TARGETING THE METABOLIC REPROGRAMMING ASSOCIATED WITH CDK4/6 INHIBITION AS AN EFFECTIVE COMBINED THERAPY IN COLON CANCER

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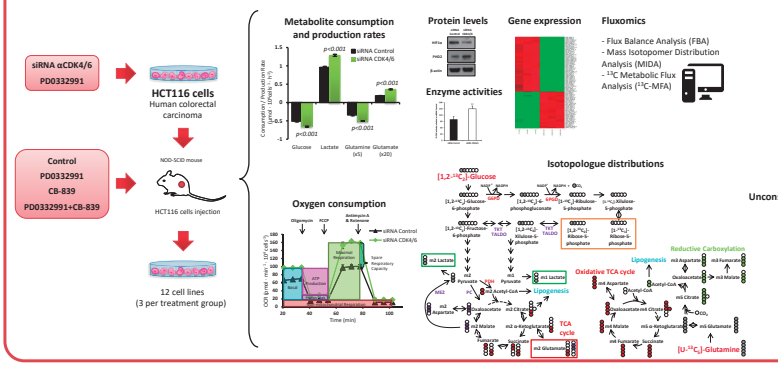
Introduction

Therapeutic resistance is currently one of the most critical challenges in cancer treatment. Accumulating evidence suggests that, under an environmental pressure such as a chemotherapeutic treatment, cancer cells engage adaptive mechanisms intended to optimize survival, therefore becoming resistant. Metabolic reprogramming is one of these relevant cell-autonomous mechanisms through which tumor cells shift their energetic dependencies to alternative pathways to bypass the selective pressures exerted by a given class of antineoplastic drugs. We hypothesize that the metabolic shift undergone by tumor cells in response to targeted therapies constitutes adaptive responses that unveil *de novo* vulnerabilities that can be targeted to overcome drug-resistant states.

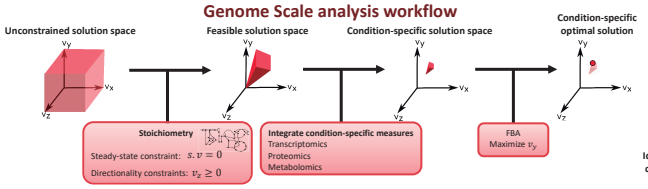
Genes that have a role in cell cycle control (checkpoints, regulation of transcription, or cell cycle progression) are frequently altered in cancer. Cyclin-dependent kinases CDK4 and CDK6 (CDK4/6) are promising targets for inhibiting cell cycle progression since their overexpression is implicated in a wide range of human cancers. Like with all targeted therapies, tumor cells eventually acquire resistance to CDK4/6 inhibition. To extend the benefits of CDK4/6 inhibition and overcome the acquired drug resistance in cancer therapy, we have analyzed the metabolic reprogramming associated with the inhibition of CDK4/6 in HCT116 colon tumor-derived cells. Our investigations have unveiled *de novo* MYC addiction as a response to CDK4/6 inhibition, allowing us to identify new targets to counteract the adaptation of tumor cells to the cell cycle targeted therapy, thus averting the acquisition of pharmacologic resistance. In particular, the inhibition of glutaminase in combination with CDK4/6 results in strong synergistic and selective antiproliferative effects on tumor cells both *in vitro* and *in vivo*. Moreover, we report that cancer cells that have been treated with the combined therapy remain sensitive to the double treatment even after chemotherapeutic exposure and exhibit a downregulated metabolism. Together, our results validate the combined inhibition of CDK4/6 and glutaminase as an effective strategy to prevent pharmacologic resistance and improve the antiproliferative effects of the single-agent treatment.

Methods and procedures

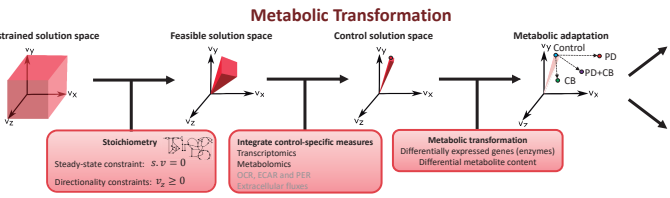
Study and characterization of tumor metabolic reprogramming



