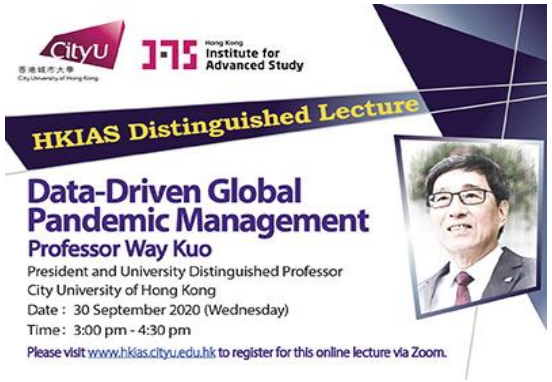
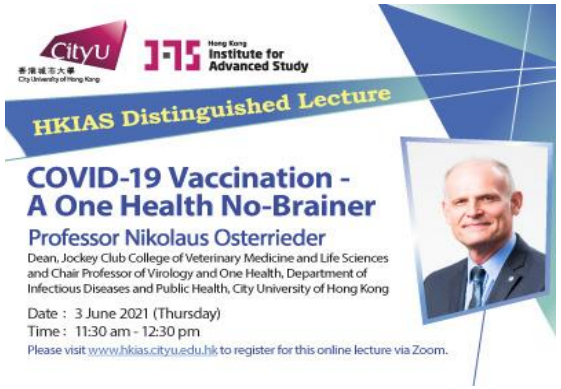
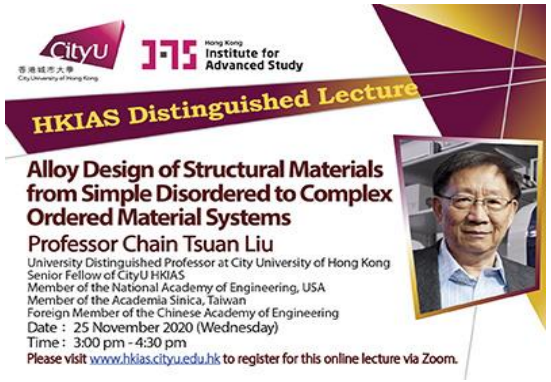
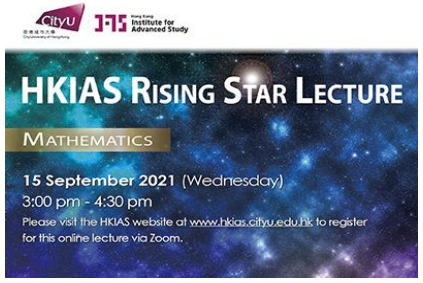
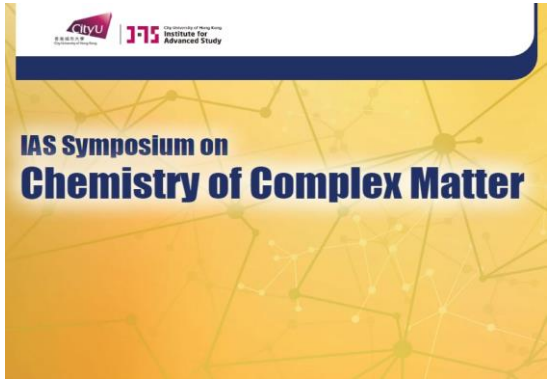
Hong Kong Institute for Advanced Study











Single Cell Analysis of Tumor Heterogeneity During Cancer Metastasis

Prof. Mengsu (Michael) Yang

Department of Biomedical Sciences
City University of Hong Kong
bhmyang@cityu.edu.hk

18 Feb 2022

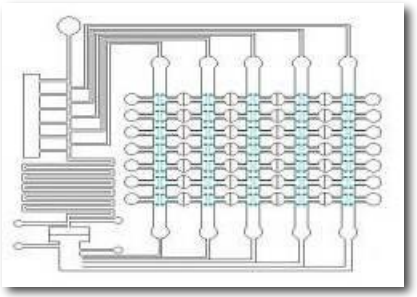
Hong Kong Institute of Advanced Studies

Research Interests: Development of biochip technology and nanomedicine for cancer research and molecular diagnostics and therapeutic applications.

Biochip Technology

Biosensors & Microfluidics

Single Cell Analysis



Anal Biochem **2017**, *409*, 2163-78.

Biosens Bioelec **2017**, *89*, 837-845.

Anal Chem **2018**, *90*, *1992-2000.*

Anal Chim Acta, **2018**, *1044*, 29-65.

ACS Sensors **2020**, *5*, 870-878.

Biosens Bioelec **2021**, *181*, 113142.

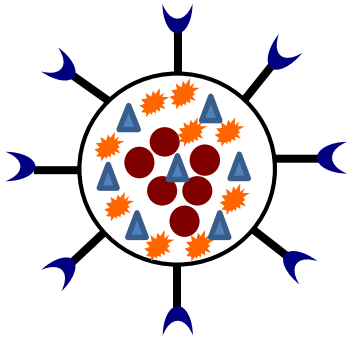
Lab Chip **2021**, *21*, 122-142.

iScience, **2022**, in press

Nanomedicine

Bio-Nano Interactions

Targeted Cancer Therapy



Adv Healthcare Mat **2017**, 1700185.

Nanotechnology **2018**, 29, 365503.

Adv Materials **2019**, 1904197.

Theranostics **2020**, *10*, 1181-1196.

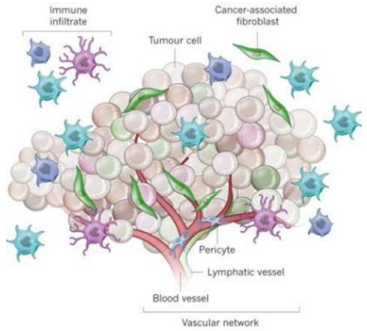
Adv Healthcare Mat **2021**, 2001658.

Nanomedicine **2021**, *16*, 1411–1427.

Cancer Biology

Circulating Tumor Cells

Migration & Metastasis



Br J Pharm **2017**, *174*, 302-313.

Nat Comm **2018**, *9*, 2359 (15pp).

Oncotarget **2020**, *11*, 1017-1036.

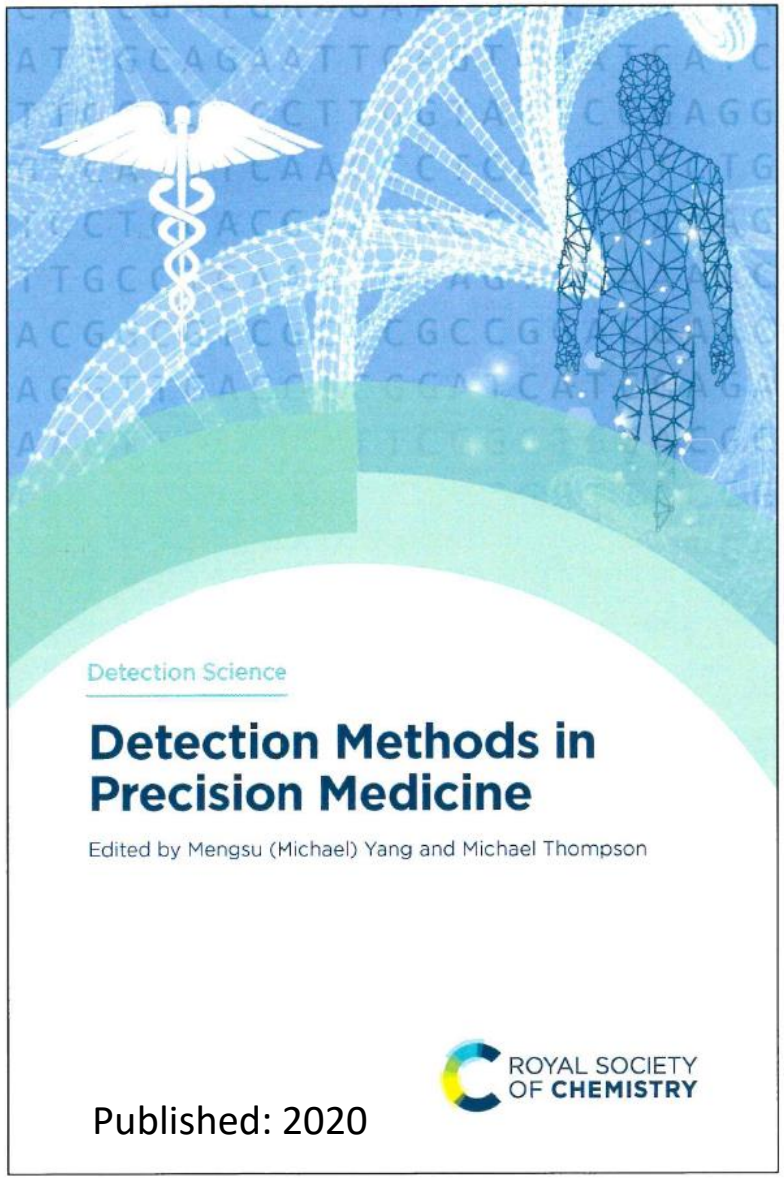
Cell Signalling **2020**, *71*, *109555.*

Oncogene **2020**, *39*, *4227–4240.*

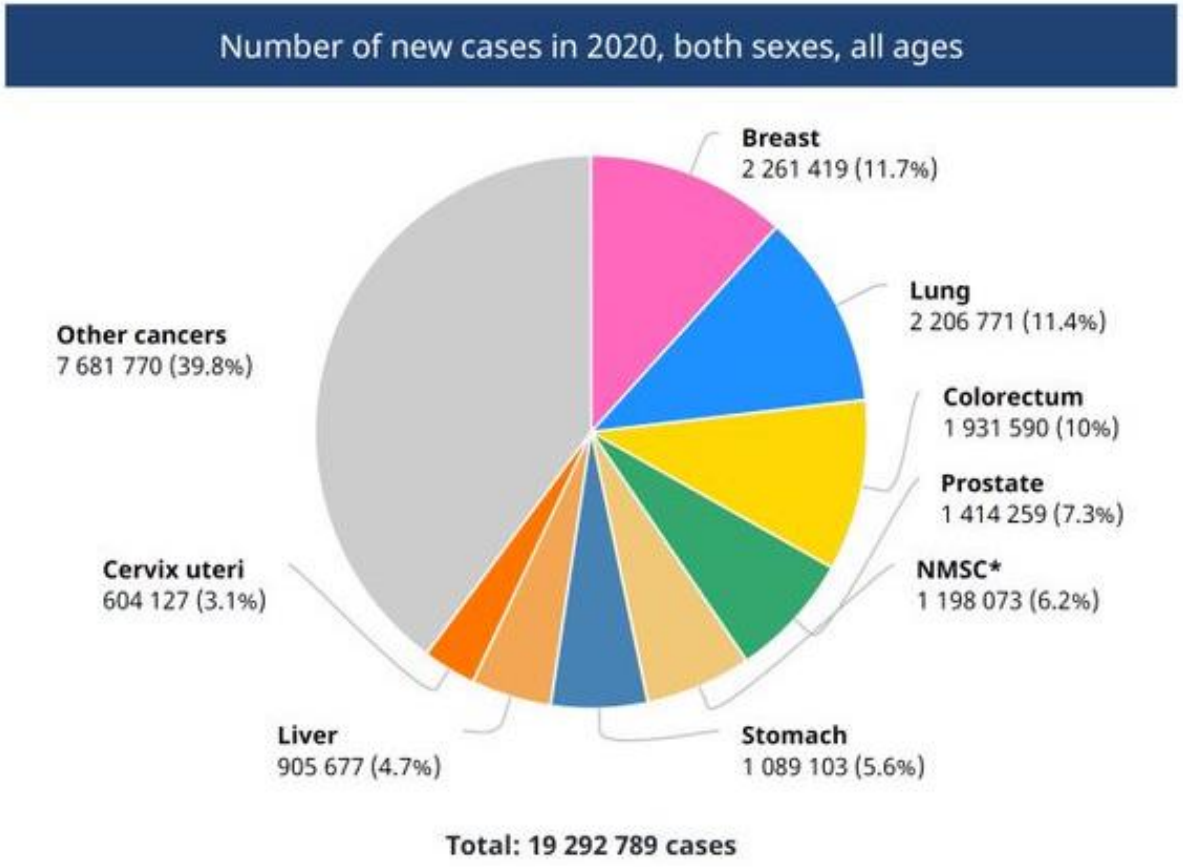
Oncogene, **2021**, *40*, 1775–1791.

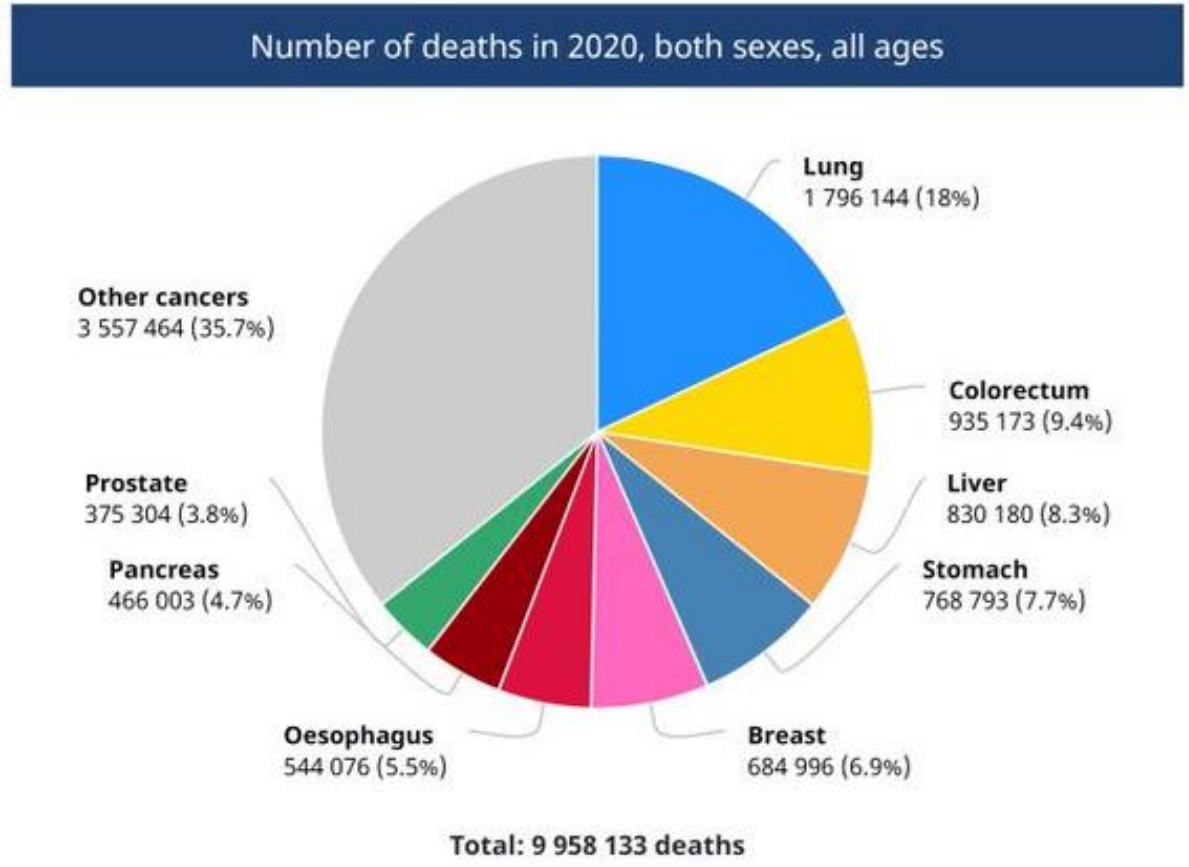
Nat Comm **2021**, *12*, 6103. (9pp)

Oncogene, **2022**, *41*, 895-906.



Cancer Incidence and Death: According to Globocan 2020, an estimated 19.3 million new cancer cases and 10 million cancer deaths occurred worldwide.





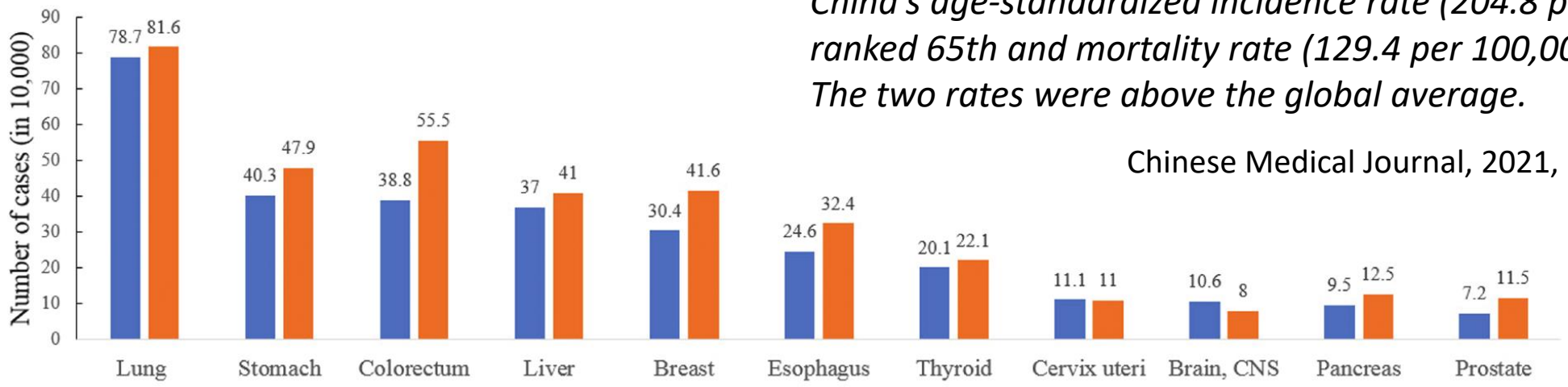
International Agency for Research on Cancer
World Health Organization

GLOBAL CANCER OBSERVATORY

#GCO365

Alarming Numbers in China: 24% of newly diagnosed cases and 30% of the cancer-related deaths worldwide in 2020.

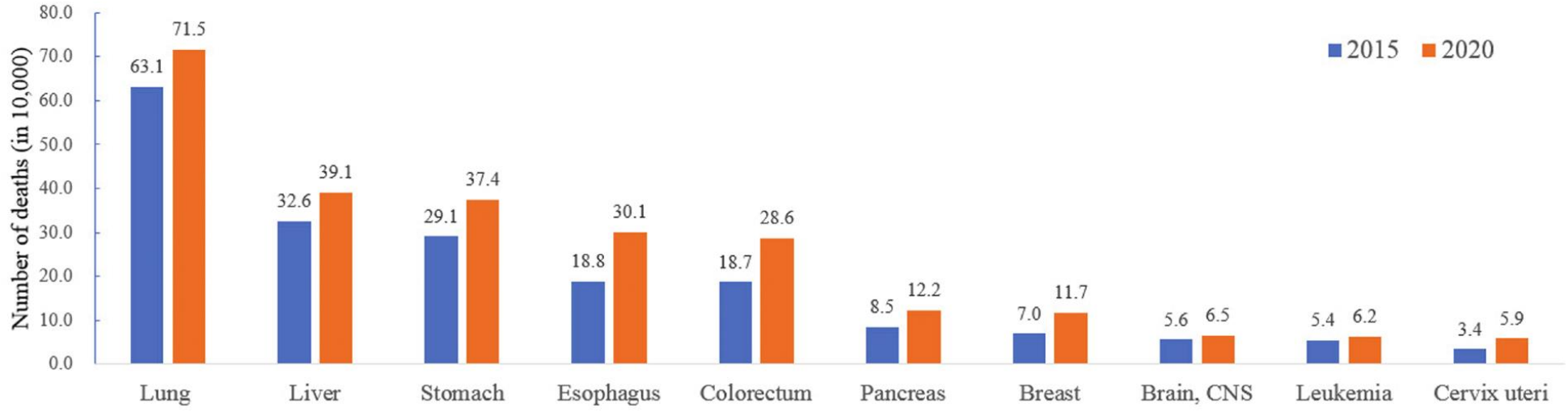
A



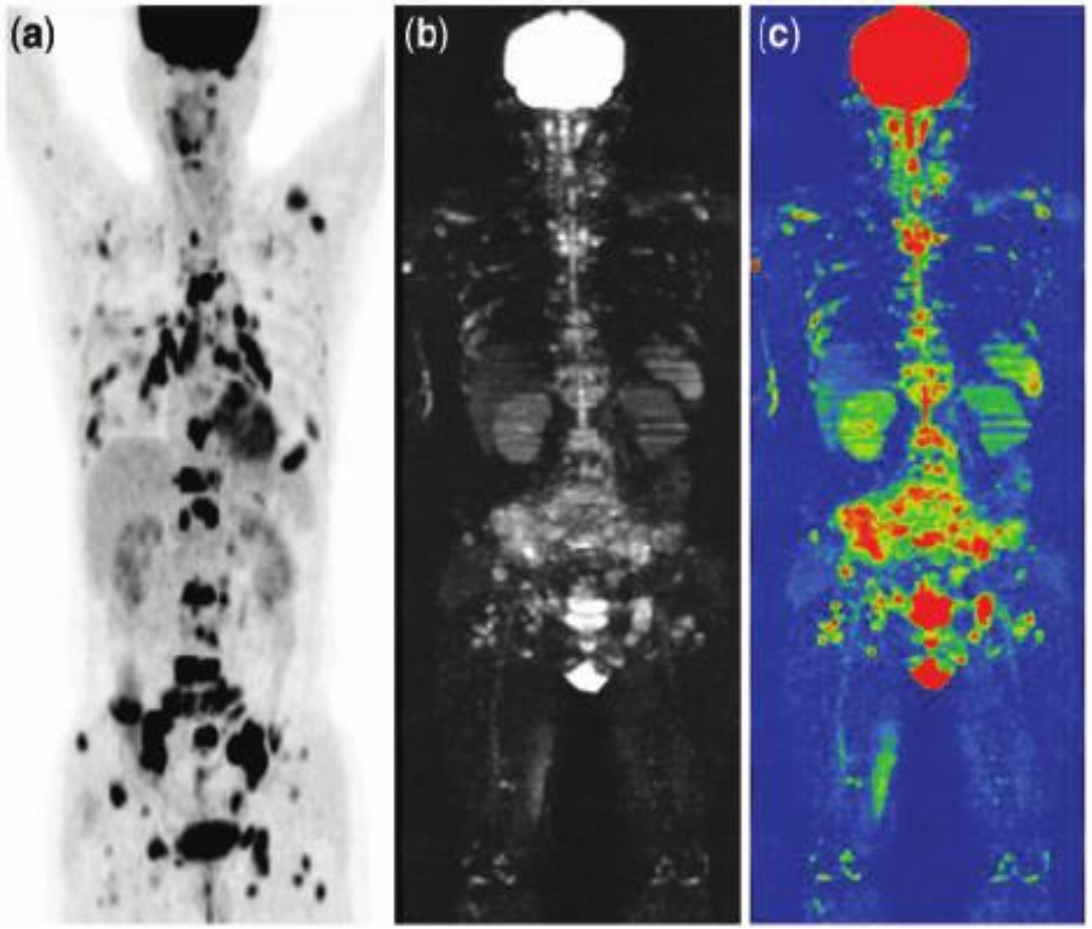
China's age-standardized incidence rate (204.8 per 100,000) ranked 65th and mortality rate (129.4 per 100,000) ranked 13th. The two rates were above the global average.

