

Potential new drug therapies in advanced renal cell carcinoma: An investigation of different combinatory drug regimens on the proliferation of cultured human renal cell carcinoma cells

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ABSTRACT

Renal cell carcinoma accounts for approximately 2-3% of all malignancies. Contrary to localized disease, which often can be cured by surgery, there is no effective treatment for metastatic renal cell cancer. Due to the lack of clinically relevant antiproliferative drugs, we investigated several combinatory regimens of recently developed drugs. Using UMRC3 cells as a model for metastatic renal cell cancer, we studied the effect of monotherapy compared to that of a combination of Imatinib, 2-Methoxyestradiol (2ME2) and/or 3-(5'-hydroxymethyl-2'-furyl)-1-benzylindazole (YC-1) on cell proliferation. Due to the different mechanisms of action of these drugs – Imatinib, a receptor tyrosine kinase inhibitor, is known to inhibit the bcr-abl fusion protein, stem-cell factor receptor c-Kit, and platelet derived growth factor receptor (PDGFR) signaling; 2ME2, an estrogen metabolite, inhibits hypoxia-induced factor-1, a key angiogenic transcription factor and stabilizes microtubules while YC-1 known as an inhibitor of platelet aggregation blocks HIF-1 - we evaluated whether an additive or even synergistic antiproliferative effect could be achieved. We identified doses for which cell proliferation is inhibited by 25%. Using these doses, we found additive inhibitory effects using a combination of Imatinib, YC-1 as well as 2ME2 and YC-1. Synergistic antiproliferative effects could be demonstrated by combining Imatinib with 2ME2 and all three drugs. Our data indicate a greater efficacy of renal cell cancer inhibition by using combinatory drug therapies compared to a single drug application. Due to the low dosage of each drug, a lower toxicity profile could be assumed. We are currently examining the mechanism of these additive and synergistic activities *in vitro* and *in vivo*.

INTRODUCTION

The hypoxia-inducible transcription factors HIF-1 α and HIF-2 α have been investigated and brought into context with tumor growth and angiogenesis previously. This has stimulated widespread interest in developing new therapeutic strategies to block the HIF-dependent pathways. Nevertheless there is still controversial data with regards to function of each HIF in regulating tumor biology in RCC. Sowter et al. suggested that down regulation of HIF transcriptionally regulated genes such as CaIX, GLUT-1 and VEGF are mediated by HIF2 α . This was demonstrated by treating renal cell carcinoma (RCC) cells (786-O cells express HIF-2 α but not HIF-1 α) with siRNA to HIF-2 α . Further it has been demonstrated that inhibiting HIF-2 α in 786-O cells, using short hairpin RNAs, leads to an impaired tumor growth *in vivo* (Kondo et al., 2003). Similarly, HIF-2 α but not HIF-1 α overexpression in VHL-defective renal cell carcinoma cells enhanced *in vivo* tumor growth (Raval et al., 2005). On the other hand there have been reports that indicate a tumor suppressor role of HIF2 α by demonstrating reduced tumor growth *in vivo* using stably transfected GS91 cells (Glioma cell line) that overexpressed HIF-2 α (Acker et al., 2005). In the present study, we investigated the function of HIF-2 α in a human metastatic renal cell line UMRC3 by suppressing HIF-2 α using dominant negative and antisense expression vectors for HIF-2 α . We further studied the effects upon HIF2 α signaling by known small molecule inhibitors for HIF-1 α .

