Characterisation of HIF-mediated genoprotection in clear cell renal cell carcinoma

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Introduction

Clear cell renal cell carcinoma (ccRCC) is the most common form of renal cell carcinoma (RCC). Patients with ccRCC often have a remarkably high resistance to traditional radiotherapy and chemotherapy. Hypoxic cores are a common marker of various tumours, which trigger a genetic cascade causing the upregulation of genes crucial to cell survival, such as angiogenesis, cellular proliferation, and altered metabolism. Previous work suggests that increased resistance to radiotherapy/chemotherapy may be due to high levels of hypoxia-inducible factors (HIFs), which under normoxic conditions are targeted for degradation by the von-Hippel Lindau protein (VHL)/E3 ligase complex. Under hypoxia, HIF levels accumulate, allowing them to activate hypoxic response elements downstream. Mutations in VHL, which are commonly seen in ccRCC patients, cause constant upregulation of HIFs. Therefore, pathways promoting cell survival are constitutively upregulated, initiating tumour formation.This project seeks to understand what pathways are involved in HIF-mediated genoprotection and whether or not high levels of HIF provide a genuine genoprotective effect in humans.

Methods

DNA damage assays, such as comet assays, clonogenics and cell titre blue assays were used to compare the DNA damage response between RCC4 VHL -/- and RCC4 VHL WT cell lines. Western blot and qPCR was used to compare protein expression between cell lines, focusing on VHL, HIFs, and DNA repair proteins

Results

The expression levels and activity of a key regulator of DNA damage response was highly upregulated in RCC4 VHL -/- cells, which correlates with high HIF expression. By knocking down HIF1a or HIF2a separately, I demonstrate that HIF2a is key for this upregulation. RCC4 VHL -/- cells show greater resistance to two DNA damaging agents: camptothecin and olaparib, both of which demonstrate anti-cancer properties. However, initial results indicate that HIF knockdown is insufficient to resensitise RCC4 VHL -/- cells to camptothecin treatment.

Discussion

Although HIF2a expression promotes expression of an important DNA repair protein, this is not solely responsible for the DNA damage resistance in RCC4 VHL -/- cells. The VHL/HIF pathway interplays with multiple pathways that also need to be modulated to see a substantial impact on DNA damage response. HIF levels in RCC4 cells may need to be altered on a long-term basis to accurately mimic patient cells. This may be achieved by CRISPR mutagenesis of HIF1a/HIF2a, which is currently being implemented. Further work will also focus on the other pathways that HIF1a and HIF2a interact with, as well as the HIF-independent roles of VHL that may contribute to the increased radiotherapy/chemotherapy resistance in ccRCC patients.