Mouse 101
Cancer Stem Cells
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Areas of Investigation in Cancer Research

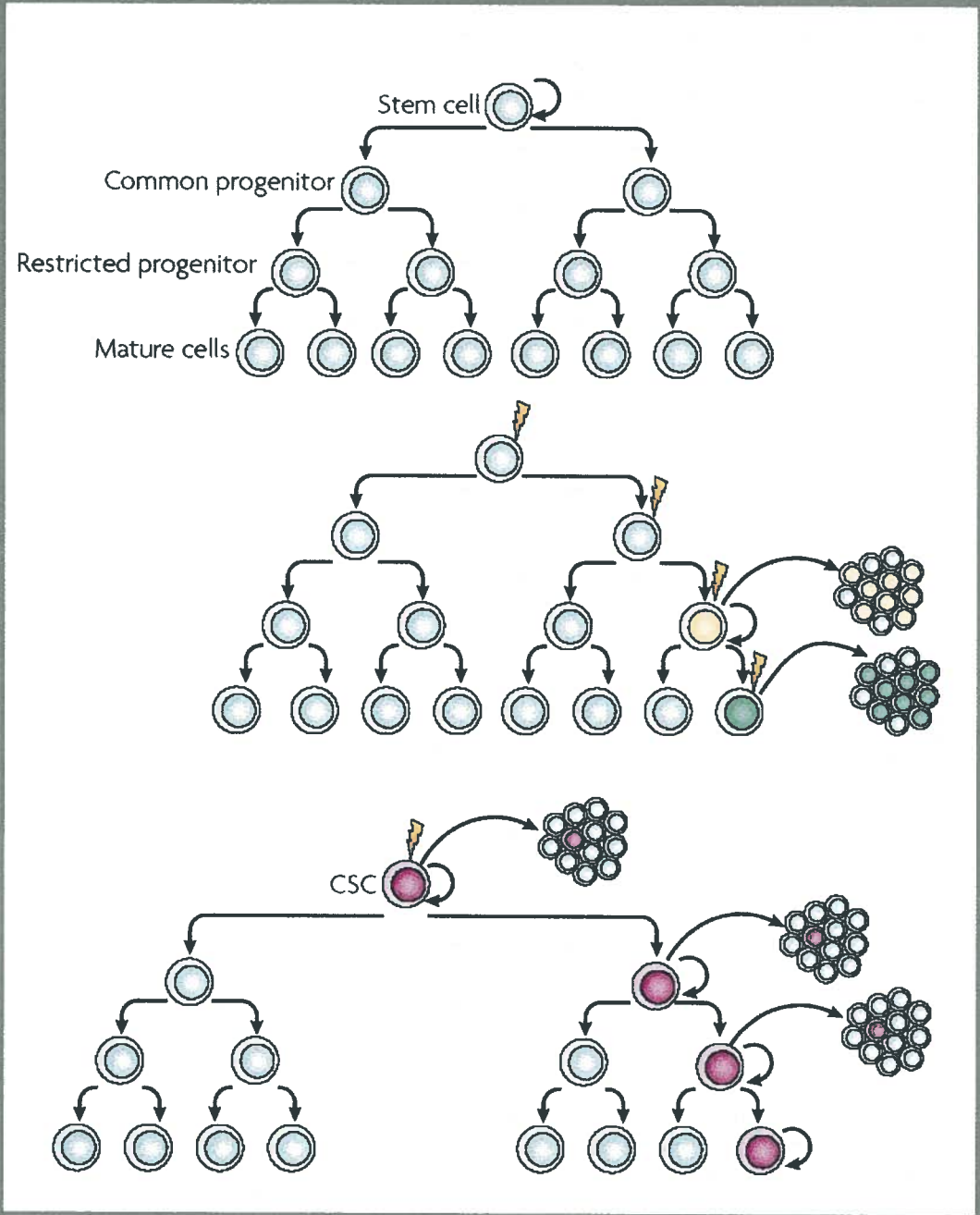
- The genetic and epigenetic basis of cancer
- The cell of origin for cancer
  - Early detection markers
  - Prevention therapies
- The mechanisms leading to progressive disease
  - Resistance to therapy
  - Metastasis
- The role of the microenvironment

