Unit in Biomedical Research in Urology

In search for good therapeutic targets to annihilate the resistance in aggressive prostate cancer

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Group Leader
Cell Signaling and Cancer Progression
Major aims of our research

- To identify PCa markers helpful for the diagnosis and prognosis of the aggressive disease.
- To understand the mechanisms implicated in PCa progression to a resistant disease. This may lead to discover more efficient therapeutic targets to eliminate the recurrent metastatic cancer.

- Discovery of the oncoprotein PTOV1, hints about its function and mechanisms of action in aggressive PCa, and in the resistance to chemotherapy.

- Models systems to study in vitro the biology of PCa and their response to treatments.
- Most common neoplasia among males in the western Countries.
- Second leading cause of death from cancer in men.

**Estimated New Cases**

<table>
<thead>
<tr>
<th>Cancer Type</th>
<th>New Cases</th>
<th>5-year Survival (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prostate</td>
<td>241,740</td>
<td>29%</td>
</tr>
<tr>
<td>Lung &amp; bronchus</td>
<td>116,470</td>
<td>14%</td>
</tr>
<tr>
<td>Colon &amp; rectum</td>
<td>73,420</td>
<td>9%</td>
</tr>
<tr>
<td>Urinary bladder</td>
<td>55,600</td>
<td>7%</td>
</tr>
<tr>
<td>Melanoma of the skin</td>
<td>44,250</td>
<td>5%</td>
</tr>
<tr>
<td>Kidney &amp; renal pelvis</td>
<td>40,250</td>
<td>5%</td>
</tr>
<tr>
<td>Non-Hodgkin lymphoma</td>
<td>38,160</td>
<td>4%</td>
</tr>
<tr>
<td>Oral cavity &amp; pharynx</td>
<td>28,540</td>
<td>3%</td>
</tr>
<tr>
<td>Leukemia</td>
<td>26,830</td>
<td>3%</td>
</tr>
<tr>
<td>Pancreas</td>
<td>22,090</td>
<td>3%</td>
</tr>
<tr>
<td>All Sites</td>
<td>848,170</td>
<td>100%</td>
</tr>
</tbody>
</table>

**Estimated Deaths**

<table>
<thead>
<tr>
<th>Cancer Type</th>
<th>Deaths</th>
<th>5-year Survival (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prostate</td>
<td>28,170</td>
<td>9%</td>
</tr>
<tr>
<td>Lung &amp; bronchus</td>
<td>87,750</td>
<td>29%</td>
</tr>
<tr>
<td>Colon &amp; rectum</td>
<td>26,470</td>
<td>9%</td>
</tr>
<tr>
<td>Pancreas</td>
<td>18,850</td>
<td>6%</td>
</tr>
<tr>
<td>Liver &amp; intrahepatic bile duct</td>
<td>13,980</td>
<td>5%</td>
</tr>
<tr>
<td>Leukemia</td>
<td>13,500</td>
<td>4%</td>
</tr>
<tr>
<td>Esophagus</td>
<td>12,040</td>
<td>4%</td>
</tr>
<tr>
<td>Urinary bladder</td>
<td>10,510</td>
<td>3%</td>
</tr>
<tr>
<td>Non-Hodgkin lymphoma</td>
<td>10,320</td>
<td>3%</td>
</tr>
<tr>
<td>Kidney &amp; renal pelvis</td>
<td>8,650</td>
<td>3%</td>
</tr>
<tr>
<td>All Sites</td>
<td>301,820</td>
<td>100%</td>
</tr>
</tbody>
</table>
Therapeutic approaches for Prostate Cancer progression

Androgen sensitive

Androgen resistant

ADT
Progression of human Prostate Cancer

Normal Prostate

Pre-malignant (HG-PIN)

Localized Tumor Androgen-independent

Normal Epithelia

HG-PIN

Localized Tumor

Metastasis

VHIR

Localized Tumor Metastasis

Pre-malignant (HG-PIN)

Normal Prostate

Pérdida de células basales

Pérdida de lámina basal

Andrógeno-independencia

Progression of human Prostate Cancer
Discovery of the oncoprotein PTOV1

Function and mechanisms of action in aggressive PCa, and in the resistance to chemotherapy
Discovery of the oncoprotein Prostate Tumor Overexpressed-1, PTOV1

PTOV1 is required for full tumor growth and metastasis of PC3 cells

Human Prostate Cancer

B) Tumor Weight

C) Metastases-Free Disease

Benedit et al., Oncogene 2001; Santamaría et al., Am J Pathol 2003; Marqués et al., Oncogene 2014; Alaia et al., Mol Cancer 2014
The expression of PTOV1 in pre-neoplastic lesions (HGPIN) found in needle biopsy is associated to the presence of cancer.
PTOV1: A NEW GENE FAMILY

