GSK Course Lecture, April 2023

Mechanisms of Metastasis

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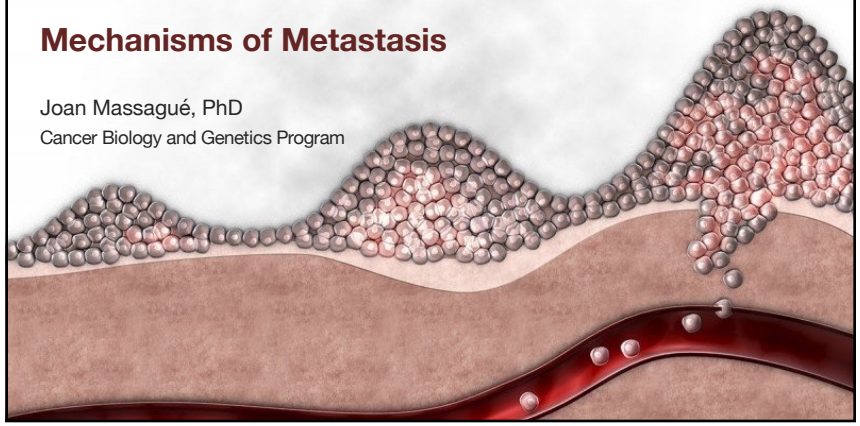
Cancer Biology and Genetics Program



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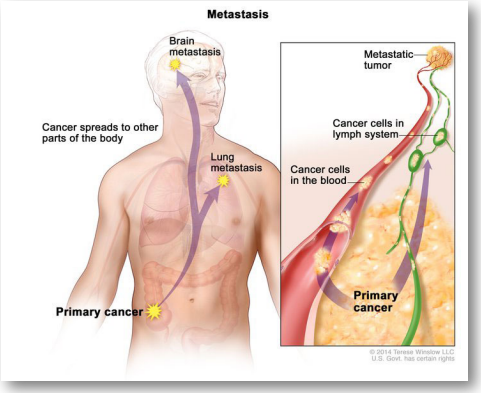
Metastasis as a medical problem

- Metastasis is the cause of majority of deaths from cancer
- Limited predictive ability to identify tumors that will metastasize
- Dormancy: metastasis may appear years after treatment of the primary tumor
- Current treatments can suppress metastasis but only temporarily.
- Understanding and targeting the basis for metastasis is a major goal of current research.



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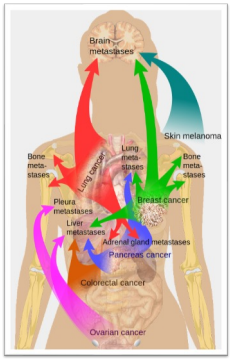
Why is it so difficult to cure metastasis?

- High tumoral load (10^{12} cells)
- Tumor heterogeneity
- Host organ heterogeneity
- Rapid development of resistance to therapy



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- Metastasis as a medical problem
- **Phases of the metastatic process**
  - Metastatic dissemination
  - Metastasis initiating cells
  - Dormancy and immune evasion
  - Organ colonization and metastatic tropism
- Tumor evolution and metastatic progression
- Metastasis as a systemic disease

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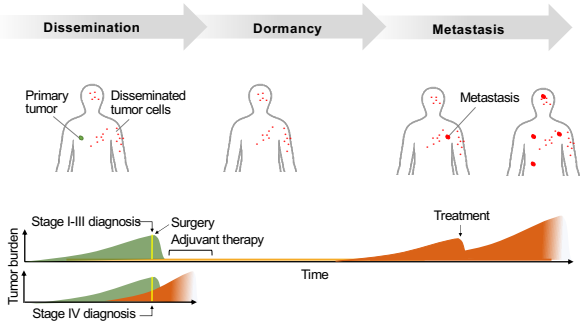
Phases of metastasis



Massagué & Ganesh *Cancer Disc.* 2021

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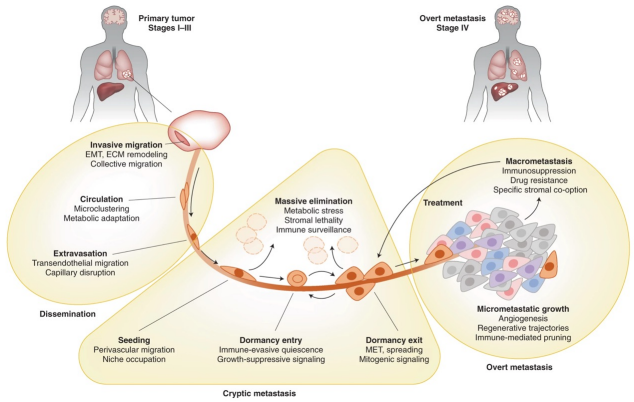
Metastasis steps and vulnerabilities



