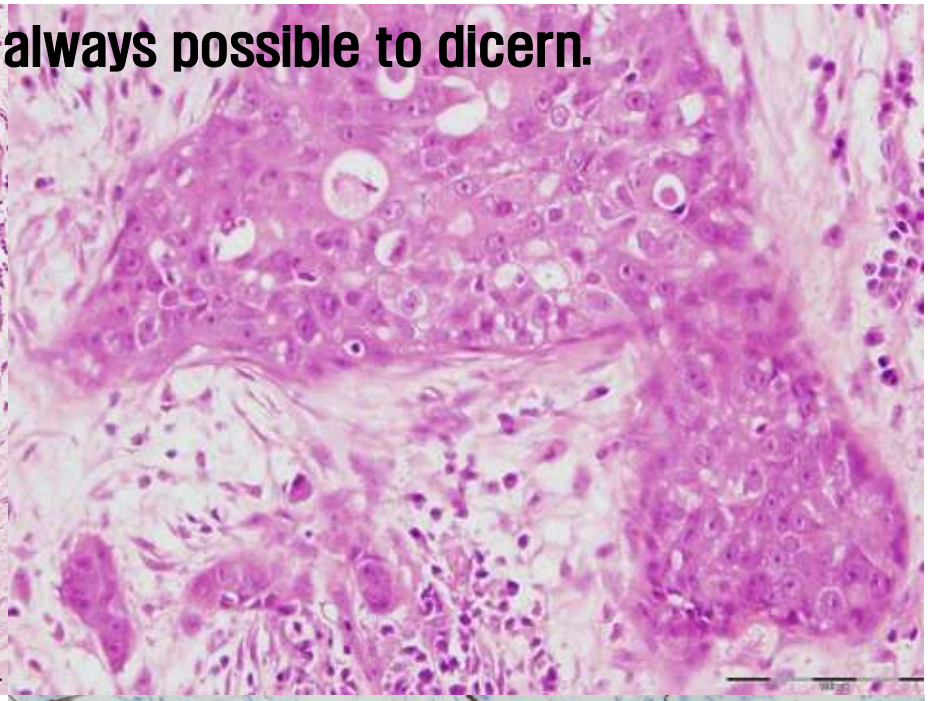


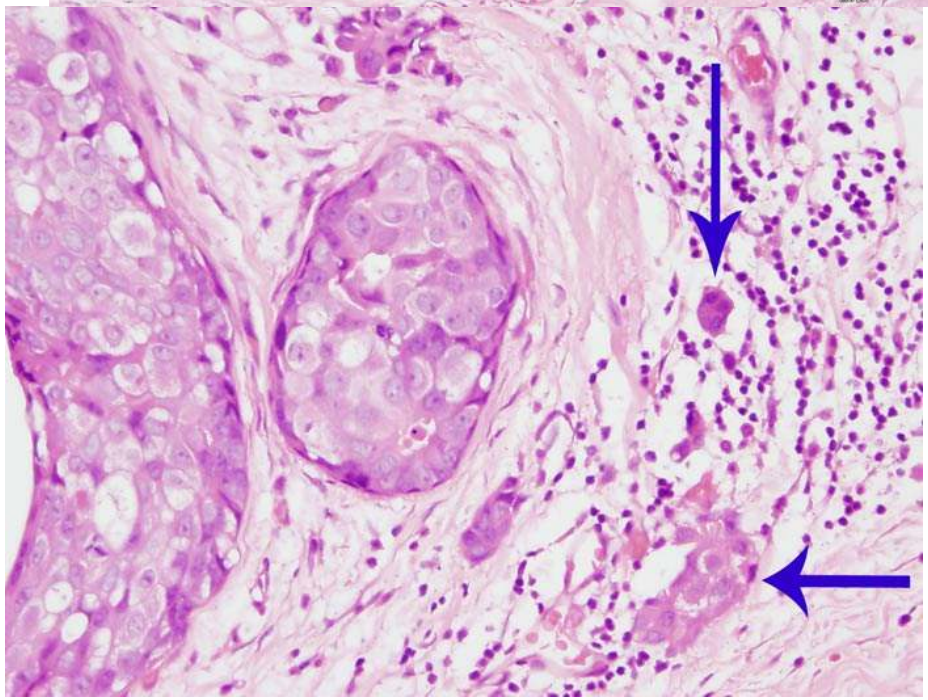


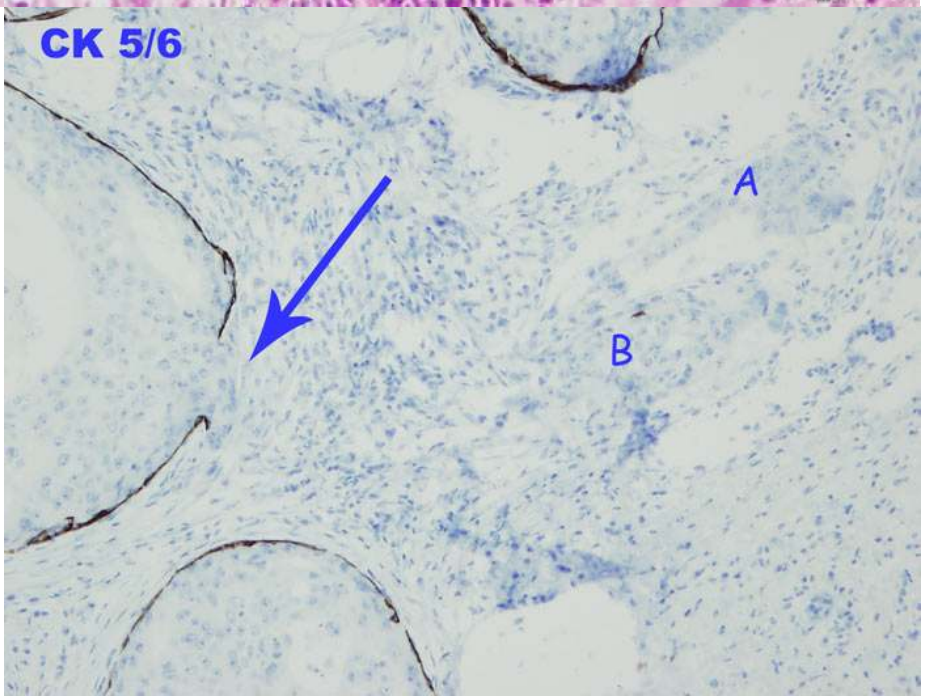


Microinvasion is not always possible to dicern.









Correlation of Cell Migration and Tissue Invasion with Metastasis

- Cell biology
  - Asymmetric polarized morphology
  - Kinetic fluctuation in receptor-ligand binding
  - Concentration gradient- independent

