Integrated analysis for quantitative predictions of drug-induced toxicity

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Objectives: Cardiotoxicity is a common adverse event caused by tyrosine kinase inhibitors (TKIs), a class of chemotherapeutic agent. Reported toxicities include left ventricular dysfunction, heart failure, and arrhythmias. TKI cardiotoxicity is thought to be induced by dysregulation of cardiomyocyte survival signaling cascades via both on- and off-target effects, but the mechanistic details remain unclear. Given the effectiveness of many TKIs in cancer treatment, elucidating molecular interactions behind these toxicities would be critical in alleviating these detrimental side effects to maintain treatment efficacy. The Drug Toxicity Signature Generation (DToxS) Center at Mount Sinai has started a large-scale project that aims to address this issue by providing a bridge between molecular changes in cells and the prediction of pathophysiological effects.

Methods: In our ongoing work we use high-throughput transcriptomic measurements using mRNA-Seq as the basis for computational analysis to generate “signatures” for TKIs. Primary human cardiac myocytes in culture are treated with TKIs, and drug-induced changes in gene expression are quantified. The gene expression profiles are then used to identify pathways involved in the drug response, to build networks of interacting cellular components, and as inputs to dynamical mathematical models that predict pathophysiological responses. My PhD project involves simulating the effects of TKI-induced changes in gene expression in several models to obtain signatures related to cellular signaling dynamics. The models that we implement are chosen based on the relevance to cardiomyocyte survival/growth/death signaling network. These include a logic-based model of cardiac hypertrophy that simulates 193 biochemical reactions with 106 ordinary differential equations (ODEs). To simulate how TKIs may influence apoptosis susceptibility, an ODE-based model with 67 species and 118 reactions is implemented. This approach has generated novel predictions about the mechanisms underlying TKI-induced cardiotoxicity.

Results: For the initial analyses, changes in gene expression in a single cell line due to three TKIs (trastuzumab, sunitinib, and sorafenib) were used. These three drugs were chosen based on the literature availability of cardiotoxicity reports. The simulations for hypertrophy and apoptosis models were examined separately to delineate the differences in the degree of the toxicity. Results from the hypertrophy model indicated that the overall risk index is in the order of sorafenib>trastuzumab>sunitinib in both basal activity (i.e. no stimulus) and when stretch is given as a stimulus. Apoptosis model result also had the same order of risk index – i.e. sorafenib as the most apoptotic inducing. To examine the mechanistic details, time course plots of selected network nodes for each model were generated. Mechanistic hypotheses that result from these simulations are as follows:

Sorafenib: Simulations suggest that the predicted strong pro-hypertrophic signaling caused by sorafenib results from the regulation pattern of the metabolic enzyme GSK3β. Originally identified as an enzyme in glycogen metabolism, GSK3β was also found to play an important role in hypertrophy prevention in cardiomyocytes. The down-regulation of GSK3β predicted by the simulations (Fig. 1A) causes an exaggerated response of other pro-hypertrophic signals in the model, as shown.

Trastuzumab: While trastuzumab is mildly pro-hypertrophic as well, the results suggest that the difference in hypertrophic tendency between sorafenib and trastuzumab could result from the maintained levels of GSK3β activity in trastuzumab.

Sunitinib: Unlike sorafenib and trastuzumab, sunitinib was predicted to be somewhat protective against both hypertrophy and apoptosis, which suggests that the toxicity could occur through some other mechanism such as dysfunction of energy homeostasis. We are currently implementing mathematical models of mitochondrial metabolism and myocyte contraction to potentially uncover mechanisms underlying sunitinib toxicity.

Figure 1: Simulation Results of Hypertrophic/Apoptotic Tendencies of TKI network Perturbation (A) Time course plot for 6 species in hypertrophy network. First 50 minutes are for basal level activity or no stimulus state and the next 50 minutes are simulated for stretch-induced state. (B) Hypertrophy index for the drugs per given stimulus. (C) Apoptosis time course plot based on the peak time of cytochrome C release given 0.0354nM
of TRAIL. TRAIL concentration was arbitrarily chosen based on the amount required for control simulation to commit death in 30 hours. (D) Based on cytochrome C release simulation, time to death bar plot is generated.

In addition to these novel insights in mechanistic details, this approach also allowed us to validate known mechanisms of cardiotoxicity for these TKIs. For instance, sorafenib and trastuzumab are both known to induce cell death via intrinsic apoptotic pathway. In both drugs, we have confirmed elevated JNK expression (Fig1A) which can lead to JNK-mediated cytochrome C release. The commitment of apoptosis was further validated with apoptosis model simulation shown in Fig1C&D where sorafenib and trastuzumab show higher tendency to commit cell death given the death signaling ligand (TRAIL).

Conclusion: In conclusion, we have demonstrated a robust quantitative systems approach in predicting drug toxicity by integrating transcriptomic data with mathematical modeling. Using this approach, we have both validated known mechanisms of cardiotoxic TKIs and generated novel insights into other possible mechanism which can be validated experimentally. In particular, we plan to apply TKIs in combination with physiological stimuli such as stretch and test model predictions using western blot or immunofluorescence. We are confident that this analyses pipeline will be an invaluable tool in delineating differences in toxicity between TKIs with similar targets as well as predicting possible toxicity of drugs in development.

References: