Introduction

FGFR3 aberrations lead to oncogenic signaling through the MAPK and PI3K pathways. Erdafitinib is a pan-FGFR inhibitor that is only currently approved for targeted therapy in advanced urothelial cancer. The efficacy of erdafitinib is short-lived as patients develop resistance within six months of treatment, emphasizing the need for effective combinational approaches to further enhance outcomes in this disease subtype.

Goal/Objective

- Overall goal of the project is to identify modifiers of erdafitinib sensitivity in FGFR3-mutated urothelial cancer and improve erdafitinib response rate using combinational approaches.
- Previous genome-scale knockout CRISPR/Cas9 screen identified CCNC/CDK8, IGF-1R/MAPK, PTPN11/SHP2 inhibitors as synthetic lethality candidates with erdafitinib.
- This study aims to validate synergism between erdafitinib and Sel120 (a CDK8 inhibitor) or SHP099 (a SHP2 inhibitor) in a erdafitinib-sensitive cancer cell line.

Methods

24h Treated with drugs 1 week Fixed and stained with propidium iodide

UC14 (FGFR3 mutated; erdafitinib-sensitive)

Captured phase and RF channels of the plates/ wells in an Incucyte machine

RNASeq data of treated UC14 cells were analyzed for gene enrichment using GSEA 4.2.3 software.

Lysates from drug-treated UC14 cells at different time points (2h and 24h) were blotted for pAKT, total AKT, pERK, total ERK, and GAPDH expression.

Fig. 1 Method workflow

Results

Growth inhibition by Erda + SHP2i / Sel120 > Erda >>> SHP2i > Sel120 > DMSO

Fig. 2 Growth inhibition by Erda, Sel120, SHP2i, and their combination in Erda-sensitive UC14 cells after 1 week of treatment

Sel120 does not synergize with Erda by combined suppression of the MAPK pathway

Fig. 3 pAKT, AKT, pERK, ERK, and GAPDH expressions in drug treated UC14 cells. Erda 1uM suppressed pERK more than Erda 10nM; No effect on pAKT at both doses; Sel120 produced no effect pERK and pAKT at 1uM; Its addition to Erda did not further suppress both.

Table 1 Down-regulated gene sets by Erda+Sel120

<table>
<thead>
<tr>
<th>Gene Set (nominal p-value)</th>
<th>ES</th>
<th>NOM p-val</th>
<th>FDR p-val</th>
</tr>
</thead>
<tbody>
<tr>
<td>FGFR signaling pathway</td>
<td>0.37, 0.34, 0.06, 0.14, 0.22, 0.31</td>
<td></td>
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<tr>
<td>Keratinocyte migration</td>
<td>0.48, 0.48, 0.12, 0.13, 0.21, 0.24</td>
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<td></td>
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<tr>
<td>Generalized hyperkeratosis</td>
<td>0.48, 0.51, 0.12, 0.08, 0.20, 0.15</td>
<td></td>
<td></td>
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<tr>
<td>Negative regulation of MAPK cascade</td>
<td>0.01, 0.00, 0.00, 0.00, 0.00, 0.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative regulation of ERK1 &amp; ERK2 cascade</td>
<td>0.01, 0.01, 0.01, 0.01, 0.01, 0.01</td>
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</tbody>
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Fig. 4 Synergism between erdafitinib and Sel120/SHP2i. The growth inhibition by the combination was divided by that of each single agent, and a value < 0.7 was considered synergistic.

Conclusions

- GSEA reveals that erdafitinib (1uM) as a single agent does not significantly suppress FGFR signaling (Table 1).
- Sel120 synergizes with erdafitinib possibly by down-regulating the hippo signaling pathway.
- Drug assay for erdafitinib + SHP2i is ongoing.
- Possible future study:
  - Drug assay for Erda/Sel120 following genetic knock-out of keratin filament genes and over-expression of hippo signaling genes in UC14 cells.

References