State-of-the-Art of Therapeutic Approaches for VHL Disease

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Coming Up With A Cure: Many Layers of Knowledge are Needed!

- Identification of the VHL Gene
- Description of VHL Protein Function
- Identifying and Characterizing Additional Genes Disrupted in VHL Disease
- Development of Relevant Model Systems

VHL Gene and Protein

- On chromosome 3p25
- 213 amino acid protein
- Binds to Elongin C/B
- Forms “VBC complex”

HIF Upregulation Alters Regulation of Reductive Carboxylation

- Modified from Stebbins and Pavletich, Science, Vol 284, 16 April 1999
- Courtesy of Othon Iliopoulos
VHL- A Regulatory Hub

- Extracellular Matrix Control
- p53 Regulation?
- Primary Cilium Function
- Angiogenesis

The VHL Life Cycle

- DNA
- HSP70 Chaperones
- HSP70 TRiC
- p53

- Protein Production
- Mature VHL Complex
- HSP70

* Roe and Youn Mol Cell 2006

Mutated VHL May Fail to Bind TRiC

- DNA
- HSP70 Chaperones
- HSP70 TRiC
- HSP90

- Protein Production
- Proteasomal Degradation

Mutated VHL May Fail to Release from TRiC if VBC Complex not Formed

- DNA
- HSP70 Chaperones
- HSP70 TRiC

- Inability to Bind Elongin C
- Proteasomal Degradation
Mutated VHL May Fail to Bind to Other Substrates

Over One Third of Mutations are Missense (Hereditary and Sporadic)

<table>
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<tr>
<th>VHL type</th>
<th>Number of families</th>
<th>Missense</th>
<th>Nonsense</th>
<th>Micro deletion (1-9 bp)</th>
<th>Insertion (1-8 bp)</th>
<th>Deletion (4-380 kb)</th>
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<td>15</td>
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*Each type of mutation was tested by Fisher’s exact test (2-tailed) for association with VHL types 1 or 2. Microdeletions/insertions (P = 0.003), nonsense (P = 0.004), and deletion mutations (P = 0.012) were predictor of VHL type 1. Missense mutations were significantly more common in VHL type 2 (P = <0.001). Fifty-three VHL families without information sufficient to classify into VHL1 or VHL2 were excluded from this analysis.

What this means is you have a full sized protein, that can possibly be fixed


Point Mutations Destabilize VHL But May Retain Functionality

And It’s Not Only VHL That is Mutated!

- Renal Cell Carcinoma:  
  - SETD2, PBRM1, BAP1

- Hemangioblastomas:  
  - HNF1B

Knowing how these genes interact will be critical to fully understanding VHL disease, develop relevant model systems and discover treatments

C-terminal Venus Tagged Proteins
Currently Evolving Treatment Paradigms

- Targeting HIF-Dependent Downstream Consequences of VHL Loss
  - HIF, HAF, VEGF Modulating Agents and Metabolism Modifiers
- Restabilizing/Refunctionalizing Mutated VHL
  - Modulators of VHL Proteostasis
- Developing Synthetic Lethal Approaches that Target the Tumor Cell
  - Modulators of Autophagy, or of co-Mutated Genes
- Targeting the Immune Microenvironment
  - Immune Checkpoint Inhibitors

Targeting Downstream Consequences of HIF Dysregulation

- VEGF = vascular endothelial growth factor receptor; EGFR = endothelial growth factor receptor; PDGFR = platelet derived growth factor receptor.
Lesion site | Number of Lesions | PR (%) | SD (%) | PD (%)
--- | --- | --- | --- | ---
Hemangioblastoma* | 21 | 0 | 19(91) | 2(9)
Renal cell carcinoma* | 18 | 6 (33) | 10(67) | 2(10)
Renal cyst | 9 | 0 | 9 (100) | 0
Retinal angiommas | 7 | 0 | 7 (100) | 0
Pancreatic NET | 5 | 0 | 5 (100) | 0
Pancreatic cyst | 3 | 0 | 3 (100) | 0

Nine out of 15 patients completed study- most came off study due to poor tolerability

Jonasch and Matin, Annals of Oncology 2011

*P=0.014

Vandetanib in VHL: Efficacy

37 patient study: One RECIST PR, some shrinkage in a number of patients.

Ram Srinivasan

Pazopanib in VHL Disease

Kim Jonasch and McCutcheon Targ Oncol 2012
Ongoing Study

- NCT01436227, a Phase II Study of Pazopanib in VHL Patients.
- Must have at least one measurable lesion.
- So far 23 out of 40 patients enrolled.

Targeting HIF

Geldanamycin, an HSP90 Inhibitor, Destabilizes HIFα
NIH Geldanamycin Study

- Geldanamycin administered to 8 patients with VHL disease and measurable RCC lesions.
- Stable disease seen in all patients.
- Trial closed due to slow accrual.