Ganesh & Massagué *Nat Med.* 2021

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Mechanisms of Metastasis

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- Phases of the metastatic process
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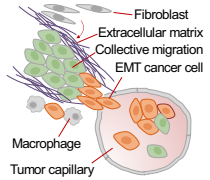
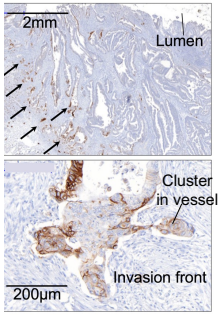
Metastatic dissemination: Intravasation



Primary colorectal cancer



Current focus: cell migration and invasion mechanisms, epithelial-mesenchymal transitions (EMTs), extracellular matrix remodeling.

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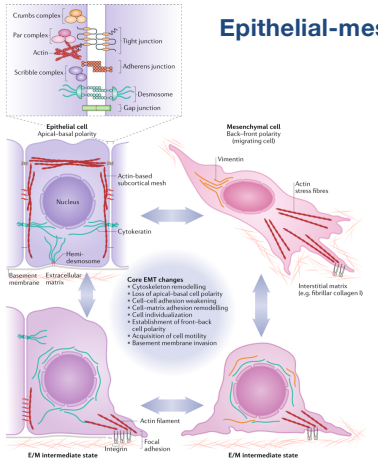
Epithelial-mesenchymal transitions (EMTs)



- Phenotypic plasticity processes in which epithelial cells lose apicobasal polarity and cell-cell adhesion, gain anteroposterior polarity and become migratory.
- EMTs play critical roles in gastrulation, morphogenesis, and wound healing
- In cancer cells, EMTs promote invasion and metastasis.
- TGF-β drives EMTs by inducing the expression of transcription factors (Snail, ZEB) that repress epithelial genes and enable mesenchymal gene expression
- Different epithelial cells manifest different EMTs ("partial", "complete").
- EMTs occur as part of broader developmental and regenerative programs
- EMT is coupled with differentiation, apoptosis or fibrosis depending on the context.

Yang et al. *Nat Revs.* 2020

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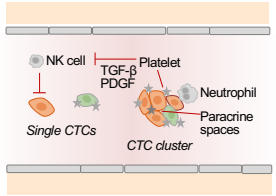
Metastatic dissemination: Circulation

Barriers
- Shear stress
- Oxidative stress
- NK cell attack

Adaptations
- MIC Clustering
- Metabolic adaptation
- Platelet coating
- Neutrophil interaction



Current focus: circulating tumor cells (CTCs), CTC microclusters, lymphatic vs hematogenous routes, oxidative stress.

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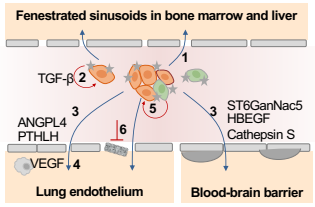
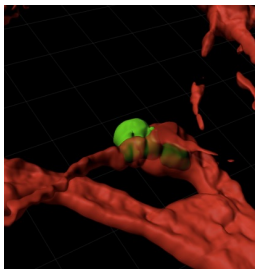
Metastatic dissemination: Extravasation

c. Extravasation

Barriers
- Endothelial junctions
- Shear stress

Adaptations
1. Fenestrated endothelia
2. Platelet TGF-β for EMT
3. CTC endothelial disjunction and motility factors
4. Monocyte endothelial permeability factors
5. Cooperation within clusters
6. Endothelial necroptosis





Lung cancer cells extravasating the the brain of a mouse.
Valiente et al *Cell* 2014

Current focus: as listed above.

