

Analysis of long non-coding RNAs expression profiles in ovarian CAF



CHARLES
PERKINS CENTRE

Fatemeh Vafae

Charles Perkins Centre

School of Mathematics and Statistics

The University of Sydney

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Academic Qualifications and Positions

- › Research Fellow,
 - Charles Perkins Centre and School of Mathematics & Statistics, University of Sydney
 - **Research topic:** *Personalised Medicine, Systems biology, Integrative Computational Modelling, Bioinformatics*
- › Postdoctoral Associate,
 - University of Toronto, University Health network, Princess Margaret Hospital
 - **Research topic:** *Integrative computational biology, Cancer Informatics*
 - Genomic cancer profiles analysis, cancer gene signature identification, PPI prediction, genome-level drug effectiveness quantification
- › Ph.D.,
 - University of Illinois at Chicago, Artificial Intelligence Laboratory
 - **Research topic:** *Artificial Intelligence:*
 - Optimization and search algorithms, evolutionary algorithms, machine learning, data mining, information retrieval
 - *Computational biology:* PPI network alignment, phylogenetic network reconstruction, genome-wide association studies
- › B.Sc., Sharif University of Technology, Computer Engineering Department



The Princess Margaret
Cancer Foundation  UHN



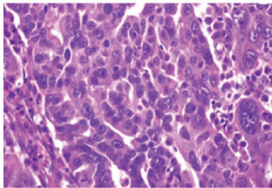
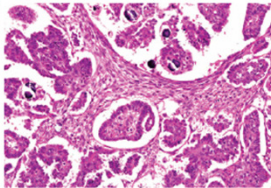
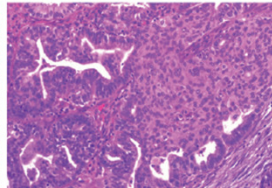
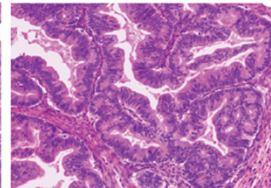
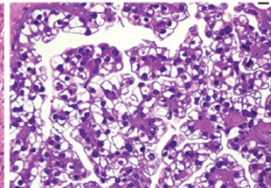
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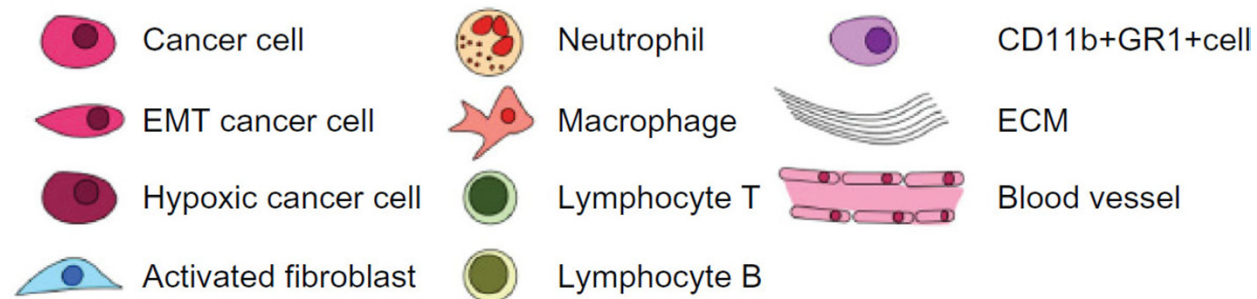
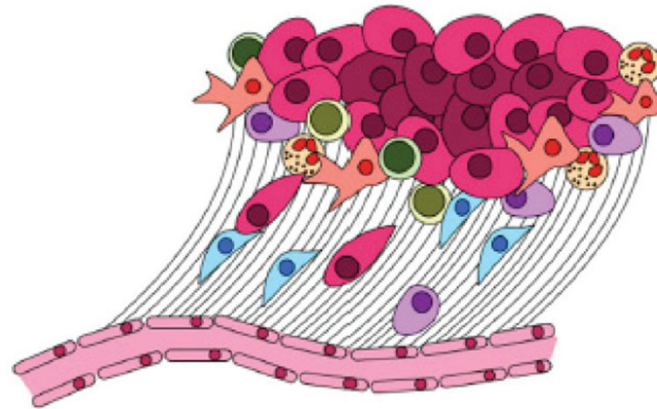
Sharif University
of Technology

Epithelial Ovarian Cancer

- > About 9 out of 10 tumours of the ovary diagnosed (90%) are this type
- > 6th most common cause of cancer death
- > 5-year survival ~ 40% (<25% in advanced disease)
- > Recurrence of chemoresistant disease common
- > Standard treatment has not changed in decades
- > Poor prognosis

	High Grade Serous	Low Grade Serous	Endometrioid	Mucinous	Clear Cell
					
Incidence	70%	<5%	10%	3%	10%
Molecular Abnormalities	<i>BRCA1/2</i> <i>TP53</i>	<i>BRAF</i> <i>KRAS</i>	<i>PTEN</i> <i>ARID1A</i>	<i>KRAS</i>	<i>ARID1A</i> <i>PIK3CA</i>

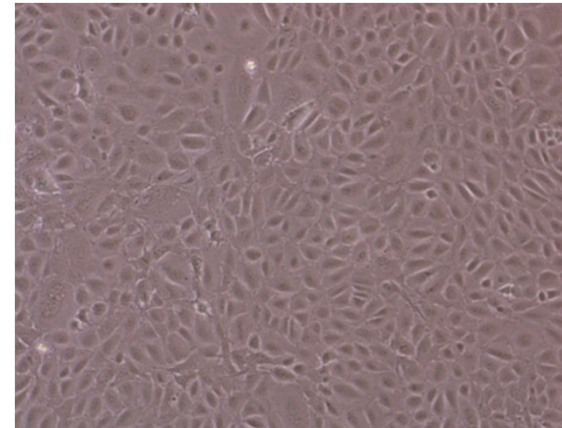
The Tumour Microenvironment



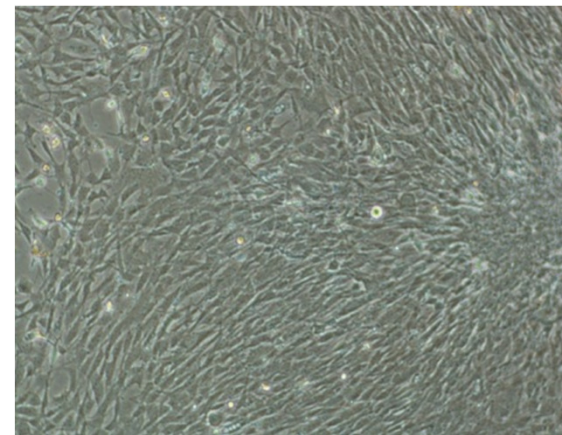
Adapted from Mayorca-Guiliani *et. al.* 2013 *OncoTargets and Therapy*

Cancer-associated fibroblasts (CAFs)

- › Produce extracellular matrix
- › In other cancers they communicate with tumour cells and other cells in the tumour microenvironment to:
 - Promote invasion and metastasis
 - Promote chemoresistance
 - Promote angiogenesis (the formation of blood vessels)
 - Help create an immunosuppressive microenvironment



