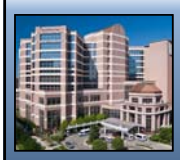


The Hypoxia Associated Factor is a Novel Target for Treatment of CC-RCC

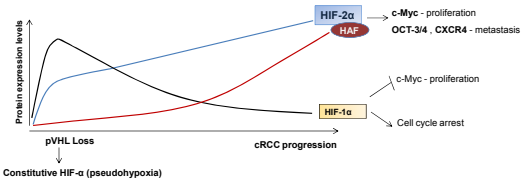
Mei Yee Koh, Ph.D

Assistant Professor
Department of Experimental Therapeutics
M.D. Anderson Cancer Center

HIF-2α is the main driver of cRCC progression

- Clear cell renal cell carcinoma (cRCC) the most common and aggressive type of kidney cancer (70-80%)
- pVHL loss seen in 60-80% of cRCC – promotes constitutive HIF activation

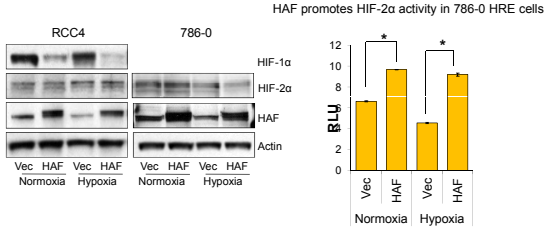
HIF switch during cRCC progression



- HIF-2α drives proliferation and metastasis - promising target for treatment of cRCC
- HIF-1α is a tumor suppressor - antagonizes c-Myc
- Isoform-specific HIF-2α inhibitors challenging due to significant similarities between HIF-1α and HIF-2α

The Hypoxia-associated factor, HAF, and the HIFs

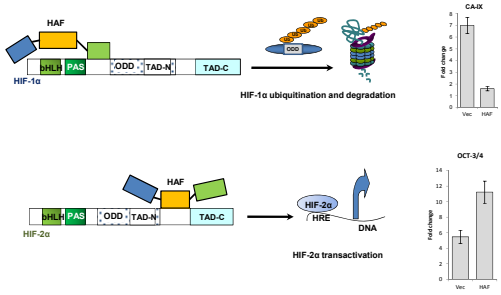
- 800 aa protein, also known as the squamous cell carcinoma antigen recognised by T-Cells, SART1, and is overexpressed in RCC



- promotes the degradation of HIF-1α and the activation of HIF-2α, thereby causing the HIF switch

HAF promotes the HIF switch

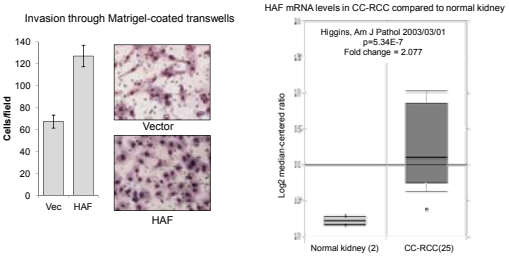
- HAF binds to HIF-1α and HIF-2α at different sites



Koh, et.al. **Cancer Research** 2011 Jun 1;71(11):4015-27; *Nat Rev Cancer. 2011 Jun;**11**(6):391.*

HAF promotes invasion and stem cell proliferation

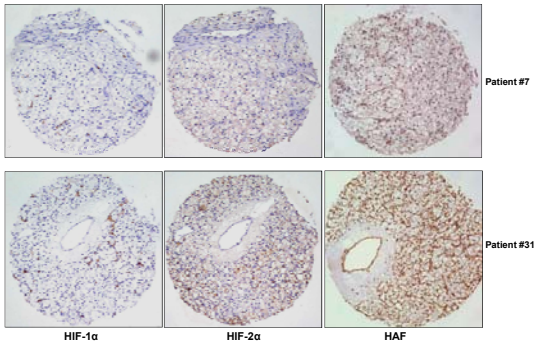
- HIF-2α specific target genes: matrix metalloproteinases (eg. MMP9) and the stem cell factor OCT-3/4.
- **Hypothesis**: HAF promotes CC-RCC aggressiveness by activating HIF-2α



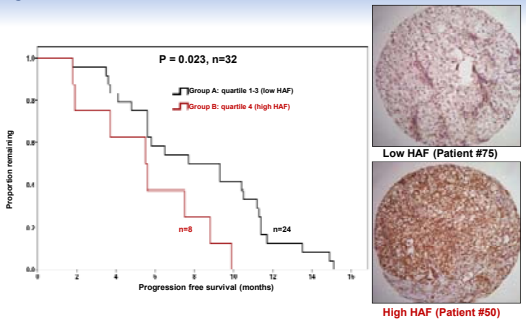
Koh, et.al. **Cancer Research** 2011 Jun 1;71(11):4015-27

HAF and the HIFs in RCC progression and prognosis

•TMAs from stage III/IV cRCC patients treated with sorafenib (GUMA08-002; 40 patients)



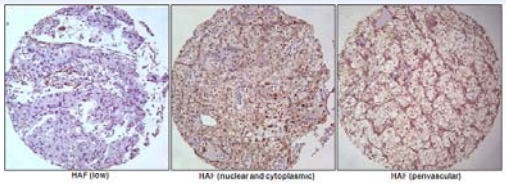
High HAF correlates with decreased PFS to sorafenib



• HAF is a potential biomarker predicting response to sorafenib.

