Prostate Cancer Stem Cells and Metastasis- What is the connection? BSA-November 3, 2009

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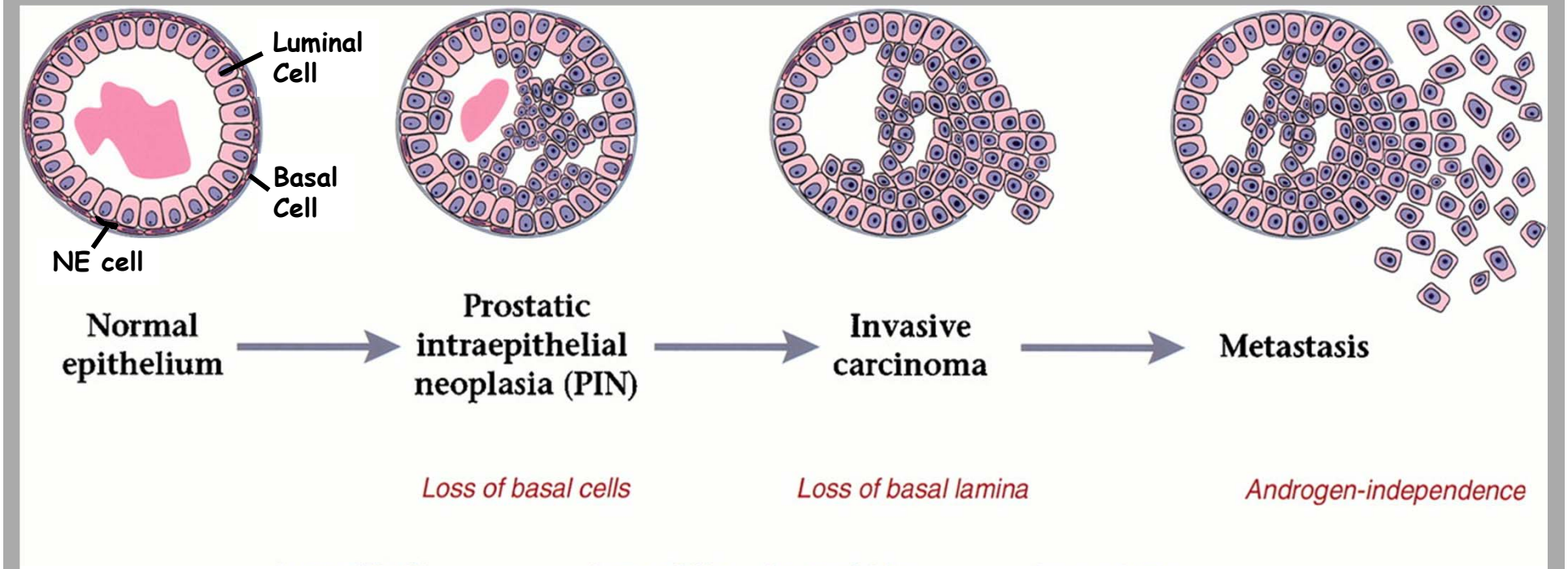
Cellular origin of metastatic PC

- The properties of metastatic PC parallel those expressed by transformed progenitor/stem cell populations
- What are the identities of prostate cancer tumor initiating cells?
- What if any are the molecular differences between tumor initiating and metastasis initiating PC cells?

