Targeting Glycolysis and Mitochondrial Function in Prostate Cancer with Carbon Monoxide Releasing Molecules

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Background: Carbon monoxide (CO) is a gasotransmitter endogenously produced during heme degradation by the enzyme heme oxygenase-1 (HO-1). CO has shown antitumor effects in various cancer models. Additionally, CO interacts with hemoproteins, including redox-sensitive enzymes, exerting secondary effects on cell metabolism, such as energy production and signaling. To enable controlled delivery, CO-releasing molecules (CO-RMs) have been developed and are under investigation as potential anticancer agents. In this study, we examine the metabolic effects of CO-RMs in prostate cancer (PCa) cells, aiming to better understand its role in modulating cancer cell metabolism as a promising therapeutic approach.

Methods: Experiments were conducted using the PC3 human PCa cell line. Intracellular CO accumulation was confirmed using the HemoCD1 probe. Cell viability after 6-hour exposure to CO-RMs (25–150 µM) was assessed by the MTS assay. In parallel, glycolytic activity was evaluated by measuring intracellular ATP levels via a luciferase-based luminescence assay, while LDH activity and lactate production were determined through enzymatic kinetics and fluorometry, respectively. Additionally, metabolic profiling was performed using the Seahorse XF Analyzer to measure extracellular acidification rate (ECAR), reflecting glycolytic flux. Mitochondrial integrity was subsequently analyzed by flow cytometry and fluorescence microscopy using the TMRE probe, and further complemented by mtDNA copy number quantification through qPCR targeting *ND1*, *COX2*, and *CYTB* genes. Finally, tumorigenic potential was assessed by 3D spheroid formation using the hanging drop method, as well as clonogenic assays.

Results: Treatment with CO-RMs resulted in a marked accumulation of intracellular CO ($p < 0.001$), without compromising cell viability. Glycolytic function was notably disrupted, as indicated by significant reductions in ATP production ($p < 0.0001$), LDH enzymatic activity ($p < 0.01$), and intracellular lactate levels ($p < 0.01$). Consistently, ECAR measurements confirmed this glycolytic inhibition ($p < 0.0001$). This metabolic effect was not replicated by the CO-RM backbone (MnS), underscoring the specific contribution of CO. Mitochondrial analysis revealed functional impairment in PCa cells induced by CO-RMs treatment, with a decline in mitochondrial integrity ($p < 0.05$) and significantly lower expression of mitochondrial genes *ND1*, *COX2*, and *CYTB* ($p < 0.0001$). Furthermore, CORM-401 reduced tumorigenic potential, as evidenced by smaller spheroid size ($p < 0.0001$) and diminished colony-forming ability ($p < 0.01$).

Conclusions: CO-RMs disrupts PCa cell metabolism by impairing glycolysis and mitochondrial function, without affecting cell viability. Their ability to suppress stem-like features further highlights their potential as a metabolic-targeting strategy in PCa.

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