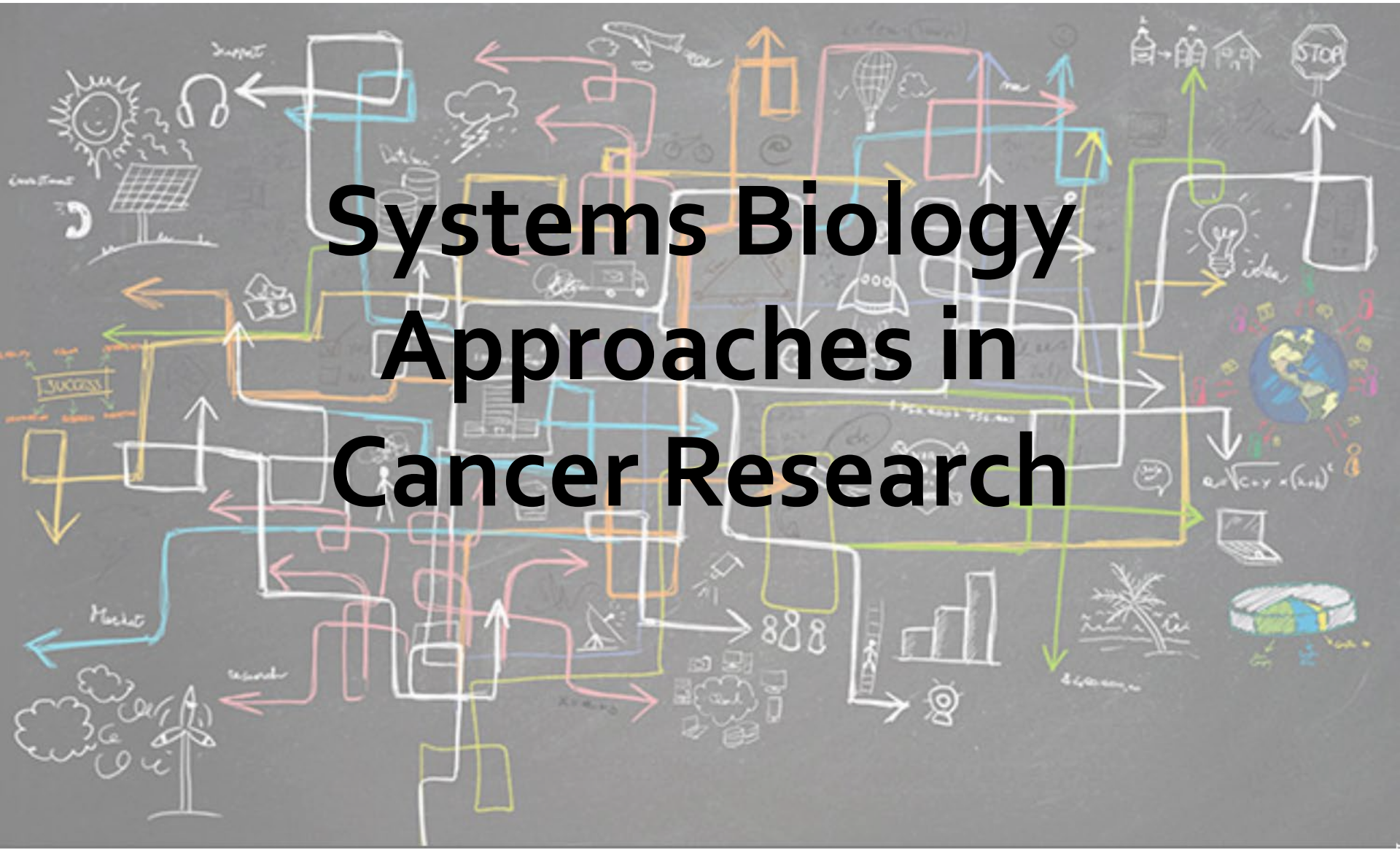
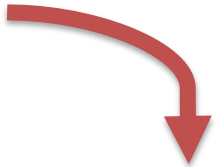

Systems Biology
Approaches in
Cancer Research

- First Principles: Genetics & Reductionism
- Cancer is More than "Just Genetics"
- Examples of the use of Big Data in Systems Biology Approaches

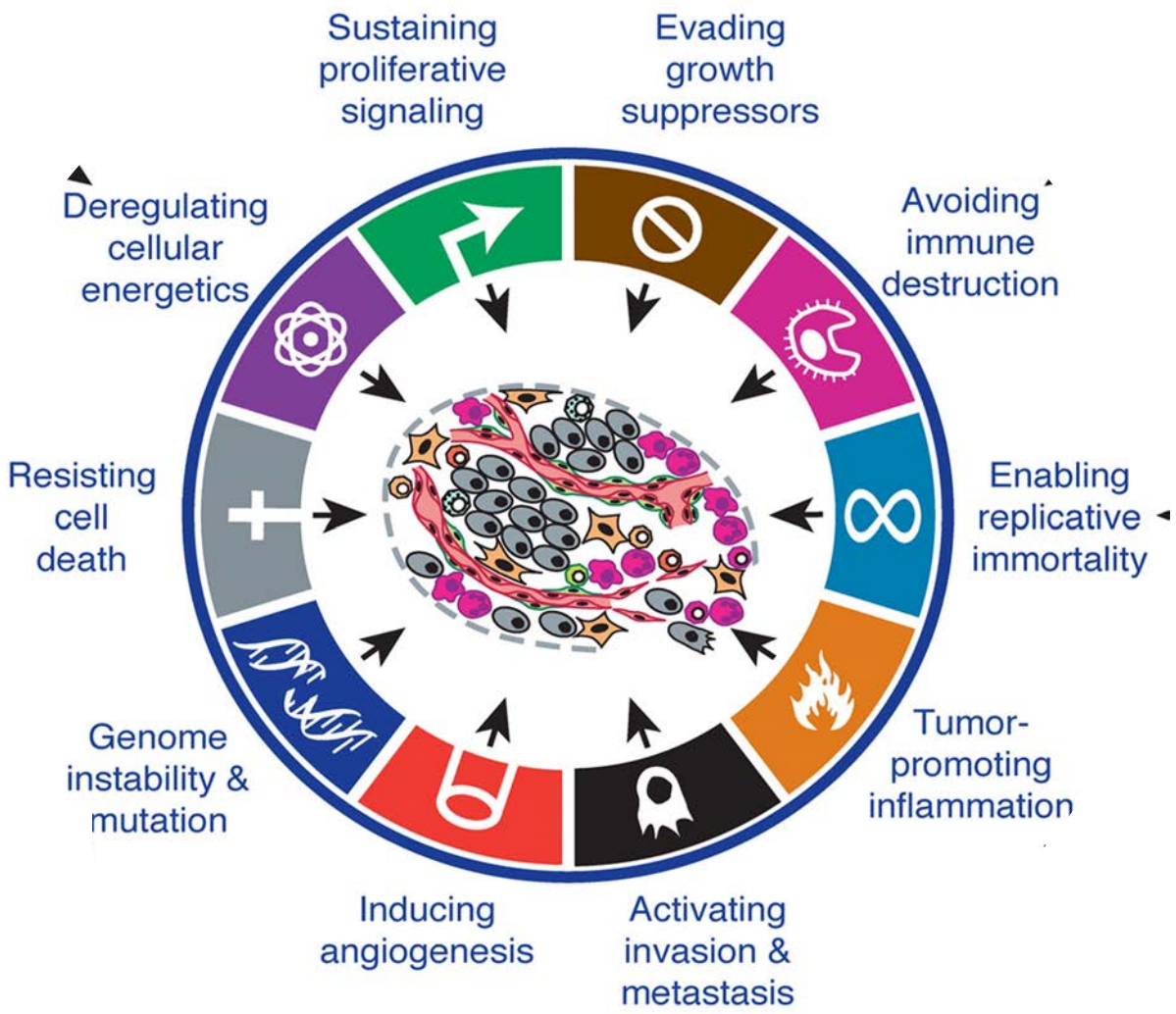
First Principles

- Cancer is a genetic disorder caused by genetic alterations to DNA
- Most mutations are acquired, some are inherited



Accumulation of mutations leads to the hallmarks of cancer
(Hanahan and Weinberg, 2011).

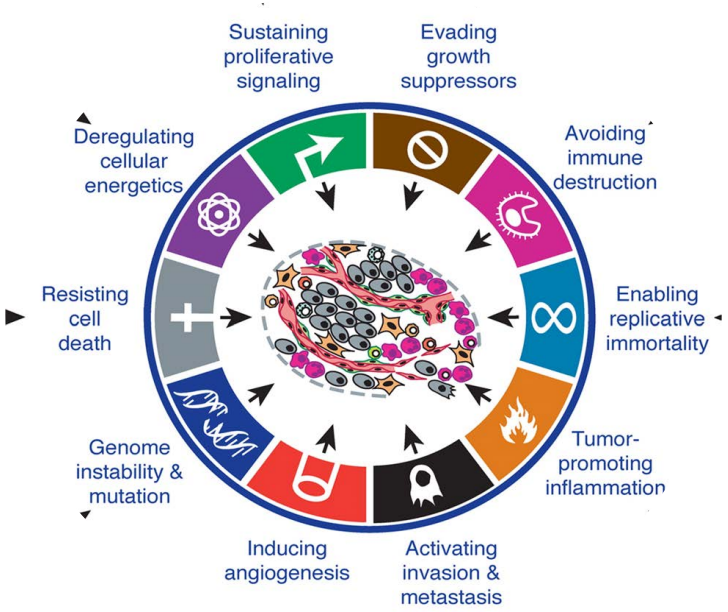
Hallmarks of Cancer



Hanahan & Weinberg. 2011. Cell.

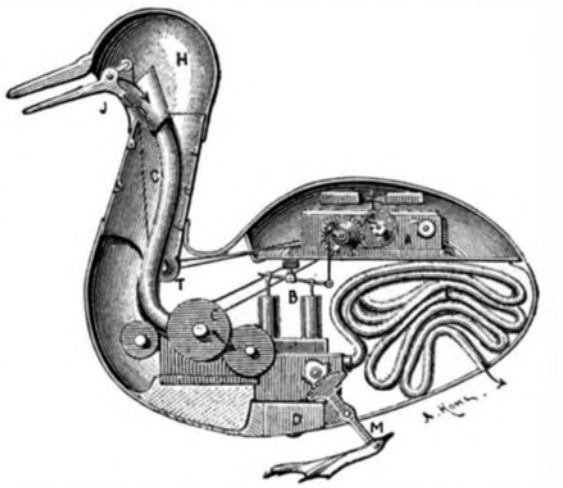
as of July 2024: 76,104 citations

“Traditional View: Cancer is a disease of uncontrolled growth due to genetic mutations”

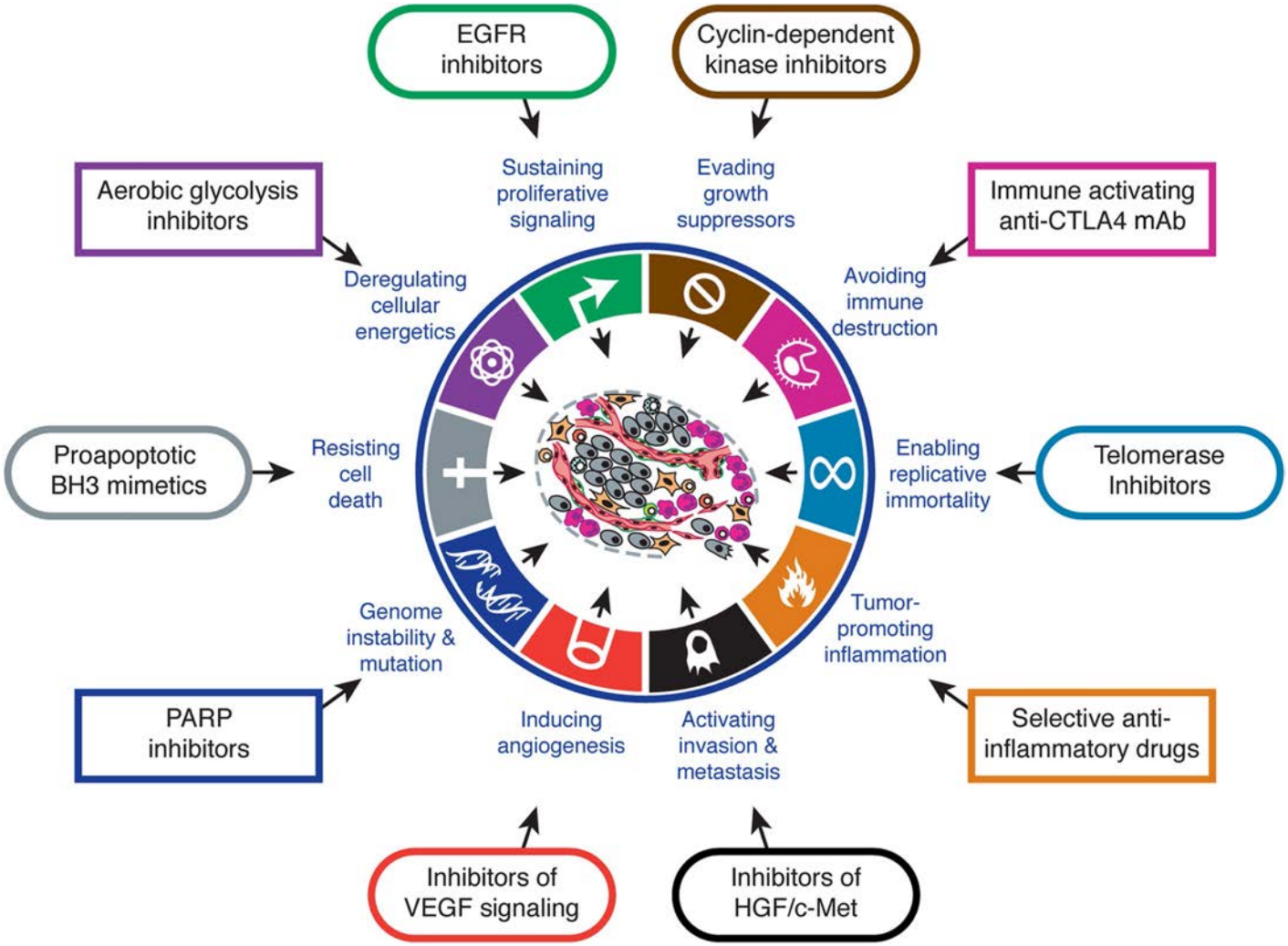


Reductionism: the predominant research paradigm

- Much like a mechanic who repairs a broken car by locating the broken part, a reductionist approach to understanding cancer aims to identify an isolatable abnormality and then develop a treatment to target that abnormality. Implicit within this approach is the motivation and approach that cancers have potential singular target(s).



Targeting Cancer



Hanahan & Weinberg. 2011. Cell.

as of May 2022: 60,017 citations

The Limits of Reductionism

- Low rate of clinical trial success

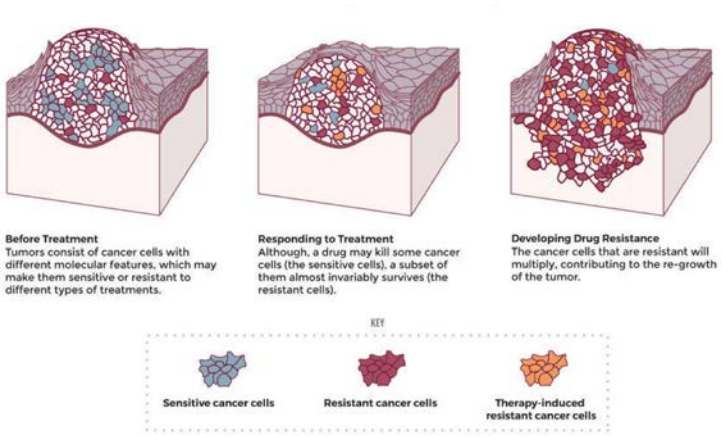
  "Cancer therapeutics currently have the lowest clinical trial success rate of all major diseases.… Partly as a result of the paucity of successful anti-cancer drugs, cancer will soon be the leading cause of mortality in developed countries.

EDITORIAL

Rethinking cancer: current challenges and opportunities in cancer research

Ross Cagan, Pablo Meyer

Disease Models & Mechanisms 2017 10: 349-352; doi: 10.1242/dmm.030007



cancer.gov

- Development of Drug Resistance

  "… nearly all current treatments face the same problem: for many patients, they ultimately stop working. Commonly known as drug resistance, this phenomenon is one of the most challenging problems facing cancer researchers and patients today."