Prostate Cancer Progression



Luminal Cells: CK8+

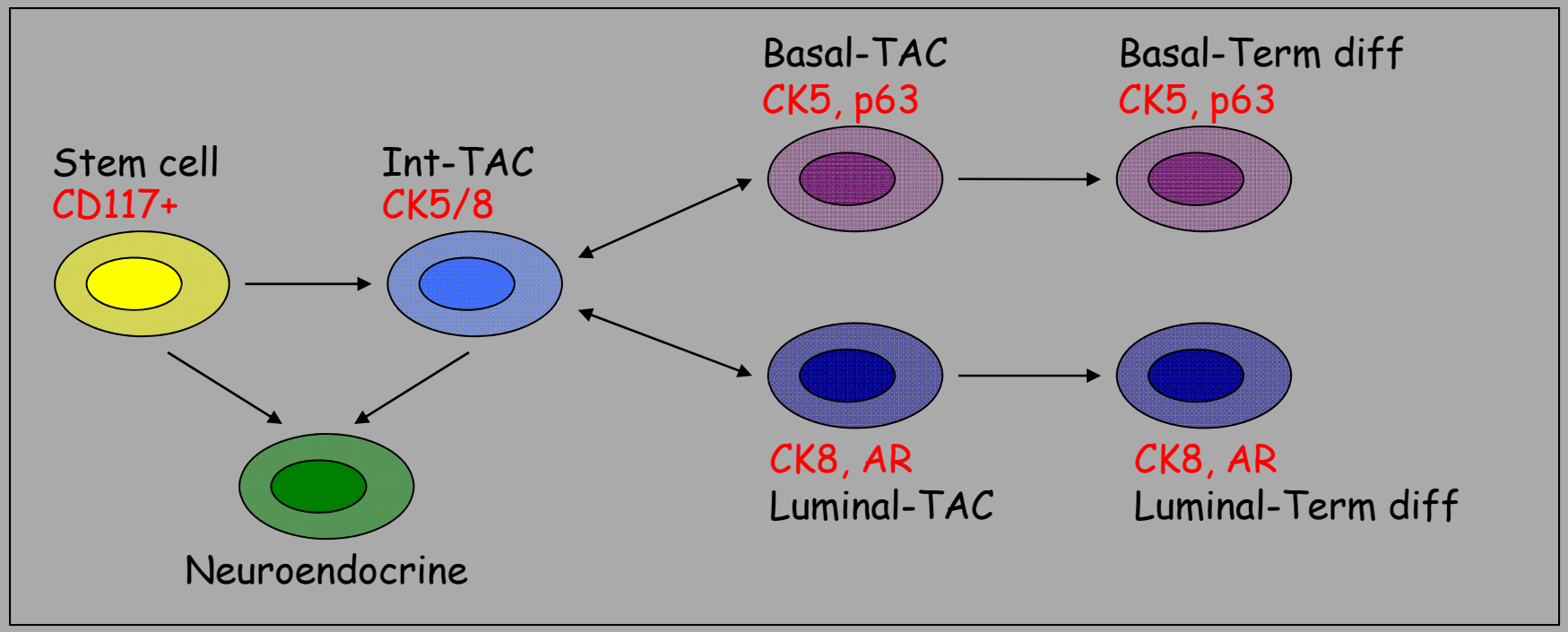
Basal Cells: CK5+, p63

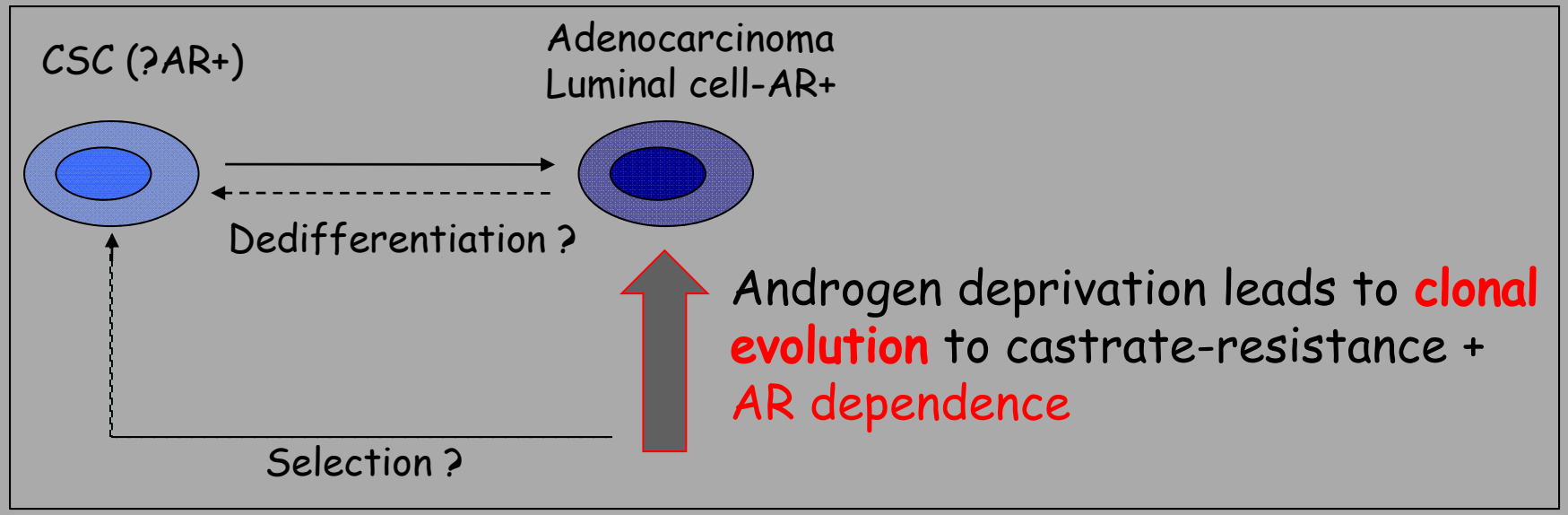
Neuroendocrine Cells: synaptophysin+, β–3 tubulin

Properties of PC Metastasis

- Poorly differentiated CK8+ carcinomas
- Metastases can demonstrate mixed lineage markers, especially luminal and neuroendocrine
- A large percentage of castrate-resistant prostate cancers express mutated AR, suggesting evolution from an AR+ cell

Lineage Maps of Normal Prostate and PC Development





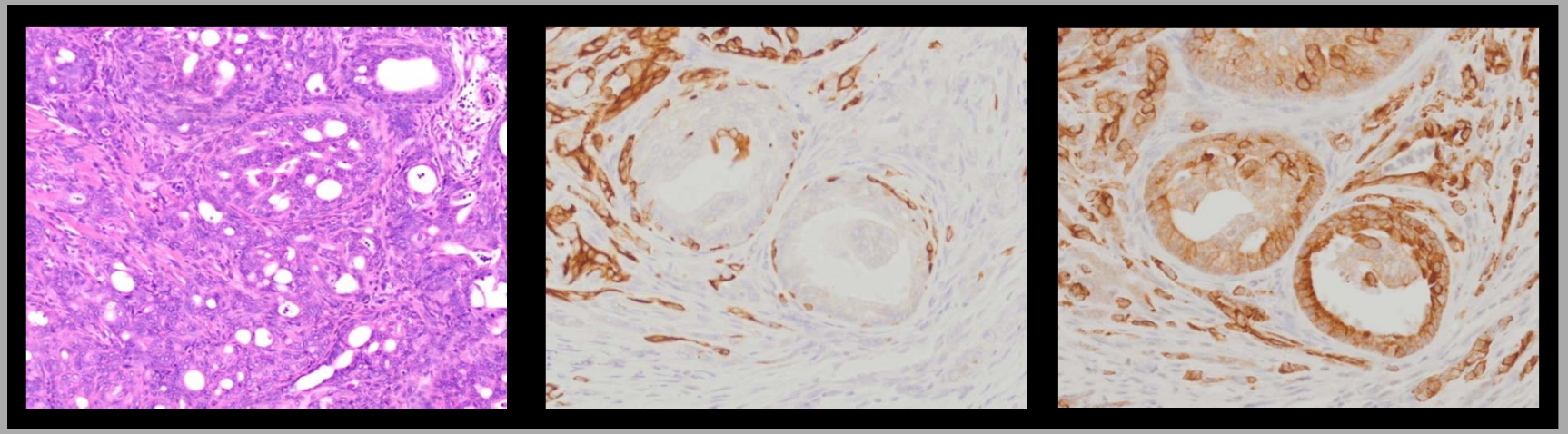
Modeling PC in the mouse: (*PbCre+*) $PTEN^{fl/fl}$,$P53^{fl/fl}$,Luc^{+}