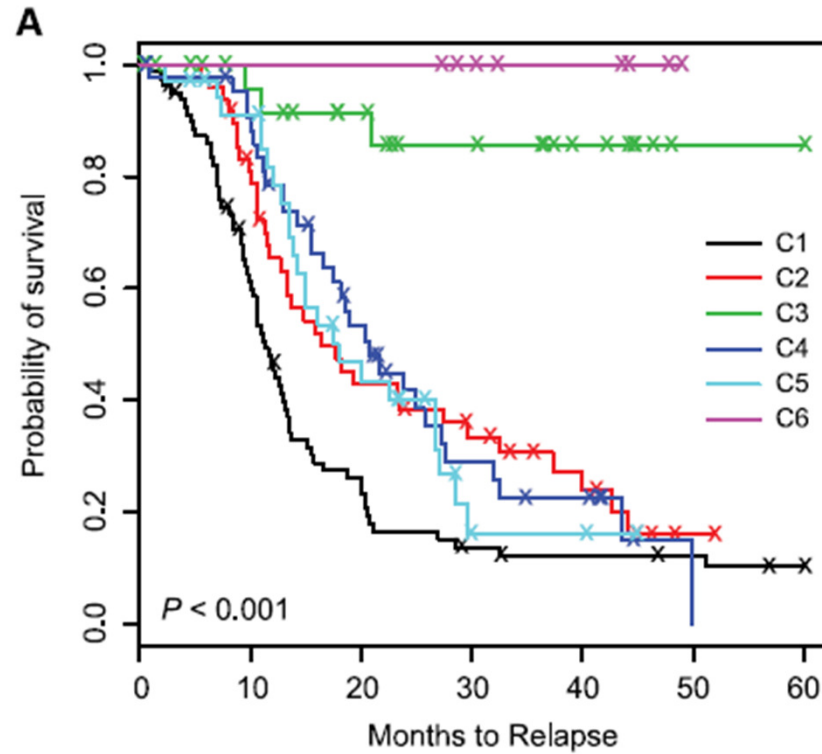
Tumour cells



CAFs

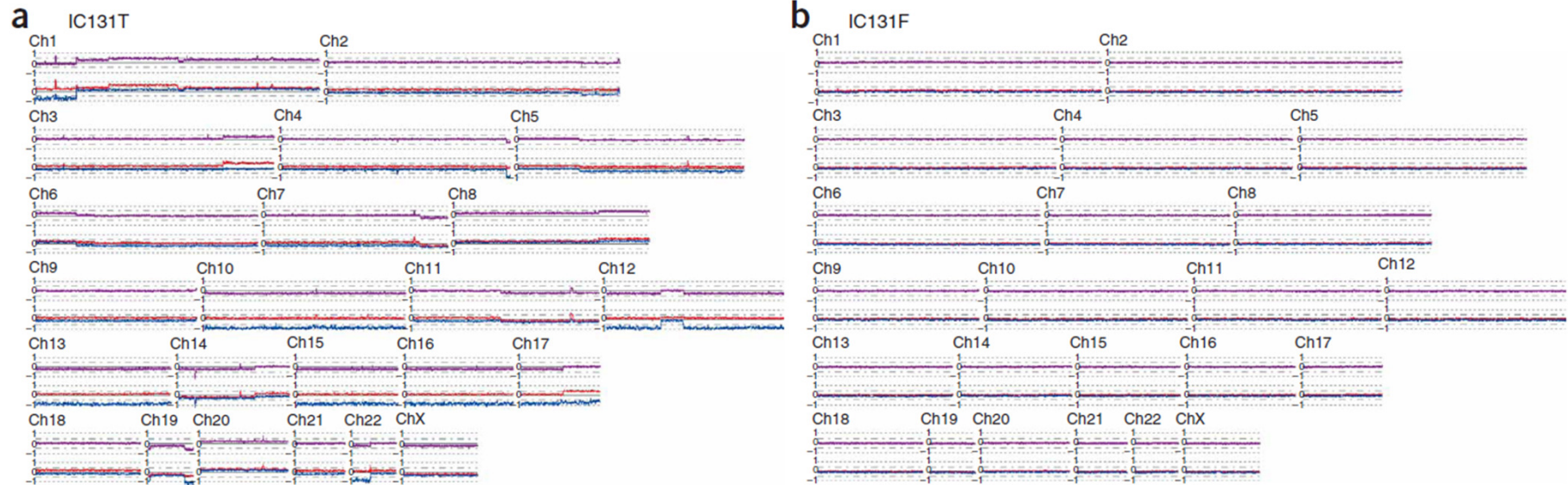


Stroma influences prognosis



Tothill RW et al 2008 *Clinical Cancer Research*

Somatic mutations in CAFs are rare

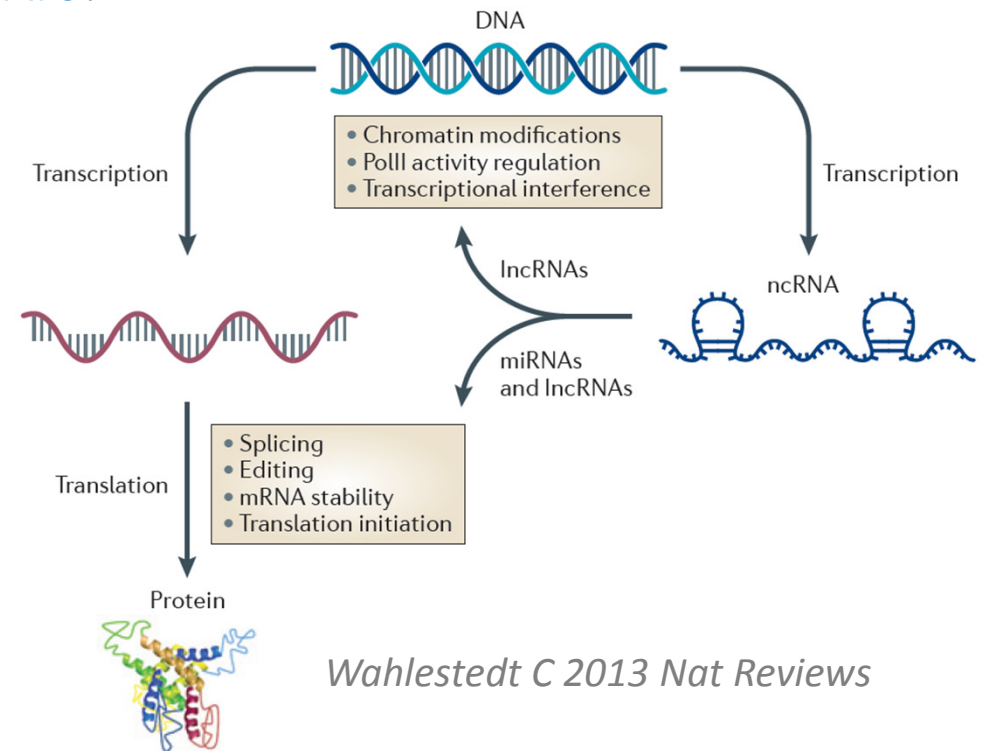


Qui W et. al. 2008 Nat Genet

Therefore other mechanisms that regulate gene expression and function may be involved

lncRNAs may contribute to the CAF phenotype

- › lncRNAs are non-protein-coding RNAs > 200 nucleotides long
- › Increasingly recognized to play functional roles in cancer.
- › Do lncRNAs play a functional role in CAFs?



Are lncRNAs differentially expressed in ovarian CAFs vs. normal ovarian fibroblasts?



DE Analysis

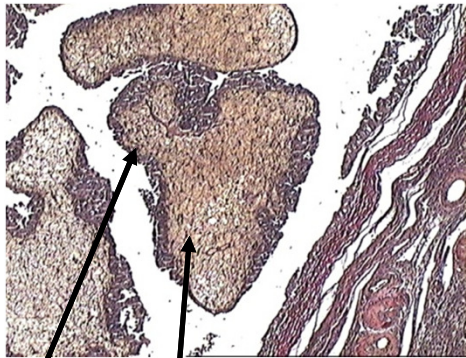
What are their potential mechanisms, functions, and pathways of relevance?