"I think the next frontier in precision genomic medicine is figuring out how to circumvent resistance"
-- Laurie Glimcher, M.D., President, Dana-Farber/Harvard Cancer Center

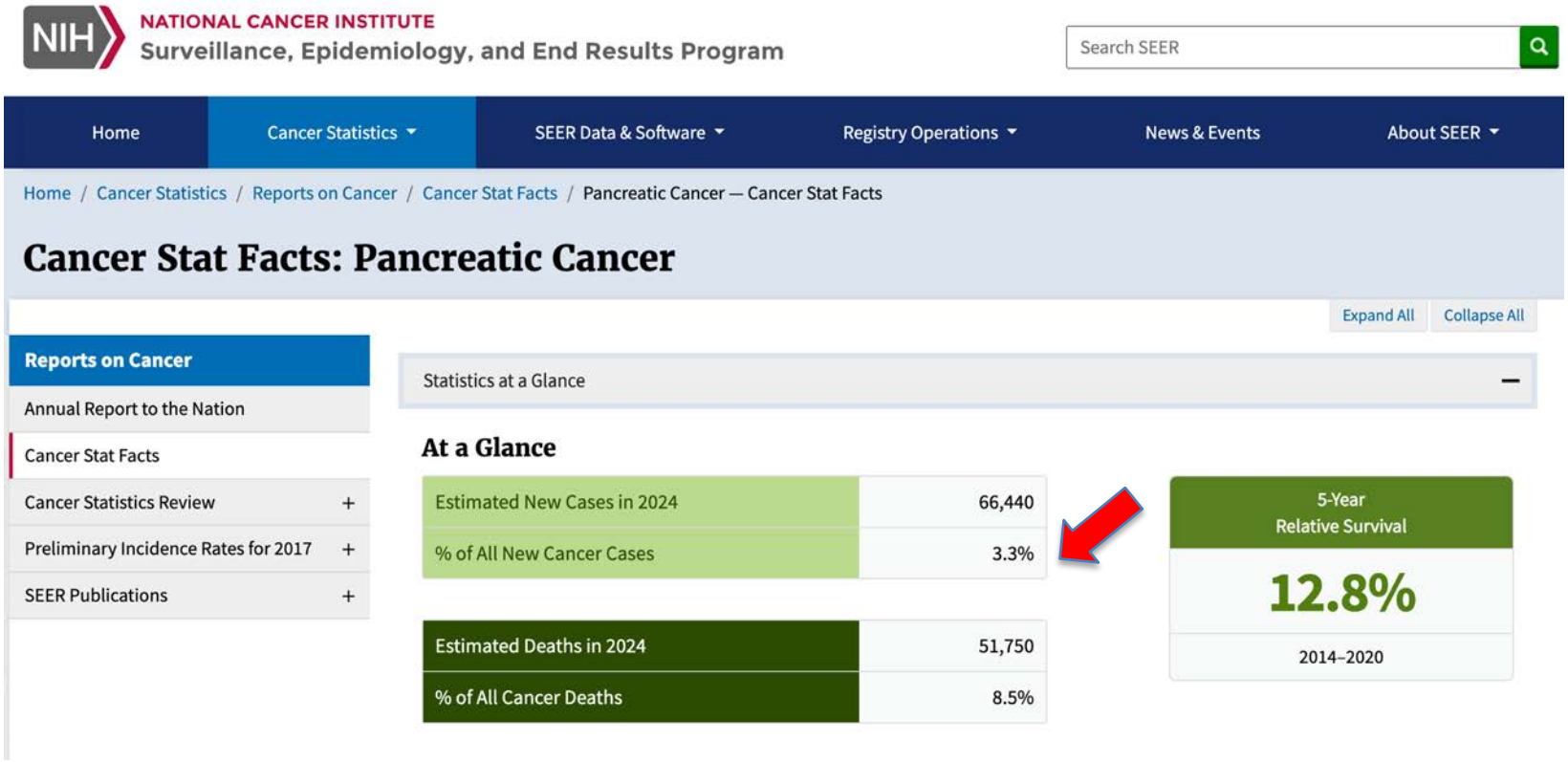
The Limits of Reductionism

Cancer is More than "Just Genetics"

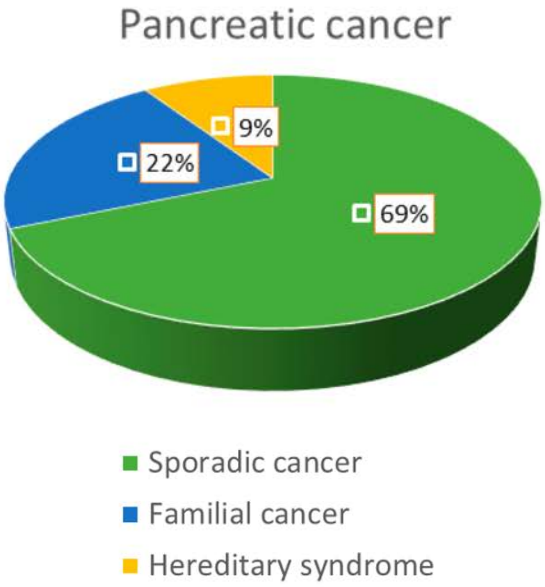
The traditional view of cancer emphasizes a genes-first process for initiation and tumorigenic development.

Recent studies however, point to "System Breakdown"…

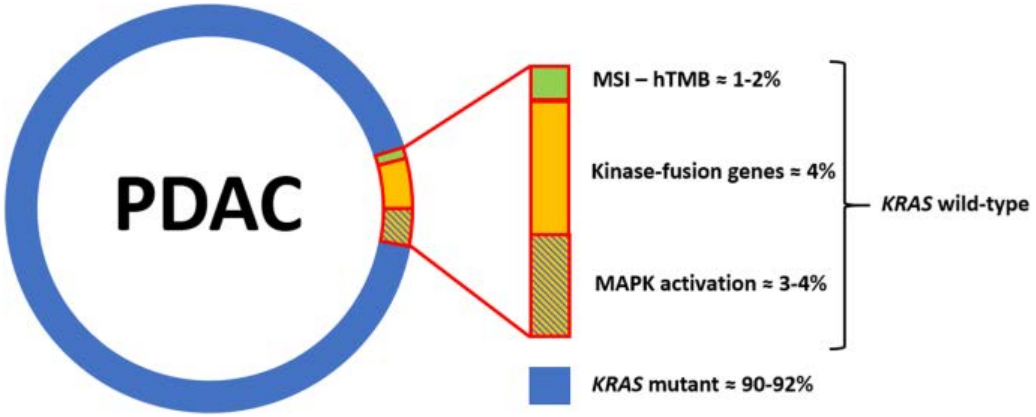
Pancreatic Cancer



Mutations in Kras Drive Most Pancreatic Cancers

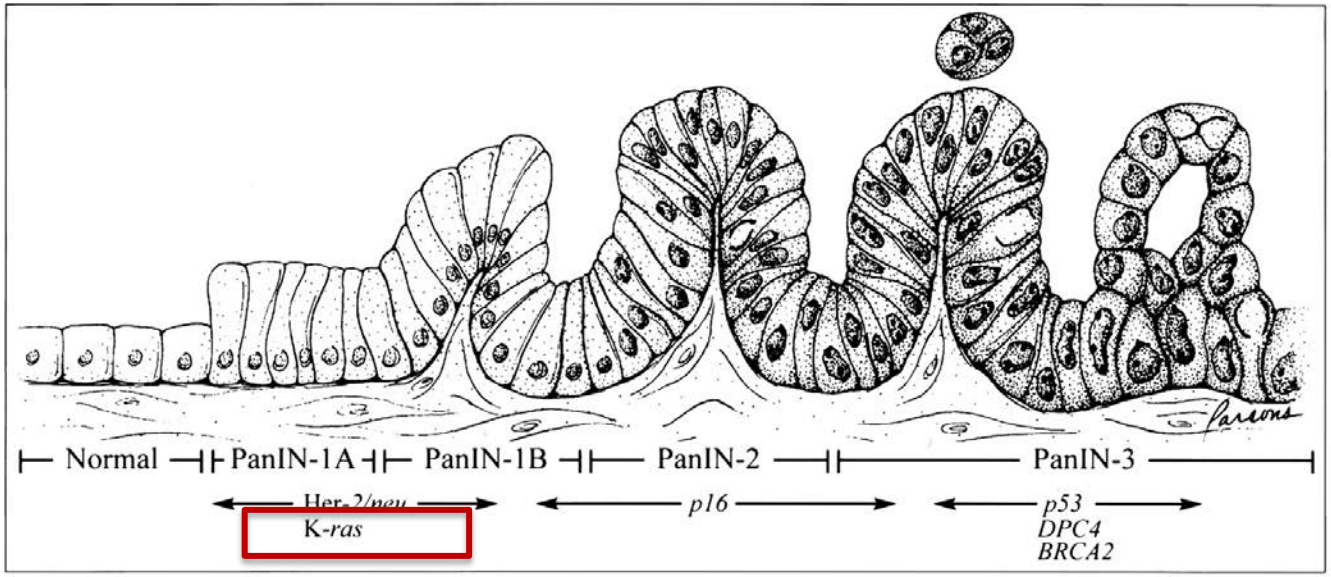


Llach et al, Cancers 2020



Luchini et al., J. Exp Clin Can Res 2020

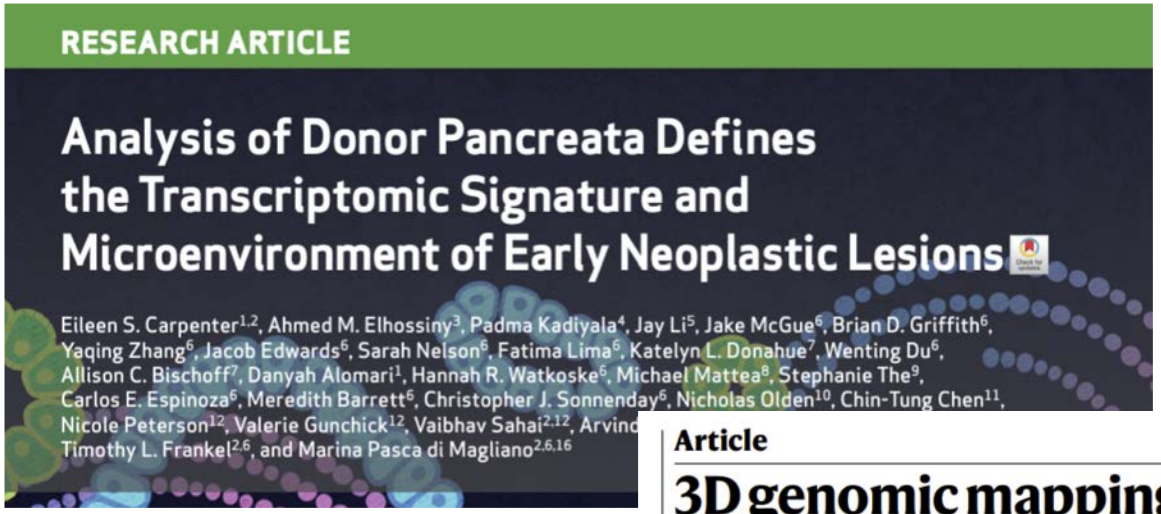
PDAC progression model



Hruban et al., Clinical Cancer Research 2000

"PanIN": pancreatic intraepithelial neoplasia

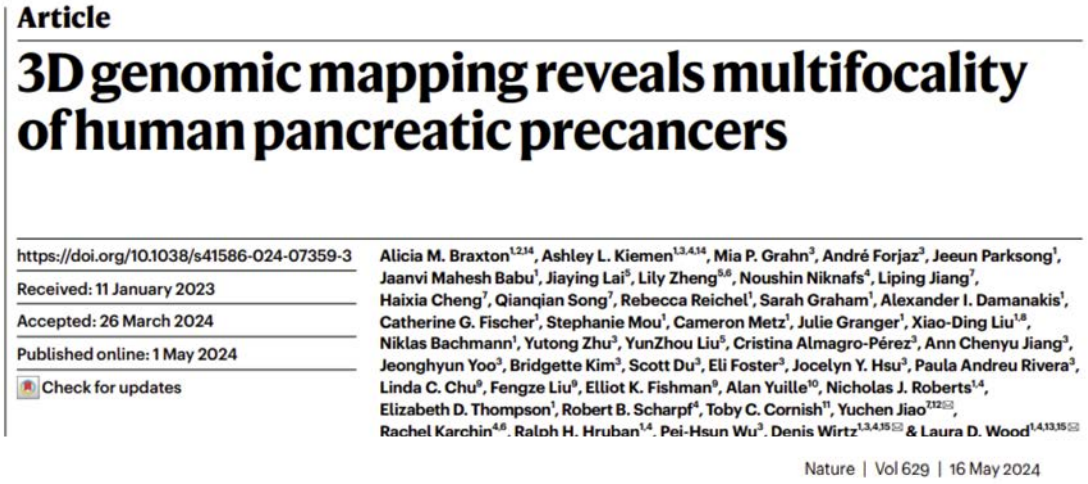
Study of Normal Adult Pancreas Challenges a Long-Held Model



RESEARCH ARTICLE

Analysis of Donor Pancreata Defines the Transcriptomic Signature and Microenvironment of Early Neoplastic Lesions

Eileen S. Carpenter[1,2], Ahmed M. Elhossiny[3], Padma Kadiyala[4], Jay Li[5], Jake McGue[6], Brian D. Griffith[6], Yaqing Zhang[6], Jacob Edwards[6], Sarah Nelson[6], Fatima Lima[6], Katelyn L. Donahue[7], Wenting Du[6], Allison C. Bischoff[7], Danyah Alomari[1], Hannah R. Watkoske[6], Michael Mattea[8], Stephanie The[9], Carlos E. Espinoza[6], Meredith Barrett[6], Christopher J. Sonnenday[6], Nicholas Olden[10], Chin-Tung Chen[11], Nicole Peterson[12], Valerie Gunchick[12], Vaibhav Sahai[2,12], Arvind Timothy L. Frankel[2,6], and Marina Pasca di Magliano[2,6,16]



Article

3D genomic mapping reveals multifocality of human pancreatic precancers

https://doi.org/10.1038/s41586-024-07359-3

Received: 11 January 2023

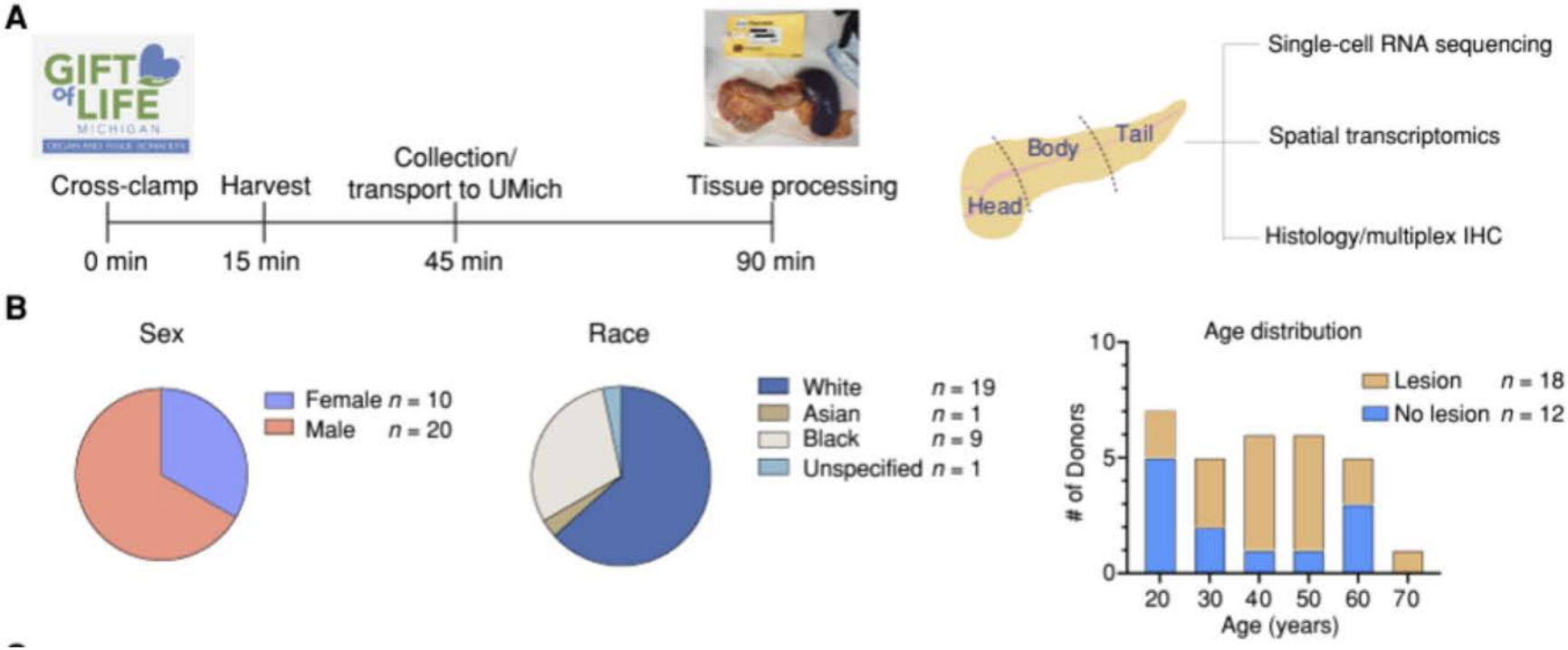
Accepted: 26 March 2024

Published online: 1 May 2024

Check for updates

Alicia M. Braxton[1,2,14], Ashley L. Kiemen[1,3,4,14], Mia P. Grahn[3], André Forjaz[3], Jeeun Parksong[1], Jaanvi Mahesh Babu[1], Jiaying Lai[5], Lily Zheng[5,6], Noushin Niknafs[4], Liping Jiang[7], Haixia Cheng[7], Qianqian Song[7], Rebecca Reichel[1], Sarah Graham[1], Alexander I. Damanakis[1], Catherine G. Fischer[1], Stephanie Mou[1], Cameron Metz[1], Julie Granger[1], Xiao-Ding Liu[1,8], Niklas Bachmann[1], Yutong Zhu[3], YunZhou Liu[5], Cristina Almagro-Pérez[3], Ann Chenyu Jiang[3], Jeonghyun Yoo[3], Bridgette Kim[3], Scott Du[3], Eli Foster[3], Jocelyn Y. Hsu[3], Paula Andreu Rivera[3], Linda C. Chu[9], Fengze Liu[9], Elliot K. Fishman[9], Alan Yuille[10], Nicholas J. Roberts[1,4], Elizabeth D. Thompson[1], Robert B. Scharpf[4], Toby C. Cornish[11], Yuchen Jiao[7,12] ✉, Rachel Karchin[4,6], Ralph H. Hruban[1,4], Pei-Hsun Wu[3], Denis Wirtz[1,3,4,15] ✉ & Laura D. Wood[1,4,13,15] ✉