- The PTEN pathway is frequently altered in human PC, especially high in metastatic PC

- Development of invasive and disseminated adenocarcinoma, but not clinically-apparent metastatic tumors

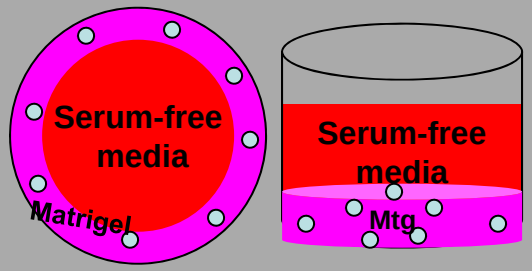
- Death from urinary outflow obstruction at ~ 6 mos.

- Proliferation of cells with intermediate ($CK5^{+}$/$CK8^{+}$) and luminal phenotypes



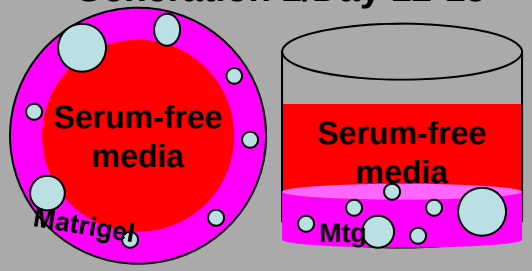
H&E CK5 CK8

Protosphere-forming assay (3-D)



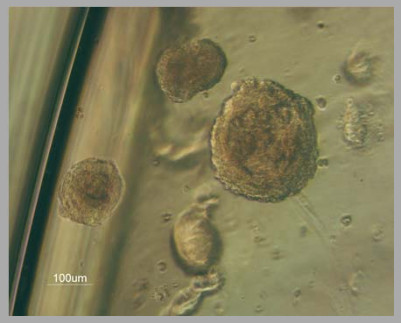
Generation 1/Day 0

Generation 1/Day 12-15



Single cell

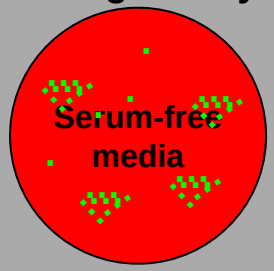
Sphere



Colony-forming assay (2-D)

