Unbiased Approaches To Identify New Targets And Combination Therapies In CRC

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Disclosures

- Ad Hoc Consulting
  - Genentech/Roche
    - Data Monitoring Committee for Roche

- *I also apologize for changes in slide formatting. My slides, my lab's slides, and the format for ICPOEP are all different.*

Unbiased Approaches To Identify New Targets And Combination Therapies In CRC

- Identification of targets in angiocrine signaling
  - Rui Wang, PhD
- High throughput screening targeting the MAPK pathway in CRC

If You Don't Remember Anything Else From This Lecture, Remember This

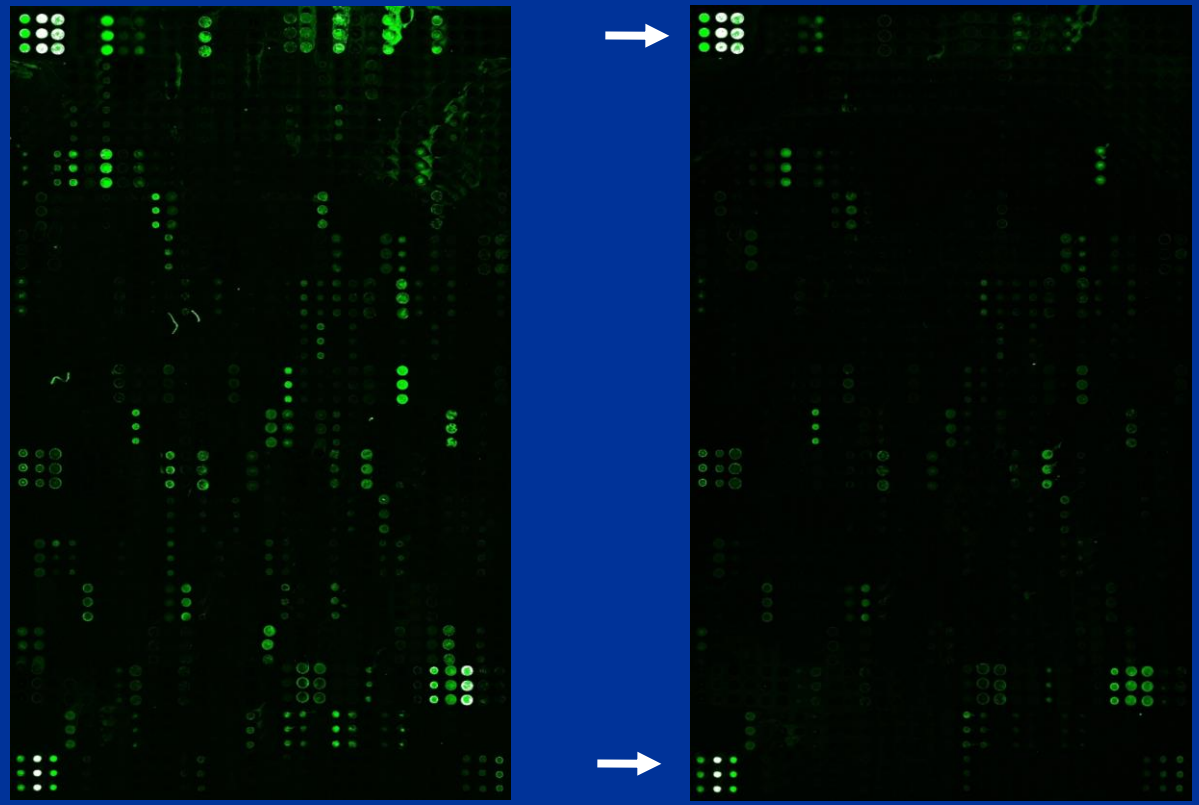
- No single agent is likely to make an impact in patients with mCRC
  - The exception is MSI-H and IO
- New multi-agent or sequential regimens can only be identified by
  - Understanding biology
    - Keeping an open mind when you acquire data
      - IJ Fidler: 1992: "*The data are the data, you can't change the data, change you hypothesis"*
  - Creating your own luck
    - Unbiased screening

Which Membrane Represents Tumor Cell Secreted Proteins?

Endothelial Cells

CRC Cells

Loading controls



Raybiotech Antibody Array

Challenging Angiogenesis
Angiocrine Signaling

- First discussed by R. Gilbertson and S. Rafii
- Angiocrine signaling = Factors released by endothelial cells that mediate the function of surrounding cells
  - Tissue regeneration
  - Cancer and the stem cell phenotype
- And challenging Precision Medicine
  - The data I will show is *independent* of the mutational status of the tumor cells

This is my own cartoon (1990's), but I only show the flow of paracrine factors in one direction.
Is this flawed thinking? (or just naïve?)

