Characterization of the pVHL interactome in human testis using high-throughput library screening

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Introduction

The von Hippel-Lindau (pVHL) tumor suppressor is a protein involved in the physiologic cell adaptation to low oxygen concentrations. Inherited mutations promoting a functional impairment of pVHL are causative of a familiar increased risk to develop cancer. As E3 substrate recognition particle, pVHL marks the hypoxia inducible factor 1α (HIF-1α) for degradation in normoxic conditions, thus acting as a key regulator of both acute and chronic cell adaptation to hypoxia. In male mice model, VHL gene conditional knockout yelds significant abnormalities in testis development paired with defects in spermatogenesis and infertility, indicating that pVHL may exerts testis-specific roles. Here we aimed to explore whether pVHL could have a similar role in human.

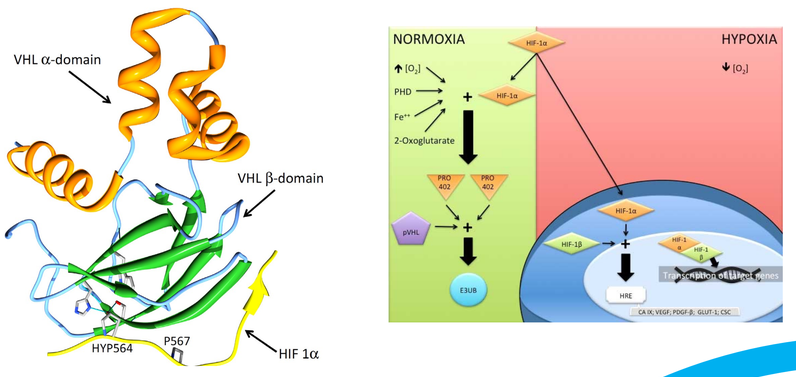

Fig.1 *Left:* Cartoon representation of the pVHL structure bound to the HIF-1A transcription factor. *Right*: schematic mechanism regulating the normoxia/hypoxia transition

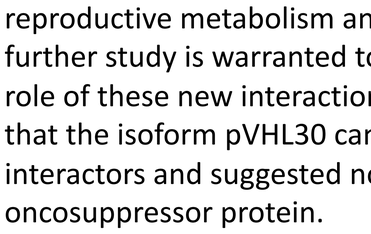

Figure 2. Western blot to confirm the expression of pVHL30 as bait in Y2H. and visualized in Western Blot. The bait protein (i.e. pVHL30) is detected by anti-Myc (line 4). C+ and Ø correspond to positive (p53-Gal4BD) and negative control (Gal4-BD) respectively.

Objective

To identify tissue-specific pVHL interactors able to explain the variability of VHL disease manifestations.

Methodologies

We combined the yeast two-hybrid (Y2H) assay, a high throughput screening approach, with in-depth bioinformatics analysis. In particular, we performed a testis-tissue library screening and identified a preliminary dataset including 61 pVHL30 interactors. Bioinformatics analysis about molecular function and subcellular localization for each interactor were performed by search against UniProtKB and Gene Ontology (GO). Cytoscape were used to construct protein-protein interaction network around the here identified pVHL30 interactors and integrated with data from from STRING and BioGRID databases. Clusters of functionally correlated proteins were identified with MCODE, while analysis of biochemical pathways, GO terms and association with human diseases was performed with Enrichr and ClueGo.

Results

Here, we identified 55 new pVHL interactors directly involved in pathways regulating spermatogenesis, cell differentiation and reproductive metabolism. Our in-depth computational investigation of these novel interactors identified multiple pVHL-specific binding motifs and demonstrated that multiple somatic mutations described in human cancers localize in these binding regions. Collectively, our findings suggest that, in addition to its role in cancer formation, pVHL may be pivotal in the correct gonads development also in human.

Conclusion

We described the testis-specific proteome around the human pVHL30 obtained by library screening. Our approach identified 55 novel pVHL30 interactors, with multiple proteins directly involved in spermatogenesis, reproductive metabolism and cancer. Albeit further study is warranted to elucidate the exact role of these new interactions, we demonstrated that the isoform pVHL30 can bind tissue-specific interactors and suggested novel roles for this oncosuppressor protein.

Reference: Falconieri A, Minervini G, Quaglia F, Sartori G, Tosatto SCE. ***Characterization of the pVHL Interactome in Human Testis Using High-Throughput Library Screening***. Cancers (Basel). 2022 Feb 17;14(4):1009. doi: 10.3390/cancers14041009

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