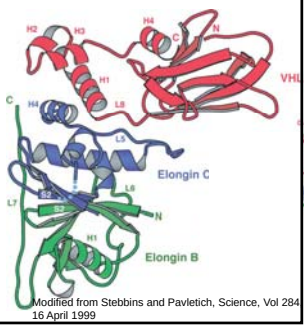
CENTER FOR PROTEIN FOLDING MACHINERY

Modulating VHL Proteostasis By Manipulating TRiC

Eric Jonasch, M.D.
UT MD Anderson Cancer Center

VHL Gene and Gene Product

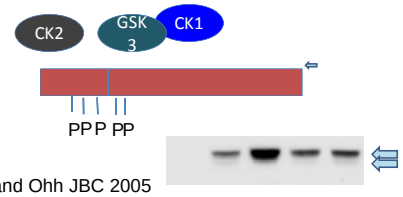
- Located on 3p25
- 213 amino acid protein
- 30 kDa Size
- Binds to Elongin C/B
- Forms "VBC complex"



Modified from Stebbins and Pavletich, Science, Vol 284 16 April 1999

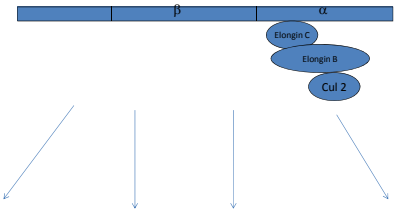
VHL Is Posttranslationally Modified

- S33, S38, and S43 are phosphorylated in the N-terminal acidic domain by casein kinase (CK) 2 and reduce fibronectin association.[1]
- S68 and S72 in pVHL30 are phosphorylated by GSK3 and CK1 respectively, which inhibits microtubule stabilization and HIF2a binding.[2]



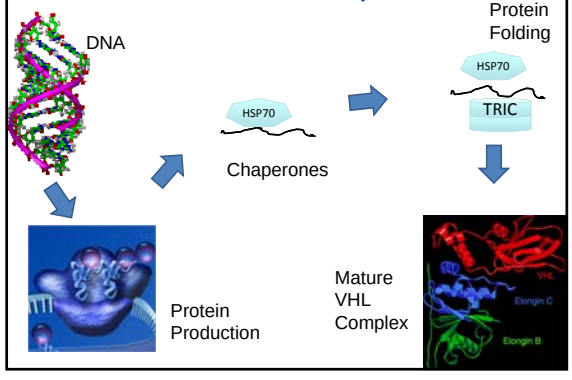
1. Lolkema and Ohh JBC 2005
2. Thoma and Krek MCB 2006

VHL: A Regulatory Hub



Ohh et al, Mol Cell, Vol 1, 959-968, 1998
Kurban et al, Cancer Res 2006; 66: (3).
Gemmill et al, Oncogene 12;24(21):3503-11.
Kim et al Clin Cancer Res 2009.
Thoma et al Nature Cell Biology Aug 2009
Kuehn et al Ca Res May 15, 2007
Pugh et al Nature Medicine 2003
Kerbel NEJM May 2008

The VHL Life Cycle



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

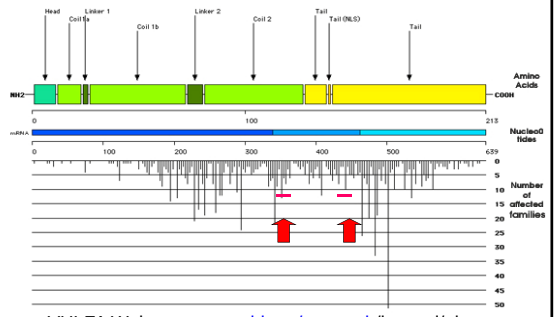
TABLE 2. Types of Mutation Found in von Hippel-Lindau Disease Without (Type 1) and With (Type 2) Pheochromocytoma[a]

VHL type	Number of families	Number of mutations found					
		Missense	Nonsense	Micro deletion (1–9 bp)	Insertion (1–8 bp)	Deletion (4–380/kb)	Splice site
1	336	72	29	31	13	53	10
2	78	61	1	0	2	2	0
Total	414	133	30	31	15	55	10

[a]Each type of mutation was tested by Fishers exact test (2-tailed) for association with VHL types 1 or 2. Microdeletions/insertions ($P = 0.019$), nonsense ($P = 0.044$), and deletion mutations (P 0.012) were predictive 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, VHL2 were excluded from this analyses.

