Metabolic plasticity in metastatic colorectal cancer in response to Palbociclib

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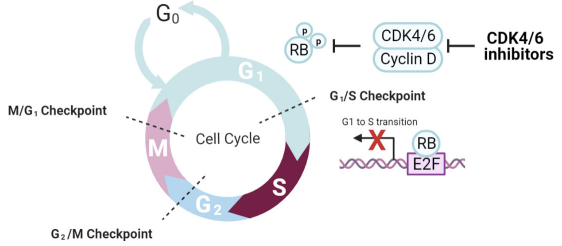
INTRODUCTION

Drug resistance and metastatic spread are the leading cause of mortality in patients with colorectal cancer. Metabolic reprogramming is a hallmark of cancer that plays a crucial role in metastasis and chemoresistance by sustaining the synthesis of the biomolecules required to maintain accelerated tumor growth and support adaptability to the microenvironment to promote cell survival. Therefore, targeting metabolic reprogramming represents an effective anti-cancer strategy (1).

Targeting the cell cycle and the metabolic reprogramming underlying the acquisition of resistance represents a novel approach in the design of combination therapies. In particular, cyclin-dependent kinases CDK4 and CDK6 (CDK4/6) are promising targets in cancer therapy since their overexpression is implicated in a wide range of human cancers. Intending to overcome the acquired drug resistance in cell cycle cancer therapy, we evaluated the effects of Palbociclib, a CDK4/6 selective inhibitor mainly used in breast cancer, on the lymphatic-derived metastatic SW620 colorectal cancer cell line. This inhibitor has demonstrated significant efficacy in several solid tumors in vivo and in vitro (2,3).

Hypothesis: Biological characterization of the metabolic reprogramming promoted by Palbociclib treatment in SW620 allows identifying metabolic vulnerabilities that can be targeted to increase tumor sensitivity.

Cell cycle regulation by CDK4/6 Inhibitors



Palbociclib affects the cell cycle regulation by inhibition of CDK4 and CDK6. This inhibition prevents the phosphorylation of retinoblastome (RB) protein by the CDK4/6-Cyclin D complex. RB-E2F complex remains attached to DNA impairing cell cycle progression by transcriptional repression of genes required for G1 to S phase transition. As a consequence, there is a cell cycle arrest in G0/G1 phase.

Figure adapted from "Cell Cycle Deregulation in Cancer", by BioRender.com (2021)

METHODOLOGY AND PRELIMINARY RESULTS

Metabolic reprogramming of SW620 cells is evaluated after short-time treatment with Palbociclib

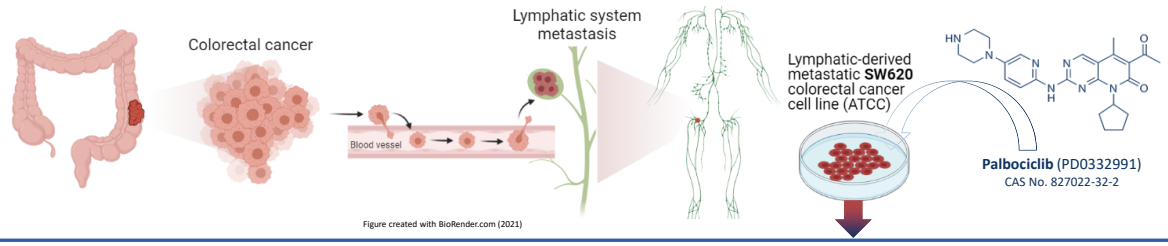
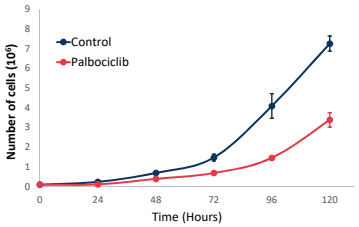


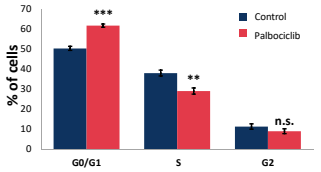
Figure created with BioRender.com (2021)

CELL PROLIFERATION



Cell proliferation is affected by Palbociclib, increasing duplication time from 18h to 22h.

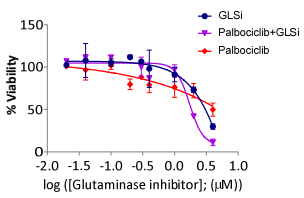
CELL CYCLE



Statistical analysis performed with T-Student is indicated as p<0.05 (*), p<0.01 (**) and p<0.001 (***)

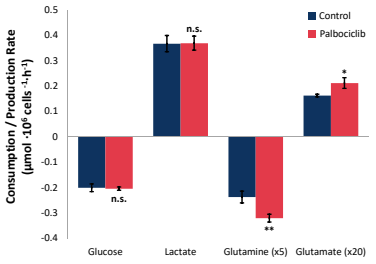
Palbociclib is promoting cell cycle arrest in G1 phase, around 10% more than control condition in agreement with drug mechanism, attenuating cell cycle progression. Consequently, less percentage of cells are displayed in S phase.