Chinese Medical Journal, 2021, 134(7), 783-791.

B

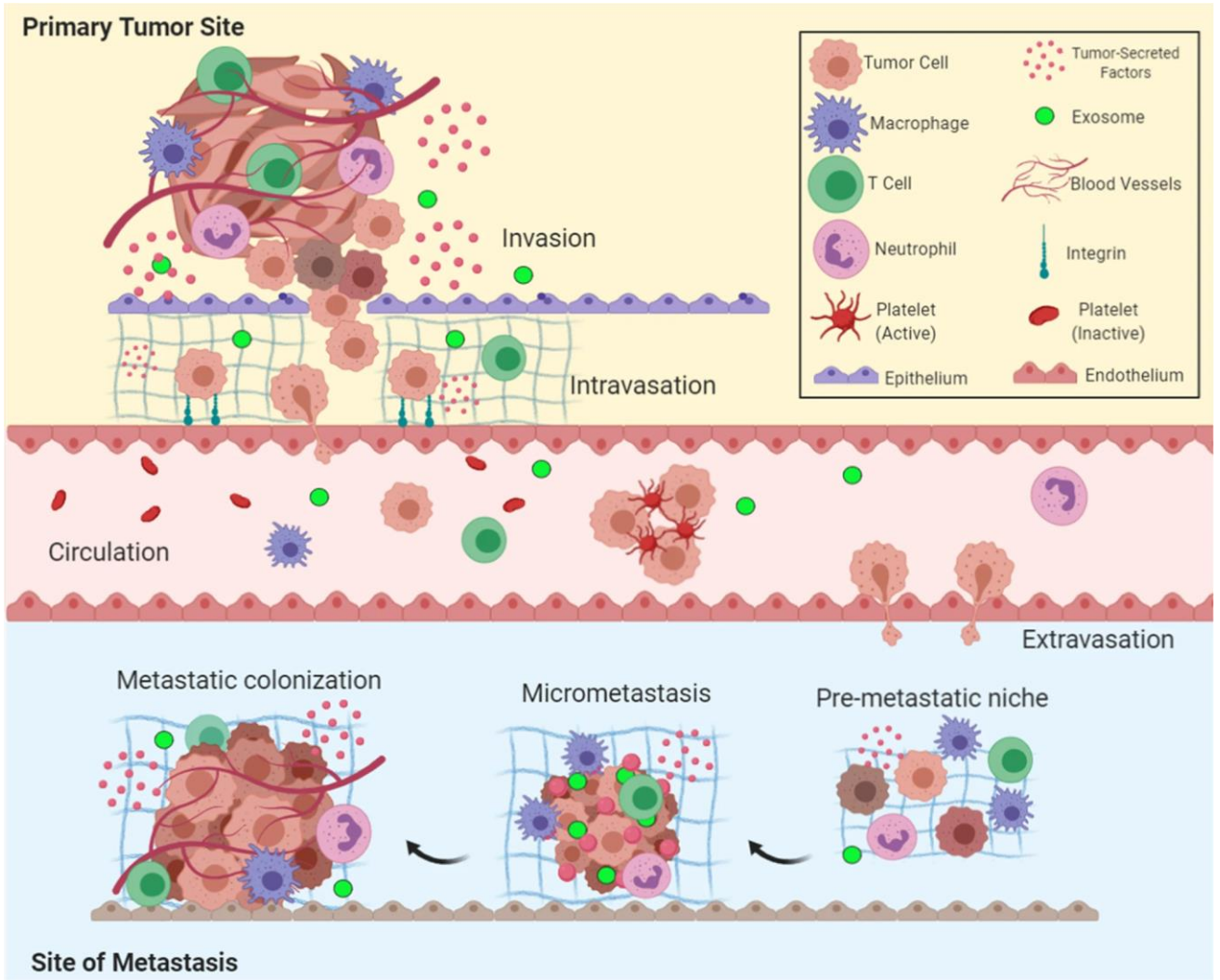


Cancer Metastasis: the primary cause of death for >90% of patients with cancer



PET/CT MRI/WB-DWI ADC

Gastroenterology Report, **2015**, 3(2), 128–135

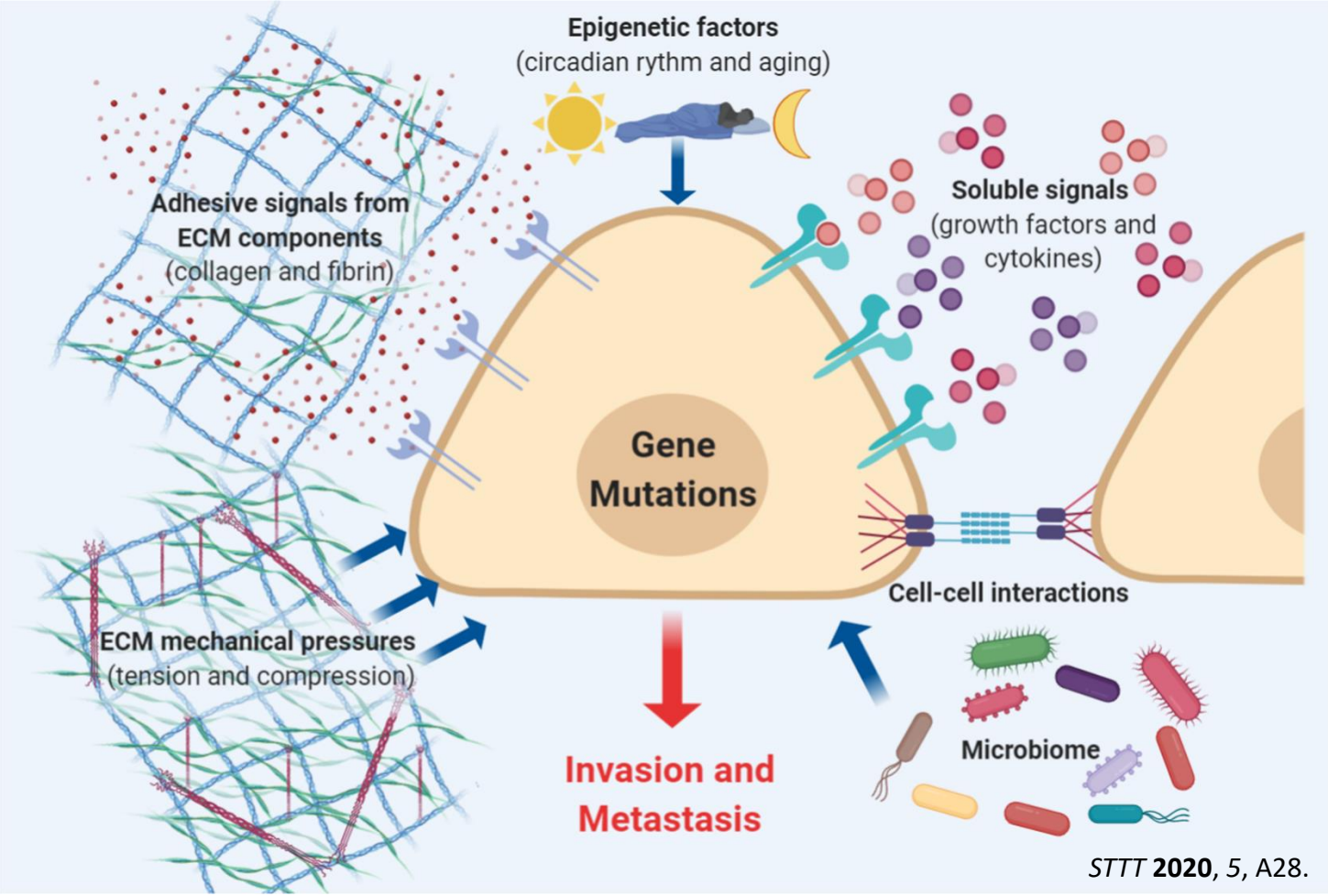


The metastatic cascade includes five key steps:
invasion, intravasation, circulation, extravasation, and colonization

Signal Transduction and Targeted Therapy, **2020**, 5, A28.

Determinants of Metastasis:

- The activation of invasion and metastasis is triggered by genetic and epigenetic factors induced by multiple environmental stimuli, including biological and physicochemical signals.
- Understanding the dynamics of this process will help identify targets for molecular therapies that may halt or possibly reverse cancer growth and metastasis.



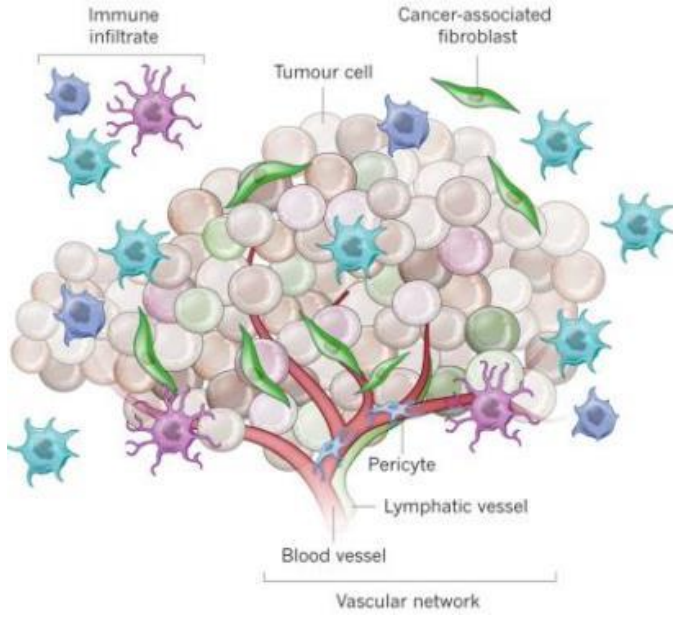
STTT **2020**, *5*, A28.

Research Challenges

in vitro modeling and study of tumor heterogeneity and microenvironment

Tumor Heterogeneity:

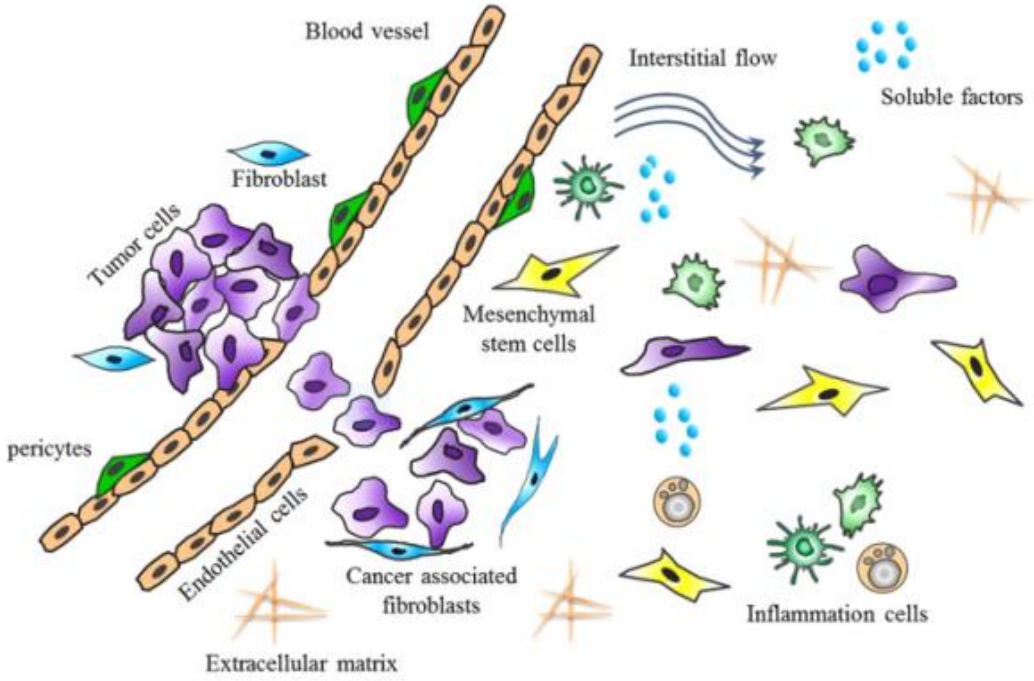
- Tumors consist of cancer cells, cancer associated fibroblasts, infiltrated immune cells…
- Cancer cells within the same tumor are heterogeneous (clones, cancer stem cells)…



Heterogeneity

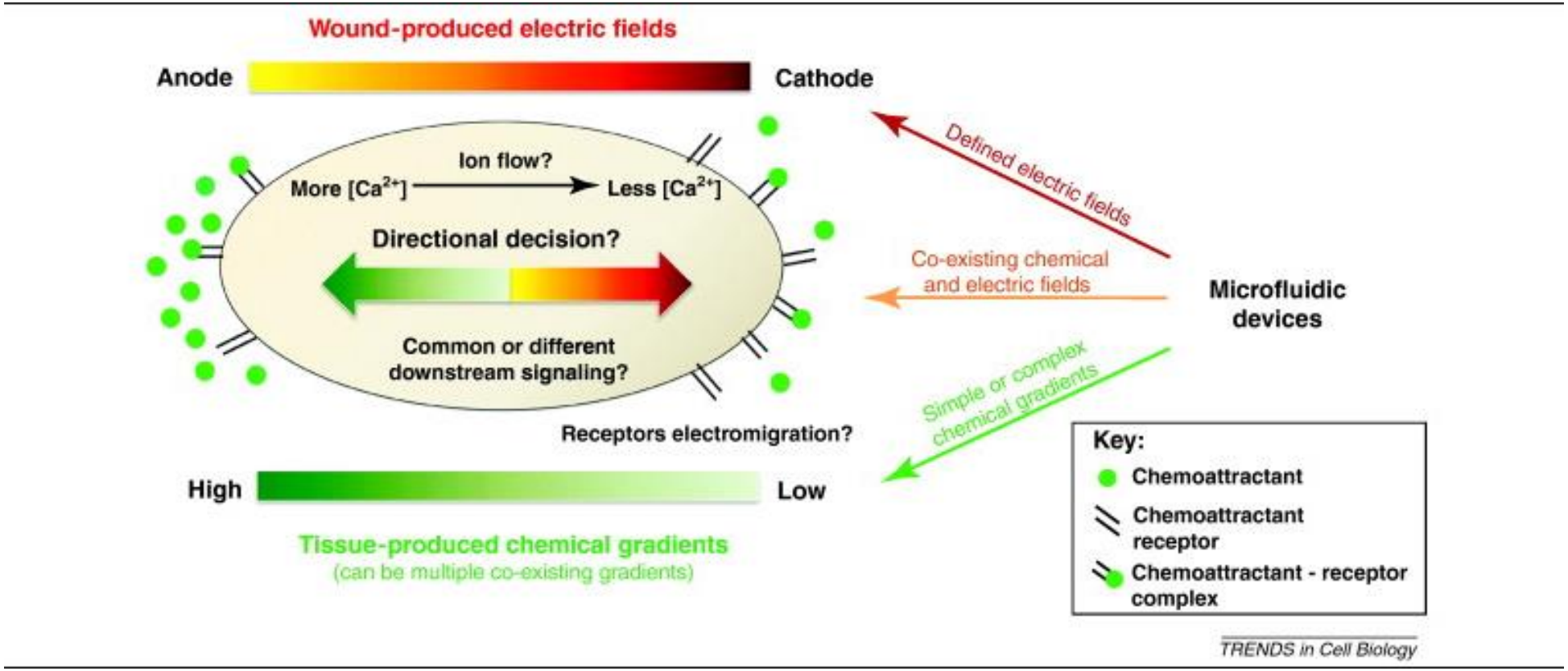
- Genetic
- Phenotypic
- Functional
- Microenvironmental

Microenvironment: including surrounding blood vessels, immune cells, fibroblasts and other cells, signaling molecules and extracellular matrix (ECM).



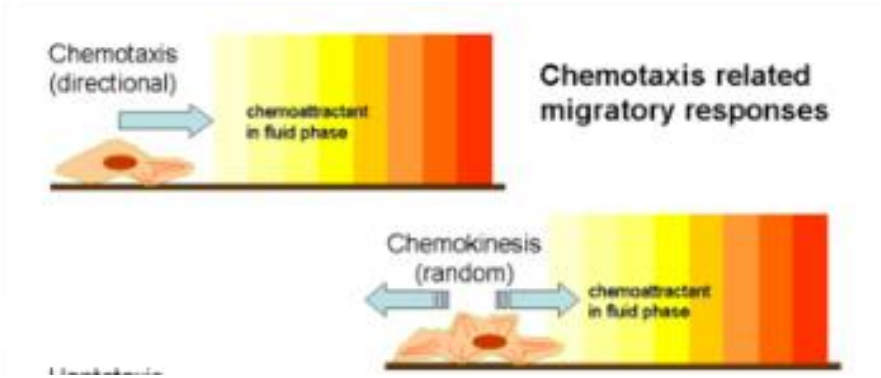
Cell Migration under Chemical/Electrical Gradients

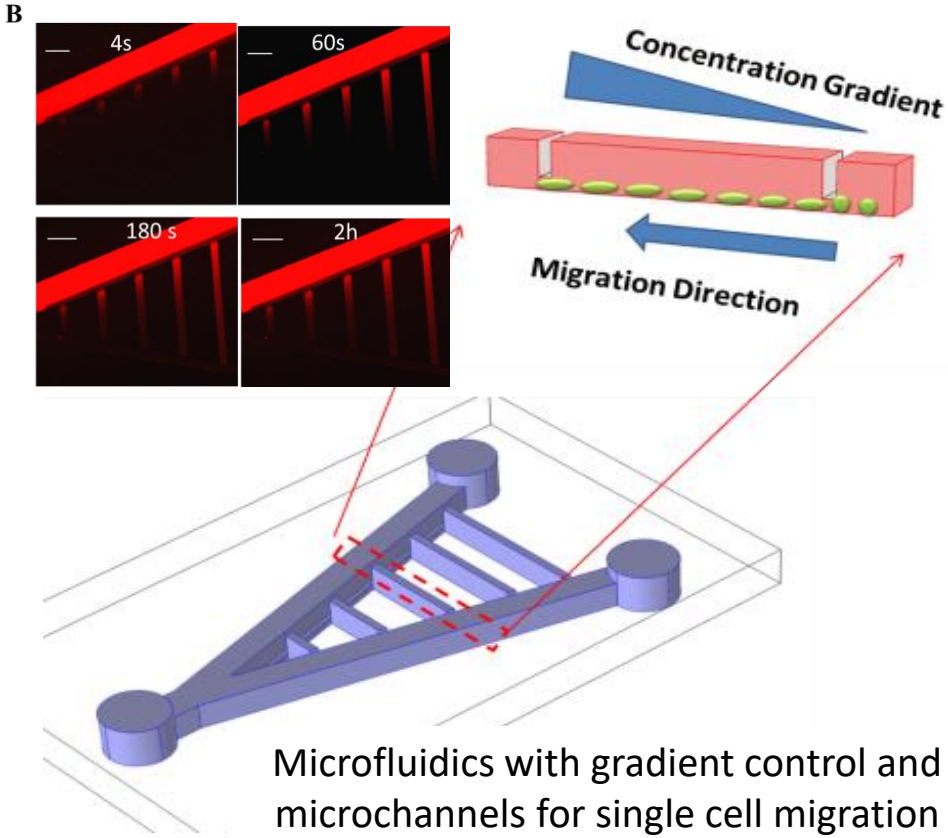
Chemotaxis/Electrotaxis plays an important role during embryogenesis, inflammation, wound healing, and tumour metastasis. However, the mechanisms at play are still poorly understood.



Trends in Cell Biology, **2011**, 21, 489-497.

Cancer Cell Chemotaxis in Microfluidics

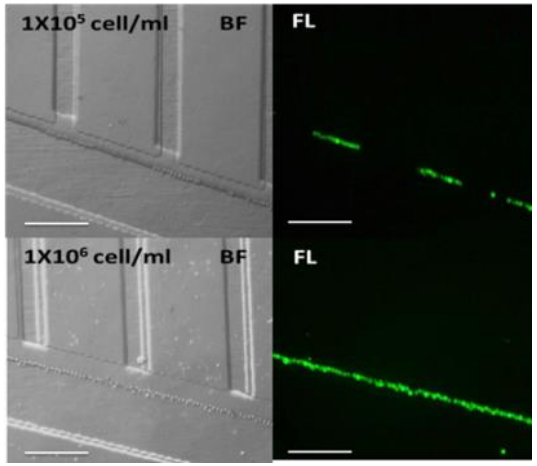


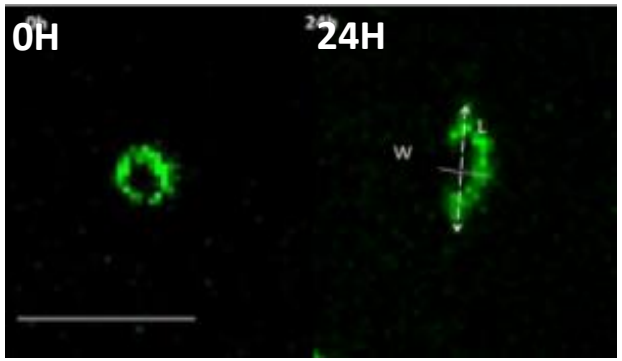


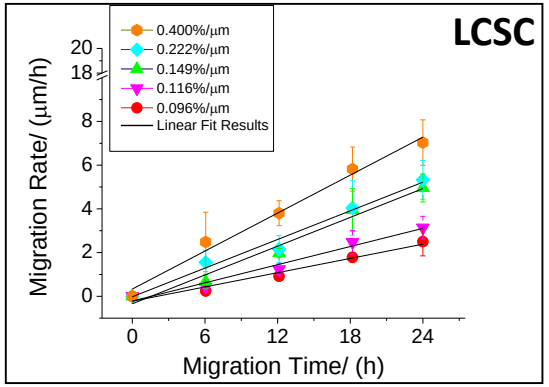
Microfluidics with gradient control and microchannels for single cell migration

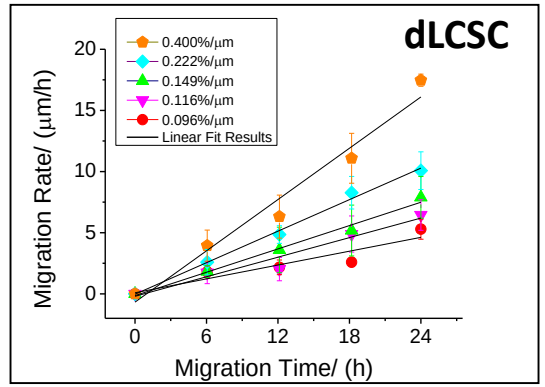
Migration, Cytoskeleton Change, and Metabolism

- Quantitative model to study chemotaxis at single cell level









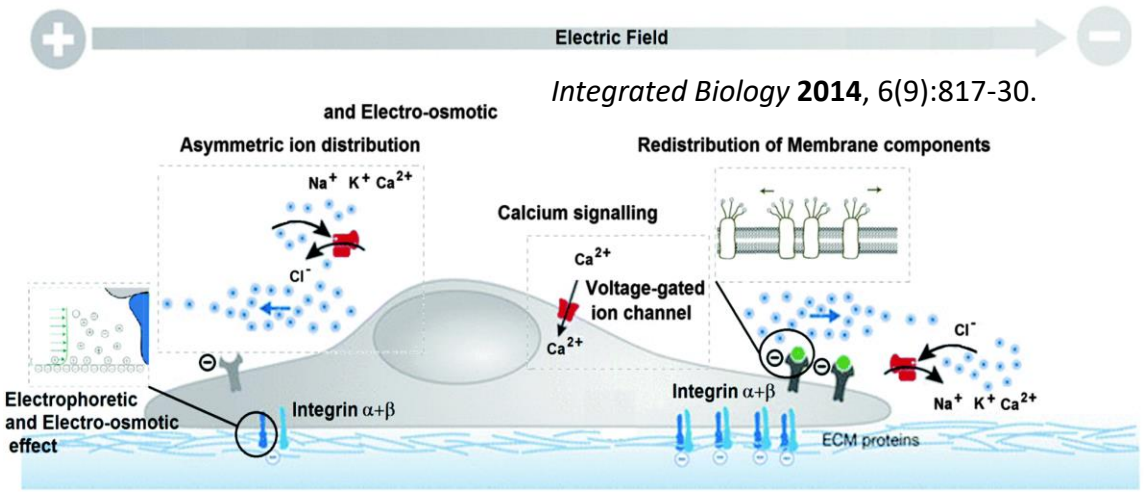
- Gradient-dependent migration, elongation and acceleration
- Association of migration rates with metabolism
- Chemotaxis mediated by Wnt/β-catenin signaling pathway

Zou Heng et al, *Anal. Chem.* **2015**, *87*, 7098-7108.