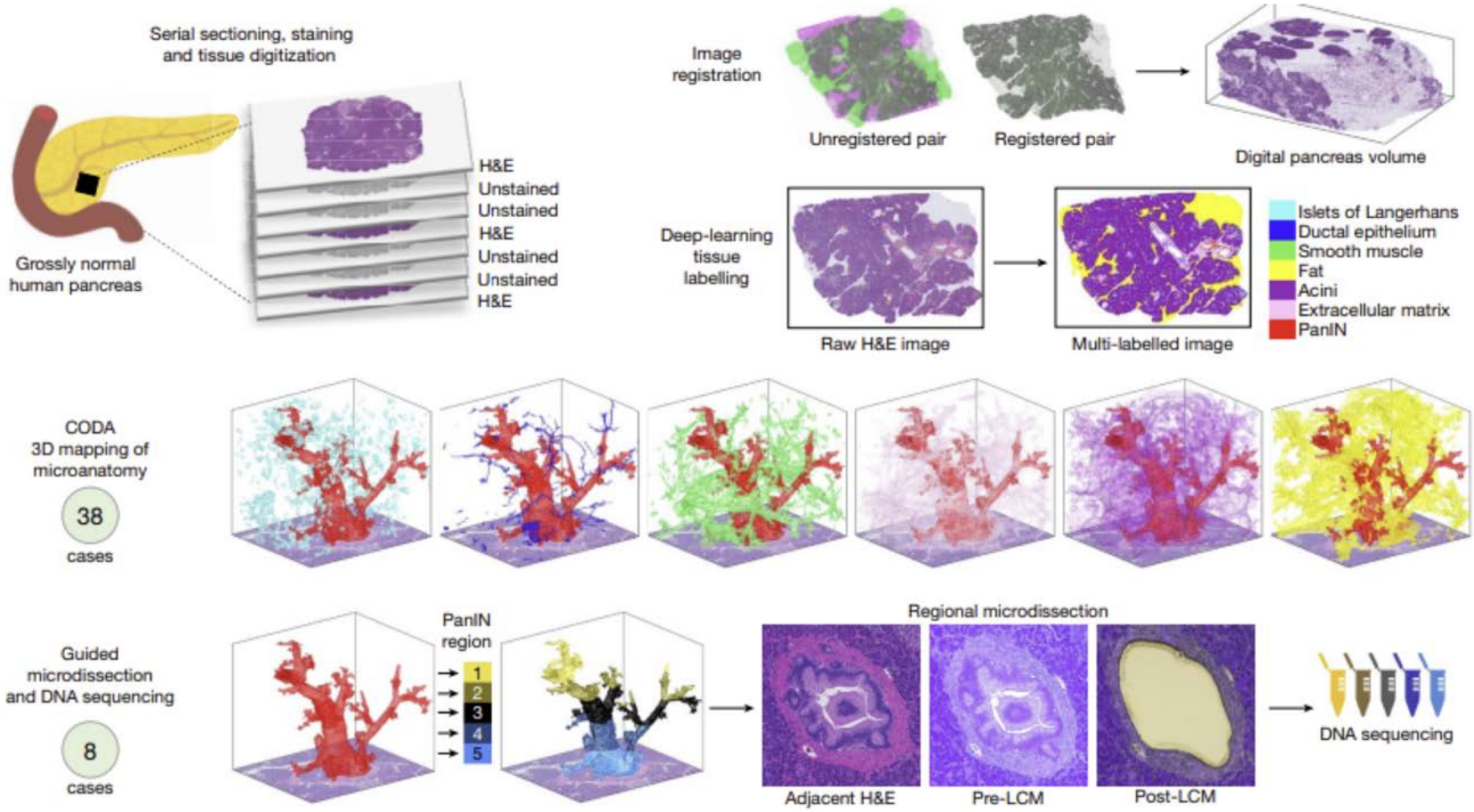
Most of us have PanINs already



Carpenter et al., Cancer Discovery 2023

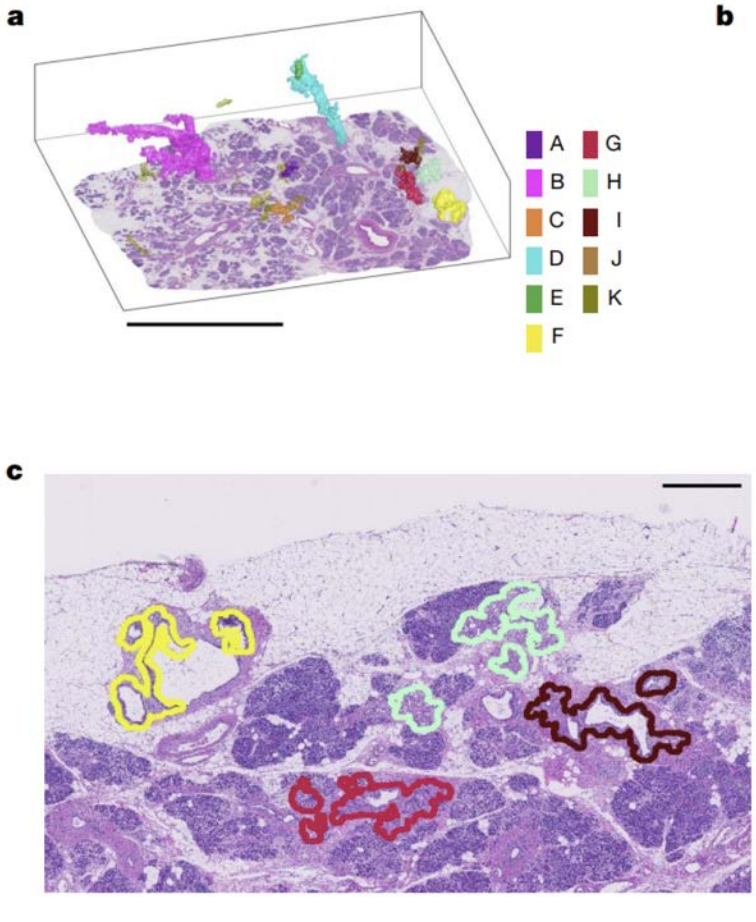
in collaboration w/ Pancreatic Disease Initiative at the University of Michigan and Gift of Life Michigan

3D Image Reconstruction using Machine Learning



Braxton et al., Nature 2024

Multiple Kras Mutations Co-exist within each patient



"…the normal intact adult pancreas harbours hundreds of PanINs, almost all with oncogenic *KRAS* hotspot mutations."

Braxton et al., Nature 2024

Farmer's Eyelid Study

TUMOR EVOLUTION

High burden and pervasive positive selection of somatic mutations in normal human skin

Iñigo Martincorena,[1] Amit Roshan,[2] Moritz Gerstung,[1] Peter Ellis,[1] Peter Van Loo,[1,3,4] Stuart McLaren,[1] David C. Wedge,[1] Anthony Fullam,[1] Ludmil B. Alexandrov,[1] Jose M. Tubio,[1] Lucy Stebbings,[1] Andrew Menzies,[1] Sara Widaa,[1] Michael R. Stratton,[1] Philip H. Jones,[2*] Peter J. Campbell[1,5*]

Bill Hinton Photography / Getty Images

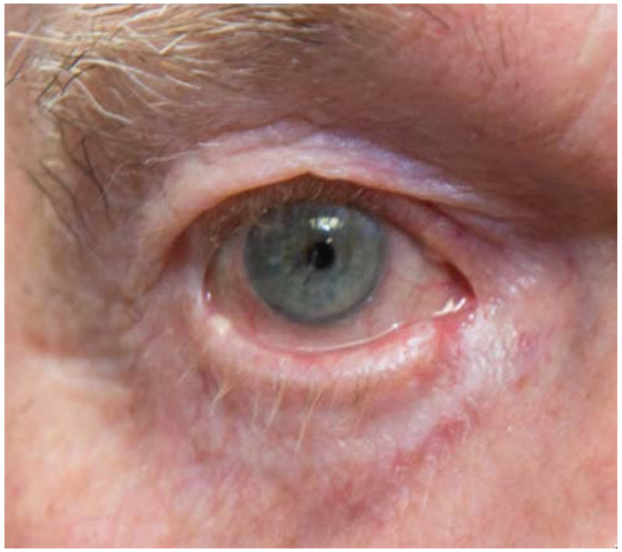
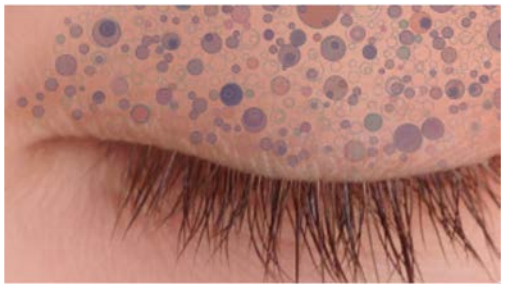
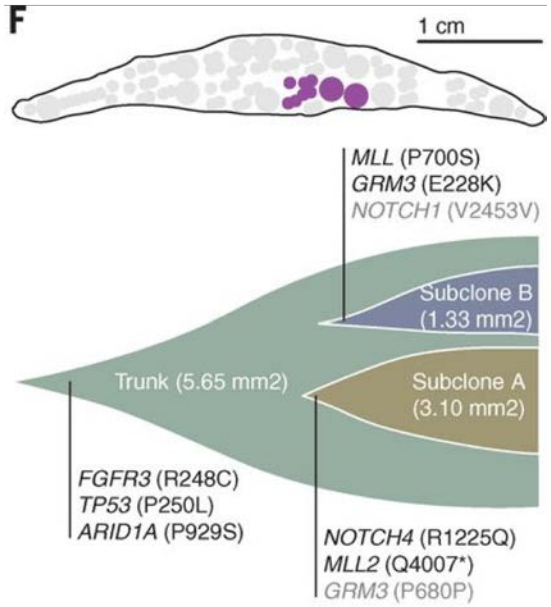
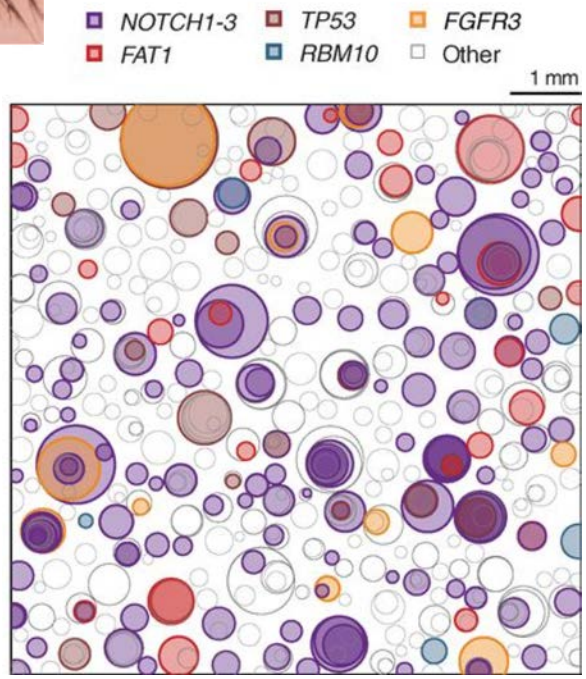


Fig. 4 Mutant clone sizes and clonal dynamics in normal skin







"Positive selection on driver mutations is strong only during the initial expansion of mutant clones"

Genetic Mutations are Not Sufficient for Cancer

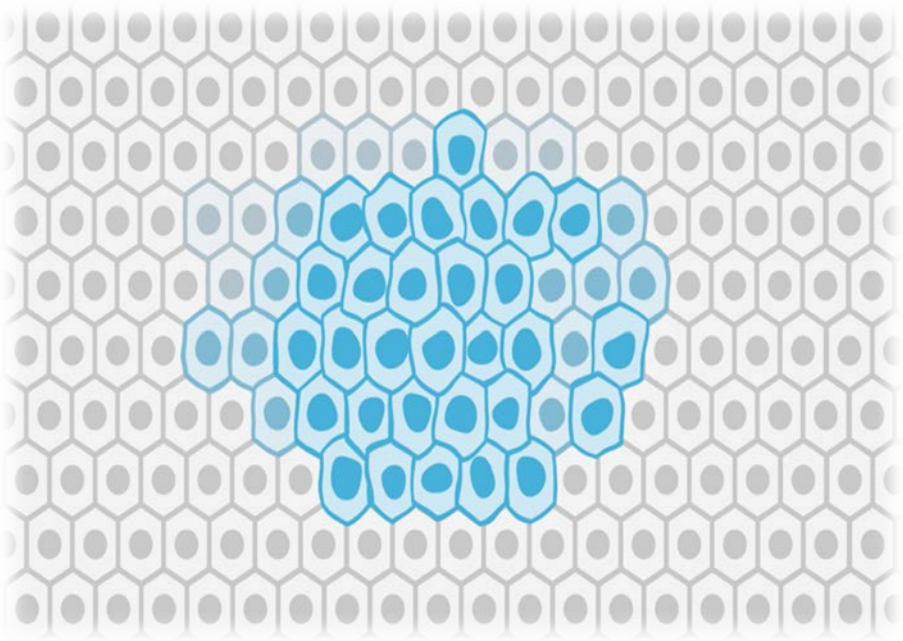
Benign conditions with oncogenic driver mutations

From: The paradox of cancer genes in non-malignant conditions: implications for precision medicine

Gene	Type of alteration	Benign or premalignant condition	Frequency of alteration in benign condition (%)	Examples of drug(s) that can potentially target the alteration	Examples of malignancies associated with this gene alteration	Mechanism
BRAF	V600E, D594V, V599E	Melanocytic nevi	70–88% [3,4,5,6,7,8,9,10,11,12]	BRAF and/or MEK inhibitors such as dabrafenib and trametanib [13, 14]	Melanoma	RAS-RAF-MEK-ERK pathway upregulation [15]
NRAS	Q61K	Giant congenital melanocytic nevi	6–14% [10, 11]	MEK inhibitors [12] such as trametinib [16]	Melanoma	RAS-RAF-MEK-ERK pathway upregulation [15]
	Q61K and Q61R	Melanocytic nevi	70–95% [17, 18]	MEK inhibitors such as trametinib [16]	Melanoma	RAS-RAF-MEK-ERK pathway upregulation [15]
FGFR3	R248C, S249C, G372C, S373C, A393E, K652E, K652M	Seborrheic keratosis	~18–85% [19,20,21,22]	FGFR inhibitors such as erdafitinib [23]	Urothelial carcinoma	Activation of the FGF/FGFR machinery [24]
	R248C, G372C, G382R	Epidermal nevi	33% [25]	FGFR inhibitors such as erdafitinib [23]	Urothelial carcinoma	Activation of the FGF/FGFR machinery [24]
PIK3CA	E542K, E545K, H1047R	Seborrheic keratosis	~16% [20]	PIK3CA inhibitors such as alpelisib [26]	Breast cancer	PI3K-AKT-mTOR pathway activation
	M1043V	Endometriosis	~4% [27]	PIK3CA inhibitors such as alpelisib [26]	Breast cancer	PI3K-AKT-mTOR pathway activation
	H1047L, H1047R	Normal esophagus mucosa	Not listed [28]	PIK3CA inhibitors such as alpelisib [26]	Breast cancer	PI3K-AKT-mTOR pathway activation
ALK	TPM3-ALK, TPM4-ALK	Inflammatory myofibroblastic tumor	~50% [29]	ALK inhibitors [30] such as alectinib [31]	Non-small cell lung cancer	ALK pathway activation [32]
NOTCH1	Loci not specified	Aging esophagus	12–80% [33]	No specific inhibitors approved	Colon cancer	Wnt-beta-catenin pathway activation [34]
KRAS	G12V or G12D	Arteriovenous malformations in brain	~63% [35, 36]	MEK inhibitors such as trametinib [16]	Colorectal and pancreatic cancer	RAS-RAF-MEK-ERK pathway upregulation [15]
	G12C, G12V, G12A, G12D, G12R	Endometriosis	~21% [27]	MEK inhibitors such as trametinib [16]	Colorectal and pancreatic cancer	RAS-RAF-MEK-ERK pathway upregulation [15]
	Q61R	Normal testis	Not listed [28]	MEK inhibitors such as trametinib [16]	Colorectal and pancreatic cancer	RAS-RAF-MEK-ERK pathway upregulation [15]
TP53	R177S, Q192L, R196*, K139R, H193Y, E224fs, N239S	Rheumatoid arthritis synovium	17–46% [37, 38]	Bevacizumab may target angiogenesis upregulation that results from *TP53* mutations [39]	Serous ovarian cancer (*TP53* mutations are common across cancers)	*TP53* is a tumor suppressor gene [40]
	Loci not specified	Aging esophagus	2–37% [33]	Bevacizumab may target angiogenesis upregulation that results from *TP53* mutations [39]	Serous ovarian cancer (*TP53* mutations are common across cancers)	*TP53* is a tumor suppressor gene [40]
CTNNB1	T41A and S45P	Desmoid tumor	88% [41]	COX-2 inhibitors [42] such as celecoxib [43], as well as sorafenib (which can suppress CTNNB1-mediated activation of the WNT pathway) [13, 14, 44]	Adrenocortical cancers	Wnt-beta-catenin pathway activation [45]
FGFR2	Y376C, P286S	Keratinocytic epidermal nevus	5–10% [46]	FGFR inhibitors such as erdafitinib [23]	Urothelial carcinoma	FGF/FGFR machinery [24]
AKT, *MAPK*, and *AMPK* pathway genes	–	Alzheimer's disease	~27% [47]	mTOR inhibitors or MEK inhibitors	Multiple tumor types	Increases tau phosphorylation

