What is epithelial-mesencyhmal plasticity and how can it help us understand metastasis?

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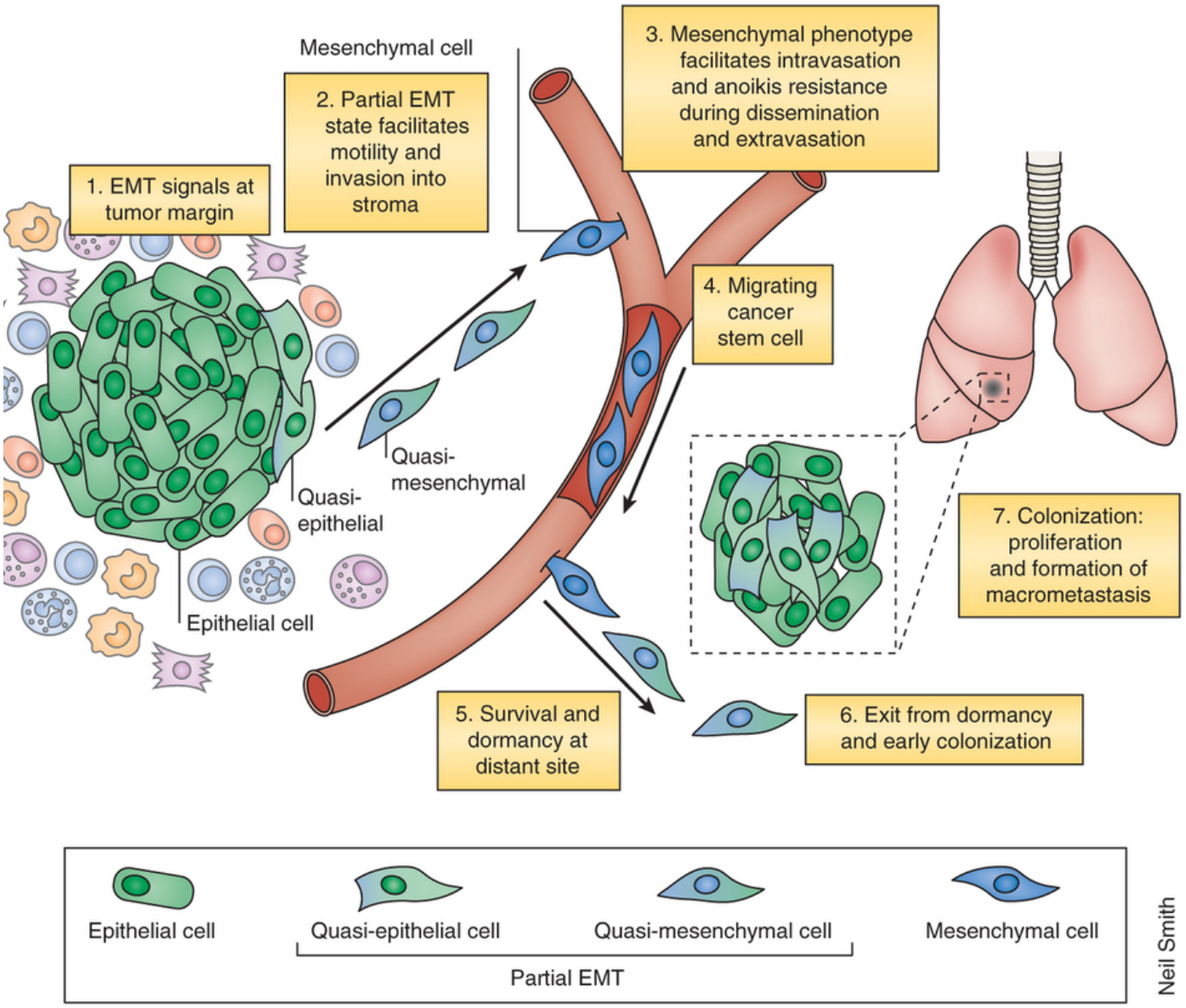
MD Anderson Cancer Center, Houston

Epithelial plasticity – a hallmark of metastasis



Tam and Weinberg, Nat Med 2013

Questions to ponder

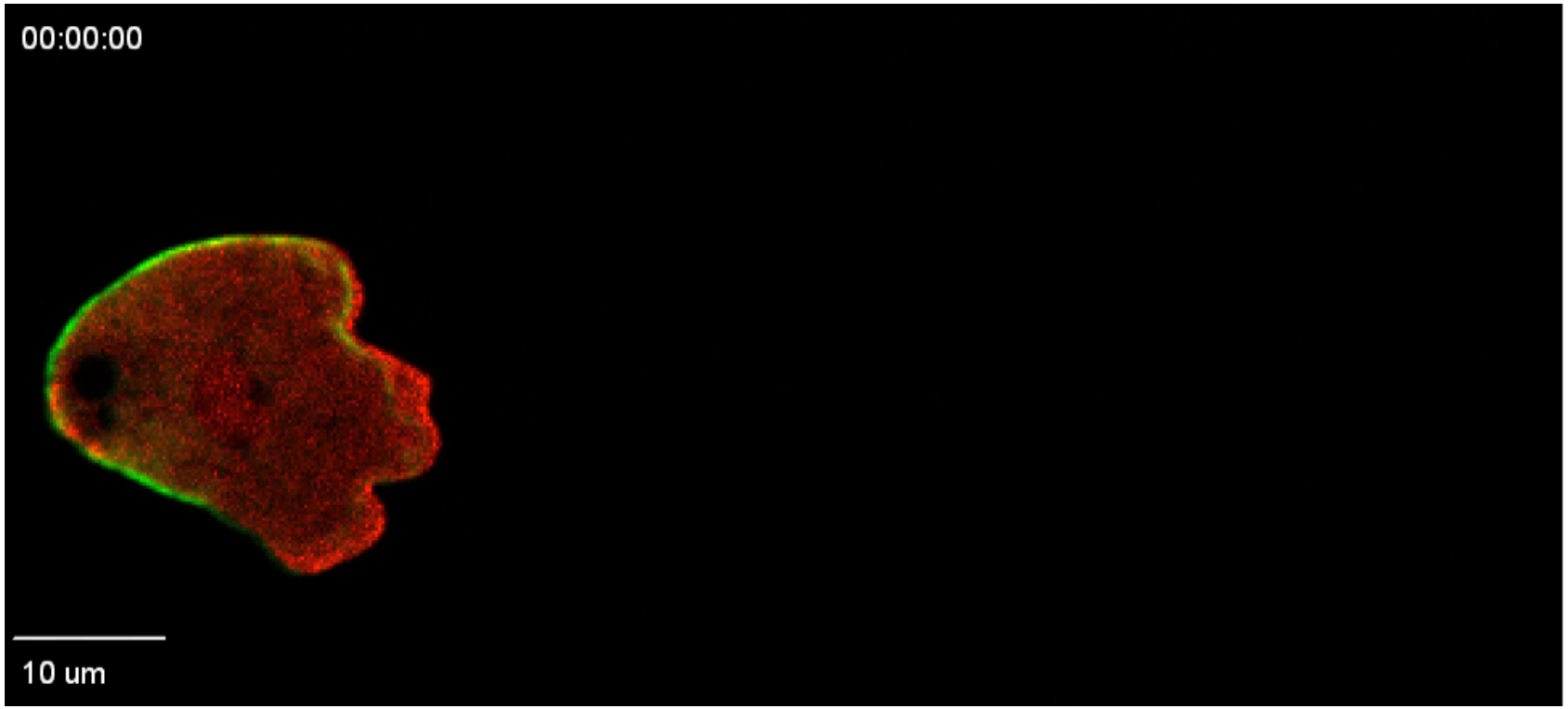
- What is the physics underlying cell motility as needed for cancer cell dispersal?
- How do cells dynamically change their "phenotype" from one state to another?
- Can understanding these issues help us better understand and treat cancer?

Questions to ponder

- **What is the physics underlying cell motility as needed for cancer cell dispersal?**
  - Active Media, A New Branch of CMT
- How do cells change their “phenotype” from one state to another?
- Can understanding these issues help us better understand and treat cancer?

The world of Active Media

Single cell dynamics



Here cell is polarized into protruding and contracting regions
by an external chemical field – “chemotaxis”, a story unto itself

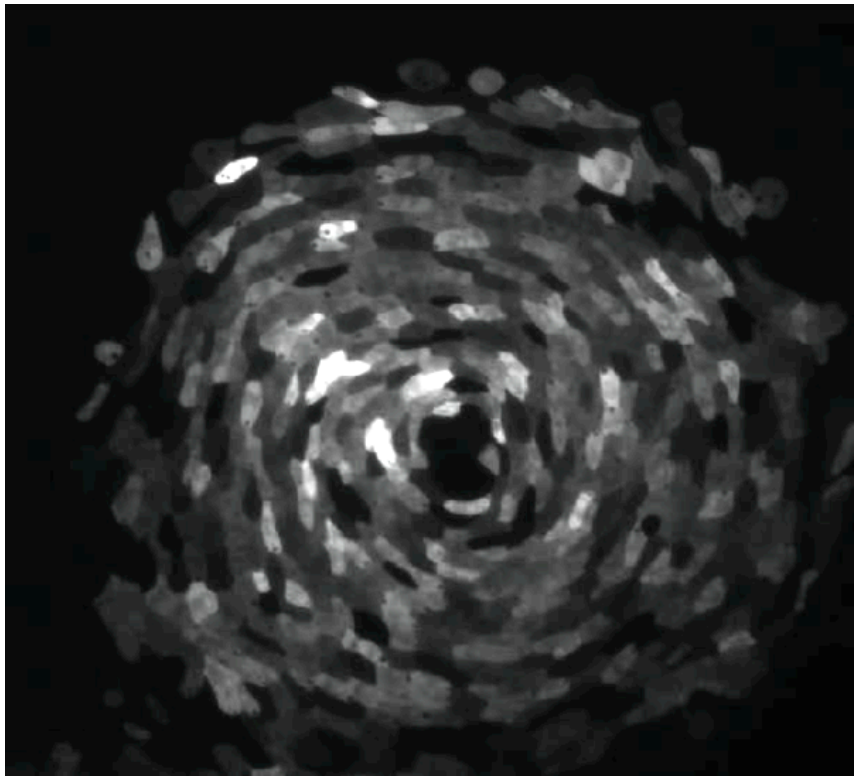
Collective cell motility

- Cells have self-propulsion just like other living systems
- Direction of the propulsive forces is determined by cell polarization
- Here polarization is determined collectively by cell-cell interactions; cells “go with the flow”
- System can order even in 2d due to non-equilibrium effects; gave rise to the field of “active media”

- <u>Seminal papers</u>

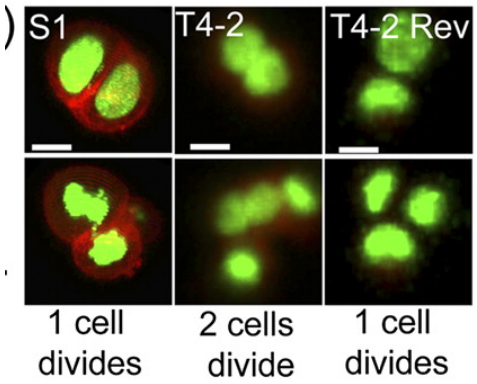
  Vicsek, Ben-Jacob et al PRL (1995)

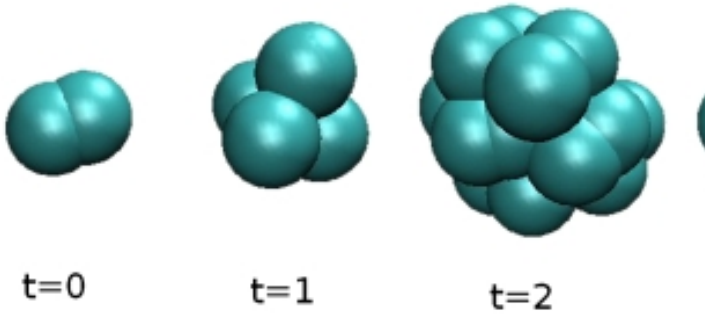
  Toner and Tu, PRE (1998)

