Determining whether DGKι is a target for VHL degradation

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Abstract

HIF1α is a transcription factor that drives hypoxia-inducible genes, allowing cells to survive in hypoxic conditions. In normoxic conditions, the von Hippel-Lindau (VHL) protein targets HIF1α for proteosomal degradation. In von Hippel-Lindau disease, mutations that affect VHL protein render it unable to bind to HIF1α, making the body susceptible to tumours by allowing cells to survive in hypoxic conditions. The enzyme diacylglycerol kinase iota (DGKι) has recently been identified as another VHL target. We hypothesize VHL regulates DGKι levels by targeting it for degradation and that this contributes to the pathogenic effects of VHL-loss in cancer. To this end, DGKι protein levels will be measured in wild type and VHL-deficient cells. To identify the VHL binding site, a number of known DGKi protein domains will be tested in protein interaction assays for their ability to interact with endogenous and recombinant VHL. Results from these experiments will help elucidate how VHL interacts with and regulates DGKι, and will lead to possible therapies.

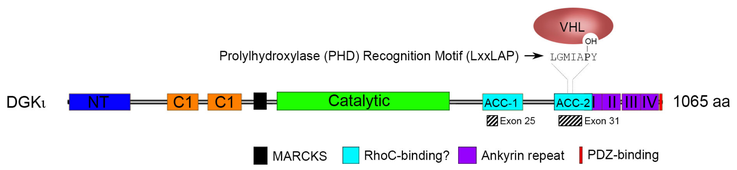

Figure 1. Regions of the DGKι sequence and functionality are indicated. The two ACC regions are pictured in blue. ACC-2 interaction with VHL is also shown.

Introduction

DGKι contains two LxxLAP motifs at residues Pro147 and Pro903 that are potential targets for PHD-catalyzed prolyl hydroxylation. Mutating these amino acid sequences prevented VHL binding, suggesting that the C-terminal LxxLAP motif is required for interaction with VHL[1]. Two antiparallel-coiled coil (ACC finger) domains are present in these regions of DGKι, and are hypothesized to be what causes this interaction.

An ACC finger domain is composed of an N-terminal loop containing a short helix (α1) and two long alpha helices (α2 and α3) forming an anti-parallel coiled coil fold. The two long helices have a leucine repeat region, which has been identified as a Rho binding region, and create a hydrophobic core that stabilizes the domain structure [1]. ACC domains are distinct from other small G proteins and exhibit characteristics of diverse effector recognition as widespread Rho effector proteins [2]. Previous studies show that the region of the ACC-1 domain of DGKι is involved in RhoC interaction [3], and that ACC coils are what allow PKA effectors to regulate catalytic activity [2]. In the case of VHL interaction, the hypothesis is that the more C-terminal domain (ACC-2) will be sufficient in the binding [1].

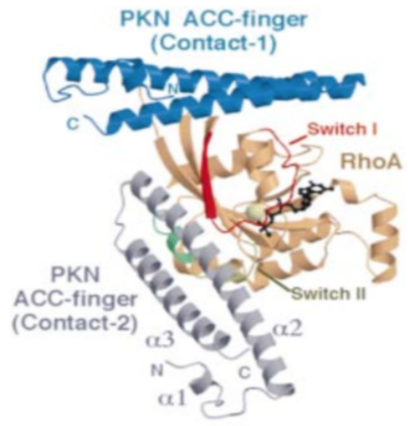

Figure 2. An example of a protein with 2 ACC-finger domains, as seen on a RhoA/PKN complex [2]. DGKι's crystallized structure has not yet been determined, but is similar in composition and domains to PKN.

Methodology

- Bacterial expression vectors of GST fused to ACC-1, ACC-2, ACC-1 + ACC-2 and a control sample of GST were prepared. When purified, a bead assay is performed (Glutathione Sepharose 4B).
- SDS-PAGE conducted (Figure 3) to analyze the size and amount of fusion proteins extracted by the beads in each sample.
- With the desired proteins present, the next experiment would be to insert the beads bound to fusion proteins into a solution with wild type and DGKι-null cells to interact with endogenous VHL.
- A Western blot would then be performed to analyze the amount of endogenous VHL that interacted with each sample.

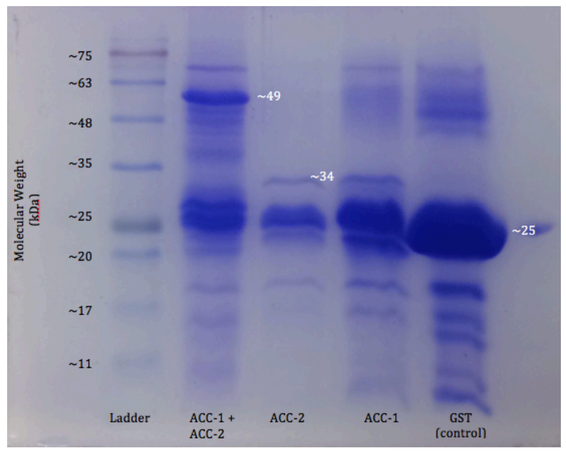

Figure 3. SDS-PAGE to analyze fusion proteins. All of the proteins seem to have expressed, as GST has a molecular weight around 28kDa, ACC-1 and ACC-2 around 34kDa, and ACC-1 + ACC-2 have a combined weight of 49kDa.

Conclusions

As DGKι's VHL binding site falls within ACC-2 [1], we hypothesize that both samples containing ACC-2 should bind to VHL and the ACC-1 and GST control samples should not bind VHL.

Determining the nature of the ACC-VHL interaction will provide further insight into how VHL interacts with and regulates DGKι. As DGKι regulates RhoC, a protein that drives cancer metastasis, VHL may also play a part in this interaction. It has been hypothesized that VHL may target RhoC for degradation, which would prevent metastasis of cancerous cells, as is demonstrated by RhoC-null cells [3]. Inhibition of the DGKι-RhoC interaction is also expected to decrease or even prevent metastasis. Further experiments would be needed to investigate these conclusions, which could lead to possible therapies.

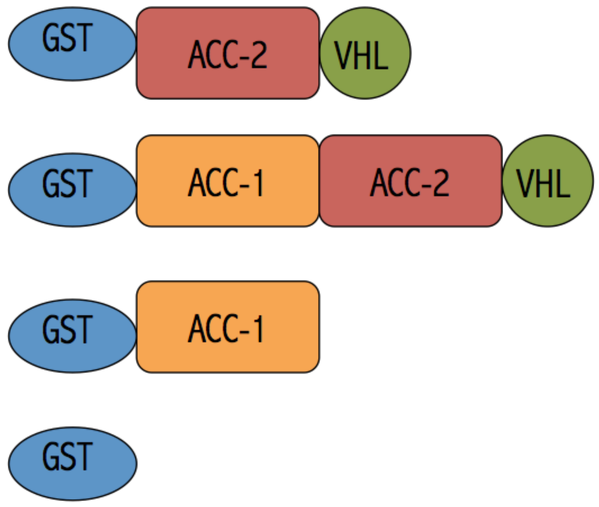

Figure 4. Expected results. Only sequences with the ACC-2 binding domain are expected to show interaction with the VHL protein.

Bibliography

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2. Maesaki et al. 1999, The structural basis of Rho effector recognition revealed by the crystal structure of human RhoA complexed with the effector domain of PKN/PRK1, Cell Press, 1999, Vol. 4, 793-803.
3. Foley et al. Diacylclycerol kinase ι regulates actin organization via Rac1 and RhoC signaling pathways (unpublished data)

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