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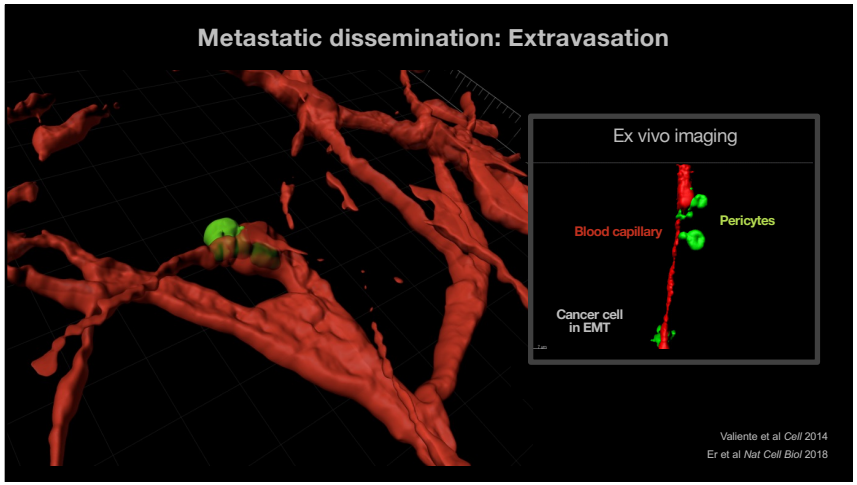
Metastatic dissemination: Extravasation



Valiente et al *Cell* 2014
Er et al *Nat Cell Biol* 2018

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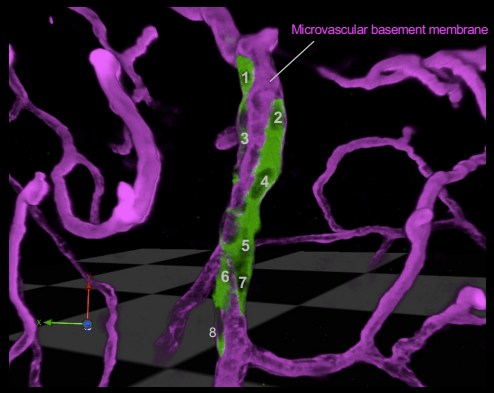
Human LUAD metastatic cells in mouse brain



Valiente et al *Cell* 2014

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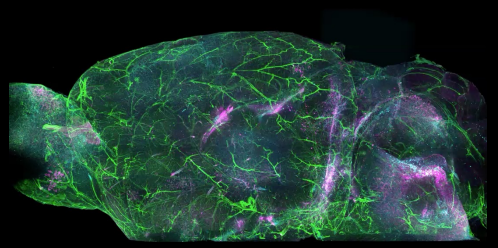
Human LUAD metastatic cells in mouse brain

H2030-BrM cancer cells (GFP)
Blood vessels (CD31)
Microglia/macrophages (IBA1)

iDISCO imaging
Luxendo MuVi SPIM light sheet
with J. Muller and K. Hadjantonakis



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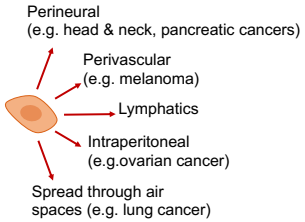
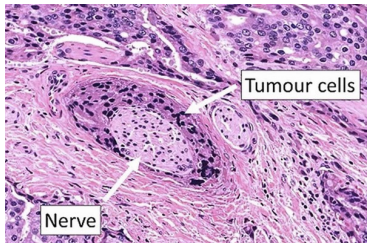
Metastatic dissemination: Alternative routes





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- Metastasis as a medical problem
- Phases of the metastatic process
  - Metastatic dissemination
  - **Metastasis initiating cells**
  - **Dormancy and immune evasion**
  - Organ colonization and metastatic tropism
- Tumor evolution and metastatic progression
- Metastasis as a systemic disease

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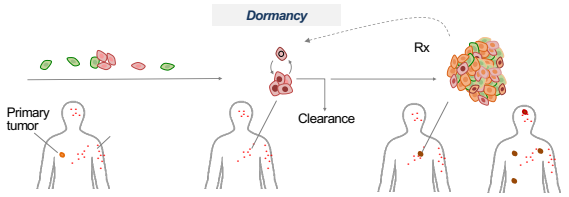

Current focus: nature of dormant MICs, dormancy-inducing signals, dormancy niches, immune evasion mechanisms, treatment resistance, MIC vulnerabilities.

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Metastasis-initiating cells (MICs)

Cancer cells that withstand multiple obstacles during metastatic dissemination and are able to regenerate the tumor in distant sites.

- What is the origin of MICs?
- Are MICs the same as tumor-initiating cells?
- How do MICs enter and exit dormancy?
- How do MICs evade immune surveillance?
- How do MICs acquire organ tropisms?
- How do MICs resist therapy?