• Ongoing studies investigating the relationship between HAF levels on tumor stage, and between HAF levels and the HIFs

HAF has distinct expression patterns in different patients



Further questions

1) **To investigate the relationship between HAF, HIFs and patient outcome in a larger dataset**

2) **To investigate the relationship between HAF and tumor progression**

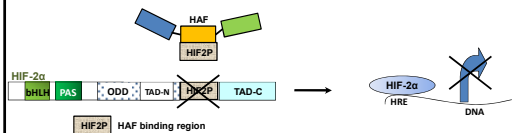
- Previous data obtained from stage III/IV RCC – need to perform studies in multistage array to investigate involvement of HAF in tumor progression

3) **To investigate the importance of HAF localization on patient outcome**

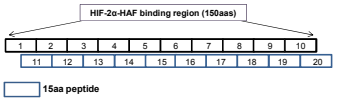
- HAF was expressed in the nucleus and cytoplasm and was also detected in the perivasculature of tumors

Inhibition of the HAF/HIF-2α axis

Strategy: To develop peptide-mimetic inhibitors of HIF-2α



Step 1: Synthesizing peptides:

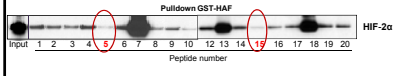


Peptide inhibitors abrogate HAF-HIF-2α binding

Positive peptides inhibit binding

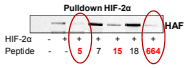
Step 2,3: Binding assays

(Input IVT HIF-2α + Immobilized GST-HAF)



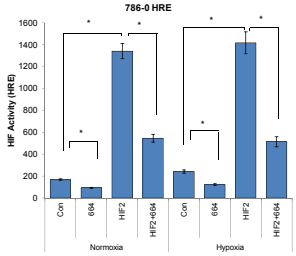
Step 4: Iterative process with shorter peptides

(Input endogenous HAF + Immobilized His_6-HIF-2α)



Peptide inhibitors decrease HIF-2α transcriptional activity

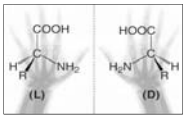
Step 5: Validation



Peptide 664 inhibits HIF-2α activity

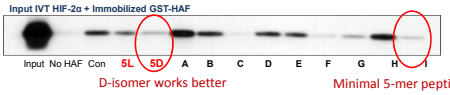
Further studies I

Amino acid chirality – only L-form exists in nature. D-form functionally similar but resistant to proteolysis



Deletion/mutagenesis: AAPLGPPVSPPHVST

- Testing ability of L-D isomers and deletion mutants to inhibit binding

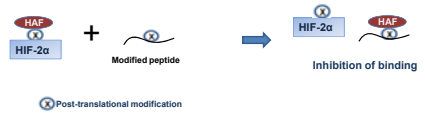


D-isomer works better

Minimal 5-mer peptide

Further studies II

Strategy to increase peptide efficacy



Functionalizing peptide for proof-of concept studies

- Attaching cell penetration sequence : HIV tat sequence, stabilization with acetyl group, FITC for imaging

Acetyl-YGRKKRRQRRRGG-peptide

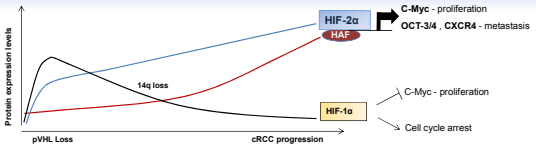
Stability | Cell penetration | peptide sequence

Fl-YGRKKRRQRRRGG-peptide

Fluorescence - imaging

- Monitoring cell uptake; HIF-2α inhibitory activity; effect on proliferation and other downstream readouts

Summary



- HAF promotes the switch from HIF-1α towards HIF-2α during RCC progression
- High HAF levels associated with decreased PFS in cRCC
- Inhibition of the HAF/HIF-2α axis is a novel approach for targeting HIF-2α driven cRCC

Future studies

Investigating HAF levels in multistage RCC TMA

- To increase patient dataset
- To investigate the relationship between HAF levels and tumor stage

We have identified a 5 residue peptide sequence that inhibits HAF/HIF-2α binding and activity

- Ongoing studies to functionalize peptide for proof-of-concept studies

Acknowledgements

Powis Lab

Galina Kiriakova (IHC)

Mena Abdelmelek (Statistical analysis)

Garth Powis

Collaborators

Peptide inhibitors

John McMurray

RCC TMA

Eric Jonasch

Federico Monzon

Lynda Corley

Funded by the MDACC Kidney Cancer MRP Project award