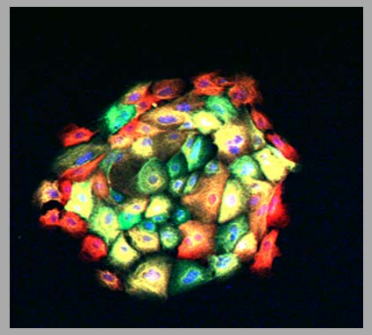
Passage 0/Day 0

Passage 0/Day 5-8

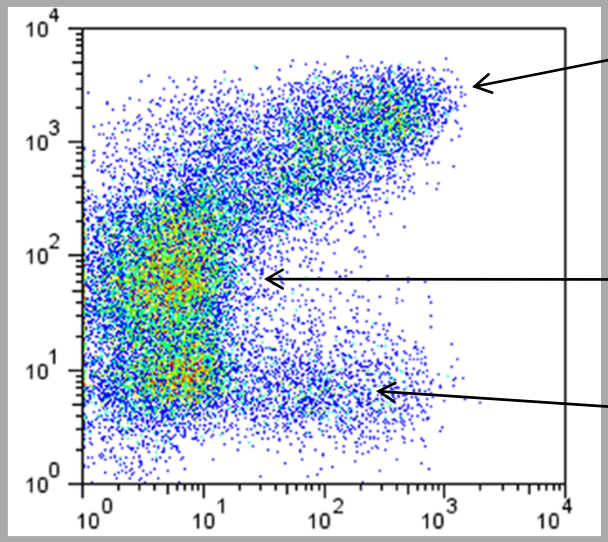


Single cell

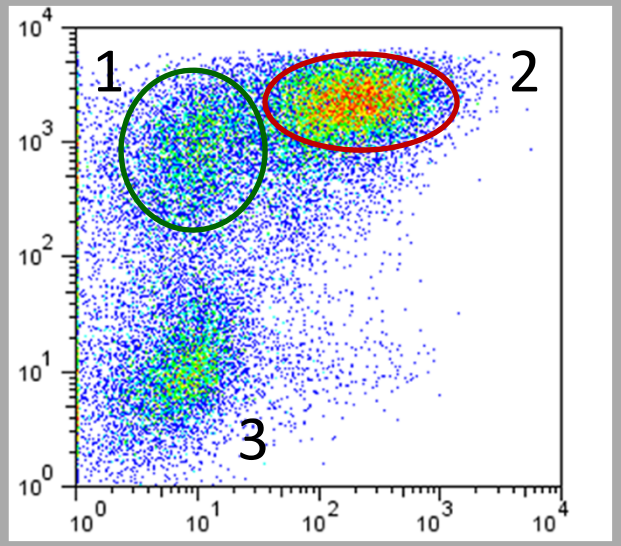
Colony



A New Progenitor Population is Observed in *PTEN$^{-/-}$P53$^{-/-}$* Prostates

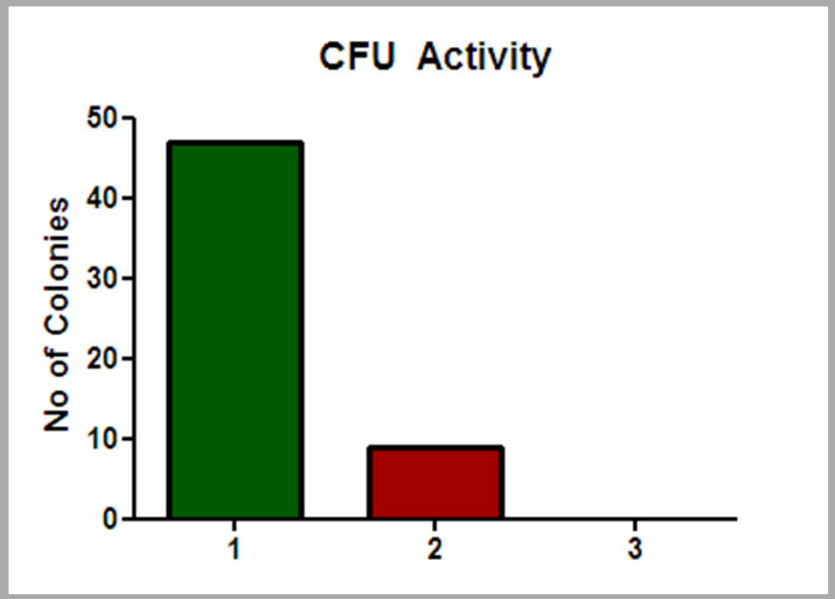


Wild Type

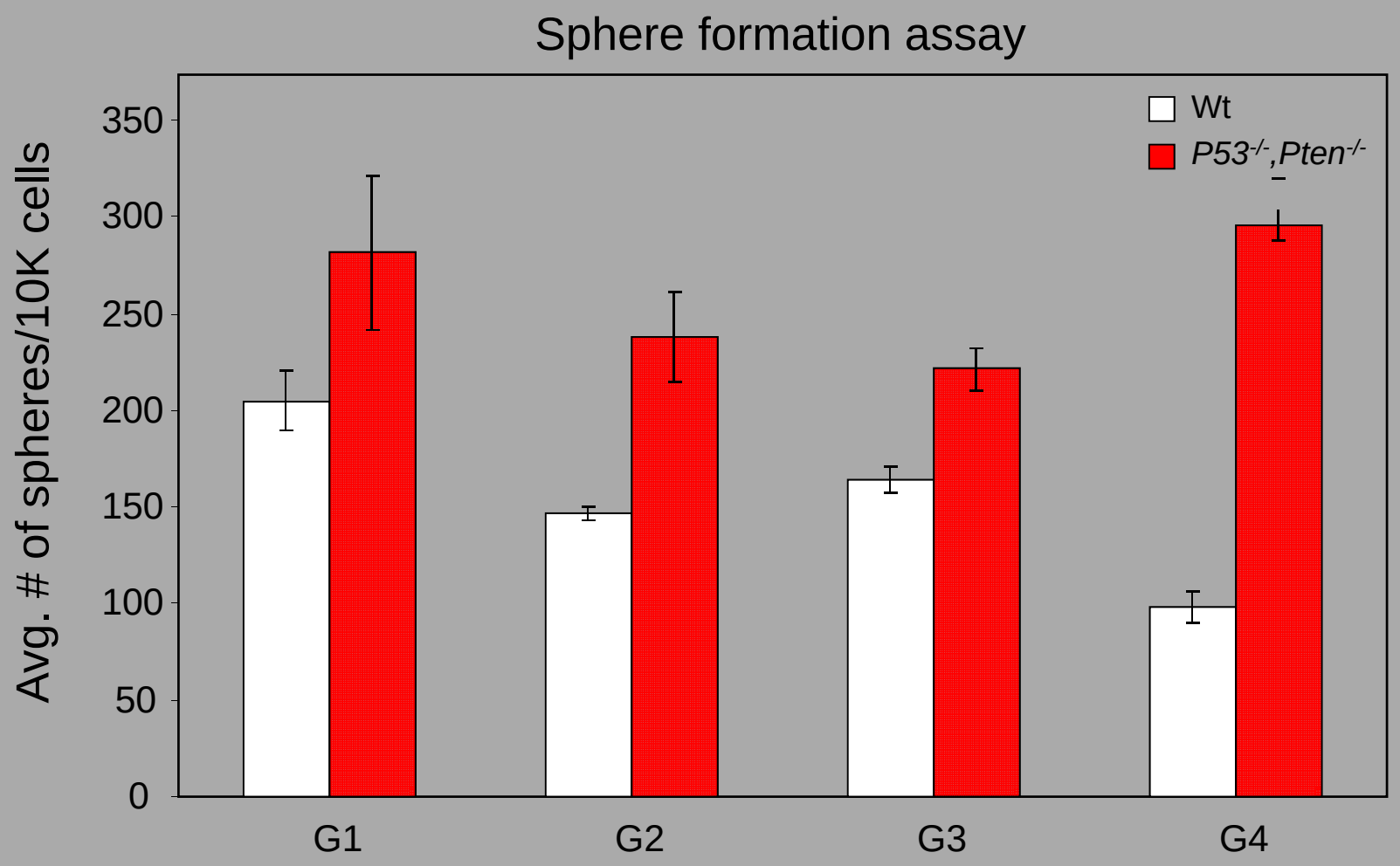


PTEN$^{-/-}$P53$^{-/-}$

CFU/SFU activity in normal prostate is a rare population that co-fractionates with basal cells