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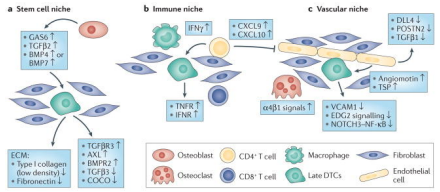
Metastatic dormancy: traditional ideas

- Growth suppressive signals inhibit the proliferation of disseminated cancer cells.
- Growth supportive signals reawaken dormant cancer cells for progression to overt metastasis.

Hypothetical dormancy-inducing niches



Sosa et al. *Nat Rev, Cancer* 2014

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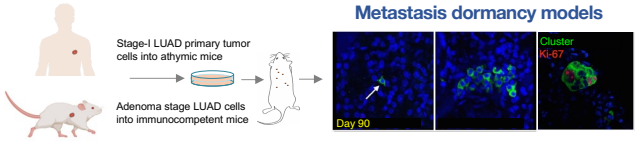
Metastatic dormancy as a dynamic process



Properties of dormant MICs

- Early-stage regenerative progenitors (SOX2+)
- Distinct from tumor-initiating cells
- Occupy perivascular niches
- Enter quiescence in response to TGF-β, WNT inhibitors
- Dormancy is an immune evasive state
- Proliferative clusters regress; infrequent outbreaks
- Aggressive outgrowth upon depletion of NK and T cells

Malladi et al., *Cell* 2016
Laughney et al *Nature Genet.* 2020
Hu et al *Nature* 2023

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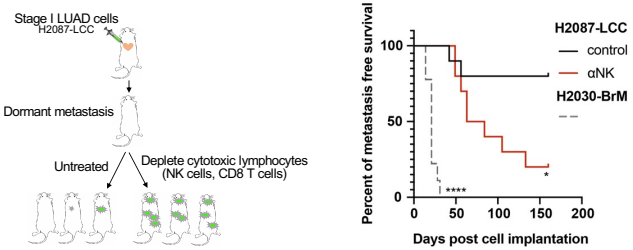
Immune surveillance prevents exit from dormancy



Malladi et al., *Cell*. 2016

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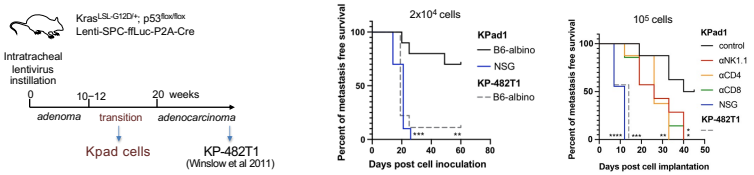
Immune surveillance prevents exit from dormancy



Hu et al *Nature* 2023

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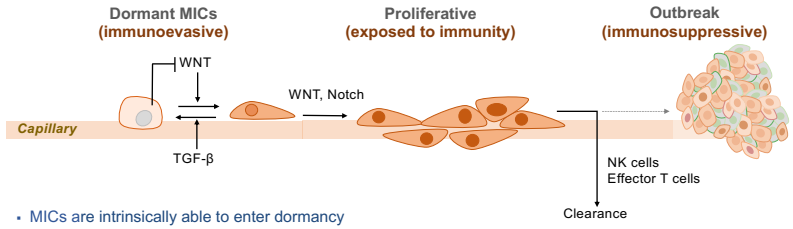
Metastatic dormancy as a dynamic process



- MICs are intrinsically able to enter dormancy
- Are immune evasive during quiescence
- Reenter the cell cycle "at their own risk"

What cell-intrinsic factors trigger immune-mediated elimination of metastatic cells upon reentering the cell cycle?

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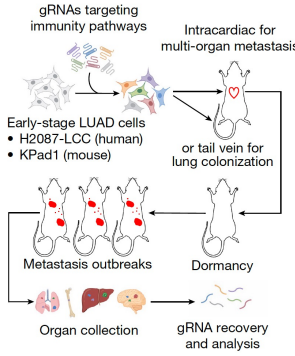
In vivo CRISPR screen for suppressors of LUAD metastatic outbreak



Hu et al *Nature* 2023

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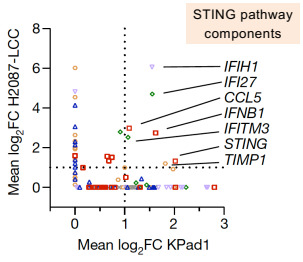
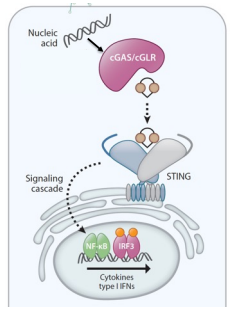
STING pathway suppresses LUAD metastatic outbreaks