    Cell migration. *Cell 1996; 84:359-69 (Bible for migration)*
- Tissue biology
  - Early onset of microinvasion: step-wise model
  - Diagnostic useful universal kit: BM disruption or formation, with different composition

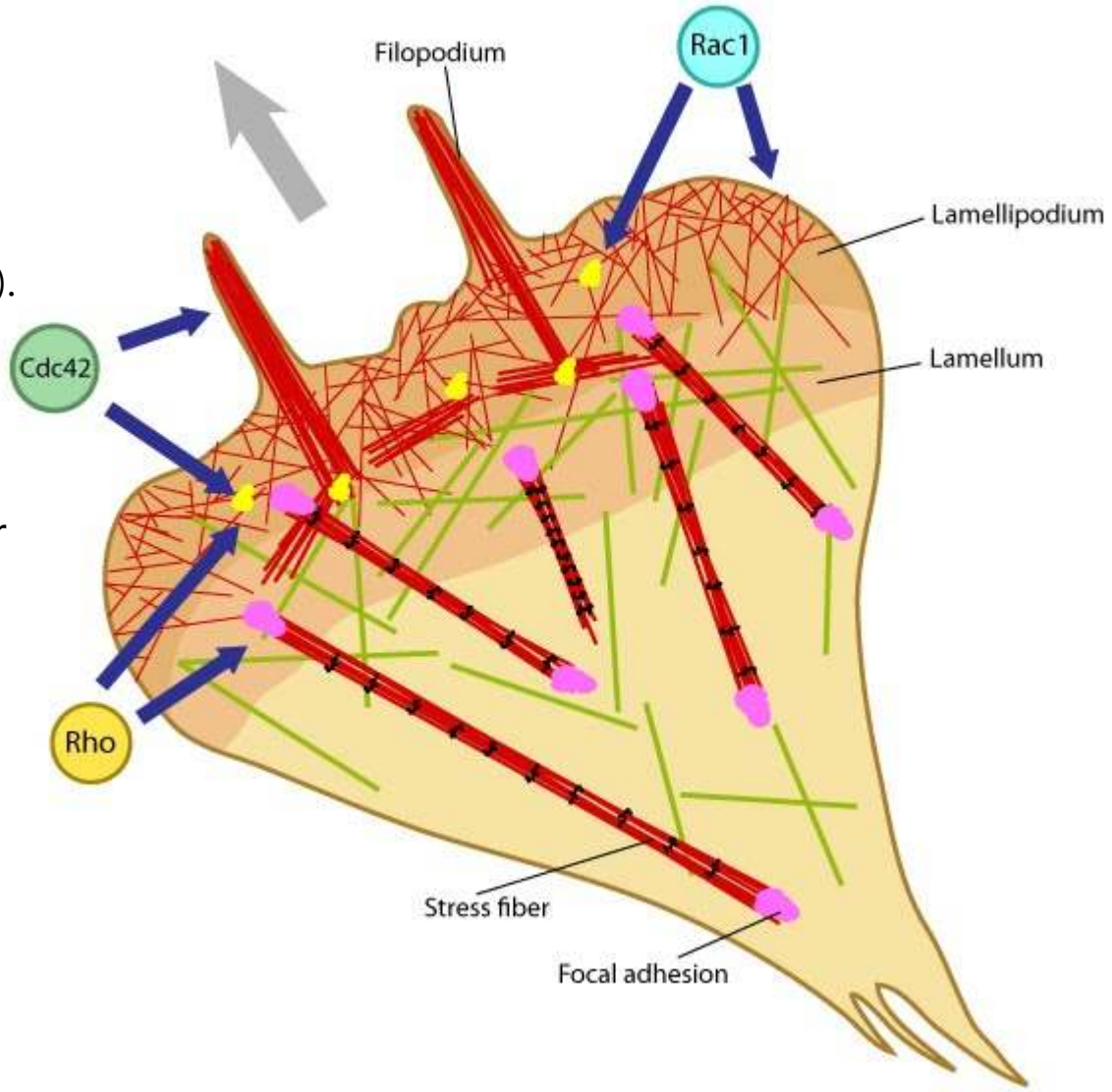
- Clinical biology
  - TNM stage reassessment
  - Aggressive non-invasive cancer
  - Biomarker to predict invasion or metastasis