Tumor Cells are Heterogeneous

- Clinically observed heterogeneity:
  - Morphological heterogeneity in some but not all types of tumors (e.g. hematopoietic-CML, germ cell tumors, colon cancer, and some brain tumors)
  - Marker expression
  - Response to therapy
  - Metastatic propensity
- Experimentally observed:
  - All the above plus
  - Tumorigenicity following transplantation

Hierarchical (Cancer Stem Cell) Models and Clonal Evolution Models

- Hierarchical
  - Tumorigenic cells give rise to non-tumorigenic cells (recapitulating differentiation)
  - Tumor cells differ epigenetically (soft-wired)
  - May be related to using stem/progenitor cell self-renewal program
- Clonal Evolution
  - Not necessarily hierarchical
  - Unstable clonal evolution gives rise to genetically distinct subpopulation (hard-wired)
- Both mechanisms can co-exist



Normal Differentiation-
Cells are produced with
Successively less
Differentiative and
Proliferative capacity

Clonal Evolution-
Heterogeneity is stable
And genetically
Determined

Cancer Stem Cells-
Epigenetically programmed
Tumorigenic cells give
Rise to non-tumorigenic
Cells

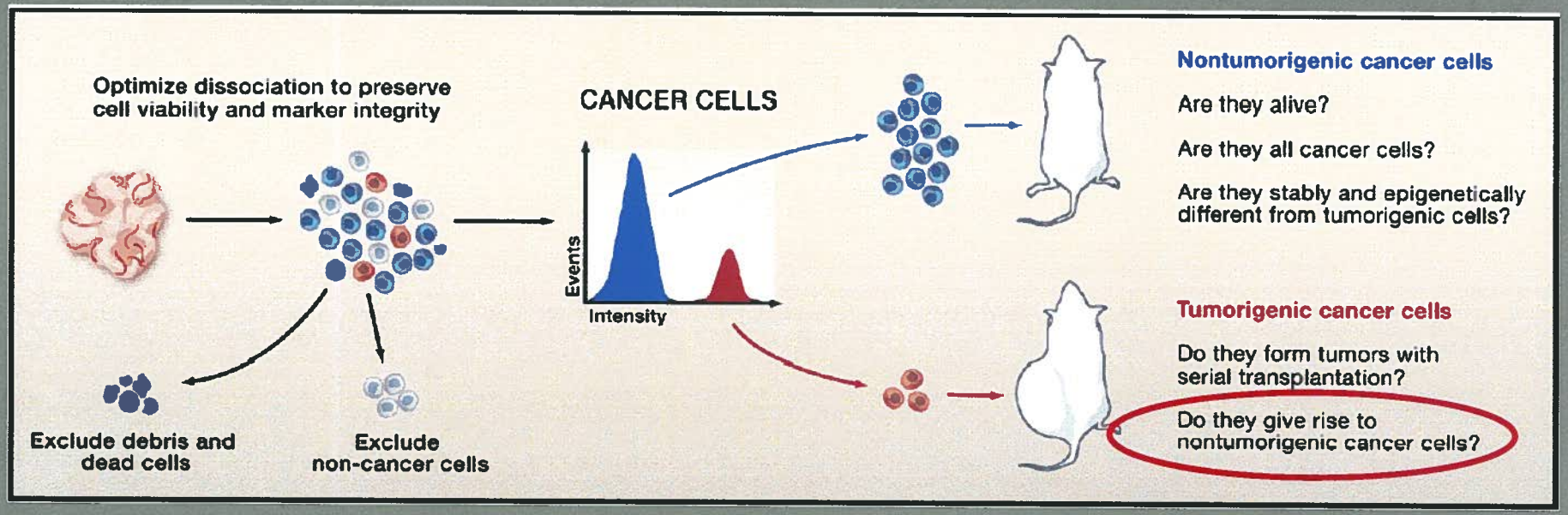
Clarifications re CSC Theory

- Does not make assumptions about the frequency of tumor initiating cells
- Does not make assumptions about the cell of origin (CSC's are <u>not</u> necessarily transformed normal stem cells)
- Does not discount the possibility of plasticity: non-CSC may convert to CSC's under the appropriate microenvironmental conditions