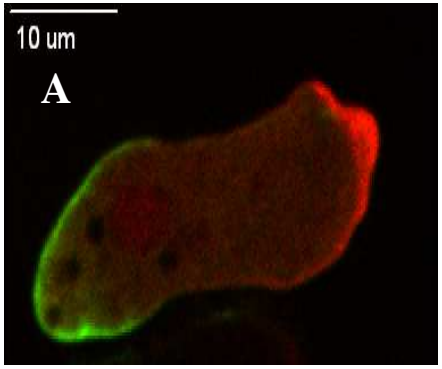


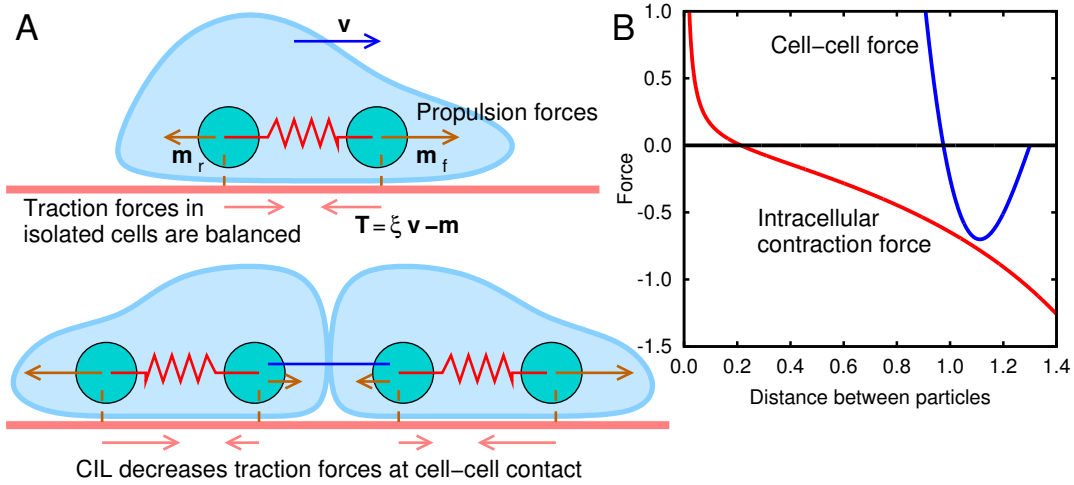
Dictyostelium rotating aggregate
Rappel, Levine et al, PRL 1999

The making of a motility model





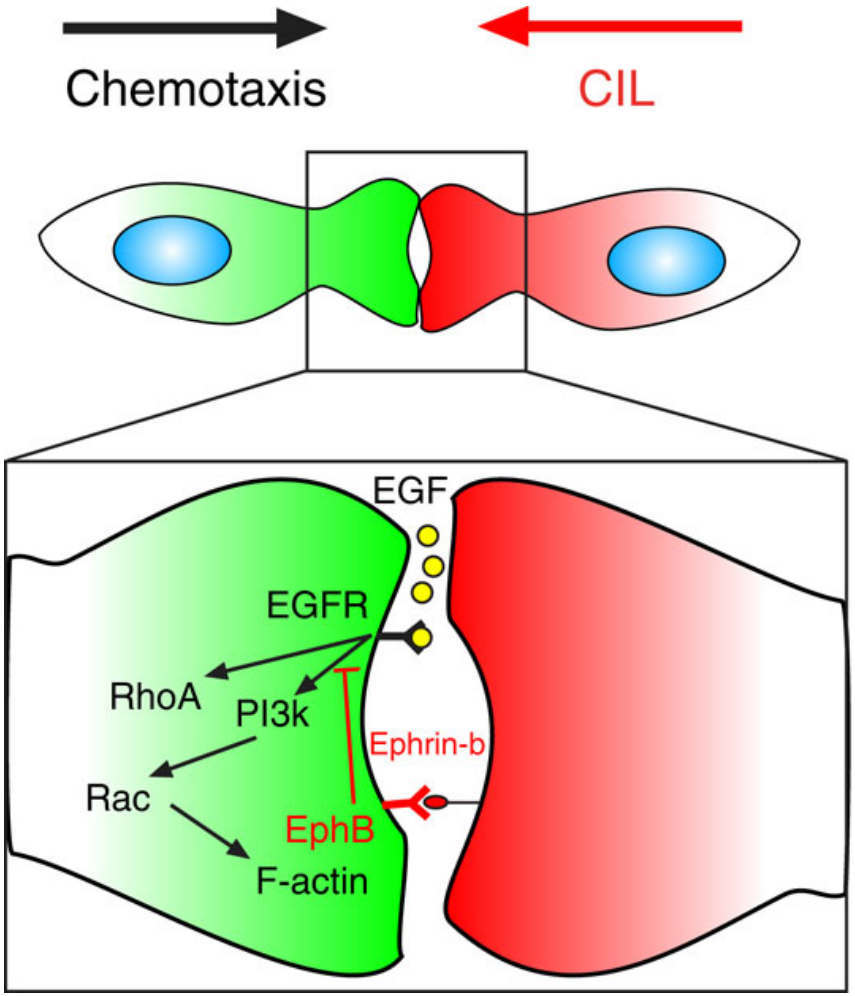




M. Basan et al PNAS (2013)

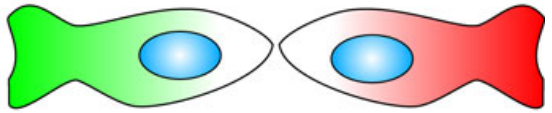
New version; Zimmerman PNAS (2016)

Lin et al, Nat Comm (2014) – another guidance mechanism!



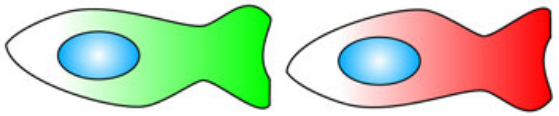
CIL > Chemotaxis

Suboptimal EGF gradients



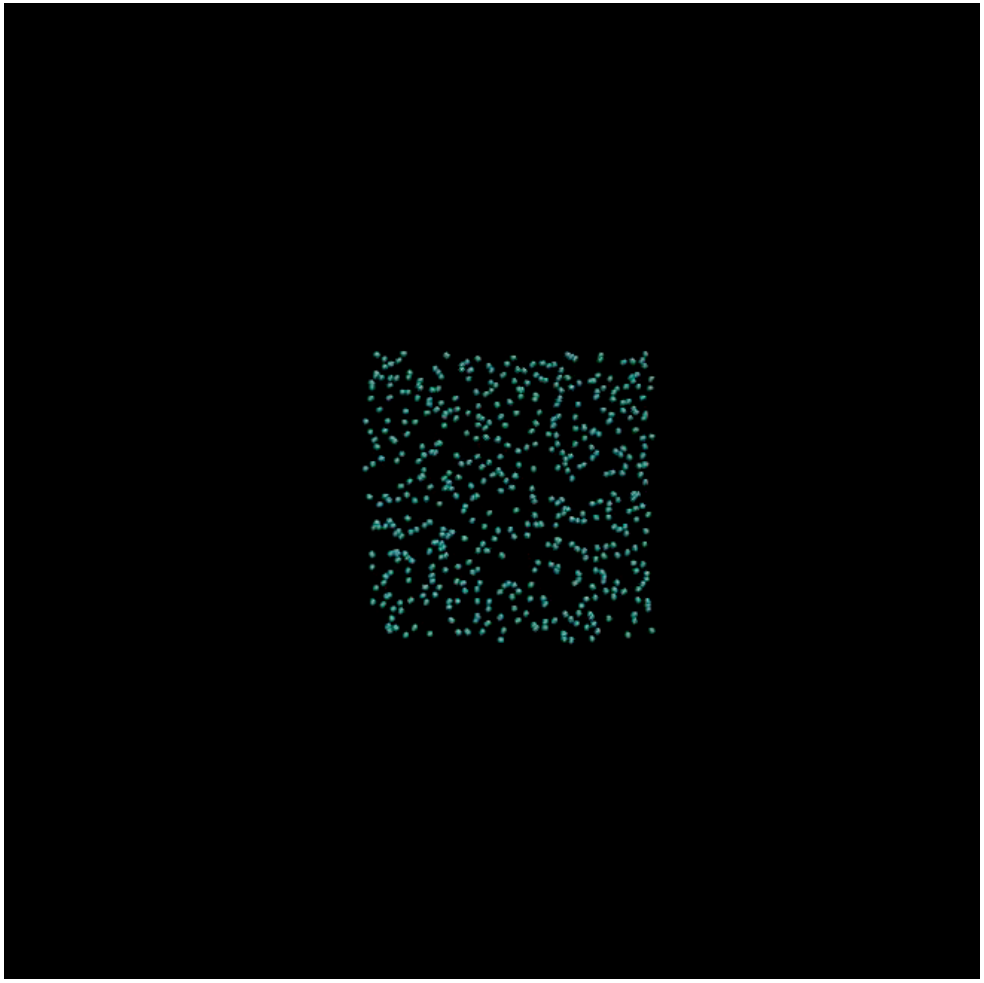
Chemotaxis > CIL

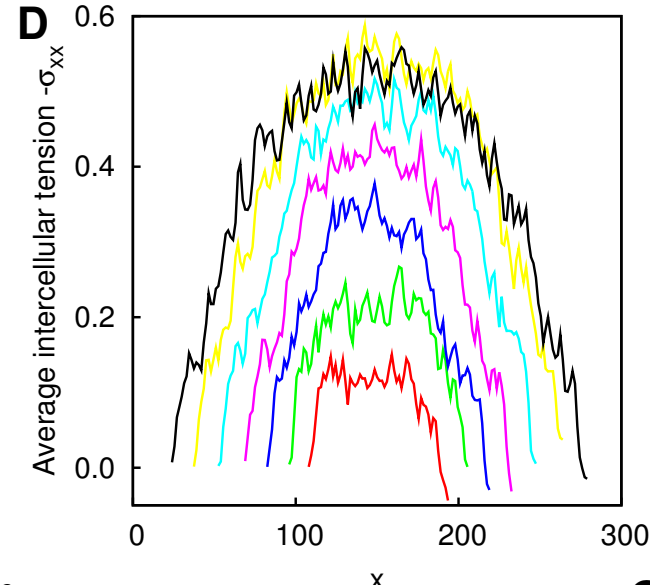
EGFR overexpression
Optimal EGF gradients
EphB3 knockdown
Unclustered ephrin-b1-fc

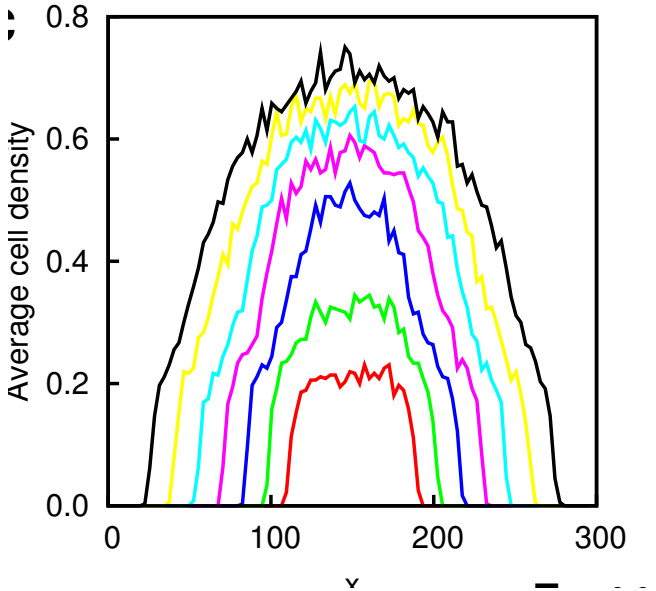


$$\partial_t \mathbf{p}^i = -\frac{1}{\tau}\mathbf{p}^i + \sigma\boldsymbol{\xi}^i(t) + \beta^i \sum_{j\sim i} \hat{\mathbf{r}}^{ij}$$

