STAT3 regulated ARF expression suppresses prostate cancer metastasis

C. E. Alken-Preisträgertreffen 2015

Jan Pencik
Prostate Cancer is the Most Frequently Diagnosed Cancer in Men

Newly diagnosed cases/year:

- 1.11 million worldwide
- 5,000 in Austria

Who is next...?
Prostate Cancer (PCa)

Prostate cancer is an adenocarcinoma

Stages of prostate cancer:

- Prostatic Intraepithelial Neoplasia (PIN),
- Invasive PCa
- Metastatic PCa
- Hormone refractory PCa

Gleason Score (GSC)

Human PCa slice (0.4 cm thick)
PSA is a Suboptimal Biomarker for PCa Detection

Great discrepancy between morbidity and mortality in PCa:
10% need systemic therapy
5% die of PCa

CHALLENGE: Finding New Biomarkers For Aggressive Forms of PCa
IL-6/STAT3 Signaling in Prostate Cancer

- Increased levels of IL-6 in serum of patients with advanced PCa
  (Nakashima et al., 2000)

- *In vitro* Stat3 stimulates metastatic behavior of human PCa cells
  (Okamota et al., 1997)

Monoclonal anti-IL-6R antibody in PCa therapy

Siltuximab (CNTO 328)
(Dorff et al., 2010; Karkera et al., 2011)

No survival advantage in PCa patients

Potential therapeutic function?

Prediction of disease progression and prognosis?
Pre-Clinical Mouse Model of Prostate Cancer

- PTEN one of most mutated/deleted genes in human cancer
- Loss of PTEN leads to hyperactive PI3K signaling and PCa development

a. Prostate Specific Pten Deletion ($Pten^{pc/-}$)

b. Complete IL-6 Deletion (IL-6$^{-/-}$)
   Kopf et al., Nature 1994

c. Conditional Stat3 Deletion (Stat3$^{pc/-}$)

IL-6/Stat3 signaling axis
Tumor suppressor? Oncogene?
Deletion of *IL-6* and *Pten* triggers progressive prostate tumorigenesis and metastatic disease.

38 weeks p.p.
Co-deletion of Stat3 and Pten Enhances Prostate Cancer Transformation and Metastatic Potential

52 weeks p.p.

(a) WT  Pten^pc/-  Pten^pc/- Stat3^pc/-

(b) mg (prostate tissue)

(c) Cumulative survival (%)

(d) Prostate, Liver, Lung
Stat3 is a Critical Regulator of the ARF–Mdm2–p53 Tumor Suppressor Pathway and Senescence
p19^{Arf} is a Direct Target of Stat3

**In vivo ChiP**

- **a**
  - CDKN2A gene encodes mouse p19^{ARF} protein

- **b**
  - *In vivo* ChiP
Loss of STAT3 and/or p14ARF Expression Predicts Early Increased Risk of Disease Recurrence in Patients with PCa

STAT3 and p14ARF are novel markers to stratify high and low-risk PCa patients

[Diagram showing percentage distributions of high and low STAT3 and p14ARF]

<table>
<thead>
<tr>
<th></th>
<th>Correlation</th>
<th>Fisher's exact test</th>
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<tbody>
<tr>
<td>STAT3 vs. GSC</td>
<td>inverse</td>
<td>p&lt;0.000001</td>
</tr>
<tr>
<td>p14ARF vs. GSC</td>
<td>inverse</td>
<td>p=0.011</td>
</tr>
<tr>
<td>STAT3 vs. p14ARF</td>
<td>direct</td>
<td>p&lt;0.000001</td>
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[Graph showing BCR free survival for different groups]

<table>
<thead>
<tr>
<th></th>
<th>Univariate analysis</th>
<th>Multivariate analysis</th>
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<tbody>
<tr>
<td></td>
<td>p-value</td>
<td>p-value</td>
</tr>
<tr>
<td>GSC ≤ 7 vs. 8-10</td>
<td>0.005</td>
<td>0.037</td>
</tr>
<tr>
<td>STAT3 Low vs. High</td>
<td>0.007</td>
<td>n.s.</td>
</tr>
<tr>
<td>p14ARF Low vs. High</td>
<td>&lt;0.000001</td>
<td>0.0002</td>
</tr>
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STAT3 and p14ARF are novel markers to stratify high and low-risk PCa patients
Loss of STAT3 and/or p14\textsuperscript{ARF} Expression is Associated with Progression to Metastatic Disease

(a) Immunohistochemical staining of STAT3 and p14\textsuperscript{ARF} in primary and metastatic PCa tissues from two patients.

(b) Bar chart showing the expression levels of STAT3 in primary and metastatic PCa tissues.

(c) Bar chart showing the expression levels of p14\textsuperscript{ARF} in primary and metastatic PCa tissues.
Genetic Alterations of STAT3 and CDKN2A are increased in metastatic PCa

Human $14^\text{ARF}$ protein is encoded by the CDKN2A gene

Technique: Whole exome sequencing

Mutations

<table>
<thead>
<tr>
<th>Gene</th>
<th>Frequency</th>
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<tbody>
<tr>
<td>TP53</td>
<td>7.4%</td>
</tr>
<tr>
<td>PTEN</td>
<td>4.7%</td>
</tr>
<tr>
<td>STAT3</td>
<td>2.5%</td>
</tr>
<tr>
<td>CDKN2A</td>
<td>2.3%</td>
</tr>
</tbody>
</table>

529 patients with Primary PCa

Novel STAT3 mutations in primary PCa

Deletions

<table>
<thead>
<tr>
<th>Gene</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>TP53</td>
<td>54%</td>
</tr>
<tr>
<td>PTEN</td>
<td>54%</td>
</tr>
<tr>
<td>STAT3</td>
<td>16%</td>
</tr>
<tr>
<td>CDKN2A</td>
<td>19%</td>
</tr>
</tbody>
</table>

37 patients with Metastatic PCa

Frequent genetic STAT3 and CDKN2A alterations in metastatic PCa

Highlighted in Nat. Rev. Urology 2015
Future Implications and Studies

Liquid biopsies:
Exome Sequencing of circulating Tumor DNA in PCa patients

Deletions > 20Mb
Deletions < 20Mb

Speicher M, Heitzer E
Future Implications and Studies

PET/MRI Characterization of PCa
Prostate-specific membrane antigen dynamics

Gleason 3 - plateau

Gleason 4 - increasing

ROC analysis of
dualtracer PET vs. loss of
STAT3/p14

AUC: 0.80 (P=0.050; n=16)

In progress: AR, Cytokines dynamics

Hacker M., Dumanic M.,
Mitterhauser M.
• STAT3 and p14ARF: New diagnostic markers for PCa

• Genetic defects of STAT3 and CDKN2A are frequent in metastatic PCa

• Genetic testing may be needed as a part of the PCa diagnosis

• Novel therapeutic approaches for this subtype of aggressive PCa
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