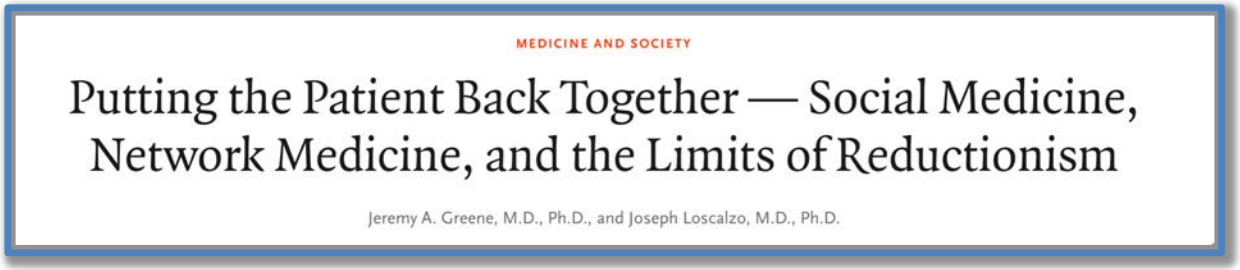
Adashek, J.J. et al. 2020. Genome Medicine

Cancer is not a disease of uncontrolled growth, but of *improperly*-controlled growth



A need for Systems Biology Approaches

December 21, 2017
N Engl J Med 2017; 377:2493-2499
DOI: 10.1056/NEJMms1706744



MEDICINE AND SOCIETY

Putting the Patient Back Together — Social Medicine, Network Medicine, and the Limits of Reductionism

Jeremy A. Greene, M.D., Ph.D., and Joseph Loscalzo, M.D., Ph.D.

*"**One disappointment of the postgenomic age is how little the Human Genome Project has taught us to date about human disease.** Only a small minority of diseases are caused by monogenic (or oligogenic) disorders. Instead, complex interactions among numerous genetic and environmental factors determine disease phenotype."*

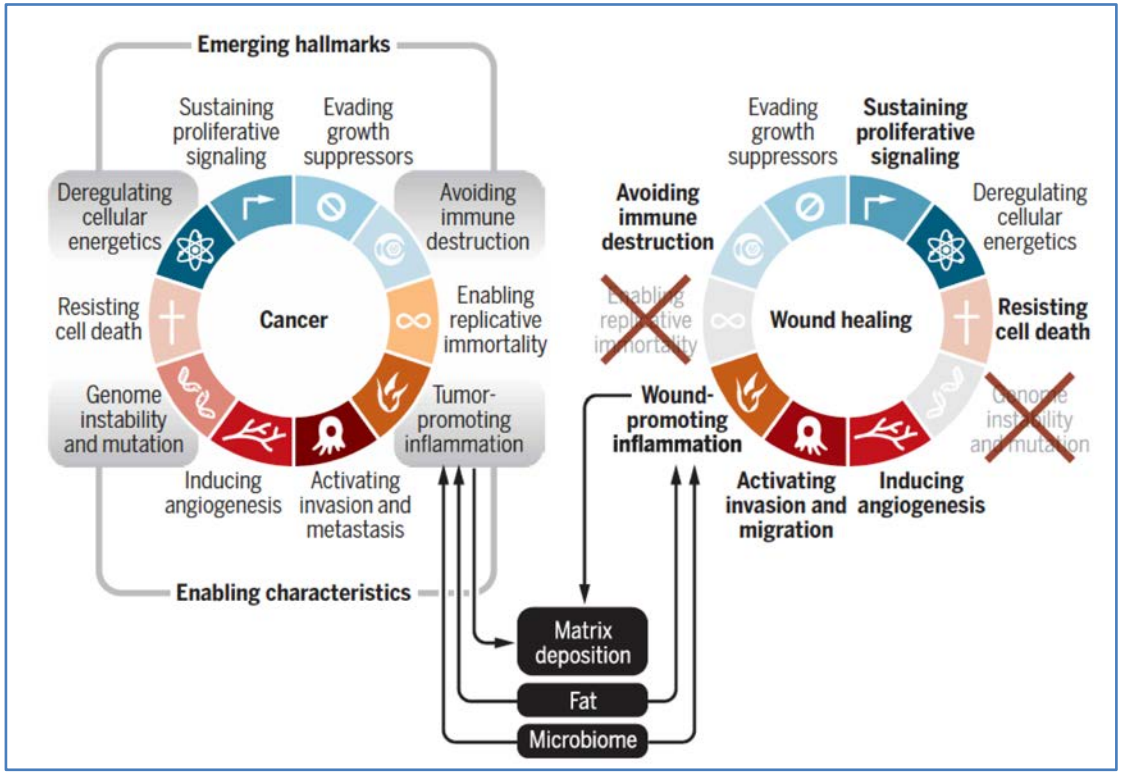
EDITORIAL

Rethinking cancer: current challenges and opportunities in cancer research

Ross Cagan, Pablo Meyer

Disease Models & Mechanisms 2017 10: 349-352; doi: 10.1242/dmm.030007

"…cancer is not just a disease of mutated genes but of dysfunctional pathways that no longer limit growth"



MacCarthy-Morrogh and Martin, *Sci. Signal.* **13**, eaay8690 (2020) 8 September 2020

Guiding Principles for Cancer Systems Biology …

Tumors inherit control strategies present in their tissue of origin

Both normal and tumor cells participate in communities

Dividing line between normal and cancer cells is not sharp

To escape control, tumor cells must work within the system

Cancer Biology Needs…

Technologies that embrace complexity (i.e. **Big Data** Generators)

Technologies that preserve spatial structures and relationships

Approaches that provide quantitative information

Modeling approaches & analysis pipelines

e.g. Models that predict emergent properties of a system

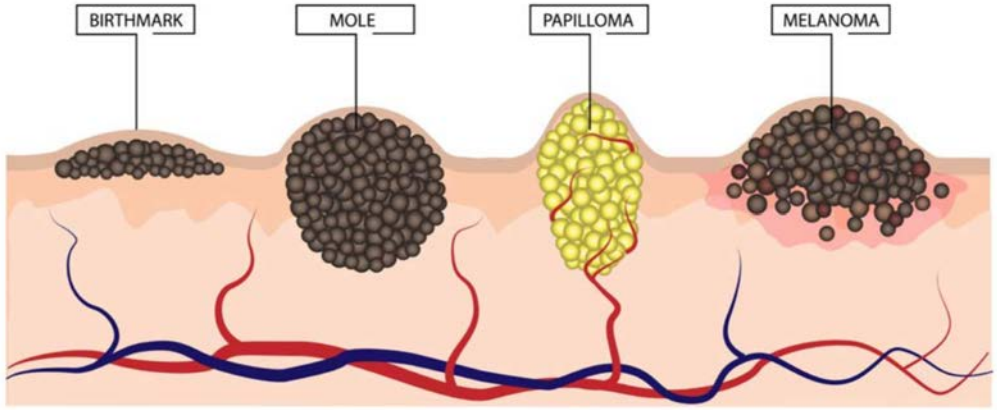
Nevi/Melanoma

Research Article
Cancer Biology, Computational and Systems Biology

Dynamics of nevus development implicate cell cooperation in the growth arrest of transformed melanocytes

Rolando Ruiz-Vega, Chi-Fen Chen, Emaad Razzak, Priya Vasudeva, Tatiana B Krasieva, Jessica Shiu, Michael G Caldwell, Huaming Yan, John Lowengrub, Anand K Ganesan, Arthur D Lander « see less

Nevi/Melanoma

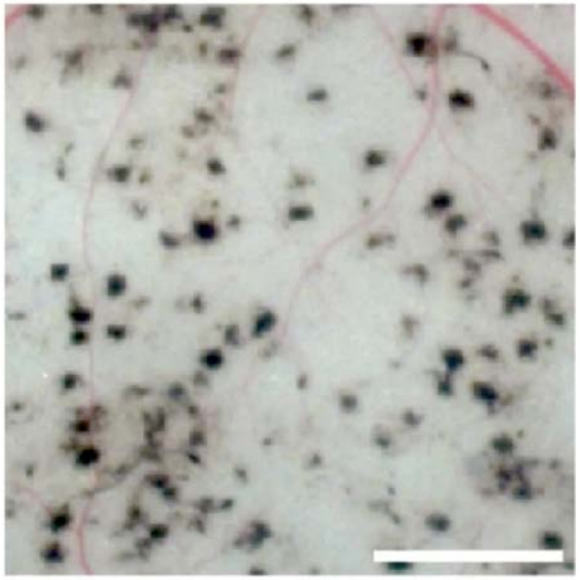


70%-90% of all nevi have $Braf^{mut}$ melanocytes
~50% of melanomas have $Braf^{mut}$

The lifetime risk for a mole in a 20 year old developing into melanoma by age 80 years is approximately 0.03% for men and 0.009% for women.

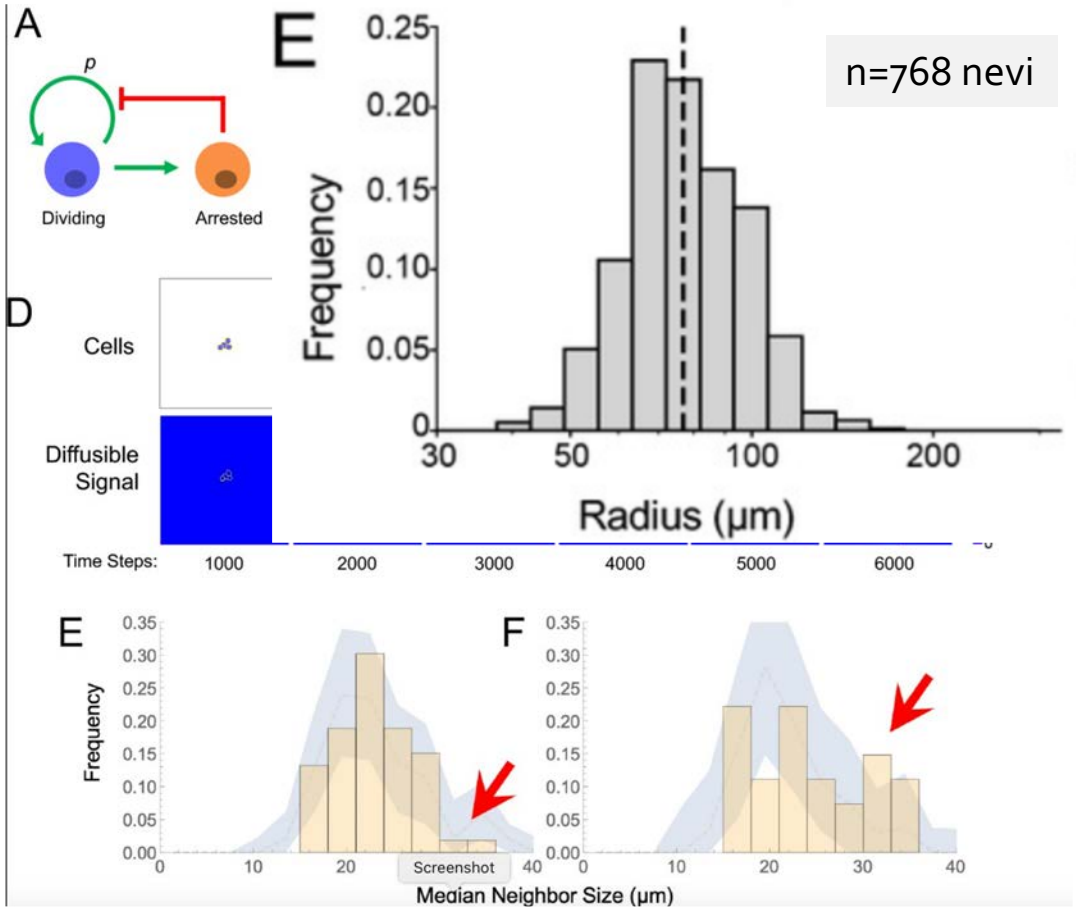
Current Model (Cell Autonomous):

- nevi melanocytes are senescent
- senescence is triggered by oncogenic mutation of Braf, ie. Oncogene Induced Senescence (OIS)



Does a collective process arrest nevi?

scRNAseq: ***no*** *OIS signatures are evident*

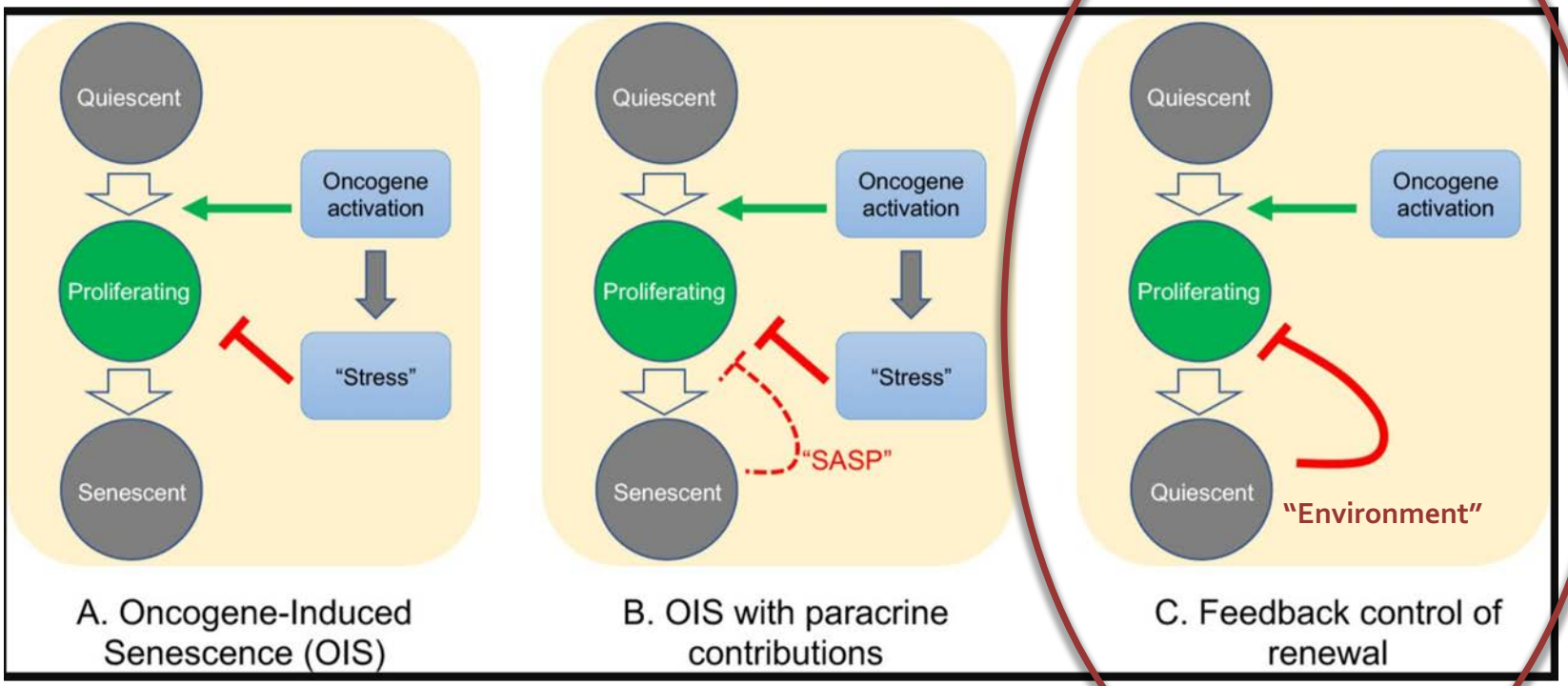


Mathematical simulation:
nevi growth and arrest is non-cell autonomous

"large nevi neighbors"

"small nevi neighbors"

