Introduction

Development of drugs targeted at specific oncogenes has revolutionized cancer care, but many patients suffer from drug-induced cardiovascular disease. Cardiovascular-related mortality is 7-fold higher in age matched pediatric cancer patients than normal individuals, and 12% of breast cancer survivors suffer heart failure within three years of treatment. Tyrosine kinase inhibitors (TKIs) are exemplars of promising anti-cancer drugs plagued by cardiotoxicity. Whether this reflects drug-mediated inhibition of the same signaling pathways as those involved in oncogenesis remains unclear. Also, unclear is how perturbations of the relevant signaling pathways ultimately lead to downstream phenotypic changes at the level of individual cardiomyocytes and of the heart as a whole. Although previous studies have provided evidence on a few proteins involved in TKI-induced cardiotoxicity, they have not taken a systems approach in which the effects of targeted inhibition are traced through downstream signaling proteins and gene expression, ultimately to phenotype. This has made it difficult to resolve the true causal factors in the observed phenotypic changes.

To investigate these issues, we are utilizing human cardiomyocytes trans-differentiated from induced pluripotent stem cells (iPSCs) to study signaling pathways involved in TKI-induced cardiotoxicity. Using multiplexed methods to measure cellular phenotypes both in single cells and in populations combined with high-throughput gene expression, we are able to phenotype. This has made it difficult to resolve the true causal factors in the observed phenotypic changes.

Hypothesis and Objective

- What are the causal signaling and gene regulations that mediate TKI-induced cardiotoxicity across dose and time, and are they similar to the targets/pathways inhibited in cancer cells?
- Through which pathways and genes do factors in the microenvironment affect TKI-induced cellular responses?
- To what extent can mechanisms and targeting strategies learned in iPSC-derived cardiomyocytes be translated into understanding of cardiac pathology in TKI-treated patients?

Methods

- iPSC-derived cardiomyocytes (Cor.4U®) were purchased from Axiogenesis and cultured following the manufacturer’s instructions.
- Immunofluorescence: Cells were fixed using 4% paraformaldehyde and stained with secondary antibodies and primary antibodies sequentially following standard methods in immunocytochemistry.
- Apoptosis: Cells subjected to different treatments were stained with Nucview488 and reverse transcribed into cDNA libraries using Ribozero magnetic beads and the Truseq RNA preparation kit (illumina). Samples were sequenced in a paired-end fashion using the HiSeq 2500 system.
- RNASeq: Total RNA was extracted from Cor.4U® cells using the RNeasy kit (Qiagen) and reverse transcribed into cDNA libraries using Ribozero magnetic beads and the Truseq RNA preparation kit (illumina). Samples were sequenced in a paired-end fashion using the HiSeq 2500 system.

Results

Table 1. TKI drugs that are associated with adverse cardiac events and their Cmax

<table>
<thead>
<tr>
<th>TKI</th>
<th>Top ranked target</th>
<th>FAERs adverse cardiac events</th>
<th>PDGFR IC50</th>
<th>EGFR IC50</th>
<th>ErbB2 IC50</th>
<th>Cmax</th>
<th>Dose/day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lapatinib</td>
<td>PDGFR, VEGFR, etc.</td>
<td>2</td>
<td>2 nM &gt; 20 µM</td>
<td>0.24 µM</td>
<td>50 mg</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sorafenib</td>
<td>PDGFR, VEGFR, etc.</td>
<td>3</td>
<td>57 nM &gt; 10 µM</td>
<td>18 µM</td>
<td>800 mg</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lapatinib</td>
<td>EGFR, ERBB2</td>
<td>4</td>
<td>10.8 nM</td>
<td>9.2 nM</td>
<td>4.2 µM</td>
<td>1250 mg</td>
<td></td>
</tr>
<tr>
<td>Erlotinib</td>
<td>EGFR</td>
<td>26</td>
<td>0.7-2 µM</td>
<td>1 µM</td>
<td>1.6 µM</td>
<td>500 mg</td>
<td></td>
</tr>
</tbody>
</table>

* Analyzed from FDA Adverse Event Reports (FAERs) database for the first quarter of 2014.

High-content Imaging and single-cell phenotypic analysis

Figure 1. IPS-derived cardiomyocytes express basic cardiomyocyte markers and are a great model for intracellular signaling

Figure 2. Lapatinib and Sorafenib induce distinct changes in mitochondrial membrane potential and caspase3 activity

Lapatinib, but no other TKIs from Table 1, decreases mitochondrial membrane potential (Left). Sorafenib causes the most significant increase in caspase3 activity among the TKIs (Right).

High-throughput phenotypic analysis at the population level

Figure 3. Different TKIs cause different changes in cell number over time

Conclusions and Future Directions

- Broad spectrum TKIs (e.g., Sunitinib and Sorafenib) are generally more toxic than target-specific TKIs (e.g., Lapatinib and Erlotinib).
- TKIs with similar targets (e.g., Lapatinib and Erlotinib) have variable potency and can induce different cellular phenotypes associated with cardiotoxicity.
- The effects of TKIs on cellular phenotypes are prolonged (up to a week).
- High-content multiplexed imaging, high-throughput gene expression assays, and single-cell and population analysis should enable us to model and validate the network mechanisms of TKI-induced cardiotoxicity.

In the future:

- Build statistical models to reveal the molecular mechanisms of TKI-induced cardiotoxicity.
- Functionally link our findings in cell culture systems to clinical data in patients.

Acknowledgement

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References