Rho-GTPases regulate actin filaments and cytoskeletal organization.

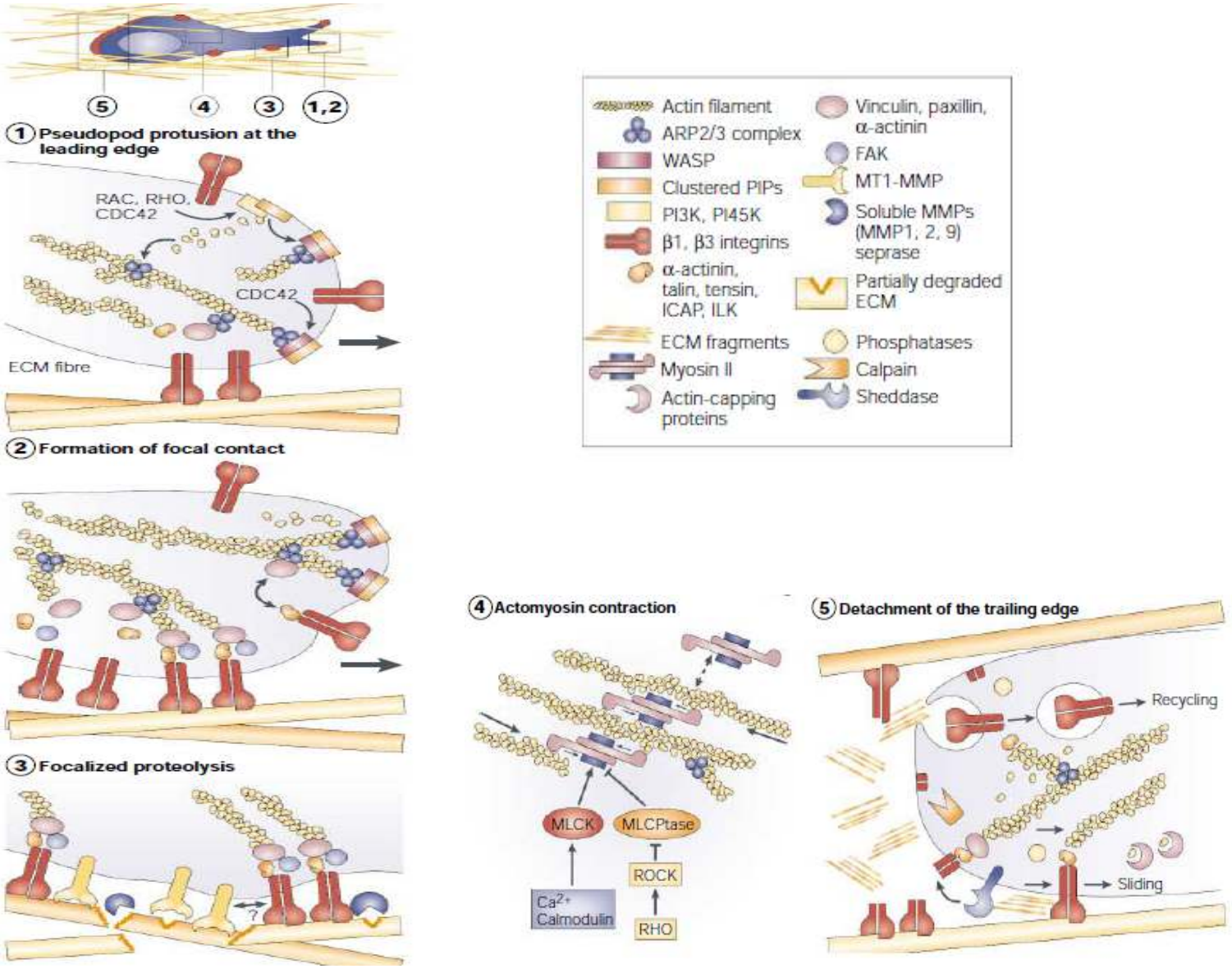
Cdc42 generally controls the cell polarity and the formation of filopodia and nascent focal complexes (shown as yellow dots).

