Novel regulation of kidney function by gastrointestinal hormones

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Abstract

The control of whole-body energy metabolism involves intricate crosstalk between multiple organ systems including the gut and the brain that play well-recognized roles in this process. However, the important contributions and mechanisms by other organs are poorly understood. Errors in metabolic regulation can lead to human metabolic diseases including diabetes and obesity that affect 75% of the population and can lead to significant comorbidities. Improving our mechanistic understanding of multi-organ metabolic crosstalk in health and disease is essential for developing highly efficient and new therapeutic approaches. We hypothesized that the kidney is a major regulator of body metabolism via a new mechanism of gut-kidney crosstalk that involves the gut hormones gastrin and cholecystokinine (CCK) activating intrarenal mechanisms to increase blood flow and filtration, progenitor cells and tissue growth factors. Macula densa (MD) cells in the kidney are chief regulators of renal blood flow and tissue remodeling. RNA sequencing and transcriptome analysis identified the gastrin/CCK receptor *Cckbr* as one of the highest expressed genes in MD versus control kidney cells. Intravital multiphoton imaging of Sox2-GCaMP6 reporter mouse kidneys revealed robust and entirely MD cell-specific calcium signaling in response to systemic gastrin/CCK injection. Using the novel MD^{geo} immortalized cell line cultured in vitro, CCK8 treatment led to 3-fold increases in MD cell synthesis of Prostaglandin E2 (PGE2)-generating COX2 and CCN1 that are established mediators of MD-induced glomerular hyperfiltration and tissue remodeling, respectively. Chronic treatment of Ren1d-Confetti mice with Darunavir, an HIV protease inhibitor significantly increased plasma CCK levels, Ren+ mesenchymal progenitor cell number and clonality, renal cell proliferation and tissue hypertrophy. This study uncovered new regulatory mechanisms of systemic metabolism especially the role of MD cells in the kidney. Repurposing the commonly used HIV drug Darunavir for kidney and metabolic diseases may provide therapeutic benefit for millions of patients worldwide.

Introduction

- Errors in metabolic regulation can lead to human metabolic diseases including diabetes and obesity that affect 75% of the population and can lead to significant comorbidities.
- **Macula densa** (MD) cells are specialized sensory epithelial cells in the kidney and are known as the chief regulators of renal blood flow and tissue remodeling.
- **Gastrin** is a hormone produced by the G cells in the lining of the stomach, primarily known to enhance gastric mucosal growth, gastric motility, and secretion of hydrochloric acid (HCl) into the stomach in response to the presence of amino acid rich food. In addition, it is also secreted into the bloodstream to act on distant organs.
- **Cholecystokinin** (CCK) is another gastrointestinal hormone which is secreted by I cells in the duodenum in response to fat rich food intake and stimulates the release of bile into the intestine and the secretion of enzymes by the pancreas. CCK is also released into the circulation and has distant organ effects.
- CCKBR is one of the two gastrin/CCK receptors that is coupled to calcium signaling and via nuclear transcription regulates tissue growth in the pancreas and stomach.
- It is well known that glomerular filtration rate (GFR) increases after food intake well before an increase in nutrient/waste levels in the plasma. Cellular/molecular understanding of gut-kidney axis is lacking.
- Darunavir (DVR) is a HIV protease inhibitor and works by decreasing HIV in the blood. It is also known to increase plasma CCK levels and prevent kidney injury via HIV-independent mechanisms, but the it's mode of action is unknown.

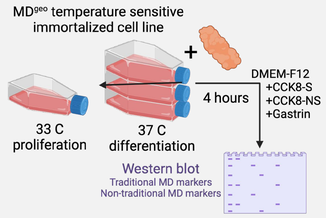
Aim

This study tested the hypothesis that gut hormones gastrin and cholecystokinine (CCK) activate MD cells in the kidney via gut-kidney crosstalk to increase blood flow and filtration, progenitor cells and the secretion of tissue growth factors.

