VHL knockdown kidney cells induce macrophage extravasation and polarization toward tumor-associated macrophage (TAM)

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No financial conflict of interests

Hoxb7-Cre driven Vhlh knockout mice model
- A mouse Vhlh conditional knockout, using Hoxb7-driven Cre that is specific for the collecting ducts and a subset of distal tubules (ascending Henle's loop).

Oncogene (2015) 34: 2631-2639

Infiltration of immune cells — inflammation

Oncogene (2015) 34: 2631-2639
• VHL inactivated cells exhibit:
  ➢ elevated levels of reactive oxygen species
  ➢ increased protein synthesis

• Increased ER stress

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VHL inactivation causes ER stress in HK-2 cells (non-cancerous human kidney cells) and primary mouse tubule cells

APY29 specifically inhibits IRE1α autophosphorylation ($IC_{50} = 280$ nM) but activates IRE1α RNase activity.

Inhibition of IRE1α autophosphorylation and p-JNK upregulation in vitro using kinase inhibitors APY29

Cancer Research (2017) 77: 3406-3416
IRE1 kinase inhibitor APY29 inhibits NFkB activation in VHL knockdown cells

<table>
<thead>
<tr>
<th>Cytoplasmic fraction</th>
<th>Nuclear fraction</th>
</tr>
</thead>
<tbody>
<tr>
<td>shVHL</td>
<td>1 2 3 4</td>
</tr>
<tr>
<td>APY</td>
<td>5 6 7 8</td>
</tr>
<tr>
<td>NFkB-p65</td>
<td>- + + -</td>
</tr>
<tr>
<td>β-actin</td>
<td>- + + +</td>
</tr>
<tr>
<td>fibrillarin</td>
<td>+ - + -</td>
</tr>
</tbody>
</table>

APY29 ameliorates fibrosis in Vhlh knockout kidney and rescues kidney function

a) Sirius red staining for collagen

b) Urine protein content

Can VHL loss-of-function epithelial cells recruit macrophages?

Macrophage extravasation

Cytokine array profile of co-culture conditioned media

What factors are secreted by VHL-inactivated kidney cells that attracted macrophages?

Bio-Plex Pro Human Cytokine bead array screening
Macrophage polarization toward tumor-associated macrophage (TAM)

- mRNA expression in migrated macrophage in the extravasation assay. These macrophages express M2/TAM markers:

<table>
<thead>
<tr>
<th>Identify</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>TNFα</td>
<td>a pro-inflammatory agent that regulates macrophage function; induces HIF1α</td>
</tr>
<tr>
<td>CXCL10</td>
<td>to increase the infiltration of inflammatory cells, including macrophages</td>
</tr>
<tr>
<td>CCR2</td>
<td>involved in monocyte/macrophage infiltration in the inflammatory response</td>
</tr>
<tr>
<td>CCL22</td>
<td>M2 macrophage/TAM derived; directly promotes tumor migratory capacity</td>
</tr>
<tr>
<td>TGFβ</td>
<td>secreted by TAM in the microenvironment, promoting tumor proliferation, migration, and invasion.</td>
</tr>
<tr>
<td>IFR3</td>
<td>activated in TAM</td>
</tr>
<tr>
<td>VEGFα</td>
<td>a cytokine produced by macrophages, is a primary inducer of angiogenesis and neovascularization</td>
</tr>
</tbody>
</table>

Macrophages in Vhlh knockout kidney comprise more tumor-associated macrophage (TAM)

Analysis of kidney macrophage population at the single-cell level—marker discovery in kidney inflammation and cancer

Conclusion

- **VHL** inactivated cells exhibit increased capacity to induce macrophage chemotaxis and extravasation, which may be correlated with secreted cytokines by the VHL-negative epithelial cells.
- Recruited macrophages tend to polarize toward the M2/TAM phenotype.
- *In vivo*, conditional Vhlh knockout kidneys show dramatically increased infiltration of macrophages. Among these macrophages, the M2/TAM type macrophages are more abundant than the M1 type.

The secreted cytokines from VHL loss-of-function cells or tumor-promoting factors released by invasive macrophages may present novel targets for anti-ccRCC therapy.
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VHL and kidney disease

- Kidney cancer is a worldwide health concern. It is predicted that from 2012 to 2020, kidney cancer will increase globally by 22 percent.
- Von Hippel-Lindau (VHL) tumor suppressor gene is closely linked with renal cell carcinoma (RCC): up to 80% of sporadic clear-cell RCC carry VHL mutations or epigenetic modifications.
- Nearly 100% of the familial ccRCC (in VHL disease) are VHL.

Can VHL loss-of-function epithelial cells recruit mavrophages?

Transwell co-culture system

Transwell co-culture system was used to mimic the extravasation process in vitro

Transwell inserts with 8-μm pore size (Corning) were coated with gelatin

Co-culture 3 cell lines for 48 hours

Macrophage (precursor THP-1 cells)
HUVEC (Human Umbilical Vein Endothelial Cell)
HK-2 cell (Human Kidney-2) with or without VHL knockdown