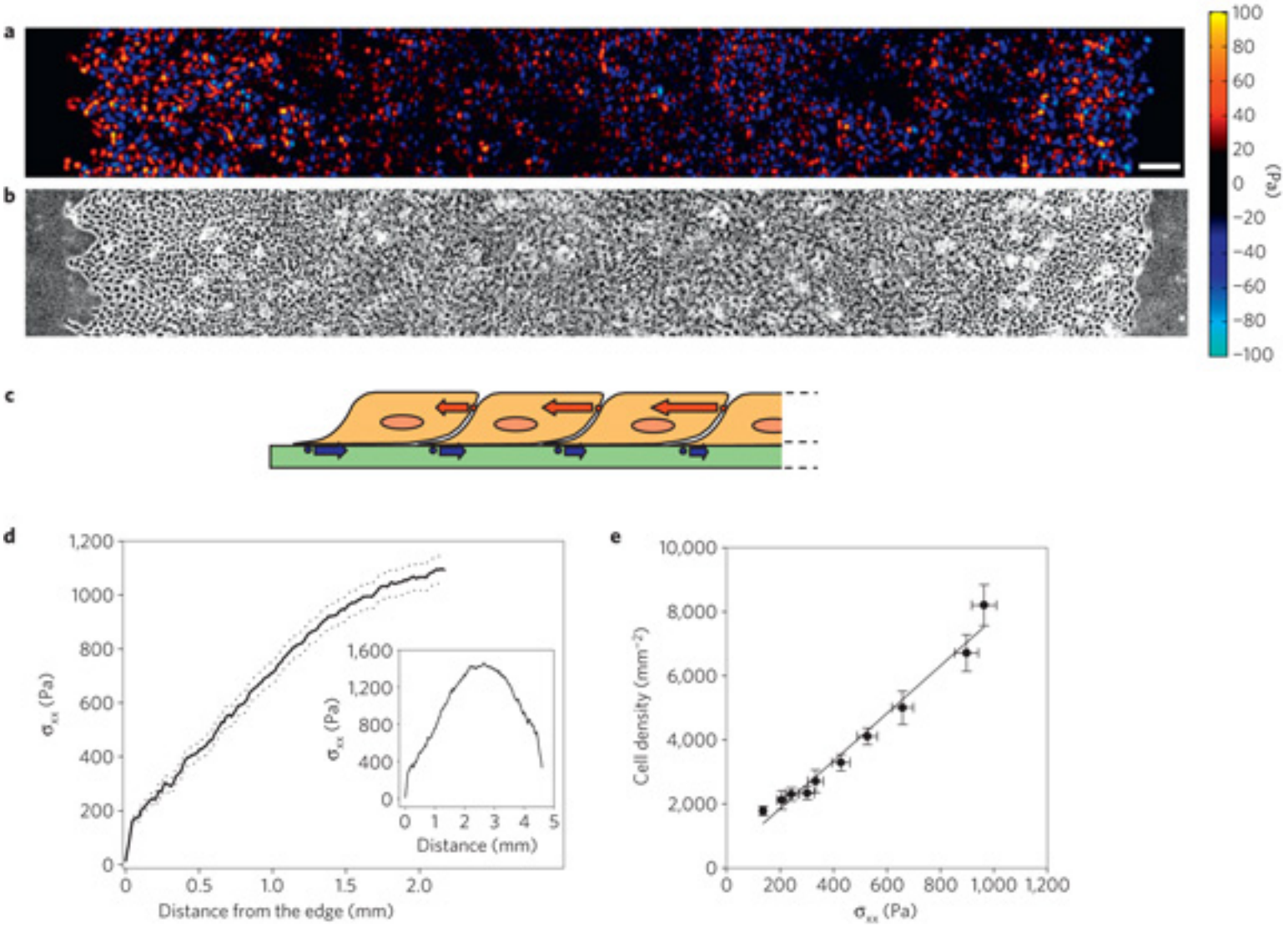
Theory of epithelial spreading





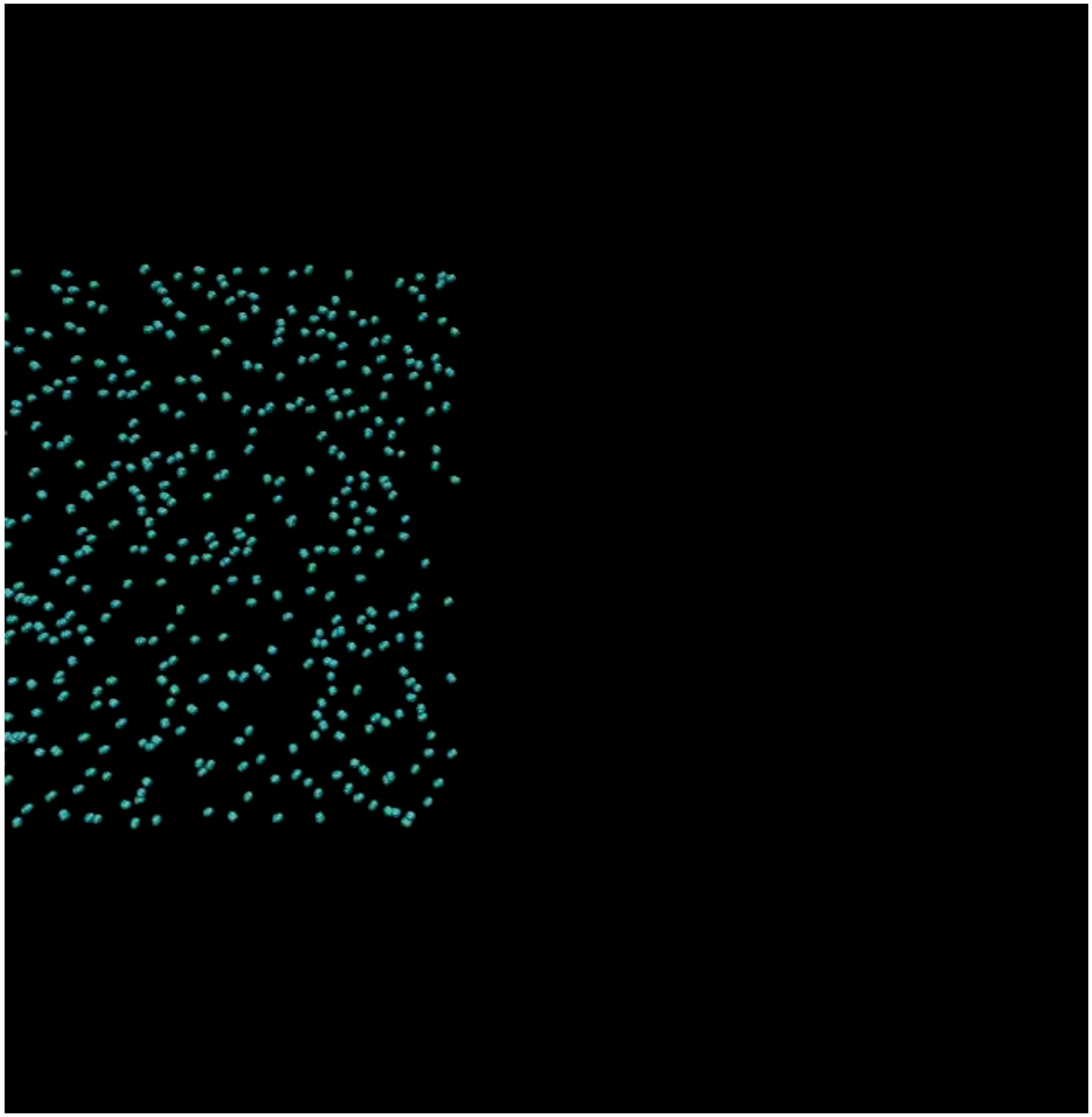


Tissues under Tension (Trepat et al)



Spreading around an obstacle

compare to Trepat experiment

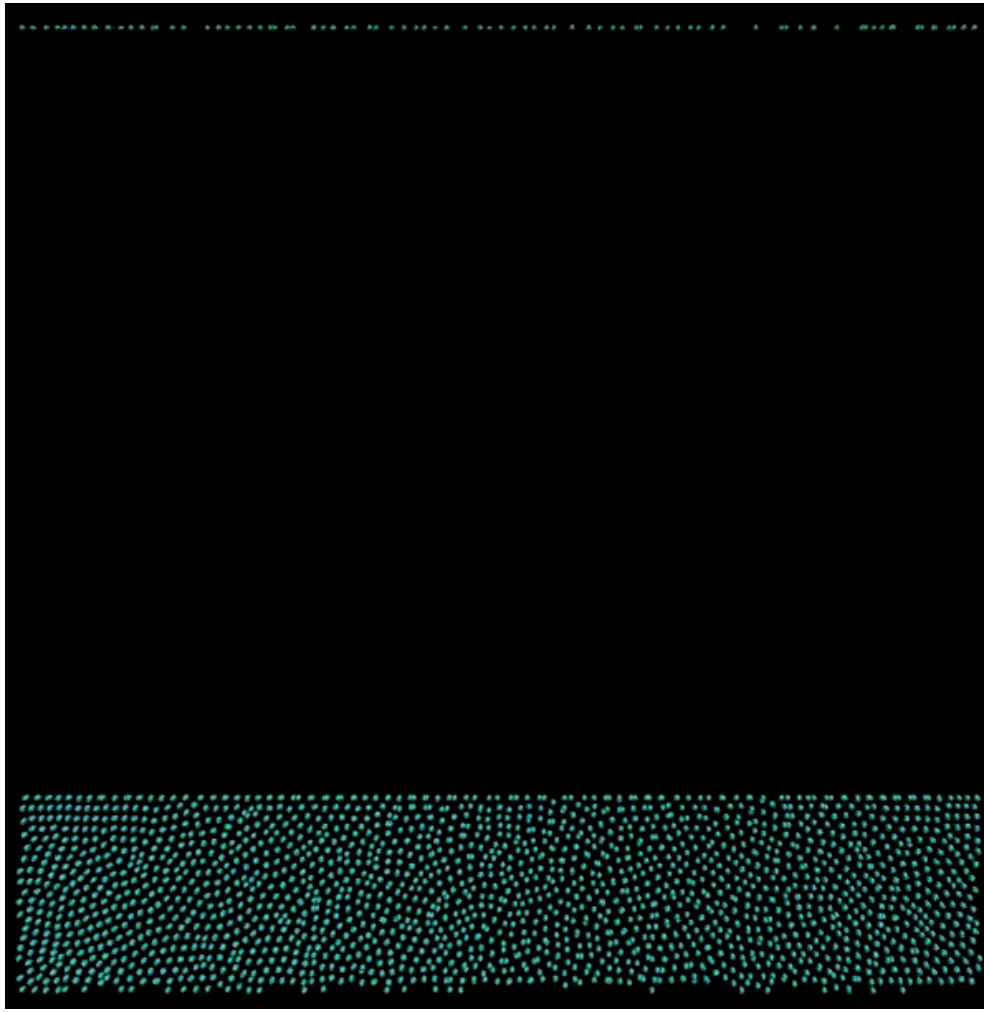


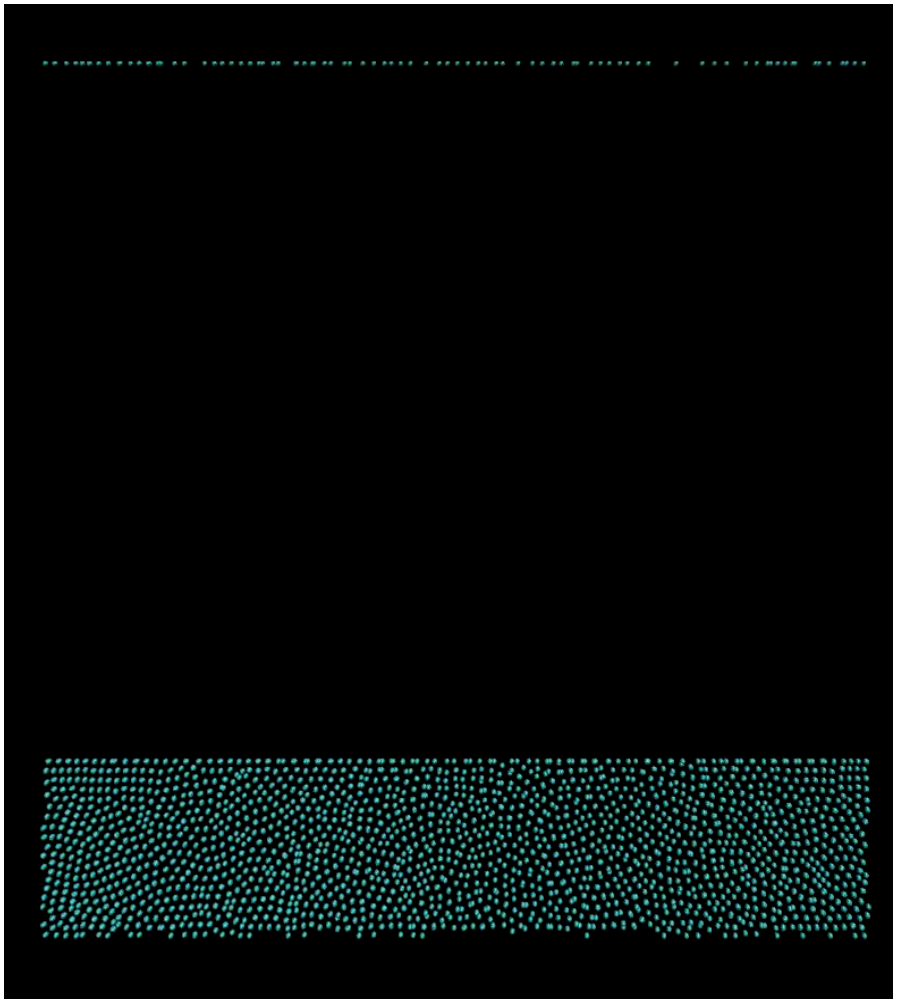
Returning to tumors

- Cancer biologists have suggested that cells at the margin become motile, involving a reduction in cell-adhesion and remodeling of their internal cytoskeleton

- What does this type of active media motility model predict for the edge of a tumor when the cells have become motile?

