Network Analysis of Proteomics of Combined Gemcitabine and Birinapant in Pancreatic Cancer Cells

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Objectives: Combinations of gemcitabine and birinapant showed synergistic effects in inhibiting the growth of pancreatic cancer PanC-1 cells [1]. In this study, we seek to understand the key pathways related to cell proliferation and apoptosis in PanC-1 cells, and to explain the mechanisms of action and interactions for combined gemcitabine and birinapant examining changes of proteins. Such data were incorporated with cell distributions and cell numbers to develop a multi-scale network model. The network model was applied in target selection, prediction of gemcitabine efficacy with different genetics, and efficacy of gemcitabine-based combinations.

Methods: PanC-1 cells were incubated with control, gemcitabine (20 nM), birinapant (100 nM) or combinations of the two. Total proteins were extracted, and mass spectrometry (MS)–based proteomics was applied for a universal identification and quantification of proteins changed after treatments. A total of 1481 significantly changed proteins were clustered by KEGG functional pathways, and relevant pathways were selected for further investigation. A total of 20 proteins were selected to represent the key pathways. Western blot analysis provided additional information of functional change of proteins. Dynamic changes of proteins were linked to cell distributions and cell numbers, the quantitative relations were built in ADAPT V software, and a multi-scale network model was developed (Figure 1).

Results: Gemcitabine activated DNA damage response (DDR) and induced DNA repair proteins, which were blocked by birinapant. Thus the cell cycle arrest effect was enhanced in the combination group. NF-κB pathways were activated by both gemcitabine and birinapant, and enhanced in combination. Both intrinsic and extrinsic apoptotic pathways were activated in combination.

The network model was tested in the presence of external TNF-α for model validation [2]. Clinical applications of the model were also explored: 1) Sobol Sensitivity analysis was applied to identify potential drug targets to increase the efficacy of gemcitabine; 2) The role of mutated p53 was assessed; 3) Model prediction indicated no beneficial interactions when combining gemcitabine and curcumin, consistent with our observations.

Conclusion: Comprehensive network model includes information for both drug actions and biological systems. It may be applied for novel drug target selection and efficacy prediction.

Reference