Rho influences cell adhesion assembly and maturation, in addition to controlling stress fiber formation and contractile activity.

Rac1 primarily controls actin assembly and adhesion in the lamellipodium.

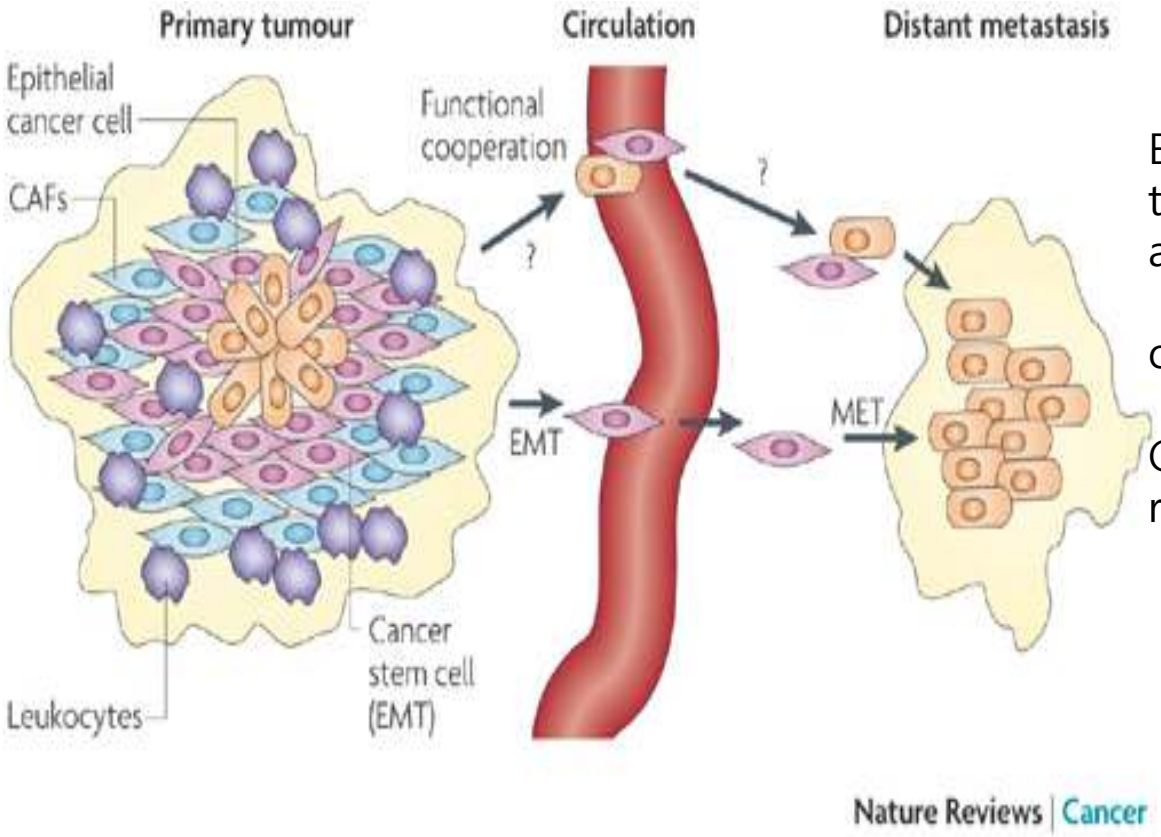


Five step of cell migration



Tumor-cell invasion and migration. Friedl & Wolf Nat Rev Cancer 2003; 3:362-74

