Genes, chromosomes, and the treatment of bladder cancer

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Disclosures

• Consulting
  – Oncogenex
  – Eli Lilly
  – Dendreon
  – Johnson & Johnson
The revolution is here....
But not yet in bladder cancer

• High-throughput genomic technologies have transformed cancer research
• Molecular alterations linked to outcomes in multiple malignancies
  – Predictive: lung, melanoma, breast, CML, colorectal
  – Prognosis: breast, oligodendrogliomas
  – Practice-changing in many malignancies
  – Not yet demonstrated utility in bladder cancer
• Bladder cancer is an attractive target for biomarker discovery and translational science
  – Abundance of resected tissue
Context: Bladder Cancer 2012

- Non-muscle invasive bladder cancer is most common form
  - 70% present with non-muscle invasive disease
    - 30% will progress to higher stage
  - 30% present with muscle invasive/metastatic disease

- Prognosis is stage-driven

- Practically:
  - Non-muscle invasive
  - Muscle invasive
  - Metastatic

BMJ 1998. 317 : 1366
No major treatment advances in 20 years

GC: median = 14.0 m (12.3-15.5 m); 13.3% censoring
MVAC: median = 15.2 m (13.2-17.3 m); 15.4% censoring
HR: 1.09 (0.88-1.34)
Log-rank $P = .44$, Wald’s $P = .66$

GCb vs. M-CAVI

HR=0.94 (95%CI: 0.72, 1.22) $p=0.64$
8.1 months (95%CI: 6.1, 10.3)
9.3 months (95%CI: 7.6, 11.3)

Gem/Cis 12.8 mo
Pac/Cis/Gem 15.7 mo
HR= 0.86 (0.72-1.03); $p=0.1$
Progress in Urothelial Cancer?

• Newer cytotoxic agents and strategies probably provide little additional benefit

• New strategies are needed
  – Customizing chemotherapy
  – Identification of effective targeted agents

• Novel approaches beyond BCG in NMIBC remain out of reach

• Personalized therapy through predictive markers is crucial but unrealized
Moving beyond therapeutic stagnation in bladder cancer

• Linkage between biological insights and clinical outcomes has not been established
• No validated novel targets
• No validated predictive biomarkers in metastatic disease
Limitations of molecular prognostic studies in UC

- Heterogeneous populations
  - Lump different biology and stages together
- Patients with non-invasive bladder cancer do better
  - Biomarkers found in those patients should predict better survival

This indicates different biology
But is this more information than the pathology report?

Lindgren et al. PLOS One 2012; 7(6): e38863
Uniform stage leads to relevant clinical outcome data

- Pathologically confirmed pT1 tumors
- FGFR3 activating mutation by SNaPshot
- FGFR3 mutation leads to improved outcomes with pT1 tumors
- Additional validation needed
Rethinking biomarker studies in UC

• Uniform cohorts are needed
• Patients in biomarker studies should have similar disease states and outcomes be determined from consistent time points
Bladder cancer genome

• Characterized by many recurrent genomic changes
  – Target rich environment
  – Many different druggable alterations
Methodologies to analyze the genome

**GISTIC 1.0**
Beroukhim et al. PNAS (2007)

**GISTIC 2.0**
Mermel et al. Genome Biol. (2011)

**Uses:**
Frequency and amplitude of events
Separates broad and focal gains and losses

**NetSig (in development)**
Zou et al., in development

**Uses:**
- all types of alterations to identify clusters of mutated genes in protein-protein networks

**MutSig**
Lawrence et al. in development

**Uses:**
- Number and types of mutations;
- corrects BMR for expression, gene footprint size etc.

Works on genes, genesets and conserved regions (intervals on the genome)

Craig Mermel, Rameen Beroukhim, Steve Schumacher, Mike Lawrence, Lihua Zou, Alex Ramos, Gregory Kryukov, Petar Stojanov
Map of recurrent gains and losses in primary tumors of patients with metastases