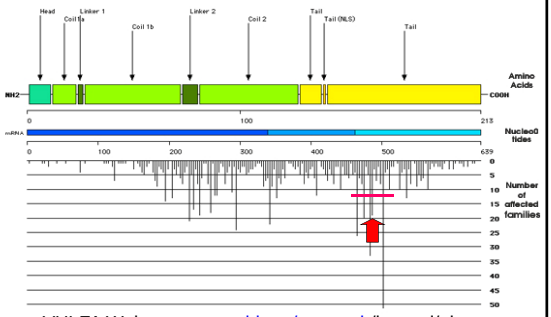
Zbar et al: Human Mut 1996;8(4):348-57

Disease Causing Mutations Occur in TRiC Binding Sites



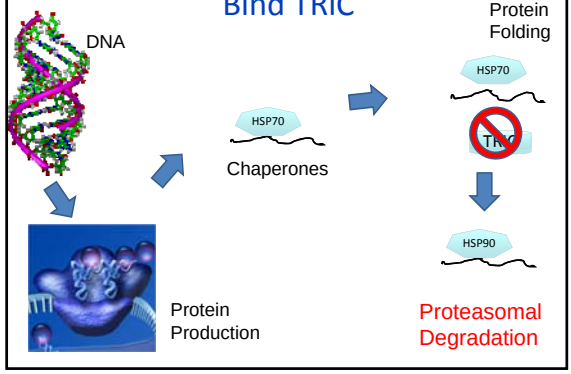
VHLFA Webpage www.vhl.org/research/beroud/php

And In the Elongin C Binding Site

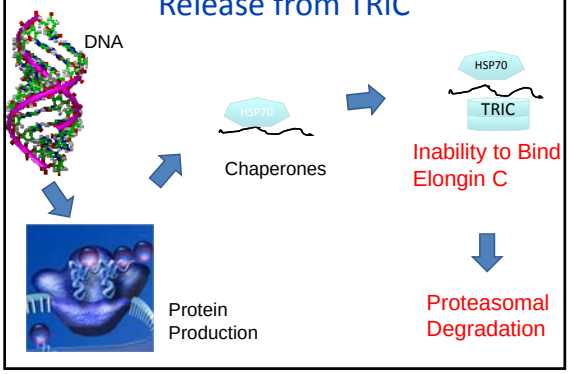


VHLFA Webpage www.vhl.org/research/beroud/php

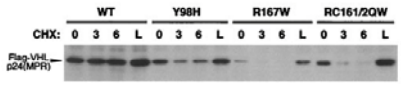
Mutated VHL May Fail to Bind TRiC



Mutated VHL May Fail to Release from TRiC

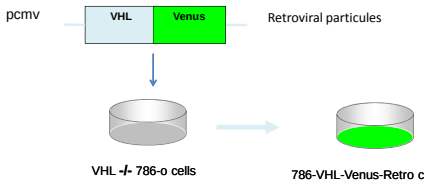


Point Mutations Destabilize pVHL

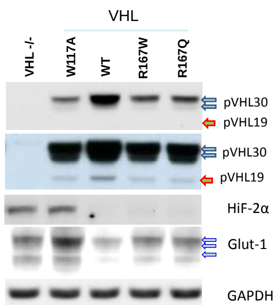


Flag tagged VHL transfected into 786-0 incubated with cycloheximide or lactacystin

Schoenfeld and Burk PNAS 2000



Comparison of mutated and the wild-type VHL protein level in 786-VHL-Venus-Retro cell lines



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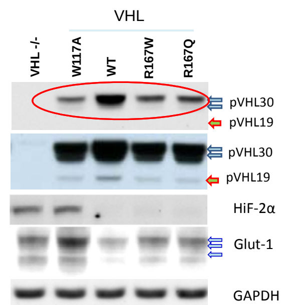