VIABILITY TEST



Considering the highest concentrations displayed, combination of glutaminase inhibitor (GLSi) plus Palbociclib promotes higher effects on viability rather than inhibitors assayed separately.

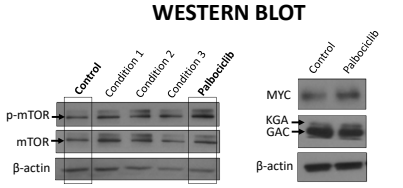
METABOLITE CONSUMPTION AND PRODUCTION RATE

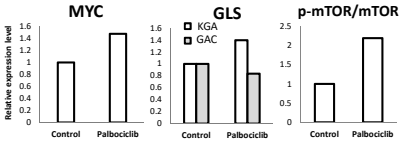


Statistical analysis performed with T-Student is indicated as p<0.05 (*), p<0.01 (**) and p<0.001 (***)

Glucose consumption and lactate production extracellular rates do not present differences with respect to control condition. However, Palbociclib is promoting an increase in glutamine metabolism.

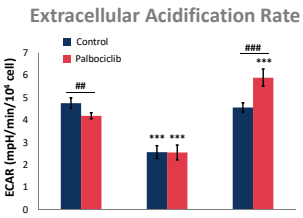
WESTERN BLOT



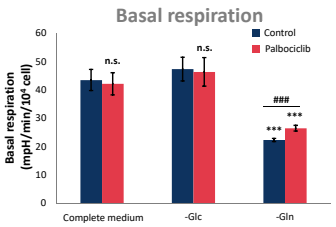


There is an increment in MYC and kidney-type glutaminase isoform (KGA) protein levels as well as mTOR activation after Palbociclib treatment. These results are in agreement with the observed metabolic phenotype.

RESPIROMETRY ASSAYS

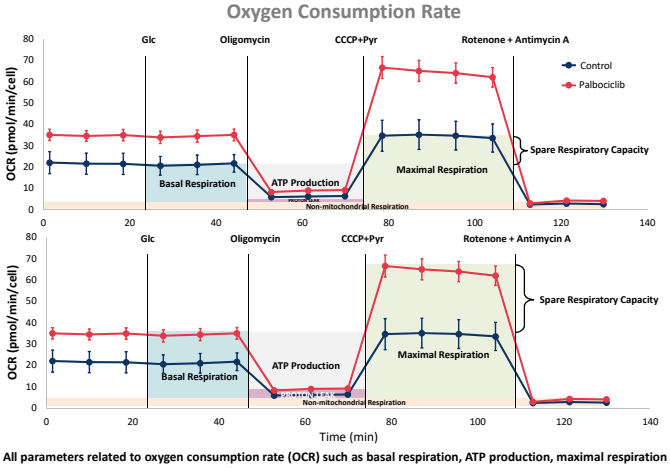


Palbociclib treatment decreases extracellular acidification rate (ECAR) in basal conditions. Glucose deprivation display no changes while glutamine deprivation promotes an increment.



The absence of glucose does not affect the basal respiration. However, it is significantly decreased by glutamine deprivation. Basal respiration with Palbociclib is less affected than in control cells, indicating that these cells are using alternative carbon sources in the absence of glutamine.

Statistical analysis performed with T-Student is indicated as Complete medium vs deprivation conditions p<0.05 (*), p<0.01 (**) and p<0.001 (***) and Control vs Palbociclib p<0.05 (#), p<0.01 (##) and p<0.001 (###)



All parameters related to oxygen consumption rate (OCR) such as basal respiration, ATP production, maximal respiration and spare respiratory capacity increase after Palbociclib treatment.

CONCLUSIONS

Results displayed an increment in glutamine metabolism and oxidative phosphorylation in concordance with our previous results with Palbociclib-treated HCT116 primary colorectal adenocarcinoma cells (4). These studies revealed that CDK4/6 inhibition induces MYC upregulation and the subsequent activation of downstream signaling, such as mTOR pathway. The combined therapy with GLS inhibitors and CDK4/6 inhibition demonstrated a synergistic effect, suggesting an effective strategy to overcome the acquired resistance of cancer cells to Palbociclib.

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ACKNOWLEDGEMENTS

This work is supported by MINECO from the Spanish Government and FEDER funds (SAF2017-89673-R, European Commission FEDER Una manera de hacer Europa)), Instituto de Salud Carlos III and Centro de Investigación Biomédica en Red de Enfermedades Hepáticas y Digestivas (CIBERHD, CB17/04/00023) and AGAUR (2017SGR1033).