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EXPLORING THE POTENTIAL ANTITUMOR EFFECTS OF METFORMIN IN PROSTATE CANCER CELLS

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Introduction

Cancer is the second leading cause of death in the U.S, behind only cardiovascular disease. The vast palette of cancer types stem from dysregulated maintenance of cellular division that lead to uncontrolled proliferation and potential metastasis[3].

During metastasis, tumor cells break off from the primary tumor site, invade the blood stream to become circulating tumor cells (CTCs), and seed new tissues for the spread of cancer[2,3,4]. Potential medications that can enter systemic circulation and preferentially affect these CTCs, based on their altered metabolic requirements, could prove to be effective treatment options.

Here, we explore the potential antitumor effects of metformin, a commonly prescribed medication for type 2 diabetes that lowers blood glucose. Metformin has been shown to inhibit protein members of the mitochondria oxidative phosphorylation (OxPhos) complex[4]. Given certain CTC's upregulation of OxPhos genes during metabolic reprogramming, we explore metformin's effect on proliferation and viability of CTCs.

Abstract

Tumor cells that undergo epithelial-mesenchymal transition (EMT) become circulating tumor cells (CTCs). While most CTCs will undergo apoptosis, the few resistant ones will adapt, acclimate and become the primary vehicle for metastasis[6,7]. Previous transcriptomic analysis showed preferential upregulation of oxidative-phosphorylation (OxPhos) genes compared to other metabolic genes in CTCs[1].

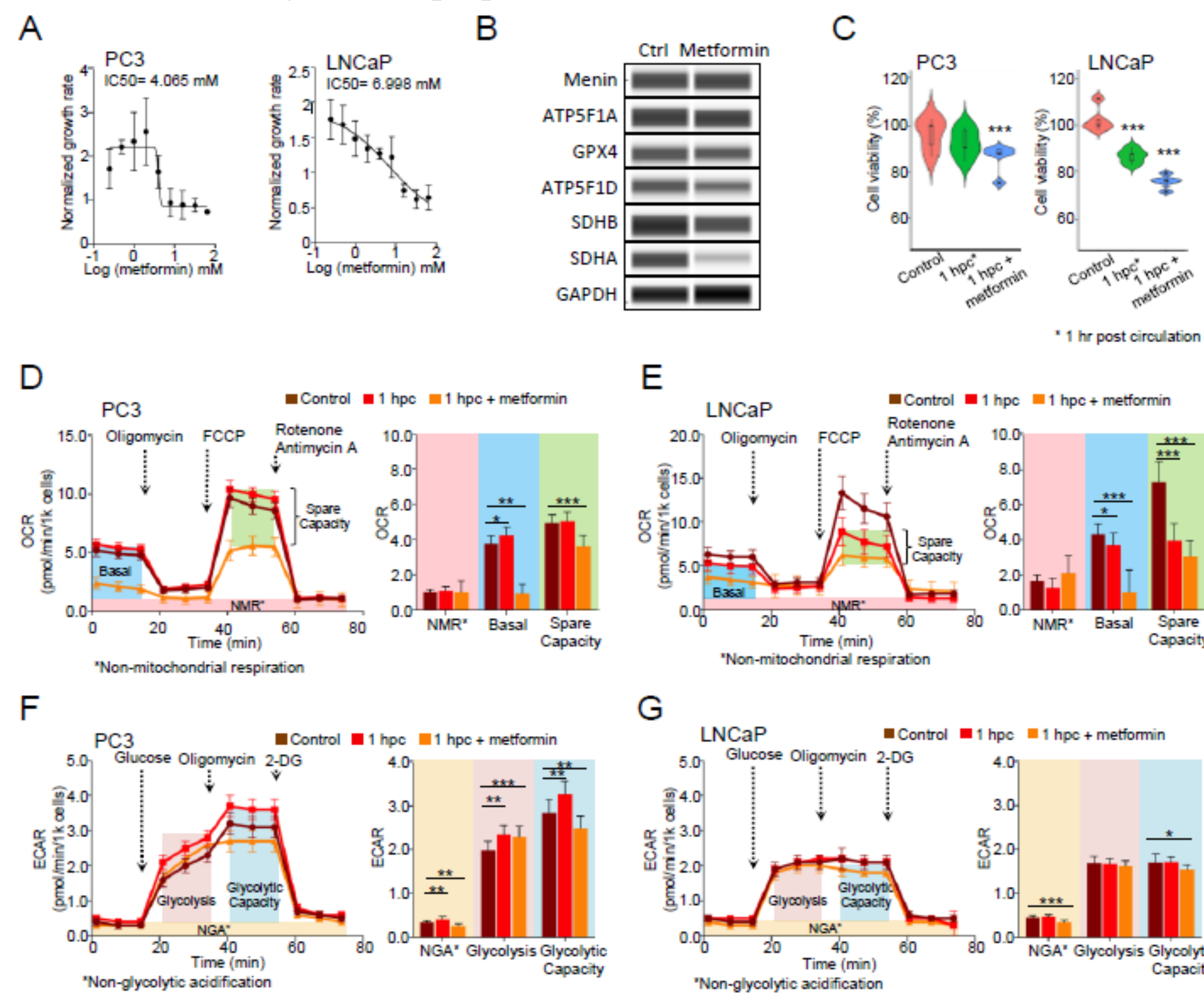
We hypothesized this reprogramming to be the main driver for CTCs adaptation to the circulating microenvironment and explored its potential therapeutic window. Metformin has been shown to inhibit protein members of the mitochondrial OxPhos complex[4]. Thus, we sought to repurpose metformin for its potential antitumor effects on two prostate cancer cell lines, PC3 and LnCaP.

