ACK1 Tyrosine Kinases: A Critical Regulator of Prostate Cancer
Learners Objectives

- How Androgen Receptor (AR) signaling is accomplished in absence of androgen
- What are the epigenetic events that takes place to achieve androgen-independent AR signaling
- What are the new therapeutic options being developed to overcome enzalutamide-resistant prostate cancer
Prostate Cancer

- Prostate cancer is second most common cancer in American men
- Almost 225,000 men are diagnosed and ~25,000 die due to prostate cancer in US every year
- Androgen receptor (AR) is a key regulator of both initiation and progression of prostate cancer to metastatic stage
Androgen Receptor Signaling

AR

TATA

ARE

PSA/hK2/TMPRSS2

+DHT

Nuclear Translocation

Coactivator

RNA Pol

Transcription

PSA/hK2/TMPRSS2

Cell survival and growth
Castration Resistant Prostate Cancer

AR + Androgen

Androgen dependent or Hormone-sensitive

Tumor Growth

18-24 M

Castration Resistant Prostate Cancer
CRPC

AR + Androgen

Tumor Growth
Second generation of anti-androgen

- Enzalutamide (Xtandi), is a new type of anti-androgen. It prevents AR entry into the nucleus, lowers PSA levels, slow the growth of tumors.
- It is effective in a subset of CRPC patients (1/3rd) and survival advantage is modest (4-6 months).
- Even the most responding patients relapsed within 2 years, often with lethal consequences.

Tran et al., *Science*, 2009
Mechanism of Enzalutamide-resistance

- AR-V7 splice variant expression has emerged to be the major cause of Enzalutamide and Abiraterone resistance
- AR-V7 lacks the C terminal ligand-binding domain (& Enzalutamide binding) and remains constitutively active as a transcription factor.

Antonarakis et al., *NEJM*, 2014
CRPC: AR dependence

- Overall, the most common genetic aberration in CRPC is AR mRNA upregulation, also reflected in AR-V7 increase in Enzalutamide resistant

- These data have opened a new paradigm:

- To achieve complete remission of CRPC, ablation of AR is the key.

- However, targeted inhibition of AR transcription with small molecule inhibitors has not yet been accomplished.
ACK1 or TNK2

- Ubiquitously expressed non-receptor tyrosine kinase

- Plays a central role in integrating signal from receptor tyrosine kinases e.g. EGFR, HER2 and IR

- ACK1 binds to AR and ACK1/AR complex translocate to nucleus activating transcription of AR target genes
Novel ACK1 inhibitor, (R)-9bMS

- We identified (R)-9bMS as a potent ACK1 inhibitor
- *In vitro* IC$_{50}$=48 nM
- Mesylate salt is highly soluble in aqueous media
- (R)-9bMS exhibit significant selectivity
- Inhibited 11 kinase out of 369 kinase (90% inhibition)
- Except ACK1 others not known to express in prostate

Lawrence. et al., *J Med Chem* 2015
(R)-9b suppresses AR expression
(R)-9b inhibits AR transcription

- VCaP
- LNCaP
- LAPC4
- C4-2B

AR/Actin Ratio

PSA/Actin Ratio
(R)-9b suppresses AR-V expression
How does ACK1 regulates AR/AR-V7 transcription?
ACK1 translocates to nucleus

- ACK1 'piggybacks' AR to nucleus
- DNA is not exposed, but bound to histones to form chromatin

- Does ACK1 phosphorylate histone to activate AR transcription?
Histone H4 phosphorylation at Tyr88

Mass Spectrometry

CRPC Sample #1

CRPC Sample #1

pY88-H4 Ab

pY88-H4 Ab+
phosphopeptide

Biotin

Antibody generation
ACK1 phosphorylates H4 at Tyr88

**In vitro Kinase assay**

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<th>H4</th>
<th>pY88-H4</th>
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**(R)-9bMS**

**Ligand**

Endogenous H4 Y88-phosphorylation

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Ligand
H4 Y88-phosphorylation is insensitive to Enzalutamide and DHT
pY88-H4 marks deposited upstream of AR
(R)-9b compromises pY88-H4 deposition
How do pY88-H4 epigenetic marks regulate AR transcription?
WDR5-MLL2 complex is an epigenetic reader of pY88-H4 marks

- pY88-H4 peptide used as a bait to identify interacting proteins:
  - **MLL2 (KMT2D)** - H3K4 lysine methyltransferase, modifies histones to activate transcription
  - **WDR5** - WD repeat domain 5
WDR5 `reads’ pY88-H4 marks, MLL2 acts as a `scribe’ to regulate deposition of H3K4me3 activating marks
(R)-9bMS overcomes Enzalutamide Resistance

LNCaP-C4-2B: 0.40 uM
VCaP: 0.45 uM
LAPC4: 0.75 uM
LNCaP: 1.75 uM
RWPE: 10.0 uM
(R)-9bMS Inhibits CRPC Growth
Epigenetic dynamics at AR locus

ACK1 Inhibitor

Mahajan K. et al., *Cancer Cell*, 2017
Conclusion

- We uncovered a new signaling nexus - ACK1 mediated H4-Y88-phosphorylation that maintains AR transcript levels in absence of androgen.

- H4-Y88-phosphorylation can be suppressed by small molecule inhibitor (R)-9bMS, compromising AR and AR-V7 transcription.

- ACK1 inhibitor sensitizes Enzalutamide-resistant CRPC cells, opening new treatment option for patients that are:
  1. not-responsive to this second generation of antiandrogen
  2. those CRPC tumors that have developed resistance.
References


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Questions
Question: What is the `third generation’ of AR inhibitor

Answer: \((R)-9bMS\)