Ankersmit Laboratory

for Diagnosis & Regeneration in Thoracic Diseases & Applied Immunology

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

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GUT July 2015

Introduction

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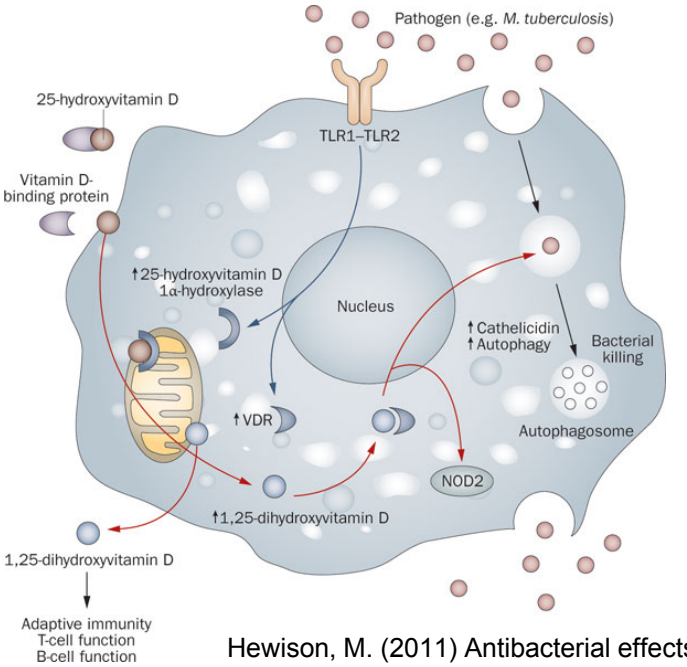
- Vitamin D and the vitamin D receptor (VDR)
  - calcium homeostasis
  - electrolyte and blood pressure regulation
  - important immunological regulators of inflammatory bowel diseases (IBD)
  - transcription factor for AMP, chathelicidin antimicrobial peptide, ß-defensing, Cyp24 hydroxylase gene
- north-south 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 homeostatis; degradation and recycling of cytosolic contents and organelles, removal of intracellular microbes, immunity against infection.



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

- IBD susceptibility genes (*IRGM*, *Nod2*, *ATG16L1*) are involved in autophagy
- deficits in autophagy pathway can impair Paneth cell function

Introduction

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- 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

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- 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

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

Methods

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- 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-resposive element transcriptional activity
- westernblot analysis
- histology
- immunoflourescence
- fluorescence in situ hybridisation (FISH)

Methods

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- 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.

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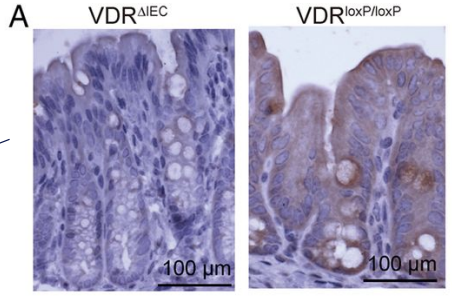
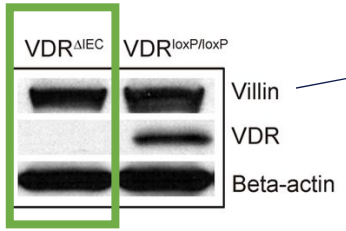
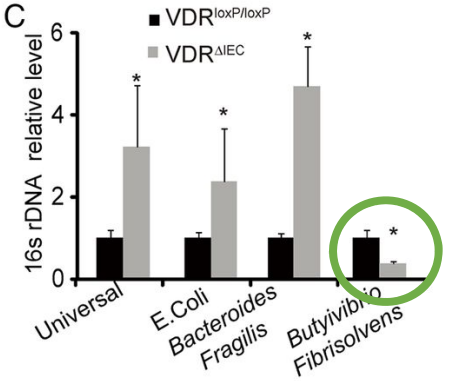
no detectable VDR expression in intestinal epithelial cells

