Precision medicine through proteomics studies in drug resistant colorectal cancer

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Drug resistance (DR) constitutes the main cause of therapeutic failure of chemotherapic agents. The inherent discontinuity between preclinical and clinical studies makes it difficult to successfully address this issue in the clinic. Indeed, lack of exploration into the DR molecular bases results in a reduced knowledge and limited strategies to address this problem. Recent technological advances allowed Mass Spectrometry to investigate DR mechanisms with a more *holistic* approach, i.e. from *bench to bedside and viceversa*. In particular, by processing thousands of proteins and tracking their changes in expression, the combination of MS proteomics with bioinformatics allows the investigation of the subcellular mechanisms triggered by drugs or a drugs combination [1] delivering concepts and results that can be transalted in the clinic. Also, the brand new invention of peptide labelling kits, like the TMT-tagging system (Thermo Fisher), makes it possible to analise multiple samples in a single MS run, detecting even smaller differences in protein concentrations. By exploiting this powerful tool, the mechanism of action of novel thymidylate synthase inhibitors (dimer destabilizers, or **Ddis**), that induce cancer cell death by targeting the enzyme at the interface of the two monomers, were investigated. Experiments of DR were conducted on HCT116 cells, using different pulse of 5-fluoruracil (5FU) and **Ddis**, we were able to achieve a first evidence on the differential behaviour of the two compounds. Unlike **5FU** and antifolates, the Ddis compound by adopting an equilibrium shift capacity, was able to delay/halt drug resistance onset. Herein, by MS proteomics, we demonstrate that the co-administration of a low-dose of Ddis with 5-FU, with respect to **5FU** only, significantly reduces the drug sentitization in HCT116 cells. Overall, we have identified a total of 5900 proteins, 19 of which differentially expressed proteins (DEPs), *i.e.* whose expression is significantly different from control cells. The DEPs were investigated with dedicated bioinformatic tools. We enlightened the strong overexpression of the RNA catabolic process (Nonsense Mediated Decay pathway), mitochondrial ATP-related metabolism, and pro-apoptotic p53 path for the drug combination, whereas **5-FU**-only effect is less significant. Interestingly, despite both treatments have evidenced TS downregulation, only in drug combination the Serine Hydroximethyl transferase (SHMT) enzyme significantly decreases, which may suggest a folate imbalance, and corroborate Ddis mechanism of action. The patwhays and proteins identified in the CRC cell lines will be evaluated in the tissues derived from patients who developed a metastatic cancer after previous treatement with drug combination containing **5FU**.The presence of **5FU** DR can be associated not only with high hTS levels, but also with SHMT and a protein profile observed in cancer cell lines proteomic studies. The transaltional approach based on MS proteomics in which a comparison is performed between cancer cell lines and patients tissues, can give insight into DR mechanism and predictive DR development. Overall, this study contributes to the demonstration that *omic* science can be a powerful strategy to correlate preclinical and clinical studies. It also demonstrates that TMT is a solid platform for precision medicine, as it can be employed to analyse patient's tissues for designing *ad hoc* treatments for each patient.

References

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