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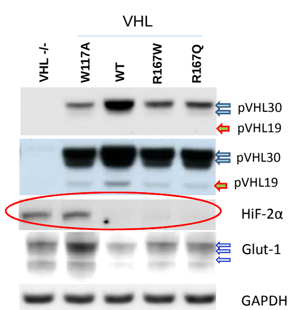
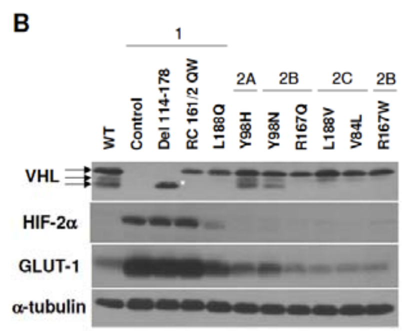
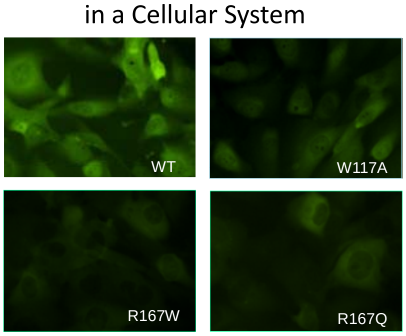




Bangiyeva *et al* BMC Cancer 2009

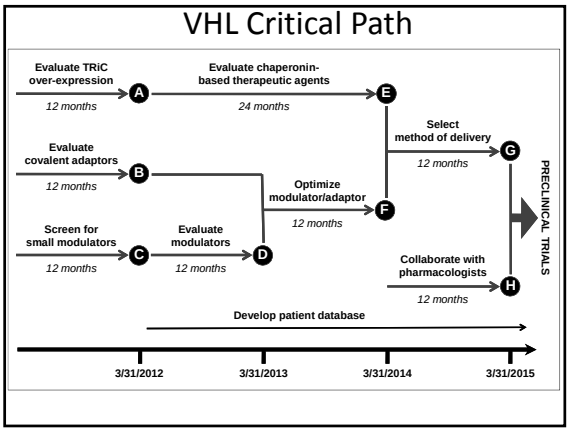
Differential Stability of VHL Can Be Measured in a Cellular System

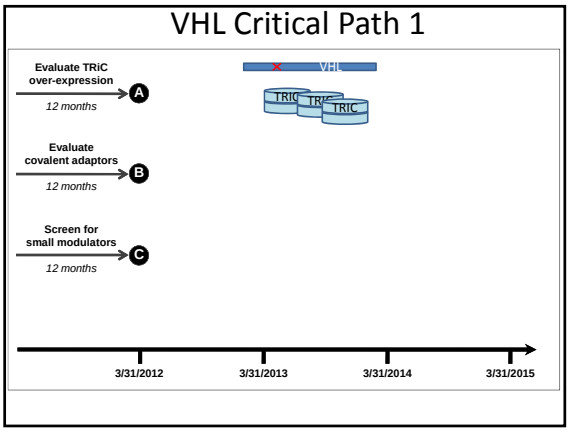


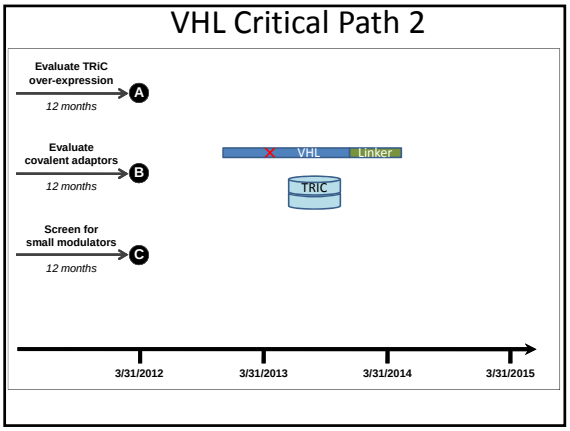
How can we normalize function of point-mutated VHL?