Experimental results/strategies supporting the CSC theory

- Measures the heterogeneity of cellular populations within tumors
  - Tumor initiation
  - Marker expression
  - Self-renewal (in vitro assays for stem cell-specific growth)
- Tumor initiating populations
  - Express markers of stem/progenitors
  - Produce tumors that contain cells expressing terminal differentiation markers

Cancer Stem Cells Separations Using Surface Markers

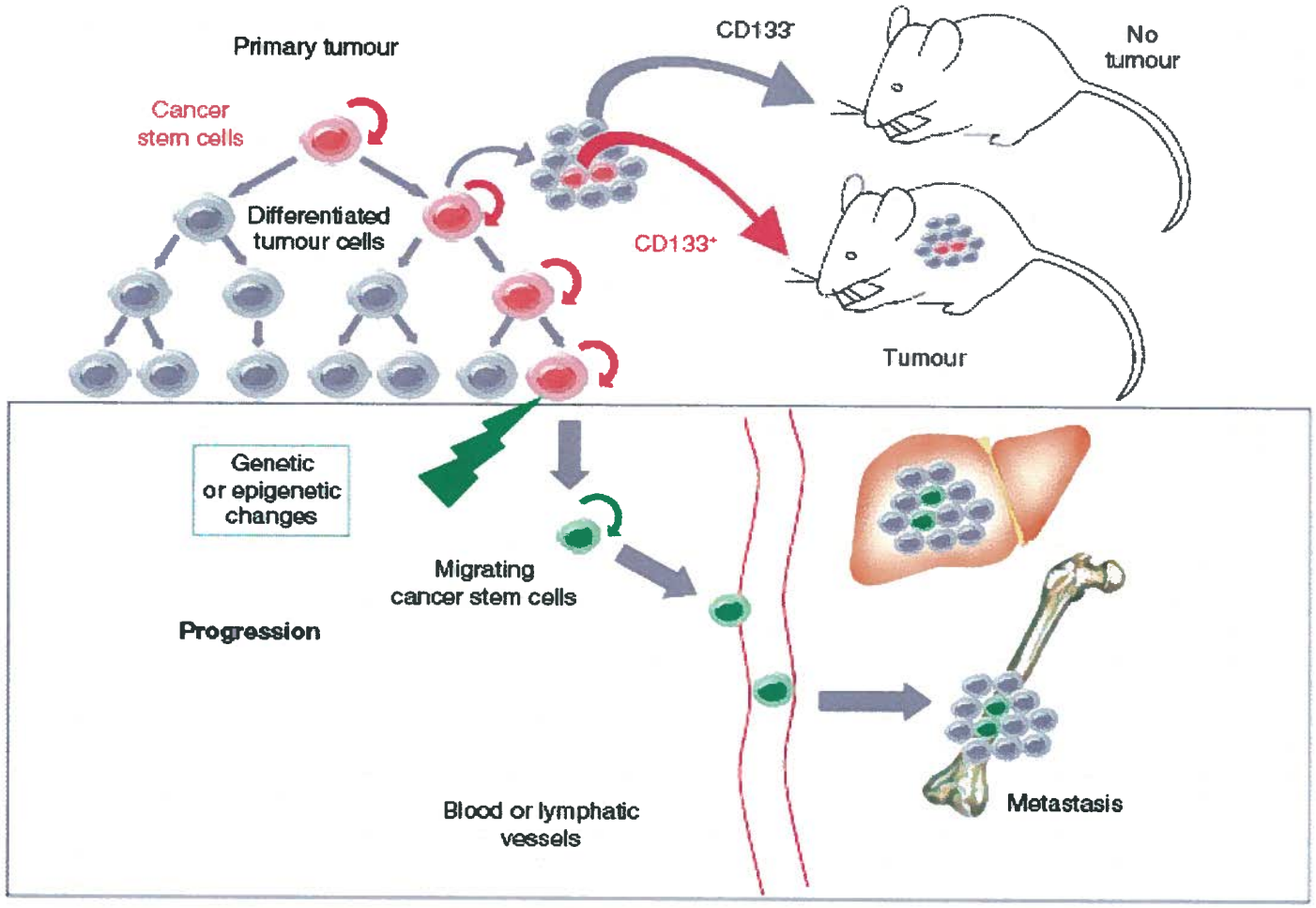


Most markers are related to Differentiation antigens- often With no specific known function

↑

Immunocompromised Mice

Hierarchical Development and Clonal Evolution Probably Co-Exist in Advanced Cancers of Certain Types



Uncertainties....

- How does the microenvironment effect tumorigenicity
  - Immune competence- immunological rejection influences tumorigenicity in vivo, inflammation can stimulate tumorigenesis
- Not all cancers are organized hierarchically
- Multiple mechanisms exist for the development of a <u>single</u> type of cancer (contributions from the initiating mutation + cell of origin + genetic background)

Using Mouse Models for Cancer Research

- Transplantation hosts
  - Xenografts (immunocompromised)
