Role of Androgen Receptor in Epithelial to Mesenchymal Transition (EMT) in Prostate Cancer Cells

Sheeba Jacob

Primate Biology Department
National Institute for Research in Reproductive Health
Mumbai, India
Challenges in Prostate Cancer Management

- Distinguishing indolent versus aggressive disease
- Treatment of androgen-independent or castration resistant prostate cancer
- Metastasis
Epithelial to Mesenchymal Transition - EMT, a Major Event in Metastasis
Androgenic Stimuli - Facilitator of Inhibitor of EMT?

- Androgens induce EMT activation in prostate cancer cells (Zhu and Kyprianou, 2010)

- Androgen deprivation leads to EMT activation in mouse harboring human PCa xenografts (Sun et al., 2012)

- Izumi et al. (2013) reported EMT activation in prostate cancer cells after AR silencing

The role of androgenic stimuli in EMT regulation remains to be established
Androgen & its Receptor (AR) as a Regulator of EMT in Prostate Cancer Cells
Queries

- Whether modulation in the levels of androgen receptor (AR) alters the invasiveness of prostate cancer cells?

- If yes, mechanisms underlying AR driven modulation in invasiveness?
Experimental Models

- Androgen dependent prostate cancer cell line (LNCaP-FGC)
- Androgen independent prostate cancer cell lines (PC3 and DU145)
Effect on Invasion of Androgen-Dependent LNCaP cells

AR silencing led to reduction in the invasiveness of androgen-dependent LNCaP cells.

*** p value < 0.001, **** p value < 0.0001
Microarray Analysis to Identify EMT/Invasion Associated Genes

6% differentially expressed genes

- Twist 2
- ZEB2 (Zinc Finger E Box Binding Homeobox 2) / SIP1
- EVT
- MMP-7
- IGF-1
- Cdc 42
ZEB2 and EMT

- ZEB2 knockdown suppressed migration and invasion in glioma cells (Qi et al., 2012)

- ZEB2 directly binds proximal E boxes within the E-cadherin gene and mediates transcriptional repression by recruiting co-repressor complexes (Postigo et al., 2003)
AR and ZEB2 Expression in Prostate Cancer and Benign Prostatic Hyperplasia

ZEB2

BPH

AR

PCa

-ve

Integrated Intensity

PCa

BPH

PCa

BPH

**

***
Androgen stimulates ZEB2 expression in androgen-dependent LNCaP cells

A. DHT concentration (nM) vs. band intensities of ZEB2 and GAPDH.

B. Western blot images showing DHT concentration (nM) vs. band intensities of AR and GAPDH at 110kDa and 37kDa.

C. Graph showing ratio of the band intensities of ZEB2/GAPDH.

D. Graph showing ratio of the band intensities of AR/GAPDH.

Androgen stimulates ZEB2 expression in androgen-dependent LNCaP cells.
ZEB2 expression in AR silenced LNCaP cells

AR positively regulates ZEB2 expression in androgen-dependent LNCaP cells
Whether the levels of ZEB2 differ in androgen dependent (less metastatic) and androgen independent (more metastatic) prostate cancer cell lines?
AR, ZEB2 and EMT in Androgen Dependent and Androgen Independent PCa Cell Lines

Relative Levels of ZEB2 Transcripts in Androgen Dependent and Androgen Independent PCa Cell Lines

- LNCaP
- PC3
- DU145

Vimentin
E-cadherin

** p value < 0.01
AR over-expression in androgen independent prostate cancer cells

ZEB2 levels?
AR over-expression led to a decrease in ZEB2 transcript levels.
ZEB2 Protein Levels in AR Over-expressing Cells

[Graph showing protein levels with different concentrations of AR and GAPDH]
Expression of E-cadherin in AR Over-expressing Androgen Independent Prostate Cancer Cells

Vector Transfected | AR Transfected
---|---
PC3 | PC3
DU145 | DU145
Expression of E-cadherin in AR Overexpressing Androgen Independent Prostate Cancer Cells

E-cadherin expression is increased following AR over-expression.
Morphology of AR Over-expressing Androgen-independent Prostate Cancer Cells

PC3

DU145

Vector Transfected

AR Transfected
ZEB2 over-expression restores invasion in AR over-expressing androgen independent prostate cancer cell lines
Migration of AR Over-expressing Androgen Independent PCa Cells and Restoration by ZEB2 Over-expression

AR over-expression leads to decrease in migration and ectopic expression of ZEB2 restores the migration of AR over-expressing cells.
Conclusion

- AR negatively regulates ZEB2 expression in androgen independent prostate cancer cells

- Expression of E-cadherin is increased in AR over-expressing prostate cancer cells

- Invasion and migration are adversely affected, following AR over-expression, in androgen independent prostate cancer cells
To summarize...

Androgen dependent

Androgenic stimuli

Cells expressing AR

EMT

ZEB2

Cells over expressing AR

EMT

ZEB2
Take Home message...

Intermittent, rather than continuous androgen deprivation therapy is more beneficial to patients with locally advanced metastatic prostate tumors.
Acknowledgement

- Dr. Geetanjali Sachdeva, Ph. D
- Mr. Sumeet Nayak (graduate student)
- Dr. Donald Tindall, Mayo Clinic, US
- Dr. Danny Huylebroeck, University of Leuven, Belgium

- Indian Council of Medical Research (ICMR)
- Department of Atomic Energy-BRNS
**In Silico** Scanning for the Presence of ZEB2 Binding Sites in the Regulatory Regions of miR200a and 200b Genes

<table>
<thead>
<tr>
<th>Gene name</th>
<th>Regions Scanned</th>
<th>E box-2 CACCTG</th>
<th>Z box -1 CAGGTG</th>
<th>Z box-2 CAGGTA</th>
</tr>
</thead>
<tbody>
<tr>
<td>miRNA 200a</td>
<td>1102243-1103242</td>
<td>-</td>
<td>-941, -418, -364</td>
<td>-</td>
</tr>
<tr>
<td>miRNA 200b</td>
<td>1101484-1102483</td>
<td>-</td>
<td>-938, -494, -182</td>
<td>-</td>
</tr>
</tbody>
</table>
miR200a and 200b Levels in AR Over-expressing PC3 Cells

miR200a and miR200b levels are increased in AR over-expressing prostate cancer cells.