Characterization of Met signaling in urothelial carcinoma of the bladder

Young Lee

Urologic Oncology Branch, National Cancer Institute
Hepatocyte Growth Factor: *mitogen, motogen, morphogen*

*mesenchymal cell expression*
- fibroblasts, glial cells, smooth muscle, macrophages

*broad target cell spectrum*
- hepatocytes, epithelial & endothelial cells, myeloid & lymphoid cells, melanocytes

*roles in development and homeostasis*
- somite migration, limb formation, organogenesis
- cell & tissue survival, tissue repair & regeneration

*roles in cancer*
- ligand & receptor overexpression, receptor mutations
- tumor growth, invasion & metastasis
Met and HGF expression in bladder tumor is correlated with poor prognosis

• Higher Met abundance in the bladder tumors is correlated with lower survival rate compared to low or no Met.
  Cheng HL. et al. (2002) J Clin Oncol 20, 1544-1550

• Higher HGF plasma levels found in muscular invasive bladder cancer.
  Wang P. et al. (2007) Urology 69, 780-784

• Phosphorylated Met is associated with poor prognosis in bladder cancer patients.
  Miyata Y. et al. (2009) Hum Pathol 40, 496-504
Ectodomain Shedding: A Common Process with Diverse Functional Roles

- Membrane anchored ligand release (EGF-R ligands, TNFα)
- Receptor downregulation (TNF-R1, TRAPS)
- Receptor activation (Notch, Tie-1, ErbB-4)
- Met shedding is increased in tumor vs normal cells
- Shed Met ECD is stable & easily measured in plasma & urine

In search of partners: linking extracellular proteases to substrates

Christopher M. Overall* and Carl P. Blobel*

NATURE REVIEWS | MOLECULAR CELL BIOLOGY
VOLUME 8 | MARCH 2007 | 245
Urinary sMet levels in bladder cancer patients are significantly higher than matched normals.

McNeil et al. Journal of Translational Medicine
Accurately selecting therapies that will be effective against a specific tumor is the ultimate goal of clinicians treating bladder cancer.

The present preclinical study was designed to interrogate potentially oncogenic HGF/Met signaling in a collection of urothelial carcinoma (UC) derived cell lines to better define the nature and extent of HGF/Met pathway involvement in bladder cancer.
Met levels in bladder cancer cell lines

Lee YL., Apolo AB., Agarwal PK. and Bottaro DP. Characterization of HGF/Met Signaling in Cell Lines Derived From Urothelial Carcinoma of the Bladder (submitted to *Exp Op on Ther Targ*)
Crizotinib inhibits HGF-activated pMet in bladder cancer cell lines

Met^low

Met^int

Met^high

Met level

Met^low
RT-4
T24M3
T24M2
TCC-SUP

Met^int
T24
UMUC5
HT1197
SW780

Met^high
J82
UMUC3
5637
HT1376
Crizotinib inhibits HGF/Met effectors in bladder cancer cells

<table>
<thead>
<tr>
<th></th>
<th>T24M2 (Met&lt;sup&gt;low&lt;/sup&gt;)</th>
<th>TCC-SUP (Met&lt;sup&gt;low&lt;/sup&gt;)</th>
<th>UMUC5 (Met&lt;sup&gt;int&lt;/sup&gt;)</th>
<th>SW780 (Met&lt;sup&gt;int&lt;/sup&gt;)</th>
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<tbody>
<tr>
<td>pAkt</td>
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<td>pErk</td>
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<td><img src="image13.png" alt="Image" /></td>
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<tr>
<td>HGF</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>PF1066</td>
<td>-</td>
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</table>

- PF1066 concentrations (μM): 5, 10, 5, 10, 5, 10, 5, 10.
Cabozantinib inhibits HGF-activated pMet in bladder cancer cell lines

Met\textsuperscript{low}  
- RT-4  
- T24M3  
- T24M2  
- TCC-SUP

Met\textsuperscript{int}  
- T24  
- UMUC5  
- HT1197  
- SW780

Met\textsuperscript{high}  
- J82  
- UMUC3  
- 5637  
- HT1376

![Graph showing the inhibition of pMet by Cabozantinib at different Met levels and cell lines.](image-url)
Cabozantinib inhibits HGF/Met signaling pathways