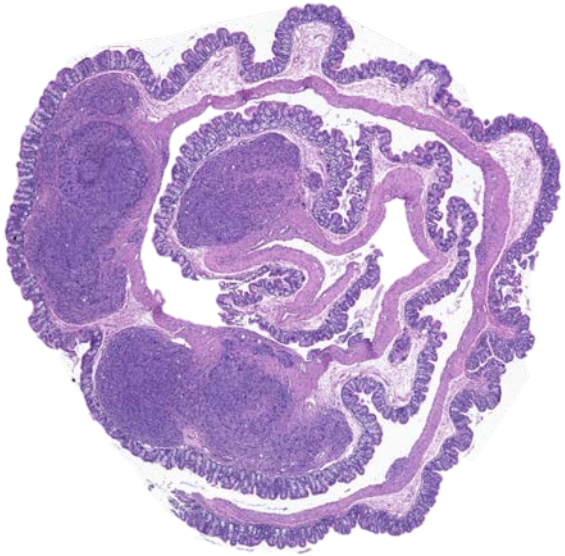
A Cell non-Autonomous Model for Nevi/Melanoma



Uncovering Minimal Pathways in Melanoma Initiation

Hui Xiao[1], Jessica Shiu[2], Chi-Fen Chen[2], Jie Wu[3], Peijie Zhou[4], Sahil S. Telang[2], Rolando Ruiz-Vega[1], Qing Nie[4, 5], Arthur D. Lander[1, 5], Anand K. Ganesan[2]

Colon Cancer Stem Cells: Patterned Heterogeneity



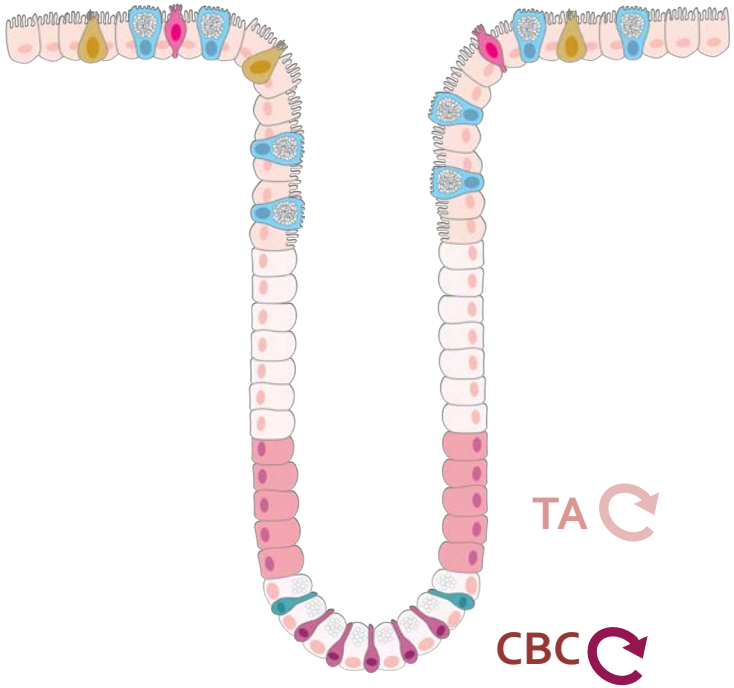
Linzi Hosohama

George Chen

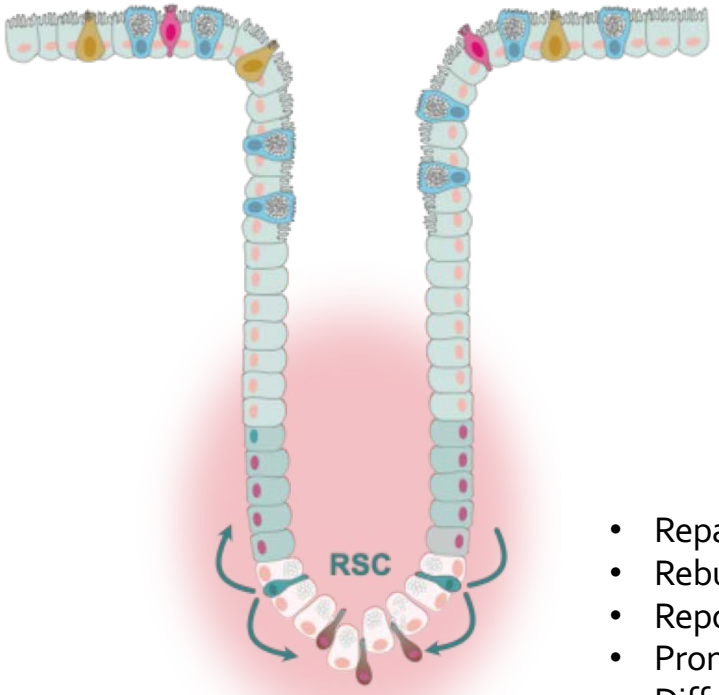
Mary Lee

John Lowengrub

Stem Cells in the Intestinal Crypt



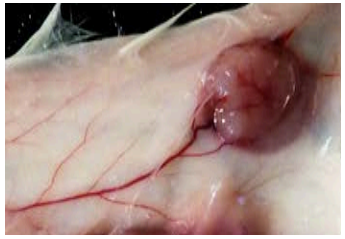
Crypt Base Columnar Stem Cells: ***LGR5+, AXIN2+, MYB+*** …



- Repair
- Rebuild
- Repopulate
- Promote Differentiation

A Turing Pattern of Wnt and Metabolism?

Anti-beta-catenin staining

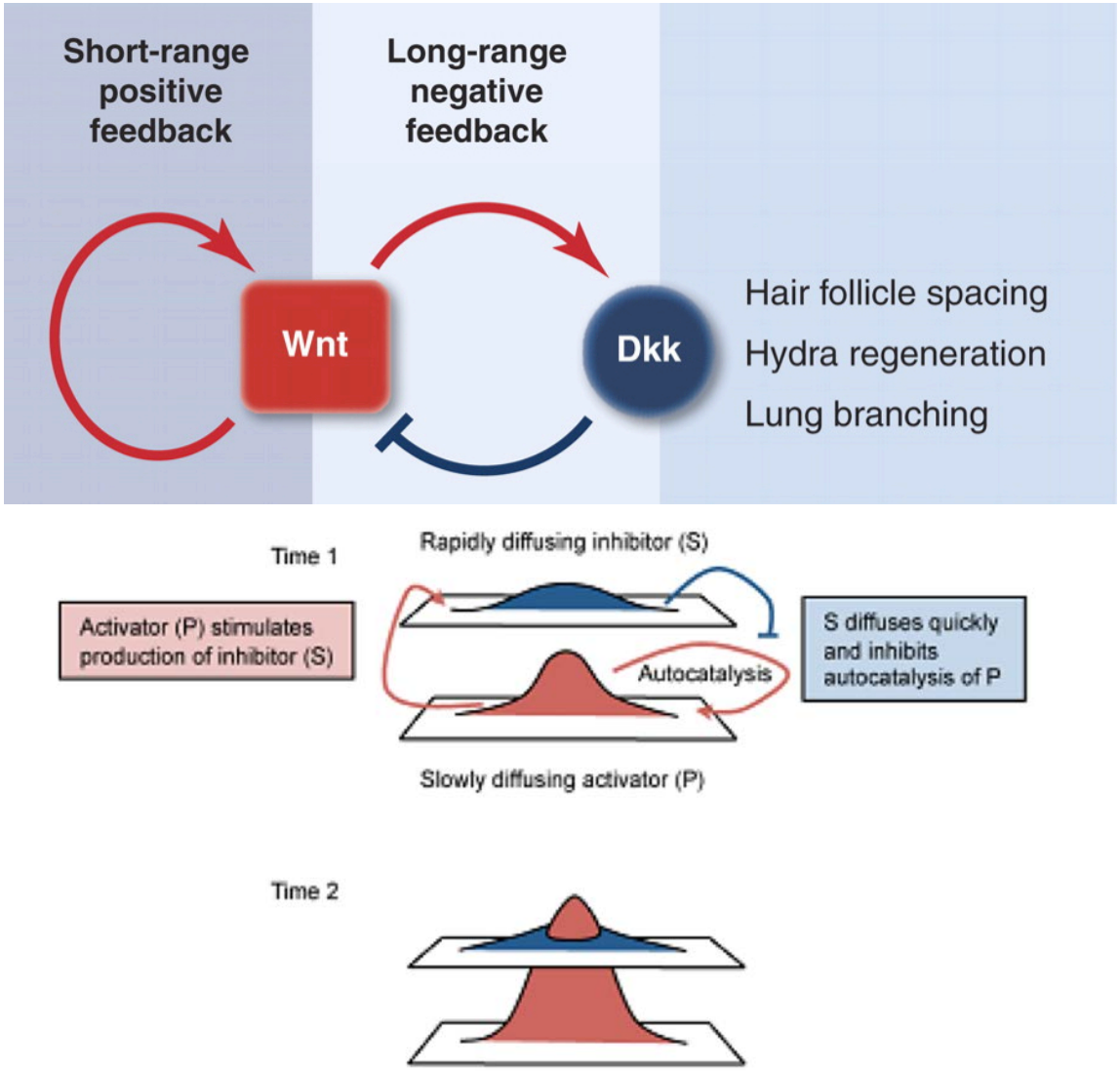


Anti-pPDH staining
"glycolysis"

Lee & Chen et al. 2017. Mol. Syst. Biol.

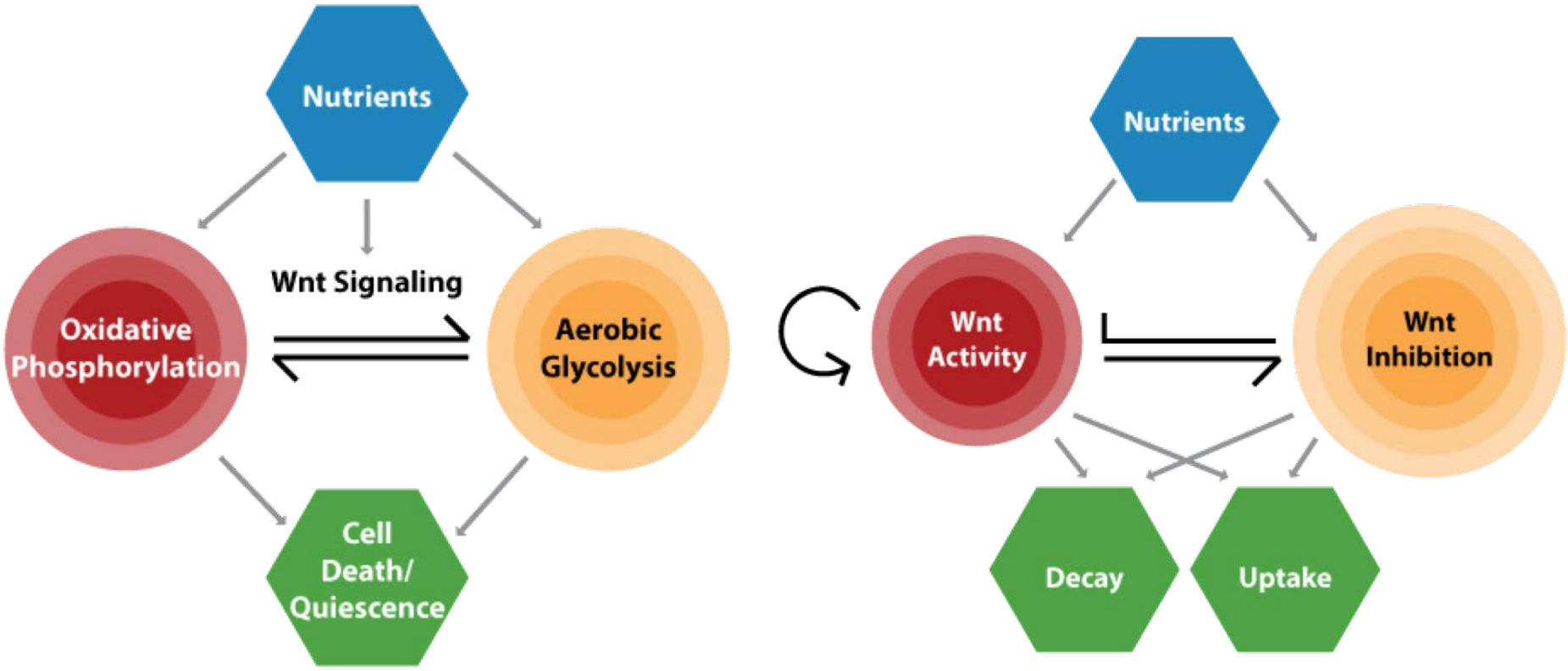
Turing Reaction-Diffusion systems

Reaction-Diffusion Modeling



Adapted from Kondo, et al. *Science*. 2010 & Gilbert, SF. *Developmental Biology.* 2000

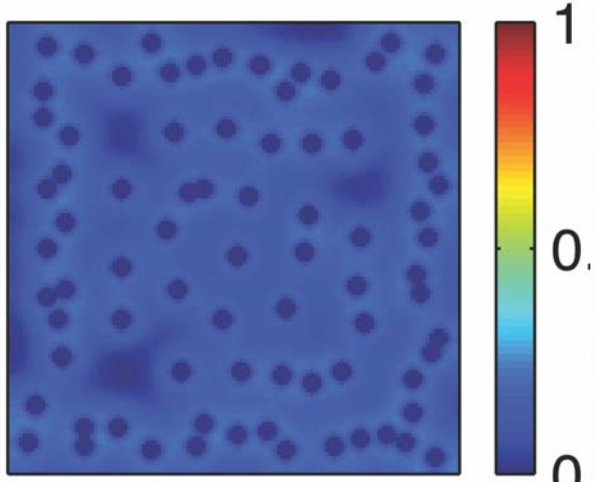
A Reaction-Diffusion model for Wnt regulation of patterned metabolism



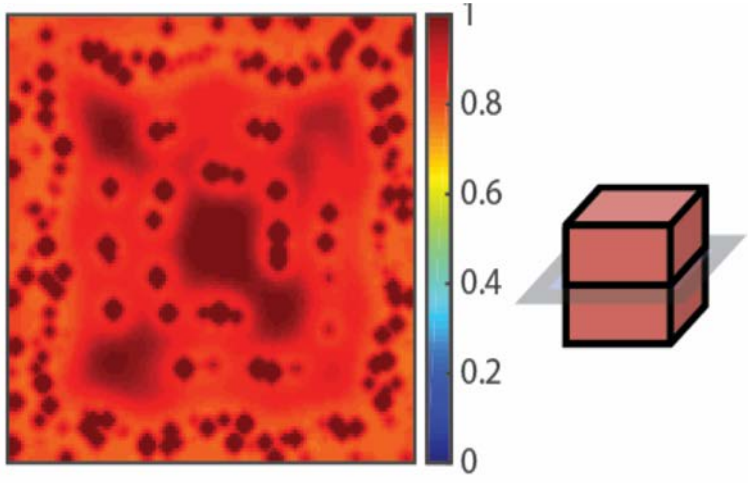
Lee & Chen et al. 2017. Mol. Syst. Biol.

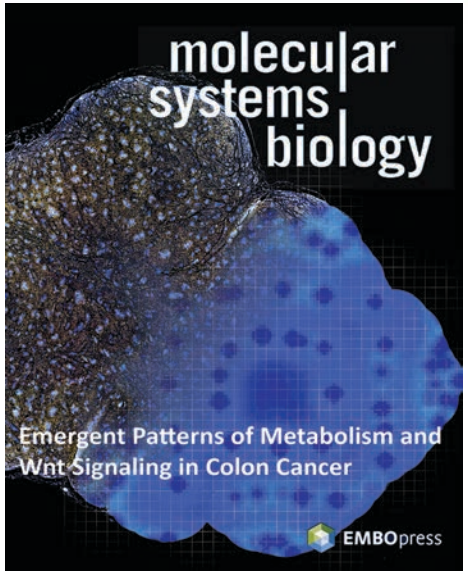
MatLab Simulations

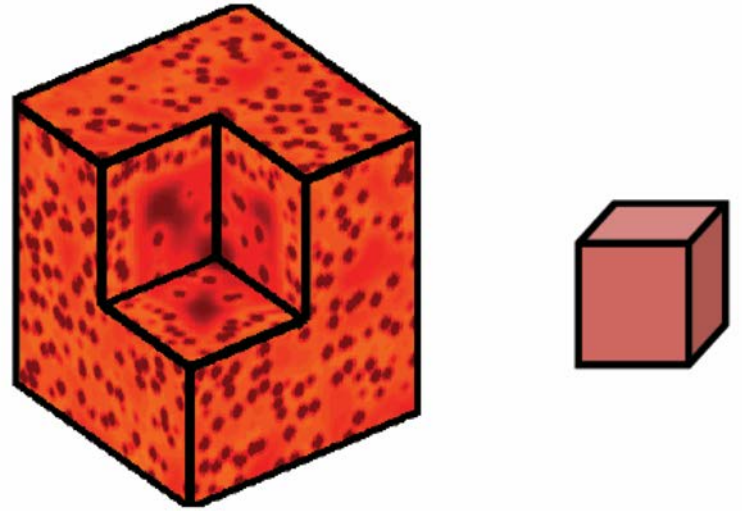
Po: Oxidative phosphorylation



Pg: Glycolysis







Lee & Chen et al. 2017. Mol. Syst. Biol.