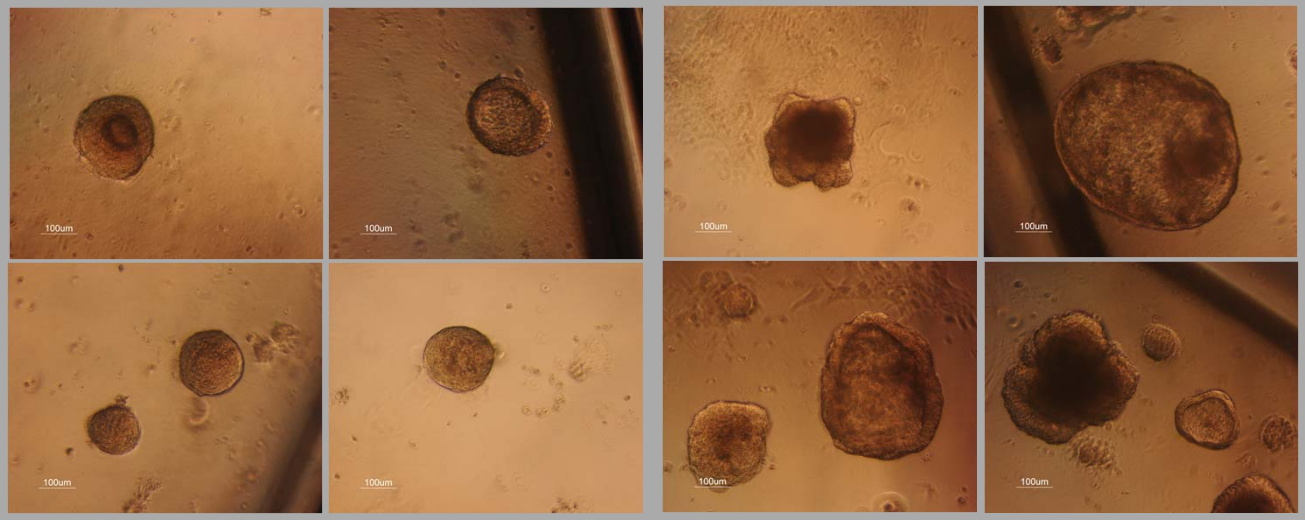


Transformed Progenitors Show Increased Self-Renewal



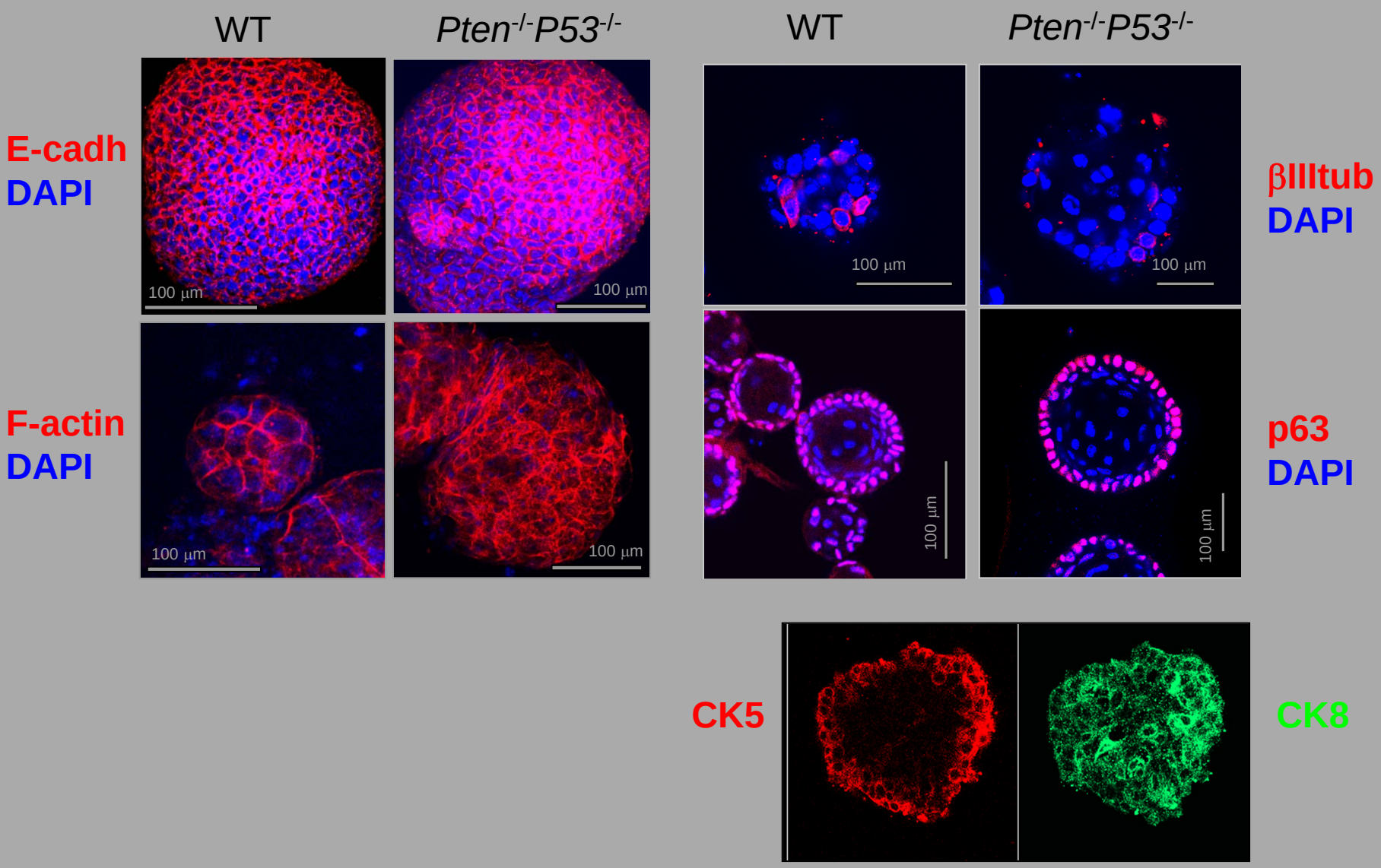
Protosphere Morphologies

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