Role of cadherin-based adhesion

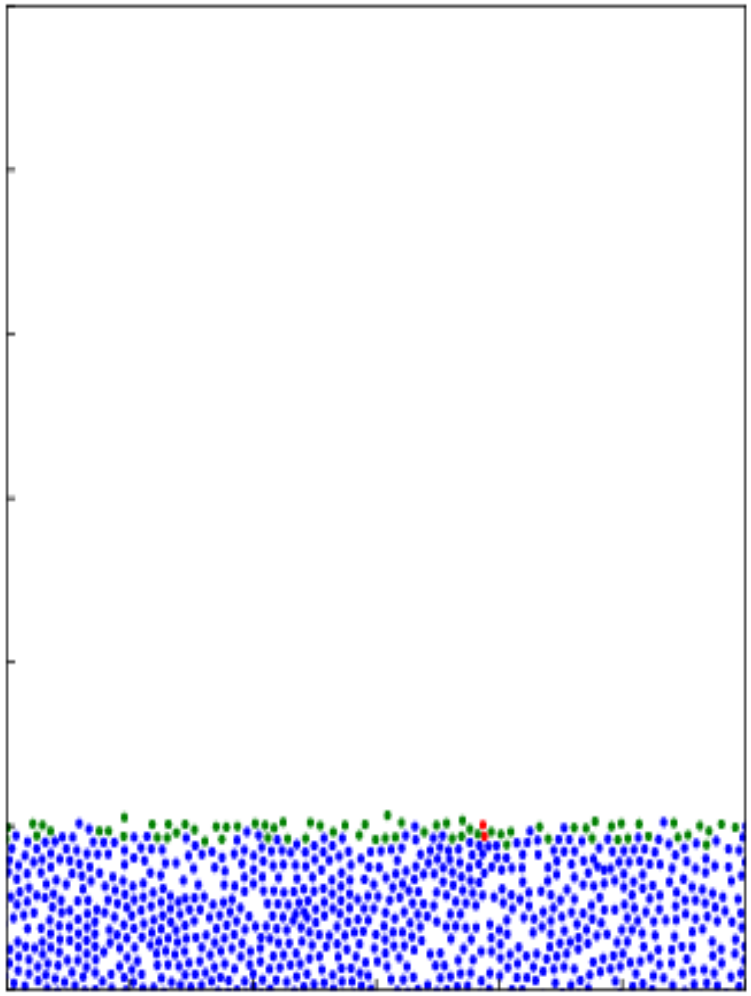




Simulation of motility, with different proteomes of M vs E/M states

Based on Basan, et al PNAS (2013); Zimmerman et al, PNAS (2016)

Under some circumstances, collective motion can lead to fingers and streaming – Yang and Levine, Physical Biology, (2020)



- Linear instability of the moving front due to curvature dependence of leader cell emergence
- May be other mechanisms such as growth or orientational ordering but these have not yet been shown to lead to stable fingers

Lessons so far

- Cells are self-propelled objects that can cooperatively organize their motion
- One can expect that some cells will move individually, other as collective objects
- This transition is controlled by biophysical parameters such as cell-cell adhesion versus the strength of self-propulsion forces

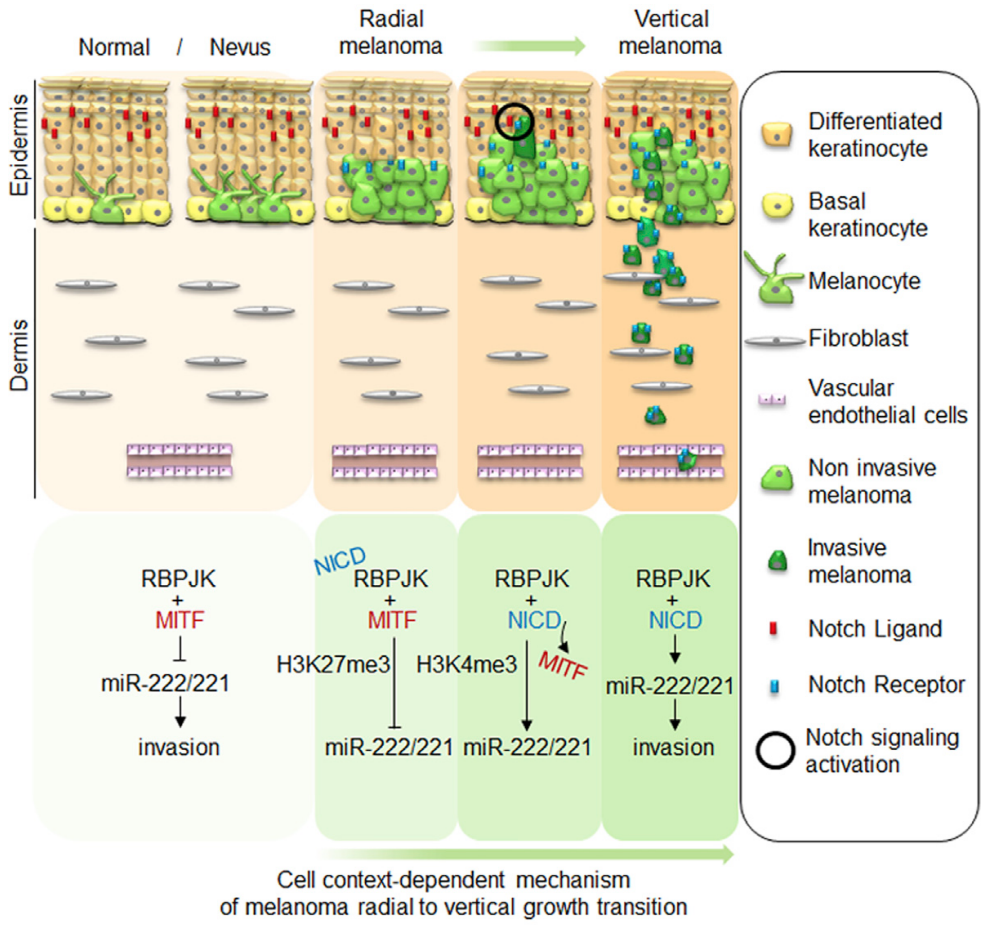
Questions to ponder

- What is the physics underlying cell motility as needed for cancer cell dispersal?

- **How do cells change their "phenotype" from one state to another?**
  - Dynamical systems and bifurcation theory

- Can understanding these issues help us better understand and treat cancer?

Focus on change in microenvironment



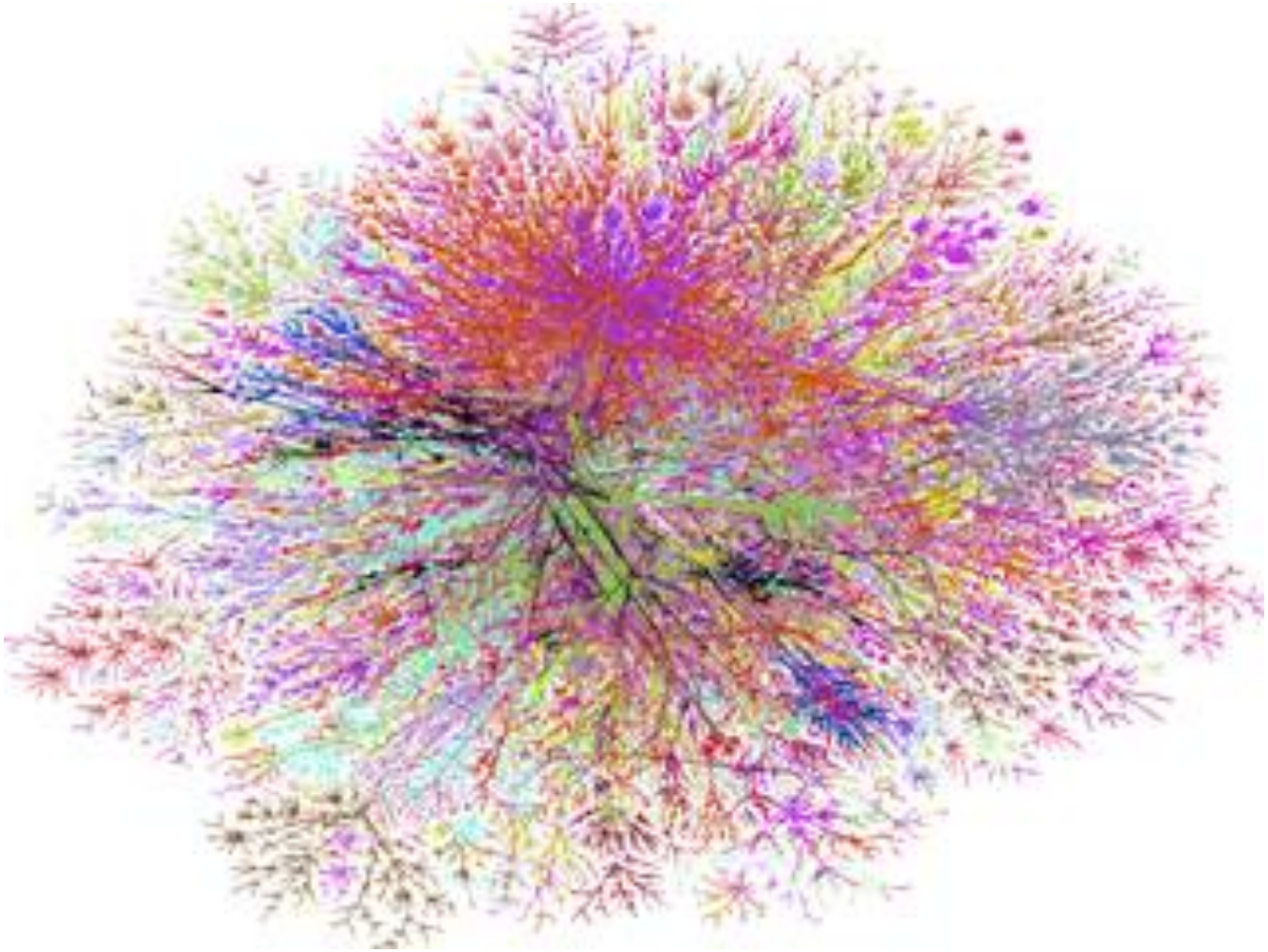
Melanoma example
Golan et al, Mol Cell (2015)

Phenotypic transition is not caused by additional mutations

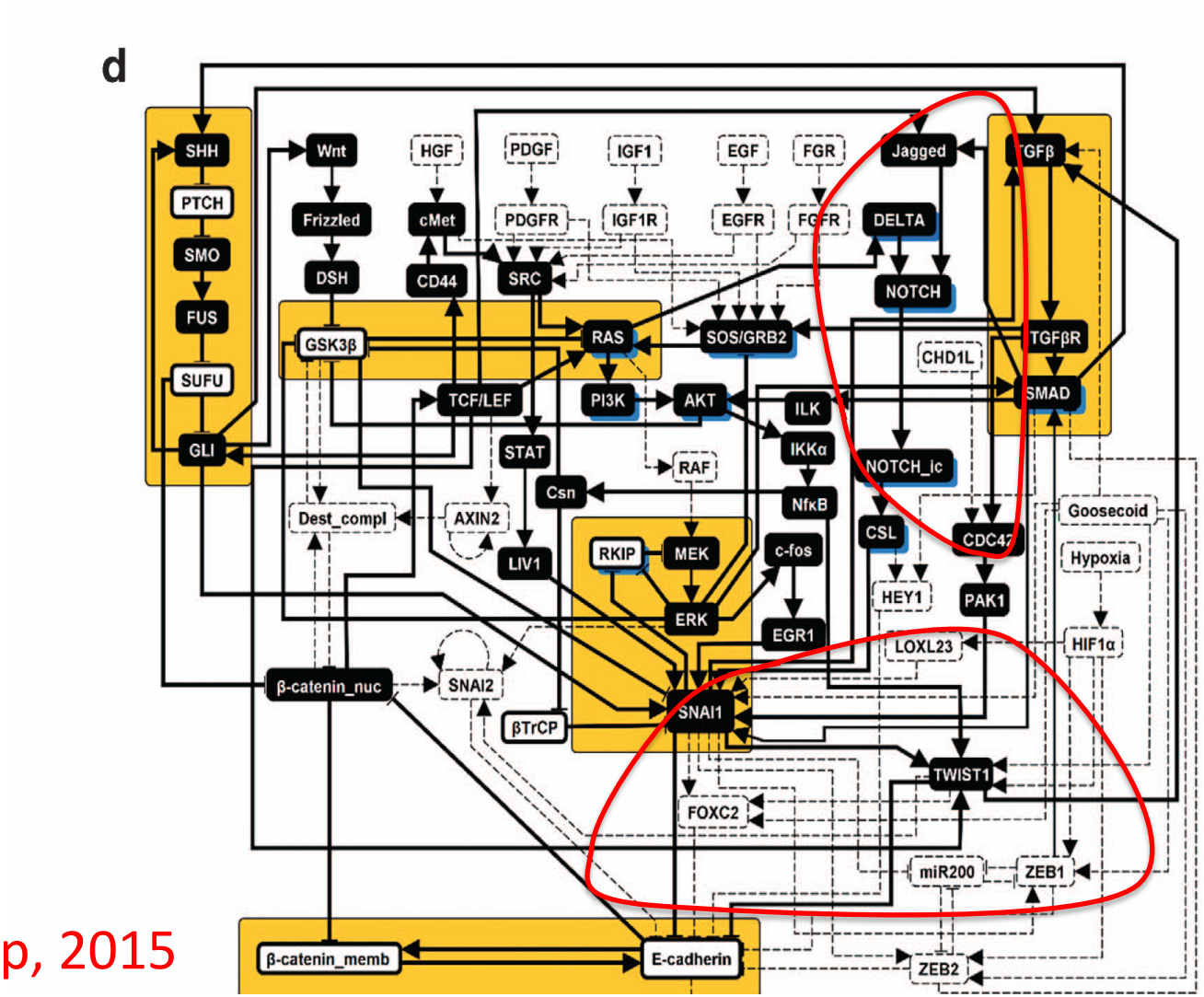
Cells become metastatic competent by being exposed to a new chemical environment; seems to be irreversible