Hu et al *Nature* 2023



Adapted from Slavik & Kranzusch, *Annu. Rev. Virol.* 2023

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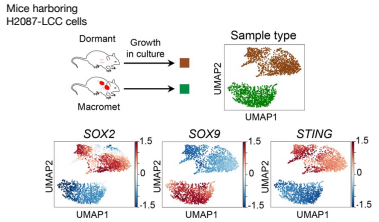
STING expression in dormant SOX2 progenitors reentering the cell cycle



Hu et al *Nature* 2023

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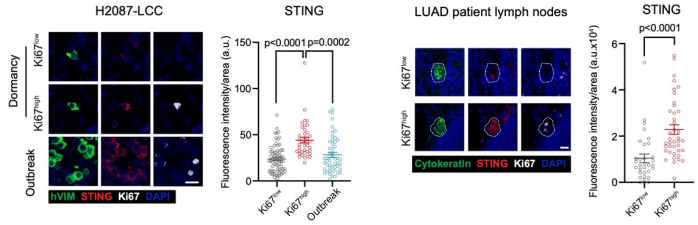
Heightened STING expression in dormant LUAD cells reentering the cell cycle



Hu et al *Nature* 2023

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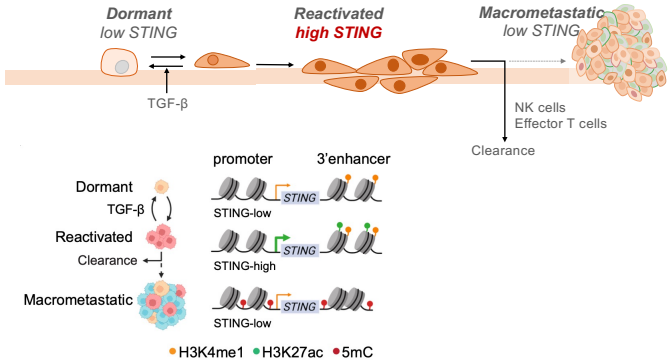
Immune surveillance prevents exit from dormancy



Hu et al *Nature* 2023

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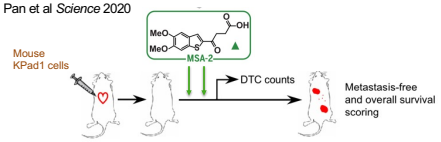
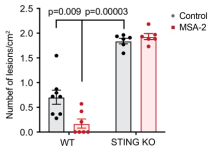
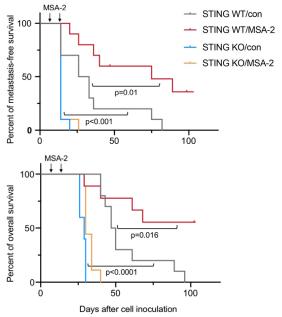
STING agonist eliminates dormant metastasis and prevents relapse

Orally available non-nucleotide STING agonist.
MSA-2 (benzothiophene oxobutanoic acid)
Pan et al *Science* 2020







Hu et al *Nature* 2023

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  - **Organ colonization and metastatic tropism**
- Tumor evolution and metastatic progression
- Metastasis as a systemic disease

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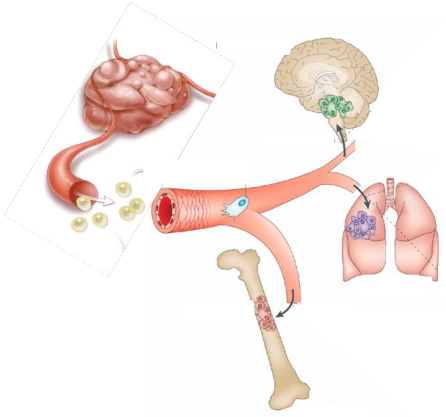
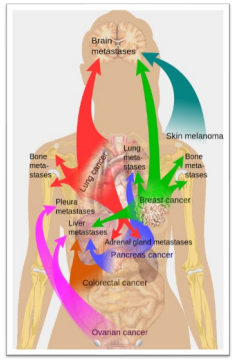
Each cancer has a stereotypical pattern of metastatic relapse