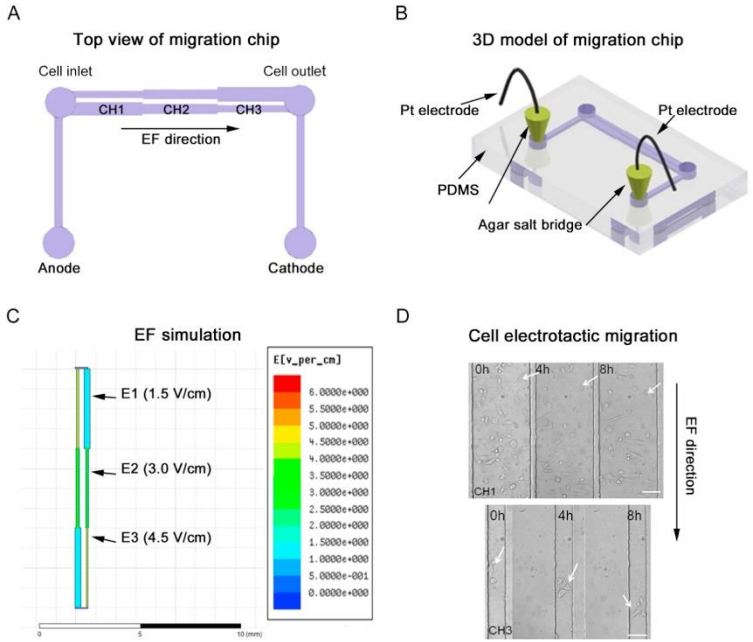
Cancer Cell Electrotaxis

Microfluidics for electrotaxis-based cell migration

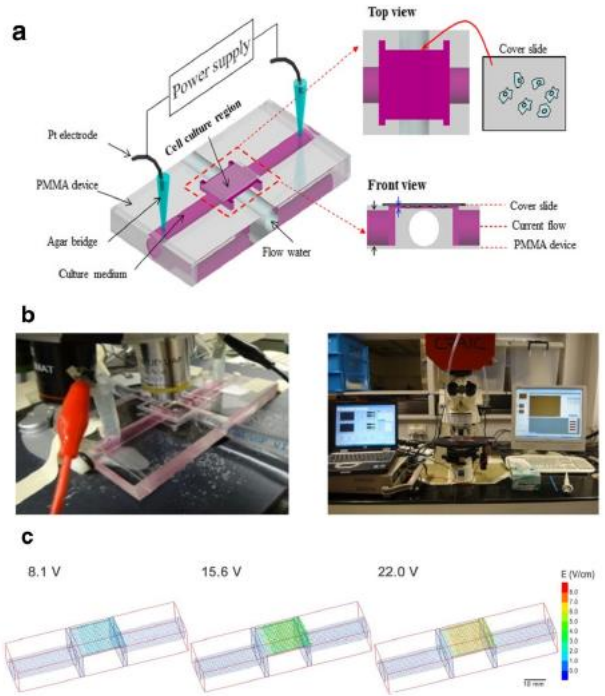
Li Yaping, Xu Tao et al
Biosens Bioelec **2017**, *89*, 837–845.
Anal Bioanal Chem **2017**, *409*, 2163–2178.
Bioelectrochem **2018**, *124*, 80–92.



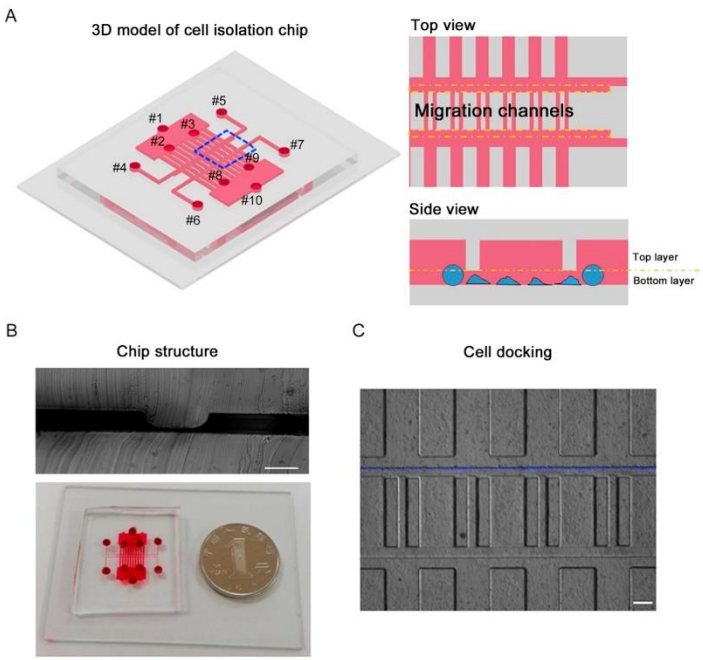
Integrated Biology **2014**, 6(9):817-30.



Microfluidic chip for <u>electric field gradient</u> generation and electrotactic migration of cells.

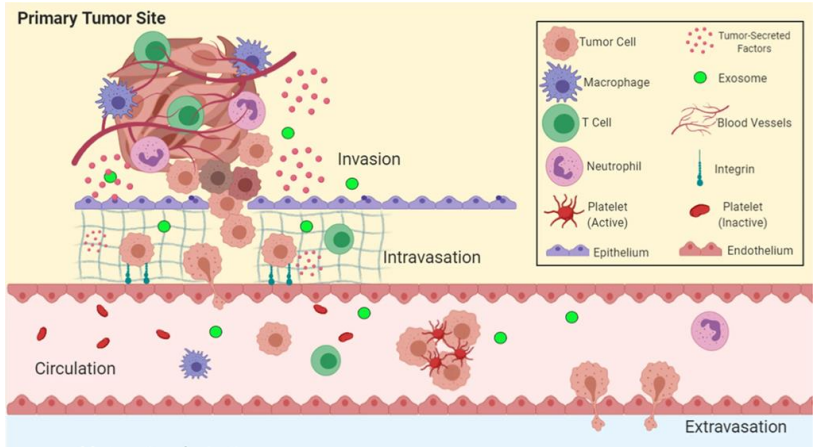


Microfluidic chip for studying electrotaxis-based <u>cell heterogeneity</u>

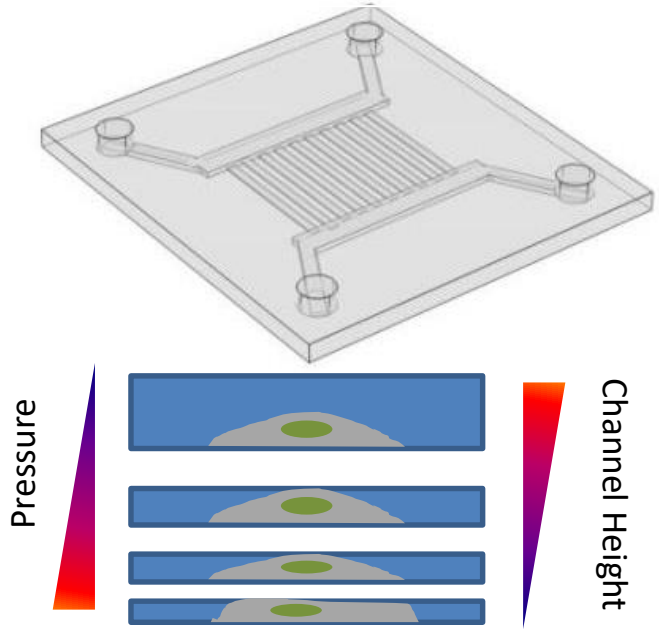


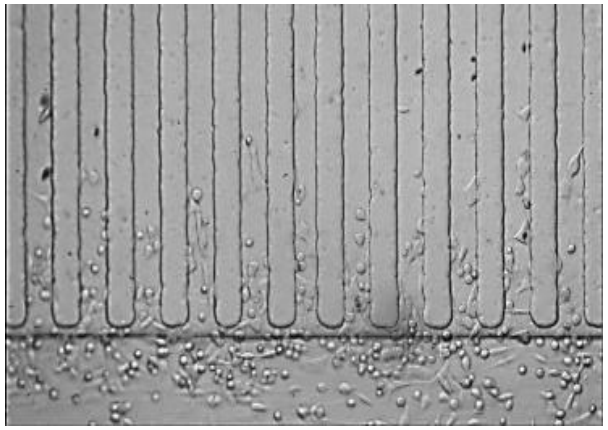
Microfluidic chip for <u>cell isolation</u> based on migratory ability.

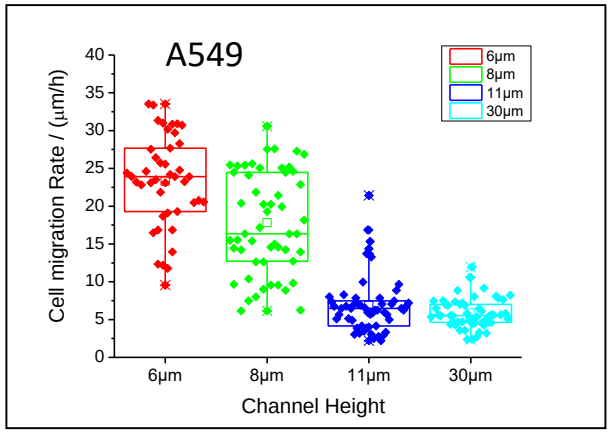
Mechanical Stress and Cell Migration

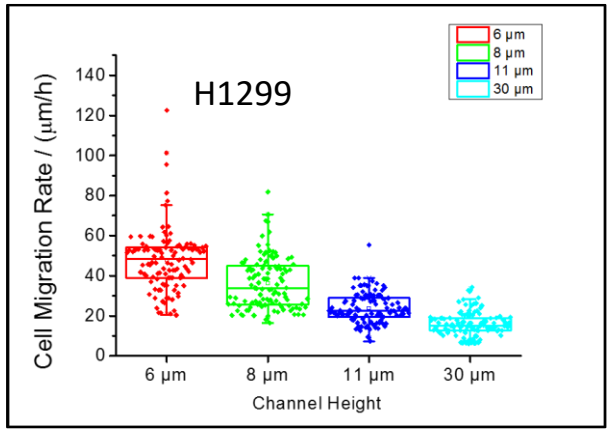


Microfluidics with controlled confined space for cell migration under stress



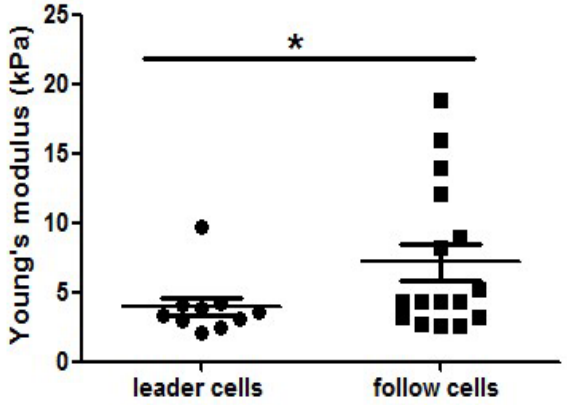


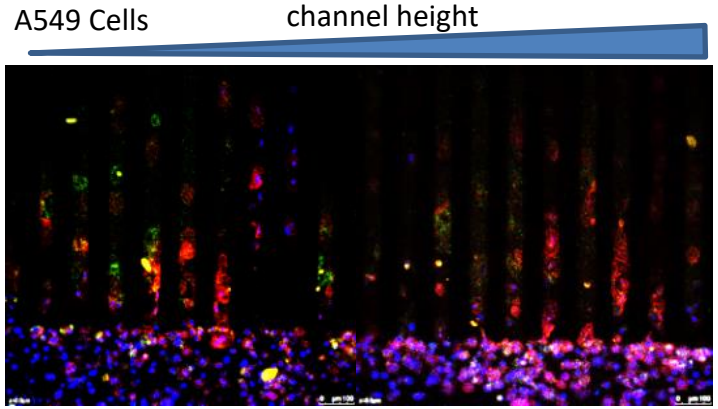




Cancer cell plasticity:

- mechanical plasticity – elasticity/stiffness
- biological plasticity – phenotypic changes





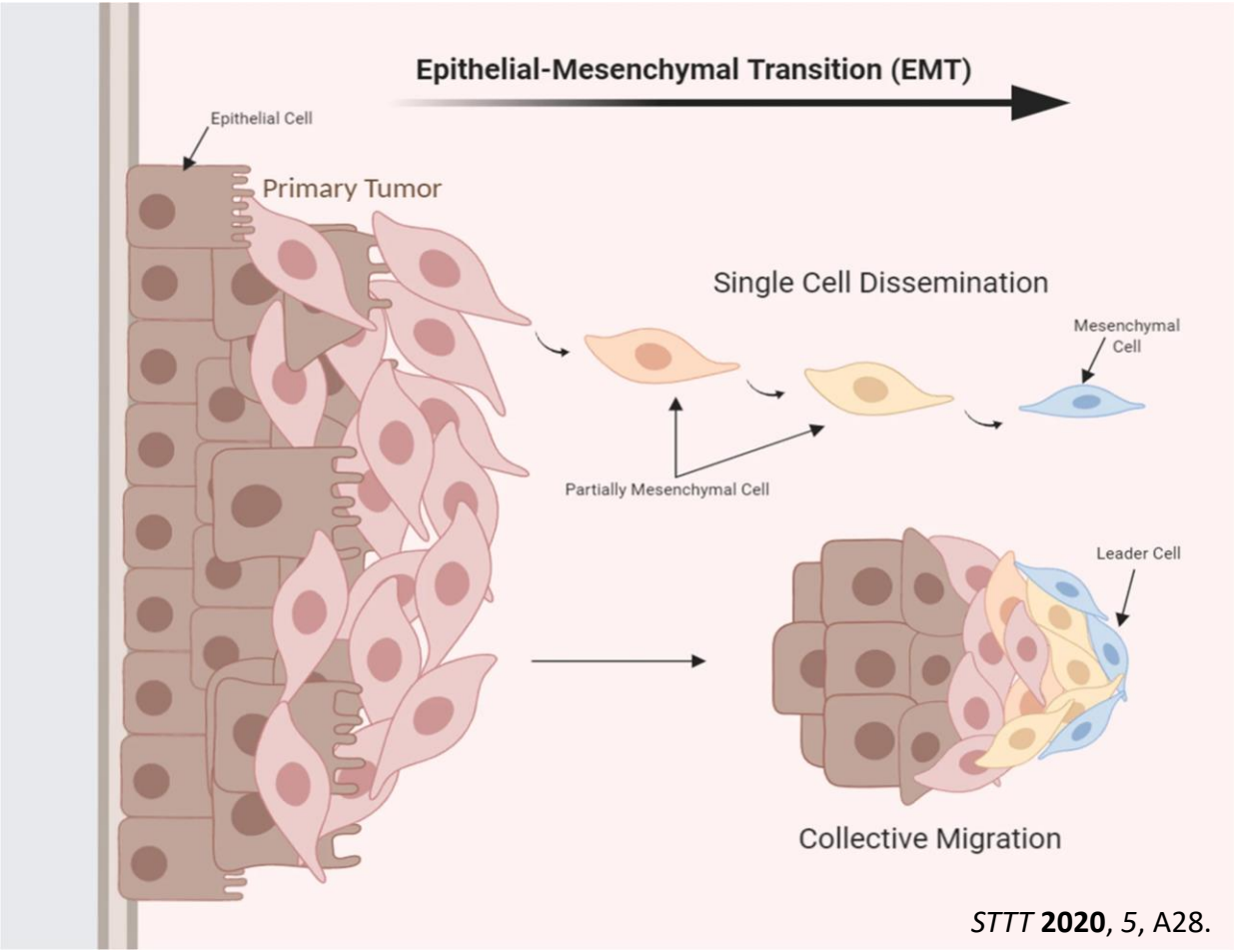
Green: N-cadherin; Red: E-cadherin; Blue: Nucleus

Migration rate in confined channels is associated with cell elasticity and EMT .

Zou Heng et al, *Anal. Chim. Acta,* **2018**, *1044, 29-65*.

Epithelial–Mesenchymal Transition (EMT)

EMT occurs through single-cell dissemination or through collective migration.



STTT **2020**, *5*, A28.

Single-cell dissemination:

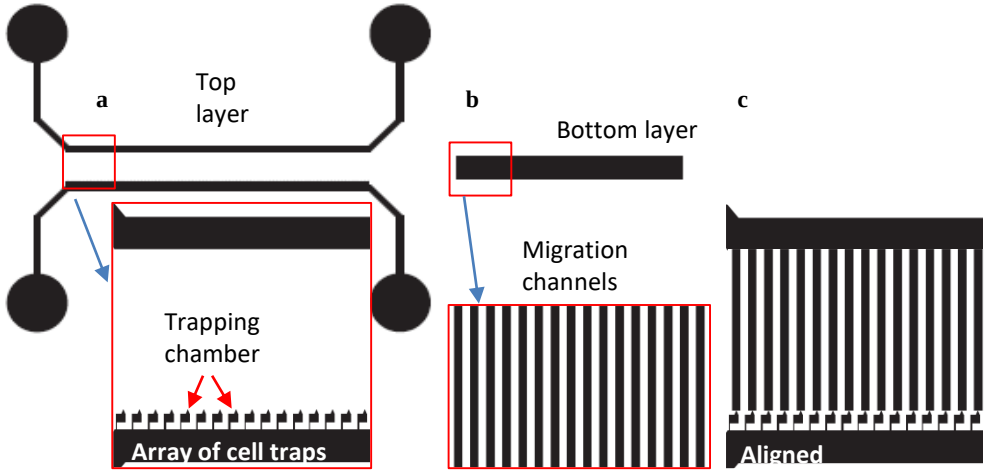
- Random or by selection?
- EMT: before or after?

Collective migration:

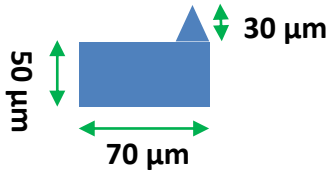
- Heterogeneity among the migrating cells?
- Plasticity: leaders vs. followers?

