ER stress in von Hippel-Lindau tumor suppressor gene mutant kidney cells and the induction of inflammatory response

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VHL knockout kidney shows extensive inflammation: macrophage infiltration

Hoxb7-driven Cre
- collecting ducts
- distal tubules

Phenotype
- pre-cancerous hyperplasia
- appearance of transformed clear cells

Metabolic abnormalities in VHL mutant cells

Loss of VHL → PI3K signaling → Excessive protein synthesis → ER stress

Severe hypoxia stress → ROS production → TCA cycle disruption

Hypothesis

VHL inactivation in kidney tubule cells induces chronic ER stress and tissue inflammation.
Unfolded protein response pathways and inflammation

ER stress

Unfolded

Misfolded

Unfolding protein response pathways and inflammation

Protein quality control

Cellular homeostasis

Inflammation

VHL knockdown causes ER stress and UPR responses in HK-2 cells

VHL knockdown increases HK-2 cell induced-chemotaxis of macrophage RAW cells

Macrophage chemotaxis assay

RAW 264.7: mouse leukemic monocyte macrophage cell line
**APY29**

APY29 specifically inhibits IRE1α autophosphorylation and TRAF2 binding, but not XBP1 splicing.

Inhibition of IRE1α autophosphorylation, p-JNK upregulation and NF-κB/p65 nuclear translocation in vitro using kinase inhibitor APY29.

APY29 attenuates the VHL inactivation induced-chemotaxis of macrophage in HK-2 and mouse primary renal tubule cells (mRTCs).

APY29 attenuates kidney injury and p-JNK upregulation in primary renal tubule cells (mRTCs) and VHL mutant mouse.

mRTCs: isolated from WT and VHL knockout mice.
VHL tumor suppressor gene mutations result in endoplasmic reticulum stress and chronic inflammation.

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**proximal tubule specific knockout**

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<th>System</th>
<th>Phenotype</th>
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<tr>
<td><strong>Phosphoenolpyruvate carboxykinase-Cre</strong></td>
<td>renal microcysts (25% of &gt; 12-month-old mice)</td>
<td>Rankin et al. 2006. Cancer research.</td>
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<td><strong>Ksp1.3-Cre</strong></td>
<td>precursor of ccRCC</td>
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<tr>
<td><strong>Ksp1.3-Cre; Pten</strong></td>
<td>hydro nephrosis in the tubule epithelium</td>
<td>Frew et al. 2008. Molecular and cellular biology.</td>
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<tr>
<td></td>
<td>1. hyper-proliferation urothelium</td>
<td></td>
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<td></td>
<td>2. enlarged kidneys</td>
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- Confirm the cyst-ccRCC correlation, did not elucidate other possible early events in ccRCC progression.
- **Ksp1.3-Cre**: expressed in distal tubules and collecting ducts, but rarely in proximal tubules
- **Pten**: Phosphatase and tensin homolog
Evidence suggested that ccRCC is of distal tubule or collecting duct origin

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<td></td>
<td>21% (+) of ccRCC</td>
<td></td>
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<td></td>
<td>14% (+) of ccRCC</td>
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<td>Human tissues</td>
<td>Distal tubule/collecting duct-specific MUC1 mucin</td>
<td>Kraus et al. Hum Pathol. 2002</td>
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<td>expression positively correlated with ccRCC progress</td>
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<td>Human tissues</td>
<td>1. Acquired cystic kidney disease-associated ccRCCs : distal tubule markers (AE1/AE3 and EMAJ)</td>
<td>Shen et al. Mod Pathol. 2005</td>
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<td>2. Classic ccRCC : proximal tubule markers (LeuM1)</td>
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Hoxb7–Cre-driven knockout produced more pronounced phenotypes with higher penetrance than the previous models

1. We backcrossed the non-BL6 mouse strains to the C57BL/6 background, which is known to influence hematopoiesis, and thus inflammatory response.

2. Hoxb7–Cre may direct Vhlh inactivation in tissues besides kidney tubules that can exert a systemic, non-cell autonomous effect.

(Martelli et al. Blood. 2005)
(Zhao et al. Dev Biol. 2004)