A Reaction-Diffusion Model Prediction

Inhhibition of Wnt signaling will trigger an increase in the diffusion range of Wnt ligands, extending their "reach"

Result: bulk RNAseq of Wnt-inhibited (dnLEF/TCF) xenograft tumors revealed sharply increased expression of SFRPs which are Wnt ligand "diffusers"

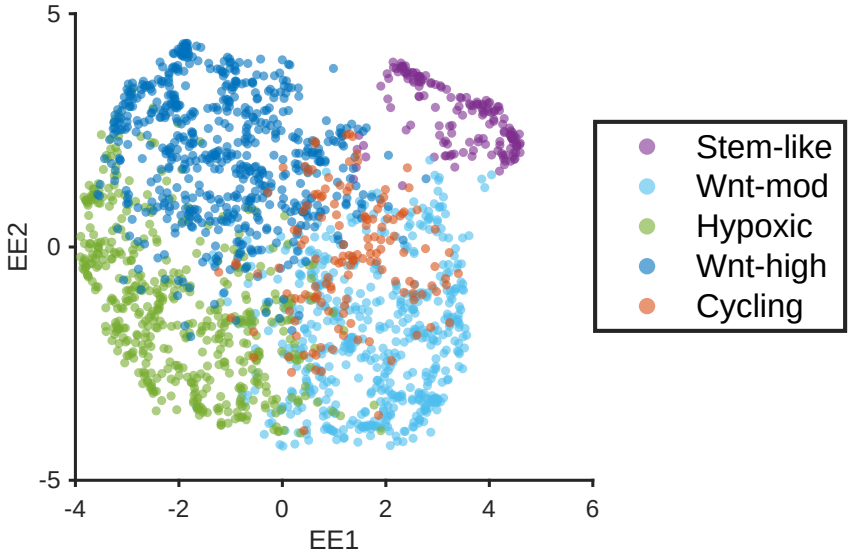
***

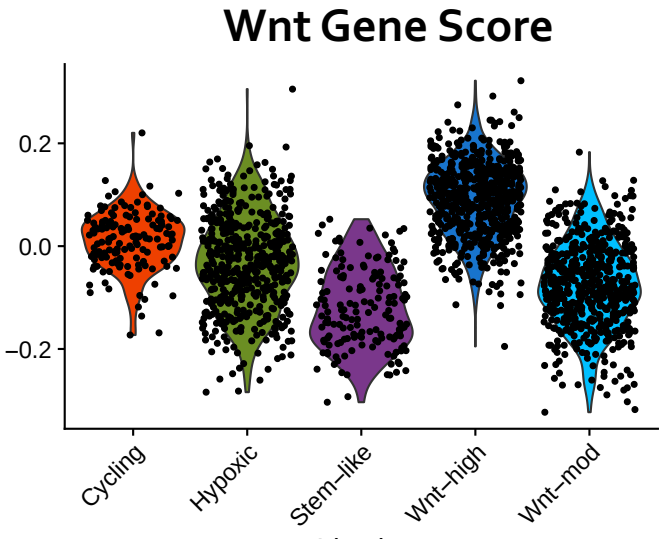
CRC relevance: Radio-Chemotherapy-treated patient rectal tumors (GEO dataset GDS3756).

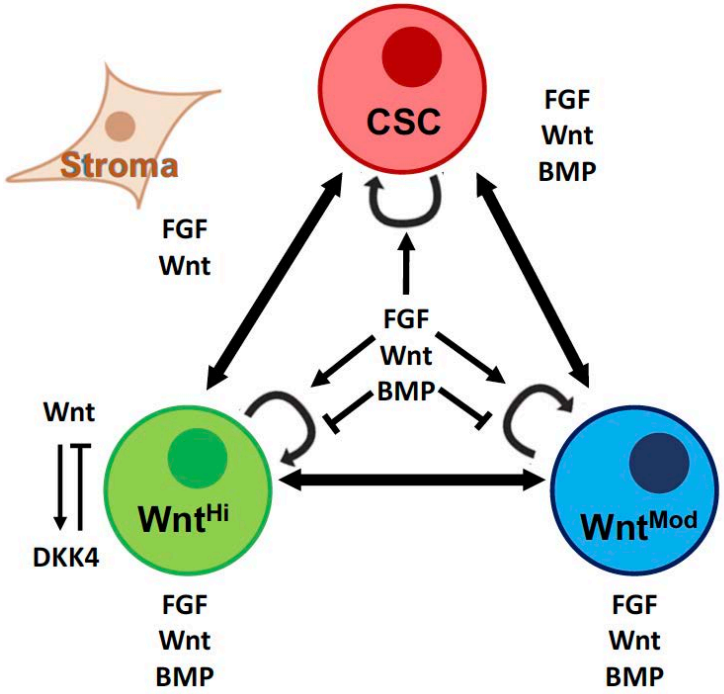
- Wnt target gene expression declined with treatment and …
- SFRP-1, -2, -4 expression increased ~5-50 fold

Lee & Chen et al. 2017. Mol. Syst. Biol.

Colon Cancer Cell Heterogeneity in SW480 Xenografted Tumors



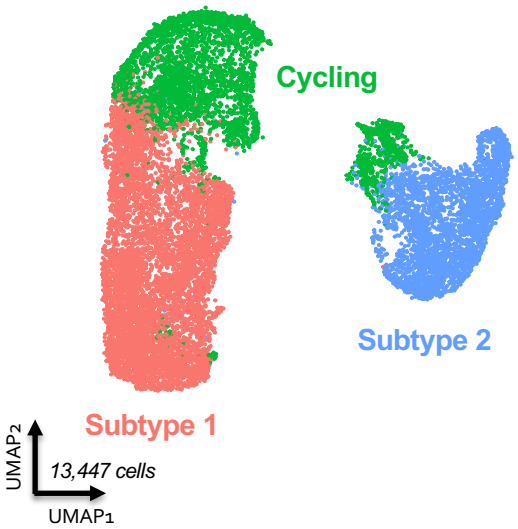




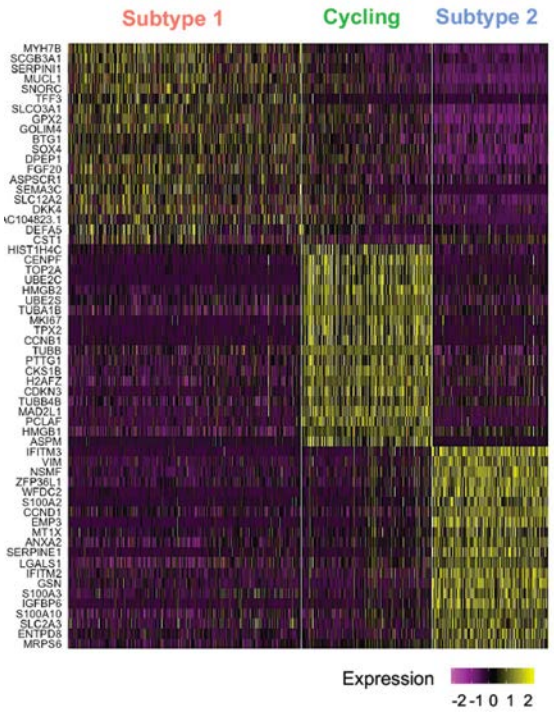
Huang et al, in preparation, 2024

Colon Cancer Cell Heterogeneity in SW480 Xenografted Tumors

Xenograft Tumor
Anti-beta-catenin staining

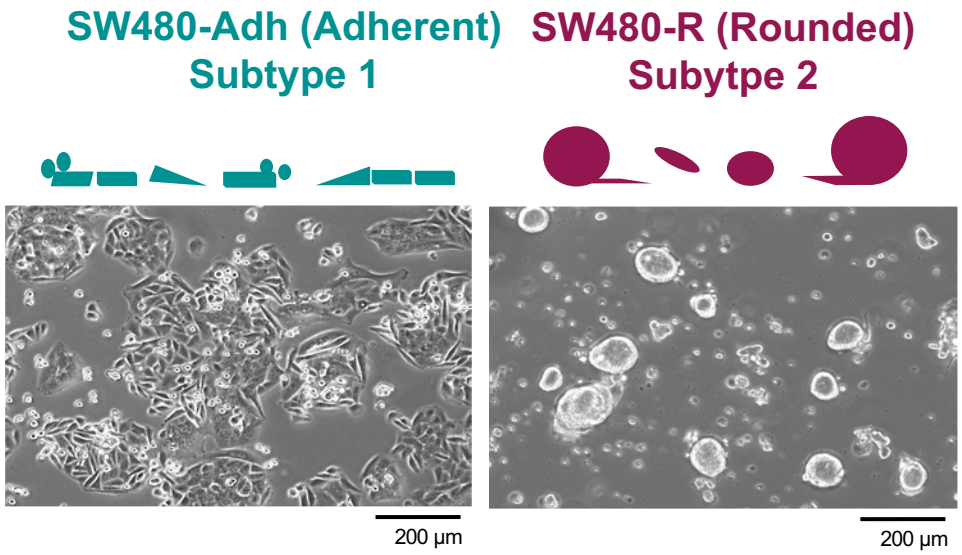


Xenograft Tumor
scRNAseq



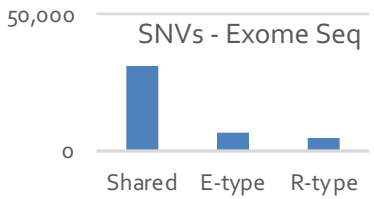
Hosohama et al., 2023, under review
bioRxiv 2024.04.25.591144; doi: https://doi.org/10.1101/2024.04.25.591144

Two CRC Subtypes Co-exist in SW480 cultures



Tomita et al. 1992. Cancer Res. 52:6840-7.

- STR Profiling - identical
- Exome Sequencing: vast majority of exome mutations are shared
  - TP53: Pro309Ser; Arg273His; Pro72Arg
  - APC: Gln1338*; Val1822Asp
  - Kras: Gly12Val

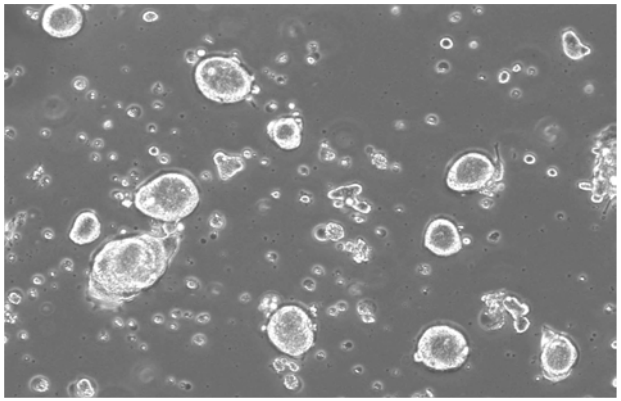


- Similar proliferation indices in vitro
- Similar Cancer Stem Cell activities in xenograft experiments (subcutaneous, orthotopic)

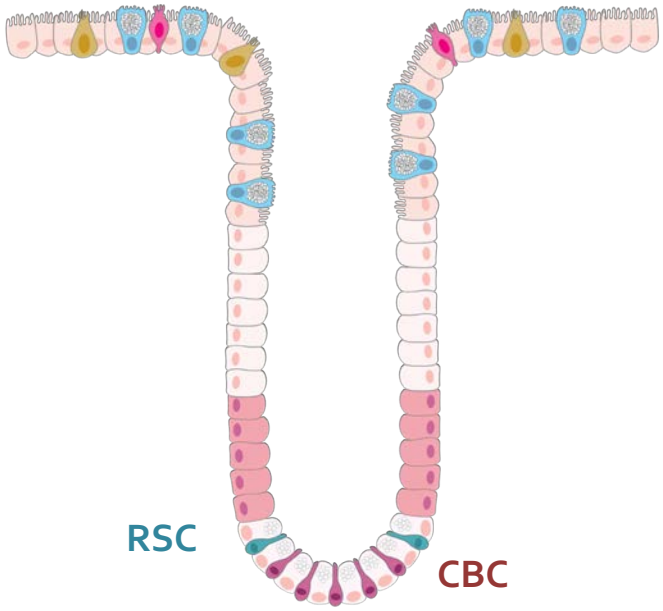
Hosohama et al., 2023, under review
bioRxiv 2024.04.25.591144; doi: https://doi.org/10.1101/2024.04.25.591144

Two Colon Cancer Stem Cell Subtypes "Model" Different Normal Stem Cell Populations

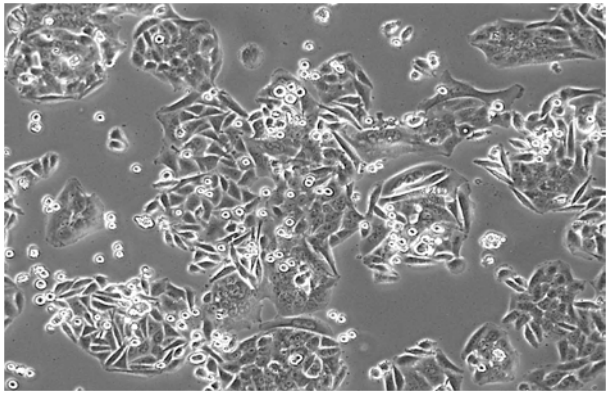
Rounded/CBC



YAP^{OFF}
MYC/MYB
LGR5
PROX1



Adherent/RSC

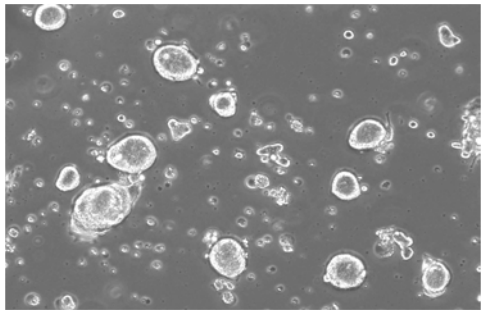


YAP^{ON}
Fetal-wounding
LGR4
Wnt5a

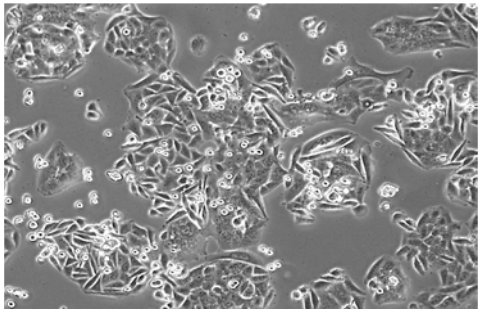
Hosohama et al., 2023, under review
bioRxiv 2024.04.25.591144; doi: https://doi.org/10.1101/2024.04.25.591144

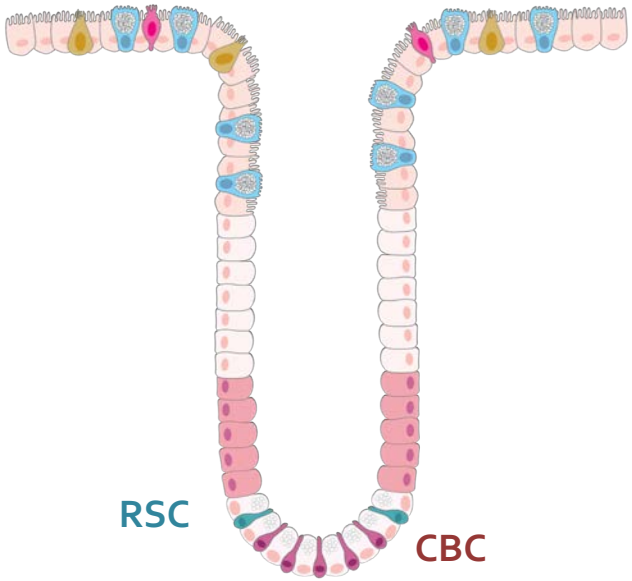
Different Intrinsic Wnt Signaling in two Colon Cancer Stem Cells

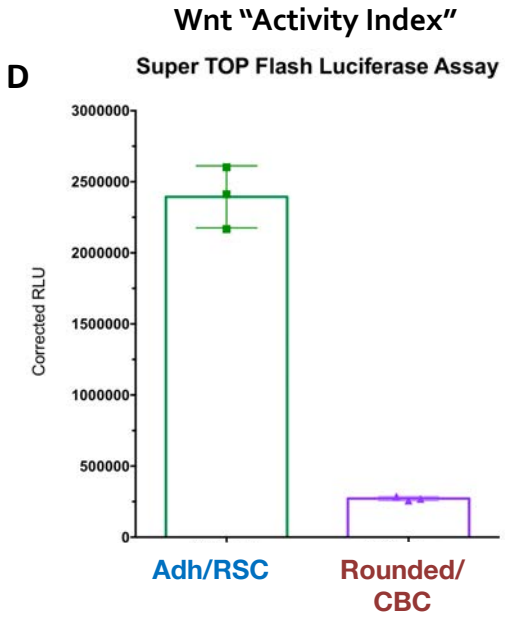
Rounded/CBC



Adherent/RSC



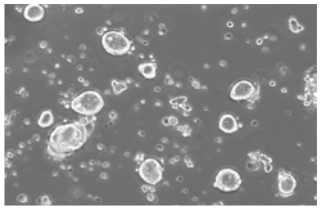




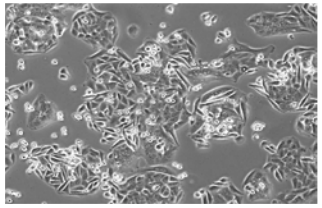
Hosohama et al., 2023, under review
bioRxiv 2024.04.25.591144; doi: https://doi.org/10.1101/2024.04.25.591144