Heterogeneity of Cancer Cells Migrating into Confined Space

Microfluidic platform for single cell migration analysis



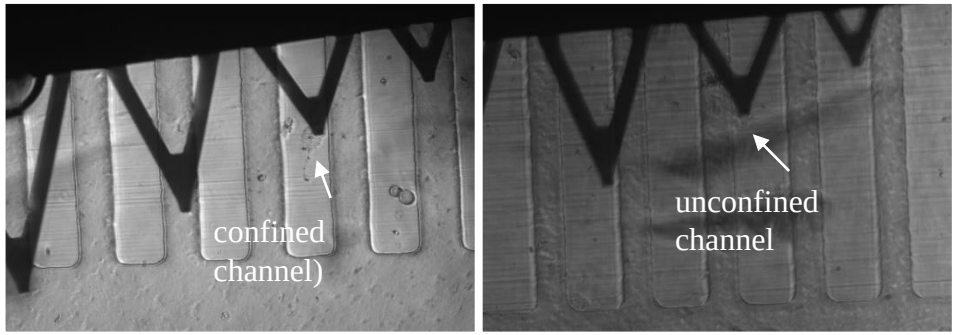
Cell trapping chambers

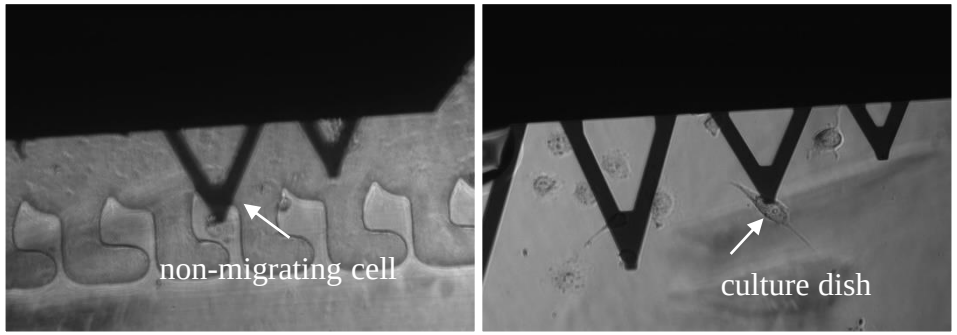


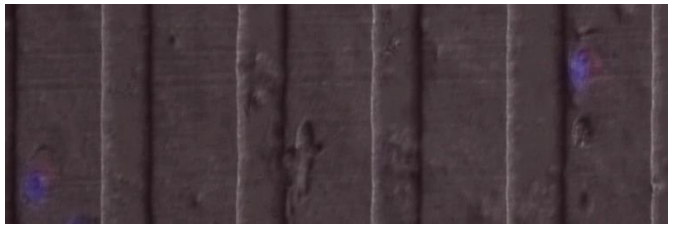
Migration channel heights

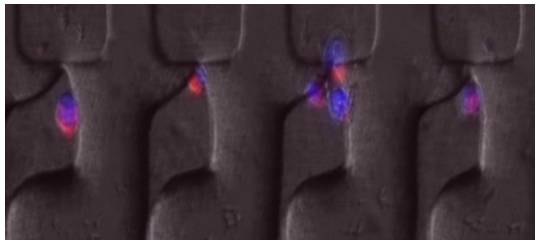
- 6 µm ≈ confined channels
- 13 µm ≈ Semi-confined channels
- 24 µm ≈ Unconfined channels

AFM-based stiffness measurement of single cells







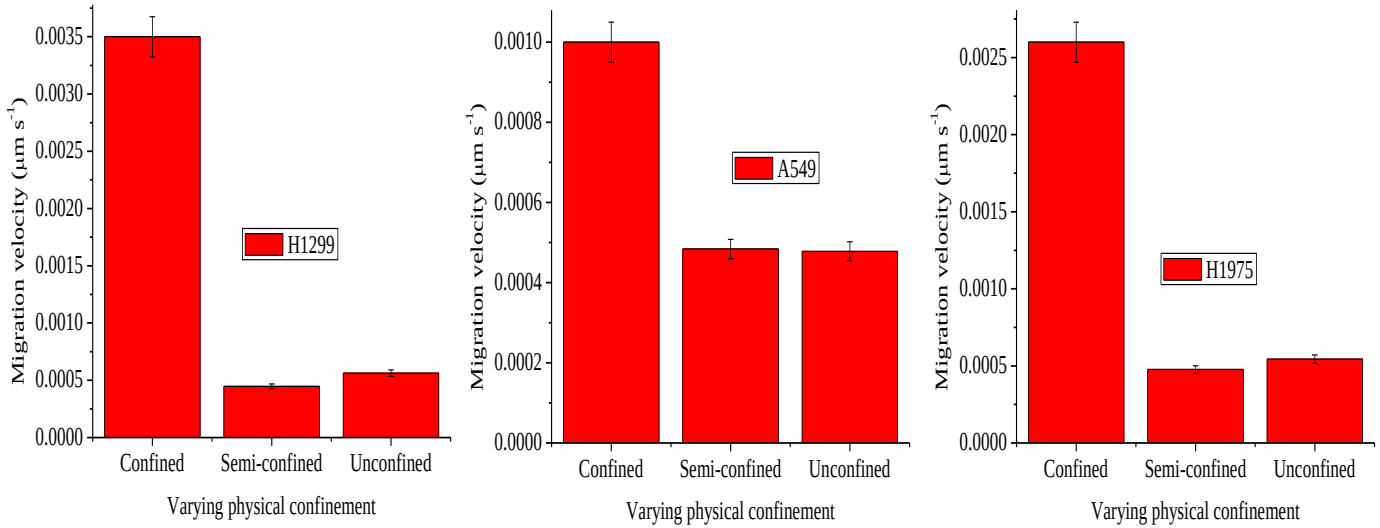


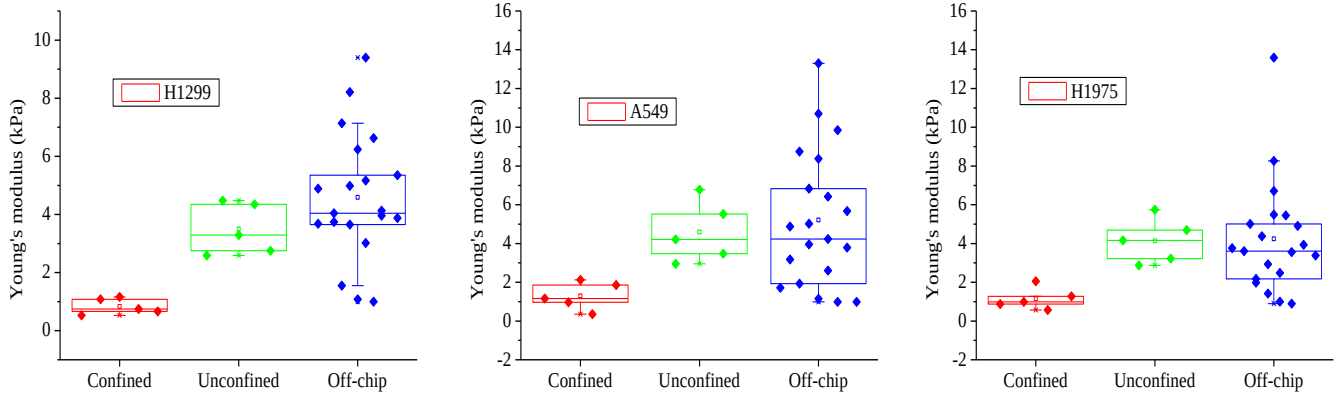
EMT-related markers staining
Single cell elasticity profiling
Single cell EMT gene profiling

Kowsar Alam et al, unpublished.

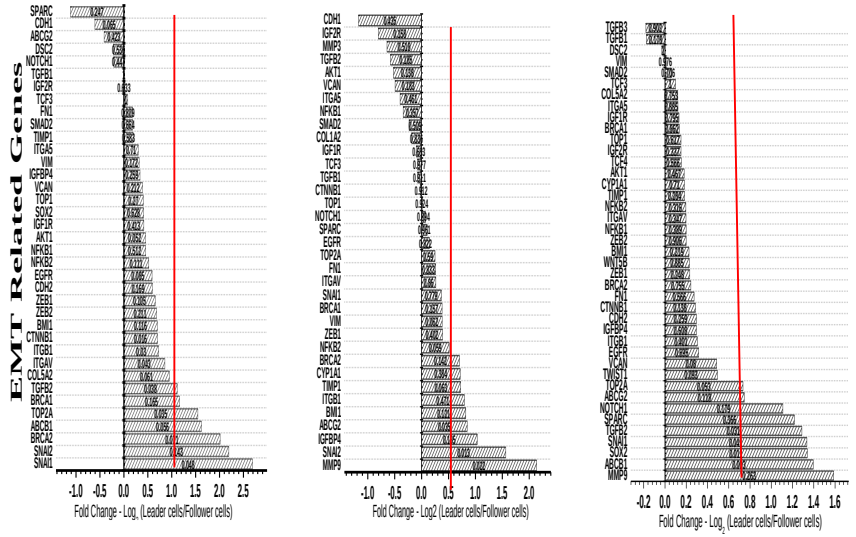
Single Cell Migration in Confined Space

Cells with more elasticity entered the confined channels and migrated faster under mechanical stress…





…with significant upregulation of EMT-related genes

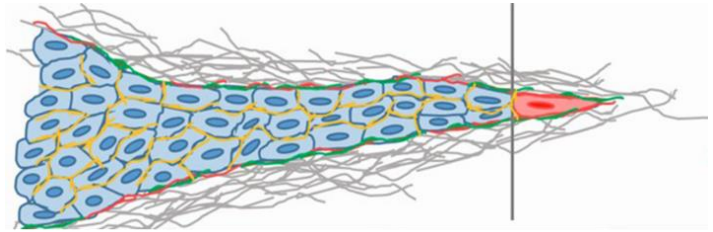


Single-cell dissemination:

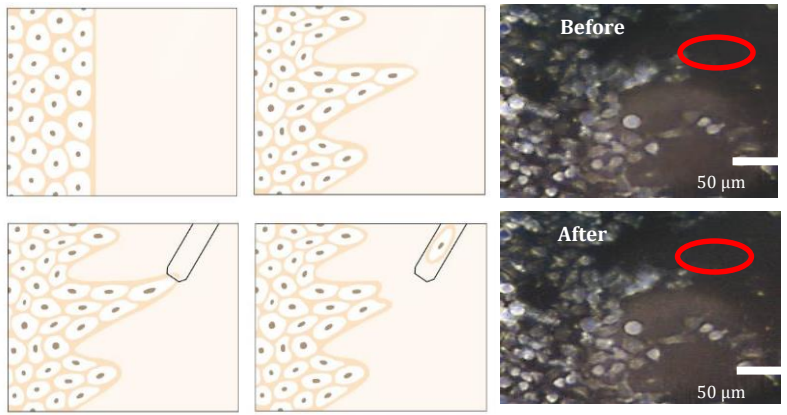
- Random or by selection? Cells with greater elasticity are preferred to migrate into confined space.
- EMT: before or after? Partial EMT before and continue to undergo EMT during the migration under confined space.

Kowsar Alam et al, unpublished.

Single Cell Transcriptional Profiling of Collectively Migrating Cells

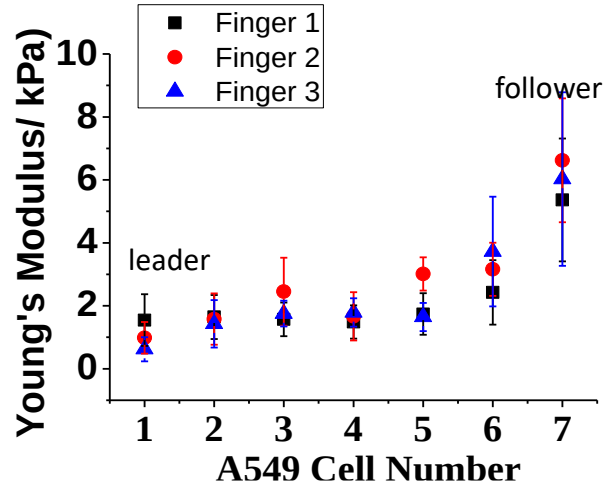


Follower cells Leader cells



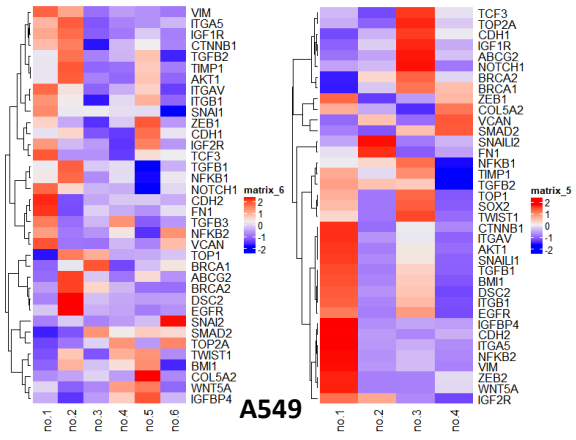
- Collective migration during metastasis
- Single-cell analysis of the migration front
- Spatial resolution of EMT-profiles

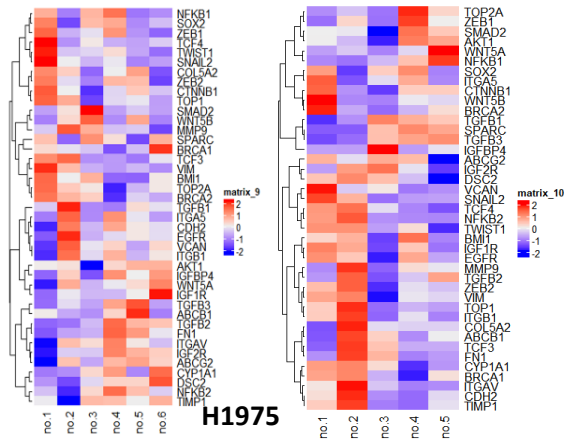
Elasticity of Fingers in Collective Migration

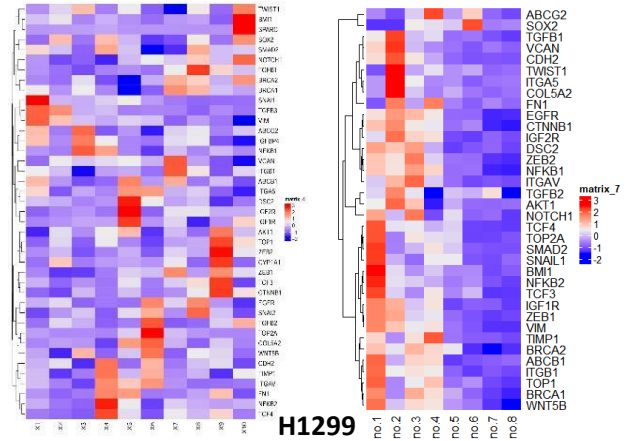


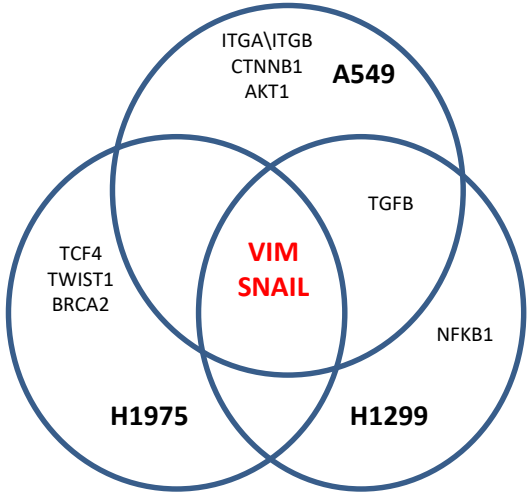
Single cell elasticity profiling with spatial resolution

Single cell EMT transcription profiling with spatial resolution





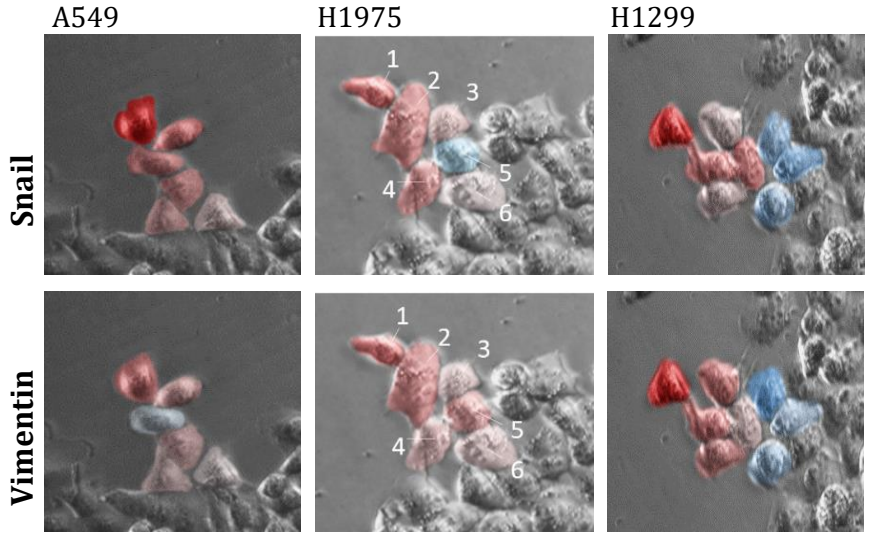




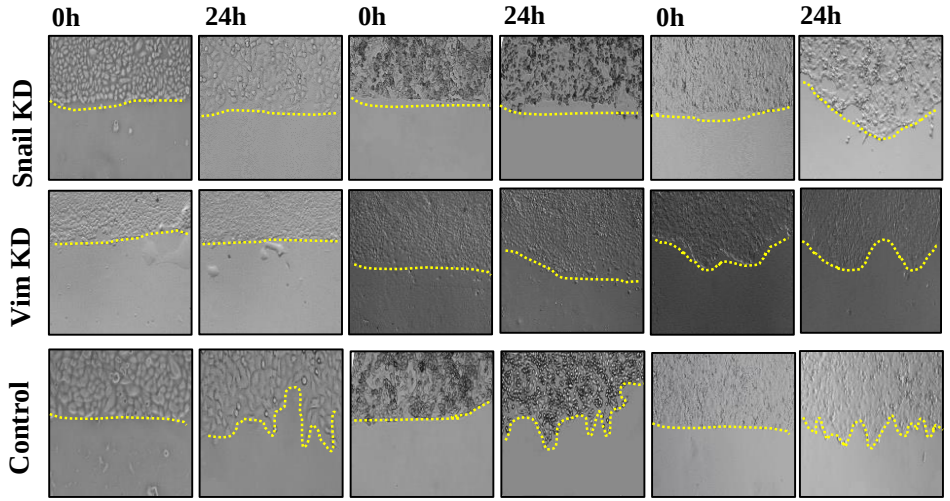
Zou Heng, Yang Zihang, et al *iScience*, **2022,** in press.

Single Cell Transcriptional and Elastic Profiles of Collectively Migrating Cells

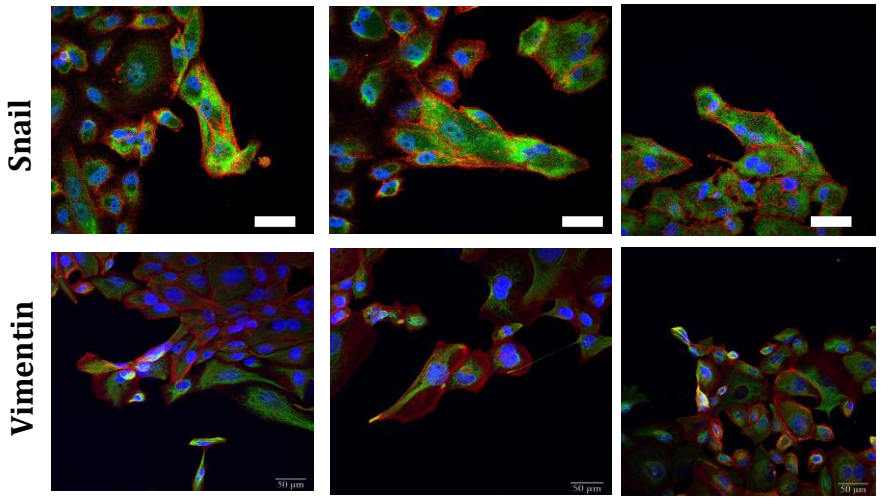
Single cell qPCR - mRNA expression pattern



Inhibition of Snail & Vimetin prevented finger formation



Immunofluorescence - protein expression pattern



Scale: 20 μm

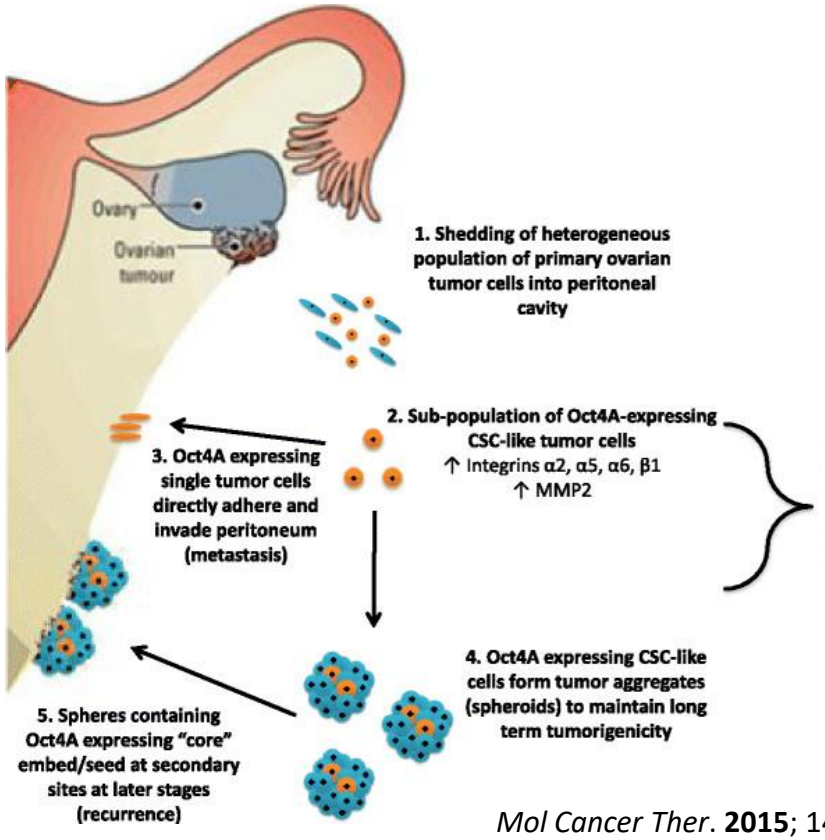
Collective migration:

- Heterogeneity among the migrating cells?
  - Yes, spatially-dependent transcription and stiffness profiles among the cells.
- Plasticity: leaders vs. followers?
  - continuous increase of phenotypic plasticity (EMT) and mechanical plasticity from follower to leader cells.

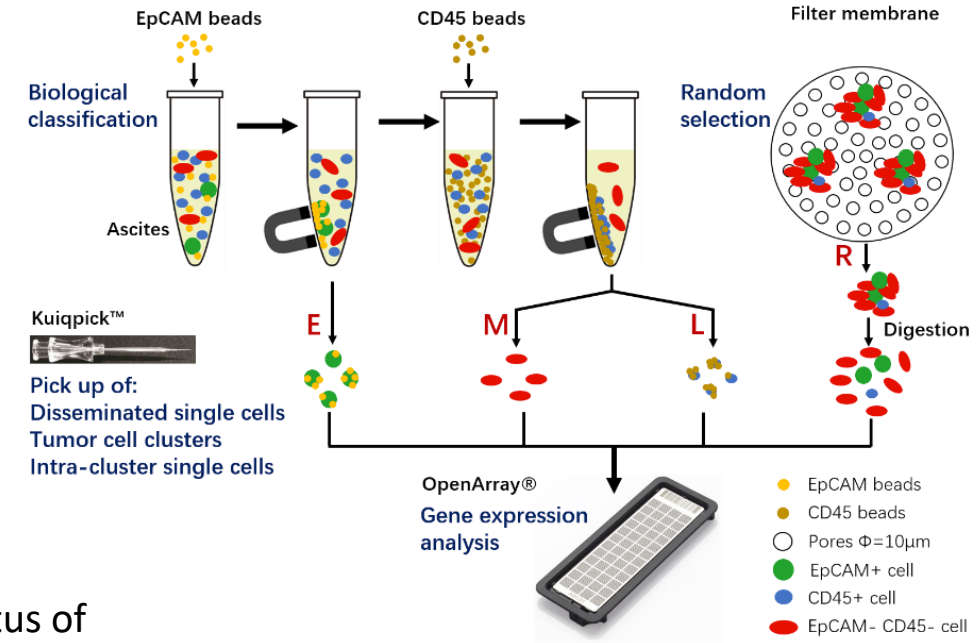
Zou Heng, Yang Zihang, et al *iScience*, **2022,** in press.

Single-cell EMT-related Transcriptional Analysis of Epithelial Ovarian Cancer Ascites

- Ascites are tumor cells or cell clusters disseminated from the primary ovarian cancer into peritoneal cavity.
- The presence of ascites correlates with the OC peritoneal metastasis and is associated with poor prognosis.

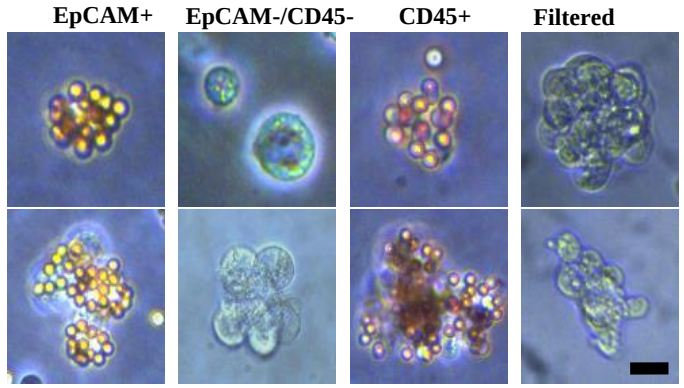


Mol Cancer Ther. **2015**; 14, 747–756.