  - Syngeneic (± genetic modification)
- Mouse models of human cancer
  - Disease process
  - Source of primary cells

Transgenic Cancer Models

- Target genetic modifications based on knowledge of human cancers to the correct tissue-specific compartment
  - Over-expression
  - Mutated gene product
  - Loss of a tumor suppressor
- Targeting tissue specific modifications
  - Cloned promoter constructs (plasmid or BAC-based)
  - Knock-out (tumor suppressor) or knock-in (mutant gene) at endogenous promoter locus

Advantages to using mouse cancer models for investigating tumor heterogeneity

- Theoretical strengths
  - Tumor development in correct microenvironment
  - Tests conservation of mechanism with <u>known</u> initiating mutations
  - Can target specific cell of origin
- Practical considerations
  - Genetic tricks to "mark" specific cell populations- useful for separation and imaging
  - Unlimited source of tumor tissue

Two Models That Illustrate the Power of Mouse Models

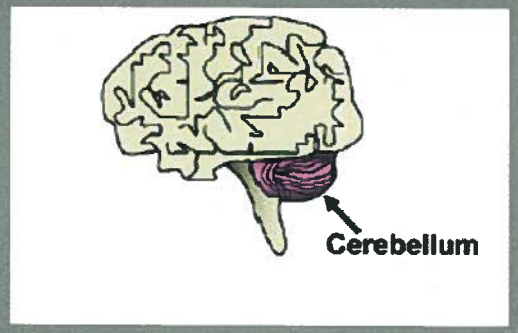
- Medulloblastoma model

Read et al., Cancer Cell 15:135-147 (2009)

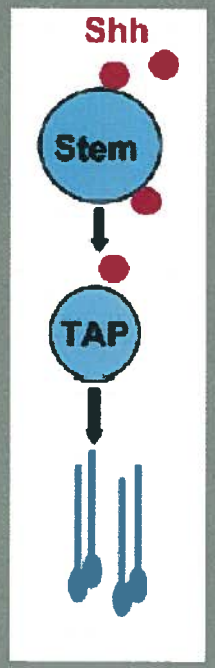
- Intestinal cancer model

Barker et al., Nature 457:608-611 (2009)