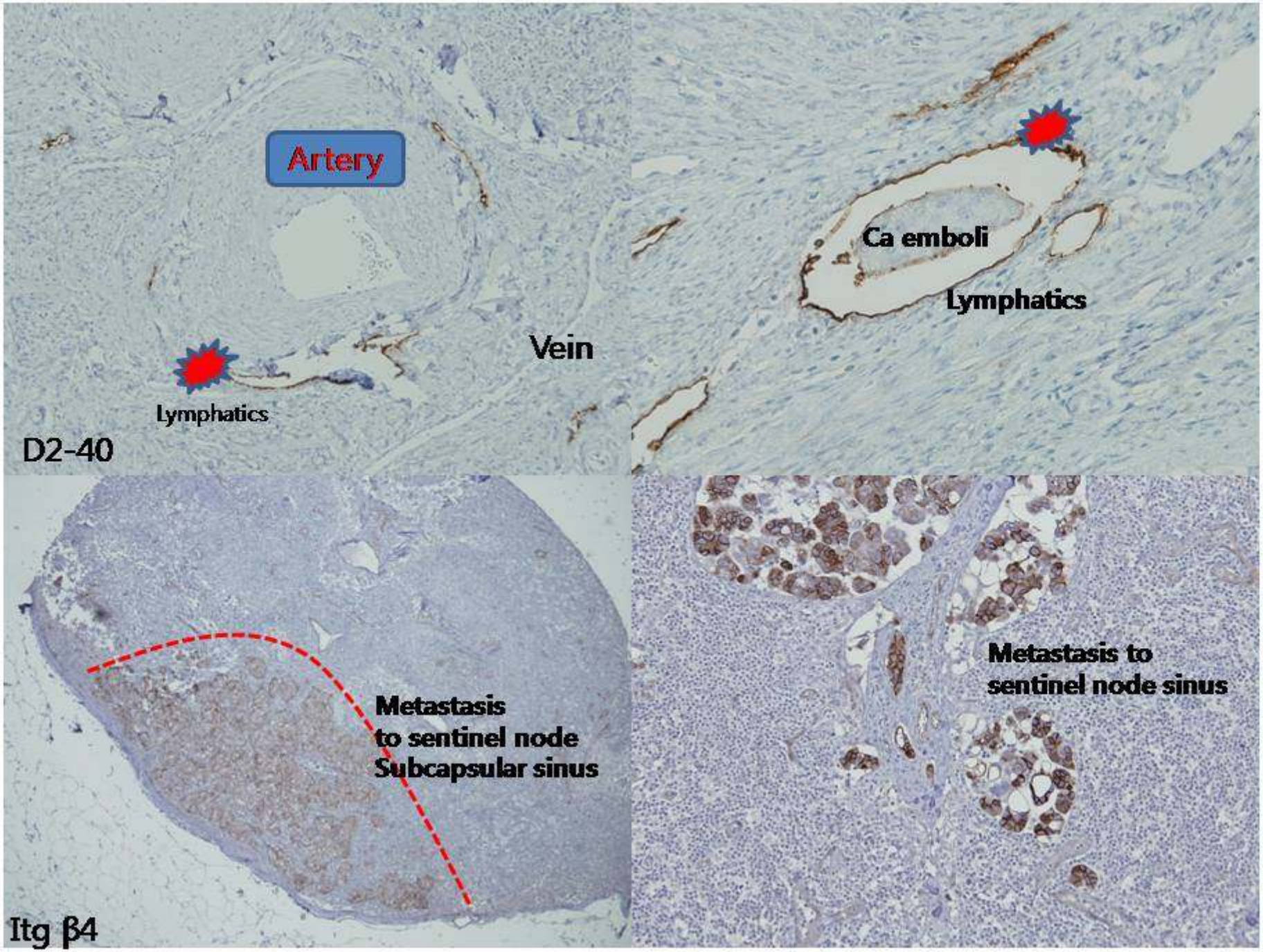
Metastasis: transitions between epithelial and mesenchymal stat es during carcinoma progression



E-cadherin (CDH1) in mets; trnsient methylation in EMT, and demethylated in MET

or loss of EMT-inducers in mets

CSC: increased until circulation, relative decrease in mets.

