# Network-based *in silico* modeling for drug repurposing and *in vitro* validation in hepatocellular carcinoma

**Esra Nalbat[1], Nurcan Tuncbag[2], and Rengul Cetin-Atalay[3]**

*[1]Cancer Systems Biology Laboratory, Graduate School of Informatics, Middle East Technical University, Ankara, 06800, Turkey*

*[2]School of Medicine, Koç University, Istanbul, 34450, Turkey*

*[3]Section of Pulmonary and Critical Care Medicine, University of Chicago, Chicago, IL 60637, USA*

## Introduction

Hepatocellular carcinoma (HCC) is the sixth most commonly diagnosed cancer and a leading cause of death worldwide. The hyperactivated cell survival signaling pathways cause resistance to conventional chemotherapeutics so that targeted therapies could extend patient survival for only a few months. Furthermore, there are limited chemotherapeutics for HCC patients due to impaired liver functions. Thus, it is vital to determine repurposed drugs and drug combinations in HCC treatment.

## Material and Method

To generate the directed network, the reported hepatocarcinogenesis-associated pathways in the KEGG database were retrieved. Drugbank for the small molecule inhibitors, those having at least one target protein in the generated network was integrated. The feedback loops in the network of KEGG pathways belonging to the HCC signaling pathways (MAPK, calcium, p53, PI3K-AKT, WNT, TGF-beta, NAFL, and Apoptosis) were illustrated that are crucial for the signaling of liver cancer as well as in resistance and in the non-target effects of drugs. The *in silico* perturbation attack strategies were used to identify the drug effectiveness, changes in both efficiencies of the signaling network and the number of feedback cycles, and the functionality of the network. We repeated the same attack strategy with combinations of drugs to calculate the effects on the network and the additive or synergistic effects of the drugs. The top 9 damaging drugs were selected to validate their bioactivities on HCC cell lines *in vitro* by NCI-SRB cytotoxicity assay. Transcriptomic analysis was performed by Nanostring nCounter system using the PanCancer Pathways panel. Synergistic drug combinations were determined by analyzing NCI-SRB results using SynergyFinder and cytotoxic effects of drug combinations were controlled by real-time cell analysis. The molecular mechanisms induced by single drugs and drug combinations were studied by PanCancer Pathways panel transcriptomic analysis, cell cycle assay, cell death assay, and western blotting.

## Results and discussion

The most damaging nine drugs (Amrinone, Thalidomide, ChloroquineP, Sunitinib, Pranlukast, Pseudoephedrine HCI, Brigatinib, Lenvatinib, and Regorafenib) were identified to assess their biological effects in hepatocellular carcinoma cells. Except for Thalidomide, Pranlukast, Amrinone, and Pseudoephedrine HCI, the selected drugs, especially Brigatinib, Sunitinib, and their drug combinations with other drugs, were remarkably effective on HCC cells. Many differentially expressed cancer-related genes and pathways were identified in the presence of drugs, with Brigatinib having the highest number of differentially expressed genes (DEGs). The most enriched signaling pathways were Wnt-beta catenin, cell cycle, and MAPK. Brigatinib differed from the rest of the drugs in the pathway scoring matrix. Apart from the previously reported genes, some of the genes (HDAC1, HDAC2, HDAC4, FOS, DAXX, LEF1, RELA, and RUNX, etc.,) that were not associated with liver cancer therapeutics before were significantly enriched in the network. The bioactive drugs induced apoptosis involving Akt pathway inactivation due to cell cycle arrest in G0/G1 phase. Furthermore, combinations of Brigatinib and Sunitinib drugs with other drugs showed synergistic effects on HCC cells by inducing apoptosis, arresting the cell cycle, and causing changes in protein expression levels.

## Conclusion

Our results have shown that our network-based *in silico* model can be exploited for drug repurposing and novel target identification.