Notch pathway plays the critical role in this transition

The World of Networks

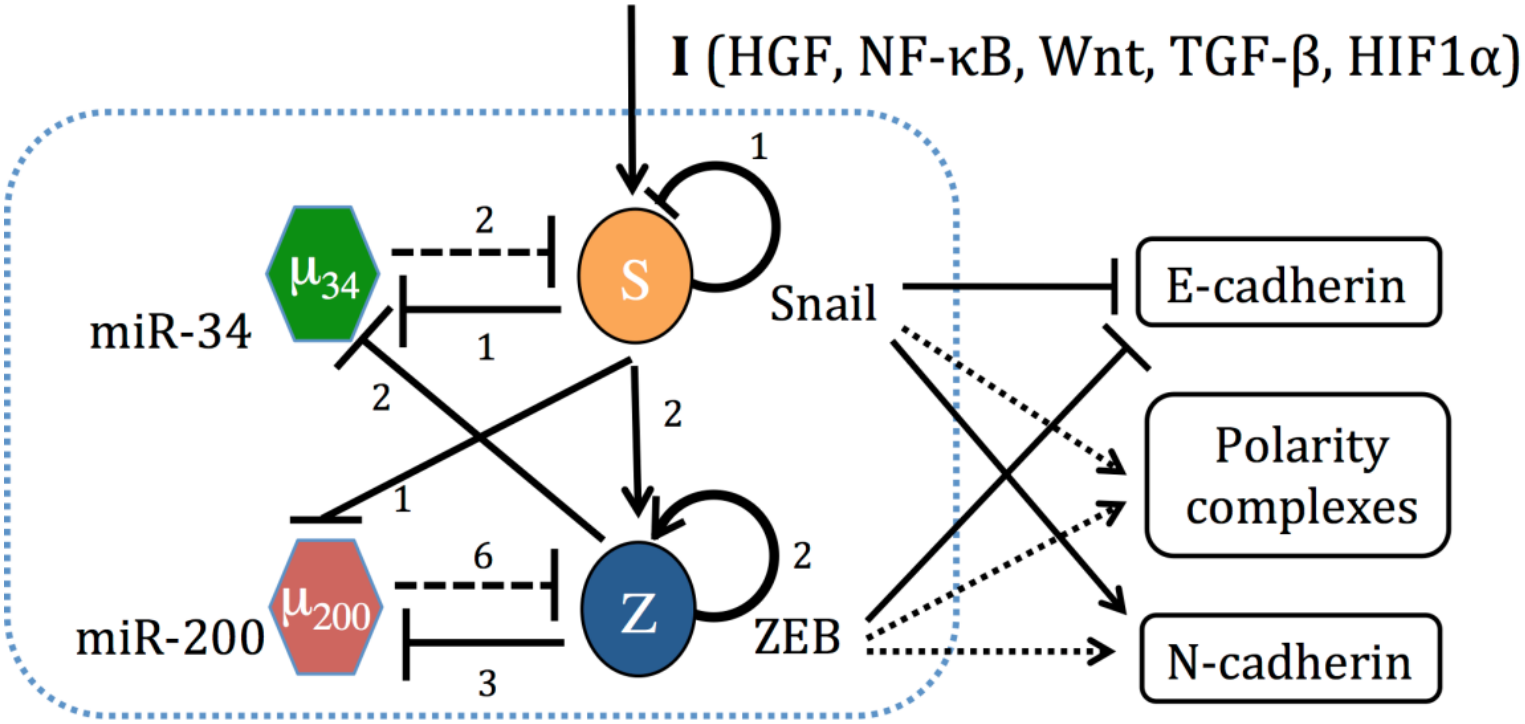


simplified EMT circuit diagram



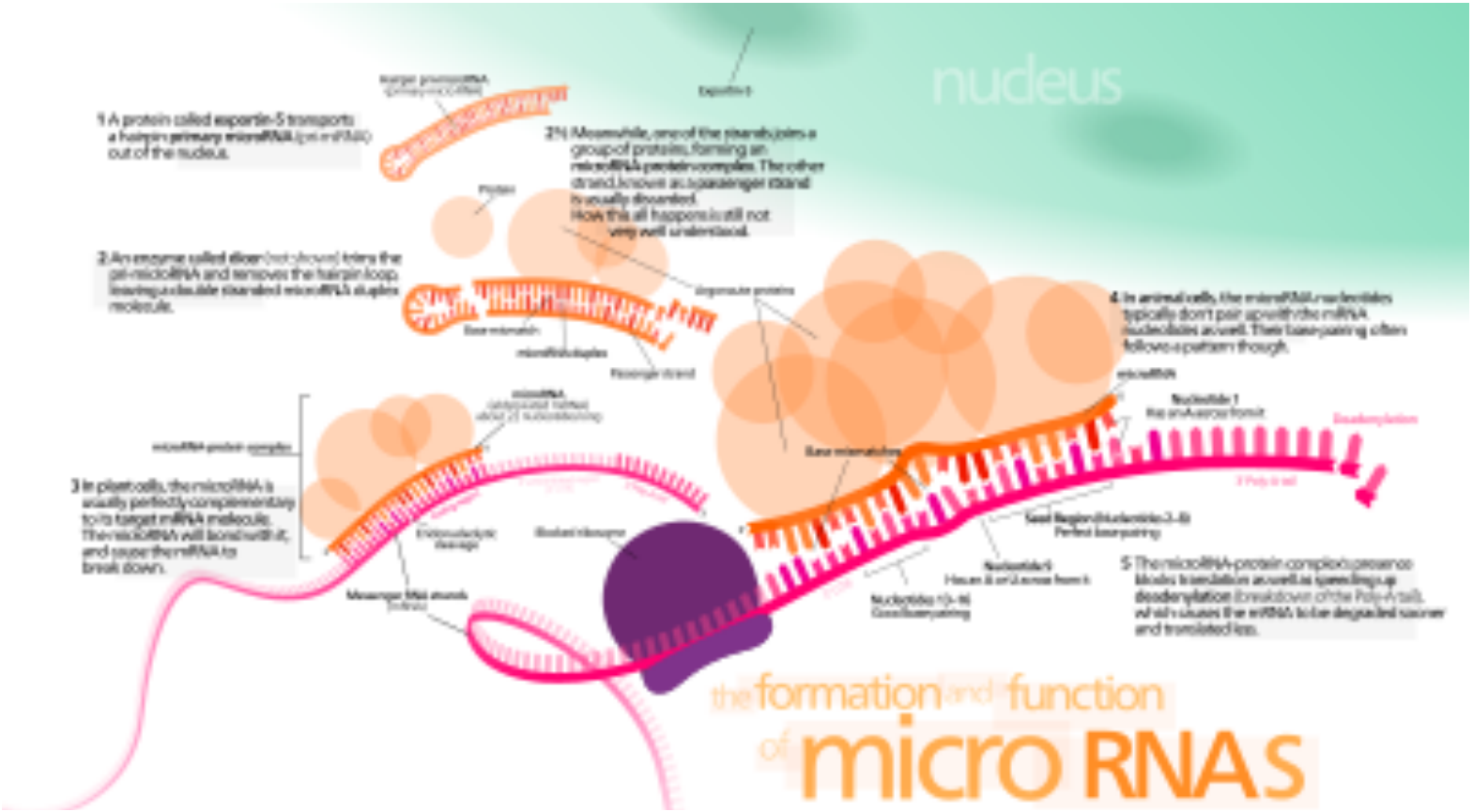
Albert group, 2015

The core EMT genetic circuit



- Each arrow is a quantitative relationship between the input and output levels
- This has been done for many transcription circuits, e.g. in microorganisms
- We needed to develop a new method for translation regulation

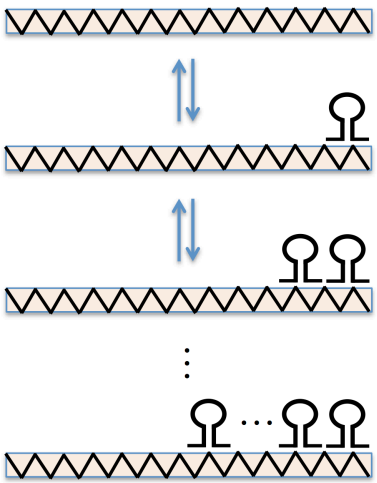
Biology since I went to High School



Levine, Erel, Eshel Ben Jacob, and Herbert Levine. "Target-specific and global effectors in gene regulation by MicroRNA." *Biophysical journal* 93.11 (2007): L52-L54

Loinger, A., Shemla, Y., Simon, I., Margalit, H. and Biham, O., 2012. Competition between small RNAs: a quantitative view. *Biophysical journal*, *102*(8), pp.1712-1721.

Generalized equations

	Population	Degeneracy	Effective Translation	mRNA Active Degradation	microRNA Active Degradation
	$M_n^0(\mu)$	C_n^0	l_0	0	0
	$M_n^1(\mu)$	C_n^1	l_1	γ_{m1}	$\gamma_{\mu 1}$
	$M_n^2(\mu)$	C_n^2	l_2	γ_{m2}	$\gamma_{\mu 2}$
	$M_n^i(\mu)$	C_n^i	l_i	γ_{m2}	$\gamma_{\mu 2}$
Total			$\sum_{i=0}^{n} l_i C_n^i M_n^i$	$\sum_{i=1}^{n} \gamma_{mi} C_n^i M_n^i$	$\sum_{i=1}^{n} i\gamma_{\mu i} C_n^i M_n^i$

These sums define

- L = translation suppression
- Y_μ = degradation increase
- Y_m = decay increase

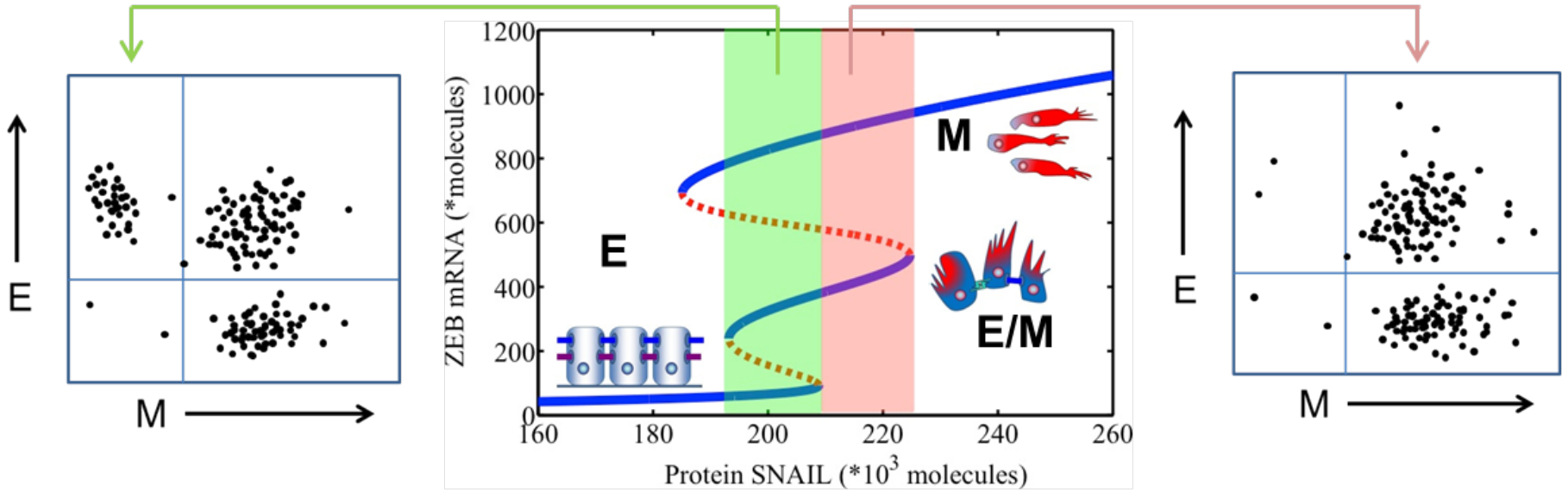
- H – standard Hill form for transcription regulation

$$\dot{\mu}_{200} = g_{\mu_{200}} H^S(Z, \lambda_{Z,\mu_{200}}) H^S(S, \lambda_{S,\mu_{200}}) - m_Z Y_\mu(\mu_{200}) - k_{\mu_{200}} \mu_{200}$$

$$\dot{m}_Z = g_{m_Z} H^S(Z, \lambda_{Z,m_Z}) H^S(S, \lambda_{S,m_Z}) - m_Z Y_m(\mu_{200}) - k_{m_Z} m_Z$$