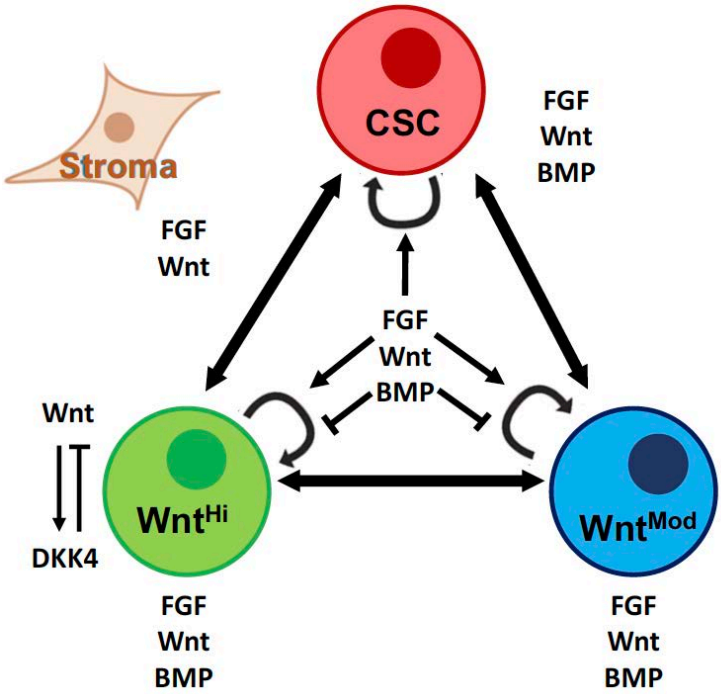
Modeling Colon Cancer Stem Cell Heterogeneity

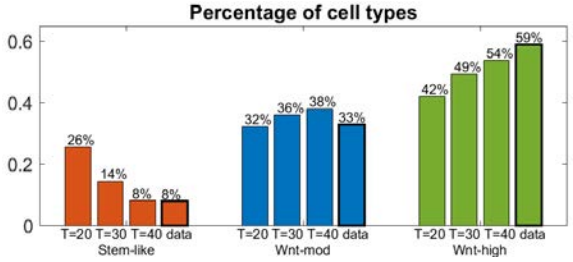
Rounded/CBC

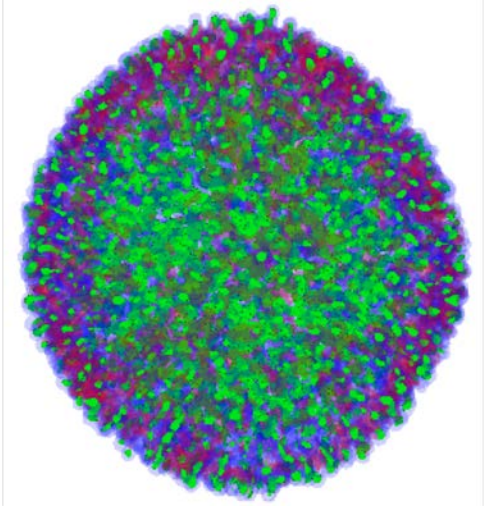


Adherent/RSC



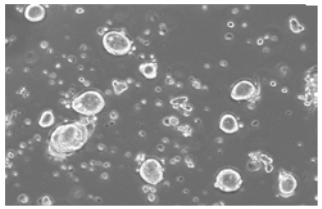




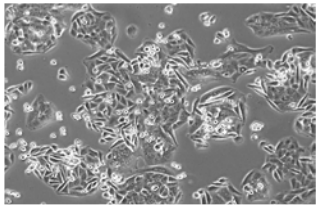


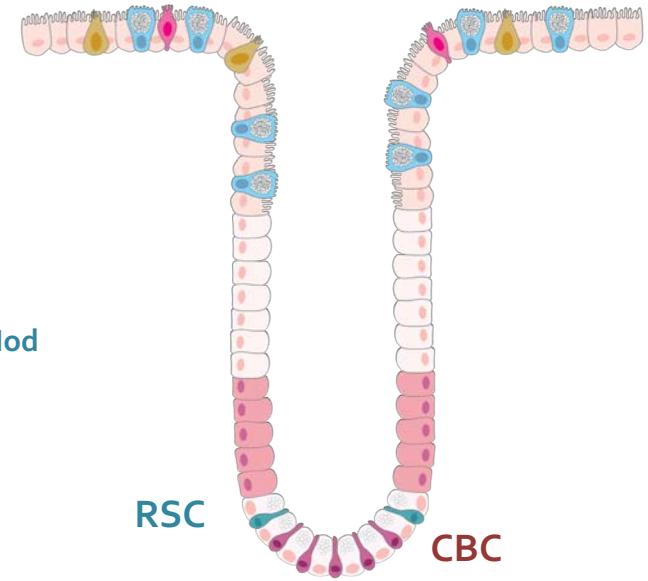
A "Switching Prediction"

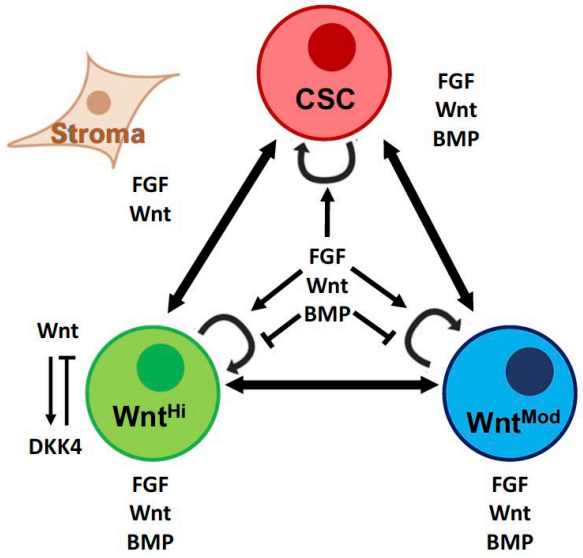
Rounded/CBC/CSC



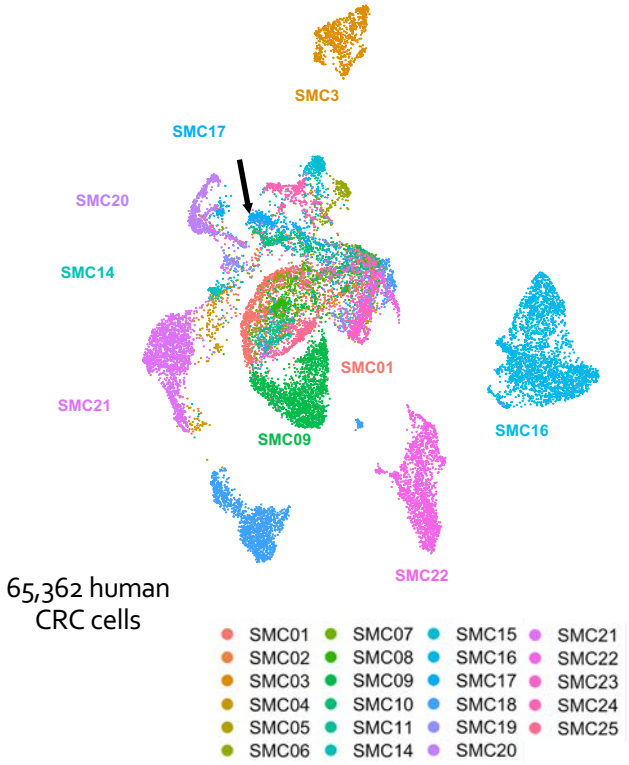
Adherent/RSC/Wnt$^{Hi\text{-}Mod}$

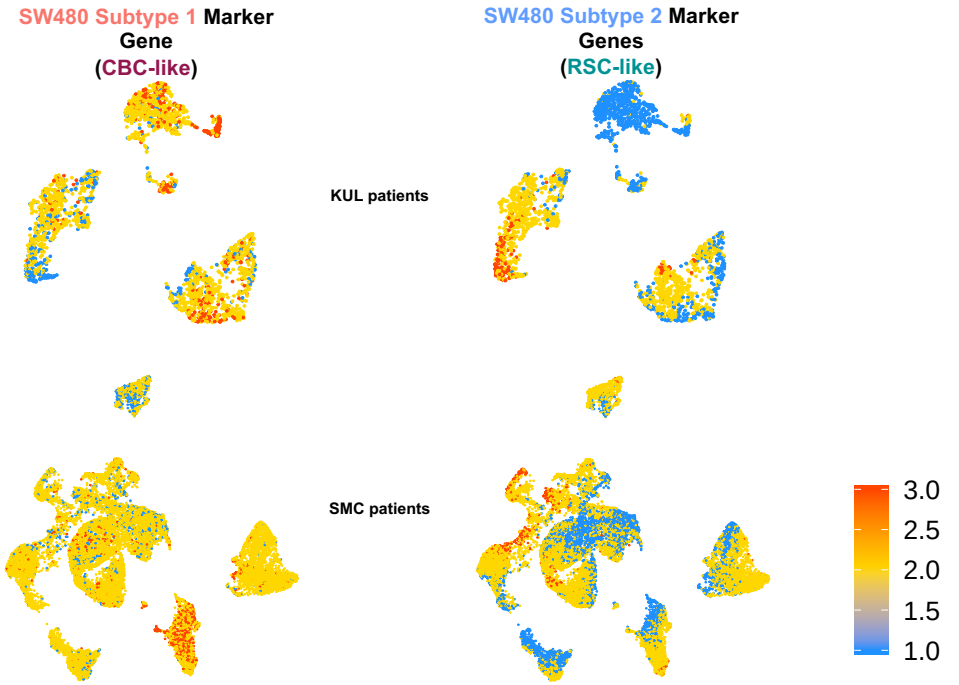






scRNAseq Study: Human CRC tumors are Heterogeneous

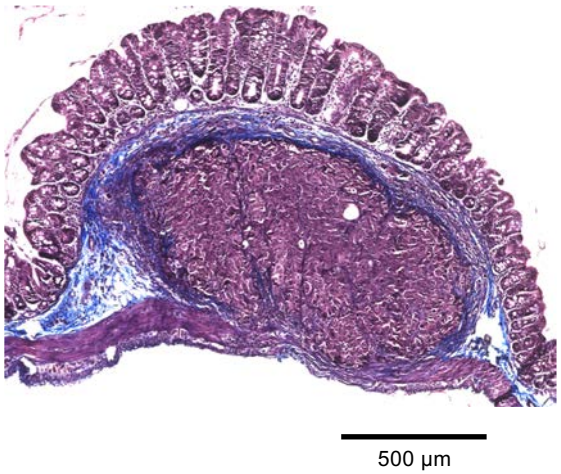




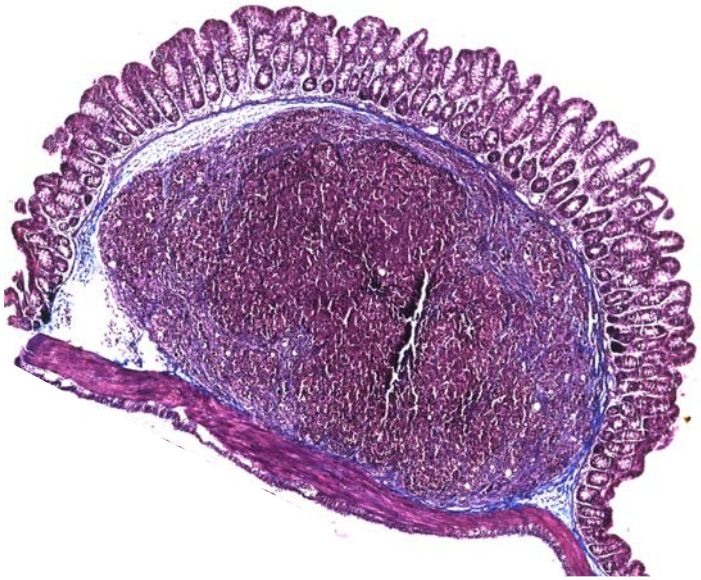
Lee H., et al. Nat Genet 2020 52:594-603. PMID: 32451460

RSC (Wnt^{Hi-Lo}) tumors are Fibrotic
CBC/CSC tumors are Necrotic

Adh/RSC



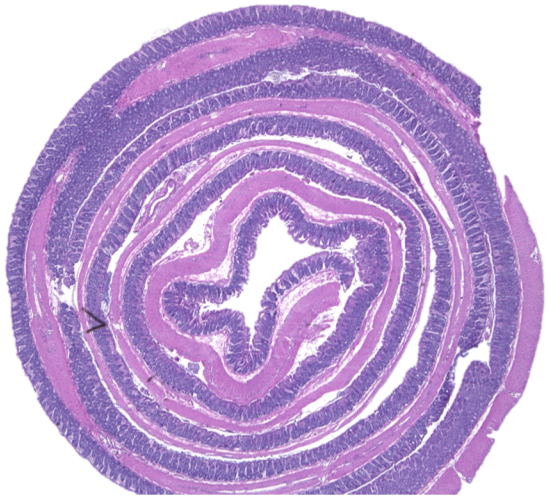
Rounded/CBC



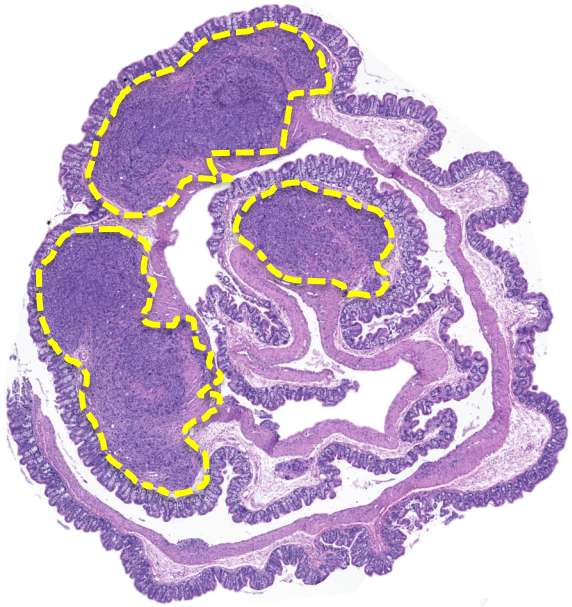
Trichrome staining (collagen fibers)

Orthotopic Tumor Phenotypes

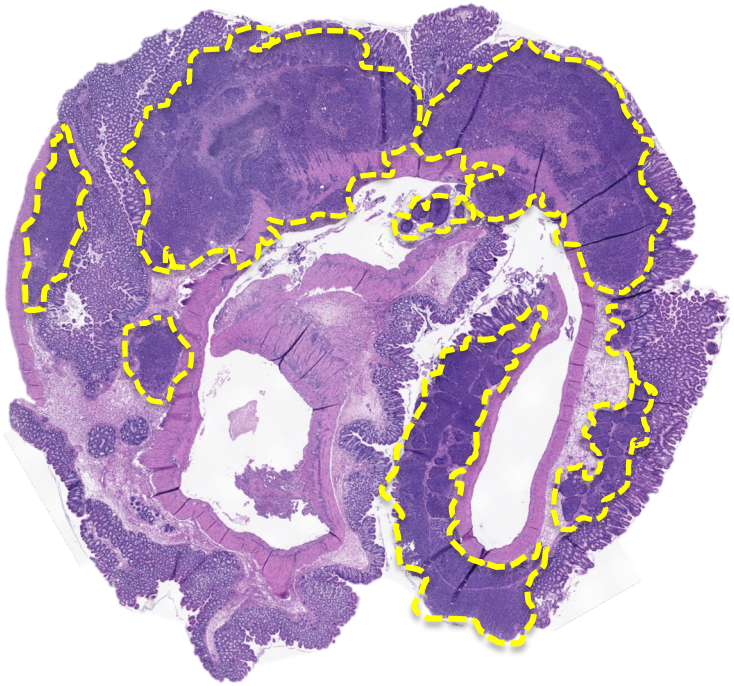
Normal Colon



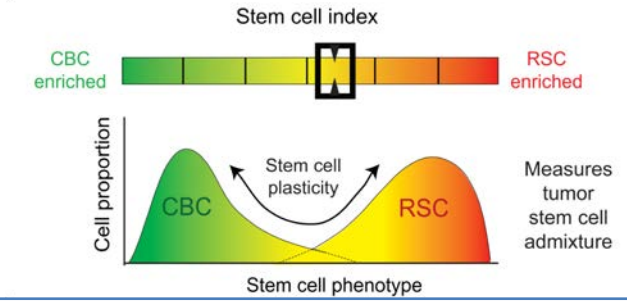
RSC ($Wnt^{Hi\text{-}Lo}$)



CBC/CSC



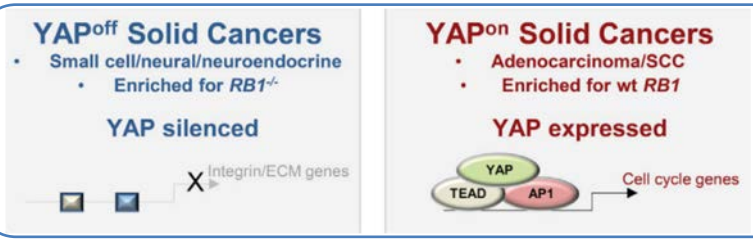
New: Binary Classification of Colorectal Cancer



