Tumor microenvironment complexity, genomics and immunity in prostate cancer

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The urgent need of risk stratification tools!
Inflammation influences prostate cancer development

In an observational study of men with a negative baseline biopsy and an elevated PSA who received routine biopsies at 2 and 4 years largely independent of PSA, those men taking either aspirin and/or NSAIDs at baseline had a lower risk of prostate cancer including high-grade prostate cancer.

Vidal et al., Clin Cancer Res 2015

Cellular components of the human prostate gland

Barron and Rowley, *Endocr Relat Cancer* 2012
Prostate gland homeostasis and reactive stroma formation in cancer

Normal prostate

Localized inflammation/remodeling

Prostatic intraepithelial neoplasia

Reactive stroma induction

Reactive stroma coevolution with cancer progression

Inflammatory cell recruitment

Barron and Rowley, Endocr Relat Cancer 2012
A suppressive microenvironment supports metastatic dissemination and colonization at secondary sites

DF Quail & JA Joyce, Nature Med 2013

Kruszinski et al, Trends in Pharmacological Sciences 2015
Evolution of the tumor microenvironment during prostate cancer progression
Influences of ECM on the metastatic cascade

Gonçalves et al, Acta Histochemica 2015

Pickup et al., EMBO Rep. 2014

Architecture of extracellular matrix during prostate cancer progression
Prostate cancer is associated with high levels of inter-patient heterogeneity and intra-patient heterogeneity

Monoclonal and polyclonal models of metastasis from a multifocal primary prostate cancer

Given its heterogeneity, clinical management of prostate cancer is challenging and requires a detailed understanding of the genetic alterations that occur in all cells, including small subpopulations
Prostate cancer is characterized by extraordinary genomic complexity

- Somatic copy number alterations
- Germline mutations
- Chromosomal rearrangement (such as translocations, insertions, duplications, and deletions) and gene fusions
Driver genes mutated in primary and metastatic prostate cancer

<table>
<thead>
<tr>
<th>AR-associated</th>
<th>PI3K pathway</th>
<th>WNT pathway</th>
<th>DNA repair</th>
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<tbody>
<tr>
<td>ETS fusion</td>
<td>PIK3CA</td>
<td>APC</td>
<td>BRCA2</td>
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<tr>
<td>TP53</td>
<td>PIK3CB</td>
<td>CTNNB1</td>
<td>ATM</td>
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<td>PTEN</td>
<td>PIK3R1</td>
<td>RNF43</td>
<td>BRCA1</td>
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<td>FOXA1</td>
<td>AKT1</td>
<td>RSPO2</td>
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<td>NCO1</td>
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<td>ZNRF3</td>
<td>MLH1</td>
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<td>NCO2</td>
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<td>MSH2</td>
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</table>

Chromatin modifier: RB1, CDKN1B, CDKN2A, KMT2C, KMT2D, CHD1, MED12, ZFHX3, ERF, GNAS, ZNF770, NKK3-1, BRAF
ERG regulates the expression of target genes associated with cancer initiation and progression.
Genomic aberration of \textit{PTEN} tumor suppressor gene are among the most common in prostate cancer

- Phosphatase and tensin homologue (PTEN) loss in radical prostatectomy samples is often concurrent with genomic rearrangements involving the ETS family transcription factors

- PTEN might be a useful prognostic biomarker to distinguish potentially aggressive Grade Group 1 or 2 tumours, which might make patients poor candidates for active surveillance programmes

- PTEN loss is associated with suppression of androgen receptor (AR) transcriptional output, and phosphoinositide 3-kinase (PI3K) inhibitors activate AR signalling, suggesting potential efficacy of combination therapies targeting the PI3K and AR signalling pathways

- Emerging studies indicate that PTEN loss is associated with alterations to cellular interferon responses in the tumour microenvironment - tumours with loss of PTEN are more likely to have an immunosuppressive microenvironment, suggesting that advanced prostate cancers with PTEN loss might be amenable to immune-based therapies
Heterogeneous immunohistochemical expression of ERG and PTEN in prostate tumours

PTEN loss is enriched among localized tumors with ERG gene rearrangement compared with those without this alteration

Jamaspishvili et al., Nat Rev Urology 2018
Algorithm for when to determine PTEN status on diagnostic biopsy material using IHC and FISH

High PSA, abnormal DRE

Biopsy

No cancer

Grade Group 1 or 2 cancer

Grade Group >2 cancer

PTEN intact (IHC)

PTEN partial loss (IHC)

PTEN ambiguous (IHC)

