Activation of Wnt/beta-catenin Signaling Promotes Castrate-Resistant PCa Progression

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Prostate cancer progression

- Pre-Neoplastic Cells
- Treatment
- Androgen Deprivation Therapy

normal → localized disease → metastasis → castration resistant

PIN → PCa

(PIN: Prostate Intraepithelial Neoplasia)
Mechanisms for CRPC

- AR-centered mechanisms
  - AR amplification
  - AR mutations
  - AR activation by growth factors
  - AR variants
  - Intra-prostate synthesis of androgens

- Pathways that bypass AR: NED

DHT: Dihydrotestosterone
AR: Androgen Receptor
ARE: Androgen Response Element
NED: Neuroendocrine Differentiation
PSA: Prostate Specific Antigen
HSP: Heat Shock Protein
NE phenotype is increased in CRPCa


Our own research has found about 40% mCRPC has NED and studies from other labs found 25% heavily treated PCa have small cell carcinoma.
Primary NE PCa is rare but aggressive
NE PCa is androgen independent: AR negative
Neuroendocrine (NE) PCa and NE differentiation (NED) in PCa

- NE PCa is rare but aggressive
- NE PCa is androgen independent: AR negative
- NE Differentiation of adenocarcinoma occurs in 40% to 100% of advance PCa
- NED is associated with poor prognosis
- NE secretions promote the androgen independent growth of a non-NE PCa

Wnt/beta-Catenin signaling is involved in PCa initiation and progression
Wnt/b-Catenin Signaling

Cyto/nuclear b-Catenin = active Wnt/b-Catenin
Active Wnt/b-Catenin is associated with advanced stage PCa

- Mutations in Wnt/b-Catenin signaling were enriched in CRPC (Grasso, et al. *Nature*, 2012)
- Nuclear b-Catenin was detected in CRPC specimens (Kumar, et al. *PNAS*, 2011)
- Wnt1 and Nuclear b-Catenin was detected in 77% of lymph node metastases and in 85% of skeletal metastases. (Chen, et al. *Cancer*, 2004)
Constitutively activate Wnt/b-Catenin signaling in mouse prostate

Deletion of Exon 3 results in constitutive appearance of nuclear b-Catenin in prostatic epithelium
Dominant Active (DA) b-Catenin

Harada et al. EMBO 18:5931, 1999

Yu et al. Prostate 2009
Selected promoters target Cre transgene at different times in prostate development
Embryonic activation of Wnt/b-Catenin by Nkx3.1-Cre
Organ Rescue: Nkx3.1-Cre/b-Catenin prostates develop PIN

Yu et al. Prostate 2009
Foxy1 and Foxy2 are expressed in prostate

Yu et al. Mol Endocrinol. 2006
Mirosevich et al. Prostate 2006
Foxy2 is expressed in rare NE cells in adult prostate
Fňoa2 is expressed in human NE PCa

Yu et al. Mol Endocrinol. 2006
Activation of Wnt/b-Catenin induces the expression of Foxa2

Yu et al. Prostate 2009
Post-natal activation of Wnt/b-Catenin by PB-Cre
Activation of b-Catenin in prostate by PB-Cre causes hyperplasia and PIN at 12 weeks

Control prostate

DA b-Catenin prostate

H&E

b-Catenin IHC

PIN: Prostate Intraepithelial Neoplasia
DA b-Catenin: Dominant Active b-Catenin
Activation of b-Catenin in prostate by PB-Cre causes HGPIN at later age (9 Months)

H&E  b-Catenin IHC

PIN: Prostate Intraepithelial Neoplasia
DA b-Catenin: Dominant Active b-Catenin

Yu et al. Prostate 2009
Fonna2 was re-expressed in DA b-Catenin mouse prostates

Control prostate                  DA b-Catenin prostate

DA b-Catenin: Dominant Active b-Catenin

Yu et al. Prostate 2009
T-antigen/DA b-Catenin mice

Lady LPB-Tag 12T-7f Develops PIN

Activated b-Catenin b-Cat$^{Δex3}$ Develops PIN

LPB-Tag + b-Cat$^{Δex3}$

Yu et al. Oncogene, 2011
Activation of Wnt/b-Catenin and T-antigen results in invasive prostate cancer