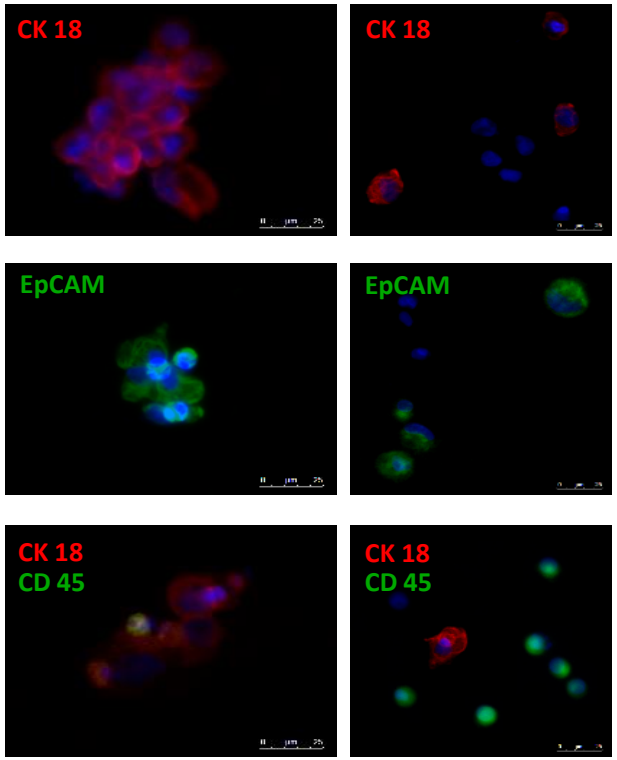
Questions:

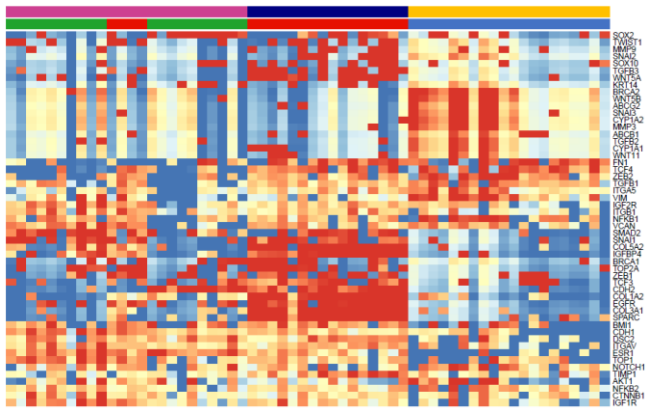
- EMT gene expression status of disseminated cells and cell clusters in ascites of EOC?
- Classification of cells based on EMT gene expression profile?
- Components in cell clusters? Intra-cluster and inter-cluster heterogeneity?



Kan TT et al, *Oncogene*, **2020**, *39(21)*, 4227–4240.

Single-cell EMT-related Transcriptional Analysis of EOC Ascites



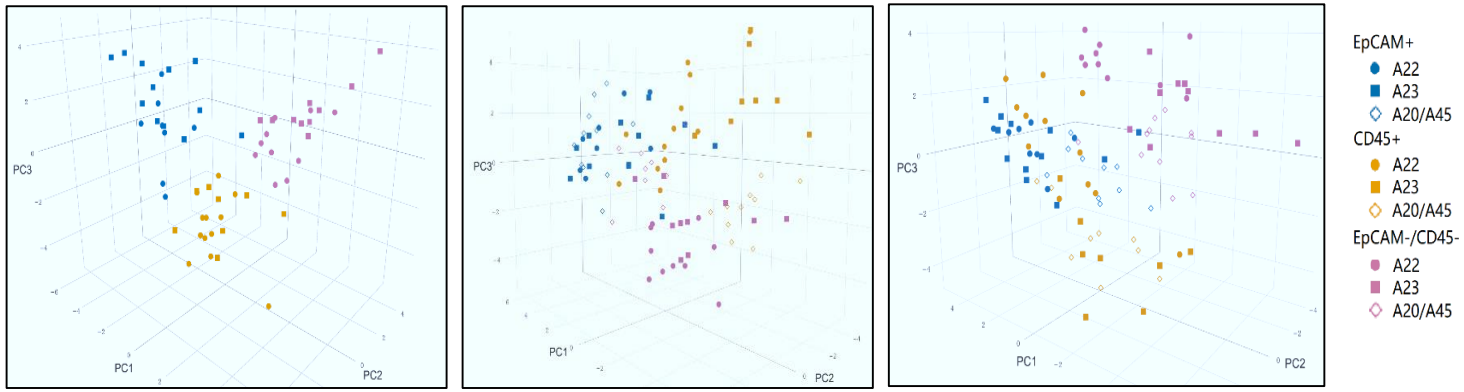


Intra-pathological Similarity and Inter-pathological Difference

A22 & A23:
clear-cell carcinoma (CCC)

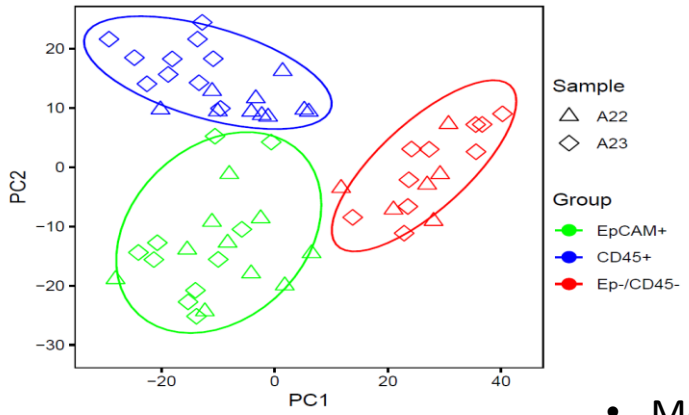
A22/A23 & A20:
CCC vs high grade serous carcinoma

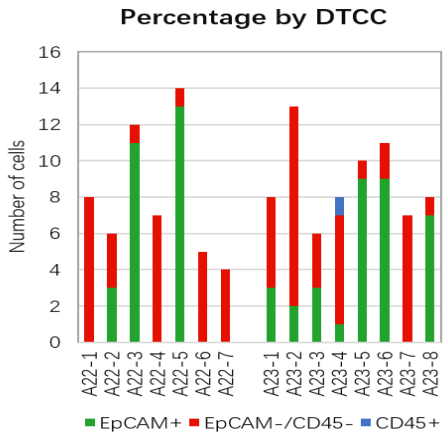
A22/A23 & A45:
CCC vs endometrioid carcinoma

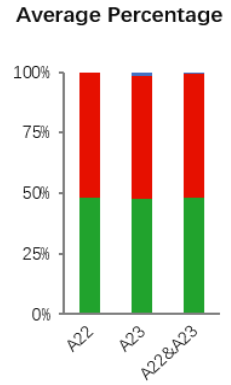


Intra-cluster and Inter-cluster Heterogeneity of DTCCs in Ascites

Classifier based on 56 DSCs







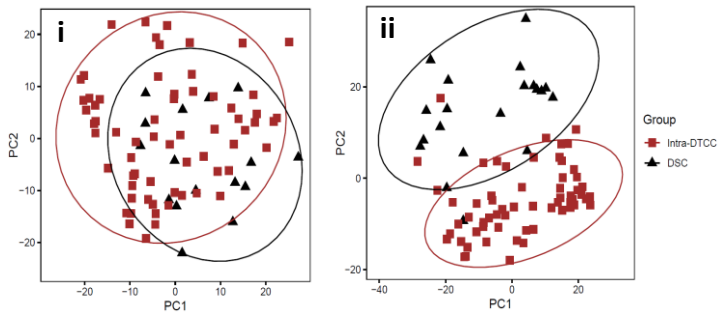
- Most were classified as E-like or CAS/M-like
- Difference between population and individual clusters

Kan TT et al, *Oncogene*, **2020**

Single-cell EMT-related Transcriptional Analysis of EOC Ascites

DTCCs are more metastatic than DTCs

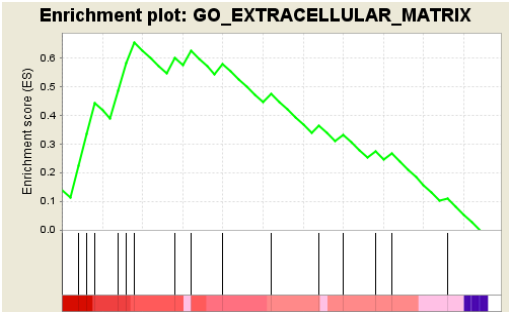
Similarity between DSCs and single cells from DSCCs



i. CSA/M-like cells (EpCAM-/CD45-): similar

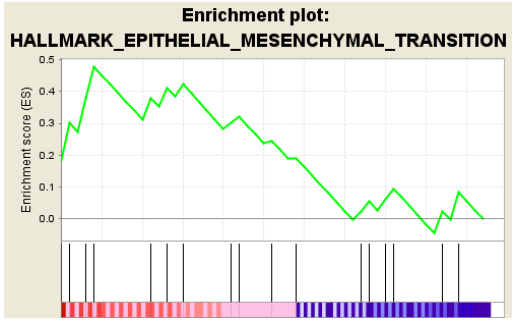
ii. E-like tumor cells (EpCAM+): different

GSEA: Difference between E-like and CAS/M-like cells in DTCCs



Up-regulation of **ECM-related genes** (COL5A2, 3A1 and 1A2) in CAS/M-like cells - **enabled strong cell-cell connection and prevent anoikis**

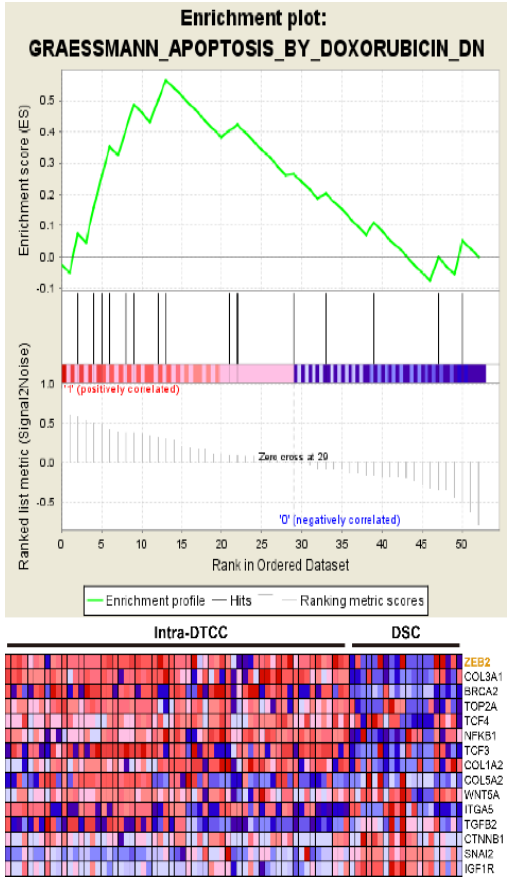
GSEA: Difference in E-like cells between DSCs and DTCCs

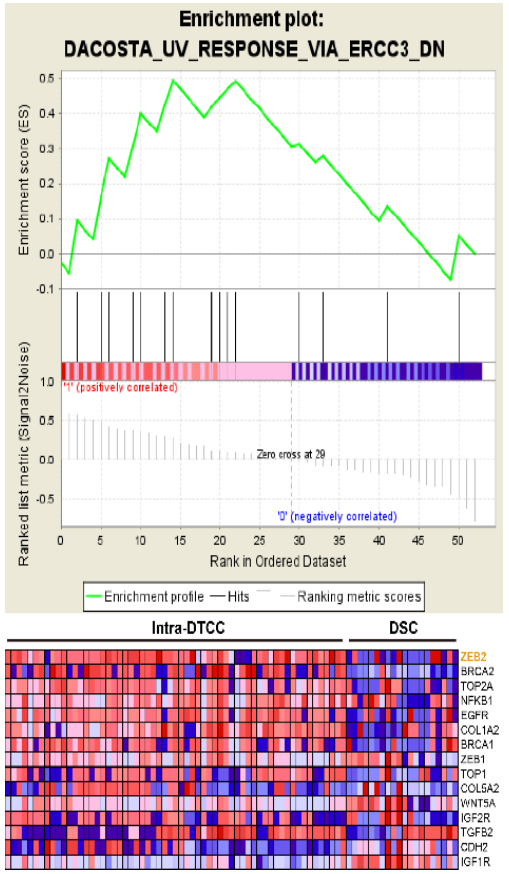


Up-regulation of **EMT-related genes** in E-like cells from DTCCs - **higher migration and invasion abilities**

EMT activation is related to chemo-resistance and DNA damage repair

GSEA: Difference in E-like cells between DSCs and DTCCs



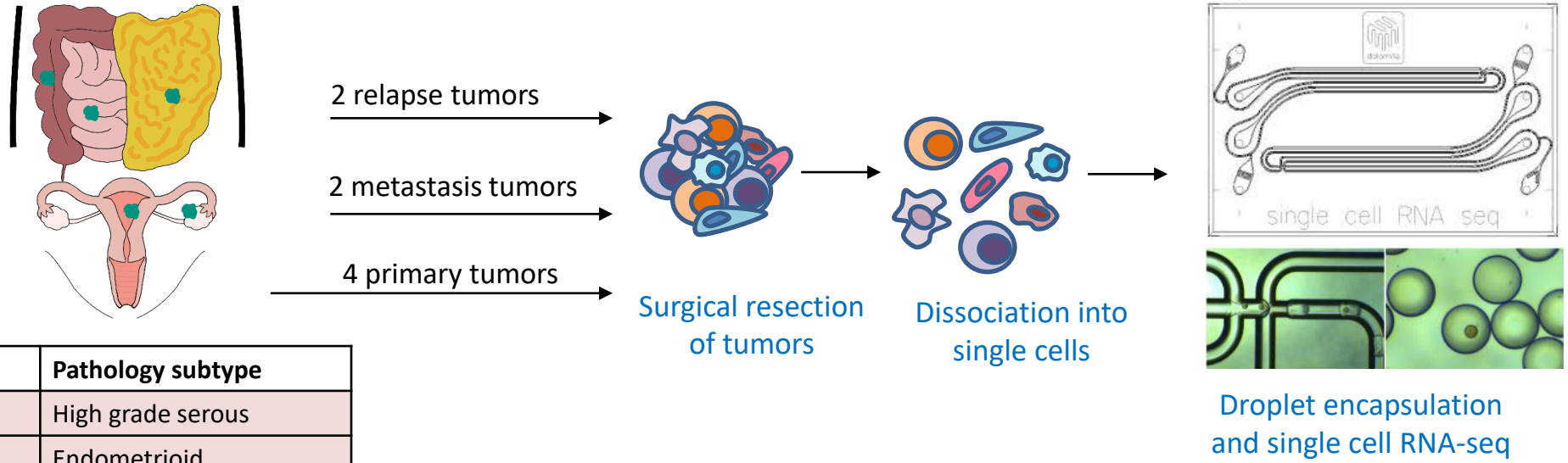


Activation of chemo-resistance and DNA damage repair programs - E-like cells in the DTCCs may be better protected from chemotherapy and radiotherapy

Kan TT et al, *Oncogene*, **2020**

Single-cell RNA-seq identified tumor subpopulation as progenitor of epithelial ovarian cancer recurrence

- Identify the initiation population of tumor cells in primary/relapsed/metastatic tumors;
- Roles of stromal cells in tumor microenvironment in relapse/metastasis;
- Biomarkers for prognosis and prediction of recurrence.



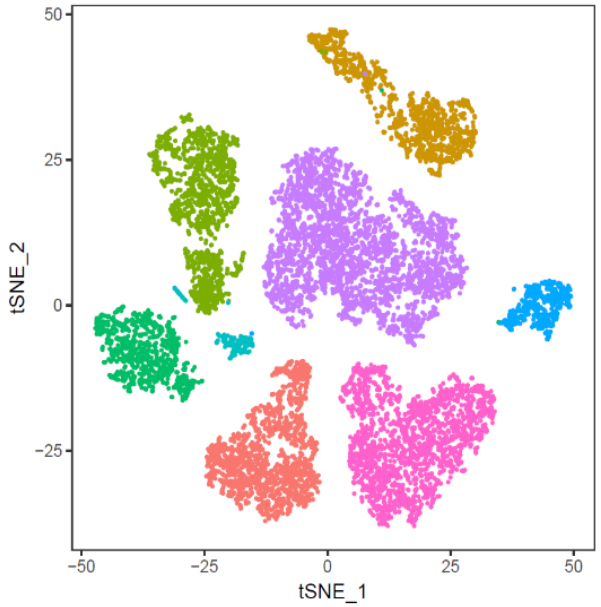
Sample	Pathology subtype
Primary-1	High grade serous
Primary-2	Endometrioid
Primary-3	Low grade serous
Primary-4	Low grade serous
Metastasis-1	High grade serous
Metastasis-2	Low grade serous
Relapse-1	Serous
Relapse-2	Low grade serous

- **Metastasis tumors:** EOC patients were diagnosed with peritoneal metastasis before chemotherapy;
- **Relapse tumors:** recurrence of cancer several years after EOC surgery and chemotherapy.

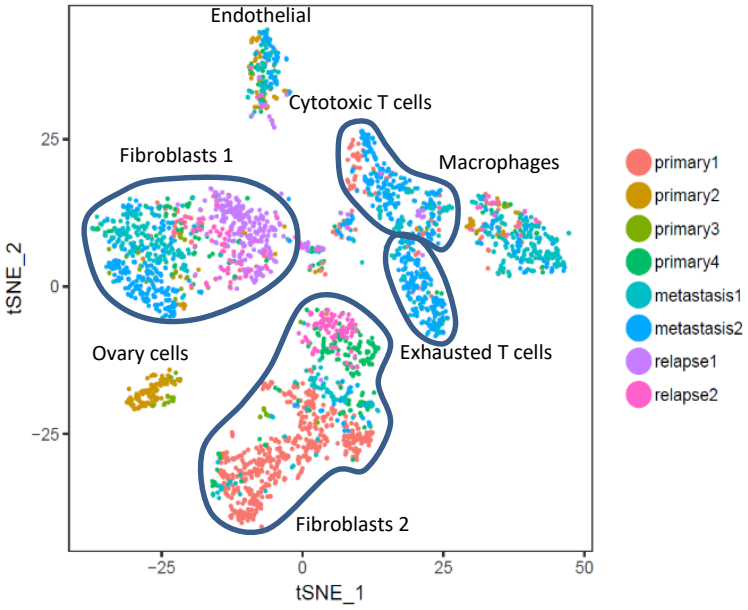
Kan TT et al, *Oncogene*, **2022,** *41(6)*, 895-906.

Tumors are different, microenvironments are similar

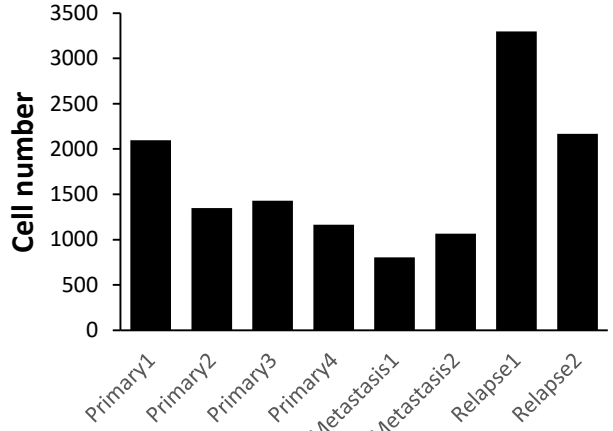
Tumor cells (10381 cells)

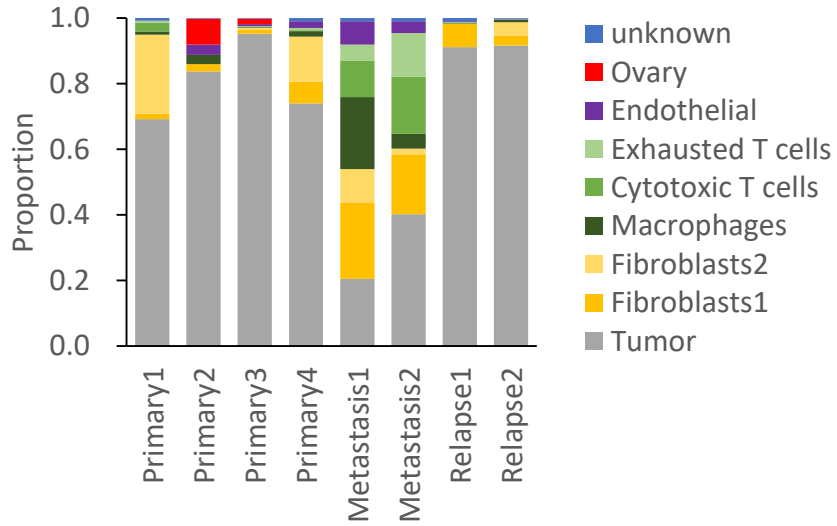


Stromal cells (3005 cells)



Cellular proportion of each tumor





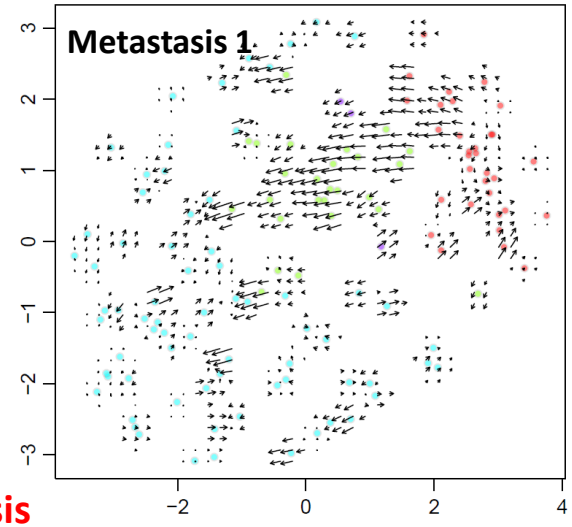
- For tumor cells, the cells were clustered according to their tumor of origin;
- For the stromal cells, each cell population contained cells from all patients, no matter primary, metastasis or relapse tumors.

