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Novel HIF-2α targeted RNAi therapeutic for renal cell carcinoma

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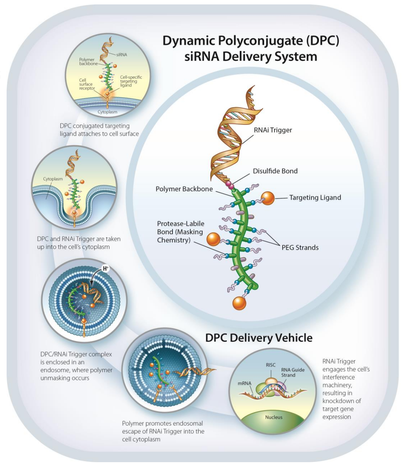
INTRODUCTION

Targeted therapy including VEGF and mTOR pathway inhibitors has dramatically transformed treatment options and outcomes for patients with metastatic clear cell renal cell carcinoma (ccRCC). However, alternate treatments are needed as resistance to these initially promising agents occurs frequently. RNAi interference (RNAi), an innate gene silencing mechanism, has been explored as a new class of therapeutics where conventional treatments are lacking or have failed. The challenge in leveraging this promising approach has been efficient delivery of an RNAi trigger (siRNA) to target tissue. Over 90% of ccRCC tumors express a mutant inactive form of the von Hippel-Landau protein (pVHL), an E3 ubiquitin ligase that promotes target protein degradation. Strong evidence supports the observation that pVHL functional loss leads to the accumulation of the transcription factor hypoxia-inducible factor 2α (HIF-2α), a tumorigenic driver of ccRCC.

METHODS

We have developed a targeted delivery platform called Dynamic Polyconjugate™ (DPC) as an RNAi-based therapeutic targeting HIF-2α for advanced ccRCC. The ccRCC-specific DPC (ITG-DPC) comprises a membrane active polymer to promote RNAi trigger endosomal release, a ligand that binds to αV-containing integrin receptors expressed on tumor cells, reversible masking to prevent polymer activity before reaching the endosomal compartment, and a potent and specific RNAi trigger to HIF-2α. The modular nature of this delivery platform allows for flexibility to optimize each functional component independently. The ligand-dependent delivery of ITG-DPC was first evaluated in cultured tumor cells and then confirmed in ccRCC tumors established in nude mice using fluorescently-labeled ITG-DPC and confocal microscopy. To validate silencing of HIF-2α as an effective therapeutic approach, an inducible shRNA to HIF-2α was expressed in ccRCC tumors established in mice that significantly silenced HIF-2α gene expression and induced tumor regression.

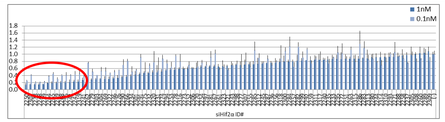
Proof-of-concept functional delivery was then obtained using optimized HIF-2α ITG-DPC in two different orthotopic RCC tumor bearing mouse models.



Schematic of ITG-DPC and target cell delivery

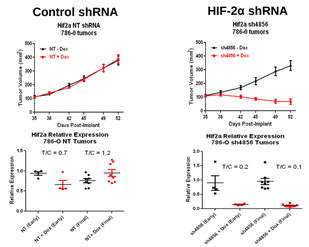
RESULTS

A. HIF-2α RNAi Trigger selection in cultured cell system



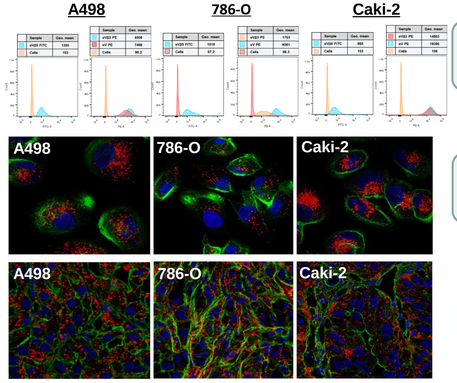
Lead sequences selected for further evaluation

B. *In vivo* target validation in SQ 786-O RCC xenograft expressing Dox inducible HIF-2α shRNA



Induction of HIF-2α shRNA expression resulted in HiF2α mRNA knockdown and tumor growth regression