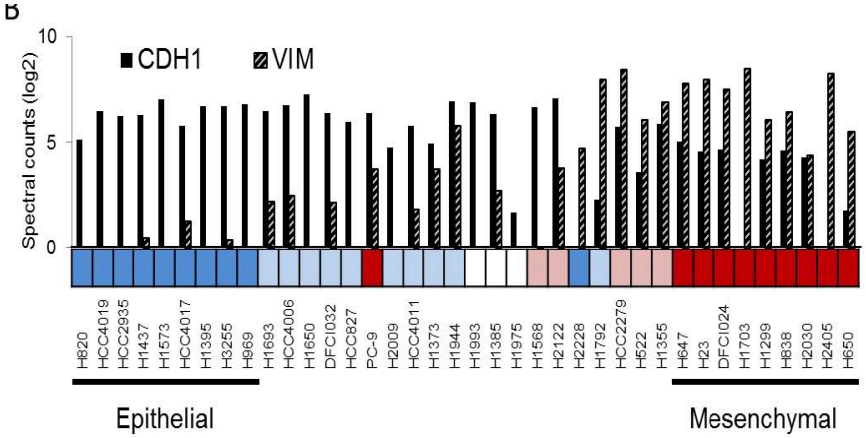
$$\dot{Z} = g_Z m_Z L(\mu_{200}) - k_Z Z$$

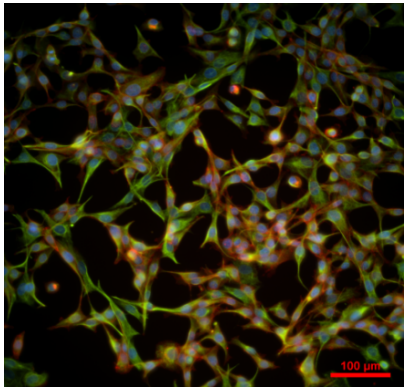
Coexistence of multiple phenotypes

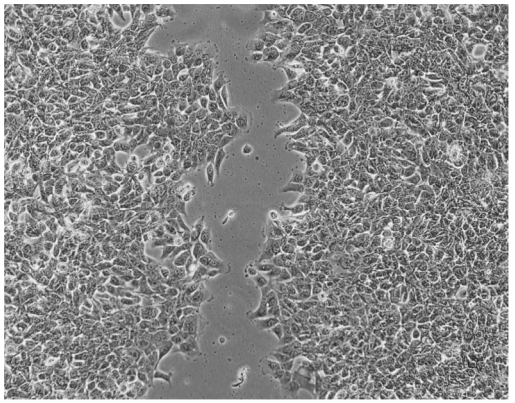


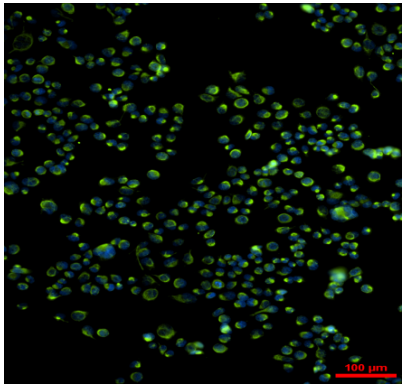
- Note that at intermediate EMT driving, population with this network is expected to be multimodal
- Other cell lines with other modulating factors (e.g. GRHL2) can create unimodal hybrid states

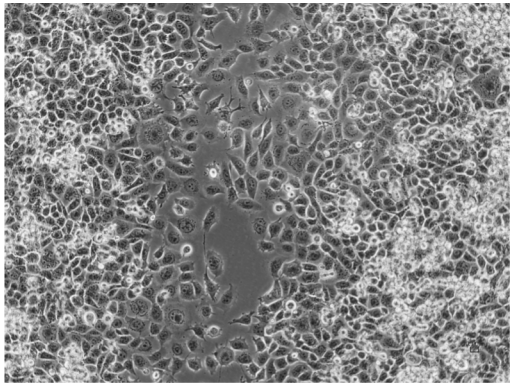
What kind of cells move collectively







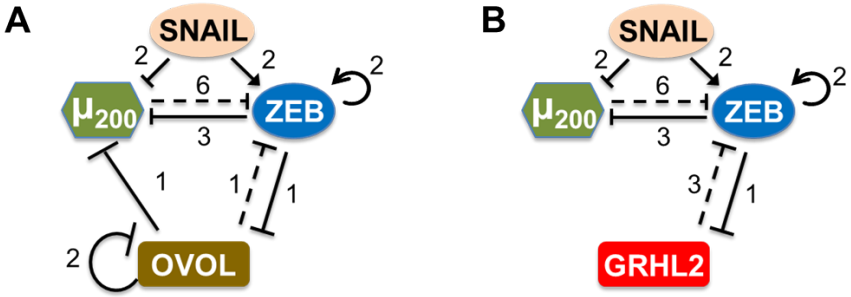




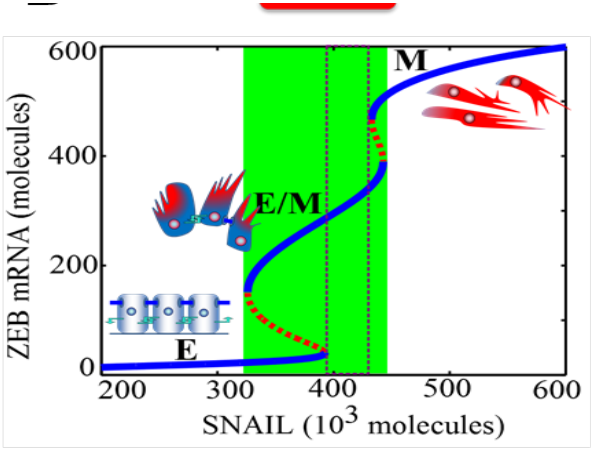
- Three types identified; E, M and E/M
- Correlates with motility
- Can be de-stabilized by knockdown of predicted stability factors

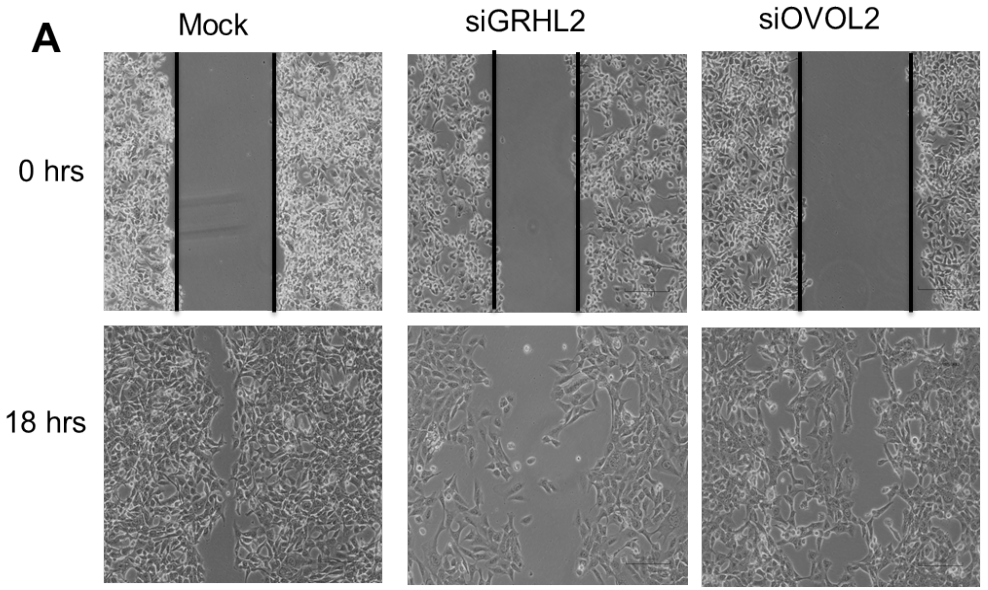
With Hanash group, MD Anderson Cancer Center

Phenotypic stability factors



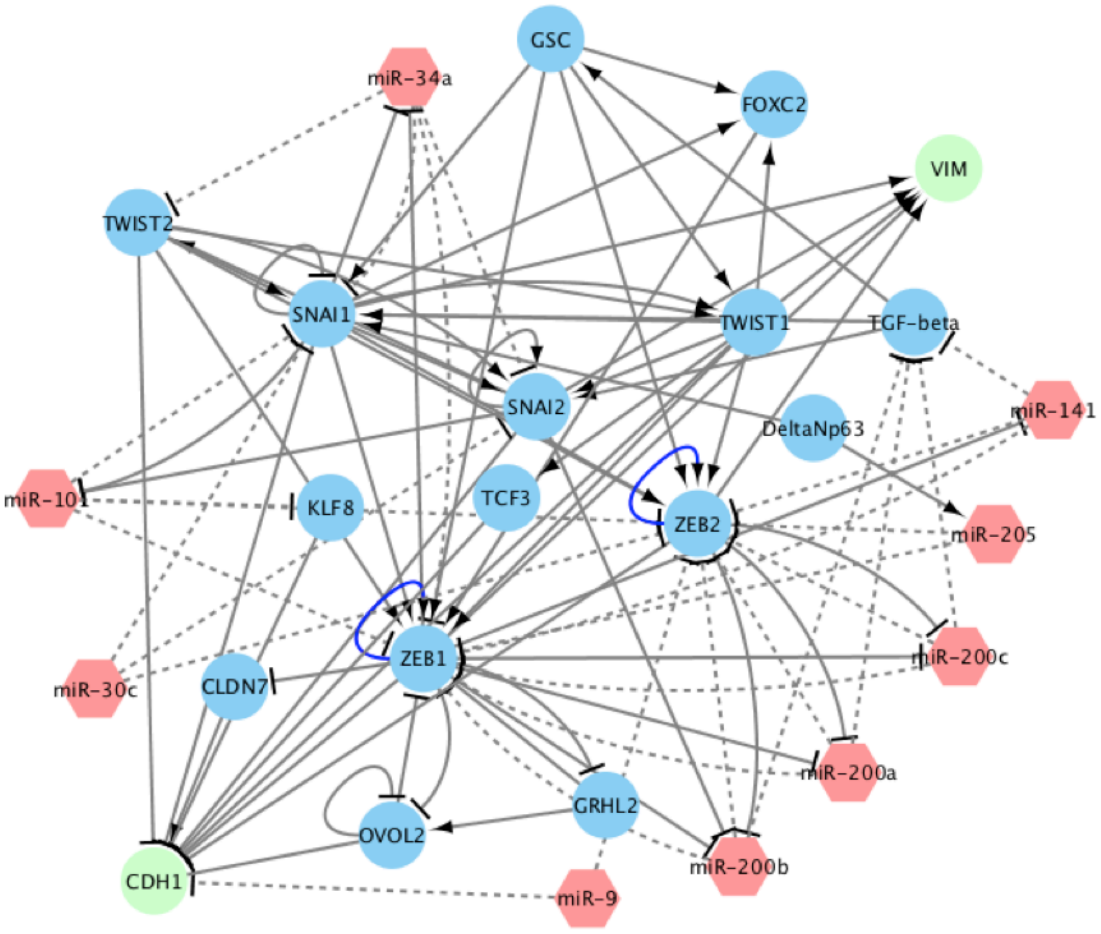
- Form of coupling to baseline circuit can predict effect of specific perturbations
- Key is increased frustration

