



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

Mei Yee Koh, Ph.D

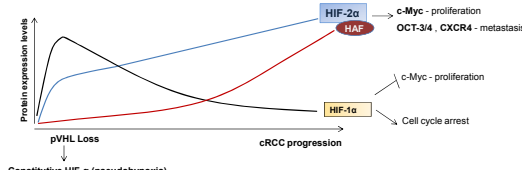
Assistant Professor
Department of Experimental Therapeutics
M.D. Anderson Cancer Center



HIF-2 α 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

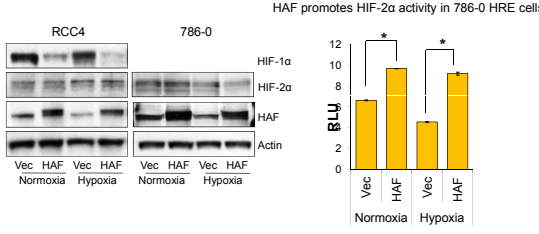


- HIF-2 α drives proliferation and metastasis - promising target for treatment of cRCC
- HIF-1 α is a tumor suppressor - antagonizes c-Myc
- Isoform-specific HIF-2 α inhibitors challenging due to significant similarities between HIF-1 α and HIF-2 α

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

HAF promotes HIF-2 α activity in 786-0 HRE cells



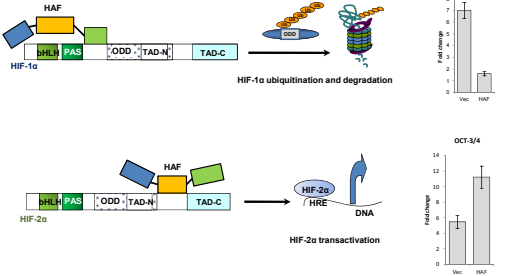
BLU

Cell Line	Condition	HIF-1 α BLU	HIF-2 α BLU
786-0	Normoxia	~6	~6
	Hypoxia	~10*	~10*

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

HAF promotes the HIF switch

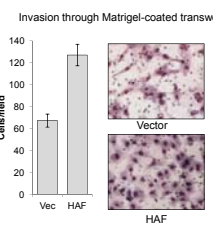
- HAF binds to HIF-1 α and HIF-2 α at different sites



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

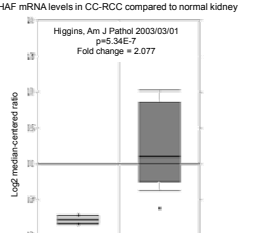
HAF promotes invasion and stem cell proliferation

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



Invasion through Matrigel-coated transwells

Condition	Cells field
Vec	~60
HAF	~130



HAF mRNA levels in CC-RCC compared to normal kidney

Sample	Log median-centered ratio
Normal kidney (2)	~0
CC-RCC(25)	~1.5*

Higgins, Am J Pathol 2003/03/01 p#5:34E-7 Fold change = 2.077

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

RESEARCH HIGHLIGHTS

HIF switch



HYPOXIA

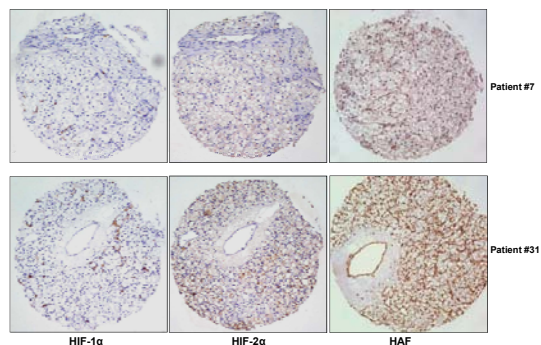
Hypoxia induces factor 1 α (HIF-1 α) and HIF-2 α are crucial mediators of the hypoxic response, regulating the transcription of both common and tissue-specific genes. Both HIF α subunits have roles in tumour development and progression, but despite the interest in their differential effects, the molecular mechanisms behind the HIF-1 α to HIF-2 α switch remains unclear. Mei Koh and colleagues have previously shown that hypoxia-associated factor (HAF, also known as SART1), an E3 ubiquitin ligase, targets HIF-1 α for proteasomal degradation. They have now provided evidence that HAF can also promote HIF-2 α .

HIF-2 α protein levels, indicating that binding is necessary for HIF-2 α .