Genome Scale analysis workflow

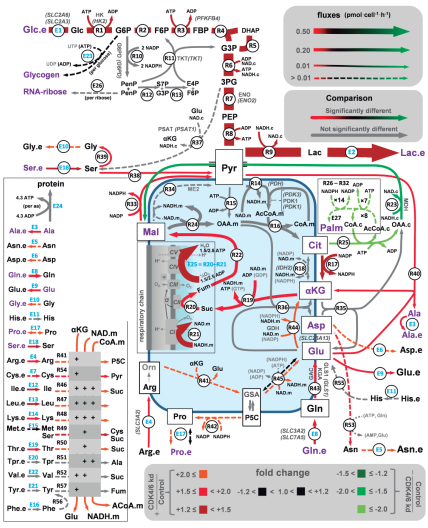


Metabolic Transformation

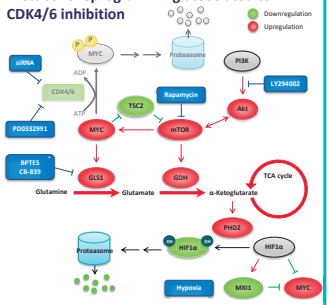


Results

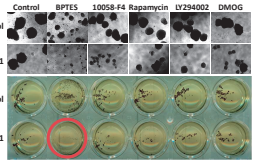
Quantitative metabolic network model



Metabolic reprogramming associated to CDK4/6 inhibition



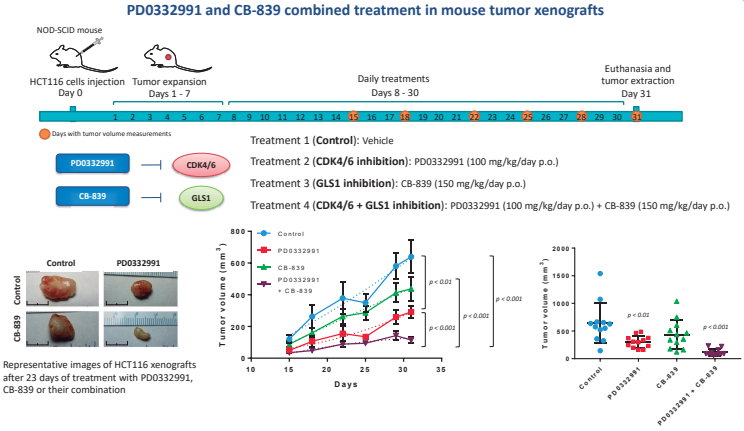
Combined treatment *in vitro*



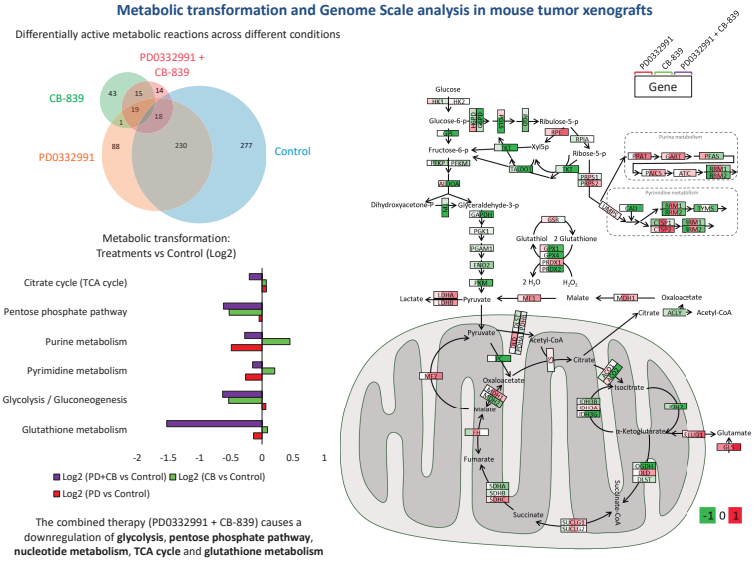
CDK4/6 depletion or inhibition sensitizes cells to inhibition of **MYC**, **glutaminase**, **mTOR** or **PI3K** or to **hypoxia**

The combined inhibition of the metabolic adaptations and CDK4/6 results in **strong synergistic and selective antiproliferative effects** on tumor cells

PD0332991 and CB-839 combined treatment in mouse tumor xenografts

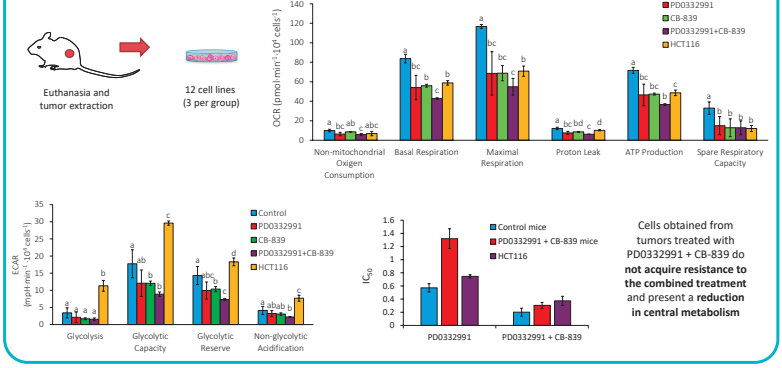


Metabolic transformation and Genome Scale analysis in mouse tumor xenografts



The combined therapy (PD0332991 + CB-839) causes a downregulation of **glycolysis**, **pentose phosphate pathway**, **nucleotide metabolism**, **TCA cycle** and **glutathione metabolism**

Metabolic characterization of cell lines derived from mouse tumor xenografts



Cells obtained from tumors treated with PD0332991 + CB-839 do **not acquire resistance to the combined treatment** and present a **reduction in central metabolism**

Conclusions

The absence of functional CDK4 and CDK6 causes a metabolic reprogramming that unveils metabolic dependences and vulnerabilities that can be targeted to overcome acquired drug tolerance and resistance by cancer cells. Targeting the metabolic reprogramming associated with CDK4/6 inhibition results in strong synergistic and selective antiproliferative effects *in vitro* and *in vivo*.

The combined inhibition of CDK4/6 and glutaminase is an effective strategy to prevent pharmacologic resistance and improve the antiproliferative effects of the single agent treatment.

Acknowledgments: *This work was supported by Agència de Gestió d'Ajuts Universitaris i de Recerca (AGAUR) – Generalitat de Catalunya (2017SGR1033), ICREA Foundation (ICREA Academia award granted to M. Cascante), Instituto de Salud Carlos III and Centro de Investigación Biomédica en Red de Enfermedades Hepáticas y Digestivas (CIBERHD, CB17/04/00023), and MINECO from the Spanish Government and FEDER funds (SAF2017-89673-R, European Commission FEDER – Una manera de hacer Europa).*