- CDKN2A
- RB1
- TSC
- CCND1, FGF3, 4, 19
- PPARγ
- E2F3
- E2F1
- ERBB2

www.esmo2012.org
Landscape of Copy Number Alterations in High-Grade Bladder Cancer

A. [Genome-wide copy number alterations heat map]

B. [Bar chart showing fraction of genome altered for different cancer types]

C. [Gene expression q-values for selected genes]
Copy number and mutation analysis reveals some mutual exclusivity between different genomic alterations in UC

Composite heatmap of copy number alterations and mutations within 97 HGUC tumor samples

Altered in 80 (82%) of cases.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Total altered</th>
<th>All 97 cases</th>
<th>Alteration Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>FGFR3</td>
<td>15%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ERBB2</td>
<td>6%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MET</td>
<td>2%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BRAF</td>
<td>2%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>KRAS</td>
<td>7%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NF1</td>
<td>4%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MYC</td>
<td>7%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PIK3CA</td>
<td>19%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PIK3R1</td>
<td>1%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AKT1</td>
<td>3%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AKT3</td>
<td>1%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PTEN</td>
<td>4%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TSC1</td>
<td>7%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TP53</td>
<td>35%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MDM2</td>
<td>5%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CCND1</td>
<td>15%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CCNE1</td>
<td>3%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CDKN2A</td>
<td>20%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>E2F3</td>
<td>21%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RB1</td>
<td>16%</td>
<td></td>
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</tr>
</tbody>
</table>

- Amplification
- Homozygous Deletion
- Mutation

Copy number alterations are putative.
A. P53 signaling

- **MDM2** 5%
- **TP53** 34%

Proliferation cell survival

37 altered samples (38%)

B. RB signaling

- **CDKN2A** 24%
- **CCND1** 14%
- **CCNE1** 5%
- **RB1** 15%
- **E2F3** 21%

Cell cycle progression

58 altered samples (60%)

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Multiple oncogenic pathways altered

A. 
- **ERBB2**: 6%
- **FGFR3**: 13%
- **MET**: 2%
- **FGFR1**: 6%

```
  ↓
KRAS  NF1
  ↓
BRAF  RAF1
```

Proliferation, cell survival, translation

B. 
**ERBB2 amplified in 6%**

```
2 mb on chr 17q
```

C. 
- **HER2 amplified**
- **HER2 non-amplified**
ERBB2 Incidence 6-11% by Copy Number Analysis

Spain

DFCI
Genetic basis of extreme everolimus sensitivity

PI3K/Akt pathway mutations confer sensitivity to Akt inhibition in high-grade UC cell lines

A. PI3K signaling

- PTEN 4%
- PIK3CA 18%
- AKT1 2%
- TSC1 7%
- MTOR

MK2206

Proliferation, cell survival, translation

B. Graph showing IC50 (uM) for different cell lines and mutations.

C. Western blot analysis showing changes in various proteins (pAKT S473, Akt, pPRAS40, pFOX-O1/3, pS6, S6 total, p-4EBP1 T37/46, 4EBP1 total) with treatment by MK2206.
Molecular targets and outcomes

- **PIK3CA mutation**
  - P=NS

- **FGFR3 by IHC in primary tumor**
  - Negative
  - Positive
  - \( p = 0.78 \)

- **FGFR3 by IHC in metastases**
  - Unmutated
  - Mutated

- **Time to Recurrence - PI3K/Akt Pathway**
  - PI3K/Akt Altered
  - PI3K/Akt WT
  - \( p = 0.05 \)

- **Time to Recurrence - TK Receptor and Downstream Elements**
  - TK Wild Type
  - TK Altered
  - \( p = 0.06 \)
Chromatin-modifying genes

- Methylation of DNA leads to conformational and structural changes
  - Histone deacetylation and changes in chromatin structure
- Altered methylation changes gene expression in cancer cells
  - Global hypomethylation
  - Focal hypermethylation
- Recently described in bladder cancer

Chromatin remodeling genes identified in UC: Most mutations are predicted to be inactivating
Clinical relevance and utility of CMG mutation unclear