HAF Differentially Regulates HIF1a and HIF2a, and is Potentially Targetable

Koh and Powis TIBS 2012

Othon Iliopoulos: VHL Models and Novel Therapeutics

Massachusetts General Hospital, Boston MA

- Zebrafish are tiny fish that can be genetically modified
- VHL mutation in zebrafish can represent aspects of human biology
- Dr. Iliopoulos will use zebrafish to discover new drugs that may rescue consequences of VHL mutation.

HIF2a inhibitors decreases hypoxia-induced erythropoiesis and angiogenesis

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Ana Metelo et al
ILOPOULOS LAB, MGH Cancer Center
Harvard Medical School

Courtesy of Othon Iliopoulos
Metabolomics

Loss of PGC-1α Induces the Clear Cell Phenotype in Renal Proximal tubule Cells

- Genetic suppression of PGC-1α results in lipid and glycogen accumulation, consistent with clear cell phenotype of renal cell carcinoma.
- Mechanism of lipid accumulation unclear
  - Increased lipid synthesis or uptake driving lipid accumulation?
  - Decreased fatty acid oxidation?

Summary

- HIF suppresses PGC-1α via activation of Dec1
- Loss of PGC-1α results in decreased OXPHOS activity and promotes clear cell phenotype
- Induction of PGC-1α induces oxidative stress, reverts metabolic phenotypes, and suppresses tumor growth
Regulation of reductive carboxylation by HIF

Loss of VHL renders RCC cells/tumors sensitive to glutaminase inhibitors in vivo

Phase I Study

• NCT02071862 “A Phase 1 Study of the Safety, Pharmacokinetics, and Pharmacodynamics of Escalating Oral Doses of the Glutaminase Inhibitor CB-839 in Patients with Advanced and/or Treatment-Refractory Solid Tumors”.

• Trial allows recruitment of patients with metastatic RCC.

Currently Evolving Treatment Paradigms

Targeting HIF–Dependent Downstream Consequences of VHL Loss

Restabilizing/Refungionalizing Mutated VHL

Developing Synthetic Lethal Approaches that Target the Tumor Cell

Targeting the Immune Microenvironment

HIF, HAF, VEGF Modulating Agents and Metabolism Modifiers

Modulators of VHL Proteostasis

Modulators of Autophagy, or of co-Mutated Genes

Immune Checkpoint Inhibitors
Genetic Titration of Mutant VHL Levels Alters Growth in Xenograft Models

Pharmacological Modulation of VHL Levels Can Augment Function

Ongoing Trial

- NCT01775930: Carfilzomib in patients with refractory RCC.

- Trial on hold after 12 patients, with tissue specific analysis to test genotype-phenotype link.
Preclinical Study: Daniel Segal-Fixing Broken VHL
Tel Aviv University, Tel Aviv

• Dr. Segal will study a candidate substance, D-Arginine, which appears to stabilize mutant VHL protein.

Currently Evolving Treatment Paradigms

1. Targeting HIF- Dependent Consequences of VHL Loss
   HIF, HAF, VEGF Modulating Agents and Metabolism Modifiers

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   Modulators of VHL Proteostasis

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   Modulators of Autophagy, or of co-Mutated Genes

4. Targeting the Immune Microenvironment
   Immune Checkpoint Inhibitors

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A Molecule Targeting VHL-Deficient Renal Cell Carcinoma that Induces Autophagy

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3Correspondence: jgiaccia@stanford.edu
DOI 10.1016/j.cell.2008.06.064

Cell Survival

Treatment with STF 62247

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   Immune Checkpoint Inhibitors
Immune Checkpoints

Tumor Cell or Antigen Presenting Cell

- Signal 1
- Signal 2

T cell

- CD28
- CTLA-4

HLA

- B7.1/2
- HLA
- Class II MHC
- B7-H1 (PD-L1)

T Cell Receptor

- LAG-3
- PD-1

But what if all you see below is immunogenic?

We need to proceed, but with caution

Past Present and Future

- Identification of the VHL Gene
- Targeting VHL Gene Deficiency
- Identifying and Characterizing Additional Genes Disrupted in VHL Disease

3p Shows Multiple Hits in RCC

- Q arm
- P arm

- PBRM1 3p21.1
- SETD2 3p21.3
- BAP1 3p21.1
- VHL 3p25

4: Pena Llopis and Brugarolas, Nature Genetics, 2012
Focal loss of chromosome 17q12,32 in hemangioblastomas

L2 Hydroxyglutarate Dehydrogenase (on 14q) Suppresses In Vitro Tumor Phenotypes

Summary

• Current therapies that can “round off the edges” of biology arising from VHL deficiency, and may in some cases be very helpful.

• Only by characterizing the additional genomic drivers of RCC, hemangioblastoma, pheo and NETs will we be able to devise appropriate model systems that can help develop curative therapy.

11/7/2014