Mouse Model of Medulloblastoma



Ptch$^{+/-}$ mice $\xrightarrow{20\%}$ Medulloblastomas tumors of cerebellum

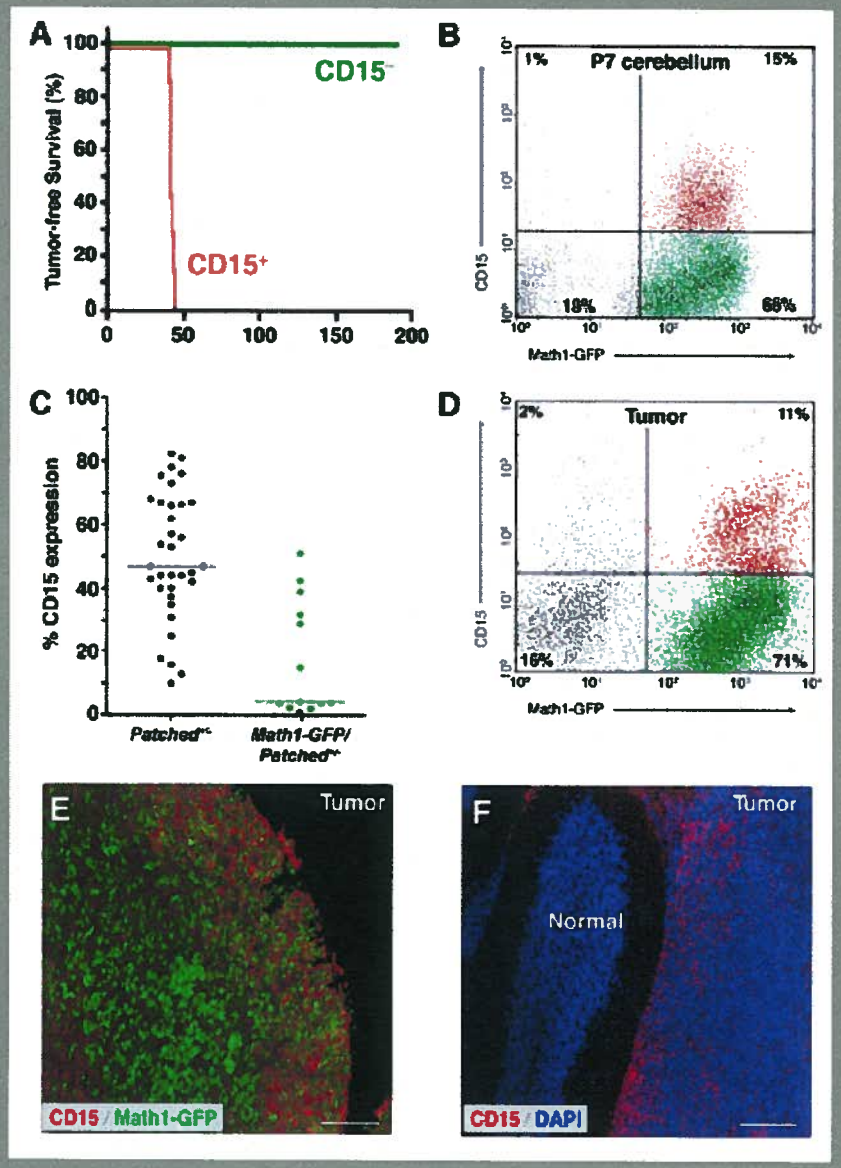


Neural stem cell (CD133+)

Granule neuron precursors (MATH1+)