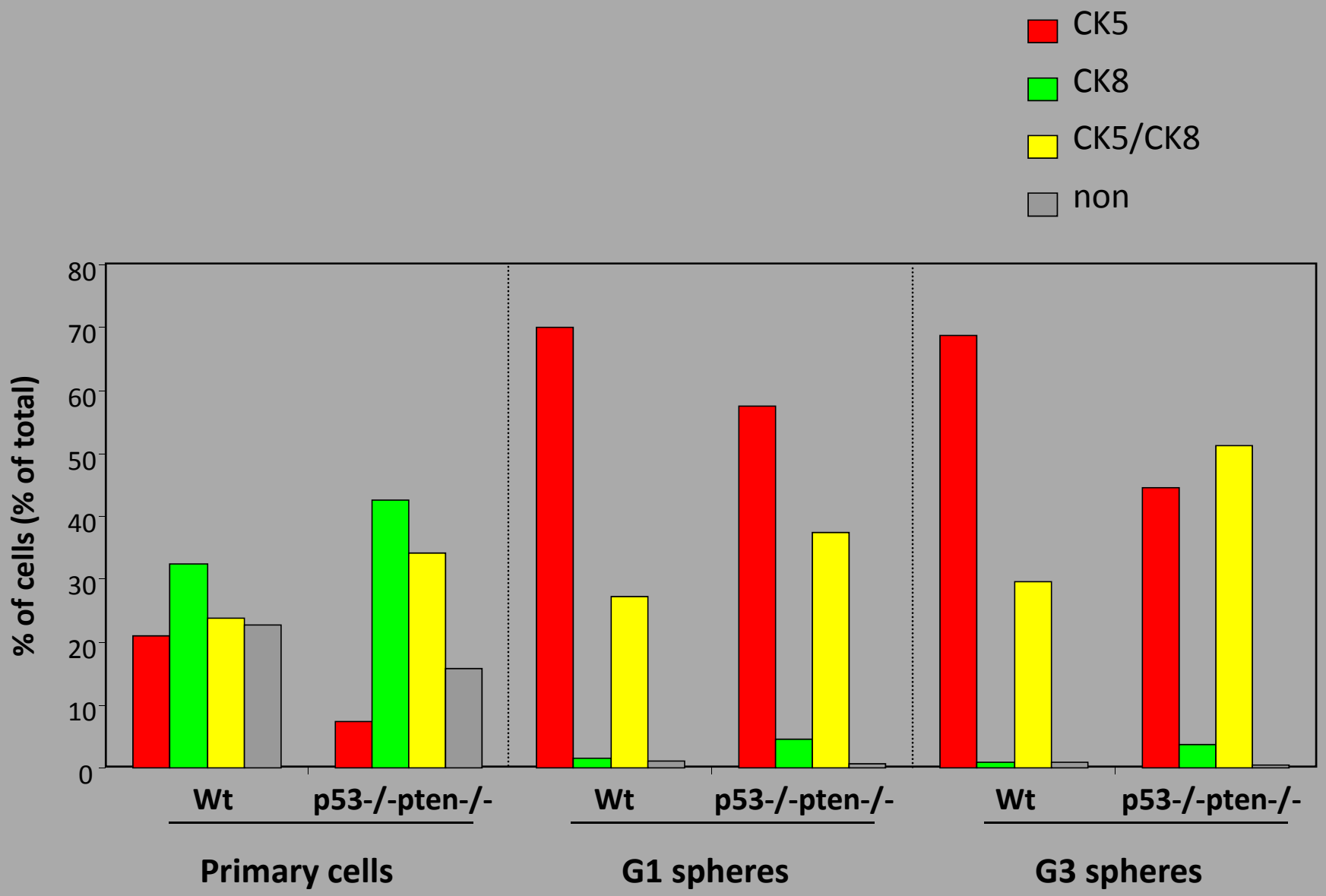


1. *Pten$^{-/-}$P53$^{-/-}$* protospheres are 3X larger in diameter
2. *Pten$^{-/-}$P53$^{-/-}$* protospheres contain 50% more cells