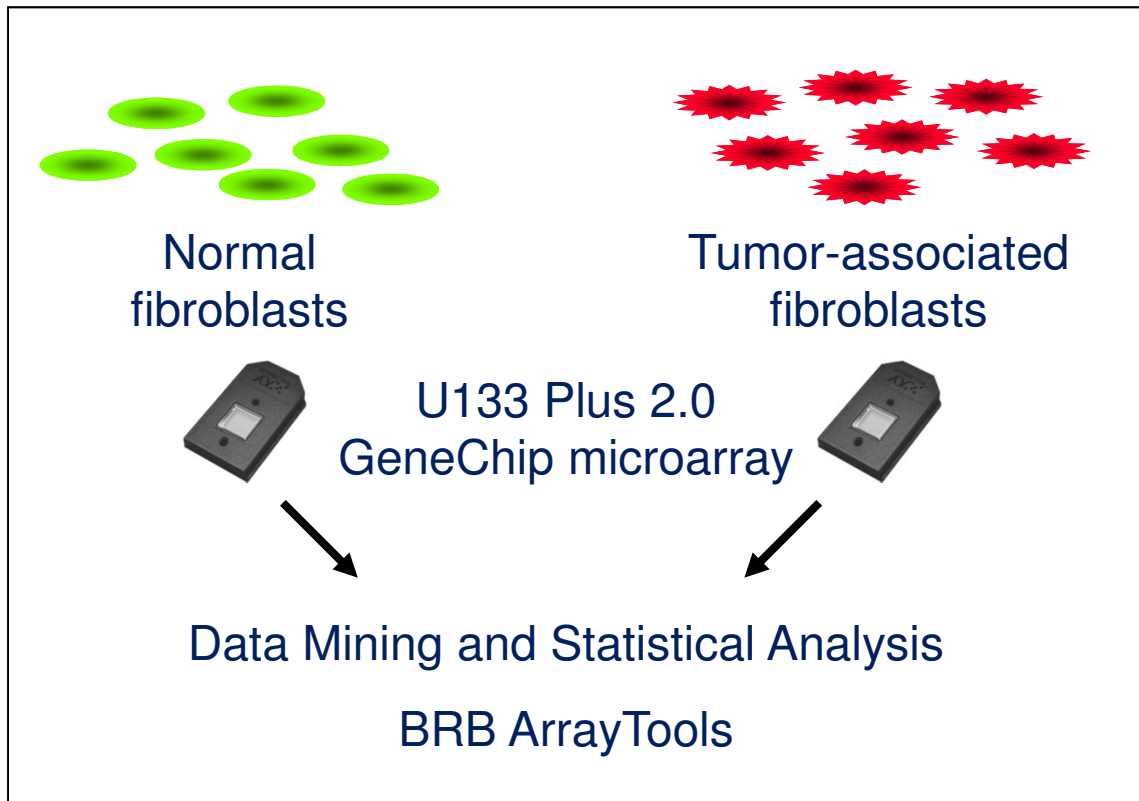
Functional Analysis



Identifying differentially expressed lncRNAs



Epithelium Microenvironment



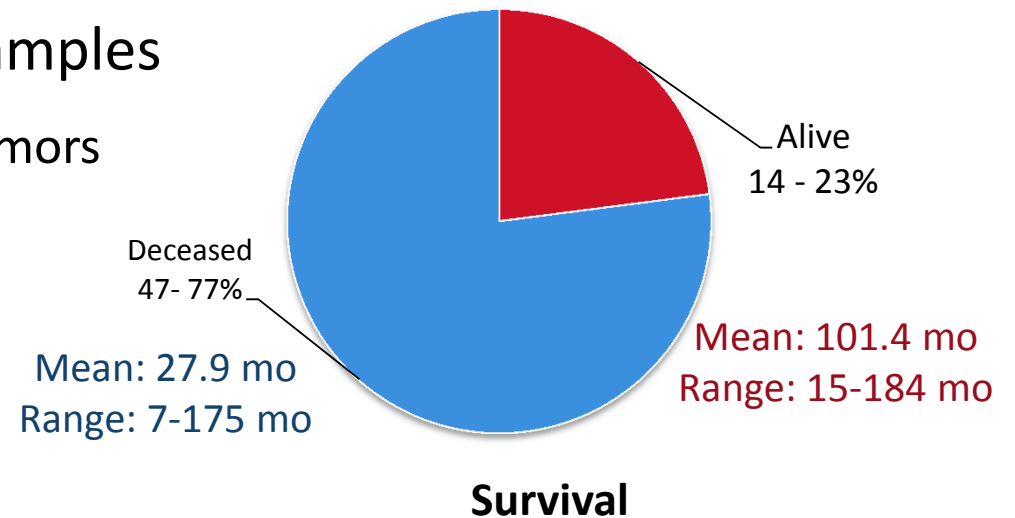
(Mok SC et. al. 2009 *Cancer Cell*)

> 79 microdissected fibroblast samples

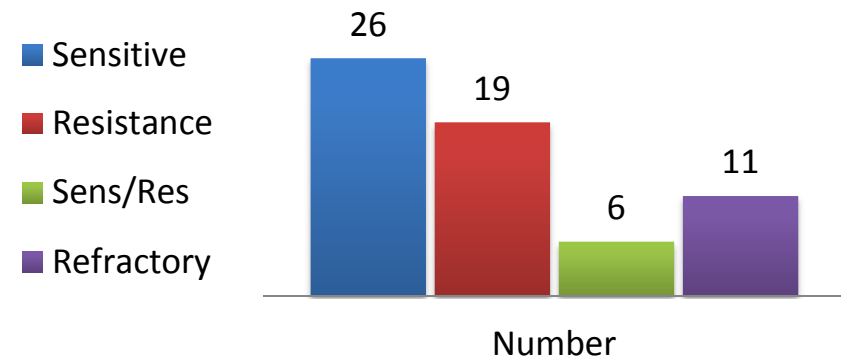
- 69 late-stage, high-grade serous tumors
- 10 normal ovarian sample

> Clinical Information:

- Survival (months)
- Chemo-response
- ...

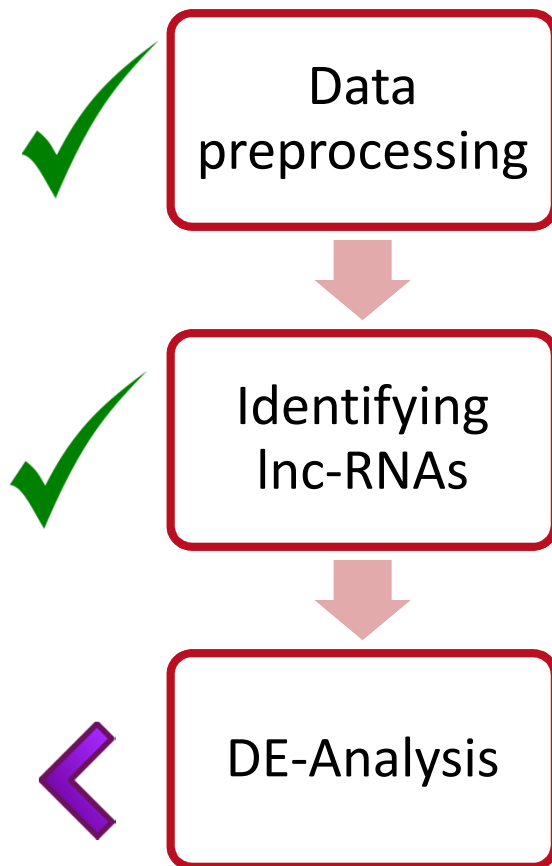


Chemo Response

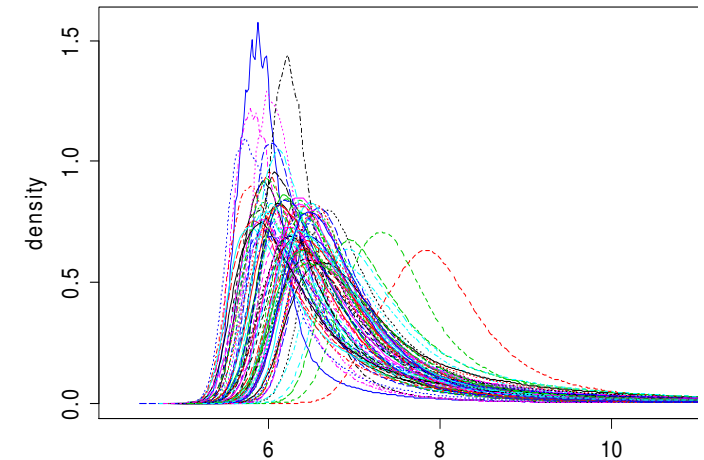




Identifying differentially expressed (DE) IncRNAs



- > Background Correction
- > Normalization
 - Robust Multi-array Average
- > Log transformation



- > Probes identified as IncRNAs from *Zhang X. et. al. 2012* were used which identifies **2,448** probes as IncRNAs

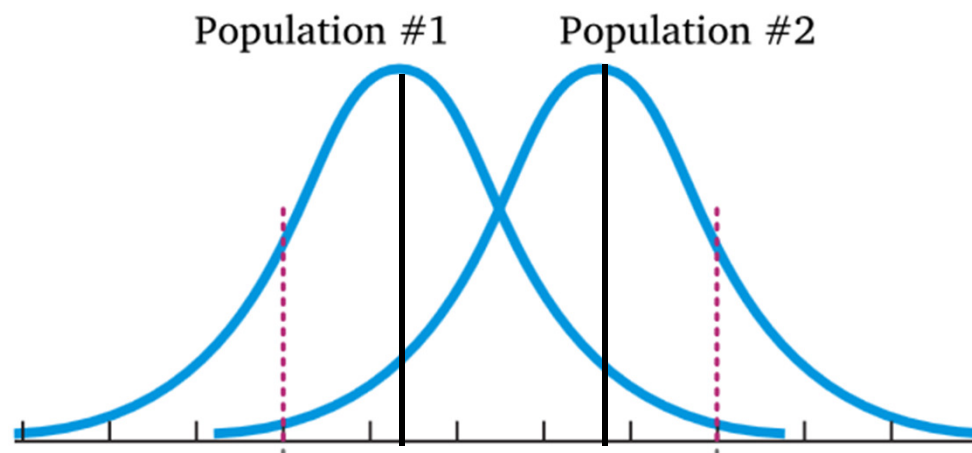
Long non-coding RNA expression profiles predict clinical phenotypes in glioma