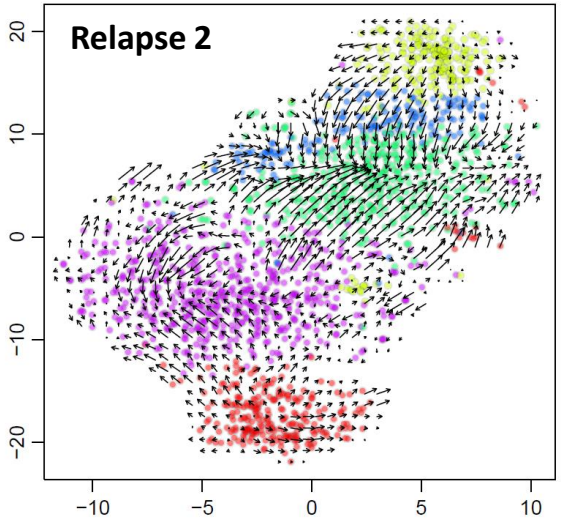
- Fibroblast was the dominate cell population of tumor microenvironment;
- Metastatic tumors contained more immune cells and less tumor cells than the other tumor types.

Kan TT et al, *Oncogene*, **2022**

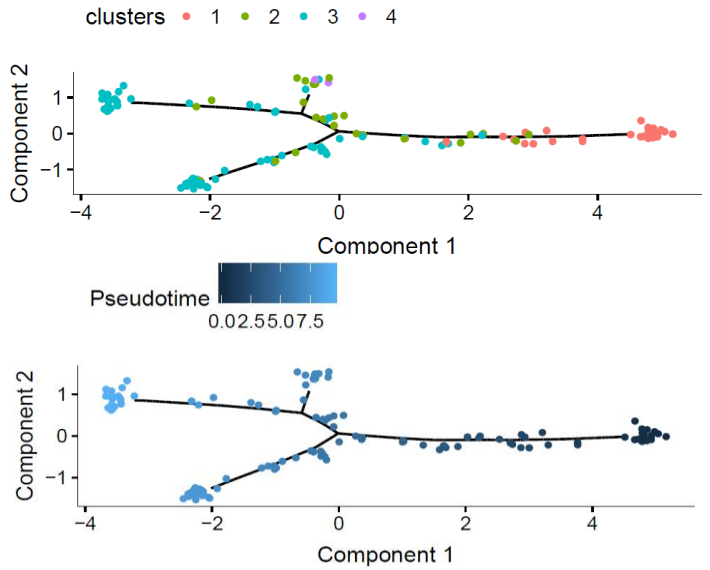
The initiation cell population of metastatic/relapsed tumors

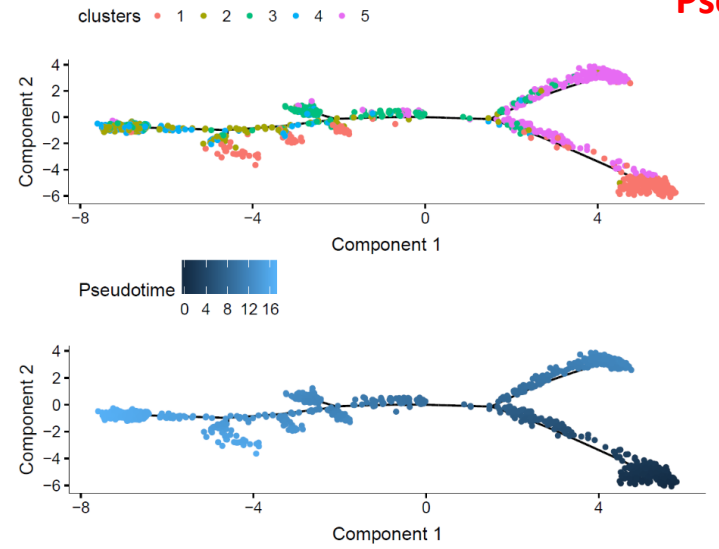
The earliest cell population were identified by the combination of the RNA velocity and pseudo-time analysis:





RNA velocity analysis

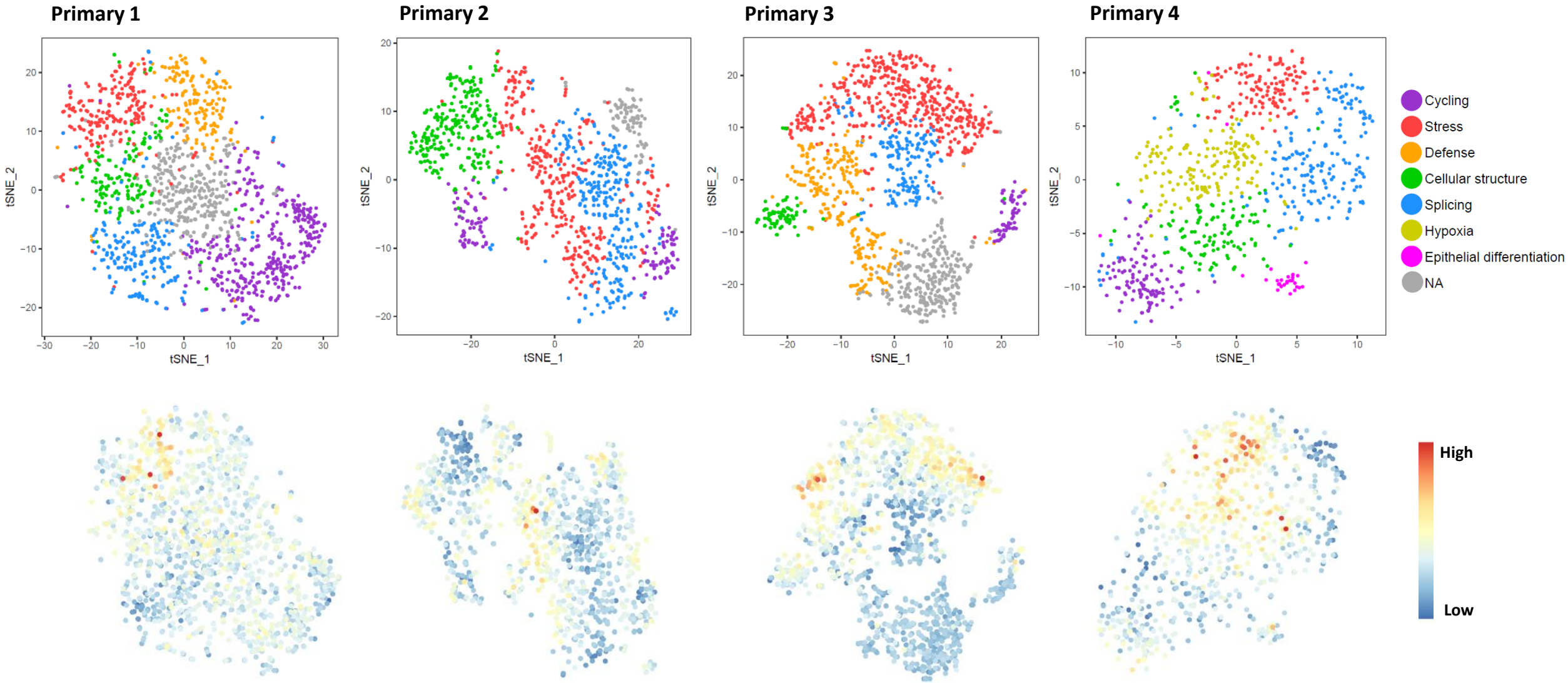




Pseudo-time analysis

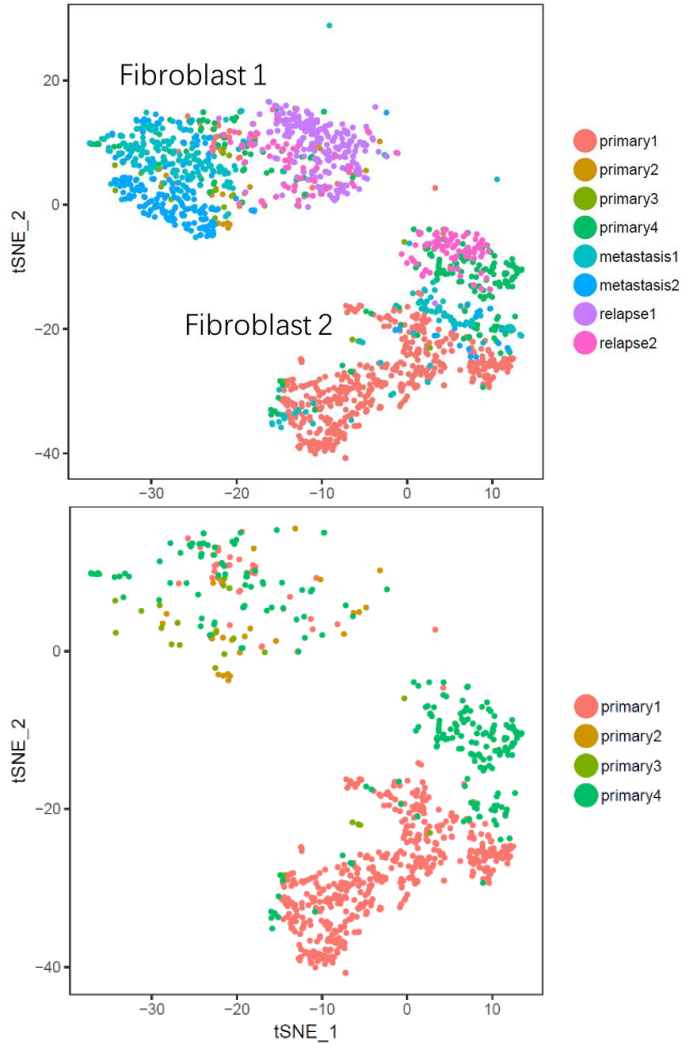
Kan TT et al, *Oncogene*, **2022**

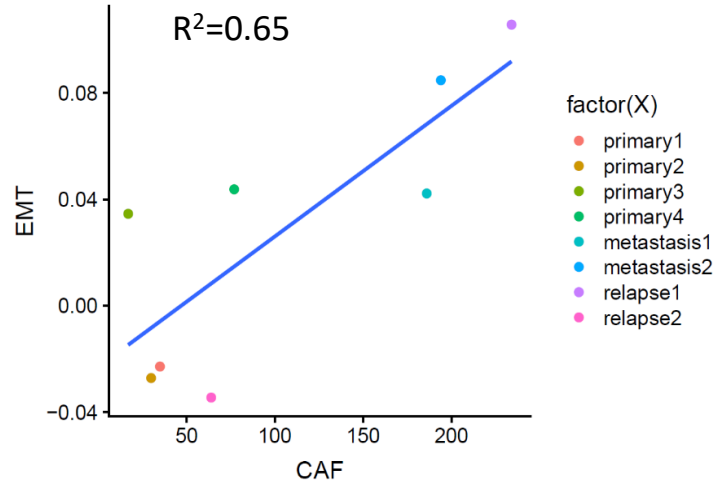
The initiation subpopulation in relapsed tumors was found in primary tumors – cancer cells with enhanced stress program

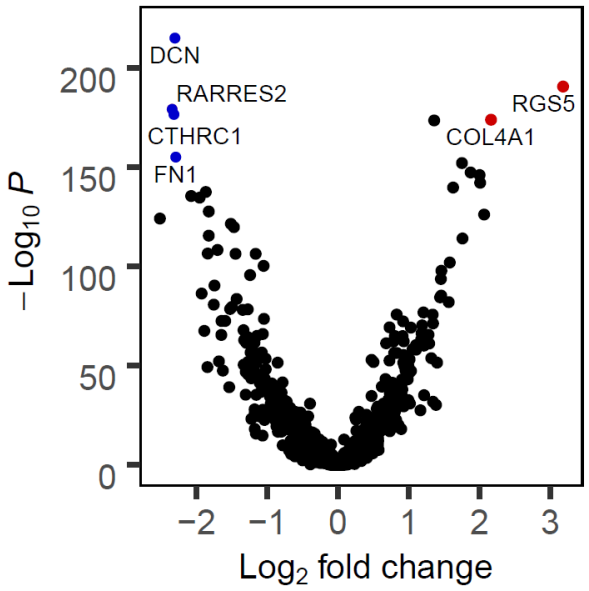


Kan TT et al, *Oncogene*, **2022**

A subpopulation of cancer-associated fibroblasts (CAF) contributed to tumor metastasis with enhanced EMT program





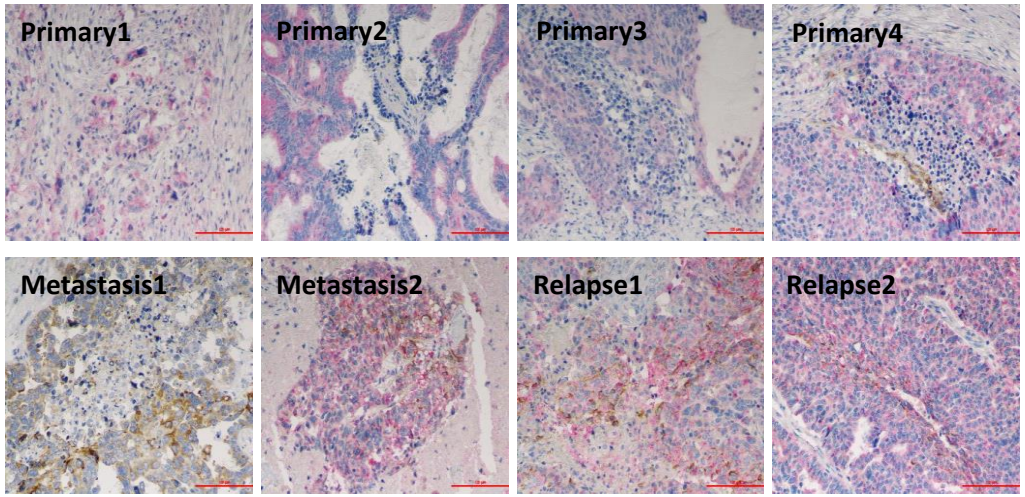


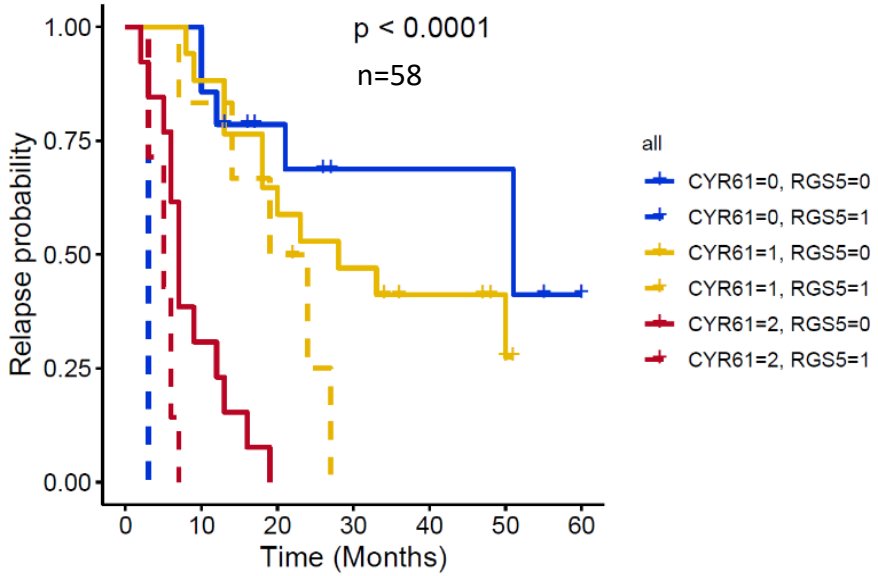
- Two populations of CAFs in TME;
- CAF1 – metastatic; CAF2 – primary.

Volcano plot of DE genes in CAF1

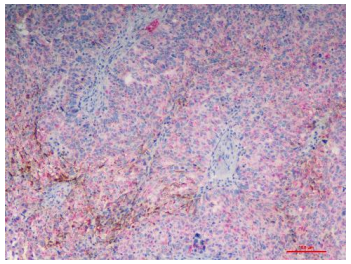
Kan TT et al, *Oncogene*, **2022**

Prognosis according to biomarkers on cancer cells and CAF cells

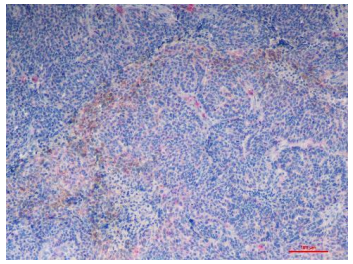




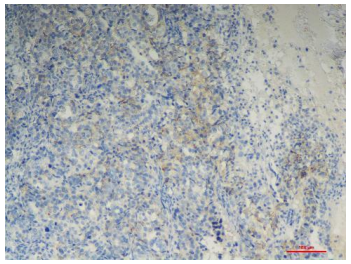
CYR61 High 2
RGS5 Positive 1



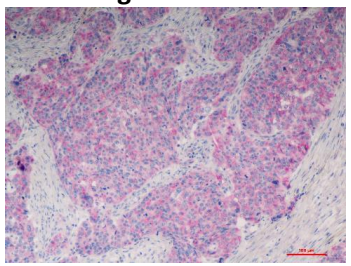
CYR61 Low 1
RGS5 Positive 1



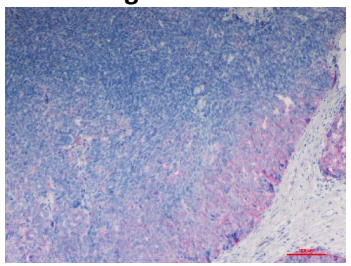
CYR61 Negative 0
RGS5 Positive 1



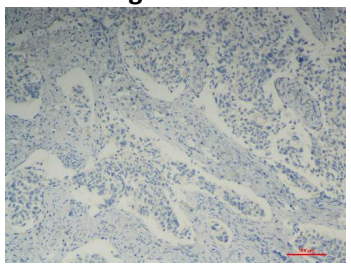
CYR61 High 2
RGS5 Negative 0

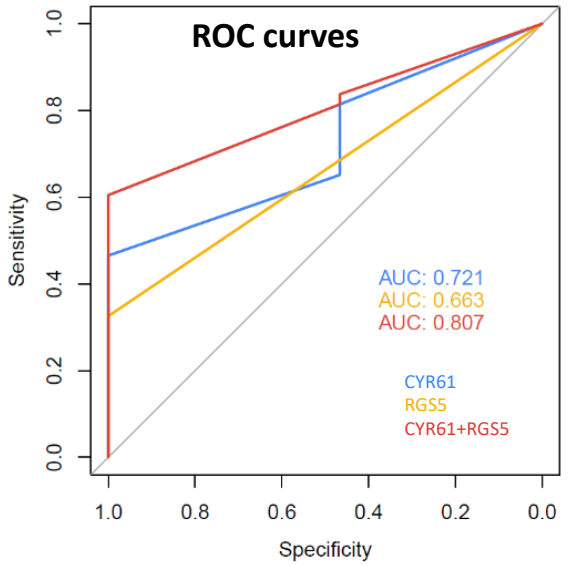


CYR61 Low 1
RGS5 Negative 0



CYR61 Negative 0
RGS5 Negative 0

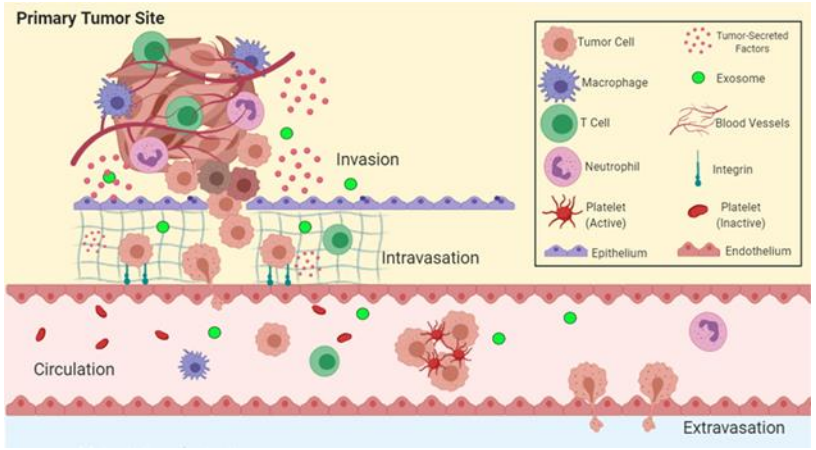


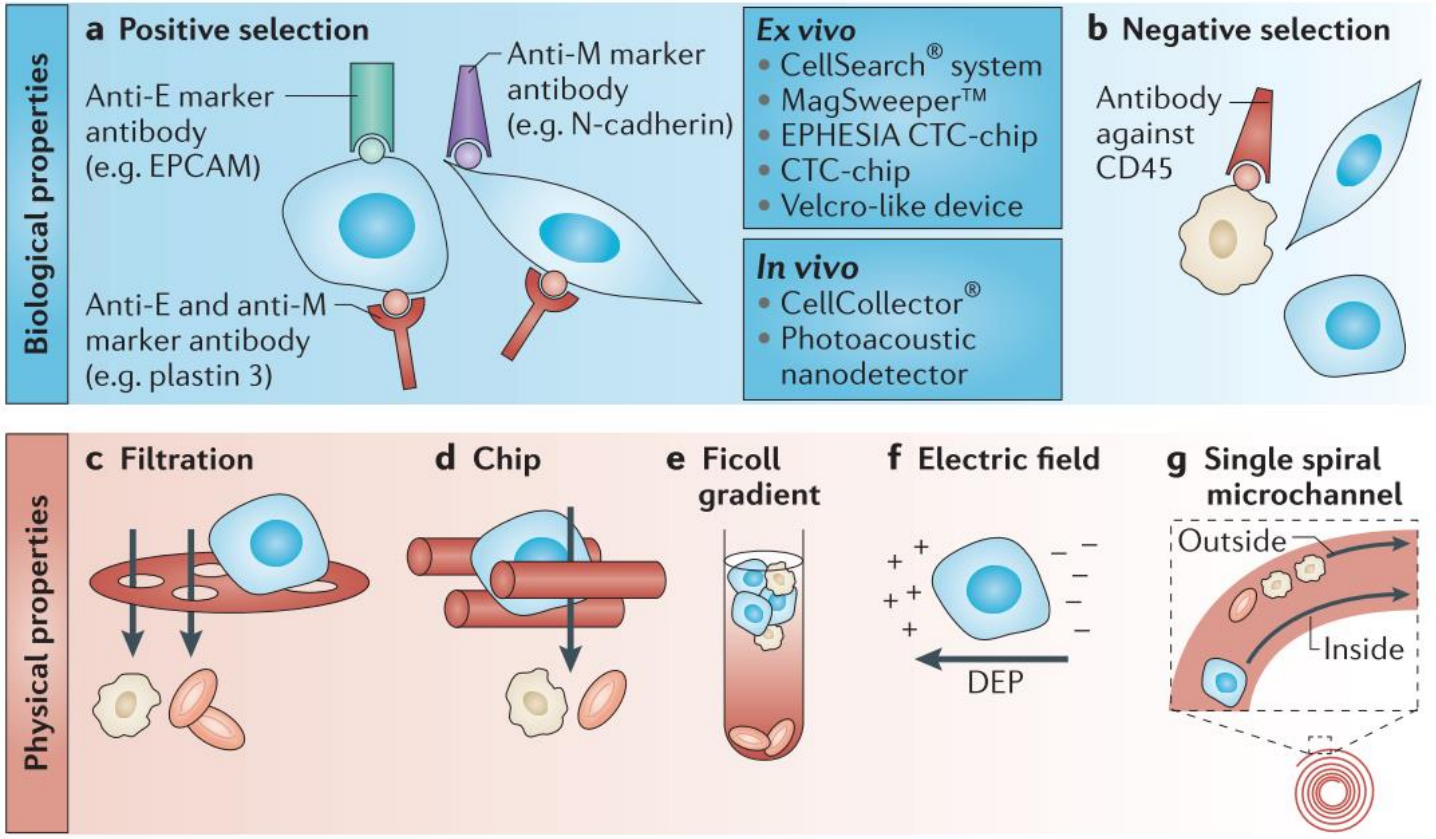


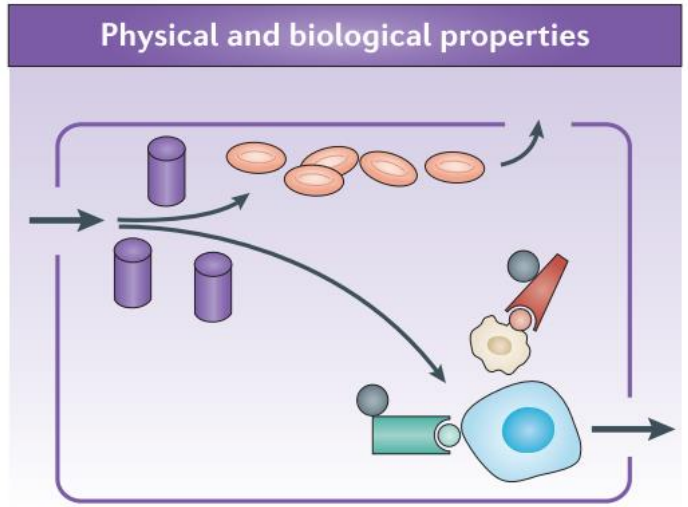
The combined CYR61/RGS5 expression scores significantly correlated with the relapse-free-survival of EOC patients.