We MUST challenge existing paradigms!

Hypothesis: There is More to The Function of Endothelial Cells Than Simply The Formation of Tubes to Deliver Blood to Tissues

Cancer Cell

Article

201
3

Endothelial Cells Promote the Colorectal Cancer Stem Cell Phenotype through a Soluble Form of Jagged-1

Jia Lu,[1] Xiangcang Ye,[1] Fan Fan,[1] Ling Xia,[1] Rajat Bhattacharya,[1] Seth Bellister,[2] Federico Tozzi,[2] Eric Sceusi,[2] Yunfei Zhou,[1] Isamu Tachibana,[1] Dipen M. Maru,[3] David H. Hawke,[3] Janusz Rak,[6] Sendurai Mani,[4] Patrick Zweidler-McKay,[5] and Lee M. Ellis[1,2,*]

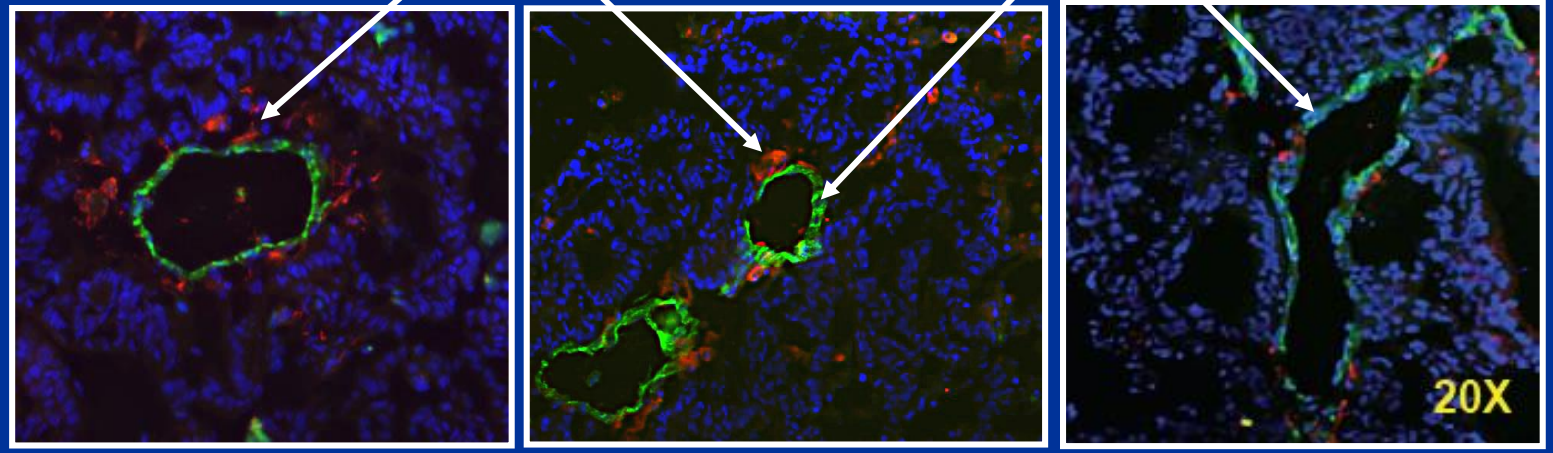
Reproducibility is another interest of mine (Nature, Lancet Oncology, others)
If your data is sound, you can build upon it.

Proximity of CD133+ Cells to Endothelial Cells in CRC Specimens

(Representative photomicrographs)

