Hypoxia inducible factor 1α in kidney cancer

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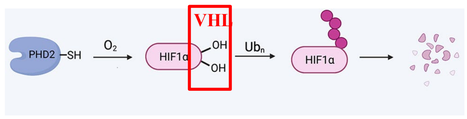
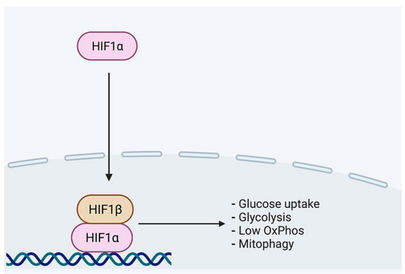
Introduction

- Renal cell carcinoma (RCC), which accounts for approximately 90 percent of kidney cancers, encompasses more than 10 different cancer subtypes that vary in their severity [1,2]. These RCC subtypes are known to be biologically distinct from each other and these differences between subtypes can be used to create targeted therapies.
- Clear cell renal cell carcinoma (ccRCC) is the most common kidney cancer. Approximately 90% of ccRCC tumors bear inactivating mutations in the von Hippel-Lindau (VHL) gene, which codes for the VHL protein [1]. In normal tissue, HIF1α and HIF2α accumulates in cells exposed to hypoxia. After oxygen levels are restored, HIF1α and HIF2α is degraded through a protein complex involving VHL protein. In ccRCC, inactivating mutations in VHL lead to accumulation of HIF1α and HIF2α.
- Renal medullary carcinoma (RMC) is a rare but aggressive kidney cancer. It is characterized by complete SMARCB1 loss. It has been found to have high expression of HIF1α [2].

Background



When oxygen is present, prolyl hydroxylase (PHD) recognizes HIF1α and HIF2α. This leads to VHL recognition and ubiquitination (Ub_n) and degradation of the protein.



HIF1α and HIF2α have to dimerize with HIF1β to act as a transcription factor. It is the dimer that leads to transcription of genes associated with glycolysis, glucose uptake, angiogenesis, and lower oxidative phosphorylation [1].

Hypothesis

Our overall hypothesis is that inhibiting HIF1α dimerization with HIF1β will lead to reduction of HIF signaling in kidney cancer and lead to reduced tumor growth.

Goals

(1) To visualize HIF1α signaling in ccRCC and RMC cell lines.
(2) To determine if current small molecules that inhibit HIF signaling have activity in ccRCC and RMC cell lines.

Result

Experiment 1: Visualizing HIF signaling

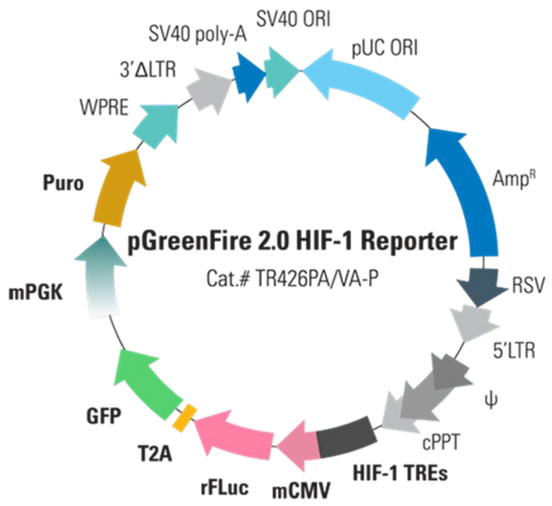


Figure 2: Vector to observe HIF1α signaling. HIF-1 transcriptional response elements (TREs) are placed upstream of a minimal CMV promoter (mCMV) which together drive co-expression of rFLuc and GFP in response to HIF-1 activity. The result is the ability to quantitatively measure HIF-1 activity using both fluorescence and luciferase activity. Used ampicillin resistance to determine which cells were transfected. The vector allowed us to determine if HIF1α was expressed in the cell. When HIF1 α was present – the transfected cells expressed GFP and luciferase.