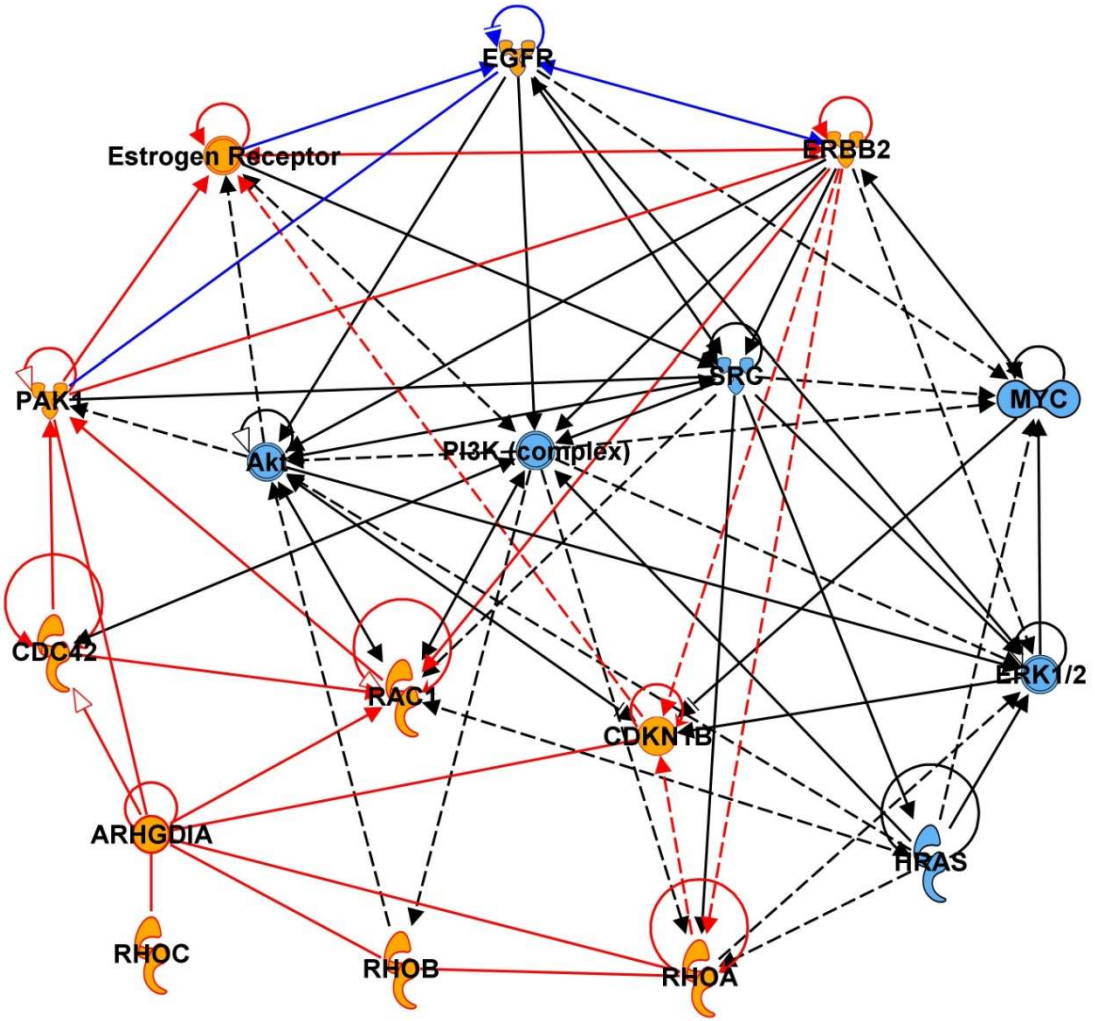

Artery
Vein
Lymphatics
D2-40
Ca emboli
Lymphatics
Metastasis
to sentinel node
Subcapsular sinus
Itg β4
Metastasis to
sentinel node sinus

Tissue validation could be confirmative step for arbitrary issues in Omcis

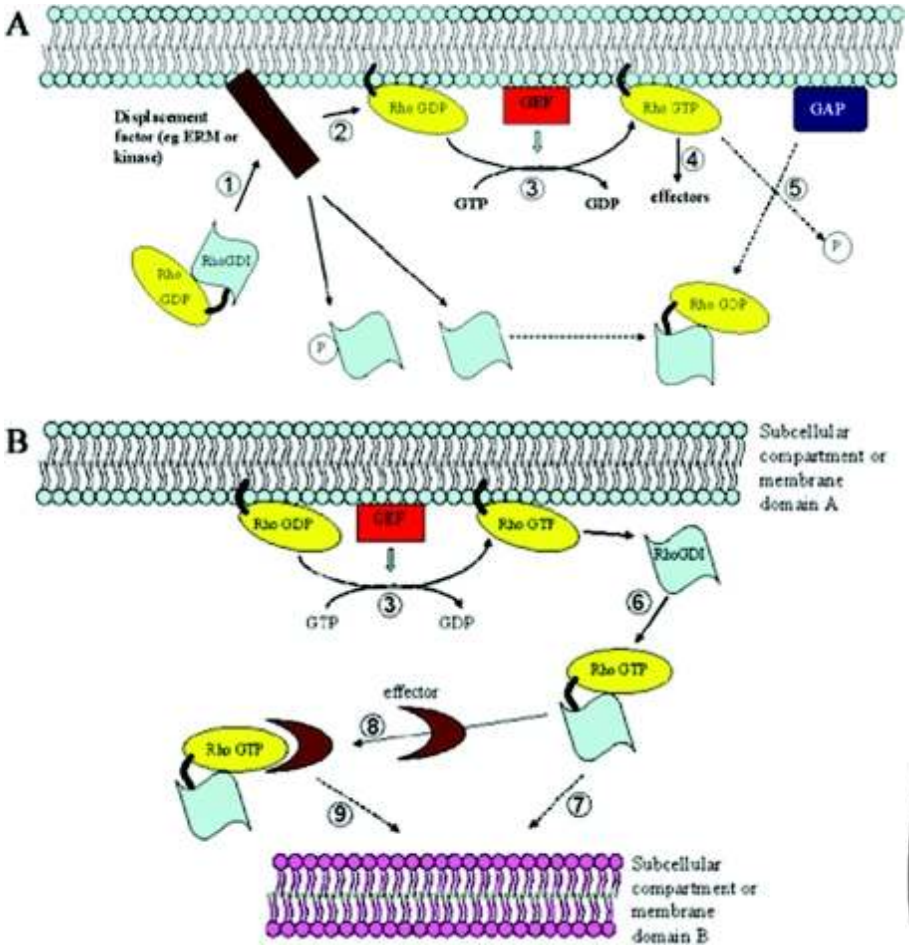
Examples of undergoing project with arguable data in omics study