CD133
Cancer Stem Cells

CD31
Vessels

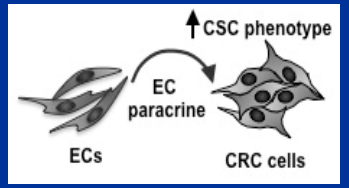


Primary Colon Cancers

Colon Cancer
Liver Metastasis

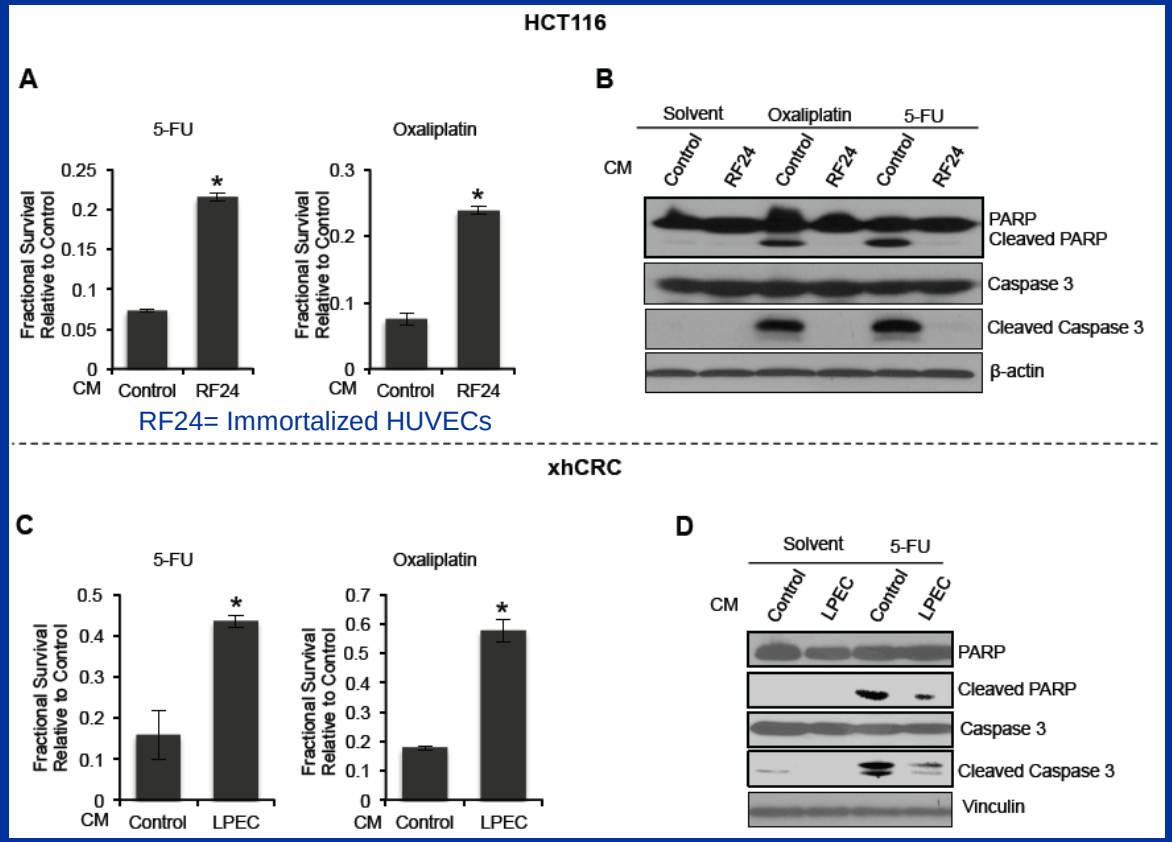
Ling Xia

Conditioned Medium From Endothelial Cells Promote Chemo-resistance In CRC Cells

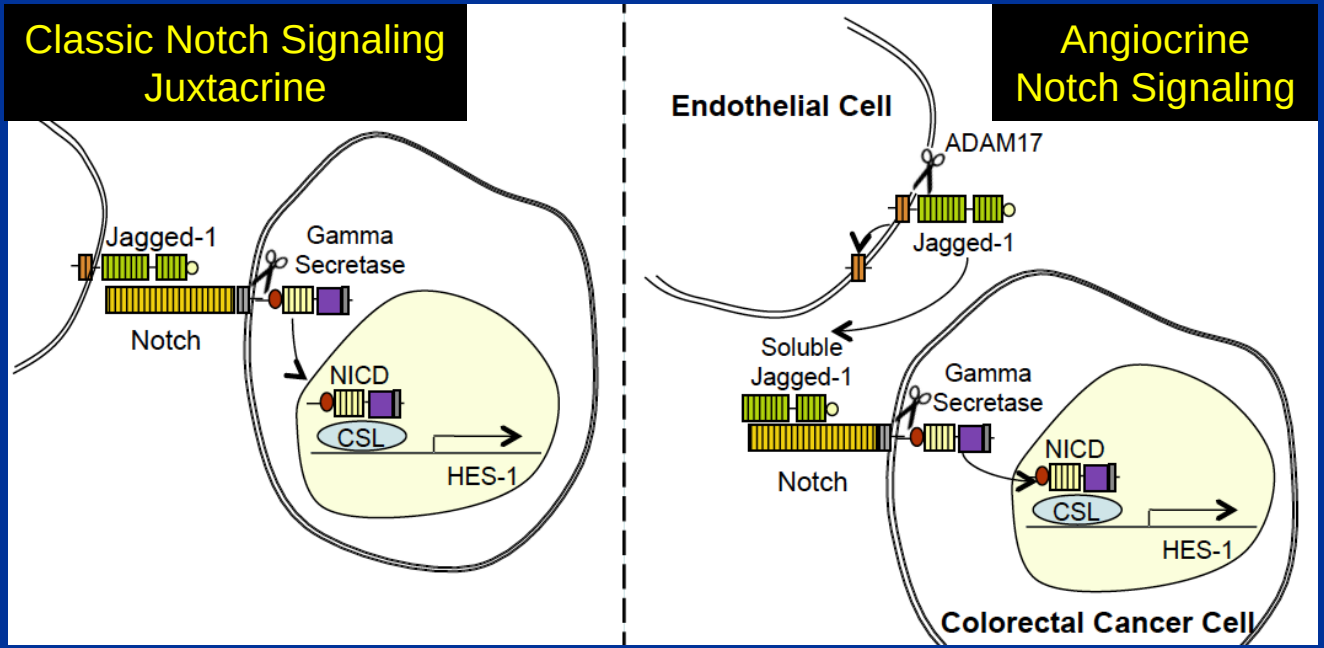


RF24/HCT116

LPEC/xhCRC



Angiocrine Signaling in CRC: Part 1



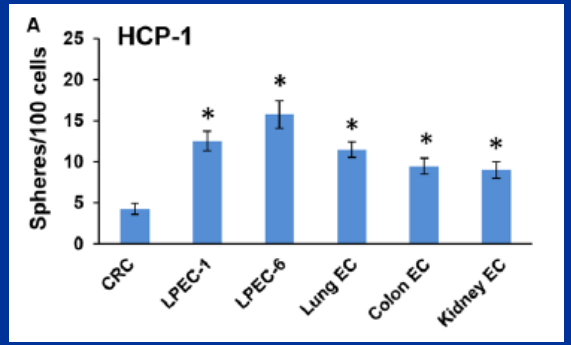
Endothelial cells secrete a truncated form of Jagged-1, cleaved by ADAM-17, that mediates cancer stem cell-ness of target colon cancer cells.

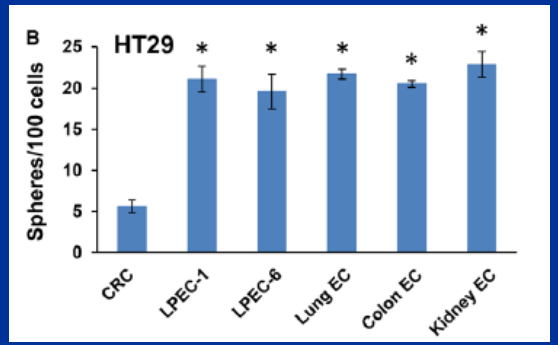
Cancer Cell, 2013

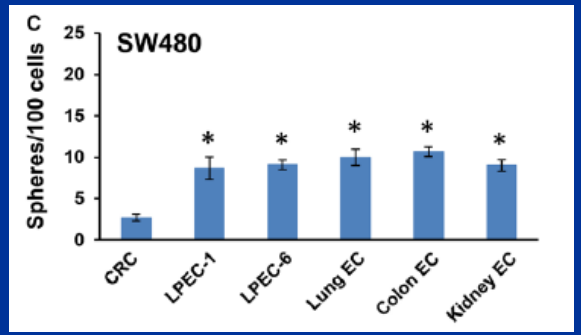
But, We Have Learned that *Biology is Not Linear*
(nothing is simple in tumor biology)

- We generated antibodies to soluble Jagged-1 and this had minimal effect on CRC growth
- Subsequent studies showed that blockade of ADAM-17 can induce only modest chemo-sensitivity in vitro
  - Wang et al. Stem Cell Trans Med, 2016
- There must be other factors that mediate the CSC-phenotype
  - Do distinct endothelial cells mediate distinct CSC pathways?
  - What other stem cell pathways are activated by angiocrine signaling?