- RT4 (Met<sup>low</sup>)
  - pAkt
  - tAkt
  - pErk
  - tErk

- T24M2 (Met<sup>low</sup>)
  - pAkt
  - tAkt
  - pErk
  - tErk

- T24M3 (Met<sup>low</sup>)
  - pAkt
  - tAkt
  - pErk
  - tErk

- TCC-SUP (Met<sup>low</sup>)
  - pAkt
  - tAkt
  - pErk
  - tErk

- UMUC5 (Met<sup>int</sup>)
  - pAkt
  - tAkt
  - pErk
  - tErk

- SW780 (Met<sup>int</sup>)
  - pAkt
  - tAkt
  - pErk
  - tErk

- J82 (Met<sup>high</sup>)
  - pAkt
  - tAkt
  - pErk
  - tErk

- HGF
  - XL184

- +
- 30
- 300

- +
- 30
- 300

- +
- 30
- 300

- +
- 30
- 300

- +
- 30
- 300
Crizotinib inhibits HGF-induced cell growth and invasion

A

RT4 Met\textsubscript{low}

UMUC5 Met\textsubscript{int}

UMUC3 Met\textsubscript{high}

5637 Met\textsubscript{high}

B

TCC-SUP Met\textsubscript{low}

UMUC5 Met\textsubscript{int}

HGF

PF1066

HGF/PF1066

*P < 0.05
Crizotinib inhibits HGF-induced cell growth and invasion

A

RT4 Met\textsuperscript{low}

UMUC5 Met\textsuperscript{int}

UMUC3 Met\textsuperscript{high}

TCC-SUP Met\textsuperscript{low}

B

TCC-SUP Met\textsuperscript{low}

UMUC5 Met\textsuperscript{int}

HGF

PF1066

HGF/PF1066

*P < 0.05
Cabozantinib inhibits HGF-induced cell growth and invasion

A

- RT4 Met<sub>low</sub>
- T24M3 Met<sub>low</sub>
- UMUC5 Met<sub>int</sub>
- J82 Met<sub>high</sub>
- UMUC3 Met<sub>high</sub>

B

- RT4 Met<sub>low</sub>
- T24M2 Met<sub>low</sub>
- UMUC5 Met<sub>int</sub>
- SW780 Met<sub>int</sub>
- J82 Met<sub>high</sub>

*P < 0.05
Cabozantinib inhibits HGF-induced cell growth and invasion

A

- RT4 Met^{low}
- T24M3 Met^{low}
- UMUC5 Met^{int}
- J82 Met^{high}
- UMUC3 Met^{high}

B

- RT4 Met^{low}
- T24M2 Met^{low}
- UMUC5 Met^{int}
- SW780 Met^{int}
- J82 Met^{high}

*P < 0.05
Crizotinib and cabozantinib inhibit HGF-induced anchorage independent growth

**A**

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<thead>
<tr>
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<th>RT-4 Met&lt;sub&gt;low&lt;/sub&gt;</th>
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<th>UMUC3 Met&lt;sup&gt;high&lt;/sup&gt;</th>
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<td>0 - - - +</td>
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<td>0 - - - +</td>
<td>0 - - - +</td>
<td>0 - - - +</td>
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**B**

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<th>SW780 Met&lt;sup&gt;int&lt;/sup&gt;</th>
<th>J82 Met&lt;sup&gt;high&lt;/sup&gt;</th>
<th>UMUC3 Met&lt;sup&gt;high&lt;/sup&gt;</th>
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<td>HGF XL184 (30nM)</td>
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<td>0 - - - +</td>
<td>0 - - - +</td>
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*P < 0.05
hHGF Accelerates SW780 Tumor Xenograft Growth in hHGF(ki)/SCID vs SCID Mice

![Graph showing tumor growth comparison between SCID and hHGF/SCID groups](graph.png)

- **SCID**
- **hHGF/SCID**

Tumor volume (mm$^3$ x 10$^{-2}$) vs days post implant.

*P < 0.05
Cabozantinib Inhibits SW780 Tumor Xenograft Growth in hHGF/SCID Mice

- ○ hHGF/SCID vehicle
- □ hHGF/SCID XL184

Start of XL184 treatment

**P < 0.01

Days post implant

Tumor volume (mm$^3 \times 10^{-2}$)
Cabozantinib inhibits pMet in SW780 tumor Xenograft in hHGF/SCID mice

**P < 0.05
Conclusions

• Met is widely expressed in urothelial carcinoma cells
  – Added HGF activated Met and downstream effectors

• Crizotinib and cabozantinib inhibited HGF-induced:
  – Met and effector activation
  – Cell invasion
  – Proliferation
  – *in vitro* anchorage-independent growth

• hHGF accelerated SW780 xenograft growth in mice; cabozantinib reversed this effect

• Phase II trial of cabozantinib in bladder cancer patients as a second line therapy (at the NCI clinical center, Dr. Apolo, PI)
Future Directions

• Determine oncogenic impact of Met-related receptor tyrosine kinases (RTKs) in bladder cancer cell lines

• Examine the effects of cabozantinib inhibition in RTKs

• Study the biopsy samples from bladder cancer patients treated with cabozantinib to identify its targets to better understand drug efficacy
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