"Targeting HIF-VHL deregulation in RCC and Hemangioblastoma"

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Identification and validation of therapeutic targets in Renal Cancer

TCGA Mutations

RCC as metabolic disease

GSEA

Zebrafish Models of driver mutations

In vivo validation

Preclinical validation

Clinical Trials

Tumor hypoxia and cancer associated mutations promote HIF activity

TUMOR HYPOXIA

LKB1
ERB
AMPK
mTOR
HIFa mRNA
ROS
EGLN
SDH
FH
HIFa-ARNT

HIF is a validated target for RCC and other cancers

Direct targeting of HIF2a with small molecules

Synthetic lethality with HIF1a/2a expression based on metabolic reprogramming

Insights into the biology of Hemangioblastomas
Identification of HIF2α inhibitors

1. Cell-based assay for HTS
2. HIF2α inhibitors
3. Specificity by GSEA
4. Mechanism of action

Zimmer M et al. Molecular Cell 2008
Metelo AM et al. JCI 2015
Noonan H et al. DMM 2017

HIF2α inhibitor decreases abnormal erythropoiesis and angiogenesis in vhl-/^-embryos

HIF2α inhibitor ameliorates pathologic angiogenesis and abnormal hematopoiesis in vhl-/^-embryos

Kidney of vhl-/^-embryos resembles premalignant ccRCC phenotype
HIF2α inhibitors downregulate HIF2α signaling IN VIVO (Mouse xenograft model)

Before Treatment After 76 Treatment Before Treatment After 76 Treatment

SV40-Luc

HRE-Luc

HIF2α inhibitors suppress RCC growth in mice (PK unknown)

Direct targeting of HIF2α with small molecule inhibitors: Peloton (PT2297) compound interrupts HIF2α-ARNT interaction

Phase 2 clinical trial in RCC and VHL

Phase 1 trials:
- Well tolerated
- Has activity in heavily pre-treated metastatic RCC patients
- Resistance linked to AQUIRED mutations in the binding pocket

Direct targeting of HIF2α with small molecules

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Insights into the biology of Hemangioblastomas

TCA metabolites link metabolism to HIF translation

IRP1 KO mice develop EPO-driven erythrocytosis and HIF2α-dependent pulmonary hypertension

IRP1

ACO1

Fe(II)-Fe(II)

5'-UTR mRNAs:
- Ferritin
- eALAS
- SDHb
- ferroportin

Translation Start

Coding message
Normal cells use Glucose for carbon source

VHL-/- and HYPOXIC cells depend on Glutamine for growth

Reductive carboxylation of GLUTAMINE derived carbons is the primary pathway for lipid synthesis under hypoxia

Citrate levels regulate Glutamine consumption: HIF1a/2a expression is necessary and sufficient to confer Glutamine dependence

Metallo CM et al Nature 2012
Gameiro et al Cell Metabolism 2013
Citrate levels regulate Glutamine consumption: opportunities for targeted therapy

GLS Inhibition suppresses growth of VHL-Deficient RCC Cells

Loss of VHL renders RCC cells/tumors sensitive to glutaminase inhibition in vivo
Phase 1a/1b CLINICAL TRIAL with oral GLS inhibitor CB-839
MASSACHUSETTS GENERAL HOSPITAL

Phase 1a All solid tumors
Phase 1b RCC (CBE or CB + Nivolumab)
TNBC (CB+Placlitaxel)
NSCLC (CB+Placlitaxel)

Effects of GLS1 inhibitors on RCC metabolism

De novo and Salvage Pathways for Pyrimidine biosynthesis

GLS Inhibition Compromises De Novo Pyrimidine Synthesis in VHL-Deficient Cells

GLS Inhibition increases ROS in VHL-Deficient RCC Cells

A

B

C

GLS Inhibition Induces DNA Replication Stress in VHL-Deficient Cells


GLS Inhibition Induces DNA Replication Stress in VHL-Deficient Cells

Treatment of VHL-Deficient Cells: combination of GLS Inhibitors with other drugs

Hydroxyurea
5-Fluorouracil
Orotate dehydrogenase inhibitor (teriflunomide)

Cisplatin
DNA synthesis
DNA damage
DNA repair
PARP inhibitor (olaparib)

Synergistic effect of GLS Inhibitors with Olaparib
GLS Inhibitors synergize with Olaparib in killing VHL-Deficient Cells *in vivo*

Resistance to GLS1 inhibitor CB-839: identification of differentially expressed genes

Direct targeting of HIF2α with small molecules

**Synthetic lethality with HIF1α/2α expression based on metabolic reprogramming**

**Insights into the biology of Hemangioblastomas**

What do we know about human HB

- *VHL*−/−
- *PDPN* expressing
- Stromal cell
- Endothelial cell
- Hematopoietic cell

- Loss of 3p25 - VHL inactivation
- Gain of chromosomes 1 and 4
- Loss of chromosomes 6, 9, 19 and 22q13

Lipid accumulation
The growth of HB is variable: lack of biomarker predicting growth

WES revealed LOH or somatic point mutations leading to the inactivation of both VHL alleles in all VHL-related HB

Loss of Chromosomes 3 and 8 are recurrent Copy Number Variation events in VHL-related HBs

Single Cell Sequencing from HB fresh tumors and HB-derived cell lines

Discovery of actionable signaling pathways
HB-derived cell lines capture HB heterogeneity

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<th>AM2</th>
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HB-derived cells generate HB in mice: a model for human HB (PDX)

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Stromal cells
Endothelial cells
Vascular tumors
Immune cells
Neuronal stem cells
Microglia/Macrophages
Hematopoietic stem cells

HB cell-derived xenografts are rich in intracellular lipids and highly vascularized

The mobile lipids are detected by $^1$H MRS at 0.8 – 1.3 ppm. They correspond to methyl (CH$_3$) and methylene (CH$_2$)$_n$ groups.

ADC measures the movement of H$_2$O molecules.
This value is higher in the presence of angiogenesis, blood vessels, tumor necrosis or edema.

Mouse model for HB
Single Cell Sequencing from HB fresh tumors and HB-derived cell lines

Surgery Tumor Excision → Tumor Dissociation → Single Cell Sorting

Cell of origin

Identification of cluster specific gene expression signatures

Identification of clusters with high expression of HIF-target gene signature

Unsupervised analysis of sc-RNAseq clusters cells in distinct subgroups
Summary

- HIF upregulation is a major oncogenic event in VHL-related tumors
- HIF can be directly targeted with small molecule inhibitors
- HIF reprograms cancer cell metabolism and renders VHL-null cells dependent on Glutamine – remains to be proven for HB
- GLS inhibitors selectively kill VHL-null cells and synergize with PARP inhibitors - remains to be proven for HB
- HB-derived cell lines recapitulate the cellular heterogeneity of the human CNS HBs
- A xenograft mouse model may guide the development of targeted therapy for CNS HB

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