Endothelial Cells From Various Organs Increased the CRC Stem Cell Phenotype







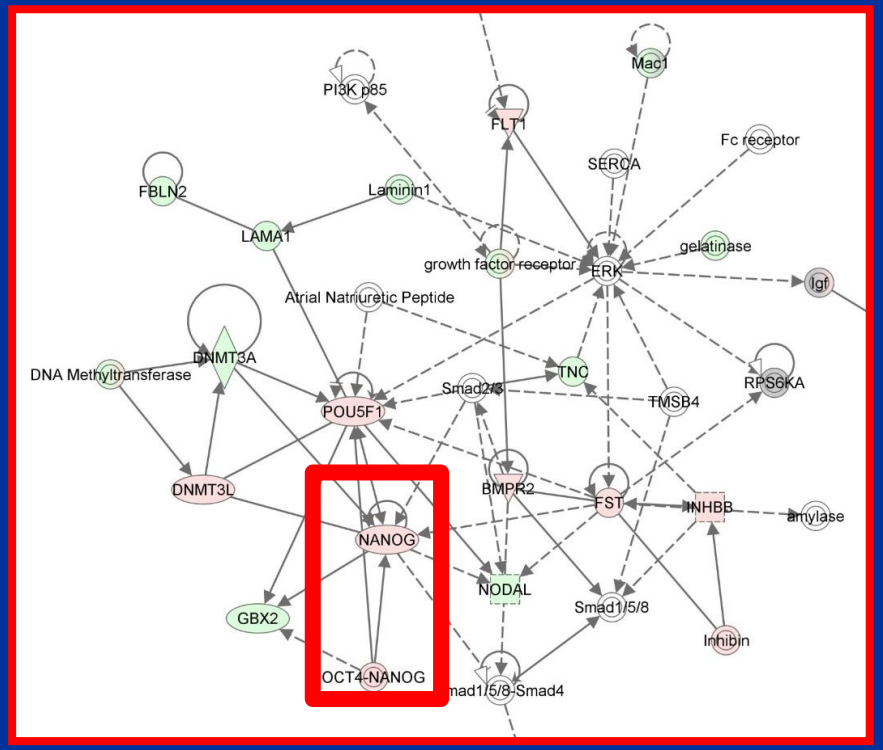
Angiocrine signaling occurs in all organs studied: Implications for other cancer types

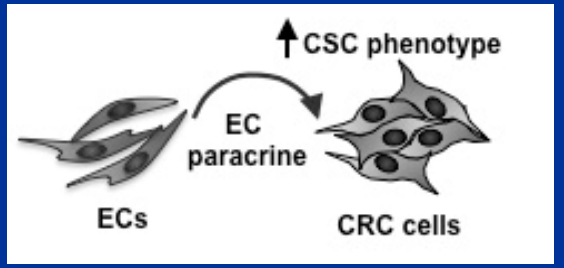
What Other Pathways Are Activated in CRC Cells by Angiocrine Signaling?