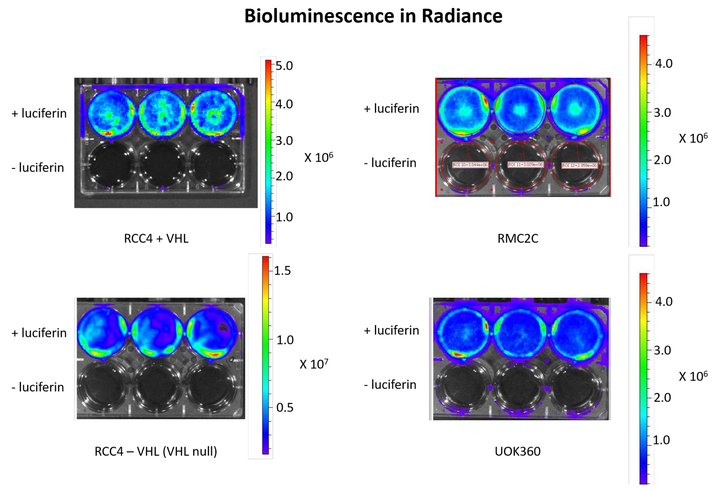


Figure 3. Bioluminescence of kidney cancer cells transfected with HIF1α signaling vector. We used two ccRCC cell lines: RCC4 with VHL (RCC4 +VHL) and RCC4 without VHL (RCC4 -VHL) [3]. RCC4 +VHL has a fully functional VHL protein while RCC4 -VHL is VHL null. The RCC4 -VHL null cell line is known to have higher expression of HIF1α than RCC4 +VHL cell line [3]. In addition to RCC4, we used RMC cell lines RMC2C and UOK360. Method: Luciferin dissolved in phosphate buffer was added to 6-well plates (25 ul per well) and incubated for 10 minutes prior to imaging. Bioluminescence was imaged used an IVIS Imager (Perkin Elmer).

Experiment 2: Validating HIF signaling

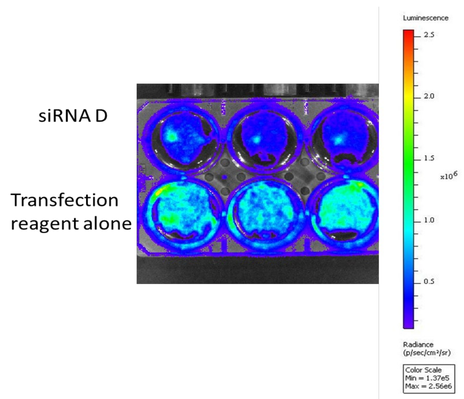


Figure 4: To validate that we were observing HIF1α expression with bioluminescence, we tested 4 siRNAs against HIF1α in RCC4 –VHL cells. We labeled the siRNAs A, B, C, and D. We observe 60% reduction in bioluminescence 48 hours after siRNA D is added to the media compared to the transfection reagent alone.

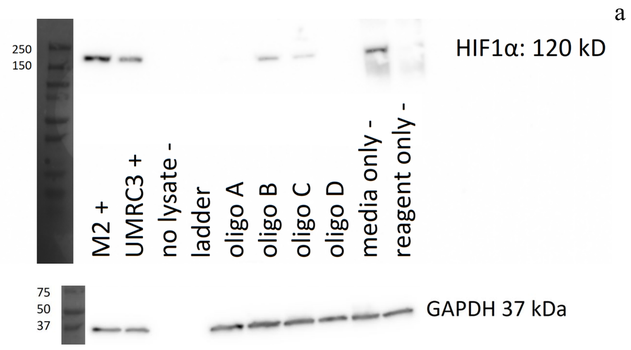


Figure 5: Western blot illustrating the reduction of HIF1α expression with siRNAs against HIF1α. We tested 4 different siRNAs (oligo A, oligo B, oligo C, and oligo D). HIF1α is not expressed when oligo A and oligo D are added to the cells. Methods: 48 hours incubation with siRNA prior to harvesting cells and lysing. Blots were visualized with 1:1000 dilution of primary antibody against HIF1α with overnight incubation at 4°C. 1:2000 dilution of the secondary antibody at RT for 1 hour.