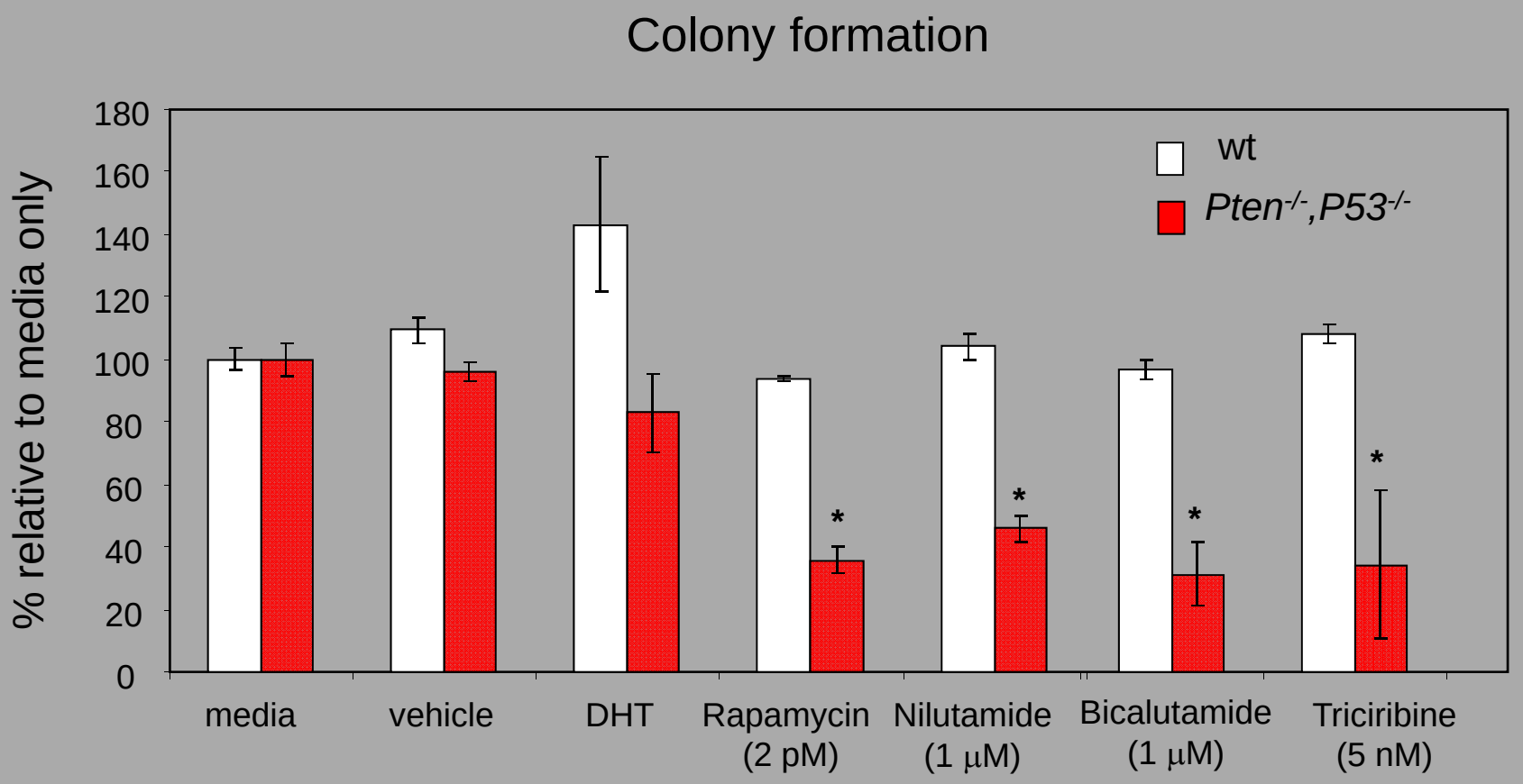
In situ Assays of Differentiation and Signal Transduction Markers