CBC vs RSC

Vasquez EG, et al. 2022. Cell Stem Cell 29, 1213–1228

RSC: Regenerative Stem Cell
CBC: Crypt Base Columnar Cell

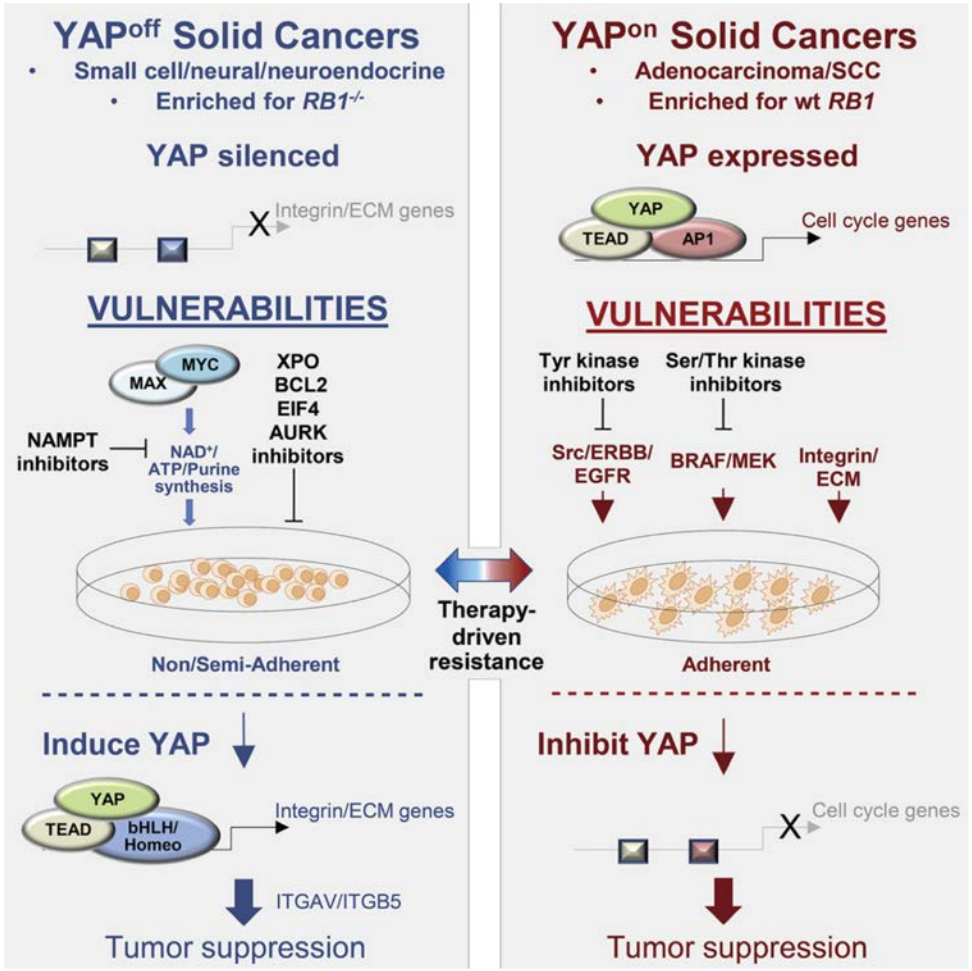


YAP^{OFF} vs YAP^{ON}

Pearson JD, et al. 2021. Cancer Cell 39:1115-1134

Binary Classification & Predicted Responses to Therapies

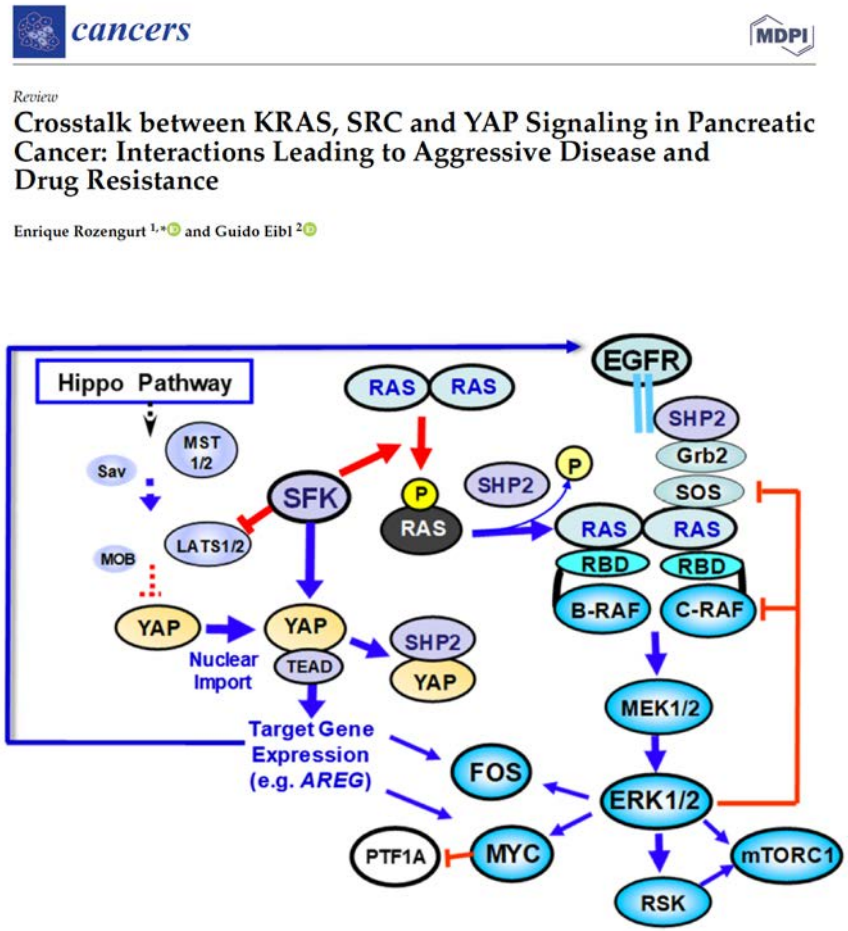
Rounded/CBC/ CSC

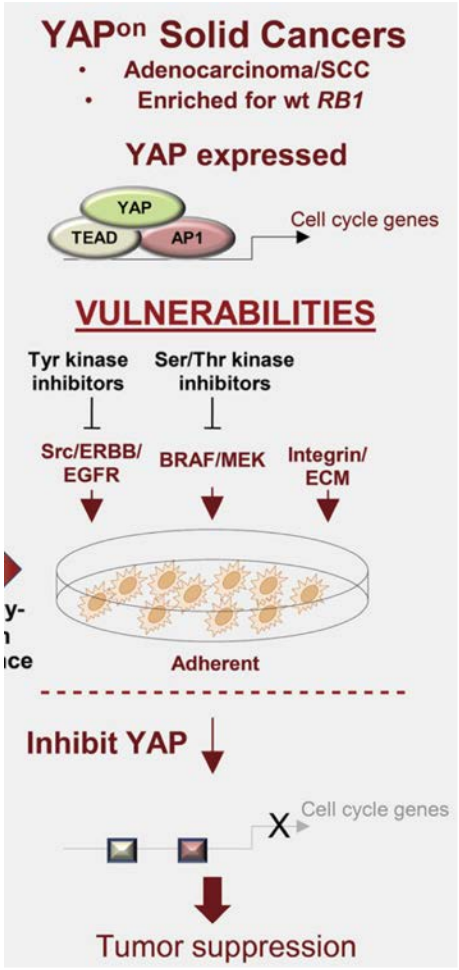


Adherent/RSC /Wnt^{Hi-Lo}

Pearson …Wrana, Goodrich, Bremner et al. 2021. Cancer Cell

Binary Classification & Predicted Responses to Therapies





Adherent/RSC /Wnt^{Hi-Lo}

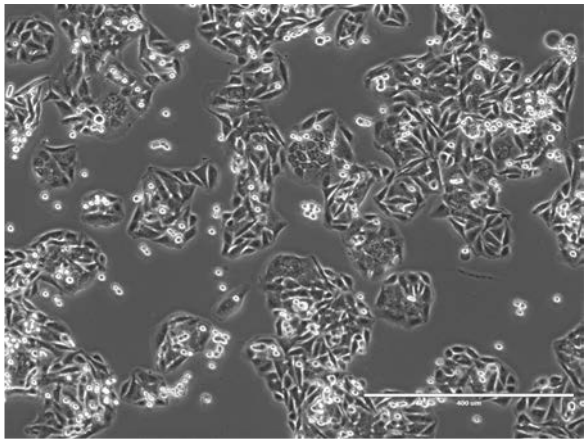
Pearson …Wrana, Goodrich, Bremner et al. 2021. Cancer Cell

Dasatinib treatment converts Adh/CBC to R/CBC phenotypes

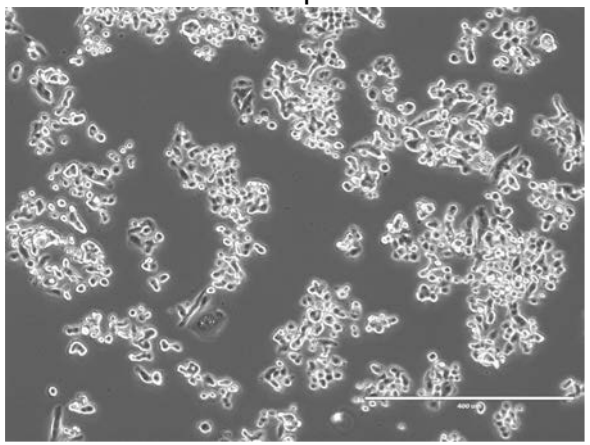
Adh/RSC

Rounded /CSC /CBC

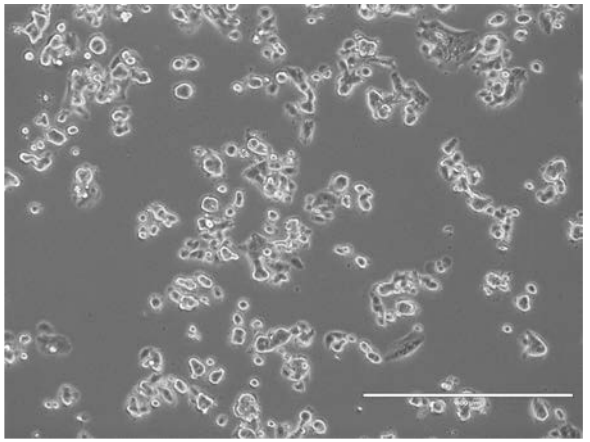
DMSO



1µM



5µM

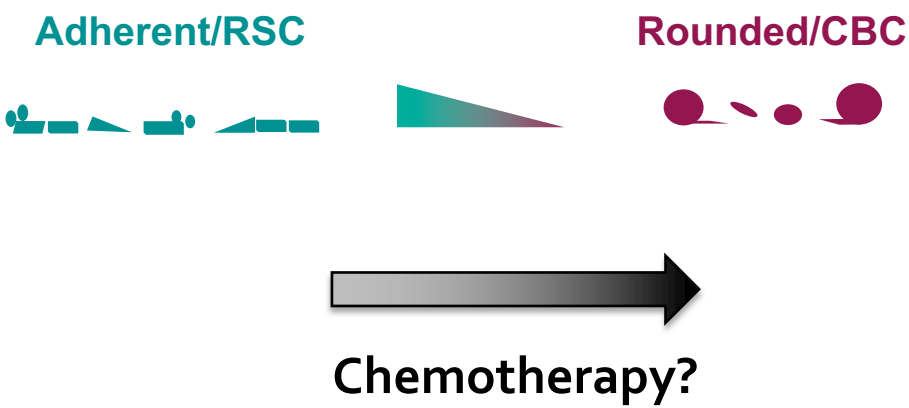


Dasatinib, 3d Rx

unpublished data

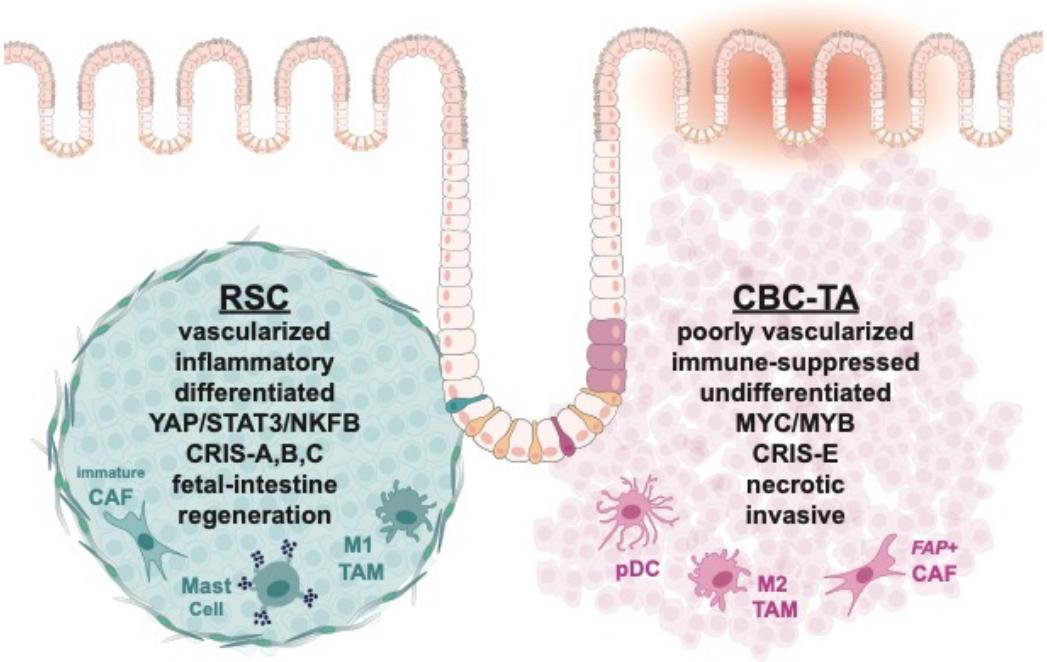
Dasatinib Rx of *in vitro* Adh/RSC cells triggers colon cancer stem cell "switching"

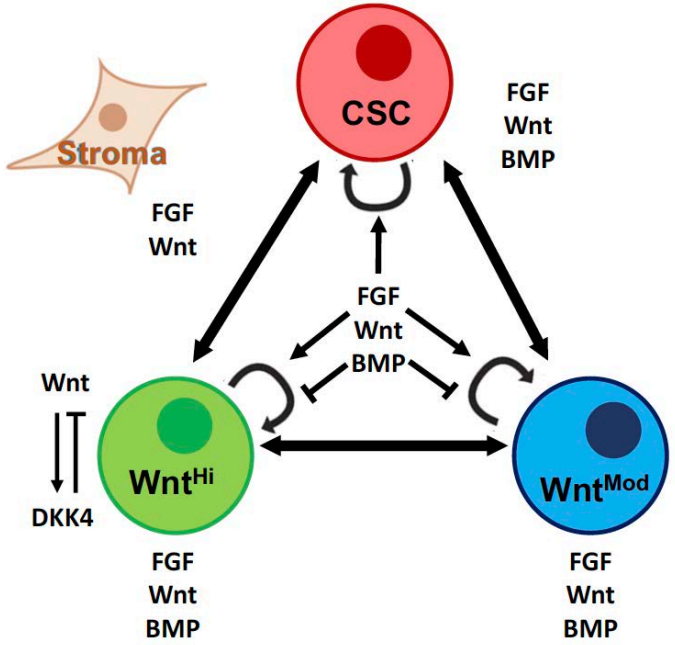
Gene Name	Decreased Expression	Increased Expression
SAA2	-4.0	
SAA1	-3.2	
MALL	-1.6	
MSX1	-1.3	
LIMS1	-1.3	
METTL9	-1.2	
ANO1	-1.2	
DCBLD2	-1.2	
TIAM1	-1.1	
DOCK9	-1.1	
PDP1	-1.1	
PDGFB	-1.1	
RHOBTB3	-0.8	
MED13L		0.8
NR4A1		1.0
KIAA1549		1.1
CEMIP2		1.1
ERVMER34-1		1.2
FOXC1		1.5
CST1		1.8
RASL11B		1.9
FAM178B		2.0
SYTL3		2.1
VIL1		2.4
SEMA3F		2.5
FSTL4		5.6



unpublished data

Summary


RSC
vascularized
inflammatory
differentiated
YAP/STAT3/NKFB
CRIS-A,B,C
fetal-intestine
regeneration
immature
CAF
M1
TAM
Mast
Cell
CBC-TA
poorly vascularized
immune-suppressed
undifferentiated
MYC/MYB
CRIS-E
necrotic
invasive
pDC
M2
TAM
FAP+
CAF


Stroma
CSC
FGF
Wnt
BMP
FGF
Wnt
FGF
Wnt
BMP
Wnt
DKK4
Wnt^Hi
Wnt^Mod
FGF
Wnt
BMP
FGF
Wnt
BMP

Colorectal Cancer Stem Cell Subtypes and Tumor Microenvironments

Shared Resources

Genomics Research & Tech. Hub
Melanie Oakes
Jenny Wu

Experimental Tissue Resource
Rob Edwards
Delia Tifrea
Kehui Wang

Optical Biology Core
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