Experimental validation of drug responses predicted by deep learning-based bioactivity models in hepatocellular carcinoma cell lines

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Introduction:
Hepatocellular carcinoma (HCC) is one of the deadliest cancers worldwide. Although it has high mortality rate, there are currently few drugs available as treatment options. Therefore, there is an urgent need for intensive basic research and clinical trials in HCC. With the utilization of computational predictive approaches, effective treatment strategies, which leverage target-based drug response predictions, can be developed. This study aims to validate the results of deep learning-based prediction method, DeepResponse, developed by "Biological Data Science Lab" at Hacettepe University, and propose small molecule inhibitors that are active against HCC cell lines.

Methodology: Predictions are filtered based on their pIC_{50} values (>6) and specificity for minimum 6 different HCC cell lines. Compounds were tested *in vitro* via sulforhodamine B (SRB) assay and real-time cell monitoring system. Annexin-V assay and Hoechst staining was performed to detect induction of cell death in HCC cell lines. PI staining was done to perform cell cycle analysis by flow cytometry. Sorafenib was used as a control for all experiments to compare and evaluate the effects of predicted drugs.

Results and discussion: Among a large group of predicted inhibitors, 3 compounds, 1 investigational drug (Eprinomectin), which was not studied on HCC before, and 2 PI3K/mTOR pathway inhibitors (Rapamycin and ZSTK474), which were previously studied on HCC cell lines, were selected for *in vitro* validation studies. pIC50 values of all drugs were consistent with the IC_{50} values evaluated with SRB assay and RT-CES analysis. Eprinomectin inhibited cell growth, induced cell death and displayed G1 arrest, and showed cytotoxic effect on HCC cell lines comparable to Sorafenib.

Conclusion: We observed that the prediction model successfully predicts specific small molecule drugs for HCC cell lines and Eprinomectin can be further studied for its molecular mechanism of action as a potential drug against HCC.