**PTOV1 / Acid-2**

- Domain A
  - NLS 1
  - KRRP
- Domain B
  - NLS 2
  - PNSRSKR

**PTOV2**

- Arc92 / Acid-1 / MED25
- DmPTOV1
- DmPTOV2

**SPOC, Ku70/Ku80**

2011

**References**

- Benedit et al., Oncogene, 2001
- Wang et al., BBRC 2001
- Lee et al., EMBO J. 2007
- Nääär et al., Nature, 1999
- Mittler et al., EMBO J. 2003
- (Acc. AC013074) predicted
- Adams et al., Science, 2000
PTOV1 Interacts with the Receptor of ACtivated protein Kinase C, RACK1

Receptor of ACtivated protein Kinase C,

- Very conserved, homologous to β-subunits of heterotrimeric G proteins.
- 7 WD repeats, protein interaction domains.
- Regulates cell spreading, focal adhesions and cell-cell contacts
- Binds to β-subunit of integrin receptor: important for focal adhesion

- Subunit of 40S ribosome
- Regulates translation initiation: its recruitment of PKC allows the formation of the 80S.

PKCβ, JNK, c-Jun, β-Integrin receptor..
PTOV1 associate with RACK1 and ribosomes after activation with growth factors
PTOV1 binds to ribosomes through RACK1 and promotes the translation of subsets of mRNAs, including c-Jun.

Increased PTOV1 produced increased translation of c-Jun protein.

The protein binds to RACK1 on 40S ribosome, but not to polysomes.

PTOV1 overexpression increased global protein synthesis.

Marqués N, Sesé M, Cánovas V, et al., Oncogene 2014
The complex PTOV1 /c-Jun RNA binds the 40S ribosomes and promotes translation of subsets of mRNAs, including c-Jun.

Increased levels c-Jun/AP1 oncoproteins

Mesenchymal gene program
(↑Snail1, N-Cad, Vim... ↓E-Cad)

Invasion and metastasis
Oncogenic PTOV1 represses transcription downstream of the Notch receptor

HES1 promoter activity

Chromatin Imm. Precipitation

In the prostate
PTOV1 is oncogenic and counteracts with NOTCH tumor-suppressor functions
The expression of hPTOV1 increases the effects of Notch loss-of-function (LOF) and suppresses the effects of Notch gain-of-function (GOF).
Coordinated expression in prostate cancer progression

<table>
<thead>
<tr>
<th></th>
<th>PTOV1</th>
<th>HEY1</th>
<th>HES1</th>
<th>c-Jun</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal (BPZ)</td>
<td>![Image]</td>
<td>![Image]</td>
<td>![Image]</td>
<td>![Image]</td>
</tr>
<tr>
<td>PCa</td>
<td>![Image]</td>
<td>![Image]</td>
<td>![Image]</td>
<td>![Image]</td>
</tr>
<tr>
<td>Metastasis</td>
<td>![Image]</td>
<td>![Image]</td>
<td>![Image]</td>
<td>![Image]</td>
</tr>
</tbody>
</table>
PTOV1 is overexpressed in several tumor types and significantly associated to high grade malignant tumors

For bladder
Low vs High  \( p = 0.026 \),
Cramer’s coeff. = 0.84
PTOV1 is an independent prognostic factor associated with cancer progression and poor survival in several neoplasias.

Abstract -
Increased PTOV1 expression is related to poor prognosis in epithelial ovarian cancer.

Does PTOV1 have a role in the metastatic resistant prostate cancer?

Original Paper
Prostate tumour overexpressed-1 promotes tumourigenicity in human breast cancer via activation of Wnt/β-catenin signalling
Yanmei Cui, Weifeng Ma, Fangyong Lei, Qingyuan Li, Yanhong Su, Xi Lin, Chuyong Lin, Xin Zhang, Liping Ye, Shu Zhongyu Yuan, Libing Song

First published: 30 May 2016
DOI: 10.1002/path.4725
Cancer cell models used to study recurrent Castration Resistant Prostate Cancer (CRPC)

Androgen sensitive
Androgen resistant (CRPC)

LNCaP-AD
Androgen-dependent AD

LNCaP-Al
PC3, Du145
docetaxel sensitive DS

PC3
Du145
docetaxel resistant DR
LNCAP
- From metastatic site: supraclavicular lymph node
- Androgen-sensitive
  - PTEN null
  - p53 WT / RB WT
  - AR positive
  - PSA positive
  - ETV1 fusion+ overexpr.

DU145
- From metastatic site: brain
- Androgen-resistant
  - PTEN +/-
  - p53 mutant/ RB null
  - AR negative
  - PSA negative

PC3
- From metastatic site: bone
- Androgen-resistant
  - PTEN null
  - p53 null/ RB WT
  - AR negative
  - PSA negative
  - ETV4+ overexpr.

