Signaling pathways, mechanisms of resistance and treatment decisions in renal cell cancer

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Signalling pathways in Renal Cell Cancer

**Signalling Pathways**

- PI3K
- PLCγ
- RAS
- Raf
- MEK
- AKT
- mTOR
- PTEN
- PI3K
- MAPK
- E2F
- Cyclin D1
- CDK
- HIF-1
- VEGF
- VEGFR1
- VEGFR2
- Endothelial cells

**Cell Proliferation**

- **Survival**
  - E2F
  - CDK

**Angiogenesis**

**Invasion metastasis**

Tortora et al., 2010
Theoretical pathways by which VEGF-targeted therapies can increase tumor aggressiveness in endothelial and cancer cells

Site of action of VEGF and VEGFR inhibitors on endothelial and cancer cells

Modified from the original Kelly R.J., Targeted Oncology 2009
Anti-VEGFR TKIs have different potency and off-target activity


Adapted from Karaman et al. *Nat Biotech*. 2008;26:127
Sunitinib primarily acts on tumor endothelium rather than via direct targeting of ccRCC cells in vivo.

Sunitinib inhibits A-498 tumor angiogenesis as soon as 12 h after treatment.

Sunitinib shows minimal effect on tumor cell proliferation and no induction of apoptosis up to 72 h after treatment in vivo.

Huang D et al. Cancer Res 2010;70:1053-1062
PRIMARY AND SECONDARY (EVASIVE) RESISTANCE

- **Intrinsic Resistance**: 2-3 months of treatment
- **Primary refractory**: 2-3 months of treatment
- **Early progressors**: 6-12 months of treatment
- **Late progressors**: 6-12 months of treatment
- **Evasive Resistance**

Modified from the original Rini B and Flaherty K, Urology Oncology 2008
Key factors in the resistance to treatment in RCC

- Hypoxia and angiogenesis
- Production of alternative pro-angiogenic factors (IL-8, FGF, HGF/MET).
- Activation of alternative signalling pathways (mTOR, PI3K/Akt etc.)
- Microenvironment
- Tumor heterogeneity
HYPOXIA AFFECTS MANY FUNCTIONS

- Genome instability and tumor progression
- Angiogenesis
- Inflammation and Immunosuppression
- Switch to anaerobic metabolism
- Resistance to radio-chemio- and Immunotherapy
- Resistance to apoptosis and autophagy
- EMT and metastasis
- Induction of “cancer stem cells phenotype”
Adaptive-Evasive Responses by Tumors to Antiangiogenic Therapies

Antiangiogenic therapy leads to transitory tumor stasis or shrinkage and reduction in tumor vascularity

Hypoxia may be a consequence of treatment

Revascularization by alternative proangiogenic factors

Increased Invasiveness

Enhanced Metastasis

Paez-Ribes et al., Cancer Cell 15, 220-231, March 2009
mTOR pathway is involved in angiogenesis, proliferation and bioenergetics.
Effects of drug sequences on growth of 786-O tumors in nude mice

Western blot analysis on tumors lysates on day 77, at the end of second treatment.
mTOR Signaling pathway and TORC

mTOR complexes

Double inhibitors of PI3K and mTORC1 (BEZ235, etc.)
- Two inhibitors in combination
- Novel catalytic inhibitors of mTORC1 and mTORC2
Other proangiogenic factors relevant in RCC and resistance to treatment

- IL-8
- bFGF and FGF-Rs
- HGF/c-MET
IL-8 mediates resistance to sunitinib in RCC

Phenotypic resistance of 786-O ccRCC xenografts is associated with elevated plasma levels of IL-8 in sunitinib-resistant mice treated with continuous dosing.

Neutralization of IL-8 resensitized 786-O ccRCC xenografts to continuous sunitinib.

Huang D et al. Cancer Res 2010;70:1063-1071
IL-8 expression is increased in human RCC tumors with intrinsic resistance to sunitinib

IL-8 scoring

<table>
<thead>
<tr>
<th></th>
<th>Progression on sunitin</th>
<th>Progression on sunitin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Yes (n=9)</td>
<td>No (n=11)</td>
</tr>
<tr>
<td>IL-8 composite score</td>
<td>112.2 ± 33.8</td>
<td>44.5 ± 31.8</td>
</tr>
</tbody>
</table>

Huang D et al. Cancer Res 2010
FGF Mediates Escape From Antiangiogenic Therapy

FGF2 is expressed by numerous tumor types and exerts its proangiogenic activity by interacting with TKRs, heparan-sulfate proteoglycans, and integrins expressed on the endothelial cell surface.