• Linkage of genotype to clinical phenotype is not clearly present

• Therapeutic implications not yet clear
  – Not easily druggable
mRNA expression: Hierarchical clustering in breast cancer defines subtypes with clinical biology

Sørlie T et al. PNAS 2003;100:8418-8423
Bladder cancer: mRNA expression yields 2 intrinsic classes of tumors

Panel A
- Green: low grade (g1/2)
- Red: high grade (g3)

- mRNA expression recapitulates morphology
- Gives little insight into prognosis of the tumor
"Meta-analysis" of multiple profiling datasets

<table>
<thead>
<tr>
<th>Gene symbol</th>
<th>Gene name</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>APOBEC3B</td>
<td>Apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like 3B</td>
<td></td>
</tr>
<tr>
<td>ATF3</td>
<td>Activating transcription factor 3</td>
<td></td>
</tr>
<tr>
<td>CCL5</td>
<td>Chemokine (C–C motif) ligand 5</td>
<td></td>
</tr>
<tr>
<td>DGCR2</td>
<td>DiGeorge syndrome critical region gene 2</td>
<td></td>
</tr>
<tr>
<td>ENDOD1</td>
<td>Endonuclease domain containing 1</td>
<td></td>
</tr>
<tr>
<td>FADD</td>
<td>Fas (TNFRSF6) associated via death domain</td>
<td></td>
</tr>
<tr>
<td>JUNB</td>
<td>Ribonuclease H2, subunit A</td>
<td></td>
</tr>
<tr>
<td>LMO7</td>
<td>LIM domain 7</td>
<td></td>
</tr>
<tr>
<td>MAP2K1</td>
<td>Mitogen-activated protein kinase kinase 1</td>
<td></td>
</tr>
<tr>
<td>MAP3K1</td>
<td>Mitogen-activated protein kinase kinase kinase 1</td>
<td></td>
</tr>
<tr>
<td>PDGFC</td>
<td>Platelet-derived growth factor C</td>
<td></td>
</tr>
<tr>
<td>PEA15</td>
<td>Phosphoprotein enriched in astrocytes 15</td>
<td></td>
</tr>
<tr>
<td>PFN1</td>
<td>Perforin 1 (pore-forming protein)</td>
<td></td>
</tr>
<tr>
<td>PPP1R12A</td>
<td>Protein phosphatase 1, regulatory (inhibitor) subunit 12A</td>
<td></td>
</tr>
<tr>
<td>PRDX1</td>
<td>Peroxiredoxin 1</td>
<td></td>
</tr>
<tr>
<td>PRMT1</td>
<td>Protein arginine methyltransferase 1</td>
<td></td>
</tr>
<tr>
<td>SLC1A5</td>
<td>Solute carrier family 1 (neutral amino acid transporter), member 5</td>
<td></td>
</tr>
<tr>
<td>TNGAIP6</td>
<td>TNF, α-induced protein 6</td>
<td></td>
</tr>
<tr>
<td>TSG101</td>
<td>Tumor susceptibility gene 101</td>
<td></td>
</tr>
<tr>
<td>TSPAN5</td>
<td>Tetraspanin 5</td>
<td></td>
</tr>
</tbody>
</table>
mRNA expression profiling

- mRNA expression profiling has identified distinct groups of tumors
- Findings associate with clinical features
- Can lead to more accurate risk stratification
- Therapeutic implications are unclear
  - MammaPrint, OncotypeDX are still not ready in bladder cancer
Translating findings to clinical practice

• No single druggable alteration characterizes the majority of tumors
  – Low mutation frequencies of multiple genes

• Potential model system for targeted therapy
  – None yet proven

• Requires trials enriched for patients to demonstrate proof of concept
Translating genomic information to bladder cancer practice

• Pre-screening patients for relevant molecular alterations

• Potentially relevant pathways
  – ERBB2
  – PIK3CA/mTOR/PTEN/AKT
  – CDK4/CCND1
  – FGFR3
  – BRAF

• Designing trials to demonstrate clinical benefit
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