Xiaoqin Zhang ^a, Stella Sun ^a, Jenny Kan Suen Pu ^a, Anderson Chun On Tsang ^a, Derek Lee ^a, Venus On Ying Man ^{a,b}, Wai Man Lui ^a, Stanley Thian Sze Wong ^a, Gilberto Ka Kit Leung ^{a,*}

^a Department of Surgery, Li Ka Shing Faculty of Medicine, The University of Hong Kong, Queen Mary Hospital, Pokfulam, Hong Kong

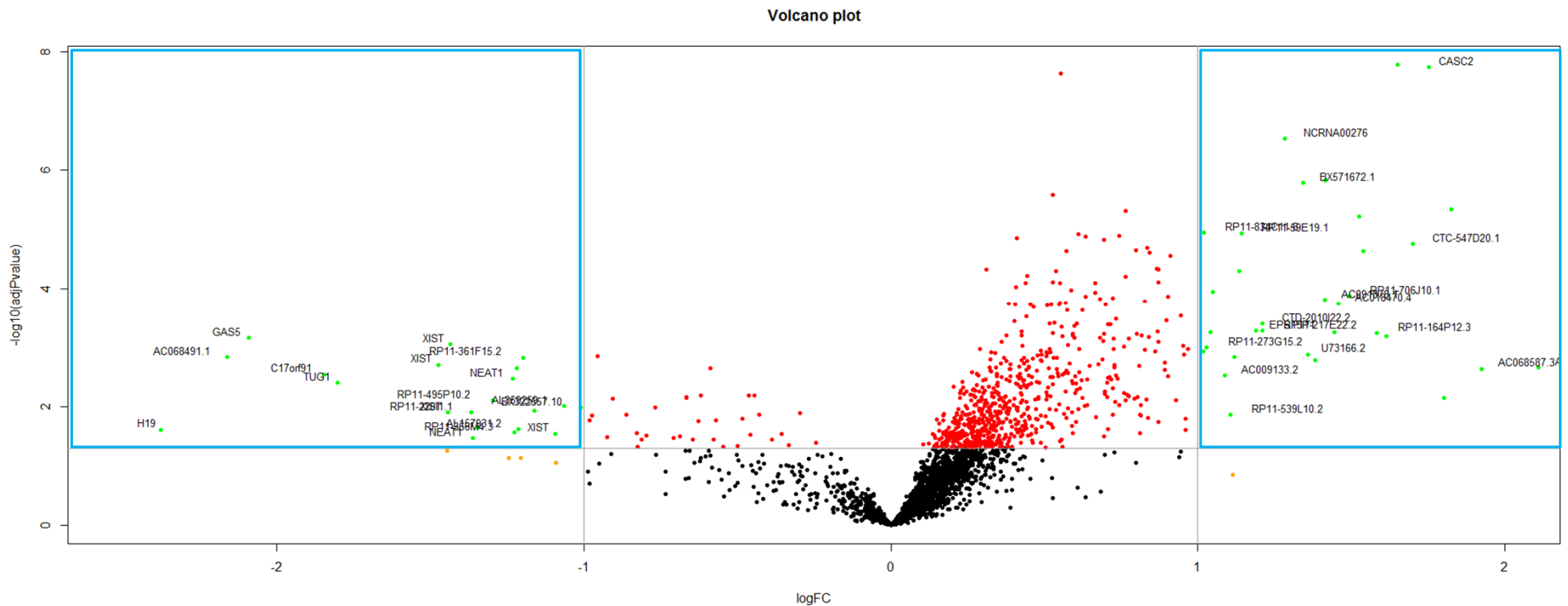
^b Department of Clinical Oncology, Li Ka Shing Faculty of Medicine, The University of Hong Kong, Queen Mary Hospital, Pokfulam, Hong Kong

- › Differentially expressed probes between cancerous vs. normal samples were calculated using *limma* ([Linear Models for Microarray Data](#)) package from [Bioconductor](#).
- › P-values were adjusted using FDR method
 - **Multiple hypothesis** problem occurs when one considers a set of hypothesis tests simultaneously (it is more likely that one incorrectly reject the null hypothesis)



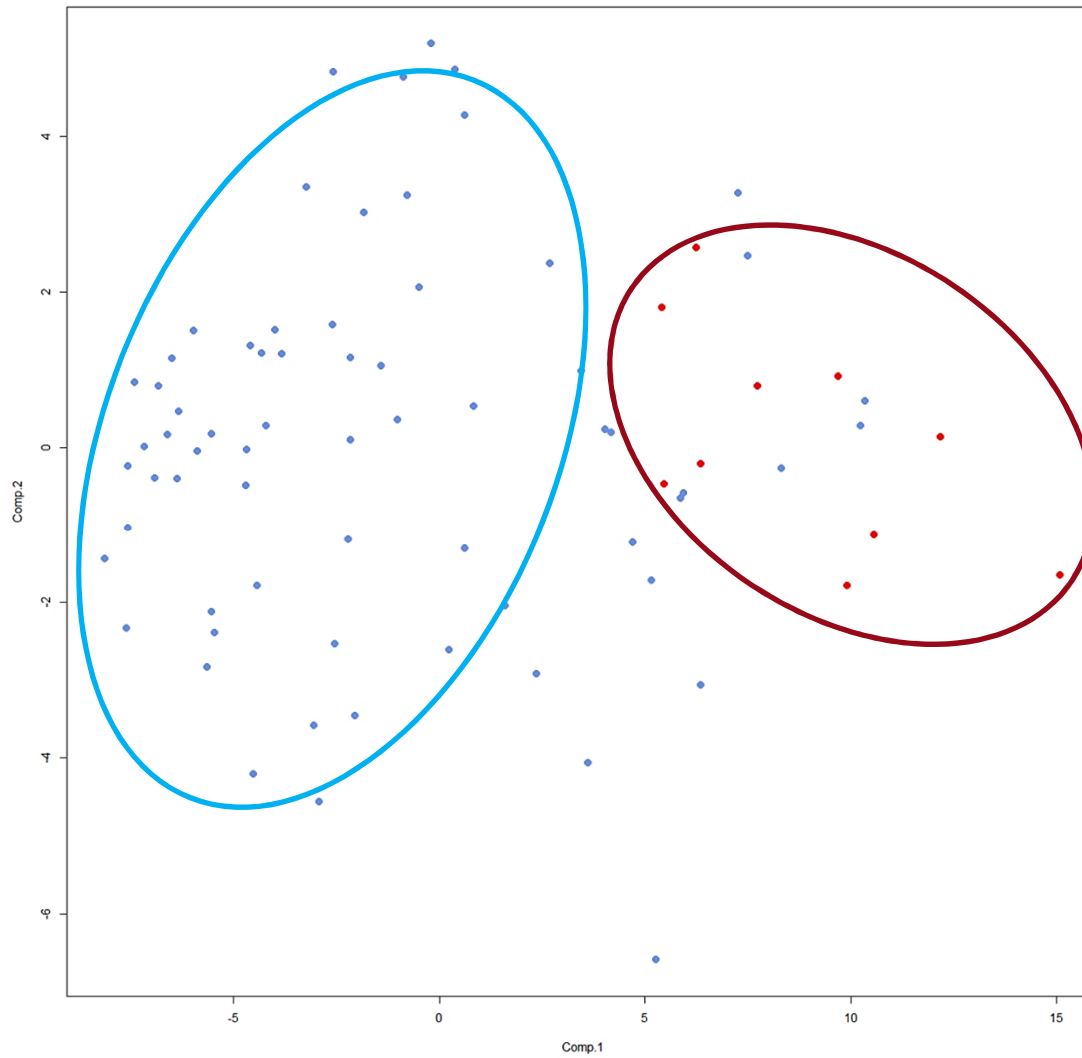
Identifying DE IncRNAs (Volcano plot)

- Significance cutoff of **fold-change >2** and a **adjusted p-value < 0.05** used to identify DE-IncRNAs in CAFs vs NFs
- 54** IncRNAs were identified





