Validation of a rhenium-based compound as potential novel anti-cancer drug and its implication in the hypoxic response

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Cancer is a major cause of death and its incidence is increasing worldwide. The development of drug resistance is a major issue. One characteristic of solid tumours is their hypoxic core. Hypoxia stabilises hypoxia-inducible transcription factors (HIFs), heterodimers composed of HIF-1α or HIF-2α isoforms together with HIF-β. They play a crucial role in cellular adaptation and regulate multiple hallmarks of cancer. Cisplatin is widely used as metal-based treatment for cancer, but the primary pitfall of the treatment is the development of drug resistance. Therefore, the transitional metal rhenium, represents an alternative. In 2020, a study found strong evidence for anti-cancer potential of a rhenium(I) tricarbonyl-based compound displaying anti-angiogenic, anti-metastatic, and tumour growth inhibition in zebrafish-human HCT116 xenografts.

The current study aimed to validate these anti-cancer properties *in vitro* by evaluating the molecular mechanisms underlying the effects of the compound on cancer progression, particularly in relation to the HIF pathway.

Our results revealed that this drug exhibited an anti-angiogenic effect by reducing vascular endothelial growth factor (VEGF) expression under hypoxic conditions in a concentration-dependent manner. Simultaneously, cell proliferation and motility were negatively affected, as observed by live-cell imaging. The efficacy of the drug in reducing cellular energy metabolism was confirmed, with a reduction in mitochondrial function and glycolysis suppression, leading to a significant decrease in ATP production. Moreover, RNA sequencing (RNA-seq) analysis suggested reactive oxygen species (ROS) generation, as reflected by the modulation of mitochondrial metabolism and the increased expression of antioxidant genes. Reporter gene assays and mRNA expression levels of redox-sensitive genes independently confirmed these observations. Intriguingly, the treatment specifically reduced HIF-2α protein levels without altering its transcriptional activity. Consistently, expression levels of specific HIF-2 target genes were reduced accordingly. Although HIF-1α protein expression levels remained unchanged, as subset of analysed HIF-1-dependent genes appeared to be decreased.

Taken together, our results demonstrate that the rhenium(I) tricarbonyl-based compound exhibits anti-tumour efficacy and highlight a potent novel anti-HIF-2α drug. These data strongly support its therapeutic potential as a novel treatment for hypoxic tumours.

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