For the study, we employed the Ibidi circulation assay to simulate physiological conditions of CTCs. After metformin treatment, we quantified cell viability, glycolysis and OxPhos activity and protein levels. When controlled for both metformin and circulation conditions, we observed reduced cell viability, decreased protein levels and some inhibition of OxPhos activity for PC3 and LnCaP.

From this preliminary data, we demonstrated the cellular effects of metformin on two prostate cancer cell lines, PC3 and LnCaP. Further study into the mechanism of action and metformin's effect on other cancer cell types will provide a more comprehensive understanding of its potential antitumor properties.

Materials and Methods

Proliferation Assay: Metformin treated cells were monitored for 72 hours and counted every 3 hours. Counted cell numbers were normalized against control condition (no metformin) and a proliferation curve was produced for metformin IC50 for both PC3 and LnCaP.
Circulation Assay: Both control and metformin treated cells, seeded at 1 million cell per circulation device, were circulated for one hour and two-hour durations and cell viability was measured afterwards.
OCAR/ECAR: The same cells from the circulation assay were then immediately seeded and prepared for OCAR and ECAR assay to measure the oxidative phosphorylation and glycolysis activities.
Western Blot: Cell samples from each of the experimental conditions were collected, lysed and prepared for western blot.



Results

(A) The (IC50) curves of metformin in PC3 and LNCaP prostate cancer cells.
(B) Capillary western blotting of OXPHOS-related proteins in control and metformin-treated PC3 cells.
(C) Cell viability results of PC3 and LNCaP prostate cancer cells before and after 1 hr in vitro flow assay with or without metformin treatment.
(D-E) Oxygen consumption rate (OCR) and OXPHOS functional profiles of PC3 and LNCaP prostate cancer cells in control, 1-hr post circulation (hpc), and 1-hpc with metformin.
(F-G) Extracellular acidification rate (ECAR) and glycolysis functional profiles of PC3 and LNCaP prostate cancer cells in control, 1 hpc, and 1 hpc with metformin.

Conclusion

Metformin has long been utilized as the main drug of choice for treating type 2 diabetes. Given the low cost, high LD50, and few adverse effects, metformin seems like a potential candidate for repurposing its usage towards CTCs treatment.

From the circulation assay, we observed clear cell viability differences between the PC3 cells under circulation with and without metformin in the 2-hour circulation condition. Furthermore, the OCR assay showed higher basal respiration and maximal respiratory capacity for the no metformin condition. This indicated some inhibition of PC3's oxidative phosphorylation when introduced to metformin. ECAR assay showed minimal elevation of glycolysis for the metformin treated cells.

LnCaP cell survival rates were similar for metformin and no metformin conditions. There was some difference between cell viability when normalized for cell count. OCR assay showed higher basal respiration and maximal respiratory capacity for the no metformin condition indicating some inhibition of LnCaP's oxidative phosphorylation when introduced to metformin. ECAR assay showed glycolysis elevation for all the cell treated conditions.

Further studies will be needed to elucidate the effect mechanism of metformin on these prostate cancer cells and whether these results can be extrapolated to other kinds of cancer cell lines.

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