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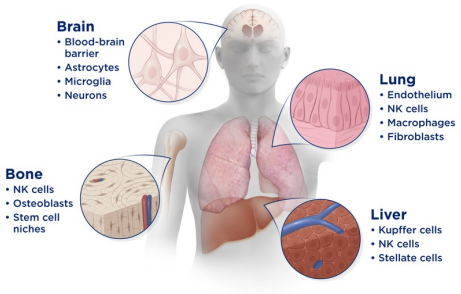
Each organ presents different barriers and opportunities

Barriers:
- Vascular walls
- Resident immunity
- Metabolic stress

Opportunities:
- Coopted stromal cells
- Microenvironmental signals



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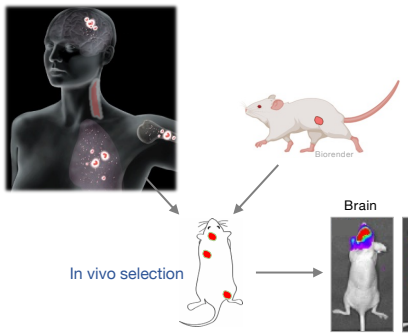
Models to study organ selective metastasis



1. Comparative omics
2. Candidate genes
3. Functional validation
4. Clinical validation

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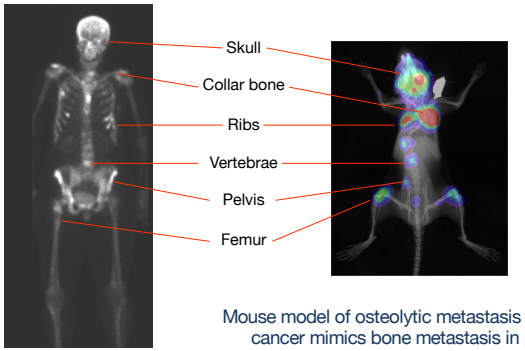
Models to study organ selective metastasis



Mouse model of osteolytic metastasis from breast cancer mimics bone metastasis in patients

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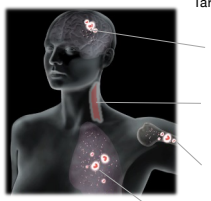
Mediators of organ-selective metastasis



Target organ	Extravasation	Seeding	Colonization
Brain	COX2 MMP1 HB-EGF ST6GALNAC5	SERPINs L1CAM	PCDH7 Conexin 43
Meninges			Complement C3
Bone		SRC CXCR4 CXCL1 L1CAM	IL11 MMP1 Osteopontin Jagged 1 CXCR4
Lung	ANGPTL4 Fascin1 COX2 MMP1 Epiregulin	Tenascin C CXCL1/2 L1CAM miR-216* miR-335*	VCAM-1

Kang et al *Cancer Cell* 2003
Minn et al *Nature* 2005
Gupta et al *Nature* 2007
Tavazoie et al *Nature* 2008
Padua et al *Cell* 2008
Zhang et al *Cancer Cell* 2009
Kim et al *Cell* 2009
Bos et al, *Nature* 2009
Nguyen et al *Cell* 2009
Oskarsson et al *Nat. Med.* 2011
Chen et al *Cancer Cell* 2011
Acharyya et al *Cell* 2012
Vanharanta *Nat. Med.* 2013
Zhang et al *Cell* 2013
Valiente et al *Cell* 2014
Obenauf et al *Nature* 2015
Malladi et al *Cell* 2016
Chen et al *Nature* 2016
Boire et al *Cell* 2017
Er et al *Nat Cell Biol* 2018

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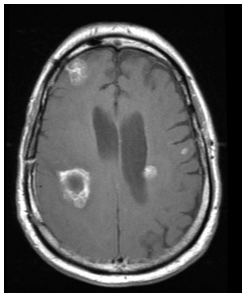
Example: Brain metastasis

- Common (>200,000/yr in US)
  - Lung cancer
  - Breast cancer
  - Melanoma
  - Colorectal cancer
  - Renal carcinoma
- Highly lethal



48 yr old with
KRAS-mutant lung adenocarcinoma

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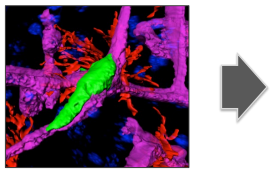
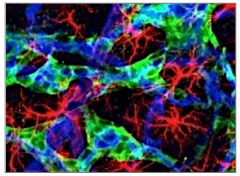
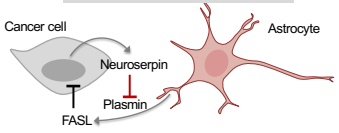
Carcinoma–astrocyte interactions in brain metastasis from LUAD and triple-negative breast cancer