Granule neurons

Identifying Tumor Initiating Cells in a Mouse Model

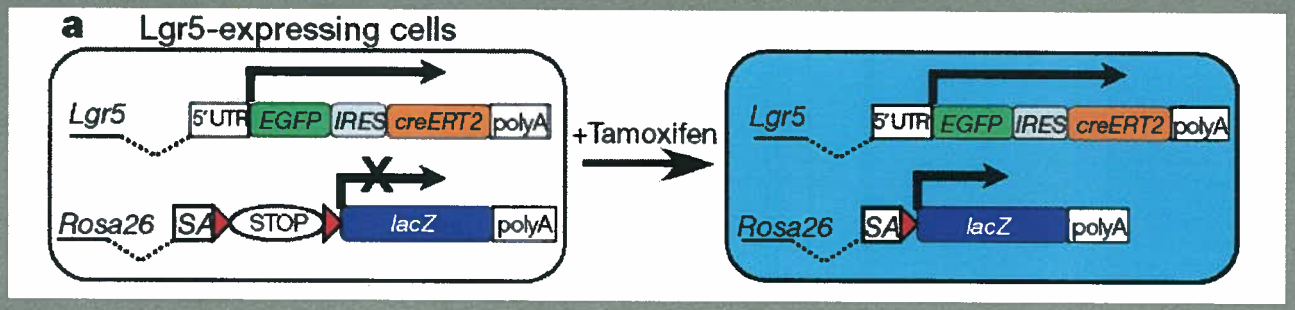


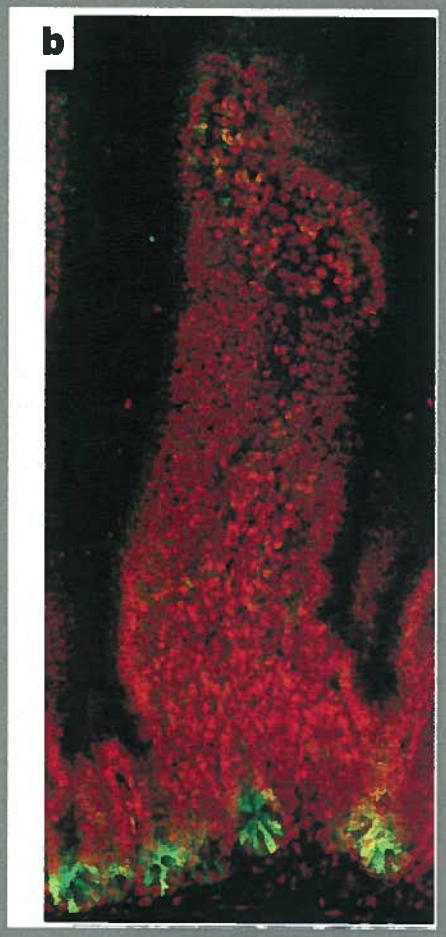
Primary tumors and normal Developing brain have a Proportion of Math1+,CD15+ Cells

Secondary tumors generated From CD15+ cells recapitulate The CD15- phenotype of the Bulk tumor

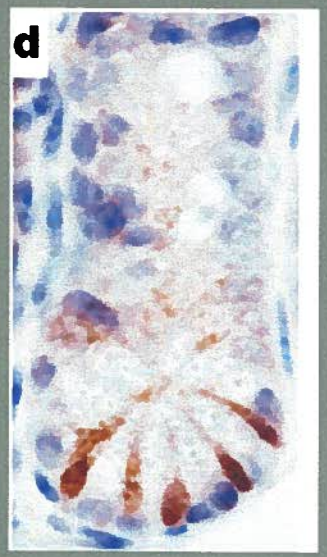
These authors conclude that A granule neuron progenitor Is most likely the cell of origin For medulloblastoma