Rui Wang, PhD

q-PCR of EC CM Treatment of Tumor Cells

Ingenuity IPA Analysis

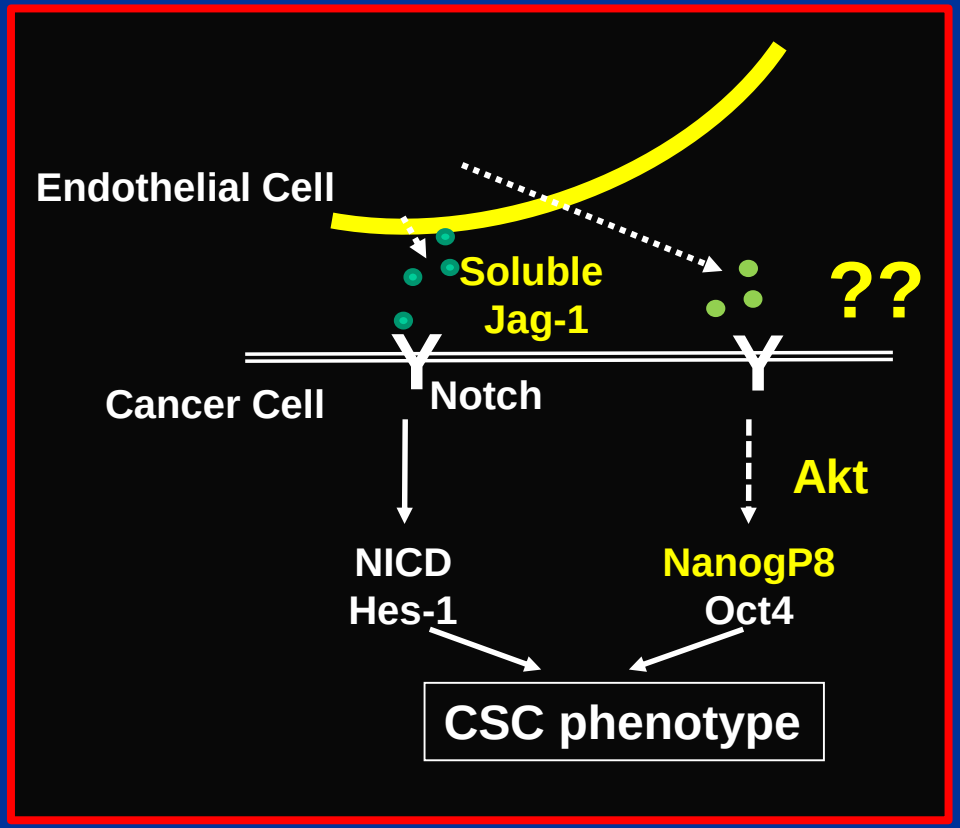




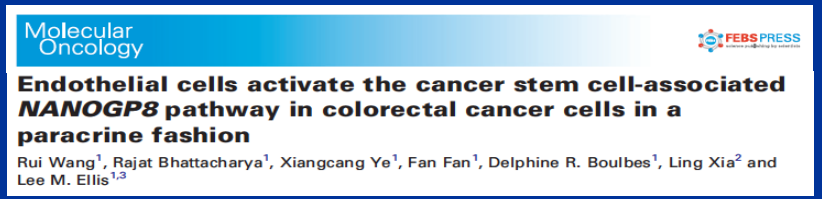
qPCR by Human Stem Cell 384 StellARray kit

Rui Wang

ECs Promote the CSC Phenotype in CRC Cells by Activating Several Pathways: NanogP8



- If you knock down Nanog in CRC cells, you decrease sphere formation (CSC-ness)
- If we block Akt, we block NanogP8 induction and sphere formation


Molecular Oncology

FEBS PRESS

Endothelial cells activate the cancer stem cell-associated *NANOGP8* pathway in colorectal cancer cells in a paracrine fashion

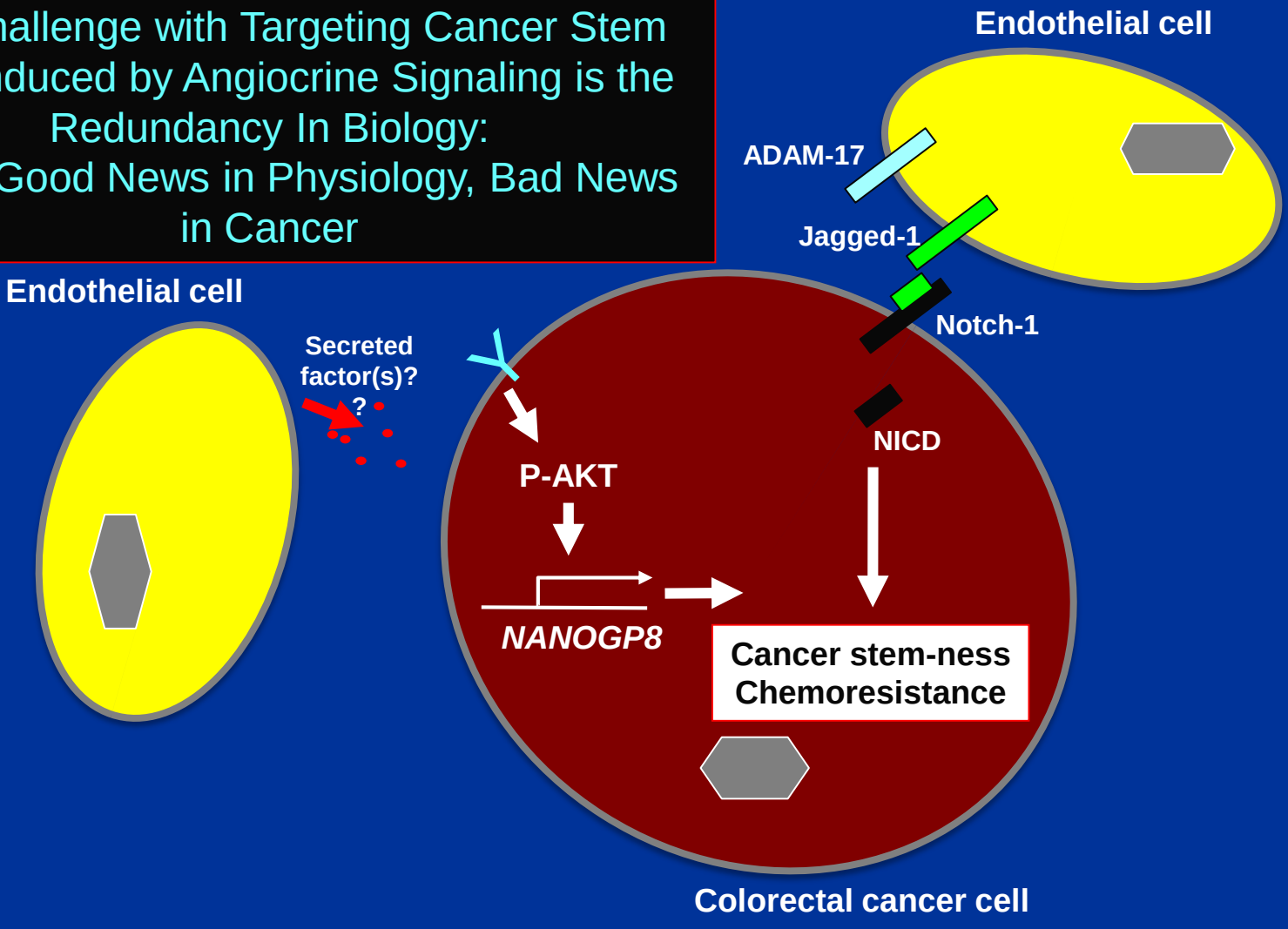
Rui Wang[1], Rajat Bhattacharya[1], Xiangcang Ye[1], Fan Fan[1], Delphine R. Boulbes[1], Ling Xia[2] and Lee M. Ellis[1,3]