Differentiation Potential in Transformed spheres



Transformed Progenitors Are Differentially Inhibited by Drugs

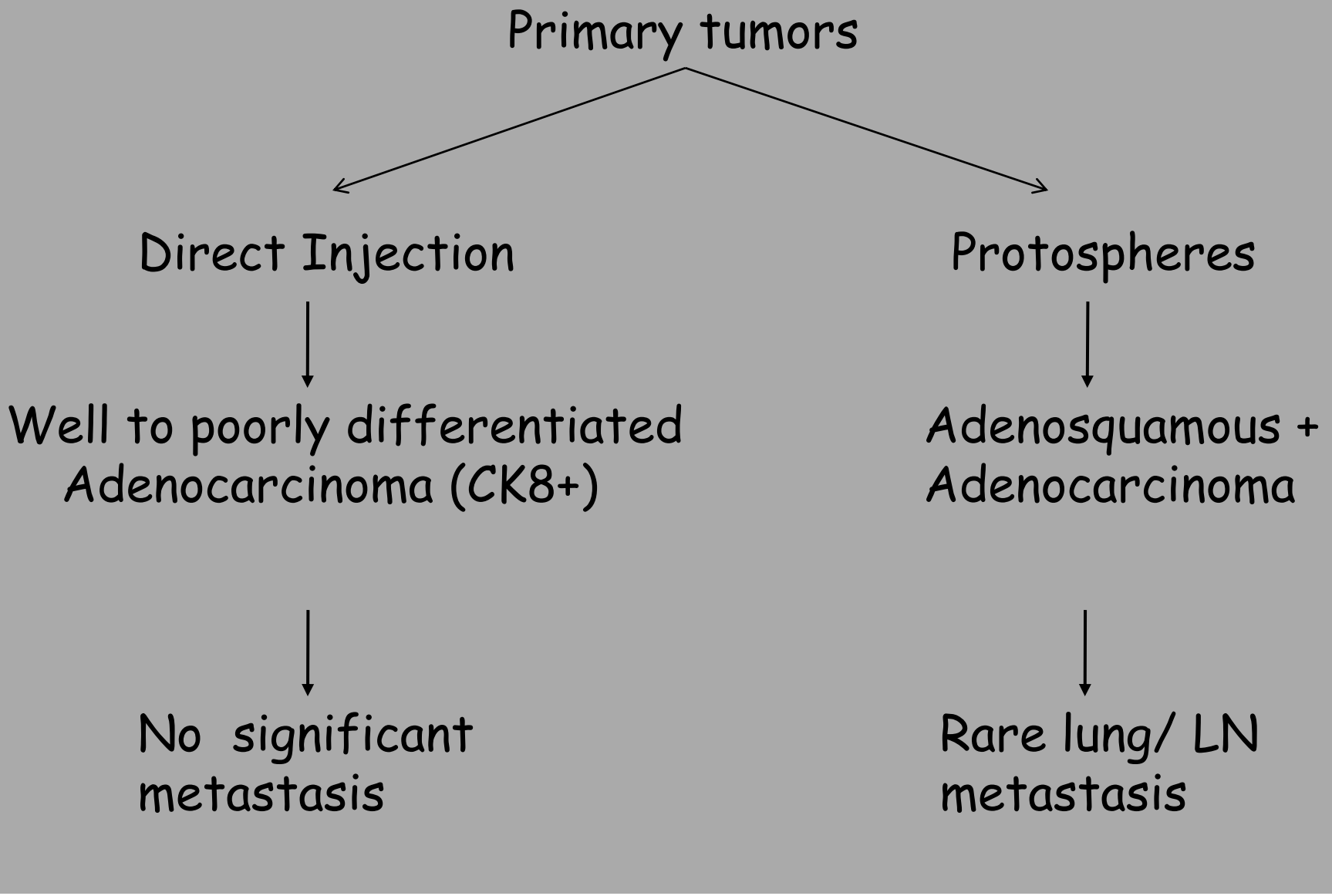


Summary

- $PTEN^{-/-};P53^{-/-}$ prostate progenitors demonstrate perturbations of cytoskeletal organization, self renewal and differentiation

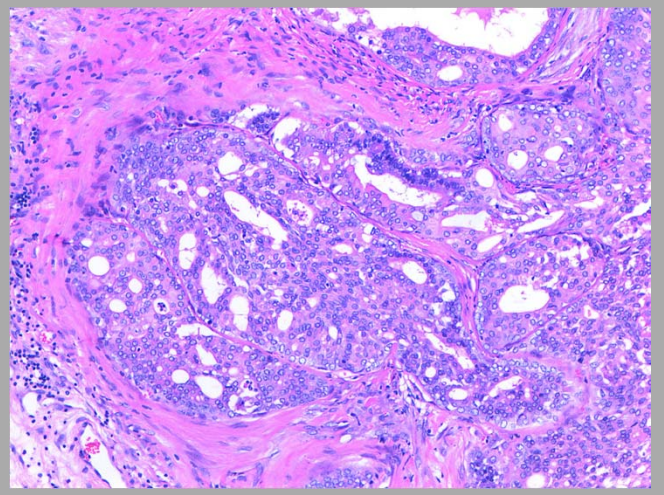
- These progenitors express altered drug sensitivity- i.e. AKT "addiction" and acquired AR dependence

Orthotopically transplanted primary tumor cells do not lead to metastatic colonization



Clonally-derived Cell Lines

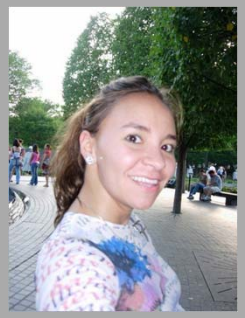
Clone 2 was derived from an orthotopic adenocarcinoma



- Immature phenotype (CK5+/CK8+/AR^{low})
- Bipotential differentiation in vivo
  - Direct injection leads to adenocarcinoma
  - Co-injection of matrigel leads to the presence of tumors cells with a basal phenotype
- Highly metastatic
- Androgen-responsive

Conclusions and Implications

- *PTEN*$^{-/-}$*P53*$^{-/-}$ progenitors express a unique phenotype relative to wt
  - ? Expansion of an existing progenitor pool
  - ? Acquired phenotype in a more mature cell
- AR-dependence of progenitors suggests that clonal evolution could be directly selected in the tumor initiating cell population
- Metastatic PC cell lines with known initiating mutations provide models for defining colonization pathways



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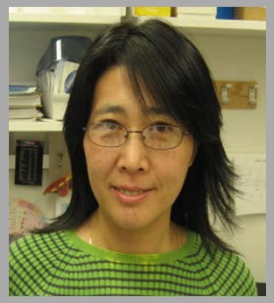

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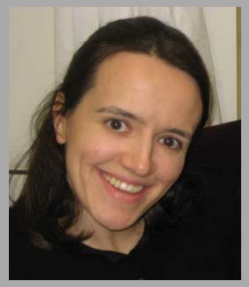

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