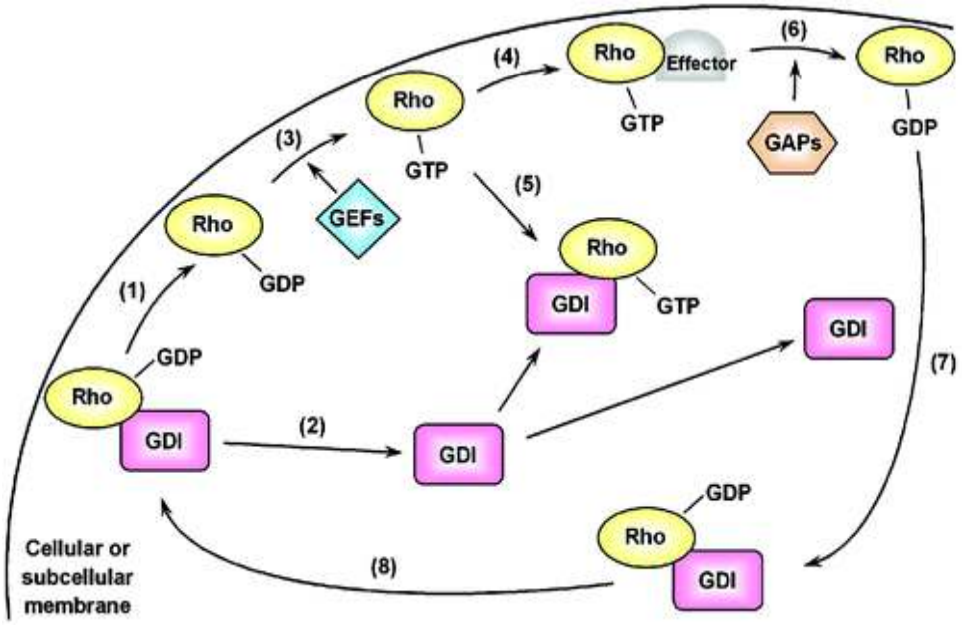
Rho-GDI in breast ca?

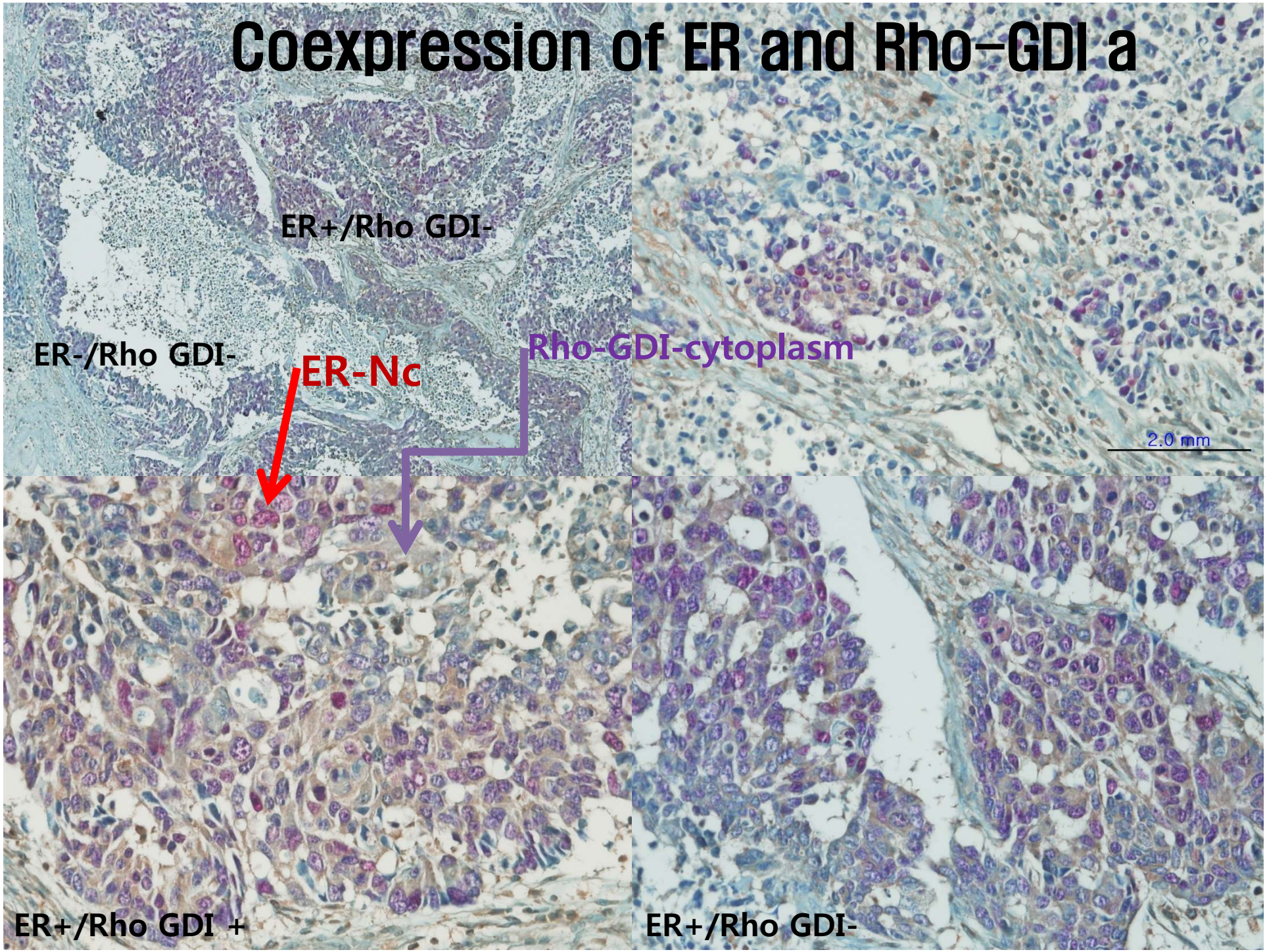
- up or down?
- ER interaction?
- ErbB2 interaction?
- T-stage ?
- N-stage ?
- metastasis?