Cancer cells (GFP)
Astrocytes (GFAP)
Capillaries (Col IV)



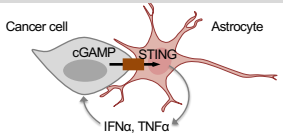


Serpins defend against killing



Valiente et al *Cell* 2014

GAP junctions for cell survival and drug resistance



Chen, Boire et al *Nature* 2016

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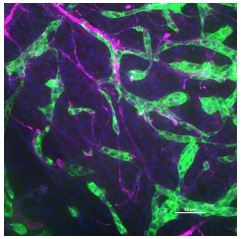
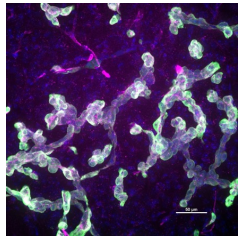
Different tumor types interact differently with the metastatic microenvironment

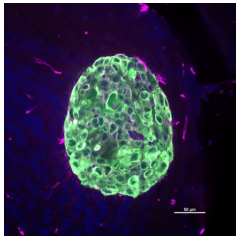
H2030
Lung adenocarcinoma



MDA231
Triple-negative breast CA



HCC1954
HER2+ breast cancer



GFP+ cancer cells
CD31 vasculature

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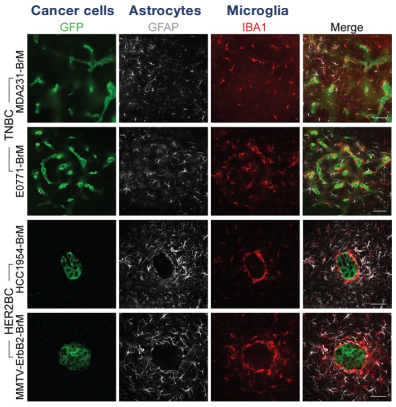
Different tumor types interact differently with the metastatic microenvironment



Siting Gan, Dana Pe'er

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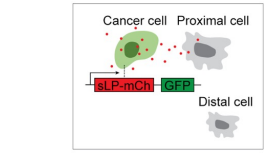
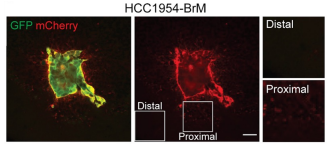
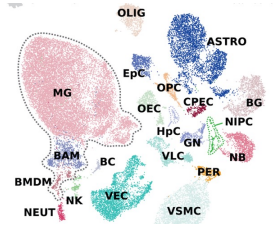
New approaches to interrogate the metastasis microenvironment

- Single-cell transcriptomic analysis of labeled neighbors







Siting Gan (w/ Dana Pe'er)

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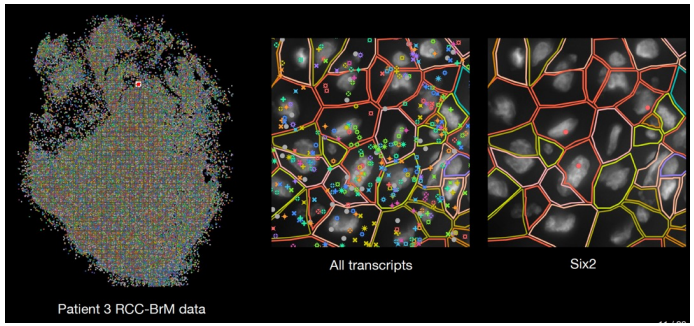
New approaches to interrogate the metastasis microenvironment

- Spatial profiling of brain metastases from renal carcinoma with 10x Xenium



Vijay Yarlagadda (w/ Christina Leslie)

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- Metastasis as a medical problem
- Phases of the metastatic process
  - Metastatic dissemination
  - Metastasis initiating cells
  - Dormancy and immune evasion
  - Organ colonization and metastatic tropism
- **Tumor evolution and metastatic progression**
- Metastasis as a systemic disease