An Intestinal Stem Cell-Dependent Knock-in Expression Model

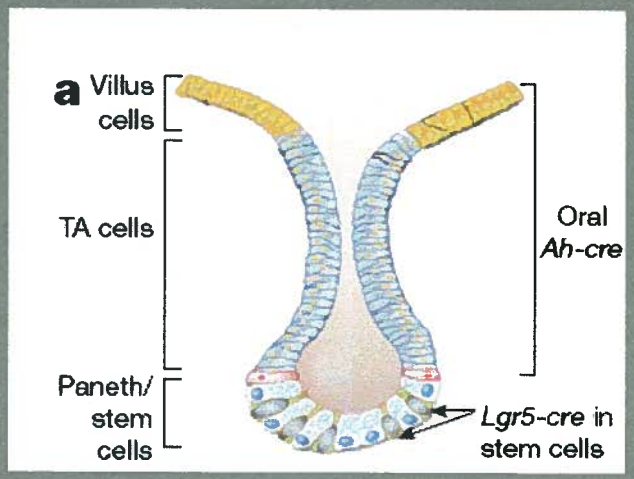


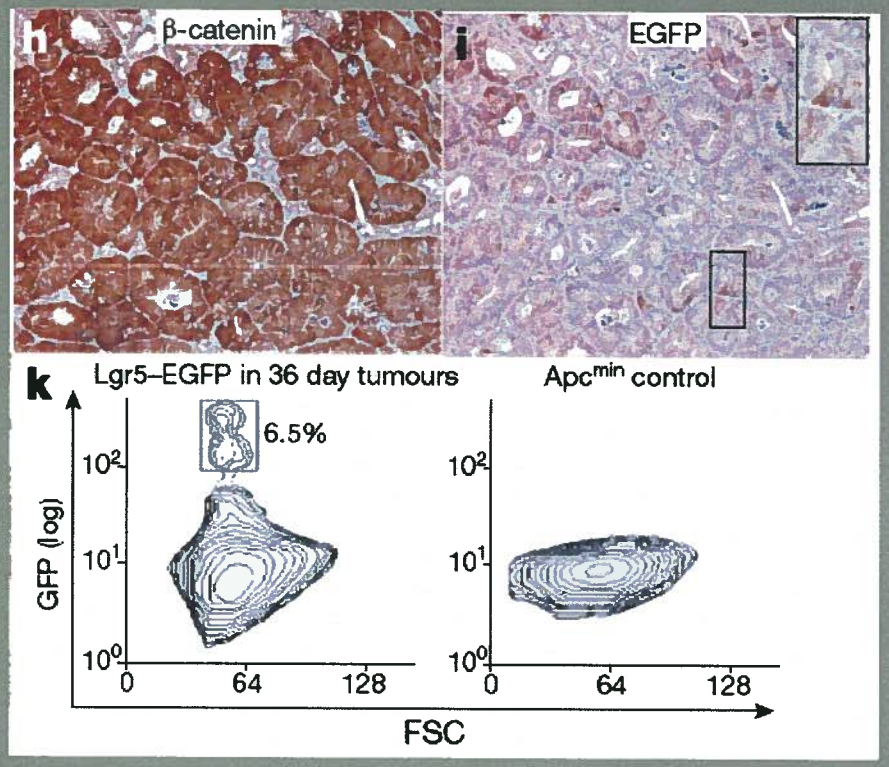


- EGFP (green) marks stem cells
- Red marks nuclei (total cells)
- LacZ marks progeny of stem cells

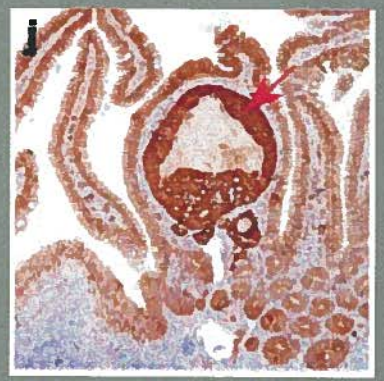


Controlled deletion of the tumor suppressor APC in mouse intestinal cells

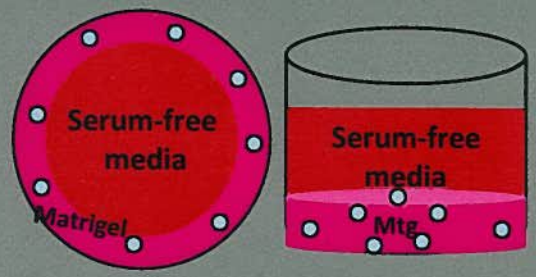




- Lgr5-cre leads to loss of APC in stem cells (lots of tumors!!)
- Ah-cre leads to loss of APC in intermediate (TA) cells (one adenoma)