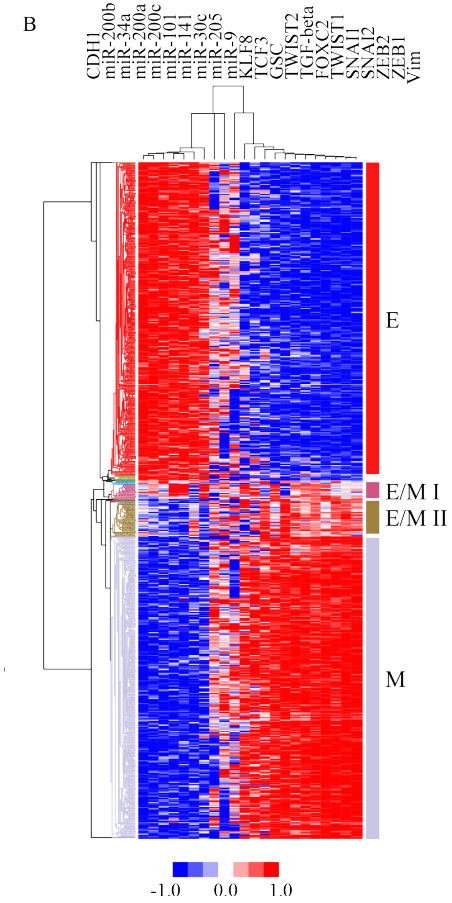




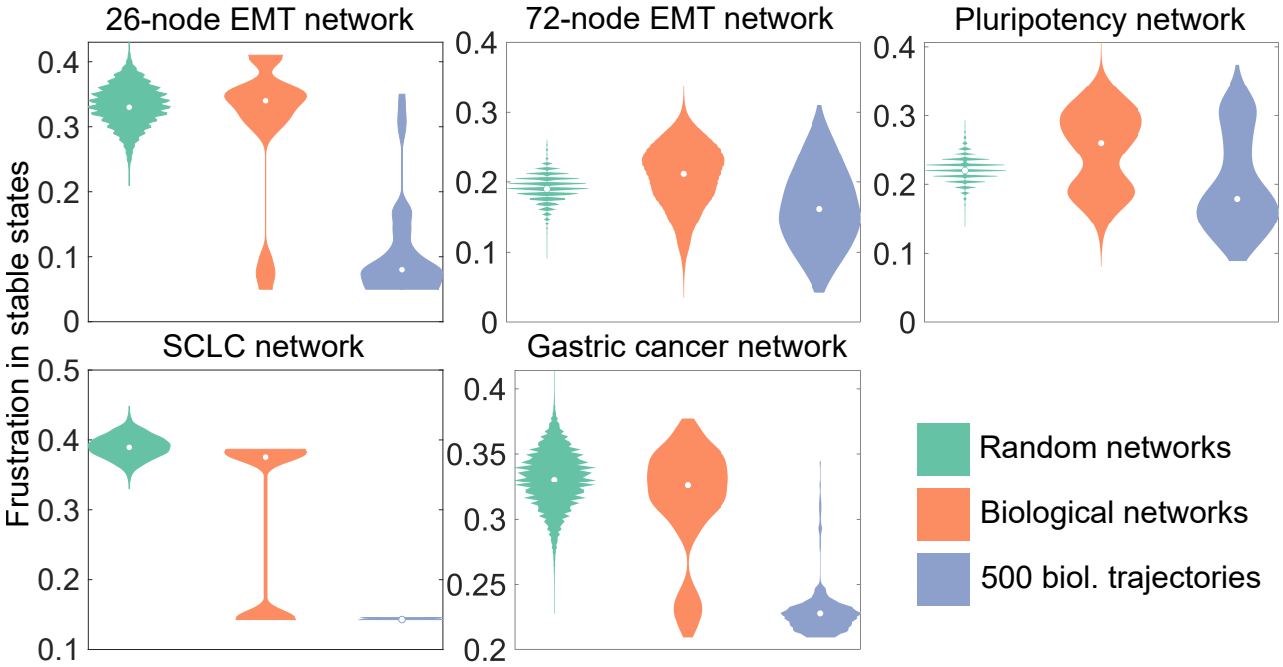
With Hanash, MDACC; Pienta (JHU) – (Oncotarget, 2016)

Results are robust upon going to more complete EMT circuit





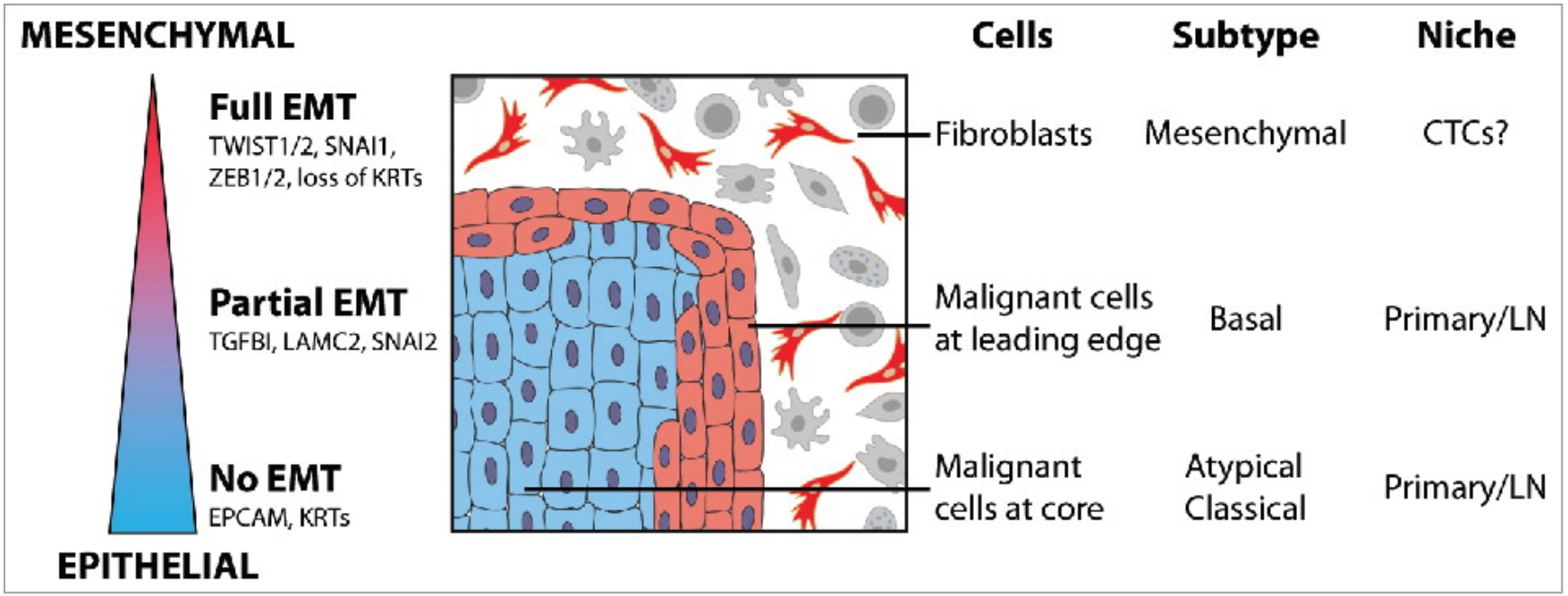
Most recent ideas



- Physiological networks are designed to have small number of ”phenotypes” with large basins of attraction
- Hybrid states specifically require expression of phenotypic stability factors. i.e. added frustration, for stabilization

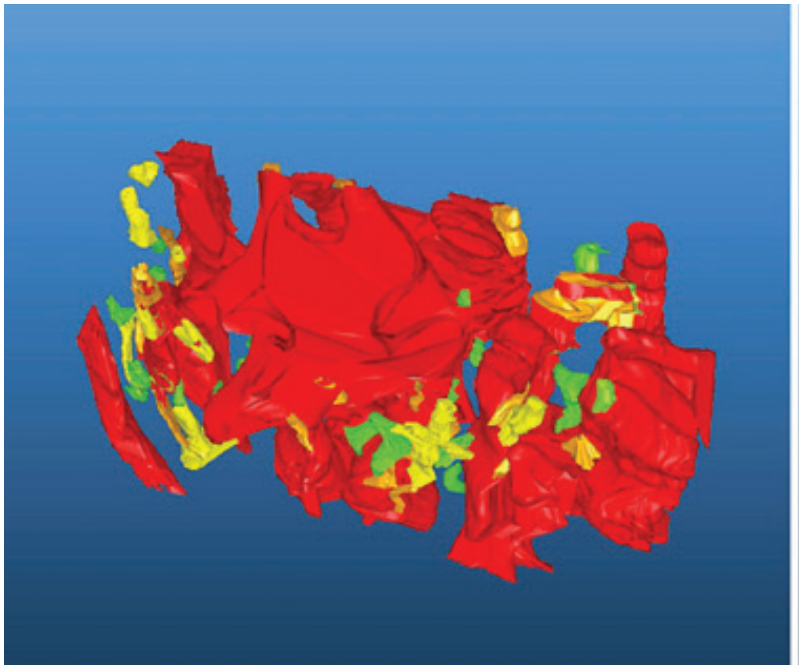
- See Tripathi, Kessler and Levine, PRL (2020)

From sequencing of head and neck tumors classification based on scRna-seq



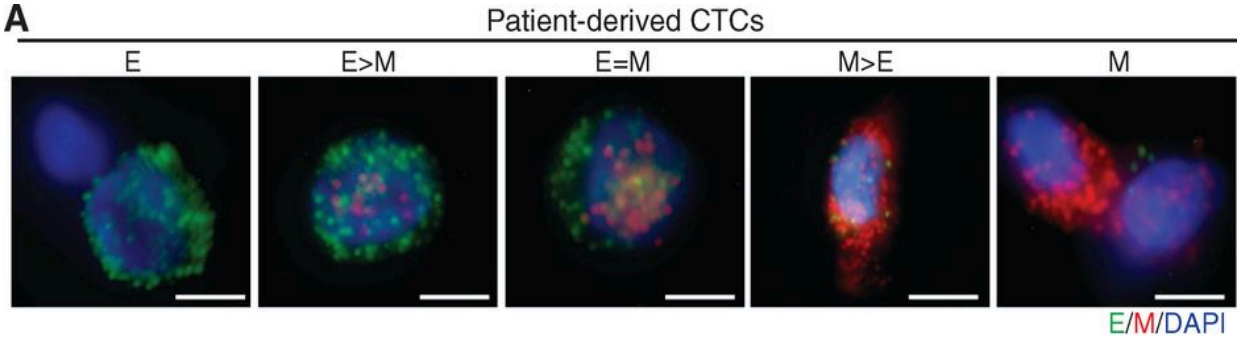
Sidharth V. Puram, Anuraag S. Parikh & Itay Tirosh (2018) Single cell RNA-seq highlights a role for a partial EMT in head and neck cancer, Molecular & Cellular Oncology, based on Cell paper (2017)

Collective motility leads to clusters



- Clusters are typically composed of several cells
- Cells in cluster express ZEB1, reduced membrane resident E-cadherin
- Hypothesized to be partial EMT phenotype

Main tumor = red, multicellular buds = green, from Bronsert et J. Path (2014))



Yu *et al*. Science 2013

At this point in the story …

- Cells can undergo motility transformation at the edge of the tumor
- This can create individually moving cells (full EMT) or collectively moving cells (partial EMT)
- Evidence that partial EMT is common and leads to the formation of clusters of metastasizing cells

Questions to ponder

- What is the physics underlying cell motility as needed for cancer cell dispersal?

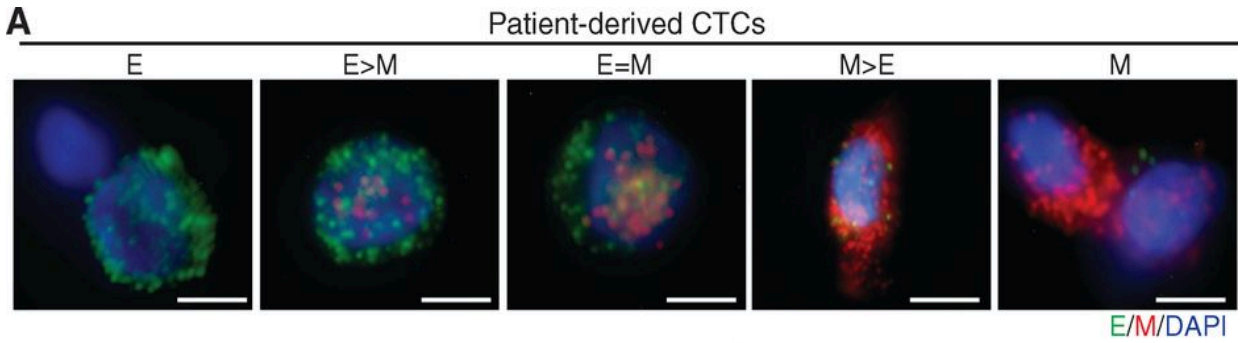
- How do cells change their “phenotype” from one state to another?