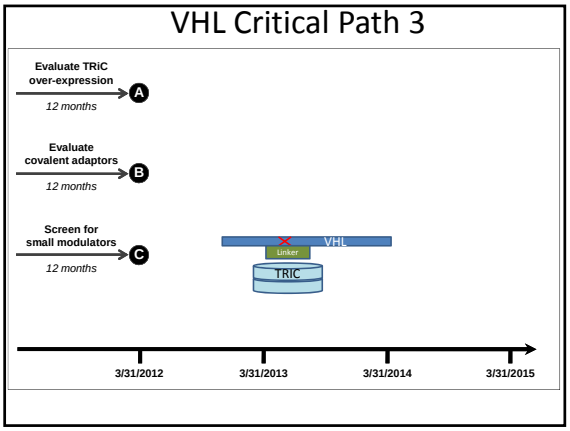
1. Raise the level of functional but unstable VHL protein

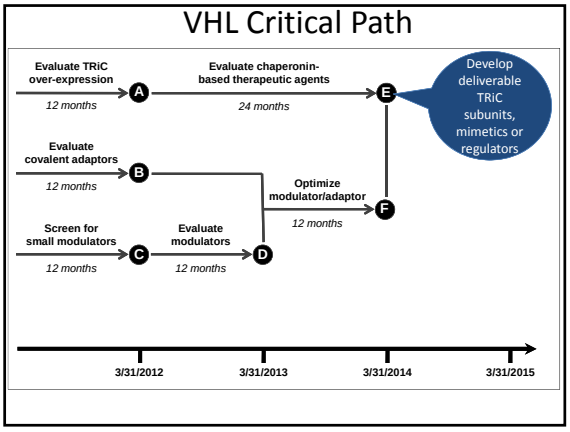
2. Refunctionalize point mutated, malfolded VHL by facilitating refolding and appropriate ligand binding

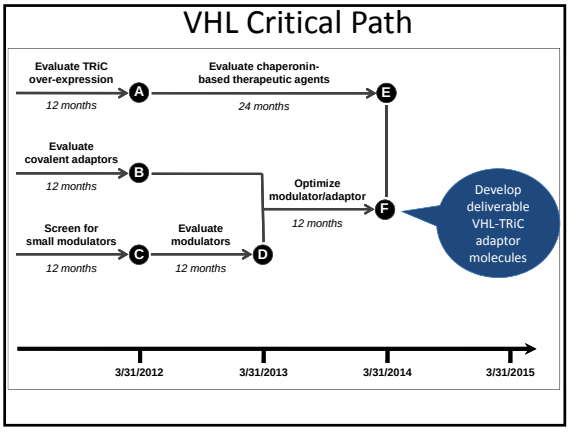

VHL Critical Path
Evaluate TRiC over-expression
A
12 months
Evaluate chaperonin-based therapeutic agents
E
24 months
Evaluate covalent adaptors
B
12 months
Select method of delivery
G
12 months
Optimize modulator/adaptor
F
12 months
Screen for small modulators
C
12 months
Evaluate modulators
D
12 months
Collaborate with pharmacologists
H
12 months
PRECLINICAL TRIALS
Develop patient database
3/31/2012
3/31/2013
3/31/2014
3/31/2015


VHL Critical Path 1
Evaluate TRiC over-expression
A
12 months
Evaluate covalent adaptors
B
12 months
Screen for small modulators
C
12 months
VHL
TRIC
3/31/2012
3/31/2013
3/31/2014
3/31/2015

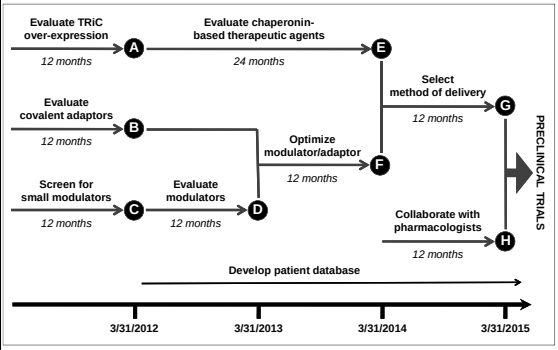

VHL Critical Path 2
Evaluate TRiC over-expression
A
12 months
Evaluate covalent adaptors
B
12 months
Screen for small modulators
C
12 months
VHL
Linker
TRIC
3/31/2012
3/31/2013
3/31/2014
3/31/2015


VHL Critical Path 3
Evaluate TRiC over-expression
A
12 months
Evaluate covalent adaptors
B
12 months
Screen for small modulators
C
12 months
VHL
Linker
TRIC
3/31/2012
3/31/2013
3/31/2014
3/31/2015

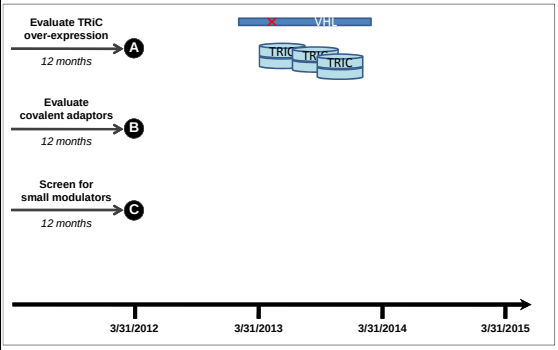

VHL Critical Path
Evaluate TRiC over-expression
A
12 months
Evaluate chaperonin-based therapeutic agents
E
24 months
Develop deliverable TRiC subunits, mimetics or regulators
Evaluate covalent adaptors
B
12 months
Optimize modulator/adaptor
F
12 months
Screen for small modulators
C
12 months
Evaluate modulators
D
12 months
3/31/2012
3/31/2013
3/31/2014
3/31/2015


VHL Critical Path
Evaluate TRiC over-expression
A
12 months
Evaluate chaperonin-based therapeutic agents
E
24 months
Evaluate covalent adaptors
B
12 months
Optimize modulator/adaptor
F
12 months
Develop deliverable VHL-TRiC adaptor molecules
Screen for small modulators
C
12 months
Evaluate modulators
D
12 months
3/31/2012
3/31/2013
3/31/2014
3/31/2015

VHL Critical Path

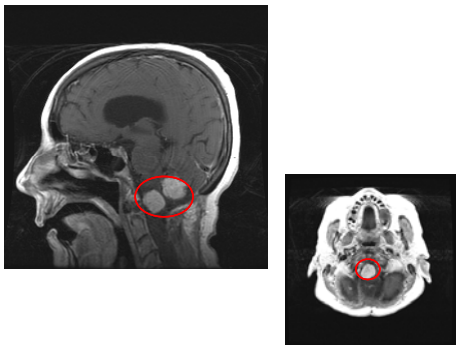


VHL Critical Path 1

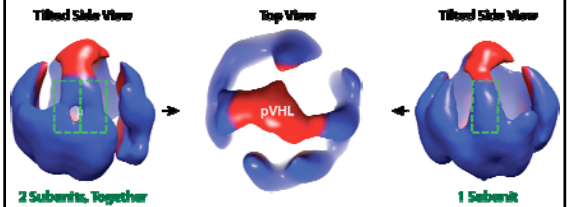


Rationale

- By understanding the effect of TRiC up- and down-modulation on the stability of mutated pVHL, we will be able to assess the therapeutic value of introducing exogenous or TRiC subunits into VHL lesions.

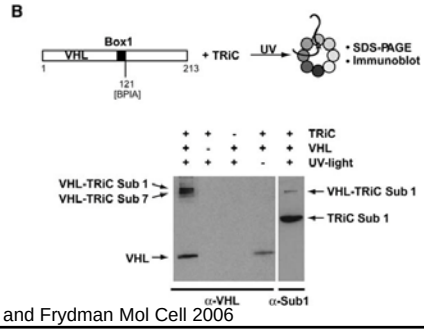


pVHL Binds to 2 Apical Regions of TRiC



Benjamin Bammes
bbammes@gmail.com

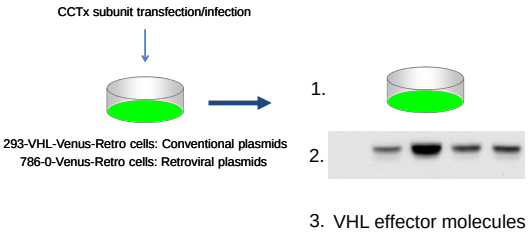
CCT1 and CCT7 are probable binding sites for pVHL



