Synergistic Targeting of Androgen Receptor Circuits in Metastatic Prostate Cancer

Epigenetic Regulation of HOXB13 in Castration Resistant Prostate Cancer

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HOXB13
Learner’s Objectives

1. The Androgen Receptor (AR) is expressed and functional in a majority of lethal castration resistant prostate cancers (CRPCs).
2. An important AR co-regulator in CRPCs is the homeodomain containing sequence specific transcription factor, HOXB13.
3. The expression of HOXB13 is epigenetically regulated by the BET bromodomain protein, BRD4.
• HOXB13 is critical for the differentiation of the ventral lobe of the prostate gland in mice.
• In human cancers, HOXB13 expression correlates with advanced pT stage, high Gleason grade, positive lymph node status, high pre-operative PSA levels, TMPRSS2:ERG fusion, PTEN deletions, AR expression, cell proliferation and early PSA recurrence.
• Co-expression analysis identified a subset of tumors with high HOXB13 and AR but low PSA expression that had a particularly poor prognosis.
• The epigenetic mechanism by which HOXB13 is regulated in AR positive prostate cancers is not known.
Mechanism of Action of HOXB13

Norris et al, Molecular Cell, 2009
HOXB13-A critical regulator of CRPC growth

**Gene expression**
- HOXB13
- C4-2B
- HOXB13 pKO

**Tumor weights**
- C4-2B
- HOXB13 pKO

**Immunoblot**
- HOXB13
- Actin

**Experiment conditions**
- Intact
- Castrated
HOXB13 direct targets in PC
HOTPAMg-Stratification of prostate adenocarcinomas from metastatic prostate cancers
Validation of HOTPAM9 in Metastatic Prostate Cancer

Graph showing gene expression levels with comparison between C4-2B Control siRNA and C4-2B HOXB13 siRNA.
BRD4 epigenetically regulates HOXB13 expression in PC

**HOXB13 gene**

![ChIP analysis of HOXB13 gene with BRD4 and IgG antibodies.](image)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>% of Input</th>
</tr>
</thead>
<tbody>
<tr>
<td>DMSO</td>
<td>0.3</td>
</tr>
<tr>
<td>JQ1</td>
<td>0.1</td>
</tr>
<tr>
<td>MA4</td>
<td>0.4</td>
</tr>
<tr>
<td>ENZA</td>
<td>0.5</td>
</tr>
<tr>
<td>ABR</td>
<td>0.2</td>
</tr>
</tbody>
</table>

**Graphs**

1. **HOXB13/ACTIN mRNA**
   - DMSO: Control siRNA, HOXB13 siRNA, BRD4 siRNA
   - JQ1: 0.1, 0.5, 1, 2.5 uM

2. **HOXB13 expression**
   - EGF, IGF, HRG: IP: HOXB13, IB: AR
   - BET inhibitor: IP: HOXB13, IB: Actin
**Detection of HOXB13 in CTCs**

<table>
<thead>
<tr>
<th>A. VCAP cells No Ab control</th>
<th></th>
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<tbody>
<tr>
<td>DAPI/CK-PE</td>
<td></td>
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<tr>
<td>CK-PE</td>
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<tr>
<td>DAPI</td>
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<tr>
<td>CD45-APC</td>
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<tr>
<td>HOXB13 FITC</td>
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</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>B. VCAP cells plus 5ug/ml HOXB13 Alexa Fluor 488 Ab</th>
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</thead>
<tbody>
<tr>
<td>DAPI/CK-PE</td>
</tr>
<tr>
<td>CK-PE</td>
</tr>
<tr>
<td>DAPI</td>
</tr>
<tr>
<td>CD45-APC HOXB13 AF488</td>
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</table>

<table>
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<tr>
<th>C. VCAP cells plus 5ug/ml HOXB13 FITC Ab</th>
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<td>HOXB13 FITC</td>
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</table>

<table>
<thead>
<tr>
<th>D. VCAP cells plus 5ug/ml Alexa Fluor 488 IgG₁ Ab</th>
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</thead>
<tbody>
<tr>
<td>DAPI/CK-PE</td>
</tr>
<tr>
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<td>DAPI</td>
</tr>
<tr>
<td>CD45-APC</td>
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<tr>
<td>IgG₁ AF488</td>
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</table>
Non-invasive Detection of HOXB13 CTCs

<table>
<thead>
<tr>
<th>Condition</th>
<th>Total Events</th>
<th>Unassigned events</th>
<th>CTCs total (n)</th>
<th>% of total events</th>
<th>HOXB13⁺</th>
<th>HOXB13⁻</th>
</tr>
</thead>
<tbody>
<tr>
<td>DMSO</td>
<td>1987</td>
<td>1310</td>
<td>677</td>
<td>34%</td>
<td>184</td>
<td>27</td>
</tr>
<tr>
<td>JQ1</td>
<td>1386</td>
<td>1323</td>
<td>63</td>
<td>5%</td>
<td>9</td>
<td>14</td>
</tr>
</tbody>
</table>

![Bar chart showing %CTCs and %HOXB13+ve for DMSO and JQ1 conditions.](image)
1. BRD4, is an epigenetic regulator of HOXB13 expression.
2. HOXB13 expression can be targeted in prostate cancers with BET bromodomain inhibitors such as JQ1.
3. We identified a subgroup of mitotic kinases as key transcriptional targets of HOXB13-a target gene signature termed as HOTPAM9 (HOXB13 Target genes separating Prostate Adenocarcinomas from Metastasis) in CRPCs.
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References


Thank you for your attention!
Which is a key epigenetic regulator of HOXB13 expression in prostate cancer cells that can be targeted with the prototype inhibitor, JQ1?

1. AKT
2. BRD4
3. c-MYC
4. FOXA1