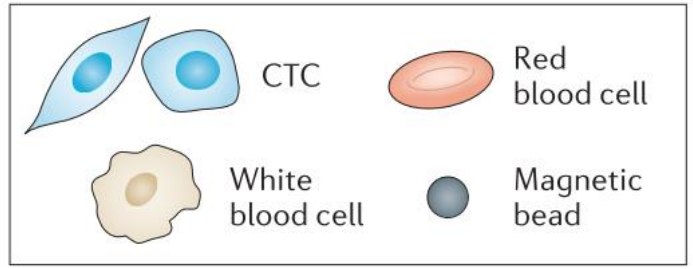
Kan TT et al, *Oncogene*, **2022**

Circulating Tumor Cells
and Techniques for CTC enrichment and isolation



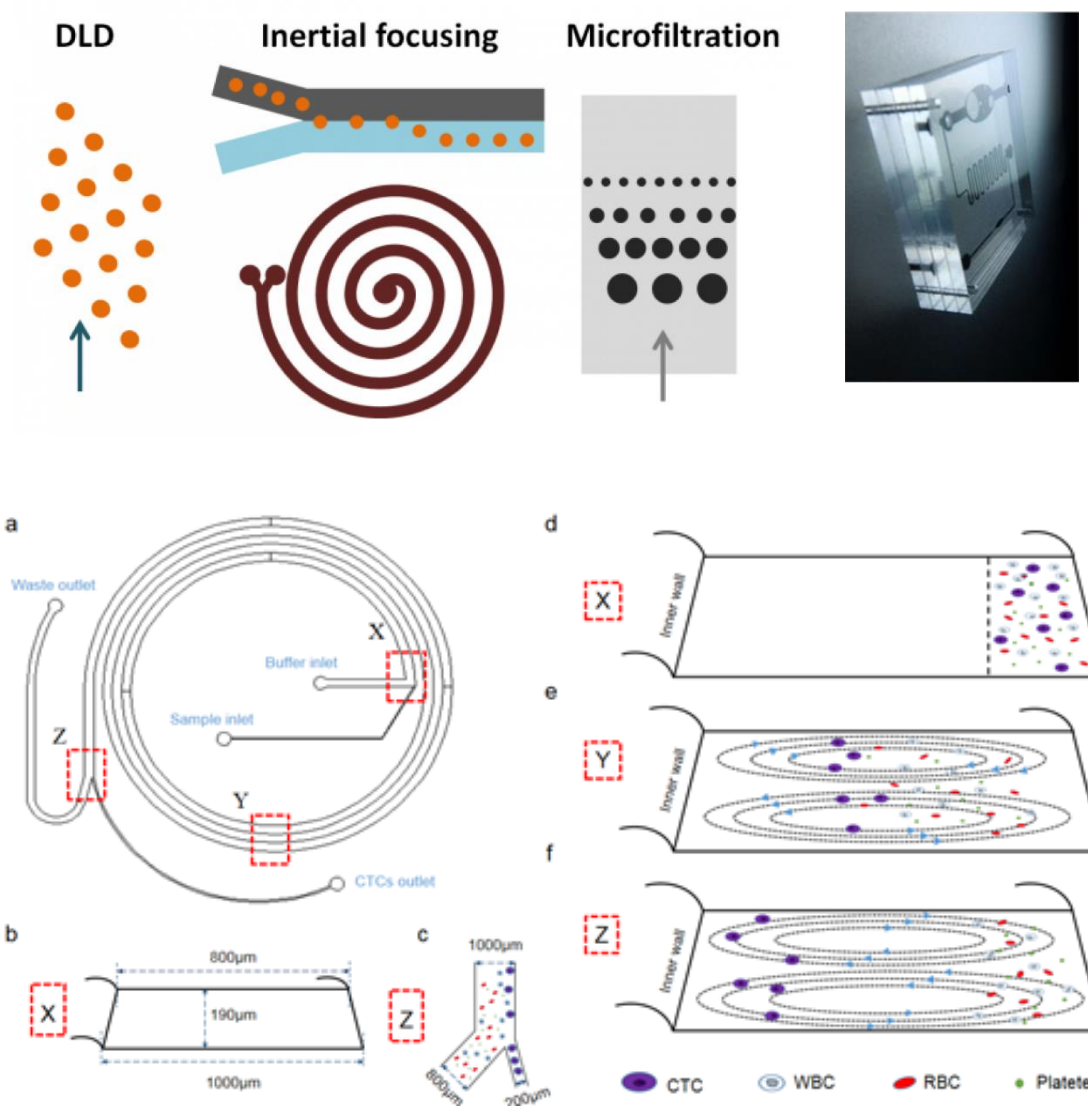




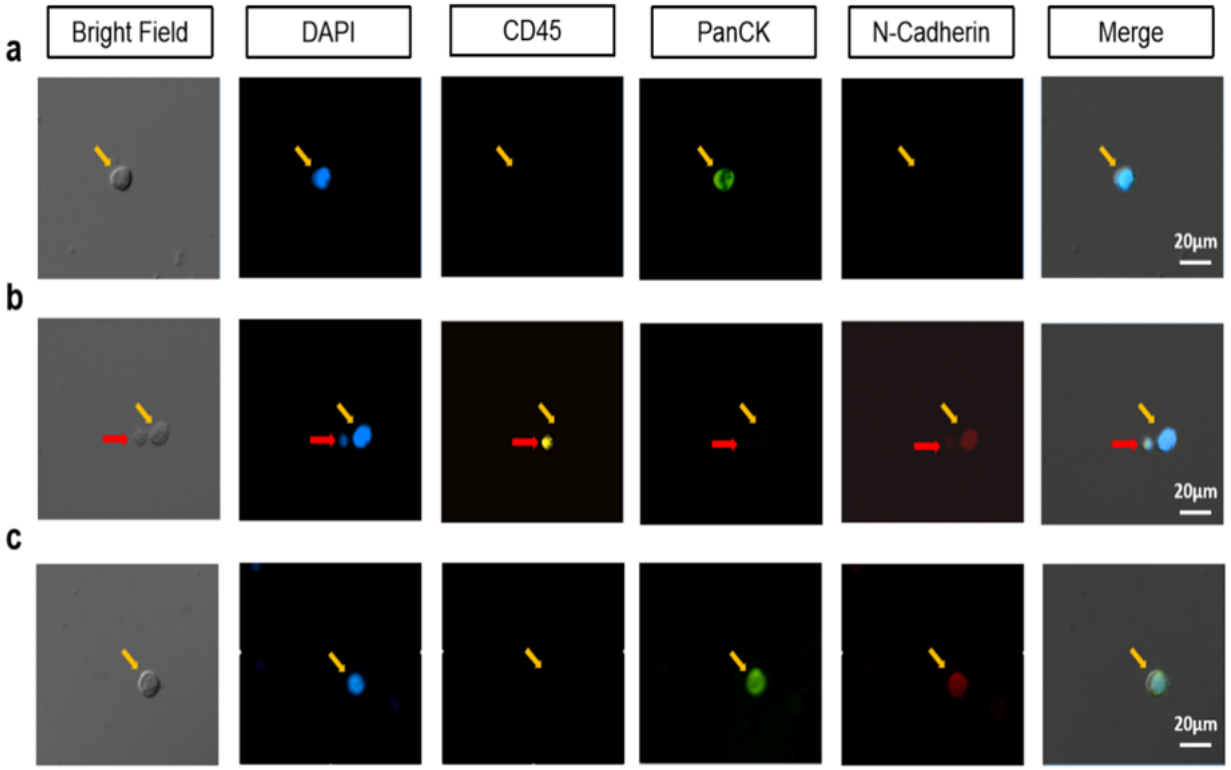


Trends in Molecular Medicine, 2010. **16**(9): p. 398-406.

A microfluidic platform for CTC enrichment, isolation and characterization



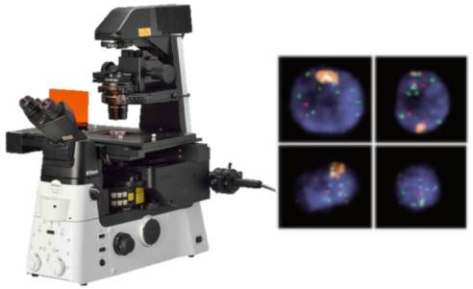
- Combined size-based separation with E/M markers.
- Capture and isolate CTCs alive for subsequent culturing



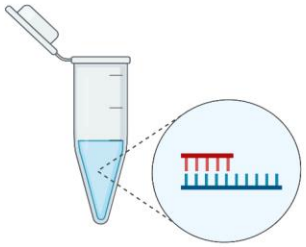
Xu Tao, Zou Heng, et al, *submitted.*

Whole-genome single cell sequencing of circulating tumor cells

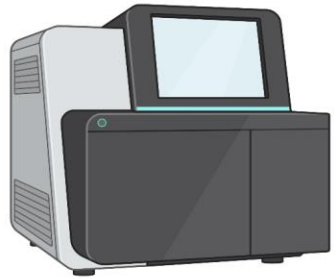
Peripheral blood



Immunofluorescence Identification

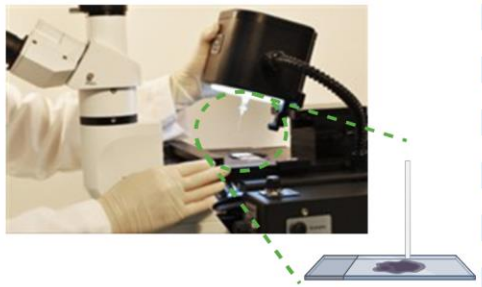


DNA Extraction



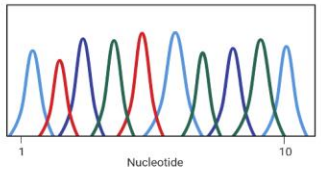
Sequencing

Microfluidic Enrichment



Single Cell Isolation

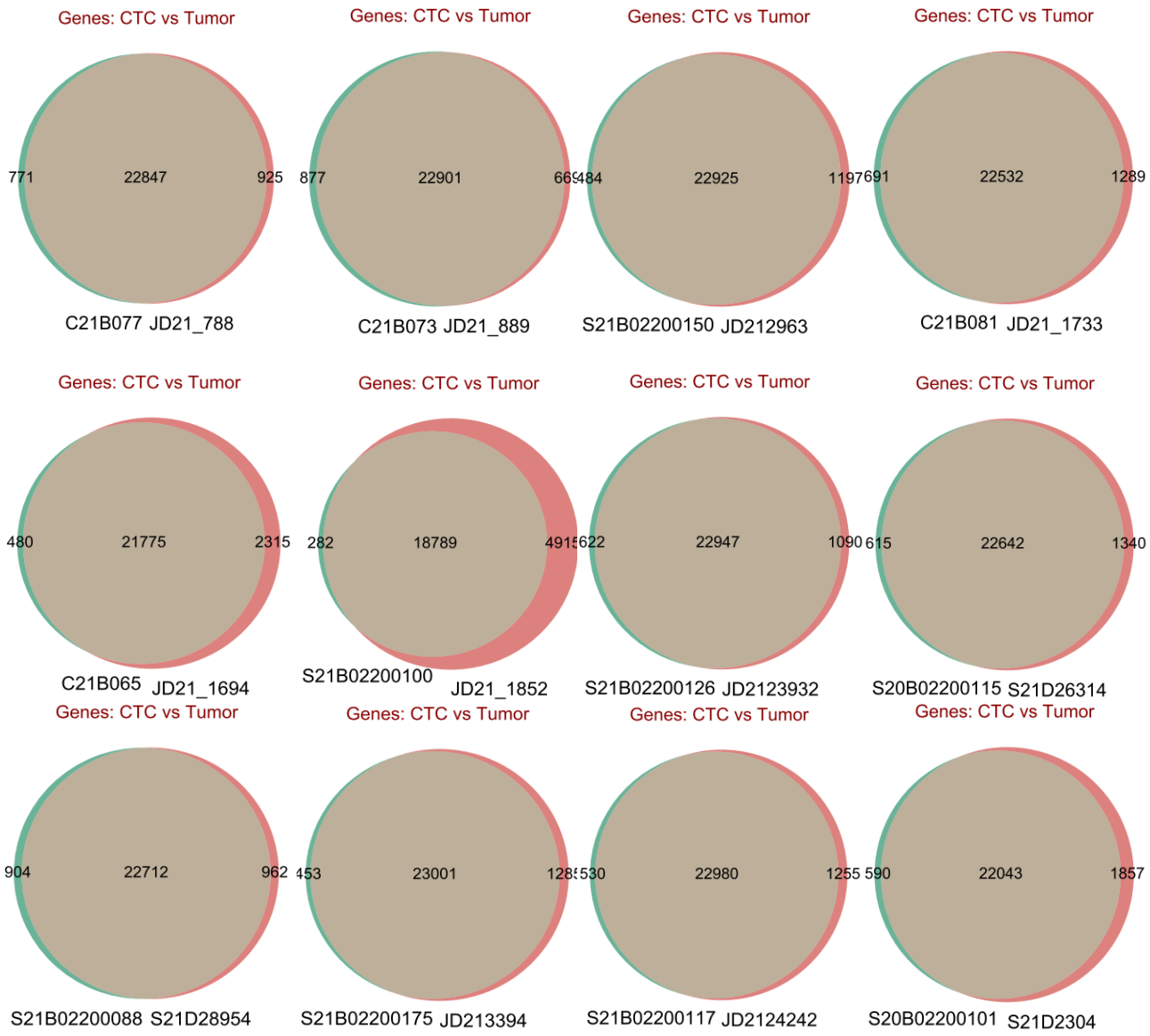
MALBAC WGA



Data Analysis

- Concordance with primary tumor?
- CTC-specific mutations?
- Novel CTC marker?

WGS reveals concordance in SNV between CTC and primary cancer

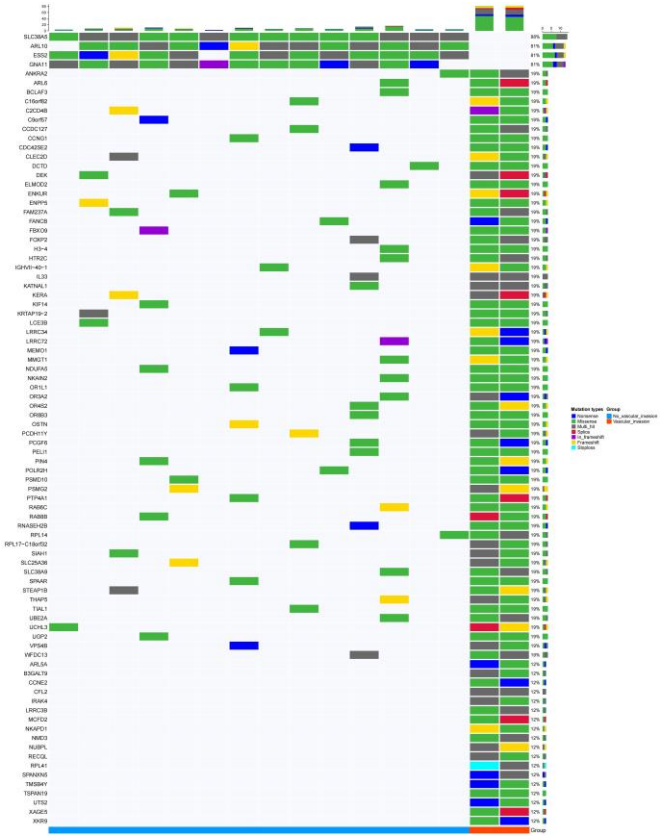


Patient NO.	CTC SNV	Overlap	Tumor snv	Concordance
1	771	22847	925	96.10886758
2	877	22901	669	97.16164616
3	484	22925	1197	95.0377249
4	691	22532	1289	94.58880819
5	480	21775	2315	90.3902034
6	282	18729	4915	79.2124852
7	622	22947	1090	95.46532429
8	615	22642	1340	94.41247602
9	904	22712	962	95.93647039
10	453	23001	1285	94.70888578
11	530	22980	1255	94.8215391
12	590	22043	1857	92.23012552
				93.33954638

Zhou Zhihang, et al, *unpublished.*

Somatic mutated genes that are associated with tumor stage

Vascular invasion

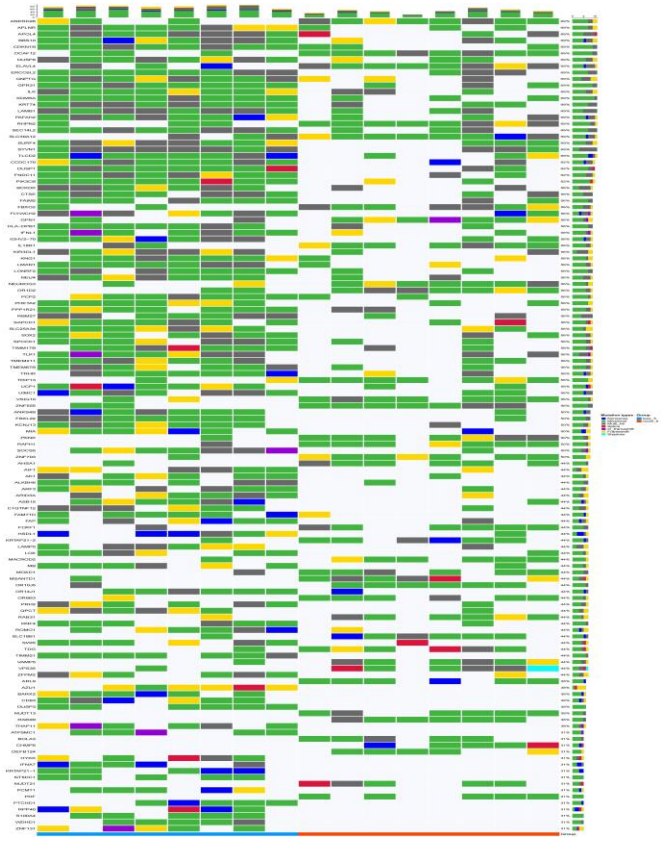


Without With

Distant metastasis

With Without

Tumor size

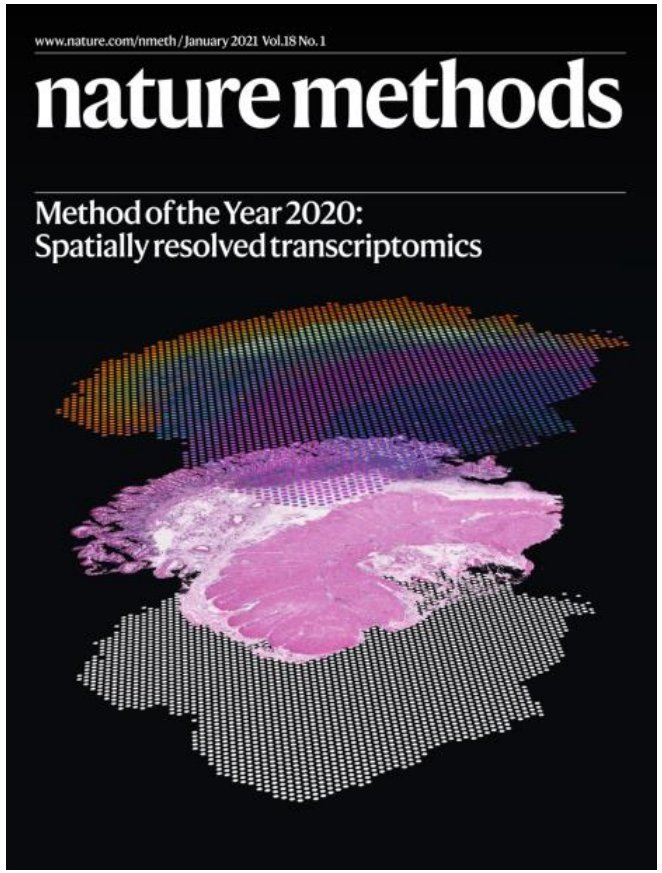


<5cm ≥5cm

- The mutated genes in CTCs are highly consistent with primary HCC, indicating CTCs can be used to reveal genomic features of tumor when biopsy is not accessible.
- WGS of CTCs could reveal novel mechanism of metastasis.

Zhou Zhihang, et al, *unpublished.*

Spatially-resolved transcriptomics: single-cell RNA seq in situ



Spatial transcriptomic, a combination of on-chip hybridization and single cell RNA-seq, allows for study of tumor heterogeneity in situ.

