Intestinal epithelial vitamin D receptor deletion leads to defective autophagy in colitis

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Introduction

• Vitamin D and the vitamin D receptor (VDR)
  • calcium homeostasis
  • electrolyte and blood pressure regulation
  • important immunological regulators of inflammatory bowel diseases (IBD)
  • transcription factor for AMP, cathelicidin antimicrobial peptide, β-defensing, Cyp24 hydroxylase gene
• north-south gradient in rates of Crohn's disease (CD) → vitamin D deficiency environmental trigger contributing to the pathogenesis of IBD
• Low vitamin D status and VDR expression in patients with IBD
• Paneth cells: innate immune responses; shaping the gut microbiota
Introduction

• autophagy: intracellular homeostasis; degradation and recycling of cytosolic contents and organelles, removal of intracellular microbes, immunity against infection.

• IBD susceptibility genes (IRGM, Nod2, ATG16L1) are involved in autophagy

• deficits in autophagy pathway can impair Paneth cell function

Hewison, M. (2011) Antibacterial effects of vitamin D
Nat. Rev. Endocrinol. doi:10.1038/nrendo.2010.226
Introduction

• studies have identified vitamin D as a potent stimulator of autophagy in *M. tuberculosis* infection and HIV infection

• however: the crosstalk among VDR, autophagy and bacteria in the gut remains unknown
Aims and hypothesis

• hypothesis: intestinal epithelial VDR is a determinant of IBD risk through its actions on the autophagy gene ATG16L1, thus determining states of paneth cells and microbial assembly in intestinal homeostasis

• investigating how intestinal epithelial VDR regulates autophagy and Paneth cells through the autophagy gene ATG16L1.
Methods

• human colorectal tissue samples from sigmoid colon:
  • 52 patients (51-83 years old) exhibiting no apparent intestinal pathology and normal mucosa
  • 30 patients with anterior resection (44-85 years old)
• animals:
  • VDR\textsuperscript{loxP/loxP} mice
  • VDR\textsuperscript{ΔIEC} mice (crossing VDR\textsuperscript{loxP/loxP} with villin-re mice)
  • IL10\textsuperscript{−/−} mice
  • all 2-3 months old
• induction of colitis with 5% dextran sulfate sodium (DSS), day 7 sacrifice
Methods

- butyrate-treated mouse model with 2% sodium butyrate for three weeks
- co-housing experiment
- cell culture with mouse embryonic fibroblasts (MEFs), human embryonic intestine INT 407, HCT116 cells and human colorectal adenocarcinoma SKCO-15 cells
- *in vitro* VDR knockdown of SKCO 15 with shRNA using cells retroviral GFP vector
- Vitamin D-responsive element transcriptional activity
- western blot analysis
- histology
- immunoflourescence
- fluorescence in situ hybridisation (FISH)
Methods

- Lysotracker staining (paneth cells)
- paneth cell counting
- real-time quantitative PCR
- real-time PCR measurement of bacterial DNA
- mucosal microbial and faecal 454 pyrosequencing
- chromatin immunoprecipitation (CHIP) assay
Established intestinal epithelial cell vitamin D receptor (VDR) knockout (KO) (VDRΔIEC) mice and their bacterial profile.

no detectable VDR expression in intestinal epithelial cells

absence of VDR leads to ecological change in bacterial profiles

relative abundance of bacteria shifted

PCoA: fecal microbial communities differ

B. fragilis associated with IBD

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VDRΔIEC mice have worse outcomes with dextran sulfate sodium (DSS)-induced colitis.

Significant loss of body weight, cecum length.

Disease activity index: fecal blood, less formed stools, more weight loss.

Severe intestinal inflammation.

Transmissibility of phenotype: increased disease activity.
Vitamin D receptor (VDR) affects patterns of Paneth cells in VDRΔIEC mice.

A) Lysozyme staining for paneth cell counting:
- D1: disordered
- D2: depleted
- D3: diffuse

B) Fewer than normal cells:
- VDRloxPloxP
- VDRstom

C) Increased abnormal paneth cells:
- Percentage of total Paneth cells (%)

D) Decreased lysozyme in VDRstom

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Vitamin D receptor (VDR) regulation of the expression levels of autophagy-related genes.

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in vivo: significant reduction of ATG16L1 protein and lysozyme

signaling adaptor that accumulates in autophagy deficient mice

starvation-induced autophagy model

complete knock down compared to partly knocked down VDR: significant lower level of ATG16L1 and lysozyme protein

VitD3 increased LC3 associated with increase of VDR

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Vitamin D receptor (VDR) expression in human intestine and colitis models.

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VDR expression is decreased in patients with ulcerative colitis

Inflamed intestine with low VDR → Increased *bacteroides* were found by FISH

Staining for ATG16L1, very low signal
Bacterial product butyrate activates vitamin D receptor (VDR) signalling pathway in human intestinal epithelial cells.

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butyrate pretreated human intestinal epithelial cells → increased expression of VDR

VDR transcriptional activity following butyrate stimulation

butyrate increased cyp24 and cathelicidin mRNA
Butyrate treatment restores vitamin D receptor (VDR) expression in colitis and inhibits inflammation.

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Butyrate increased VDR and ATG16L1 protein in IL10^-/- mice

Suppression of cytokine IL-6

Butyrate treatment increased normal paneth cell ration

Enhancing VDR expression by bacterial product is able to stimulate autophagy-marker ATG16L1, restores paneth cells, reduces inflammation
Discussion

• previous studies reported link between autophagy and IBD \([1,2]\)

• intestinal epithelial VDR regulates autophagy and Paneth cells through the autophagy gene ATG16L1, thus changing the microbiome profile.

• low levels of VDR correlate with decreased ATG16L1 in the intestine of patients with IBD and in an experimental colitis model.

• VDR KO is decreasing lysozyme

• dysbiosis, including decreased abundance of *Butyrivibrio*, in VDR\(^{\text{IEC}}\) mice increase risk for colitis

Discussion

• administration of butyrate increases intestinal VDR and ATG16L1 expression and suppresses inflammation in an experimental colitis model

• possible therapies:
  • treating mice with butyrate
  • enhancing intestinal VDR expression
  • faecal transplantation

• VDR as a clinical biomarker?
My opinion

- they offered a wide range of methods to proof their concept
- was written in easy and understandable style
- importance of Vitamin D an VDR receptor in several processes of the body
- comprehensive therapeutic options
- proceed with large animal studies and clinical studies
END. Thank you for the attention!