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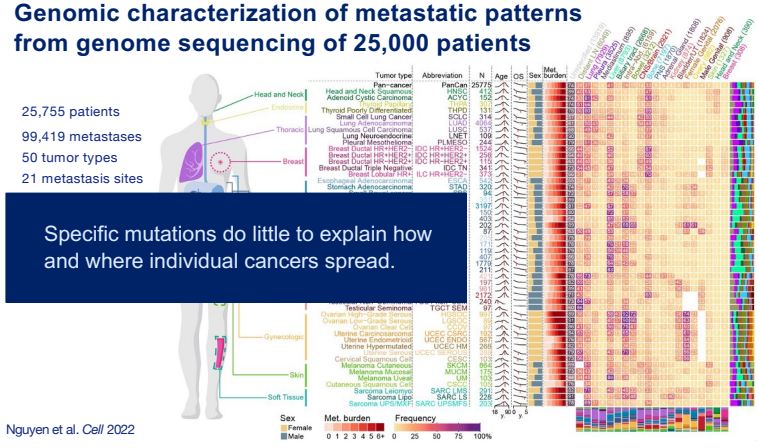
Genomic characterization of metastatic patterns from genome sequencing of 25,000 patients

25,755 patients
99,419 metastases
50 tumor types
21 metastasis sites

Specific mutations do little to explain how and where individual cancers spread.



Nguyen et al. *Cell* 2022

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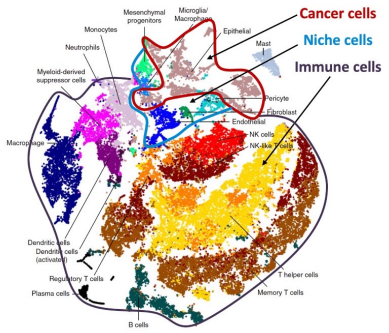
Single-cell transcriptional landscape of primary and metastatic human lung adenocarcinoma (LUAD)



Laughney et al., *Nat. Med.* 2020

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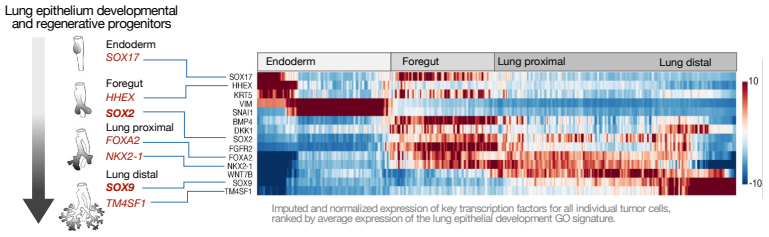
Metastasis: a continuum of developmental and regenerative phenotypes under selective immune pruning

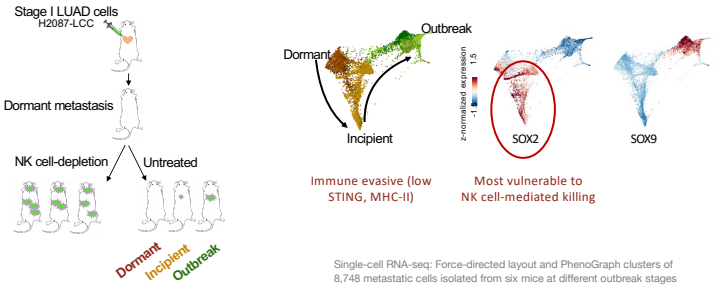


The continued development of metastatic progenitors is a major source of **intra-tumoral heterogeneity**

Laughney et al., *Nat. Med.* 2020

45

Immune surveillance prevents exit from dormancy



Single-cell RNA-seq: Force-directed layout and PhenoGraph clusters of 8,748 metastatic cells isolated from six mice at different outbreak stages

Laughney et al., *Nat. Med.* 2020

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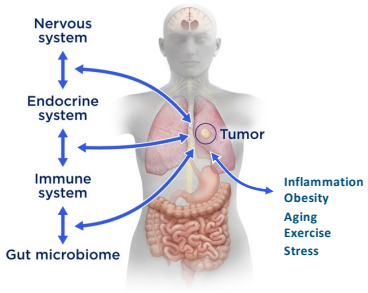
Systemic influences on metastasis

- Tumor host tissue and whole-body determinants shape cancer relapse and its response to therapy



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Summary

- Metastasis as a problem: *metastasis is the main cause of death from cancer*
- Phases
  - Invasion and metastatic dissemination: *many mechanisms identified*
  - Metastasis initiating cells: *malignant regenerative progenitors with high resilience, plasticity, and immune evasive powers*
  - Dormancy: *a proactive process; experimentally tractable; target of adjuvant therapy*
  - Organ colonization: *mediators identified; precision medicine has limited success; residual MICs eventually drive relapse*
- Tumor evolution and metastatic progression: *metastases as a developmental continuum; epigenetically driven; scant evidence of metastasis mutations*
- Treating metastasis: *paradigm-shifting approaches are requited*

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