Spiess and Frydman Mol Cell 2006

CCTx Knock-In

CCTx Knock-In: Strategy



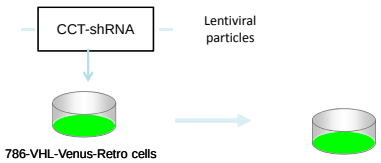
3. VHL effector molecules

CCTx Knockdown

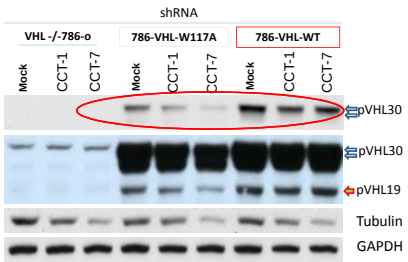
CCTx Knockdown: Rationale

- Downregulation of CCTx subunits in RCC lines will inform regarding relative dependence of VHL isoforms on TRiC stabilization.

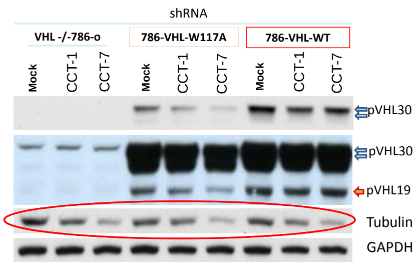
Strategy



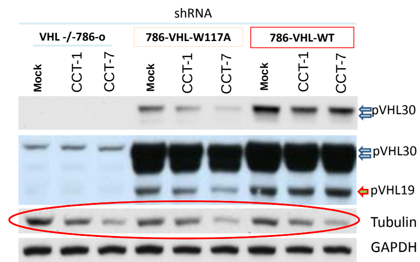
CCT1 or CCT7 Knock-Down



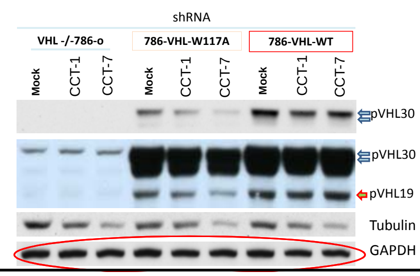
CCT1 or CCT7 Knock-Down



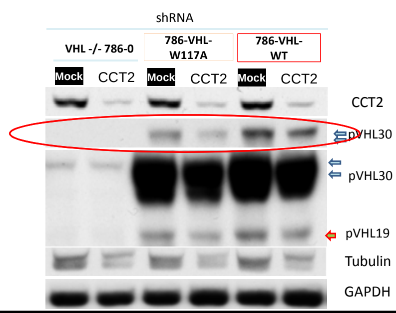
CCT1 or CCT7 Knock-Down



CCT1 or CCT7 Knock-Down



CCT2 Knock-Down



Next Steps

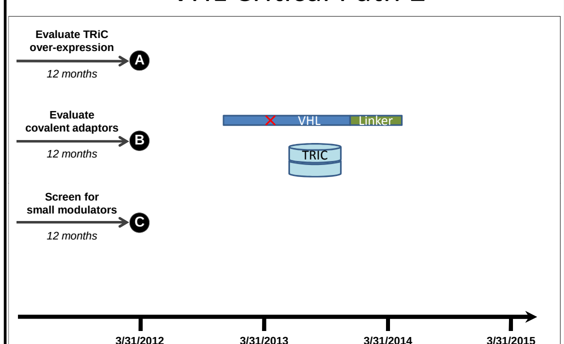
Further explore effect of CCTx knockdown on TRiC client and nonclient proteins in 786-0 and other cell lines:

- Evaluate effect of CCTx knockdown on global TRiC levels
- Evaluate effect of multiple CCTx knockdown
- Generate box 1 plus 2 mutant VHL constructs

↕

CCTx knock-in studies

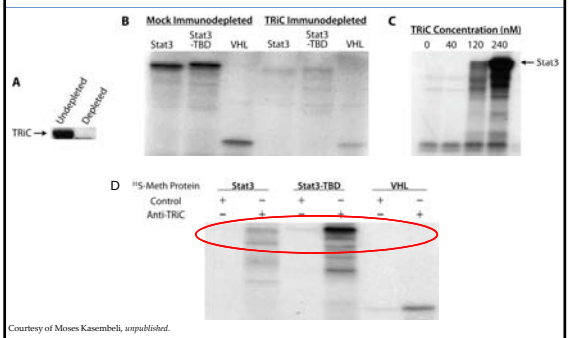
VHL Critical Path 2



Rationale

- Covalent linkage of TriC binding domains (TBD) will provide information on ability of TBD to influence VHL isoform association with TRiC.