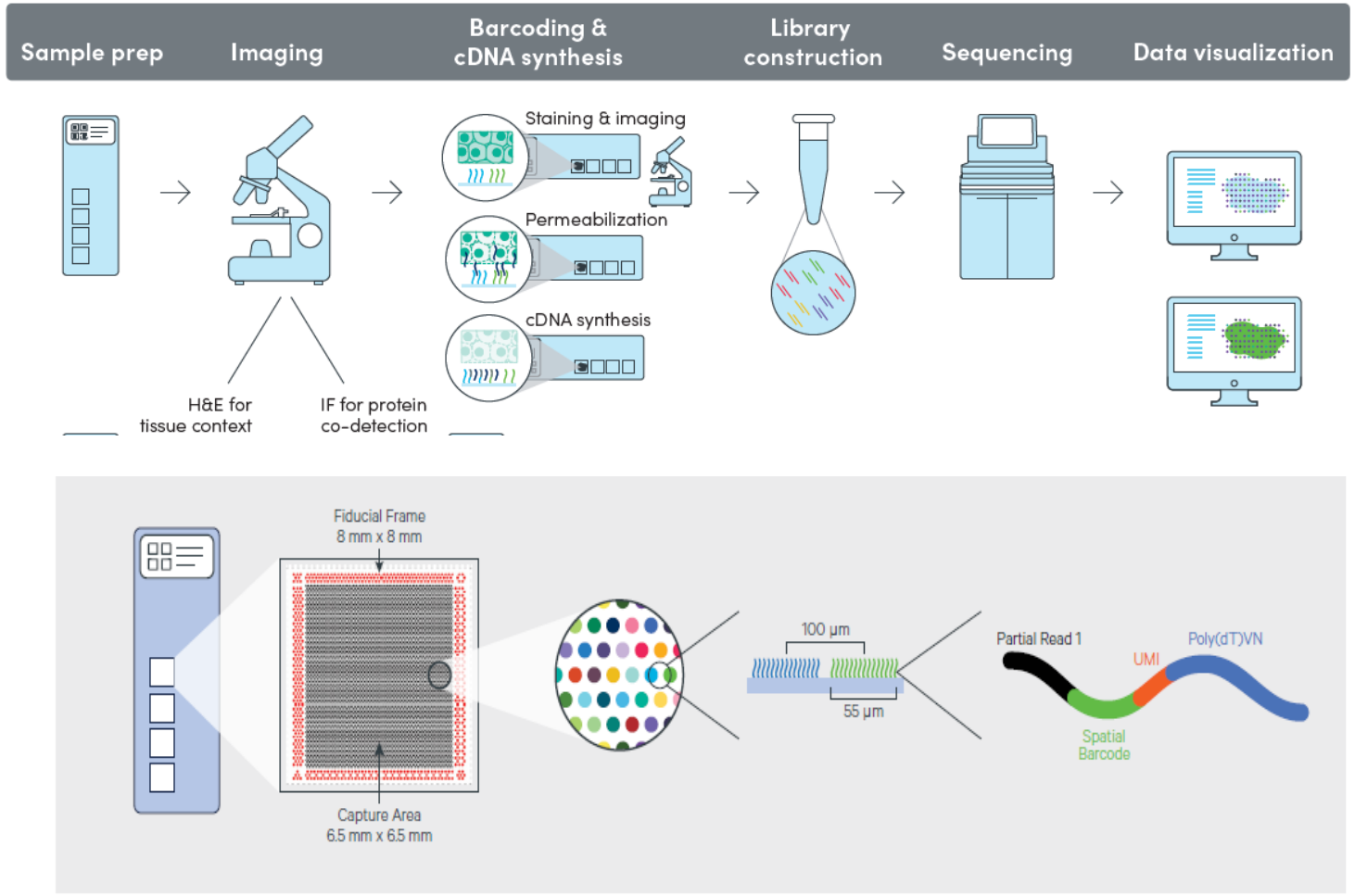


Figure 1. Composition of Visium Spatial Gene Expression Slide. Each slide can contain either two or four Capture Areas with approximately 5,000 barcoded spots, containing millions of spatially barcoded capture oligos. Released tissue mRNA binds to these oligos, enabling capture of gene expression information.

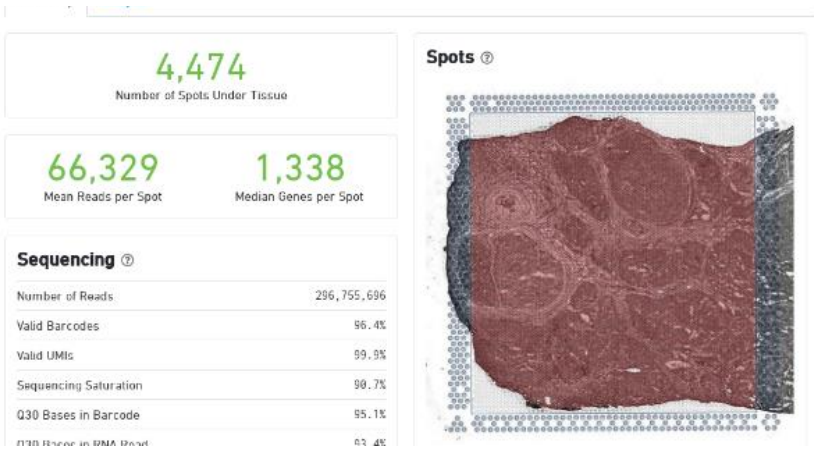
Spatially-resolved transcriptomics of liver tumor sections to reveal novel metastasis-related genes

Case 1: Tumor center

Case 2: Tumor center

Case 1: Tumor margin/frontier

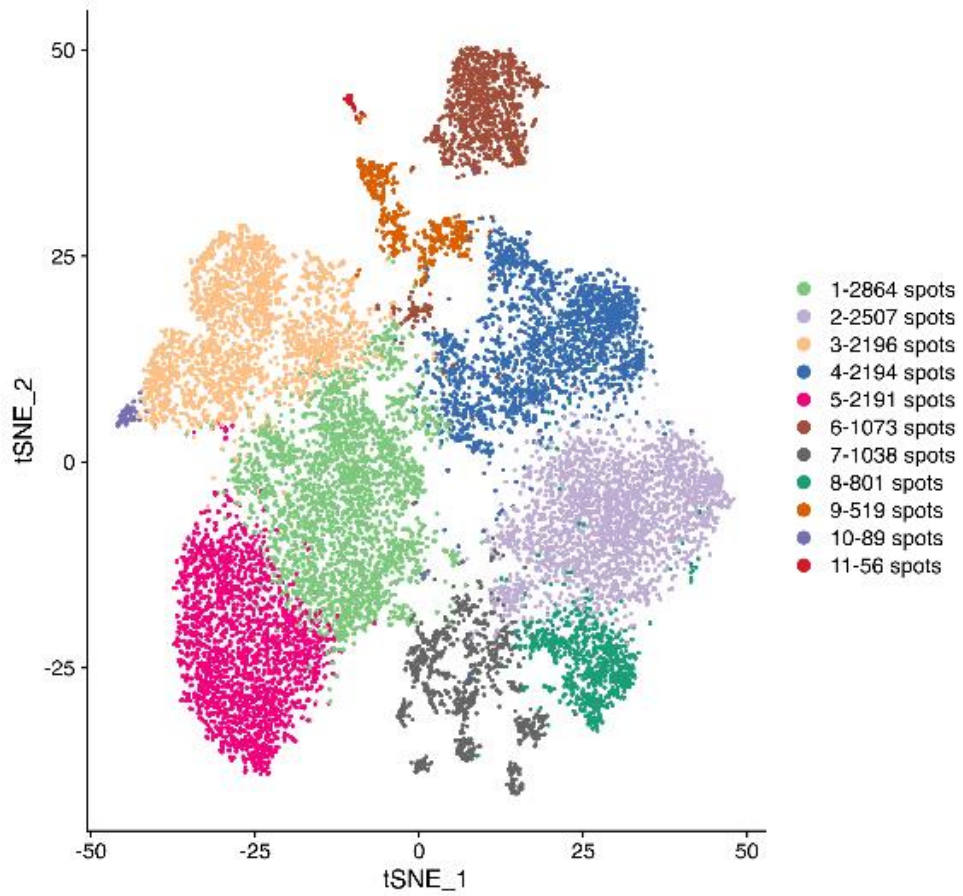
Case 2: Tumor margin/frontier



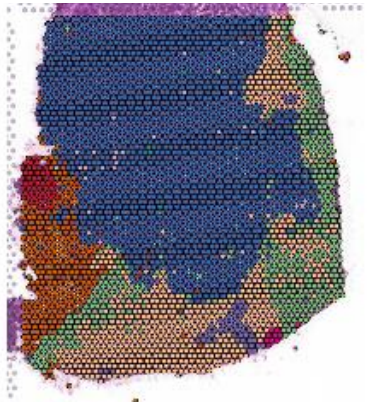
Kelvin Ng, Zhou ZH, et al *unpublished.*

Cell clusters are spatially mapped to tissue sections to reveal heterogeneity

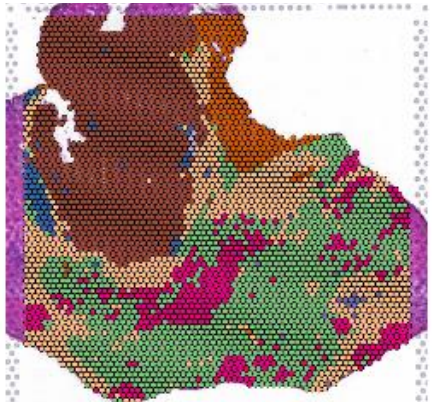
The samples are comprised of 11 clusters. More heterogenous in surrounding tissue than tumor.
HE staining confirms the blue, purple, green and brown regions belong to the tumor regions.



Case 1: Tumor center

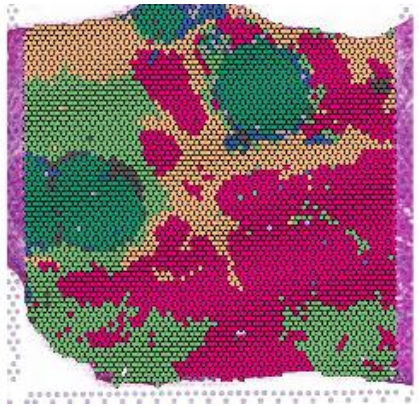


Case 1: Tumor margin/frontier



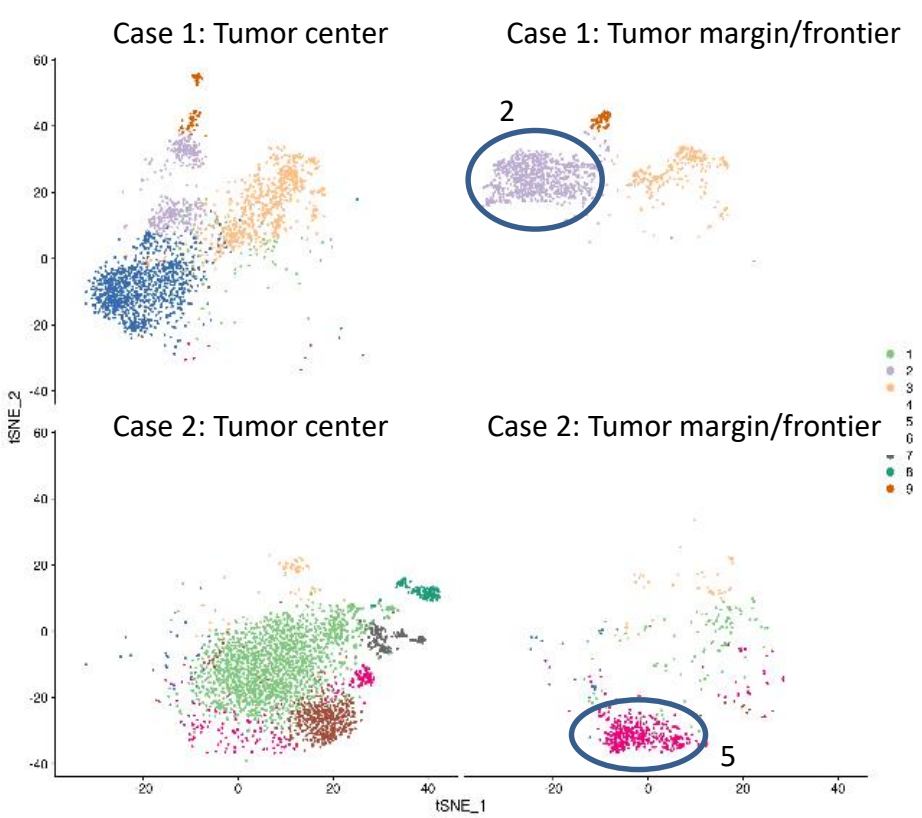
Case 2: Tumor center

Case 2: Tumor margin/frontier

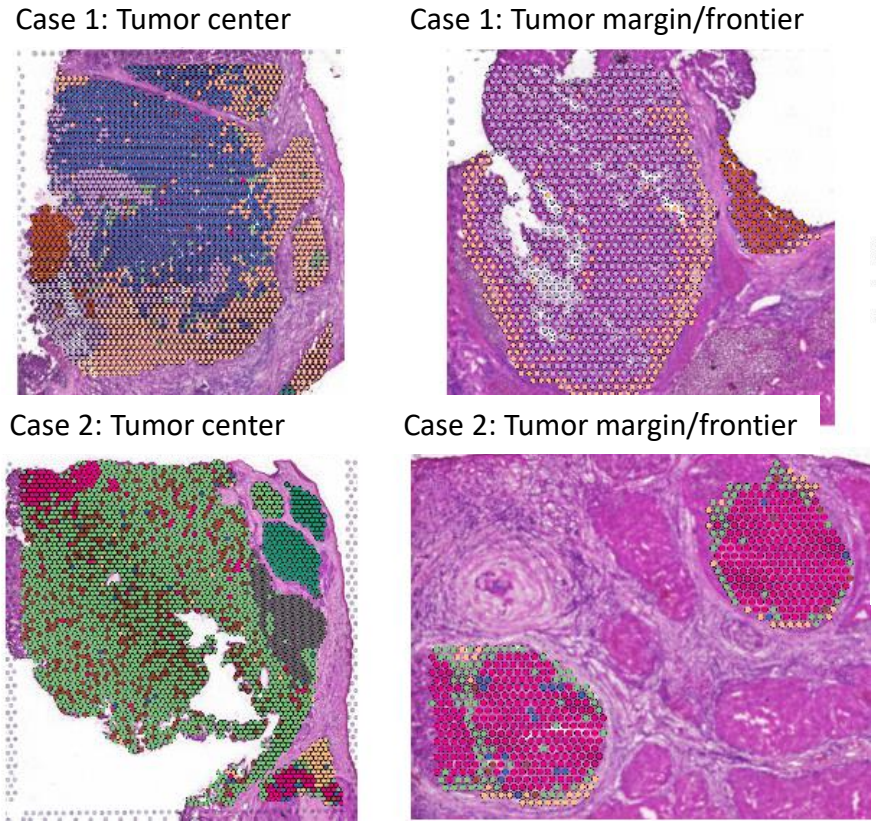


Kelvin Ng, Zhou ZH, et al *unpublished.*

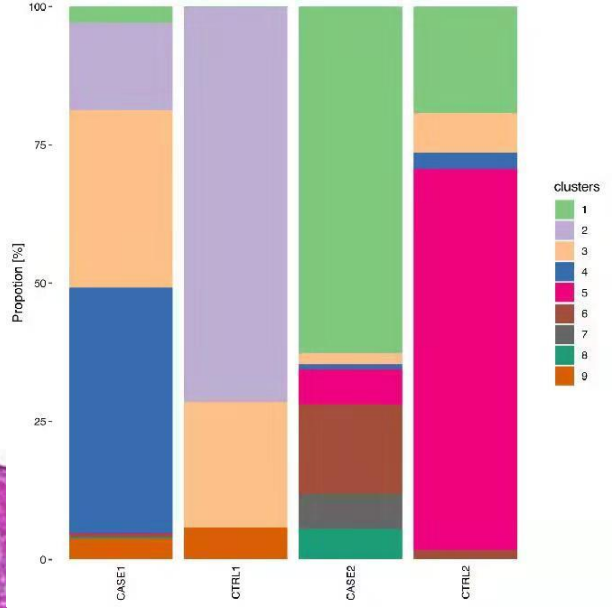
Differentiation of dominant cell types in tumor centers and margins



Clusters of cells from tumor regions



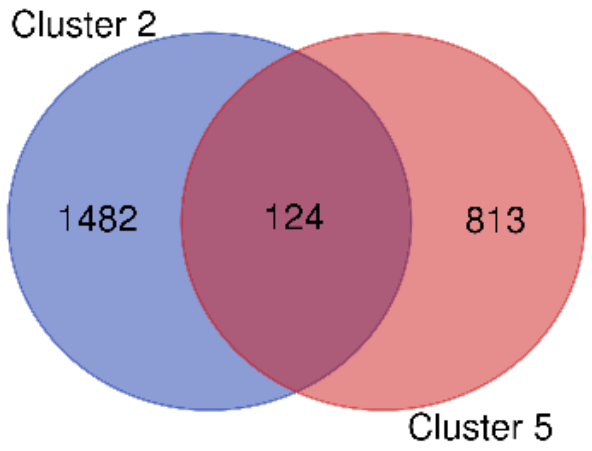
Mapping of cell clusters in tumor regions

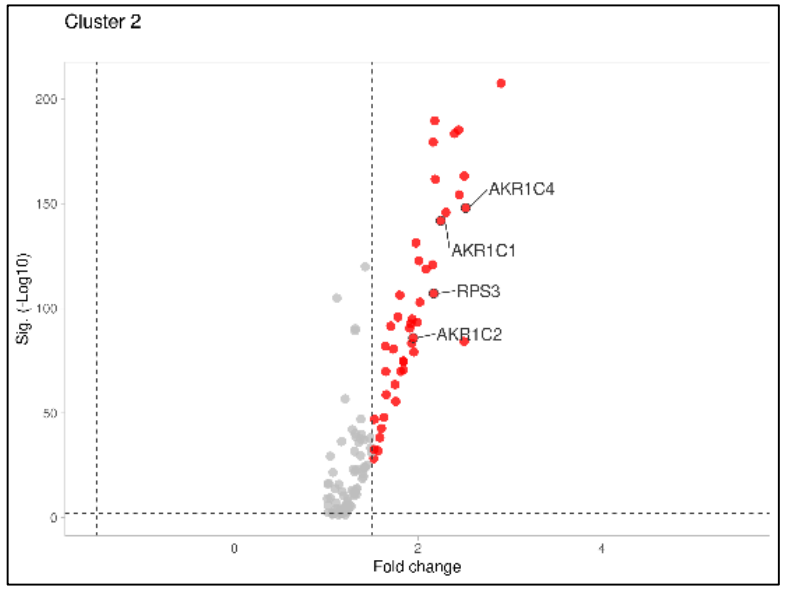


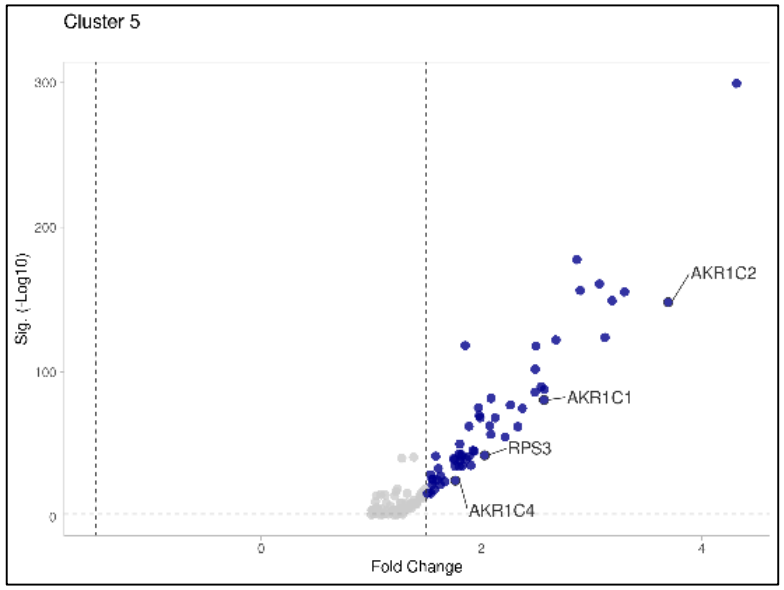
Cluster 2 and 5 are dominant cell types in the tumor margins, which may be more aggressive cell types.

Kelvin Ng, Zhou ZH, et al *unpublished.*

Upregulated genes found in cluster 2/5 may be drivers of metastasis



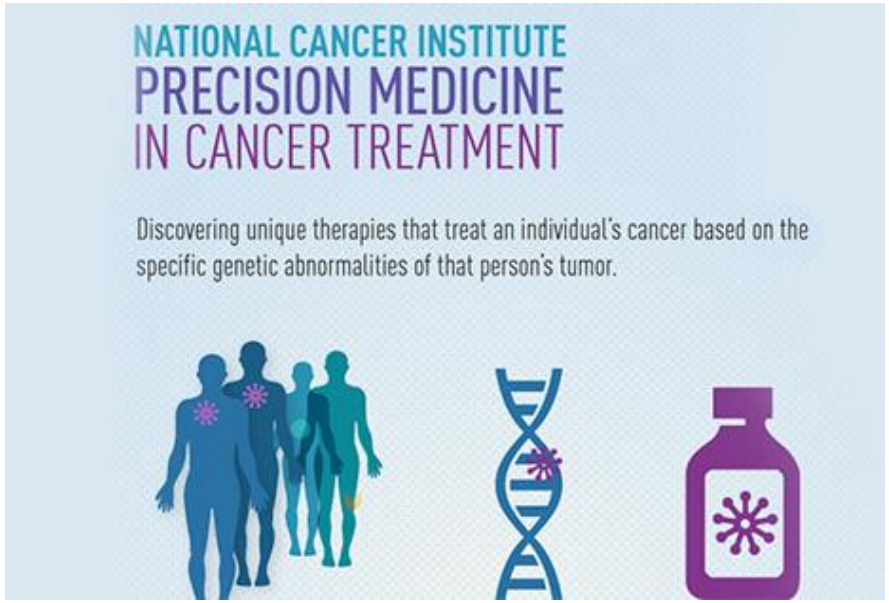




A number of upregulated gene families were identified in both Clusters 2 and 5, related to cell metabolism and ribosome biogenesis.

Kelvin Ng, Zhou ZH, et al *unpublished.*

Winning the War on Cancer Together



Acknowledgements

Funding

- Research Grants Council
- Innovation & Technology Fund
- National Key Research Fund (973)
- Guangdong Provincial Sci & Tech Fund
- Shenzhen / Futian Sci & Tech Fund
- Dr. Yeung Kin-Man
- Tung Foundation
- City University of Hong Kong

Collaborators

- Dr. Joseph Au, Queen Elizabeth Hospital
- Dr. Stanley Leung, Aventists Hospital
- Prof. Alice Wong, Queen Mary Hospital
- Prof. Shengtao Zhou, West China Hospital
- Prof. Shubin Wang, PKU Shenzhen Hospital
- Prof. Andrew Yen, Cornell University

bhmyang@cityu.edu.hk

https://www.cityu.edu.hk/bms/profile/michaelyang.htm


楊建文學術樓
Yeung Kin Man Academic Building
Lift 1, 2, 6-12, 18
Mail Room
Lift 3 - 5
Lift 16 - 17
4.5 m


Lawrence
Amy Fong
易长青中山大学
San
zyang
婉晴
Edwin
Lily苏丽
徐涛
小程序用户
May76543210
Michael Yang
yuefeng
傅华阳
jackie
Dan
HunterHUANGMinghui
叶盛
HenryTsou
Jocelyn