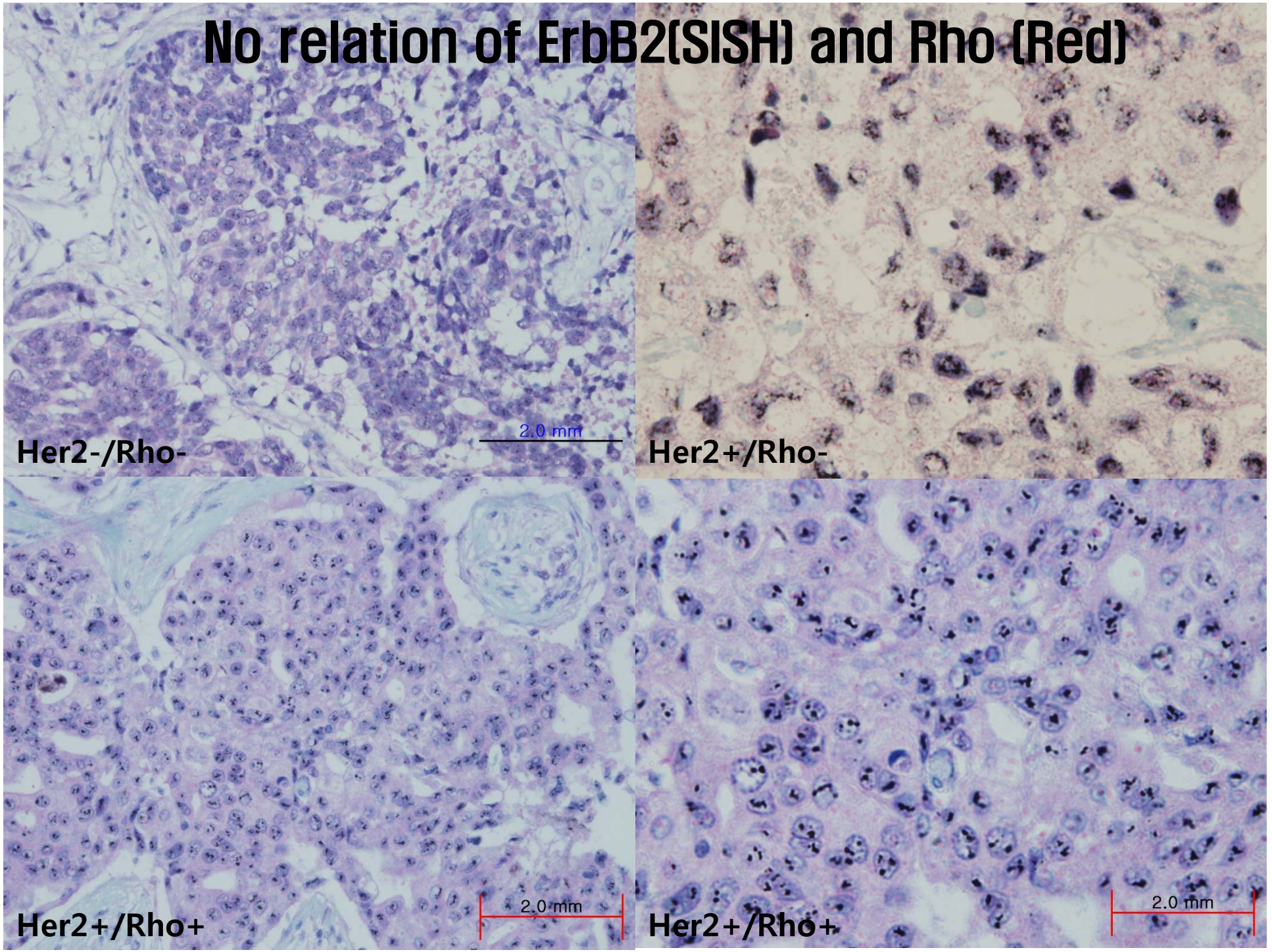
Rho GDI as regulator of cell migration






Coexpression of ER and Rho-GDI a
ER+/Rho GDI-
ER-/Rho GDI-
ER-Nc
Rho-GDI-cytoplasm
2.0 mm
ER+/Rho GDI +
ER+/Rho GDI-

No relation of ErbB2(SISH) and Rho (Red)



Take Home Message

Omics as magic-screening tools with innumerable gems,

but should be purified to be a diamond.

All your idea make yourself **innovative** and **inspired**!

Any innovation move **science** one step forward!

Discipline yourself through your **invention**!

Be a **physician scientist**
beyond medical skill master