Stat3-TBD Interacts with TRiC *in vitro*

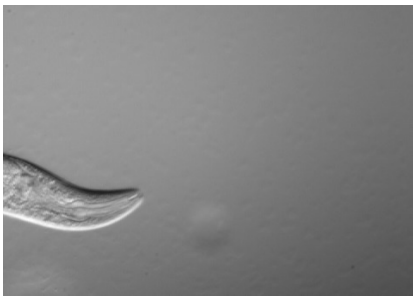


Courtesy of Moses Kasembeli, *unpublished*.

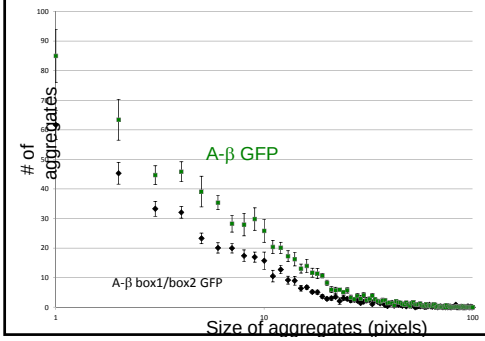
Benjamin Bammes
bbammes@gmail.com

C. elegans as an experimental system:

Kaveh Ashrafi

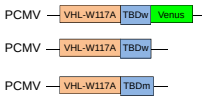


Engineering the box-1/box-2 domain into A-β GFP causes reduction of sizes of aggregates



Strategy

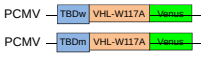
TBD fused downstream of VHL mutant: Completed, pending analysis



TBDw: TBD domain of wild type VHL

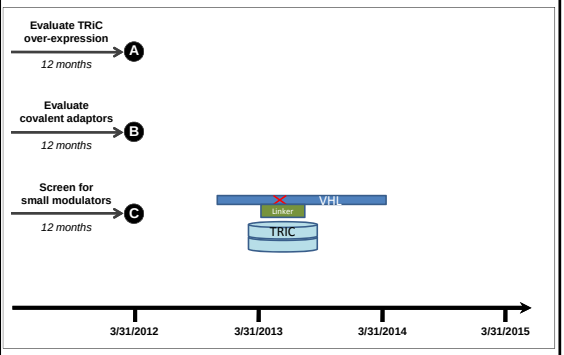
TBDm: TBD domain of VHL-W117A.

Fusions with TBD fused at the N-terminal of VHL:



We will compare the mRNA and protein levels of the two fusions in 786-0 cells.

VHL Critical Path 3



Rationale

- Noncovalent modulators of TRiC-VHL interaction will potentially aid folding of mutant pVHL, and restore functionality.
- Once promising pharmacophores are discovered, further refinement will lead to development of therapeutics for VHL and RCC.

Strategy

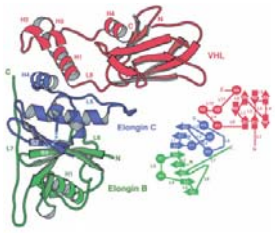
1. Use in silico techniques to generate bivalent molecules that will bind to pVHL and to TRiC.
2. Perform unbiased search for compounds that restore VHL-TRiC interaction/restore functionality of pVHL
3. Develop a "molecular toolbox" to assess impact of candidates on VHL proteostasis, in particular the effect on VHL-TRiC interaction