Lu J., et al, Cancer Cell. 2013
Wang R., et al, Mol. Onc., 2017

Our Approaches to Understanding Angiocrine Signaling

- Fractionation and *luck* *(unbiased)*
  - Jagged-1/Notch, ADAM-17
    - (Cancer Cell, Stem Cell Trans Med)
- RT-PCR Pathway Analysis *(unbiased)*
  - NanogP8 and Akt
    - (Mol Oncology)
- Hypothesis: If we already found several pathways activated by angiocrine signaling, I bet there are more!!
  - RTK Arrays on tumor cells treated with EC CM *(unbiased)*

The Challenge with Targeting Cancer Stem Cells Induced by Angiocrine Signaling is the Redundancy In Biology: This is Good News in Physiology, Bad News in Cancer



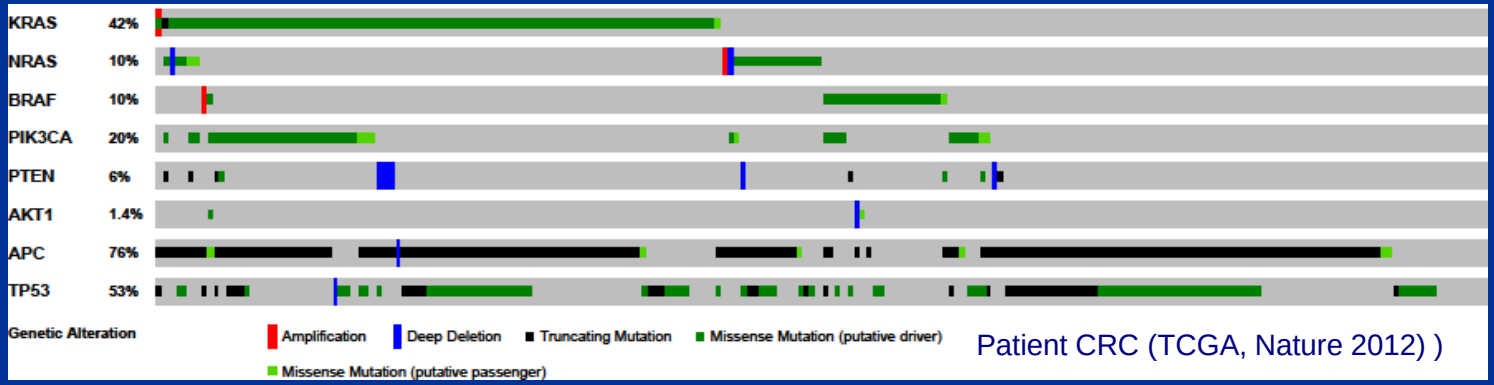
In Summary, Endothelial Cells Use Multiple Pathways To Enhance Survival Of Tumor Cells: Notch, NanogP8, Akt, and more *TBD*

- This is independent of the mutational status of the cells
- As always, tumor cells are **smart**
  - Combination and sequential therapies are not only important for genetic alterations, but also for the targeting of the tumor microenvironment