- **Early phase**: VEGFR2 blockade transiently stops tumor growth and decreases vascularity.
- **Late phase**: Reactivation of angiogenesis after anti-VEGFR2 therapy through FGF activation leads to tumor progression.

FGF2 is a potent mediator of endothelial cell resistance to sunitinib

HUVEC cells

Sunitinib inhibits VEGF- but not FGF2-stimulated endothelial cell proliferation

PD173074 inhibits FGF2- but not VEGF-stimulated endothelial cell proliferation

PD173074 prevents effect of FGF2 on sunitinib-induced retraction of tubules

- Inhibitors of FGF or FGFRs
- Multikinase inhibitors of FGRs and VEGFRs (Dovitinib, etc.)

JC Welti et al., Oncogene 2010
HGF and its receptor MET

- One of the most frequently genetically altered RTKs in human cancers (activating mutations, amplifications)
  - **Activating mutations**
    - Hereditary papillary RCC: 100%, sporadic papillary RCC (13%)
    - HNSCC: 10%
    - NSCLC (8%) and SCLC (13%)
Resistance to Sunitinib and Lysosome sequestration

- Resistance by an adaptive mechanism: increased intracellular lysosomal sunitinib sequestration.
- Upon entering acidic organelle such as a lysosome, sunitinib becomes protonated and cannot cross back membranes

Sunitinib-resistant cells are cross-resistant to pazopanib and erlotinib but are sensitive to sorafenib and everolimus.

Gotink KJ. Et al. AACR 2012
Tumor, vessels, surrounding stroma, inflammatory and immune cells: The Tumor Microenvironment

Tortora G., 2012
Circulating Bone Marrow–Derived cell populations that stimulate or amplify tumor angiogenesis

CXCL12/SDF-1 - CXCR4 Axis and expression in RCC and normal kidney

Tumor cells (arrows) and vascular cells (arrowheads).

Burger e Kipps, Blood, 107, 1761 (2006)

### Biomolecular markers under investigation

<table>
<thead>
<tr>
<th>Category</th>
<th>Markers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adhesion molecules</td>
<td>cadherin-6, E-cadherin, MUC1-EMA, ICAM-1, VCAM-1, ELAM-1, KSA)</td>
</tr>
<tr>
<td>Inducers of immune-suppression</td>
<td>HLA class 1, IL-6, IL-8, IP-10, MIG, MIP1β, B7-H1, B7-H4, CD44</td>
</tr>
<tr>
<td>Growth factors receptors</td>
<td>VEGFR-3, TGFβR-II</td>
</tr>
<tr>
<td>Hypoxia-induced molecules</td>
<td>CAIX, CAXII, CXCR-4, HIF-1α, VEGF, IGF-I</td>
</tr>
<tr>
<td>Markers of proliferation</td>
<td>Ki-67, PCNA, Ag-NORs</td>
</tr>
<tr>
<td>Cell cycle regulatory proteins</td>
<td>p53, bcl-2, PTEN, cyclin A, Akt, p27</td>
</tr>
<tr>
<td>Others</td>
<td>VHL, mTOR, ribosomal protein S6, survivin, IMP3, caveolin-1, PCR, vimentin, fascin, seric amiloide A, NGAL, IGF-1</td>
</tr>
</tbody>
</table>
Detection of prognostic and predictive biomarkers

- Immunohistochemistry
- Circulating in plasma or serum
- Polymorphisms (SNPs)
Image-guided biopsies of large tumours might not be representative of the entire primary and may be misleading for a personalized therapeutic approach.
Only 55% of all somatic mutations (70/128) from the primary tumor were detected in a single biopsy sample.

When multiple samples from the same tumor were taken from different regions, only 34% of mutations were detected in all samples – Number falls to 31% if pre-treatment and metastases are included.
No Relationship Between \textit{VHL} Gene Mutations and Treatment Outcome with Cytokines, Temsirolimus, Pazopanib


No relationship between \textit{VHL} gene mutation status and RR and PFS in patients treated with initial sunitinib, sorafenib, bevacizumab, or axitinib

Carbonic Anhydrase IX (CAIX) is not a predictive marker for IL-2 response


CAIX Is Neither Predictive Nor Prognostic for Sunitinib or Sorafenib Response

Choueiri TK. Prognostic and Predictive Factors in RCC, ASCO-GU 2011

CAIX Does Not Appear to Be Predictive for Response to Temsirolimus

VEGF as a Predictive Tool in Sunitinib-treated Patients

Sunitinib-treated patients with baseline VEGF <707 pg/ml had prolonged median PFS vs those with VEGF >707 pg/ml


