ADAPTATION TO NITRIC OXIDE EXPOSURE LEADS TO RESISTANCE TO HYPOXIA IN A549 LUNG CANCER CELLS

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Hypothesis: Lung tumor cells adapted to high levels of nitric oxide (NO) will exhibit increased resistance to hypoxia and express increased HIF-1α protein expression when compared with analogous cells grown without NO.

Objectives: It is known that patients with tumors that express high levels of NO portend a worse outcome over patients with low expressing tumors. To better model these aggressive tumors, we have adapted cell lines to high nitric oxide (HNO) levels. These HNO cell lines grow more aggressively than their corresponding parent cells (without additional NO). We predict that clinical tumors in HNO microenvironments will grow better under hypoxic conditions than tumors in low NO microenvironments. As such, we expect that HNO-adapted cells will have a higher expression of HIF-1α, a transcription factor that is commonly upregulated under hypoxic conditions.

Methods: A549, a human lung adenocarcinoma cell line was used in this study. A549-HNO and A549-Parent cells were tested under hypoxic conditions by exposing the cells to varying concentrations of $CoCl_2$. (Cobalt chloride treatment mimics hypoxic conditions in the lab.) Migration and MTT growth assays were performed in the presence of Cobalt. HIF-1α expression was measured using immunohistochemistry of cytospins. Gene chip analysis (Illumina) was conducted for both cell lines.

Results: A549-HNO cells treated with cobalt chloride grew better and migrated faster than parent cells at a range of concentrations (0.05-1 mM). Gene chip analysis showed a significant increase in HIF-1α expression in A549-HNO relative to A549-Parent. Imunocytospins confirmed higher HIF-1α expression in the A549-HNO cells.

Conclusions: These results suggest tumors growing in microenvironments containing high levels of NO will be better able to survive and thrive in hypoxic conditions than tumors growing in low NO environments. The HIF-1α gene is a potential therapeutic target for decreasing the aggressiveness of HNO expressing tumors.

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