Intestinal acidification sensed by pH-sensing receptor GPR4 contributes to fibrogenesis

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pH-sensing G protein-coupled receptors

- The family of pH-sensing GPCRs
  - GPR4
  - T-cell death associated gene (TDAG8) or GPR65
  - Ovarian cancer G protein-coupled receptor 1 (OGR1) or GPR68
  - G2A or GPR132
- sense extracellular $H^+$ → various signaling cascades

Cluster of histidines at the extracellular surface
Hydrogen-bonding between His residues stabilize the receptor

at low pH protons bind to His residues
conformational change of receptor

EC$_{50}$ – pH 7.4
fully activated at pH 6.8
minimally active at pH 7.6 – 7.8

**Hypothesis: Gpr4 represents a potential target in intestinal fibrosis?**

The proton-activated receptor GPR4 modulates intestinal inflammation

GPR4 deficiency alleviates intestinal inflammation in a mouse model of acute experimental colitis

Gpr4 depletion or inhibition decreases intestinal fibrosis
Positive correlation in mRNA expression between **GPR4**, **αSMA** and **COL1A1**

- **GPR4** comp to **GAPDH**
- **αSMA** comp to **GAPDH**
- **COL1A1** comp to **GAPDH**

(R^2 = 0.880, P < 0.000001)  
(R^2 = 0.887, P < 0.000001)
Decreased TGFβ and COL1A1 upon depletion of Gpr4 in chronic DSS-induced colitis

**Graphs:**
- **Collagen layer thickness [µm]:**
  - WT: 10, 20, 30, 40
  - Gpr4−/−: 10, 20, 30, 40
  - *p = 0.08

- **Hue > 220 area / total tissue area:**
  - WT: 5×10^4, 1×10^5, 2×10^5
  - Gpr4−/−: 5×10^4, 1×10^5, 2×10^5
  - *p = 0.08

- **Hyp [µg/mg]:**
  - WT: 0.0, 0.5, 1.0, 1.5
  - Gpr4−/−: 0.0, 0.5, 1.0, 1.5

**Images:**
- Colon 20x Sirius red
- Colon 20x Hue > 220

**Protein comparison to β-Actin (normalized to WT):**
- COL1A1
- VIMENTIN
- TGFβ1

**Legend:**
- WT
- Gpr4−/−
GPR4 inhibitor prevents pH-induced differentiation of primary human fibroblasts

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<td>pH</td>
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<tr>
<td>rTGFβ</td>
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<td>GPR4 inhibited [µM]</td>
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GPR4 inhibitor prevents pH-induced differentiation of primary human fibroblasts

DAPI
αSMA
p-SMAD3

GPR4 inhibitor [µM] relative to GAPDH

pH

GPR4 inhibitor [µM] relative to GAPDH

GAPDH

TGFβ1

αSMA

GAPDH

pH

DAPI
αSMA
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