Methods

Cell culture studies
- Immortalized MD^{geo} cell line
- Western Blot analysis of the expression of traditional (Cox2) and novel (CCN1) MD cell specific factors

Mouse studies
- MD single cell RNA seq and transcriptome analysis
- Sox2-G6 mouse model combined with in vivo multiphoton (MPM) microscopy for calcium imaging and functional validation
- Ren1d-Confetti mouse model + Darunavir (DVR) treatment for 1 week – to study tissue remodeling/growth
- Immunohistochemistry to analyze changes in tissue composition: native Confetti expression, Ki67, pERK, Fos immunostaining.



- Image analysis - Leica LAS X or Image J image analysis software
- Statistical analysis –GraphPad Prism
- Illustration: Biorender

Results

CCKBR is specifically expressed in MD cells in the kidney

- MD cell bulk and single cell RNA Seq and transcriptome analysis showed that CCKBR is highly enriched in MD cells compered to control kidney cells.

A MD Bulk RNA seq

Rank	Gene ID	Fold change
39	Nt5c1a	10.38
40	**Cckbr**	**10.24**
41	Slc5a1	10.21

B MD Single cell RNA seq

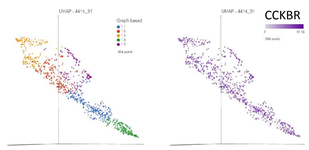
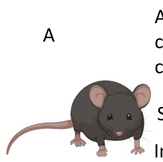


Figure 1 Comprehensive study of Cckbr expression in MD cells. A: MD cell bulk RNA seq-based transcriptome analysis found Cckbr as the top #40 highest expressed DEG. **B**: UMAP analysis of MD cell scRNA seq-based transcriptome identified Cckbr expression in all MD cells.

Intravital MPM imaging revealed robust and highly MD specific effects of gastrin and CCK

A



All kidney cell types express the calcium sensitive GCaMP6 and the calcium insensitive tdTomato

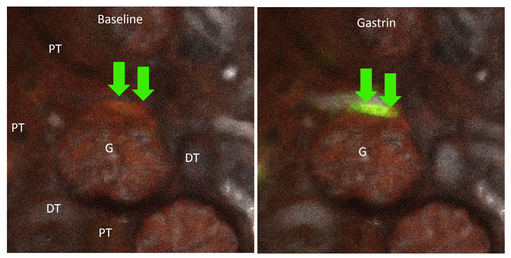
Sox2-GCaMP6/tdTomato

Increased GCaMP6 fluorescent intensity

Increased intracellular calcium

- Sox2-GCaMP6/tdTomato mouse model is a great tool for comparative analysis of cell physiology.
- Robust $[Ca^{2+}]_i$ increase specifically in MD cells compared to any other cell type in the kidney in response to intracarotid gastrin injection.
- Gastrin (and CCK) specifically activates MD cell function in the kidney in vivo.

B



C

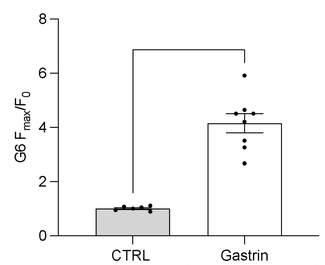
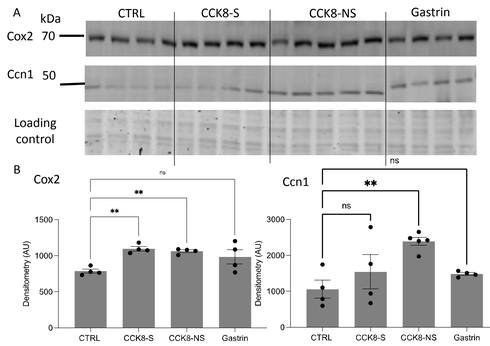


Figure 2 A: Schematic of the Sox2-GCaMP6/tdTomato mouse model. **B**: Representative in vivo MPM image of a glomerulus with the surrounding tubular segments including the macula densa (green arrows), proximal (PT) and distal tubules (DT) at baseline and after intracarotid (ic.) gastrin injection. Note the robust increase in GCaMP6 fluorescence intensity (green) exclusively in MD cells in response to gastrin. **C**: Statistical summary of MD cell calcium signaling in response to ic. gastrin injection. Statistical analysis was performed by using student T test, p<0.05 is considered statistically significant. **** p< 0.0001.