'Documentation status' from Barbieri et al, Eur Urology 2013
PTOV1 overexpressed in CRPC cells sensitive to docetaxel promotes their resistance to chemotherapy

**Du145**
- DS: 2.4 nM
- DR: 4.7 nM

**PC3**
- DS: 3.9 nM
- DR: 9.1 nM

***

**Du145**
- IC\textsubscript{50}: 2.0 nM DS-GFP
- IC\textsubscript{50}: 3.9 nM DS-HAPTOV1
- IC\textsubscript{50}: 5.3 nM DR

**PC3**
- IC\textsubscript{50}: 4.8 nM DS-GFP
- IC\textsubscript{50}: 5.6 nM DS-HAPTOV1
- IC\textsubscript{50}: 7.2 nM DR

***

KRT18: 0.03

Actin: 1

1       2.7

1       3.5

1       0.05

1       0.03

IC\textsubscript{50}: 2.0 nM DS-GFP
IC\textsubscript{50}: 3.9 nM DS-HAPTOV1
IC\textsubscript{50}: 5.3 nM DR
IC\textsubscript{50}: 4.8 nM DS-GFP
IC\textsubscript{50}: 5.6 nM DS-HAPTOV1
IC\textsubscript{50}: 7.2 nM DR
The overexpression of PTOV1 induces an increase of expression of genes associated to resistance to chemotherapy
Higher levels of PTOV1 increase the spheroid forming capacity and expression of stemness genes
The KD of PTOV1 arrested cells at the G2-M phase. Apoptosis is observed. DR cells are more sensitive.
The expression of **PTOV1, ALDH1A1, CCNG2** and **MYC** is significantly increased in human prostate tumors.

PTOV1 represents a good target to eliminate aggressive prostate cancer cells and potential CSCs-like subpopulations.
Models systems to study in vitro the biology of human prostate tumors and their response to treatments
Generation of models from cell lines and human tumors

- **Cell line models sensitive/resistant to treatments**
- **PCa Biopsies / RP tumors**
  - Induction of resistant tumors in vitro by androgen deprivation (ADT)
  - Functional selection of CSCs-like by 3D cultures (Spheres)
  - Primary tumors, Cell lines and spheres
  - In vitro assays for specific inhibitors aimed to eliminate CSCs populations
  - Screening for more specific targets to eliminate CSC populations
  
  - CTL
  - ADT
  - Subcutaneous injection - Spheres - Parental cells
  - Omics

- **Tumors**
Enriquecimiento de células tumorales con capacidad de crecer no-adheridas formando esferas (CSC)
**Modelo celular LNCaP**: el estrés por la falta de andrógeno resulta en un enriquecimiento de CSC-like

Andrógeno Dependientes

Andrógeno Independ. 

Las células AI expresan niveles más altos de Marcadores de CSC
Modelo celular LNCaP: Andrógeno Dependientes y Andrógeno Independiente formación de esferas
LNCaP AD y AI: expresión de marcadores de ‘stemness’
Modelo LNCaP: las células derivadas de esferas son resistentes a dosis altas de Docetaxel

Esferas AD

Esferas AI
Modelos celulares Du145 y PC3 (CRPC), sensibles y resistentes a docetaxel: formación de esferas

Du145- DS  Du145- DR  PC3- DS  PC3- DR

Adherentes  Docetaxel-resistentes  Docetaxel-resistentes

G2
Enriquecimiento progresivo de la capacidad de formar esferas (capacidad tumorigenica)
Prostaspheres cultures are more resistant to DOCETAXEL compared to parental cells grown in adherent conditions.

**A**

Du145 DS

**B**

Du145 DR

**C**

PC3 DS

**D**

PC3 DR
Prostatospheres cultures are more resistant to CABAZITAXEL compared to parental cells grown in adherent conditions.
Prostate tumor explants cultured in vitro in adherent conditions: Androgen deprivation (ADT) induces a resistant culture with CSC-like phenotypes.
Prostate tumor explants cultured *in vitro* in NON-adherent conditions: spheres formation from androgen-dependent and -independent tumors

**PR1 – Radical Prostatectomy**
Prostate tumor explants cultured *in vitro* are selected for cells able to grow as spheres: Androgen Independent cells have a higher capacity to grow as spheres.

**Andrógeno Dependientes**

**Andrógeno Independiente**

PR1 – Radical Prostatectomy
Prostate tumor explants cultured *in vitro* in adherent conditions: Androgen deprivation (ADT) induces a resistant culture with CSC-like phenotypes.
Thank you!

Past members of the lab:
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