A New Approach to Targeting the Androgen Signaling Axis in Prostate Cancer

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The Problem of Treating Prostate Cancer

- **First line therapies:** Radical prostatectomy, external beam radiation therapy, brachytherapy, cryotherapy
  - PC progression, PC recurrence, harmful genitourinary side effects

- **Treatment of advanced PC:** Androgen deprivation therapy (ADT) using surgery or GnRH agonists, antiandrogens (flutamide, bicalutamide or nilutamide)
  - Progression to CRPC (castrate recurrent PC), Various acute and chronic side effects on non-target tissues

- **Newer treatments:** Cabazetaxel; Alpharadin; Abiraterone acetate; Denosumab; Sipuleucel-T
  - Only some improvement
Classical Mechanism of Transcriptional Signaling by the Androgen Receptor
What happens to AR signaling during prostate tumor progression?

- Early stage PC is dependent on androgen for growth. Therefore it is sensitive to antiandrogen drugs.

- Advanced PC continues to be dependent on AR but is apparently independent of androgen. Therefore advanced PC is insensitive to antiandrogens.

- In advanced PC, AR supports growth by acting independent of androgen or by becoming hypersensitive to androgen.

- Advanced PC is also called castrate recurrent PC or CRPC.
The AR in androgen-independent cells is localized in the nucleus in the absence hormone.

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Resistant Cells

Sensitive Cells
How does growth signaling by AR become insensitive to androgen deprivation?

Some Proposed Mechanisms:

- Overexpression of AR
- AR mutations
- Phosphorylation of AR by overactivation of MAPK, PI3K/AKT and PKC pathways

BJU Int. 2005 Jun;95(9):1320-6.
Importance and limitations of targeting the androgen/AR signaling axis

- There is strong evidence that both early stage PC and advanced PC are dependent on AR for growth:
  - Castration, antiandrogens and androgen synthesis inhibitors are very effective

- Resistance mechanisms restore AR signaling
  (e.g., Changes in AR or coregulators, hypersensitization to androgen; signaling cross-talk)

- Current drugs have undesirable side effects in normal tissues
Our approach: Find a better way to block growth signaling by AR in prostate cancer cells

Rationale: Androgen is essential for normal function of prostate and other tissues. Prostate tumor cells reprogram androgen/AR signaling to primarily reactivate only its growth promoting function.

Goal: Identify mechanisms of AR action that are only required for tumor growth but not required by normal adult tissues.

Significance: Such mechanisms could serve as tumor specific drug targets.
Gene activation by AR in androgen-independent cell models is similar to advanced clinical tumors

Top 5% genes up-regulated in advanced PC (n=19)
- AR induced genes (androgen-independent) in LP50 cells
  - 424
  - 81
  - 512
  - 16% overlap
  - (p=4×10^-7)

Top 1% genes up-regulated in advanced PC (n=19)
- AR induced genes (androgen-independent) in LP50 cells
  - 71
  - 28
  - 565
  - 28% overlap
  - (p=2.7×10^-6)

Gene ontology of overlapping genes: Cell division – P = 2.7 X 10^-18
Classical Mechanism of AR Action:

Binding of AR to ‘androgen response element’ (ARE)

AR: Androgen Receptor
ARE: Androgen Response Element
\( \downarrow \): Androgen
**Question:** In androgen-independent prostate tumor cells, does AR bind to ARE without the need for androgen?
Interaction of AR with ARE requires androgen even when AR supports growth independent of androgen

Promoter Activity Assay

Chromatin binding

* P< 0.0001
DNA binding defective AR can support tumor cell growth

**Bar Graph 1:**
- **Endogenous AR mRNA (Fold):**
  - Ctrl sh
  - AR sh
  - mut AR + AR sh

**Bar Graph 2:**
- **[3H] Thymidine incorporation (CPM x 10^4):**
  - Ctrl sh
  - AR sh
  - mut AR + AR sh

**Notes:**
- Mut AR: - - +
- AR shRNA: - + +
- *P < 0.001
Both wtAR and mutAR Bind at Hormone-Independent Chromatin Sites

Promoter Tiling Array
Hormone-Deplete LP50 Cells
AR ChIP-chip Peaks:
307 (FDR <0.05)
114 (FDR < 0.01)

* P< 0.001
AR activates two sets of genes:
1. hormone-dependent 2. hormone-independent

Hormone-independent genes activated by AR: Strongly support cell division

LP50 cells

C81 cells

C4-2 cells

1274 17 97 525 18 93 794 1858

Androgen-independent Androgen activated
Conclusions 1

In PC cells that are insensitive to hormone deprivation:

1. Androgen induces many classical target genes

2. A large set of genes primarily supporting cell growth is activated by AR independent of hormone

3. Hormone-independent gene expression and proliferation are supported by AR through non-classical mechanisms
Question: If not through ARE, then how does AR interact with growth genes?
**CASSICAL**

Direct binding of AR to DNA

- AR: Androgen Receptor
- ARE: Androgen Response Element
- ▼: Androgen

**NON-CLASSICAL**

Indirect binding of AR through a tethering protein

- X: Tethering protein (Elk1)
- X binding sequence
Previously well established AR tethering proteins:

- HoxB13 – prostate development
- C/EBPα – tissue differentiation and tumor suppression
  (tethering to C/EBPα was insensitive to antiandrogens)

Are there genetically redundant AR tethering proteins that are essential for PC growth?
Elk1

- ETS family transcription factor
  TCF subfamily: Elk1, Elk3, Elk4

- Represses many genes

- Activated by phosphorylation (MAPK); mediates induction of immediate early genes

- Genetically and functionally redundant

- Expressed in the clinical spectrum of PC
Further studies from our laboratory

*Elk1 is an AR tethering protein in both hormone-dependent and CRPC cell models*

- Promoter analysis plus loss and gain of function experiments
- Co-immunoprecipitation experiments
- Mammalian two-hybrid experiments
- Binding to Elk1 and transactivation are hormone-independent and insensitive to antiandrogens
Elk1 tethers AR to Elk1 binding DNA sequences in the chromatin
Elk1 enables activation by AR of a large set of genes with principally growth supporting functions.

Gene Ontology: Primarily Support Cell Cycle / Mitosis
Benjamini $P$ value $1.6E^{-14}$
Relationship between Gene Regulation by Elk1 and Androgen
The cooperative gene activation by Elk1 and AR bypasses the classical (phosphorylation) mechanism of activation of Elk1.

Additional evidence: Depletion of SRF did not affect Elk1-dependent gene activation by androgen.
Both early stage PC and CRPC cells are addicted to Elk1 to support androgen- or AR-dependent growth, clonogenicity and tumorigenicity.

This is the first demonstration of the addiction of any type of cancer cells to Elk1.
Elk1 is required for androgen-dependent growth
Androgen-independent growth requires Elk1
(C4-2 cells)
Elk1 is required for clonogenic growth (C4-2 cells)

2D colony formation assay

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Soft agar clonogenicity assay

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Cells seeded (0.25ml gel)

ELK1

AR

GAPDH

ELK1 shRNA

R1881

-  -  +  +
AR-independent growth is not regulated by Elk1

**DU145 cells**

![Graph showing viable cell counts over days for DU145 cells with different ELK1 and GAPDH shRNA conditions.]

**PC-3 cells**

![Graph showing viable cell counts over days for PC-3 cells with different ELK1 and GAPDH shRNA conditions.]

The graphs illustrate the effect of Elk shRNA and control shRNA on viable cell counts over time for DU145 and PC-3 cells, demonstrating that AR-independent growth is not regulated by Elk1.
Depletion of Elk1 results in loss of tumorigenicity of C4-2 (CRPC) cells
AR does not cooperate with Elk3

Promoter Activation by androgen (RLU x 10⁴)

- Vector
- Ectopic Elk1
- Ectopic Elk3

(Elk1)₂-TATA-Luc
Elk3 expression is low in PC model cell lines compared to primary normal prostate epithelial cells.
Elk3 expression is low in clinical prostate tumors compared with normal prostate

Singh et al, Cancer Cell 2002, 1:203-209
Conclusions 2

1. Growth signaling by AR in both hormone-dependent PC and CRPC requires tethering of AR by Elk1.

2. Elk1 does not have such a critical role in normal cells where it may be substituted by other proteins (eg., Elk3) that do not interact with AR.

3. The cooperative action of Elk1 and AR is hormone-independent (unless hormone is needed for nuclear localization of AR).

4. Disruption of the interaction between Elk1 and AR should selectively suppress the growth of prostate tumors without the need for androgen ablation treatment.
Mapping the AR binding region in Elk1

- **Full-Length** (1-428)
- **Deleted DBD** (87-428)
- **Activation Domain** (307-428)

- 307-317 (a.a. seq: ISQPQKGRKPR)
- 287-297 (a.a. seq: PAVMDTAGQA)
The A/B domain of AR fully retains the ability to bind to Elk1 and transactivate the target promoter.
AR peptide 156-323 selectively blocks Elk1 dependent gene activation by androgen

Fold Activation by R1881

pCDH = Control
P1 = AR peptide 1-200
P2 = AR peptide 156-329
P3 = AR Peptide 306-450
P4 = AR peptide 475-555
AR Peptide 156-329 inhibits growth of PC cells
FUTURE DIRECTION

To develop small molecule inhibitors of the Elk1-AR interaction
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