Results

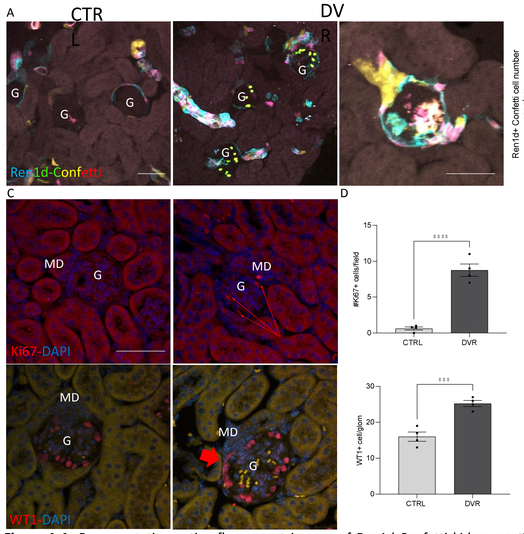
Gastrointestinal hormones (gastrin and CCK) increase MD cell function in vitro



The addition of gut hormones **gastrin** and **CCK8** in cultured MD cells significantly **increased:**
- the expression of **Cox2, traditional MD cell/function** marker to increase blood flow and GFR
- the expression of **Ccn1**, **non-traditional MD cell factor of tissue growth**

Figure 3 A: Western blot analysis of changes in the expression of cyclooxygenase 2 (Cox2) and Ccn1 in cultured MD cells in response to CCK8-S, CCK8-NS, and gastrin treatment compared to control. **B**: Statistical summary of western blot analysis of Cox2 and Ccn1 expression in cultured MD cells. n=4-5, p<0.05 was considered statistically significant using ANOVA, ns: not significant, *-**** p<0.05-0.0001.

Darunavir increases endogenous tissue remodeling

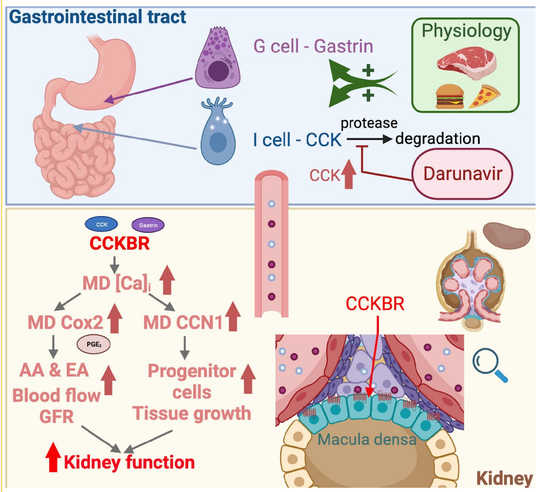


Treatment with HIV protease inhibitor DVR for 1 week increased:
- the number of Ren1d+ progenitor cells and the number of unicolor cell clones in the glomerulus.

Treatment with HIV protease inhibitor DVR for 1 week increased:
- the number of Ki67+ cells / field.
- the number of WT1+ cells/ glomerulus.
- the number of WT1+ cells at the vascular pole – at the base of the macula densa.

Figure 4 A: Representative native fluorescent images of Ren1d-Confetti kidney sections from control and DVR treated mice. Note the increased number of Ren1d+ progenitor cells and the unicolor multi-cell clones in the glomerulus in DVR treated animals compared to control. **B**: Statistical summary of Ren1d+ progenitor cells/glomerulus in control and DVR treated animals. **C**: Representative immunofluorescence images of Ki67 (top) and WT1 (bottom) mouse kidney sections from control and DVR treated animals. Note the increased number of Ki67+/WT1+ cells (red nucleus) in the DVR treated animals compared to control. **D**: Statistical summary of Ki67+ (top) and WT1+ (bottom) cells in control and DVR treated animals. n=4, p<0.05 was considered statistically significant using ANOVA, ns: not significant, *** p<0.001.

Summary and Conclusion



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