Rini BI et al. JCO 2008 Aug 1;26(22):3743-8
Lower baseline levels of sVEGFR-3 and VEGF-C are associated with longer PFS to sunitinib in RCC patients refractory to bevacizumab

Decrease of sVEGFR-2 correlates with PFS and tumor response to Pazopanib

Hutson et al. J Clin Oncol 2008;26(15S)
Prognostic or predictive plasma cytokines and angiogenic factors for patients treated with pazopanib for metastatic renal-cell cancer: a retrospective analysis of phase 2 and phase 3 trials

Hai T Tran, Yuan Liu, Amado J Zurita, Ying Lin, Katherine L Baker-Neblett, Anne-Marie Martin, Robert A Figlin, Thomas E Hutson, Cora N Sternberg, Rafael G Amado, Lini N Pandite, John V Heymach

Phase 2 trial of pazopanib in metastatic renal-cell carcinoma (n=225)²

Stage 1: Initial CAF candidate screening (n=129)
- 17 CAFs assessed: Decision Biomarkers BioChips
- Correlations: PFS and tumour shrinkage
- Study population: good vs poor responders
- 5 candidate CAFs identified

Stage 2: CAF candidate confirmation (n=215)
- 9 CAFs assessed: SearchLight and ELISA
- Correlations: PFS
- Study population: available phase 2 samples
- 7 candidate CAFs confirmed: interleukin 6, interleukin 8, VEGF, HGF, TIMP-1, E-selectin, and osteopontin

Phase 3 trial of pazopanib vs placebo in metastatic renal-cell carcinoma (n=435)¹⁰

Stage 3: CAF candidate validation (n=344)
- 7 CAFs assessed: CLIA-certified SearchLight
- Correlations: PFS and OS
- Study population: available phase 3 samples

CAFs associated with PFS in the pazopanib group:
- Interleukin 8, HGF, osteopontin, and TIMP-1

Prognostic markers of PFS in the placebo group:
- Interleukin 6, interleukin 8, and osteopontin

Predictive markers (pazopanib vs placebo)
- PFS: Interleukin 6
- OS: Interleukin 6, interleukin 8, osteopontin, VEGF, and CAF signature

IL-6, IL-8, VEGF, HGF, Osteopontin, TIMP-1
Cytokine and angiogenic factors (CAFs) associated with PFS in the randomised phase III trial

A cytokine and angiogenic factor (CAF) analysis in plasma for selection of sorafenib therapy in patients with metastatic renal cell carcinoma

Baseline biomarkers with significant predictive effect on PFS

<table>
<thead>
<tr>
<th>CAF</th>
<th>Baseline concentration</th>
<th>Median PFS (months)</th>
<th>Hazard ratio</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>SOR</td>
<td>SOR + IFN</td>
<td></td>
</tr>
<tr>
<td>Osteopontin</td>
<td>Low</td>
<td>3.8</td>
<td>11.1</td>
<td>0.36</td>
</tr>
<tr>
<td>Osteopontin</td>
<td>High</td>
<td>7.7</td>
<td>3.9</td>
<td>1.94</td>
</tr>
<tr>
<td>VEGF</td>
<td>Low</td>
<td>7.7</td>
<td>11.1</td>
<td>0.33</td>
</tr>
<tr>
<td>VEGF</td>
<td>High</td>
<td>5.7</td>
<td>4.2</td>
<td>1.57</td>
</tr>
</tbody>
</table>

Predictive value of CAF index based on the baseline signature of six biomarkers (sCAIX, Osteopontin, VEGF, TRAIL, Collagen V, and sVEGFR2)

<table>
<thead>
<tr>
<th>CAF index (cut-off)</th>
<th>Signature positive (≥cut-off)</th>
<th>Signature negative (&lt;cut-off)</th>
<th>P value for Interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(HR (95% CI))</td>
<td>(HR (95% CI))</td>
<td></td>
</tr>
<tr>
<td></td>
<td>SOR + IFN versus SOR</td>
<td>SOR + IFN versus SOR</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>1.63 (0.82–3.24)</td>
<td>0.16 (0.05–0.53)</td>
<td>0.0005</td>
</tr>
<tr>
<td>4</td>
<td>2.25 (1.02–4.96)</td>
<td>0.20 (0.08–0.55)</td>
<td>0.0002</td>
</tr>
<tr>
<td>5</td>
<td>10.8 (1.17–99.6)</td>
<td>0.48 (0.25–0.91)</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