Yu et al. Oncogene, 2011
Fossa2 is expressed at the invasive front of PCa
DA b-Catenin/T-antigen mouse prostates display increased NED

Yu et al. Oncogene, 2011
Question: Can active Wnt/b-Catenin signaling promote castrate-resistant growth?

Control mice

Castration

prostate regress

2 weeks later

DA b-Catenin mice

Castration

prostate continue growing?
Activation of Wnt/b-Catenin results in castrate-resistant prostate growth

Control prostate  DA b-Catenin prostate

BrdU IHC

Intact

2 weeks after Cx

DA b-Catenin: Dominant Active b-Catenin

Yu et al. Prostate 2009
Activation of Wnt/b-Catenin regulates AR signaling differentially at early and late age.

At 12 weeks:
- Nkx3.1: AR target gene

At 9 months:
- AR
- Nkx3.1: AR target gene

Yu et al. Prostate, 2009
AR & AR signaling are down-regulated in T-antigen/DA b-Catenin mouse prostates

Tag: reporter of androgen signaling

Foxa2: target gene of Wnt/b-catenin

Yu et al. Oncogene 2011
Candidate: Foxa2 mediates Wnt/b-Catenin induced castration resistance
Foxya2 is a downstream target of Wnt/b-Catenin signaling

b-CatΔex3 kidney tumor with Wilm’s features

prostate

kidney
Fossa2 was detected in human metastatic CRPC

<table>
<thead>
<tr>
<th>Expression Pattern</th>
<th>Percentage</th>
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<tbody>
<tr>
<td>Foxa2+ Syn+</td>
<td>21%</td>
</tr>
<tr>
<td>Foxa2+ Syn-</td>
<td>8%</td>
</tr>
<tr>
<td>Foxa2- Syn+</td>
<td>21%</td>
</tr>
<tr>
<td>Foxa2- Syn-</td>
<td>50%</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>100%</strong></td>
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</table>
Expression of Foxa2 is sufficient to drive androgen-independent cell growth \textit{in vitro}

**NT1 cells**

<table>
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<tr>
<th>EV</th>
<th>Foxa2</th>
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Focha2

B-Actin

Cell Proliferation Assay

- EV - DHT
- EV + DHT
- a2 - DHT
- a2 + DHT

**NT1:** T antigen immortalized prostate epithelia
AR positive, androgen-dependent
DA b-Catenin induced the expression of Foxa2 and enabled androgen-independent prostatic cell growth in vitro

NT1: T antigen immortalized prostate epithelia AR positive, androgen-dependent
DA b-Catenin but not Foxa2 induced NED in NT1 cells

- Foxa2 is not sufficient to induce NED
- Both the expression of DA b-Cat and Foxa2 down regulate AR
- ongoing: to study how b-Cat and Foxa2 down-regulates AR
Summary

- Activation of Wnt/b-Catenin promotes CRPCa progression
- Activation of Wnt/b-Catenin induces the expression of Foxa2 and down-regulate AR and AR signaling
- Activation of Wnt/b-Catenin induces NED in PCa
Acknowledgments

**LSUHSC Shreveport**
Shu Yang  
Dr. Hari Koul’s lab  
Dr. Runhua Shi  

**Vanderbilt University**
Dr. Robert Matusik’s lab  
Dr. Simon Hayward’s lab  
Dr. Andrew Yi  
Dr. Justin Cates

**University of Washington**
Dr. Eva Corey  
Dr. Robert Vessella  
Dr. Colm Morrissey

**Funding:**
FWCC/Foundation Legacy Funds  
LSUHSCs Research Council  
DOD New Investigator Award