In Silico Design: Vijay Pande and Veena Thomas

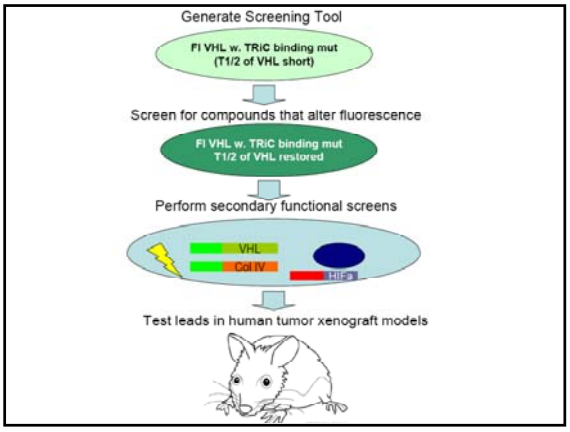
Stapled peptide approach:

-H4 helix in Elongin C could be a suitable motif for a stapled peptide to recruit VHL.

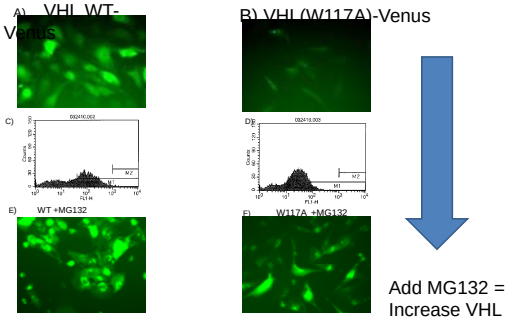
-Design of TRiC binding end- being performed in parallel for Htt project.



Veena Thomas and Vijay Pande



Proteasome Inhibition Raises Mutant VHL Levels



Phase II Trial of Bortezomib for Patients With Advanced Renal Cell Carcinoma

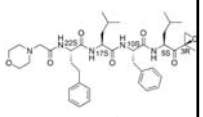
G. Varuni Kondagunta, Beverly Drucker, Lawrence Schwartz, Jennifer Bacik, Stephanie Marion, Paul Russo, Madhu Mazumdar, and Robert J. Motzer

Results
Of the 37 assessable patients, the best response was a partial response in four patients (11%; 95% CI, 3% to 25%) and stable disease in 14 patients (38%; 95% CI, 23% to 55%). The four patients with partial response experienced response durations of 8, 8+, 15+, and 20+ months. Grade 2 or 3 sensory neuropathy was present in 10 patients (53%) overall. One patient in the 1.5 mg/m^2 group had grade 3 sensory neuropathy; no grade 3 sensory neuropathy was seen in the 1.3 mg/m^2 group.

New Mechanisms of Action for Therapies in VHL and RCC: An Unmet Need

- Antiangiogenic agents do not directly target tumor cell, and resistance eventually develops
- mTOR inhibitors are not highly efficacious

Carfilzomib (Onyx)



- Tetrapeptide ketoepoxide-based inhibitor specific for chymotrypsin-like active site of 20S proteasome.
- Better tolerated than bortezomib- only minimal peripheral neuropathy.
- Phase I study contained RCC patients- partial responses and disease stabilization seen in nearly half the patients.

Clinical and Preclinical Studies to be launched at MD Anderson

- Phase II study in clear cell (VHL deficient) RCC
  - Assess mutational state of patient tumors
  - test hypothesis activity of carfilzomib is highest in tumors with point-mutated pVHL
- Preclinical studies
  - Human xenograft tumors with various VHL mutations in nu/nu mice

Summary

- Ongoing research will further elucidate role of TRiC and proteostatic pathway regulation in modulating disease state in VHL deficiency.
- Phase 2 clinical trial to be launched within next six months on basis of data generated from Nanomedicine program.

Lab Team

Zhiyong Ding, Shanshan Bai, Meng Gao and Peter German

Acknowledgements

NIH Roadmap Team
- Richard Fisher
- Jeffrey Schloss
- Jennifer Mehren

Nanomedicine Group
- Wah Chiu
- Judith Frydman
- William Mobley
- Vijay Pande
- Tanja Kortemme

Lab
- Zhiyong Ding
- Peter German
- Shanshan Bai
- Meng Guo

Clinic
- Surena Matin
- Marcia Holloway
- Maisha Amusa