Experiment 3 (Soft agar colony assay):

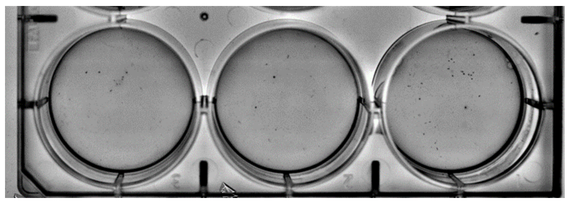


Figure 6: Tested colony formation in the presence of drug using soft agar plates. Tested the ability of RMC cell line UOK360 to generate colonies in soft agar. Unfortunately, we were not able to visualize a large number of colonies even on control plates (media and DMSO).

Method. We made three 6 well plates (**plate 1:** DMSO and media, media only. **Plate 2:** cells treated with PT2399. **Plate 3:** cells treated with PX-478). Mixed 8 ml of 2x media with 2 ml of FBS (20%), and 5 ml of 3% agarose solution to get 1% agarose solution. Added 2 ml per well. Then added 6.5 ml of normal DMEM media with glutamine and 400,000 cells and put 1 ml in each well. Lastly Added 1.5 ml of media with drug on top in each well and changed twice a week.
12 ml of media (50 uM PX-478, add 3 ul of 200 mM solution)
12 ml of media (2 uM PT2399, add 2.4 ul of 10 mM solution)
6 ml of media (add 1.5 ul of DMSO). After 18 days, removed media and added 0.1% of iodonitrotetrazolium chloride (0.1%). Waited 48 hours took image and used Azure 300 brightfield on yellow background plate to take the image.

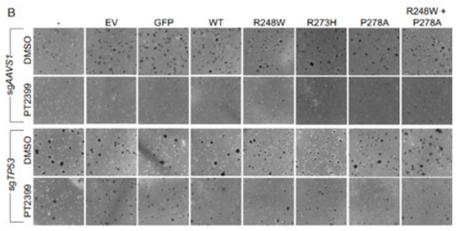


Figure 7: Soft agar assay in ccRCC cell 786-O using HIF2α inhibitor (PT2399) [4]. We were expecting a similar result from our soft agar experiment.

Conclusion

- We were able to generate transfected cell lines that express luciferase and GFP when HIF1α is expressed.
- We were able to illustrate that luciferase activity was dependent on HIF1α expression using siRNA D knockdown.
- We are in the process of determining if HIF1α inhibition reduces cell proliferation in soft agar colony assay. We are still optimizing the number of cells to use in the assay.

Future Direction

We are testing other methods to assess if HIF1α inhibition affects cell growth. Such as:

- We are in process of viral transfection of sgRNA against HIF1α . This would create ccRCC and RMC cell lines that have reduced HIF1α expression.
- Determining if we can observe reduced cell proliferation in soft agar assays with luciferase/ bioluminescence readout.
- Not discussed in the poster is the mRNA display work being done by Nasir Uddin to generate a cyclic peptide that will inhibit the dimerization of HIF1α with HIF1β.

Limitations

We are still in the process of proving our hypothesis. We are currently tested 4 cell lines and all work as been done in cell culture.

References

[1] Hoefflin R, Harlander S, Schäfer S, et. al. HIF-1α and HIF-2α differently regulate tumour development and inflammation of clear cell renal cell carcinoma in mice. Nat Commun. 2020 Aug 17;11(1):4111.
[2] Msaouel P, Malouf GG, Su X, et al. Comprehensive Molecular Characterization Identifies Distinct Genomic and Immune Hallmarks of Renal Medullary Carcinoma. Cancer Cell. 2020;37(5):720-734.e13.
[3] Maxwell PH, Wiesener MS, Chang GW, et. al. The tumour suppressor protein VHL targets hypoxia-inducible factors for oxygen-dependent proteolysis. Nature. 1999 May 20;399(6733):271-5.
[4] Stransky LA, Vigeant SM, Huang B, West D, Denize T, Walton E, Signoretti S, Kaelin WG Jr. Sensitivity of *VHL* mutant kidney cancers to HIF2 inhibitors does not require an intact p53 pathway. Proc Natl Acad Sci U S A. 2022 Apr 5;119(14):e2120403119.

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