C. Targeting ligand validation and tumor model selection

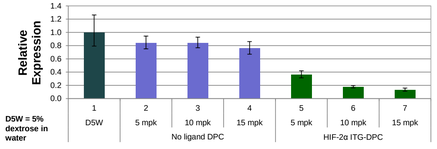


Integrin (αvβ3, αvβ5) expression profiling by FACS

In vitro ITG-DPC cell binding and internalization

In vivo ITG-DPC tumor delivery

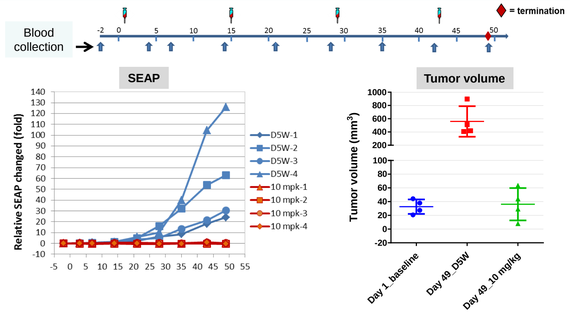
D. Ligand and dose dependent HIF-2α gene silencing in A498-SEAP* orthotopic RCC tumor model

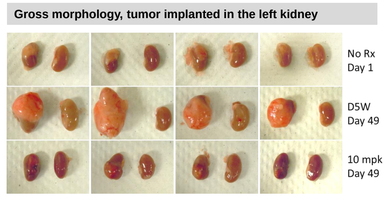


D5W = 5% dextrose in water

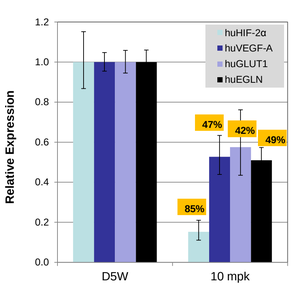
***A498-SEAP cells were stably transfected with a SEAP (secreted embryonic alkaline phosphatase) expression construct. Serum collected from tumor bearing mice can be used to monitor tumor growth**

E. Tumor growth inhibition after 4 bi-weekly doses in orthotopic A498-SEAP ccRCC tumor bearing mice

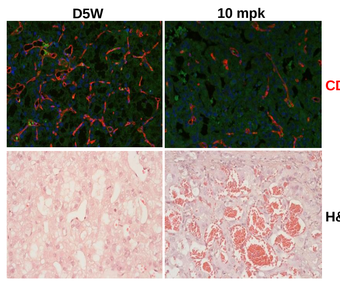




Inhibition of HIF-2α and regulated genes

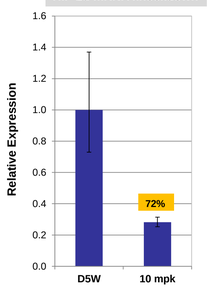


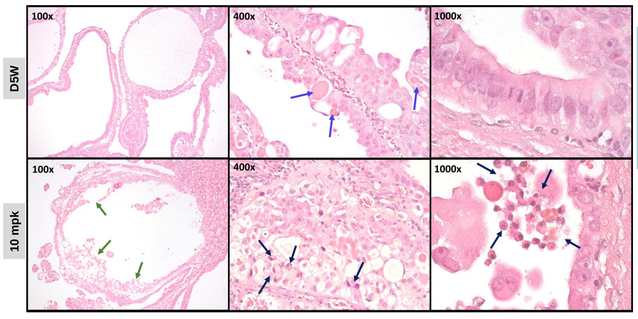
Tumor and blood vessel degeneration



- **Tumor volumes (calculated with surface measurements) were mostly unchanged in the treatment group, suggesting tumor stasis**
- **However, histological examination of tumor sections revealed extensive internal tumor destruction**
- **Down-regulation of HIF-2α regulated pathway genes including VEGF-A, and the corresponding reduction in tumor-associated CD31 positive neovascularization, corroborated on-target effects of HIF-2α gene silencing.**
- **Treatment was well tolerated**

F. QW x2 HIF-2α ITG-DPC promotes tumor degeneration in orthotopic Caki-2 papillary RCC tumor bearing mice





- **Dosed on day 1 and day 8**
- **Tumor HIF-2α expression level and histopathology on day 14**
- **Significant tumor degeneration was observed in treatment group**

Summary and Conclusions

- ***In vivo* ligand dependent delivery of ITG-DPC to RCC tumor bearing mice was demonstrated**
- **Silencing HIF-2α expression by RNA interference results in reduction of HIF-2α regulated genes, promotes tumor cell death and structural degeneration in two different orthotopic RCC tumor models**
- **HIF-2α specific RNAi-based-therapeutic has the potential to radically impact the late-stage RCC treatment paradigm**

Acknowledgement

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