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

Kathleen Kelly, Ph.D.
Cell and Cancer Biology Branch, CCR, NCI
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

Provinces of Prostatic Carcinoma:
- Normal epithelium
- Prostatic intraepithelial neoplasia (PIN)
- Invasive carcinoma
- Metastasis

Loss of basal cells → Loss of basal lamina → Androgen-independence
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

Stem cell
CD117+

Int-TAC
CK5/8

Neuroendocrine

Basal-TAC
CK5, p63

Basal-Term diff
CK5, p63

CK8, AR
Luminal-TAC

CK8, AR
Luminal-Term diff

CSC (?AR+)

Adenocarcinoma
Luminal cell-AR+

Dedifferentiation ?

Androgen deprivation leads to clonal evolution to castrate-resistance + AR dependence

Selection ?
Modeling PC in the mouse: (PbCre+) PTEN^{fl/fl}, P53^{fl/fl}, Lu{c^+}

• 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
Protosphere-forming assay (3-D)  Colony-forming assay (2-D)

- Generation 1/Day 0
  - Serum-free media
  - Matrigel

- Generation 1/Day 12-15
  - Serum-free media
  - Matrigel

- Passage 0/Day 0
  - Serum-free media

- Passage 0/Day 5-8
  - Serum-free media

- Single cell
- Sphere

- Single cell
- Colony
A New Progenitor Population is Observed in PTEN⁻/⁻P53⁻/⁻ Prostates

CFU/SFU activity in normal prostate is a rare population that co-fractionates with basal cells.
Transformed Progenitors Show Increased Self-Renewal

Sphere formation assay

- Wt
- P53⁻/⁻, Pten⁻/⁻
Protosphere Morphologies

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

WT  Pten\textsuperscript{-/-}\text{-P53\textsuperscript{-/-}}

E-cadherin
DAPI

F-actin
DAPI

β\textit{III} tubulin
DAPI

P63
DAPI

CK5

CK8
Differentiation Potential in Transformed spheres

- CK5
- CK8
- CK5/CK8
- non

Wt, p53/-, pten/-

Primary cells, G1 spheres, G3 spheres

% of cells (% of total)
Transformed Progenitors Are Differentially Inhibited by Drugs

Colony formation

% relative to media only

media, vehicle, DHT, Rapamycin (2 pM), Nilutamide (1 μM), Bicalutamide (1 μM), Triciribine (5 nM)

wt, Pten-/-, P53-/-
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.

Primary tumors

- Direct Injection:
  - Well to poorly differentiated Adenocarcinoma (CK8+)
  - No significant metastasis

- Protospheres:
  - Adenosquamous + Adenocarcinoma
  - Rare lung/LN metastasis
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

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