Principle Component Analysis

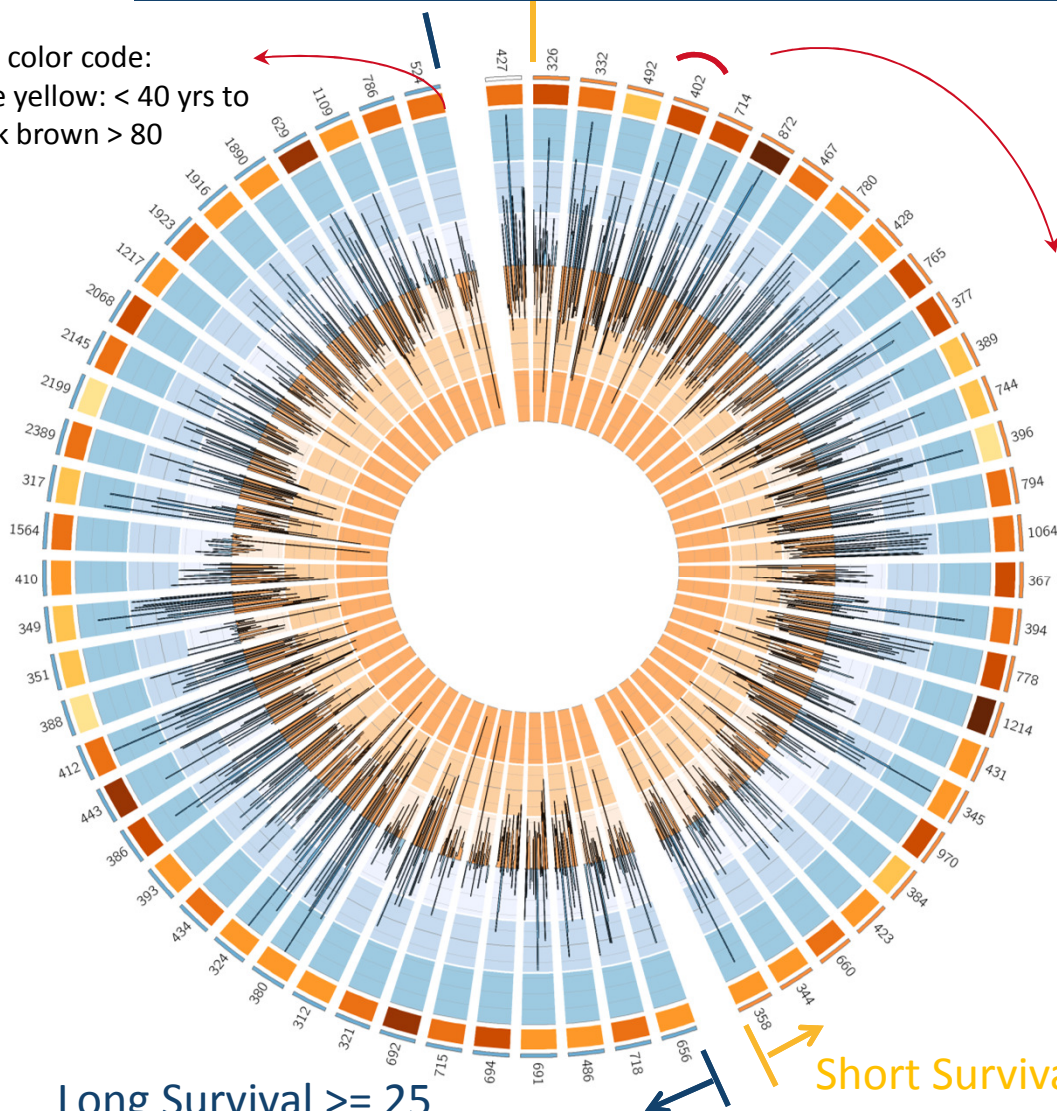


lncRNA	Proposed functions/ interactions
XIST	X-chromosomal inactivation, downregulated in female cancers including ovarian, possible association with chemoresistance
H19	Downregulated in metastatic ovarian cells compared to nonmetastatic, targets TGF β 1
TUG1	Regulated by TP53 and FSHR, decreased in NSCLC
NEAT1	Assembly of nuclear paraspeckles, target of HIF2, poor prognostic factor in breast cancer
GAS5	Decreased expression is a poor prognostic factor in cervical and colorectal cancers
CASC2	Decreased in endometrial cancers
MALAT1	Decreased in metastatic ovarian cells compared to nonmetastatic, associated with metastasis in numerous cancer types
MEG3	Decreased in gastric cancer
CRNDE	Increased in colorectal cancer, regulated by mTOR signaling



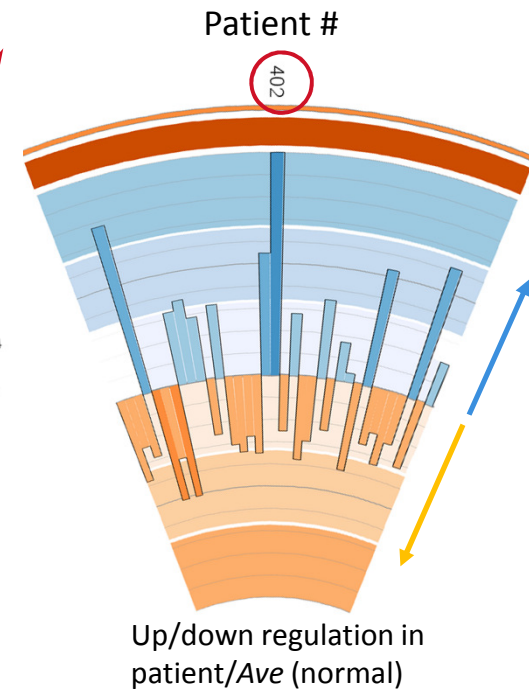
Distribution of deregulation of chosen lncRNAs across each sample

Age color code:
pale yellow: < 40 yrs to
dark brown > 80



From Left to right

- AC004951.6
- AC009133.2
- AC018470.4
- AC068491.1
- AC068587.3
- AC091878.1
- AC092620.2
- AL157931.2
- AL359259.1
- BX322557.10
- BX571672.1
- C17orf91
- CASC2
- CTC-547D20.1
- CTD-2010122.2
- EPS15P1
- GAS5
- H19
- NCRNA00276
- NEAT1
- RP11-164P12.3
- RP11-217E22.2
- RP11-220I1.1
- RP11-273G15.2
- RP11-361F15.2
- RP11-366M4.3
- RP11-38P22.2
- RP11-495P10.2
- RP11-539L10.2
- RP11-59E19.1
- RP11-706J10.1
- RP11-834C11.6
- TUG1
- U73166.2
- XIST



Long Survival ≥ 25

Short Survival ≤ 21

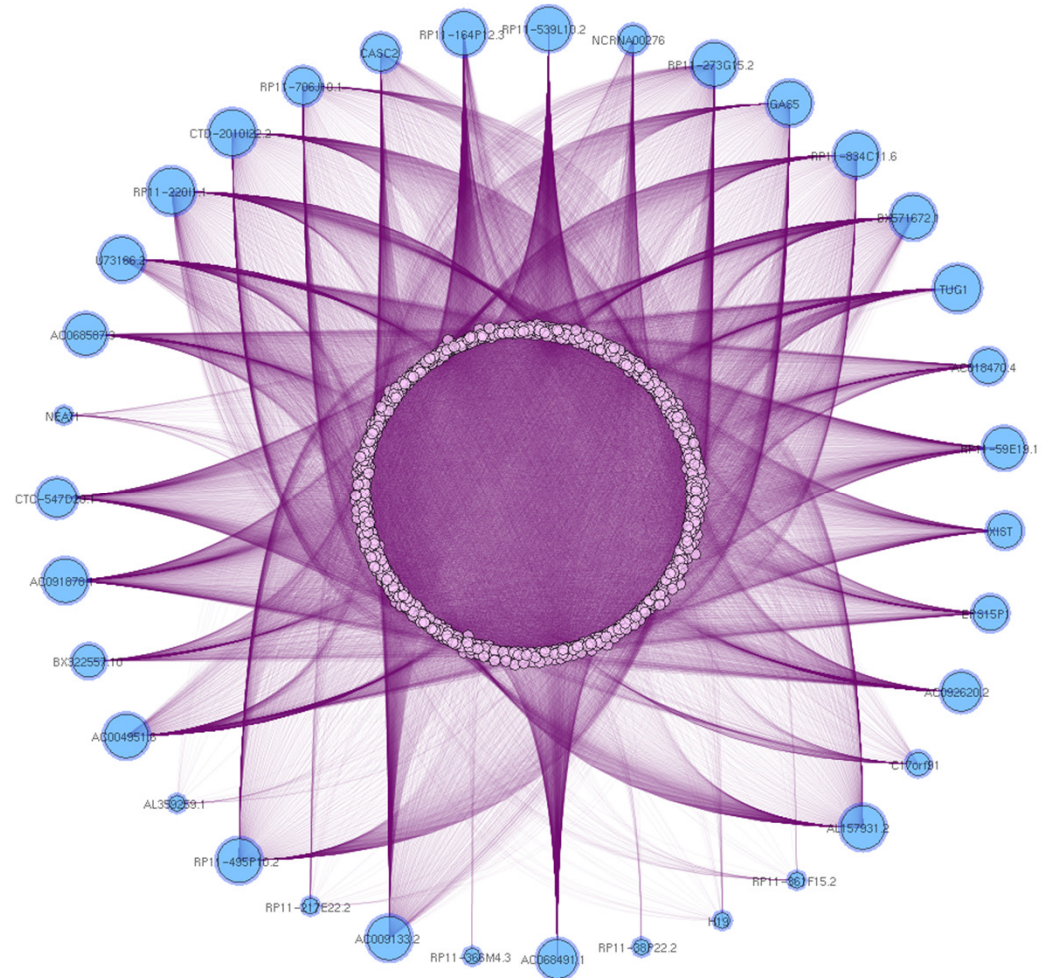
- > What are the potential mechanisms of significantly de-regulated lncRNAs, their functions, and pathways of relevance?