- **Can understanding these issues help us better understand and treat cancer?**
  - Key insight relates to “tumor initiation potential”

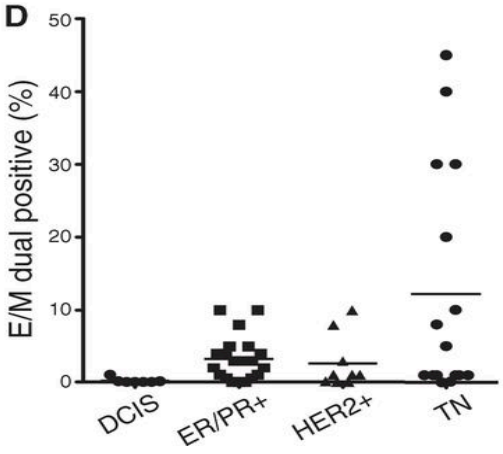
The World of Cancer Research

THE UNIVERSITY OF TEXAS
MD Anderson Cancer Center
Making Cancer History®

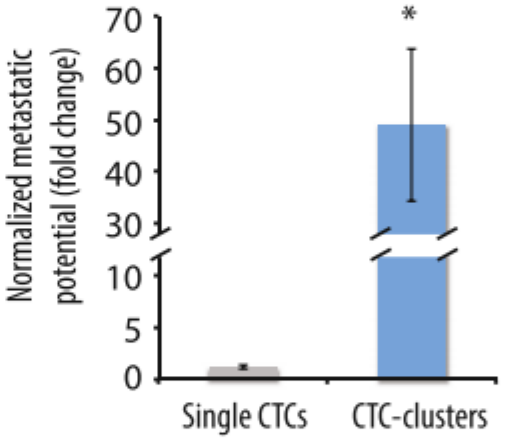
Hybrid Clusters Seem to be More Metastatic

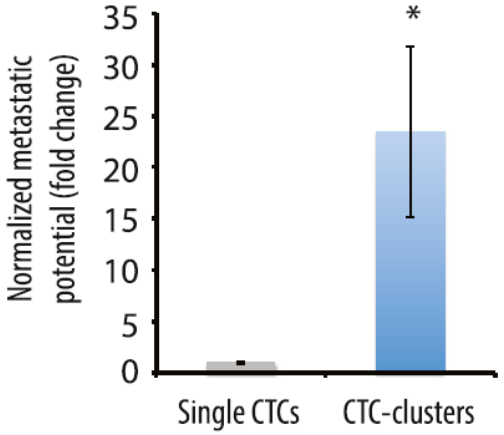


Clusters of CTCs co-express epithelial and mesenchymal features



Yu *et al*. Science 2013





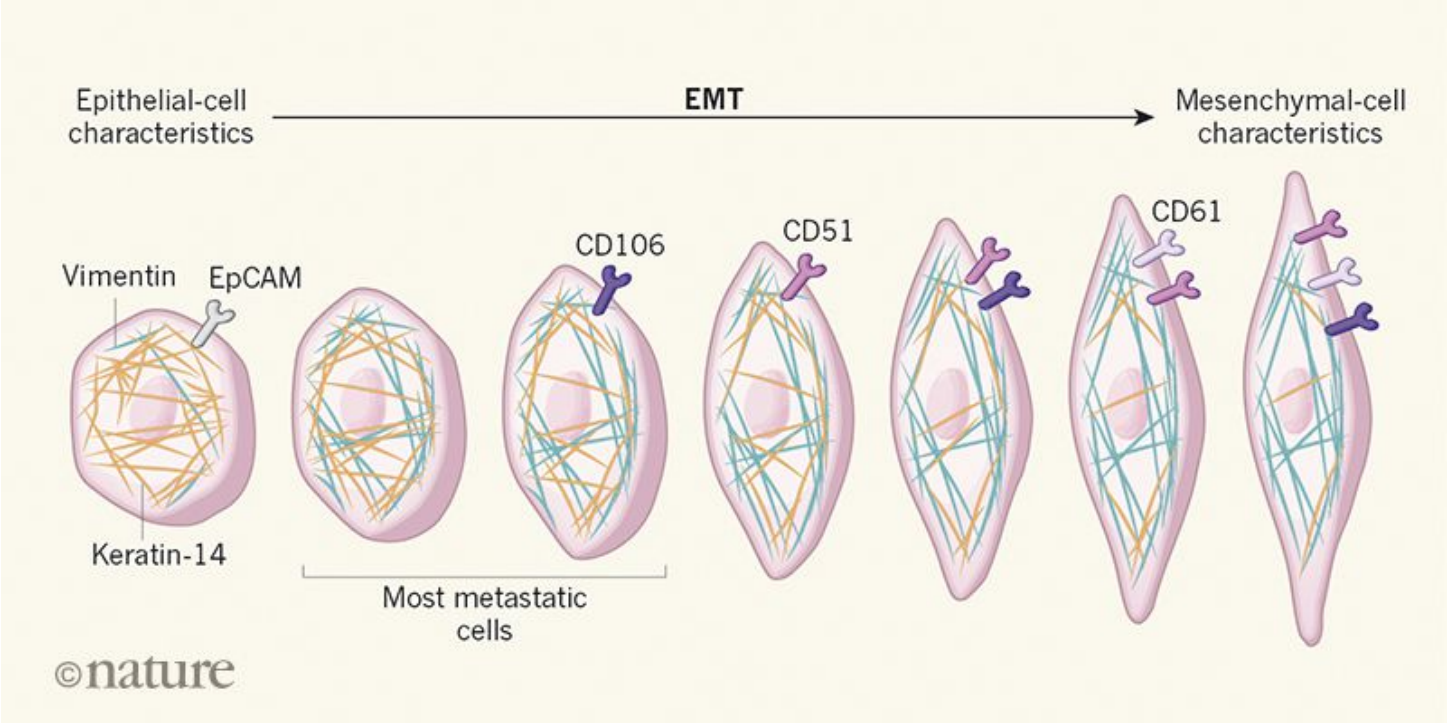
Clusters of CTCs are associated with poor prognosis, have more metastatic potential and are more apoptosis-resistant

Aceto *et al*. Cell 2014
Ewald group, PNAS 2016

More recent evidence

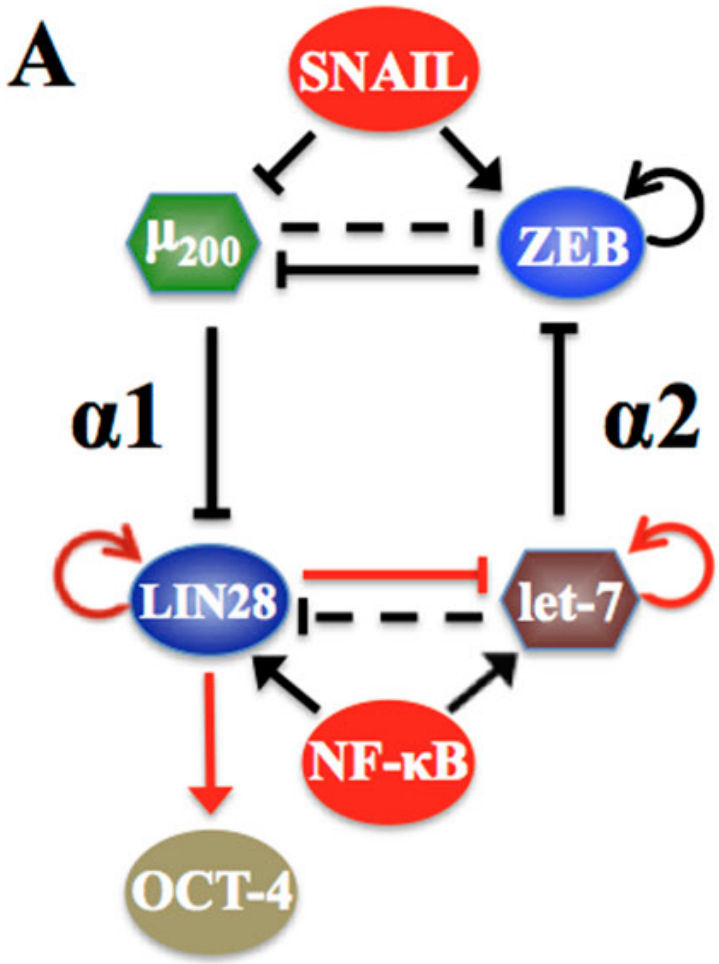
Identification of the tumour transition states occurring during EMT

Pastushenko et al, Nature 556, 463 (2018) – GEM model of SCC



"It was particularly exciting to observe that, in contrast to what one would expect, the tumor cells in the early stage of **EMT with intermediate epithelial and mesenchymal hybrid phenotype,** rather that tumor cells that underwent complete EMT, are the most metastatic populations," comments Ievgenia Pastushenko, the first author of the study.

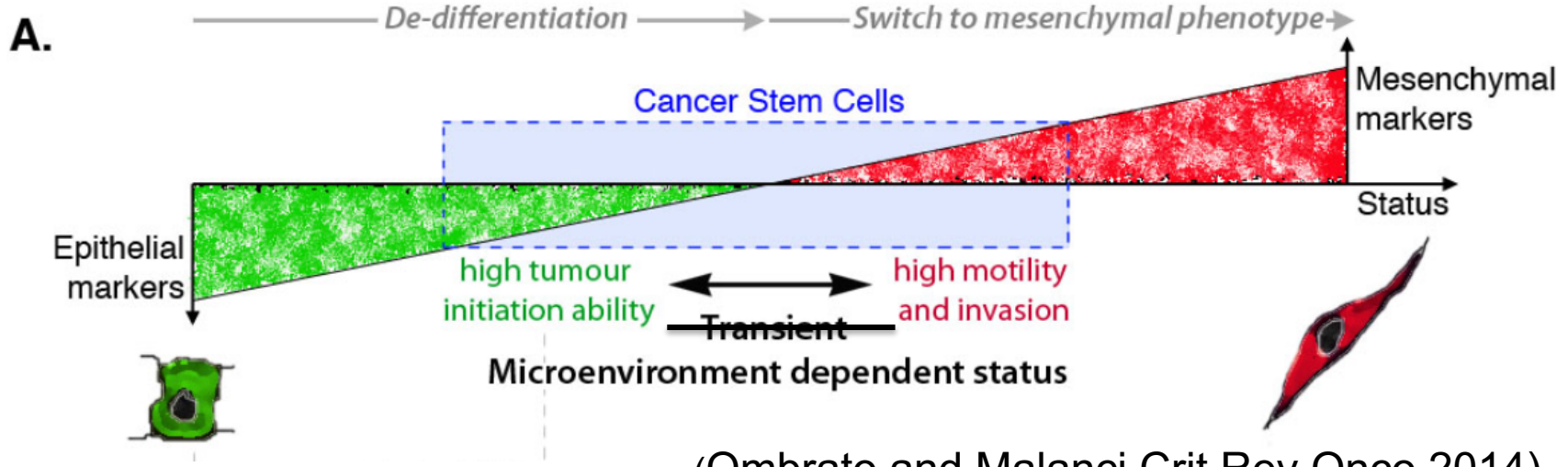
EMT coupling to "stemness"; the breakdown of modularity



- The E/M state can be more likely to become stem-like than either the E or M states
- This is strongly dependent on state of the network
- Can evaluate statistical correlation between different states

Jolly et al, J. Roy Soc Interface (2014), Oncotarget (2015); with Mani grop, MDACC; Pienta group at JHU

Why are hybrid cell clusters more metastaic?

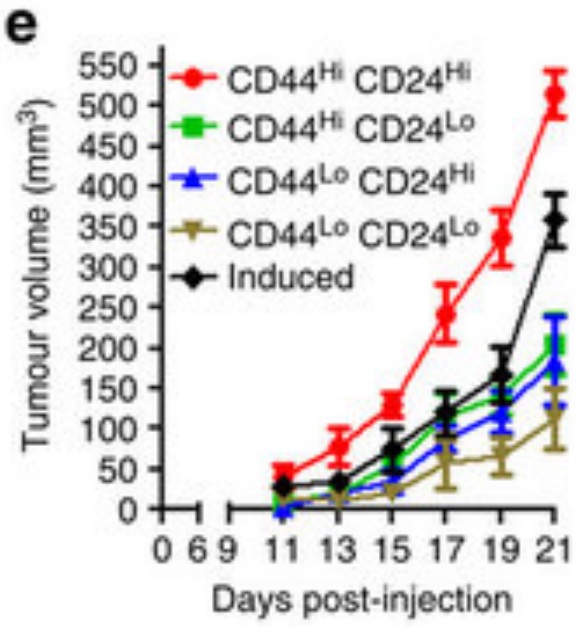


(Ombrato and Malanci Crit Rev Onco 2014)

Coupling the modules of EMT and stemness: A tunable 'stemness window' Jolly et al, Oncotarget (2015); JR Interface (2014)

"….growing evidence that a cell that has only undergone partial EMT is best positioned to acquire stem cell properties."

Pattabiraman and Weinberg 2016



Hybrid cells can initiate more tumors in vitro

Goldman *et al.* Nat Comm 2015

Results keep on coming

Acquisition of a hybrid E/M state is essential for tumorigenicity of basal breast cancer cells

Cornelia Kröger, Alexander Afeyan, … and R. A. Weinberg

PNAS published ahead of print March 25, 2019
https://doi.org/10.1073/pnas.1812876116

Showed that hybrid subpopulation of HMLER cells the most tumorigenic and this cannot be matched by mixing E and M cells; plasticity at the single cell level is absolutely the key.

New paradigm: Treatments must target hybrid, plastic cells in order to prevent metastasis. This is hard because these cells are naturally resistant to many different types of treatment.

The take-home message

- Dynamical network models predict new types of cell phenotypes, hybrid E/M states
- These cells move collectively in vitro and in vivo lead to metastasizing clusters, as seen in pathology images and in the bloodstream
- These clusters can be a major contributor to the growth of new secondary tumors and hence are a priority for proposed treatments

Summary

- "Many worlds" interpretation of how to make progress in this type of problem
  - Soft-matter physics + information processing via networks + cancer biology
- This talk: role of hybrid phenotypes in getting the right combination of properties to effectively metastasize
- Have we made progress?
  - At a scientific level, yes
  - At a clinical level, ???





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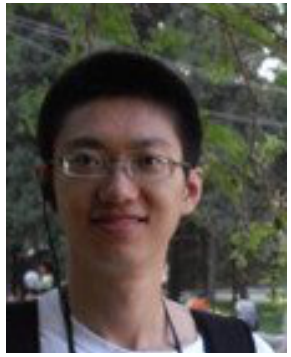

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