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Assessing Hepatoxicants based on High-throughput Quantitative SILAC Proteomics and Causal Biological Networks

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ABSTRACT

Drug-induced liver injury can result in the termination of compounds in preclinical development, as well as withdrawal of marketed drugs. Identification of the signaling pathways and proteins involved in injury is an important step towards establishing assays that could be utilized to identify potential hepatotoxicants early in the drug discovery process. In this study we used high throughput quantitative mass spectrometry proteomics involving Stable Isotope Labeling with Amino acids in Cell culture (SILAC) leveraged by a recently developed systems biology approach (Causal Reasoning Engine, CRE) to investigate the effects of hepatotoxic compounds on the cellular proteome of HepG2 cells. Cells were treated with various concentrations of nefazadone, nimesulide, nomifensine, or glafenine, all of which cause hepatotoxicity in humans. Buspirone and rosiglitazone were used as comparator compounds not associated with hepatotoxicity. In comparison to more traditional proteomic analysis tools CRE results provided detailed molecular hypotheses that condense into biological networks and collectively explain a significant number of the measured protein changes. The CRE hypotheses demonstrate that magnitude of response is not necessarily the differentiating factor between DILI and non-DILI compounds but rather the biological processes implicated. Differentiating CRE molecular hypotheses implicate lipid and glucose metabolism, inflammation, oxidative stress and DNA damage as consistent major in vitro discriminating factors. Overall, this paper provides evidence that SILAC data coupled with the CRE analysis method can provide context and new insight into variation of stress responses in hepatotoxic versus non-hepatotoxic compounds even within the same therapeutic class. Drug-induced toxicities can result in stopping preclinical development of a compound, or even in the withdrawal of compounds once they are marketed. What is lacking is a means of identifying early in the drug discovery process the potential safety liabilities of compounds. By the time a compound is tested in vivo, it is usually too late to change the compound profile and remove the toxicity. Testing compounds in in vitro cellular models is a widely used approach that can help identify potential toxicities, but this approach is limited by the endpoints that are measured The application of proteomics is a powerful approach for investigating mechanisms of compounds. Initially this technique was applied primarily to profiling the mechanism of action of compounds, but has subsequently been applied to toxicology research [24,25]. Compound toxicity is often manifest at a phenotypic level, with little or no indication of underlying mechanisms, and thus a major advantage of a proteomic approach is the broad, unbiased view it provides. Protein levels are measured directly, in contrast to transcriptomic approaches, where mRNA levels do not always correlate with protein expression due to post-transcriptional changes which may affect protein abundance.

Keywords: DILI; Causal reasoning; SILAC