PTEN complete loss (IHC)

Perform PTEN FISH

No loss (PTEN+/+)

Hemi loss (PTEN−+/+)

Homo loss (PTEN−/−)

Prognosis reflects clinicopathological variables

Prognosis reflects PTEN status and clinicopathological variables

Jaspishvili et al., Nat Rev Urology 2018
Flowchart of genomic prostate cancer biomarkers
Driver genes mutated in primary and metastatic prostate cancer

- AR-associated
- PI3K pathway
- WNT pathway
- DNA repair

Modified by Linch et al. Annual Oncol 2017
Using DNA repair defects as a therapeutic target: PARP inhibitors

Robinson et al., Cell 2015
Mismatch repair genes in metastatic CRPC

Proposed relationship between MSI status and immunologic response
Response to immunotherapy in the context of DNA damage
The best example is MSI-instable colon cancer
Milestones in the clinical development of checkpoint blockers

2014
Anti-PD-1 (Nivolumab & Pembrolizumab) approved for irresectable stage IV melanoma

2015
Anti-PD-1 (Nivolumab & Pembrolizumab) approved for NSCLC

2015
Combination of Anti-CTLA-4 (Ipilimumab) & Anti-PD-1 (Nivolumab) approved for irresectable stage IV melanoma

2017
Anti-PD-1 (Pembrolizumab) approved for solid tumors with MSI-H & MMR abnormalities

2011
Anti-CTLA-4 (Ipilimumab) approved for irresectable stage IV melanoma

2015
Anti-CTLA-4 (Ipilimumab) approved for ‘high risk’ melanoma following surgery

2016
Anti-PD-L1 (Atezolizumab) approved for metastatic urothelial carcinoma

2017
Anti-PD-L1 (Avelumab) approved for Merkel cell carcinoma

2016
Anti-PD-L1 (Atezolizumab) approved for NSCLC

2017
Third Anti-PD-L1 (Durvalumab) approved for advanced bladder cancer

Including Prostate cancer

Rotte et al., Ann Oncol 2017
Oncology meets Immunology

The new era of immune checkpoint therapy
Activation of T cells requires two signals

1. TCR signal only
   - Antigen + MHC + TCR
   - No T cell proliferation

2. Positive costimulation
   - B7 + CD28
   - T cell proliferation
   - Cytokines

Tumor or epithelial cells

APC's (dendritic cells, macrophages)

Sharma and Allison Science 2015
Multiple co-stimulatory and inhibitory interactions regulate T cell response
Anti-CTLA-4 (ipilimumab) + radiation therapy in castration-resistant prostate cancer (CRPC)

Kwon et al., Lancet Oncology, 2014
Why do some patients respond and others do not?

Schema for Analysis of Baseline and Longitudinal Tumor, Blood, and Other Samples

Molecular analysis
- Mutational load
- Driver mutations
- Gene expression

Immune analysis
- CD8
- PD-L1
- Clonality

Tumor
PBMC
Microbiome (fecal)
Microbiome (oral)

Baseline
Early-on treatment
Progression

Sharma et al. Cell 2017
Prostate cancer:
Evaluating response to immune checkpoint therapy on a pre-surgical clinical trial

Clinical trial for patients with localized disease (N=20)

Presented By Padmanee Sharma at 2018 Genitourinary Cancers Symposium: Translating Evidence to Multidisciplinary Care
Converting a ‘cold’ to ‘hot’ prostate tumor microenvironment

Gao et al., Nature Medicine 2017
Cold tumors and hot tumors

Inflamed tumor

Non-inflamed

β-catenin-driven immune escape

Spranger, Gajewski, Oncoimmunology 2016

Gajewski et al., Nature Immunology 2013
Clinical impact of the immune contexture in different primary tumors

Cancer classification based on tumour-microenvironment-related parameters (Immunoscore)

Galon, J Pathol 2014
# Immune infiltrates in untreated prostate cancer compared to other tumor types

<table>
<thead>
<tr>
<th>Tumor Type</th>
<th>CD8+ T cells</th>
<th>TLS</th>
<th>Treg cell</th>
<th>M</th>
<th>M1</th>
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Legend:
- 💚 Positive prognosis
- 🟢 Mostly positive prognosis
- 🟢 No effect on prognosis
- 🟢 Mostly negative prognosis
- 🟢 Negative prognosis

Data points:
- 🟢 0–100 patients
- 🟢 100–1000 patients
- 🟢 1000–10000 patients

*Fridman WH et al., Nat Rev, 2017*
Additional inhibitory pathways in prostate tumor microenvironment

Top 10 signaling pathways in response to ADT + ipilimumab treatment

- CTLA4 Signaling
- Antigen Presentation
- OX40 Signaling
- Autoimmune Diabetes
- Dendritic/NK Crosstalk
- ICOS Signaling
- TCR Signaling
- Interferon Signaling
- Nitric-Oxide Signaling
- CD28 Signaling

Selected immune gene expression after ADT + ipilimumab treatment

- Fold Change

Gao et al., Nature Medicine 2017
Additional inhibitory pathways in prostate tumor microenvironment

Gao et al., Nature Medicine 2017
PD-L1 expression in immune infiltration in prostate cancer

Gao et al., Nature Medicine 2017
VISTA expression in prostate cancer and melanoma

Gao et al., Nature Medicine 2017
VISTA and PD-1/PD-L1 are potent inhibitor of human T cell responses

Gao et al., Nature Medicine 2017
Ipilimumab-enhanced T cell response to conventional prostate cancer antigens and neoantigens

Presented By Padmanee Sharma at 2018 Genitourinary Cancers Symposium: Translating Evidence to Multidisciplinary Care
Conclusions

- Prostate cancer is poorly-infiltrated by T cells but an increase in T cell infiltration into prostate tumors may lead to expression of compensatory inhibitory pathways (PD-1/PD-L1, VISTA) that suppress anti-tumor immune responses.

- T cells from patients with prostate cancer are capable of recognizing mutated antigens expressed by prostate tumors and ongoing studies will help to determine whether neoantigens-specific T cell responses correlate with anti-tumor responses.

- Combination therapy will be necessary to improve anti-tumor immune responses with potential clinical benefit in patients with prostate cancer.
Biomarkers of resistance to ICB at the convergence of tumor intrinsic and extrinsic mechanisms in cancer

Tumor intrinsic

Tumor extrinsic

CONVERGENCE in the TME

Hypoxia

ECM

Rigid matrix

Inflammation

Mesenchymal traits
Mesenchymal traits resulting from tumor intrinsic and extrinsic factors influence T-cell trafficking and function determining resistance to ICB treatment.
Gene Oncotype DX Genomic Prostate Score

**Genes Associated with Worse Outcome**
- Stromal Response
  - BGN
  - COL1A1
  - SFRP4
- Proliferation
  - TPX2

**Genes Associated with Better Outcome**
- Androgen Signaling
  - AZGP1
  - FAM13C
  - KLK2
  - SRD5A2
- Cellular Organization
  - FLNC
  - GSN
  - GSTM2
  - TPM2

**Reference Genes**
- ARF1
- ATP5E
- CLTC
- GPS1
- PGK1

GPS (scaled 0–100) = \{Stromal Response Group\} − \{Androgen Signaling Group\} − \{Cellular Organization Group\} + \{Proliferation Group\}

Each gene is individually weighted in the final algorithm.

*Dall’Era M et al., University of California*
The tumor microenvironment

Pre-clinical data suggest that hypoxia leads to a mutator phenotype, chromosomal rearrangements and metastases.
Microenvironment: stroma or epithelium as a metastatic predictor?

Stroma-derived metastasis signature (SDMS) includes genes related to cellular movement and migration, and in the modulation of cell-cell and cell-matrix interaction.

Our stroma-derived metastasis signature can predict the metastatic potential of early stage disease and will strengthen decisions regarding selection of active surveillance versus surgery and/or radiation therapy for prostate cancer patients. Furthermore, profiling of stroma cells should be more consistent than profiling of diverse cellular populations of heterogeneous tumors.

Mo, Wang, Collins (Vancouver)-Eur Urology 2018
Integrated DNA-based and microenvironment-based indices

Co-occurrence of hypoxia and genomic instability predicts patient outcome

100-loci DNA signature, which measures genomic instability and is enriched for lipid metabolism genes, outperforms previously published RNA-based prognostic signatures for prostate cancer

Lalonde et al, Lancet Oncology 2014
Tumor progression and mechanical forces
The Co-occurrence of hypoxia and genomic instability

Northey, Przybyla, and Weaver. Cancer Discovery 2017
Take home message

The complexity of genomic alteration, epithelial-stroma interaction and immune cell contexture dictates a multidisciplinary approach to identify risk stratification indices and targets for novel therapies.
Organotypic tumor spheroid

Tumor Immunology and Immunotherapy Unit

Regina Elena National Cancer Institute, Rome, Italy