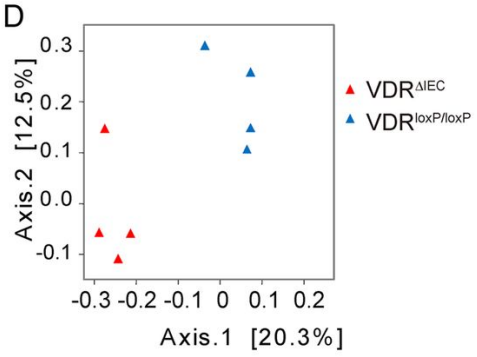




marker for IECs

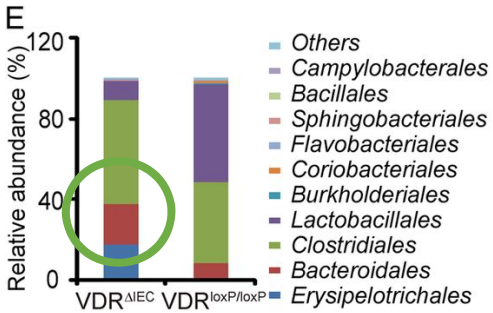
absence of VDR leads to ecological change in bacterial profiles

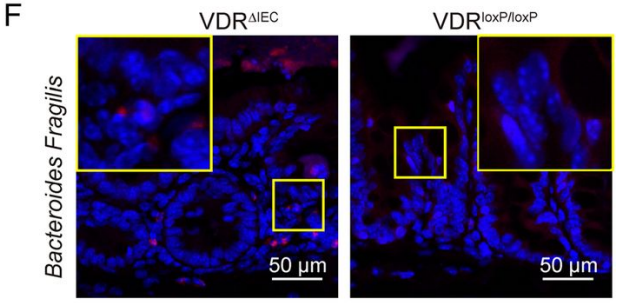




PCoA: fecal microbial communities differ

relative abundance of bacteria shifted





B. fragilis associated with IBD

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

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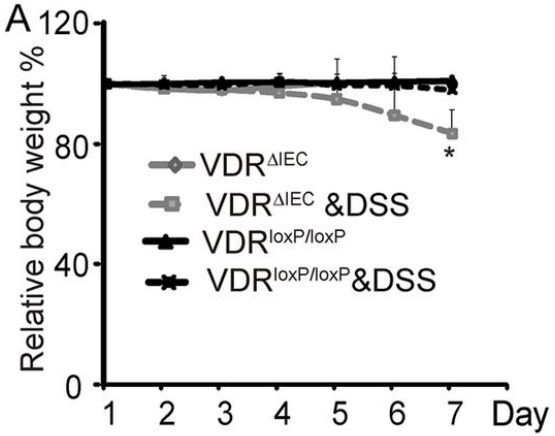
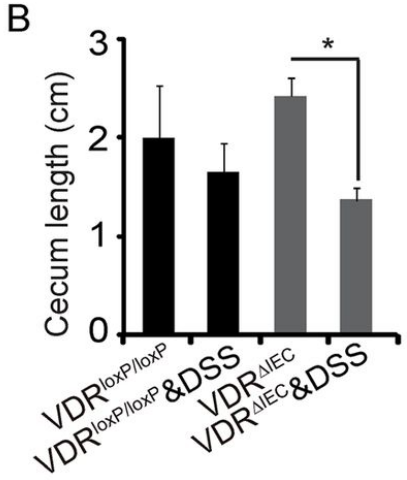
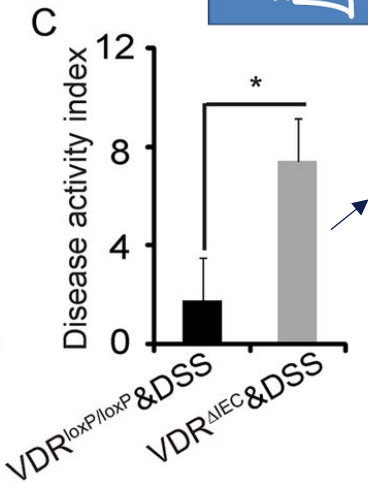
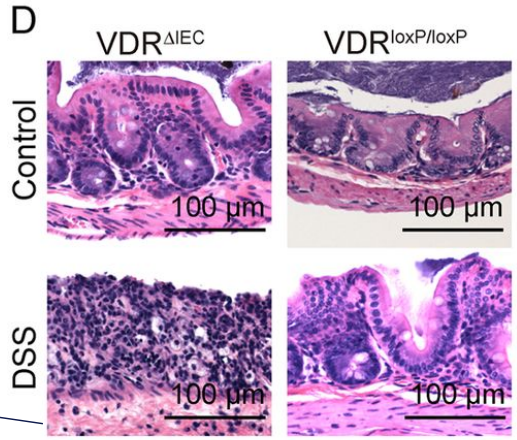
significant loss of body weight, cecum length