How to do this when our knowledge on the functions of ncRNAs is pretty limited?

Looking into the functions of **highly correlated genes**

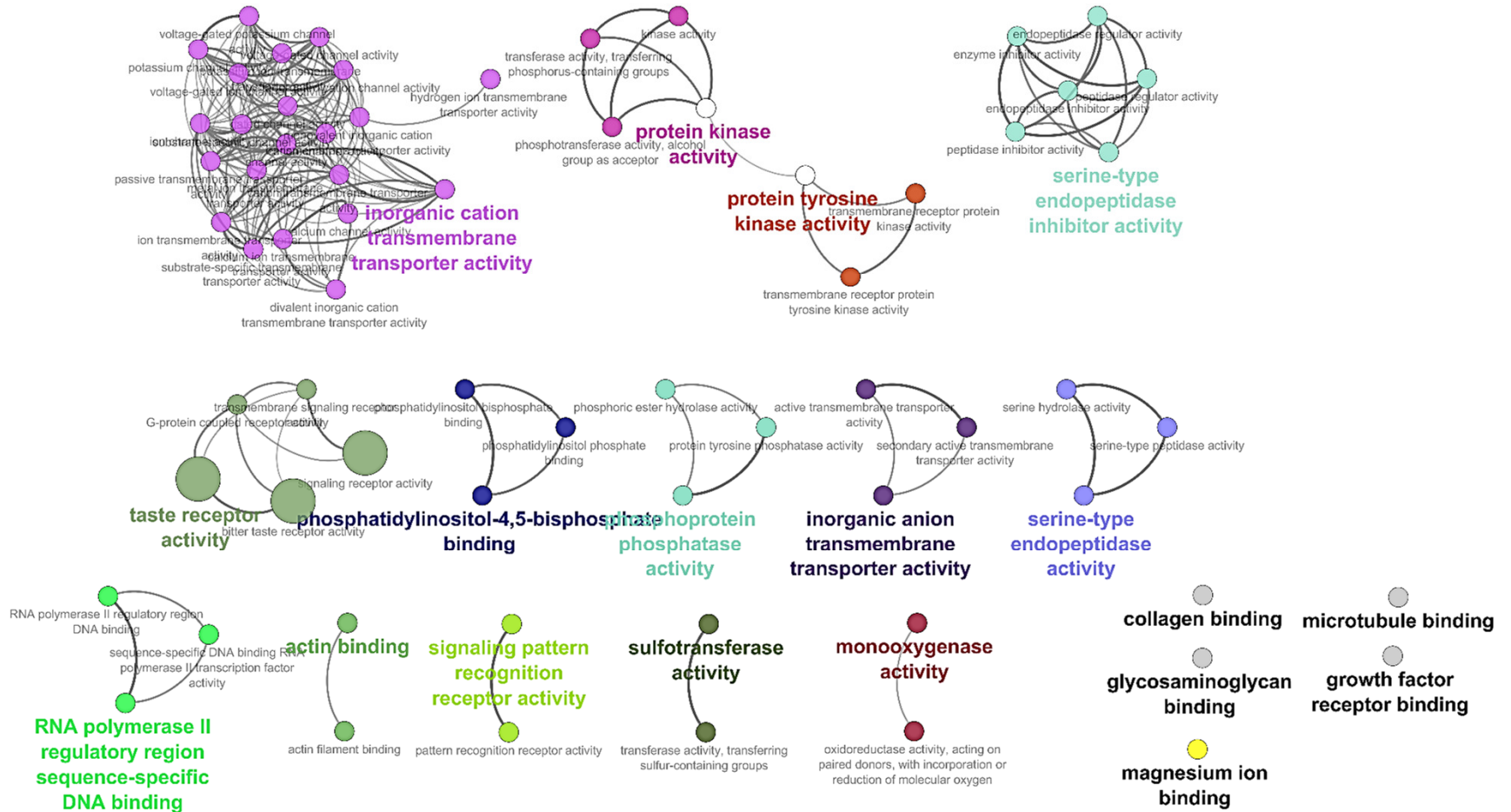


- > Network of genes highly correlated ($|\text{corr}| > 0.7$) with the DE lncRNAs
- > What are the **molecular functions over-represented** by the correlated genes?
- > What are the **enriched pathways**?





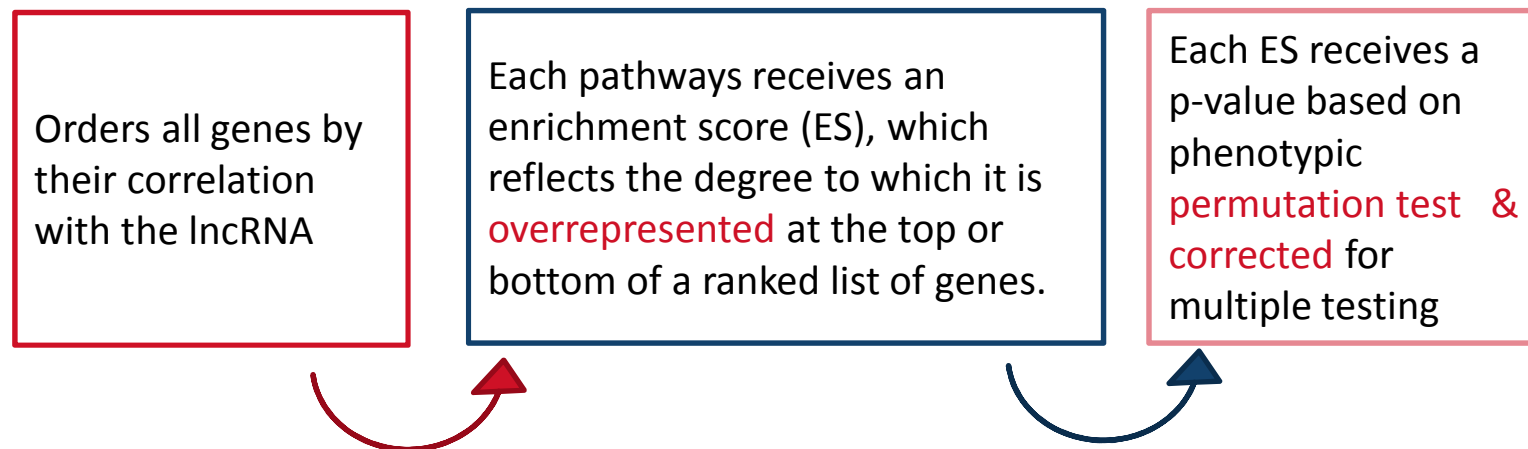
Functional Analysis



- > **GSEA** is used here to determine whether an **a priori defined set of genes** (i.e., **KEGG** pathways) shows statistically significant concordance with each of DE lncRNAs.

Subramanian, Aravind, et al. PNAS 102.43 (2005): 15545-15550.

