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An integrated proteomics and bioinformatics approach uncovering the anti-cancer mechanism of anti- thymidylate synthase drugs inhibitors in human colorectal cancer cell line

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Abstract

Background: Thymidylate synthase (TS) targeting drugs are the most widely used drugs in anti-cancer therapy. However, treatment with classical catalytic-pocket directed TS inhibitors with other DNA damaging drugs, usually induce TS over-expression and the related onset of tumor drug resistance. To avoid such adverse phenomena, we adopted a change of strategy and sought compounds that, unlike all known anti- TS drugs, alter the stability of hTS homodimeric enzyme. With the objective of proteomic and bioinformatic studies for the understanding of the biological mechanisms in the investigation of anti-proliferative drugs, these experiments were conducted to investigate the time-dependent effects on the whole proteome of human colorectal cancer cell line, HCT116 after being treated with the inhibitor 1— (IC50 on HCT116 35.5M) a thymidylate synthase (TS) inhibitor compound. The whole cell mass spectrometry (LC MS/ MS label-free quantification) differential proteomic study followed by bioinformatics analysis was performed in treated versus untreated cells. Applications of the proteomic and bioinformatic studies encompass experimental design, sample management and global data interpretation to allow to understand this mechanism of action.

Purpose: The aim of this study was to identify role of anti- TS drugs inhibitor 1 in regulating cancer cell response and provide some insights into the molecular mechanisms through a proteomics-bioinformatics approach, conducted to investigate the time-dependent effects on the proteome of HCT116 human colorectal cancer cell line after being treated with the Inhibitor 1. Our attention on biological processes involved in protein metabolism cellular component organization or biogenesis, DNA repair, DNA replication, DNA metabolism, purine nucleobase metabolism, biological regulation, the cell cycle, and apoptosis. In order to explore the relevance and mechanics of these drugs, the use of well characterized colorectal cancer cell lines HCT116.

Experimental Design: Inhibitor 1 was administered and cells were harvested at three different time points. These conditions are considered for the preparation of samples for mass spectrometry analysis. The selected times points for harvest will be at time 0h, 6h, and 12h. Times based on previous

Inhibitor 1 trials on ovarian and colorectal cancer cell lines. Inhibitor 1, was determined to be a racemic mixture that equilibrated with a half-life of 56 minutes and has shown to induce apoptotic cell death. Two biological replicates of each sample were prepared and ran three times each. (technical replicates). A standard drug, 5-fluorouracil (5-FU) was used as for reference. An untreated group with only H2O and treated group of 48h for 5-FU for a total of 14 samples were prepared for LC-MS using filter aided sample preparation (FASP) under these conditions with MS analysis performed on High-Definition (HD) ultra-high resolution (UHR) QTOF mass spectrometer (Bruker) at University of Milan Bicocca.

Results: Preliminary results provided 2,887 (1,453 nontreated; 1,434 treated) statistically significant identified proteins from two conditions, non-treated (DMSO) and treated (Inhibitor 1) HCT116 cells after 12h. Differential proteomic approach was used in identifying the most significant differentially expressed proteins (DEPs) within these conditions and reduced the list to 276 modulated proteins. To identify the role of anti-TS inhibitor 1 in regulating cancer cell response, we created a statistically significant protein – protein interaction network (PPI) using STRING that grouped together the differentially expressed proteins, then included proteins from former proteomic experiments previously reported and identified to be associated to the relevant pathways and biological process mainly involved in the mechanism of action of inhibitor 1. A further reduced panel of 46 proteins, including TYMS (Thymidylate synthase).

Conclusions: These preliminary results suggest the network and proteins involved of the mechanism of action of inhibitor 1. Given the full scope from every condition set of this experiment will enhance the current list of proteins, but also validate the proteins now identified. This working set of proteins may be further investigated as potential drug targets, and to generate combinations strategy. Additional bioinformatics analyses and validation by western blot analyses will be carried out to better identify and characterize the cellular response induced by these inhibitors.

Biography

Kevin I Muñoz, is a student in University of Modena and Reggio Emilia, Italy.

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