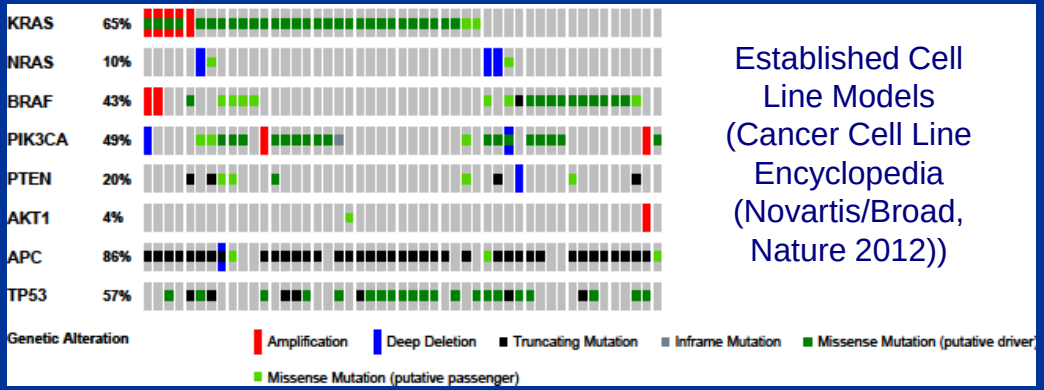
Unbiased Approaches To Identify New Targets And Combination Therapies In CRC

- Identification of targets in angiocrine signaling
- High throughput screening targeting the MAPK pathway in CRC
  - Rajat Bhattacharya, PhD

Presence Of Various Oncogenic Mutations In Patients With Colorectal Cancer And CRC Cell Lines



Patient CRC (TCGA, Nature 2012))



Established Cell Line Models (Cancer Cell Line Encyclopedia (Novartis/Broad, Nature 2012))

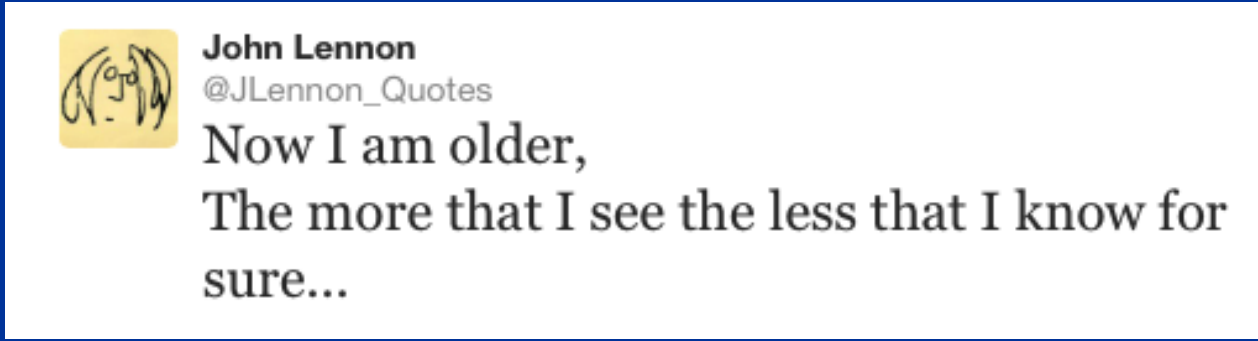
CRC cell lines and their mutational status			
Cell Line	KRAS	BRAF	PIK3CA
RKO	WT	V600E	H1047R
HCP-1	G12D	WT	H1047R
HCT116	G13D	WT	H1047R
HT29	WT	V600E	P449T
SW480	G12V	WT	WT
SW1417	WT	V600E	WT

Unbiased High Throughput Screening Identified Potential Combination Therapies for Ras Mutated CRCs

- Validation, validation, validation!!
  - 3-D modeling was challenging in our core facility due to the poor penetrance of drugs *and* reagents to detect apoptosis
    - CRC cells stick together and form tight spheres
    - We are working on this
  - Grants submitted to study *in vivo* tumor growth with cell lines and PDXs

Advantages of Unbiased Screening

- You remove your own bias and you go with the data
  - Your preconceived thoughts don't get in the way
- The UNEXPECTED findings in research are the most exciting findings



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Ling Xia

Science is a TEAM effort!!!!

Collaborators

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Patrick Zweidler-McKay, MD, PhD (Notch)
Sendurai Mani, PhD (CSCs)
David Hawke, PhD (Proteomics core)
Janusz Rak, PhD (Microvesicles)
Dipen Maru, MD (Pathology)

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