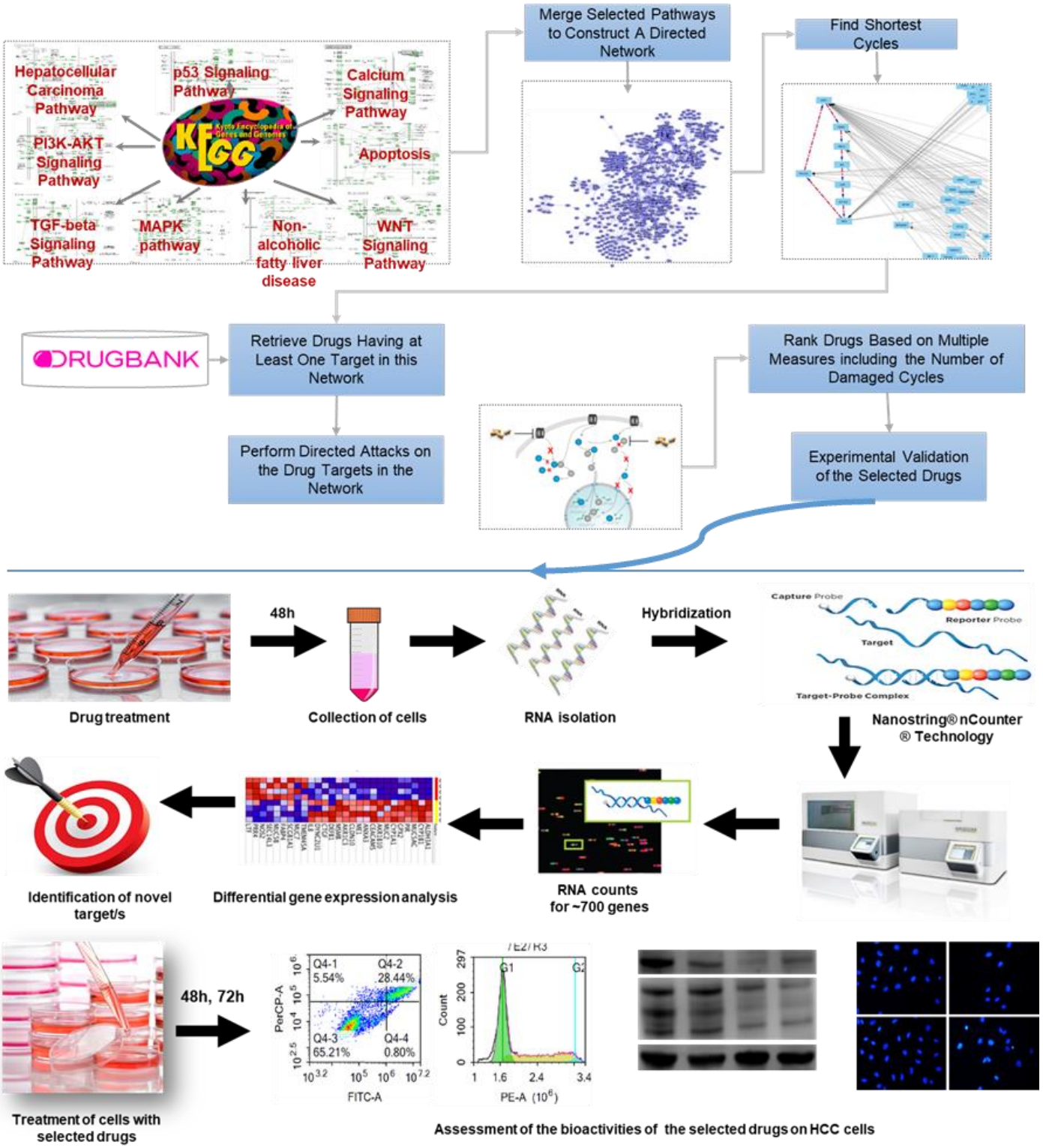


Figure 1. Computational and experimental workflow of *in silico* model