### Genetic Variations in Angiogenesis-Related Factors

#### SNPs as Predictive Factors in RCC

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Association With Outcomes</th>
</tr>
</thead>
</table>
| VEGF SNP 936  | In patients receiving sunitinib\(^1\)  
  - VEGFr2 SNP 936 associated with tumour shrinkage  
  - Presence of combined VEGFr2 SNP 889 and 1416 associated with improved OS, whereas presence of VEGF SNP 936 CC genotype in combination with VEGFr2 SNP 889 GG genotype associated with increased risk of death |
| VEGFr2 SNP 889|                                                                                                                                                        |
| VEGFr2 SNP 1416|                                                                                                                                                       |
| IL8 2767      | In patients receiving pazopanib  
  - OS longer in patients carrying  
    - Wild-type IL8 2767 AA genotype compared with wild-type IL8 2767 TT genotype\(^2,3\)  
    - Wild-type FGFR2 IVS2 + 906C>T CC genotype compared with FGFR2 IVS2 + 906C>T TT variant genotype\(^2\)  
    - Wild-type VEGFA-1154G>A GG genotype compared with VEGFA-1154G>A variant GG genotype\(^2\)  
  - Variant genotypes of NR1IR (-25385C>T) and of FLT4 (1480A>G) polymorphisms associated with reduced OS\(^2\) |
| FGFR2 IVS2 + 906C>T|                                                                                                                                                       |
| VEGFA-1154G>A |                                                                                                                                                        |
| NR1IR         |                                                                                                                                                        |
| FLT4          |                                                                                                                                                        |
| ABCB1         | In patients receiving sunitinib\(^4\)  
  - Presence of A-allele in drug transporter ABCG2 associated with a trend toward prolonged PFS and OS  
  - Presence of TCG in ABCB1 haplotype (3435C/T, 1236C/T, 2677G/T) significantly |
| ABCG2         |                                                                                                                                                        |

VEGFR2 SNPs 889 and 1416 may predict Overall Survival in mRCC patients receiving sunitinib

VEGFR SNPs, 889 and 1416 were marginally associated with overall survival (p=0.13, 0.17)

The combined genotype remained statistically significant after adjusting for prognostic factors (p=0.03 for each factor)
SNPs in IL8, FGFR2, VEGFR3, VEGFA, and NR1I2 Associated with OS in Pazopanib-Treated RCC Patients

<table>
<thead>
<tr>
<th>GENE</th>
<th>SNP (NCBI)</th>
<th>P Value</th>
<th>Allele Frequency Caucasians, %</th>
<th>Allele Frequency Asians, %</th>
<th>Allele Frequency Blacks, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL8</td>
<td>rs1126647</td>
<td>0.003</td>
<td>39</td>
<td>32</td>
<td>6</td>
</tr>
<tr>
<td>IL8</td>
<td>rs4073</td>
<td>0.01</td>
<td>40</td>
<td>35</td>
<td>83</td>
</tr>
<tr>
<td>FGFR2</td>
<td>rs2981582</td>
<td>0.01</td>
<td>42</td>
<td>25</td>
<td>52</td>
</tr>
<tr>
<td>VEGFR3</td>
<td>rs307826</td>
<td>0.04</td>
<td>7</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>VEGFA</td>
<td>rs1570360</td>
<td>0.05</td>
<td>25</td>
<td>17</td>
<td>3</td>
</tr>
<tr>
<td>NR1I2</td>
<td>rs3814055</td>
<td>0.03</td>
<td>41</td>
<td>27</td>
<td>27</td>
</tr>
</tbody>
</table>

NR1I2 encodes a key regulator for CYP3A4 expression affecting Pazopanib clarance

Xu CF et al., J Clin Oncol. 2011 29(18):2557-64
Conclusions

• Several factors involved in signaling and resistance to treatment, that may also have a potential role as predictive biomarkers, have been identified in RCC.
  • Tumor, Plasma and Genetic markers
• Validation of these markers is ongoing, with several noteworthy recent results.
• Additional challenges remain:
  • Standardize sample collection and analysis
  • Consider always tumor heterogeneity
  • Make an effort to collect large amount of tumor samples and matched response data to have reliable results.
### Histotypes and kinome in RCC

<table>
<thead>
<tr>
<th>Type</th>
<th>Clear Cell</th>
<th>Papillary Type 1</th>
<th>Papillary Type 2</th>
<th>Chromophobe</th>
<th>Oncocytoma</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gene</td>
<td>VHL</td>
<td>Met</td>
<td>$^{c}$Kit</td>
<td>$^{c}$Kit</td>
<td>BHD</td>
</tr>
</tbody>
</table>

| %     | 75% | 5% | 10% | 5% | 5% |

Kinome expression (74/518 kinases with >3-fold differential expression vs. normal kidney) (Teh, ASCO 2007 and Lancet Oncology 2010)