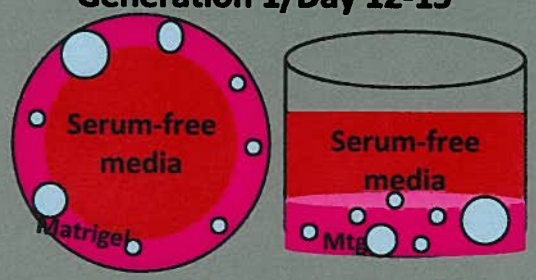


Protosphere-forming assay (3-D)



Generation 1/Day 0

Generation 1/Day 12-15



Single cell

Sphere

Colony-forming assay (2-D)

Passage 0/Day 0

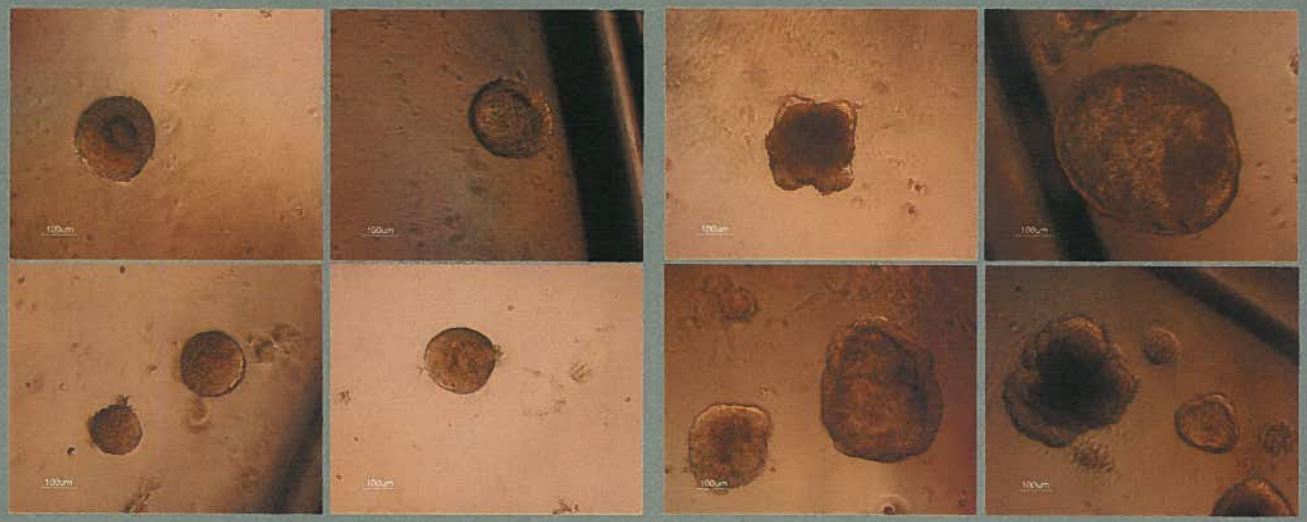
Passage 0/Day 5-8

Single cell

Colony

Protosphere Morphologies

WT G1/D12 | *Pten*$^{-/-}$*P53*$^{-/-}$ G1/D12



1. *Pten*$^{-/-}$*P53*$^{-/-}$ protospheres are 3X larger in diameter
2. *Pten*$^{-/-}$*P53*$^{-/-}$ protospheres contain 50% more cells

Next Generation Questions

- Which tumor types follow a hierarchical (CSC) model
- What are the mechanisms (i.e. developmental histories) associated with specific gene mutations/cells of origin
- What are the epigenetic (or genetic differences between tumorigenic and non-tumorigenic cells
- Can more robust markers be identified that are useful in diagnosis/prognosis of human cancers