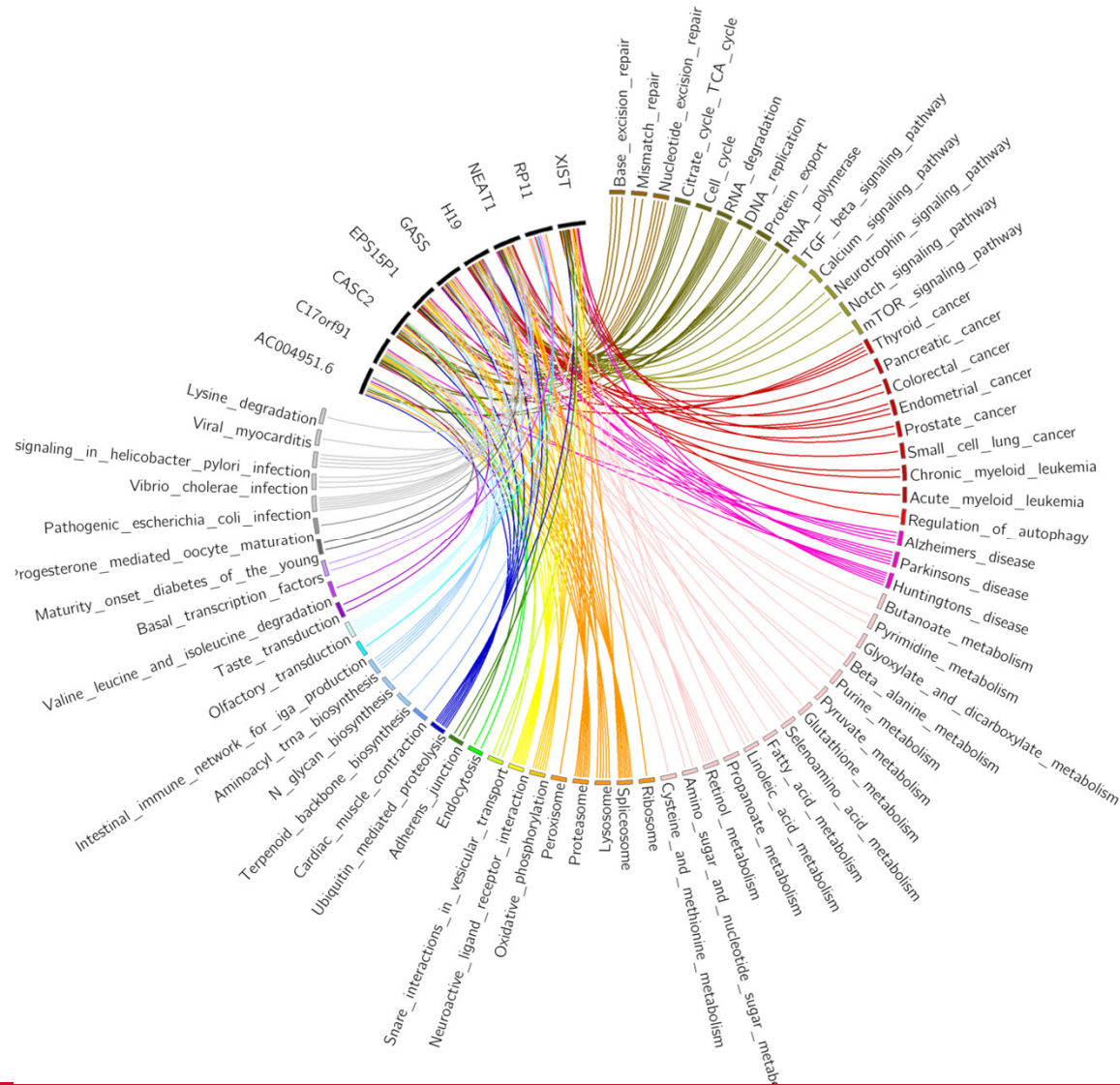
- > GSEA for each lncRNA:



Pathways whose corrected p-value <0.05 were selected



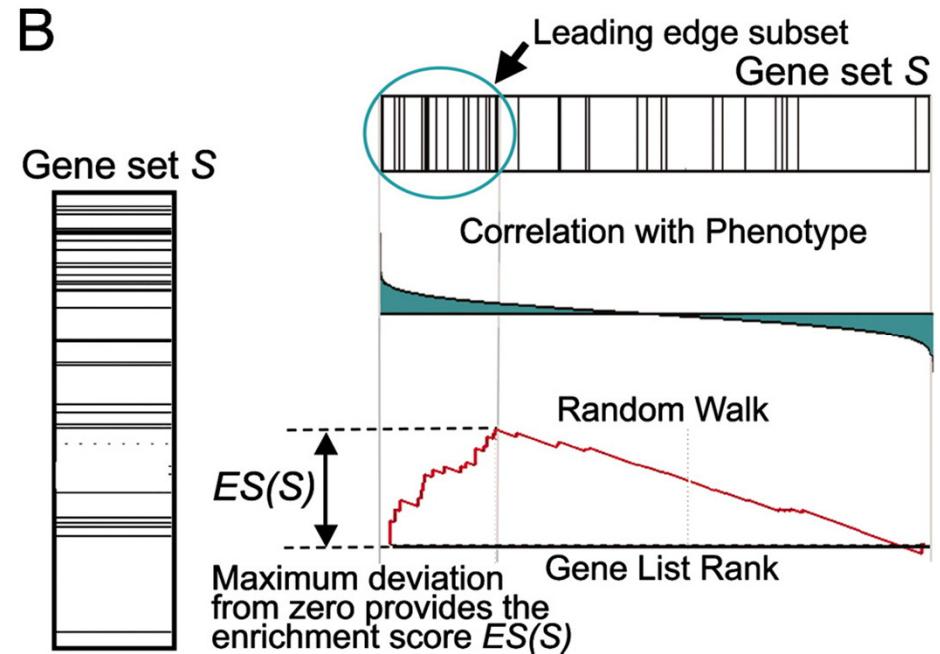
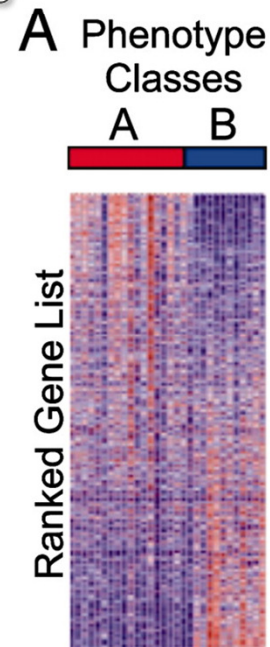
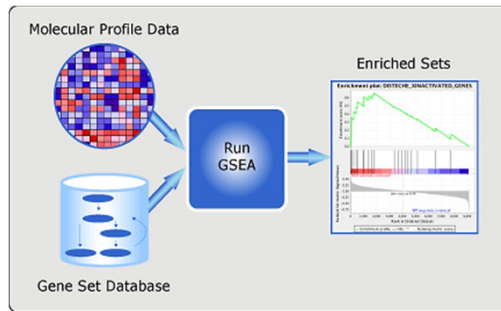
Pathways overrepresented by



Funding

- › Bill Walsh Lab
- › Kolling Institute for Medical Research
- › Charles Perkins Centre
- › Garvan Institute/TKCC
- › Functional Genomics Lab
- › Harvard Medical School





Subramanian, Aravind, et al. *PNAS* 102.43 (2005): 15545-15550.

