PIAS1 is a determinant of poor survival and acts as a positive feedback regulator of AR signaling through enhanced AR stabilization in prostate cancer

Martin Puhr PhD

Department of Urology
Division of Experimental Urology
Medical University of Innsbruck
PIAS - Protein Inhibitors of Activated STAT

- are regulated by endogenous inhibitors
  - SOCS (Suppressor of cytokine signaling)
  - PIAS (Protein inhibitors of activated STATs)
  - PTPs (phosphotyrosine phosphatases)

- PIAS family:
  PIAS1, PIAS2 (PIASx), PIAS3, PIAS4 (PIASy)

- PIAS1 inhibits p53/p73 activation
- PIAS1 is implicated in DNA damage response (e.g. Rad51 recruitment)

Nature Reviews Immunology

DNA binding

SUMO E3-ligase

Inhibits DNA binding of target proteins

Sub-cellular localization, target protein stability through sumoylation
• PIAS1 expression is significantly higher in malignant areas in comparison to benign areas of prostate cancer tissue.
• PIAS1 down-regulation leads to an G1-cell cycle arrest through increased expression of p21.


• PIAS1 is elevated in docetaxel resistant cell lines and in docetaxel treated patients.
• Prolonged PIAS1 knockdown results in elevated apoptosis in parental and docetaxel resistant cells *in vitro* and in reduced tumor growth in CAM assays and mouse xenografts *in vivo*.


The used cell lines for the *in vivo* experiments lack AR. ?Clinical significance?
Assess the interaction of PIAS1 and AR signaling with special focus on a possible therapeutic approach

TMA analysis of PIAS1 and AR expression in PCa tissue
Collaboration with Prof. Van Leenders (Rotterdam), and Prof. Kristiansen (Bonn)

Investigate the mechanism of PIAS1-AR interaction \textit{in vitro}
Internal Collaboration with Julia Höfer and Zoran Culig
PIAS1 correlates with AR (marker for decreased relapse free survival)

**Patient tissue**

<table>
<thead>
<tr>
<th>PCa tissue (Innsbruck cohort)</th>
<th>AR staining tumor</th>
</tr>
</thead>
<tbody>
<tr>
<td>PIAS1 staining tumor</td>
<td>correlation Pearson 0.674, significance (2-sided) 2.1E-14, N 99</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>PCa tissue (Bonn validation cohort)</th>
<th>AR staining tumor</th>
</tr>
</thead>
<tbody>
<tr>
<td>PIAS1 staining tumor</td>
<td>correlation Pearson 0.591, significance (2-sided) 2.8E-21, N 211</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>PCa tissue (Rotterdam validation cohort)</th>
<th>AR staining tumor</th>
</tr>
</thead>
<tbody>
<tr>
<td>PIAS1 staining tumor</td>
<td>correlation Pearson 0.773, significance (2-sided) 2.9E-89, N 443</td>
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</table>

<table>
<thead>
<tr>
<th>PCa tissue (all 3 cohorts)</th>
<th>AR staining tumor</th>
</tr>
</thead>
<tbody>
<tr>
<td>PIAS1 staining tumor</td>
<td>correlation Pearson 0.707, significance (2-sided) 3.5E-115, N 753</td>
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**Cell lines**

<table>
<thead>
<tr>
<th>PCa cell lines</th>
<th>AR</th>
</tr>
</thead>
<tbody>
<tr>
<td>PIAS1</td>
<td>correlation Pearson 0.843, significance (2-sided) 0.001, N 12</td>
</tr>
</tbody>
</table>

**Cell lines**

- Blue line: PIAS1 IR score ≤ 4
- Green line: PIAS1 IR score > 4
- Censored
- (end of follow-up)

**Progression free survival (months)**

- Total: n [total] 735, n [relapse] 155
- low PIAS1 (IRS ≤ 4): n [total] 211, n [relapse] 30
- intermediate/high PIAS1 (IRS > 4): n [total] 524, n [relapse] 125

- Log Rank (Mantel Cox): p = 0.011
PIAS1 is elevated in PCa
(PIAS1 expression correlates with AR)
PIAS1 is an AR target gene (PIAS1 is influenced by R1881 and AR knockdown)
PIAS1 binds to AR
(reduced proteasomal degradation of AR)

**DUCaP**

<table>
<thead>
<tr>
<th>binding sites (chr)</th>
<th>start-end</th>
<th>fold enrichment</th>
<th>gene</th>
<th>distance from gene</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>Chr15:66098671..66099176</td>
<td>37.21</td>
<td>PIAS1</td>
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<tr>
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<tr>
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<td>Chr15:66121679..66122353</td>
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<tr>
<td>5</td>
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<td>PIAS1</td>
<td>25487</td>
</tr>
</tbody>
</table>
PIAS1 knockdown results in reduced AR activity

Luciferase measurement

Postulated mechanism

Cytoplasm

Nucleus

AR binding (protein stabilization)

transcription of target genes

PIAS1

PIAS1

PSA

FKBP5

↑ proliferation

p21
PIAS1 knockdown leads to apoptosis in AR-/+ cells

Puhr et al. 2014
PIAS1 knockdown with ADT
(more effective than single drug treatment)
• PIAS1 expression increases with PCa tumor progression
• PIAS1 correlates with AR expression and is a marker for reduced relapse-free survival
• PIAS1 knockdown results in in elevated apoptosis *in vitro* and *in vivo*
• PIAS1 knockdown enhances efficacy of novel anti-androgens

PIAS1 might be a promising target to improve PCa therapy in combined treatment approach

- Translate findings to other malignancies (breast, bladder cancer)
- Assess the role of PIAS1 in cancer radio-therapy response; successful “Herta-Firnberg” grant application (Julia Höfer)
- Gain more mechanistic/functional data for PIAS1 inhibitor development
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