RESULTS

HIF-2 α expression and HIF regulated genes identified in ccRCC tumor tissues

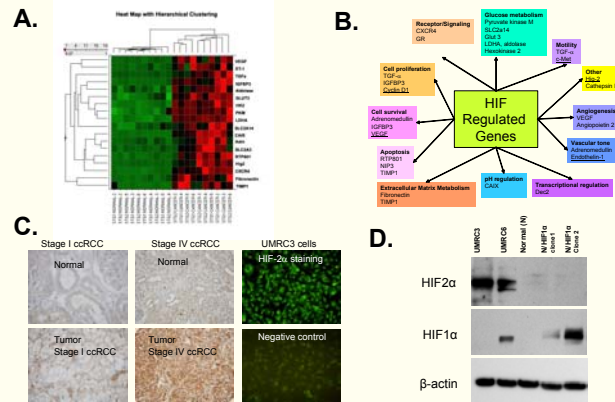
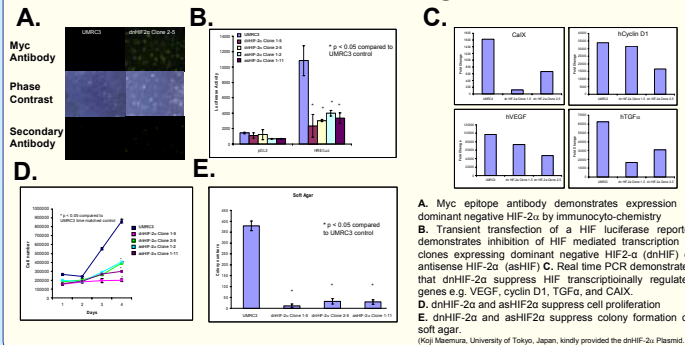


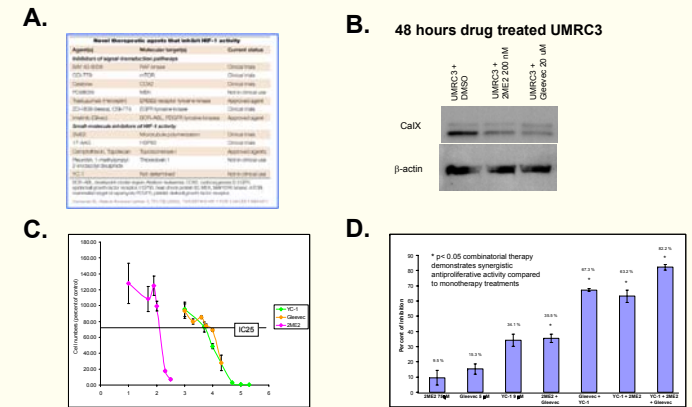
Figure 1. HIF regulated genes are expressed in Stage 1 ccRCC. **A.** Gene array analysis of patient matched normal tumor tissues identified HIF regulated genes **B.** Cartoon of HIF genes and cancer related pathways **C.** Immunohistochemistry demonstrating HIF2 α expression in ccRCC formalin fixed tissues and human ccRCC cell line **D.** Western analysis demonstrating HIF2 α expression in UMRC3 cells, a human metastatic ccRCC cell line.

Characterization of Dominant Negative HIF-2 α clones



A. Myc epitope antibody demonstrates expression of dominant negative HIF-2 α by immunocytochemistry **B.** Transient transfection of a HIF luciferase reporter demonstrates inhibition of HIF mediated transcription in clones expressing dominant negative HIF-2 α (dnHIF) or antisense HIF-2 α (ashIF). **C.** Real time PCR demonstrates that dnHIF-2 α suppress HIF transcriptionally regulated genes e.g. VEGF, cyclin D1, TGFA, and CAIX. **D.** dnHIF-2 α and ashIF2 α suppress cell proliferation **E.** dnHIF-2 α and ashIF2 α suppress colony formation on soft agar. (Kojima, University of Tokyo, Japan, kindly provided the dnHIF-2 α Plasmid.)

Drugs that antagonize HIF signaling



A. Table describing drugs that nonspecifically inhibit HIF1 α . **B.** Drugs inhibit HIF mediated transcription **C.** HIF antagonist inhibit cell proliferation in a dose responsive fashion **D.** Using concentrations of drugs at their calculated IC25 values, combinatorial therapy demonstrates synergistic inhibition of cell proliferation.

RESULTS & CONCLUSIONS

1. Attenuation of HIF-2 α in a human metastatic ccRCC cell line that expresses only HIF-2 α results in down-regulation of HIF transcriptional activity, cell proliferation and colony formation.
2. Drugs known to inhibit HIF-1 α also block HIF-2 α mediated signaling.
3. Synergistic antitumor activity is demonstrated by combinatorial therapy using HIF antagonist.

A controversy in the literature suggests that HIF-2 α can act as a tumor suppressor or play a critical role in tumor progression. Our data suggest that HIF-2 α promotes tumor aggressiveness.

