Loss of VHL Activates SFMBT1-SPHK1 Oncogenic Signaling in Kidney Cancer

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HIF2α dependence varies across ccRCC cell lines

HIF2α Dependent

ccRCC Cell Lines Remained Insensitive to HIF2α sgRNA and Inhibitor

HIF2α Independent

Can we identify additional therapeutic avenues in ccRCC regulated by VHL?

How?

VHL and P-OH HIF1α-564 Binding

Hydroxylation of HIF1α on proline residue 564 (p-OH HIF) primes its binding with VHL ubiquitin E3 ligase complex (including pVHL, Elongin B and C, VBC)

Based on the work from Kaelin, Ratcliffe and Pavletich
A Genome-Wide In Vitro Expression Cloning Strategy

- ~17,000 cDNA ORF bacterial stock
  - 24 unique cDNA/pool, 700 pools
  - 96 well plate, 8 plates
  - Mini-Preps
  - GST-VHL complex
  - Run SDS-PAGE & autoradiography
  - 35S Labeled Proteins
  - 700 pools (24 genes/pool)
  - WT HIF Peptide
  - P-OH HIF Peptide

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<th>IVT Protein</th>
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<td>SFMBT1</td>
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SDS-PAGE & Autoradiography

1: WT HIF peptide
2: p-OH HIF peptide
SFMBT1 Depletion Leads to Decreased ccRCC Growth
Integrated Analyses for SFMBT1 Regulated Events in ccRCC

1. Contain SFMBT1, H3K4me3 and H3K27ac binding peaks
2. Genes that are positively regulated by SFMBT1
3. Genes that show elevated expression in ccRCC patient tumors
4. Genes that predict worse prognosis in ccRCC patients with higher expression
VHL-SFMBT1-SPHK1 Signaling Drives Oncogenesis in ccRCC

786-O
HIF2α Dependent

UMRC2
HIF2α Independent

A VHL Proficient

B VHL deficient or Hypoxia

Mol Cell, 2020, PMID 32023483
Aims: Determine the functional significance of deregulated SFMBT1-SPHK1 signaling in *VHL*-deficient renal cancer. Determine if SPHK1 activated in ccRCC drives critical NF-κB oncogenic signaling. Determine the therapeutic potential of targeting SFMBT1-SPHK1 signaling axis in renal tumorigenesis upon *VHL* loss.
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Thank you for your attention!