Academic Qualifications and Positions

- › Research Fellow,
 - Charles Perkins Centre and School of Mathematics & Statistics, University of Sydney
 - **Research topic:** *Systems biology, Integrative Computational Modelling, Bioinformatics*
- › Postdoctoral Associate,
 - University of Toronto, University Health network, Princess Margaret Hospital
 - **Research topic:** *Integrative computational biology:*
 - Genomic cancer profiles analysis, cancer gene signature identification, PPI prediction, genome-level drug effectiveness quantification
- › Ph.D.,
 - University of Illinois at Chicago, Artificial Intelligence Laboratory
 - **Research topic:** *Artificial Intelligence:*
 - Optimization and search algorithms, evolutionary algorithms, machine learning, data mining, information retrieval
 - *Computational biology:* PPI network alignment, phylogenetic network reconstruction, genome-wide association studies
- › B.Sc., Sharif University of Technology, Computer Engineering Department



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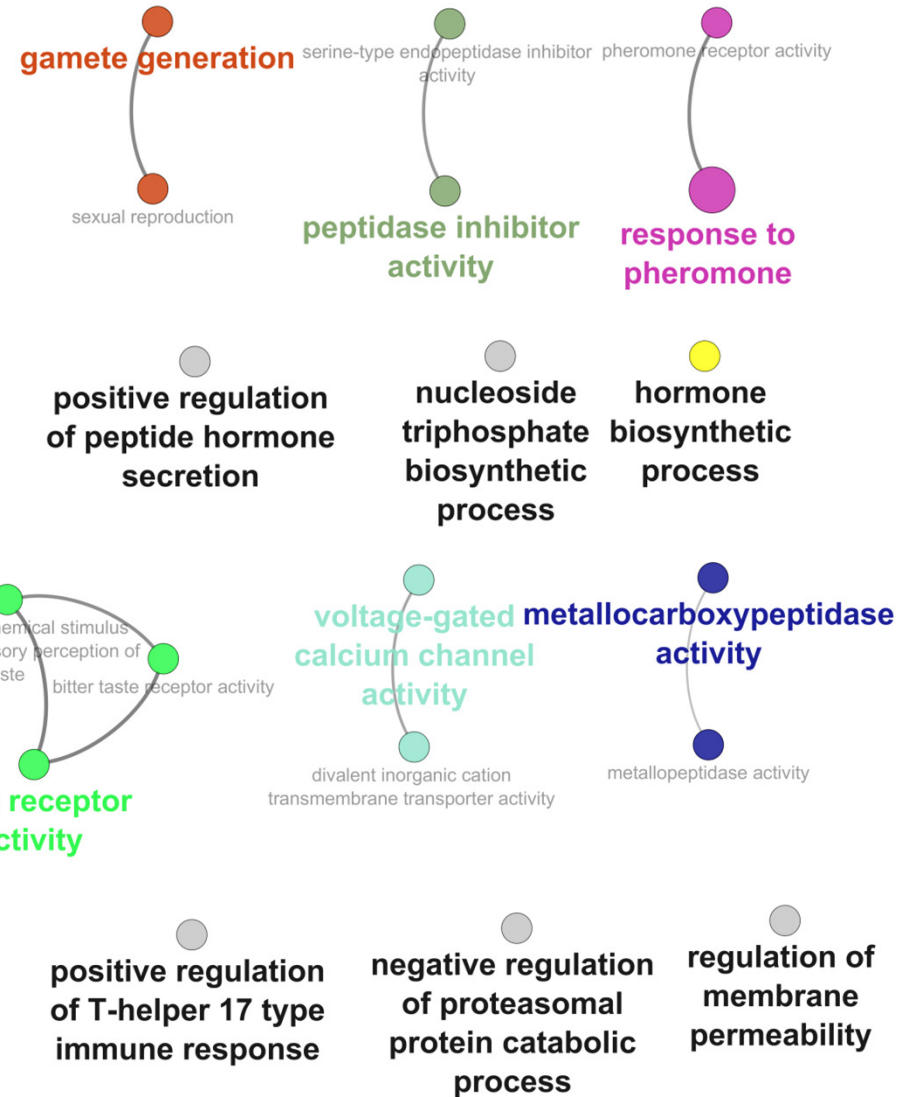
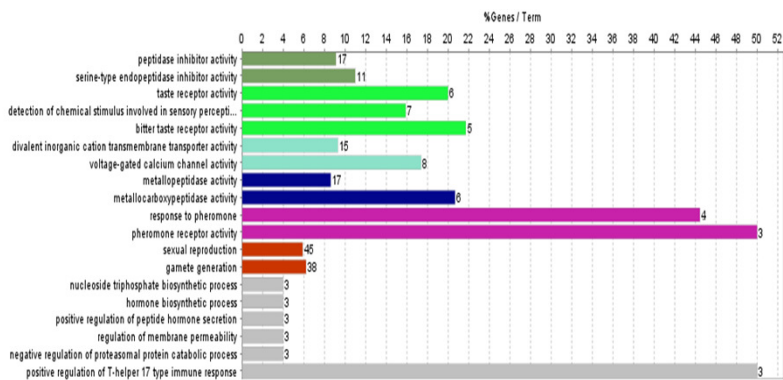
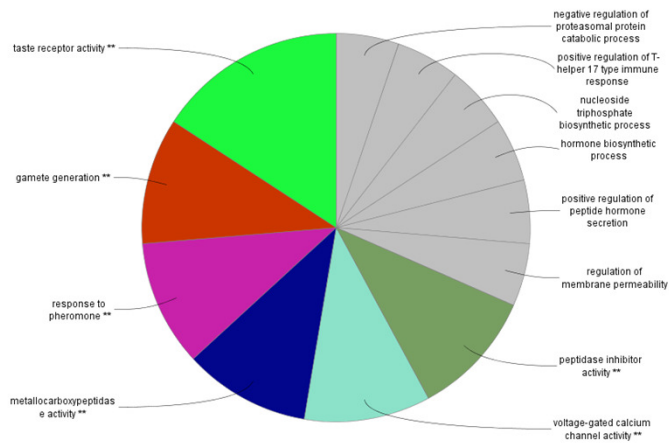
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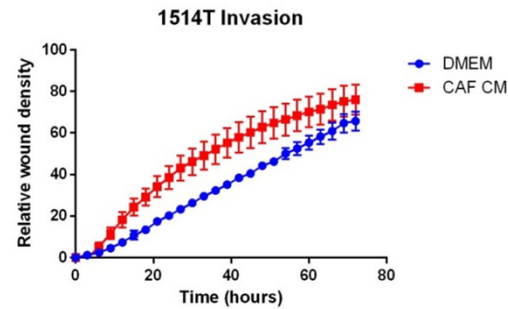
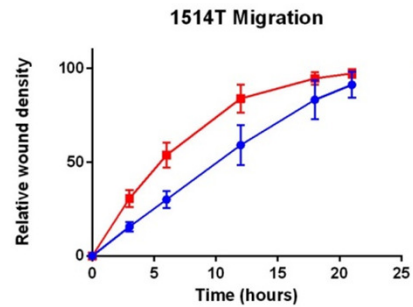


Functional Analysis

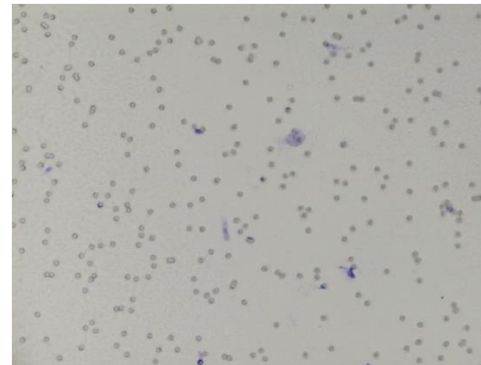
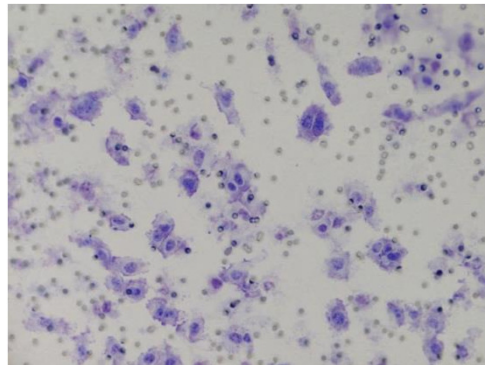




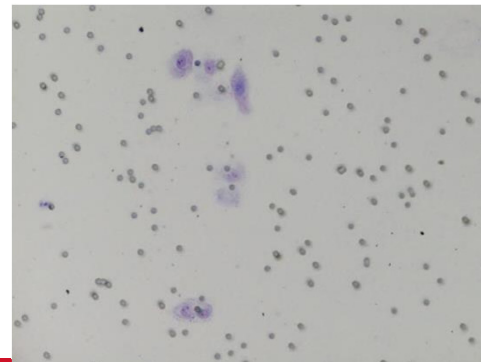
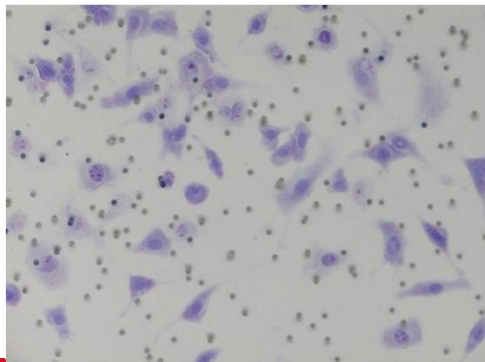
CAFs in ovarian cancer



migration



invasion



What genetic factors contribute to CAF function?