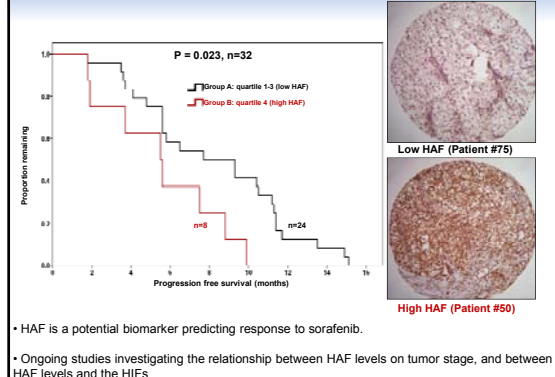
HIF-2 α may also promote stem cell maintenance in glioblastoma. Indeed, HAF overexpression in U87 cells decreased the levels of stem cell markers, particularly in the subpopulation of floating (rather than adherent) cells. Stimulation of human stem cell proliferation in solid-type U87 cells in non-adherent culture also induced HAF expression, and the stem cell population was reduced by expression of HAF cDNA. How does HAF affect tumour growth in vivo? Intracranial injection of adherent HAF-overexpressing U87 cells in nude mice did not affect survival. By contrast, the injection of high and low numbers of pooled floating and adherent cells that expressed HAF enhanced tumour progression and initiation, respectively, indicating that

HAF and the HIFs in RCC progression and prognosis

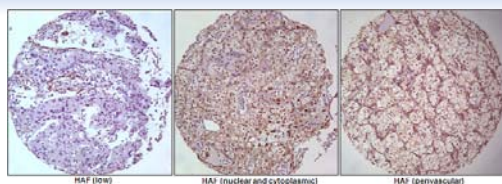
• TMAs from stage III/IV cRCC patients treated with sorafenib (GUMA08-002; 40 patients)



High HAF correlates with decreased PFS to sorafenib



HAF has distinct expression patterns in different patients

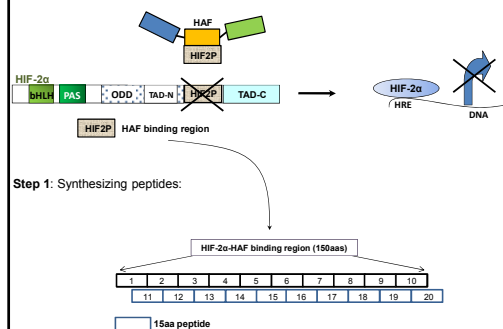


Further questions

- 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 perivascularity of tumors

Inhibition of the HAF/HIF-2α axis

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

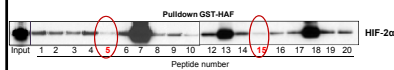


Peptide inhibitors abrogate HAF-HIF-2α binding



Step 2,3: Binding assays

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



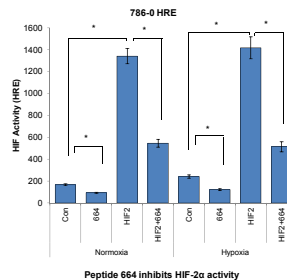
Step 4: Iterative process with shorter peptides

(Input endogenous HAF + Immobilized His₆-HIF-2α)



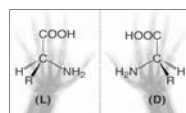
Peptide inhibitors decrease HIF-2α transcriptional activity

Step 5: Validation



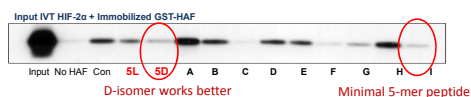
Further studies I

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



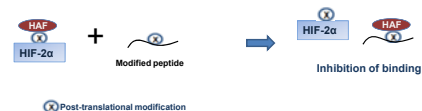
Deletion/mutagenesis: [AAPLGPPVSPPHVST](#)

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



Further studies II

Strategy to increase peptide efficacy



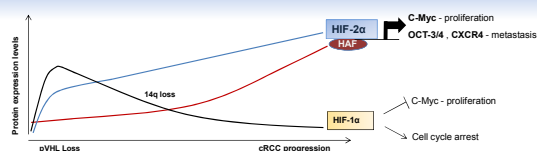
Functionalizing peptide for proof-of concept studies

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



- 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

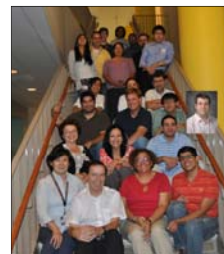
Galina Kiriakova (IHC)
Mena Abdelmelek (Statistical analysis)
Garth Powis

Collaborators

Peptide inhibitors
John McMurray

RCC TMA

Eric Jonesch
Federico Monzon
Lynda Corley



Funded by the MDACC Kidney Cancer MRP Project award