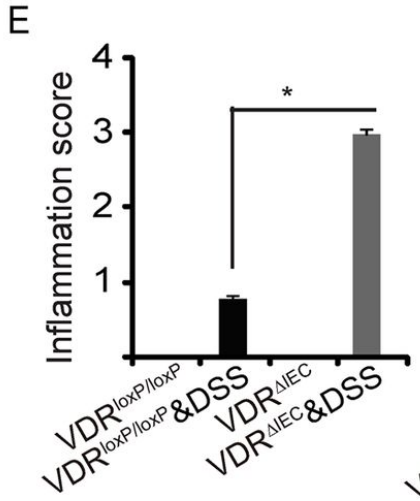


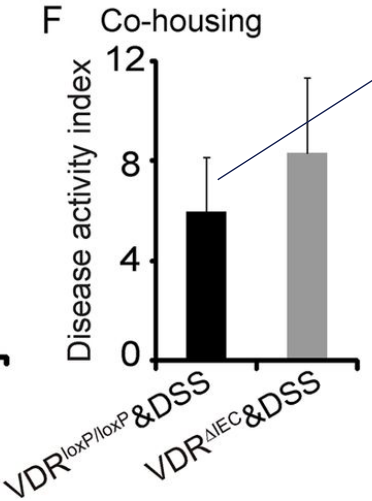


significant increase of disease activity index: fecial blood, less formed stools, more weight loss



severe intestinal inflammation





transmissibility of phenotype: increased disease activity

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Vitamin D receptor (VDR) affects patterns of Paneth cells in VDRΔIEC mice.

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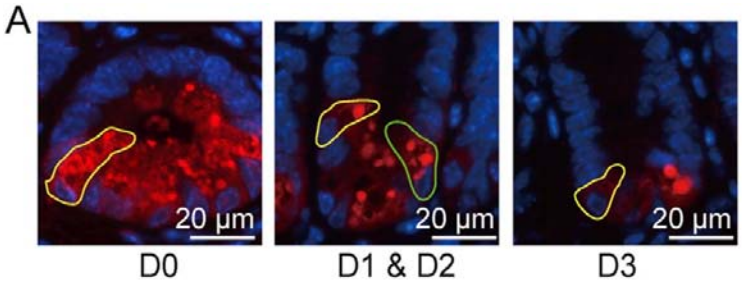
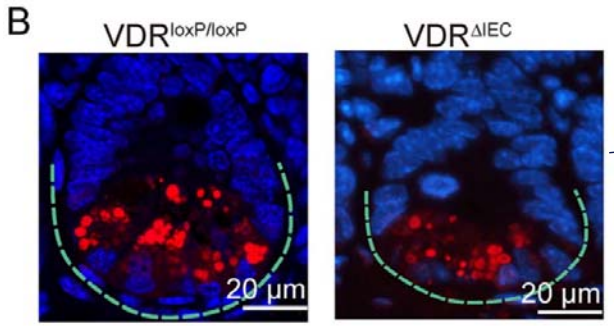
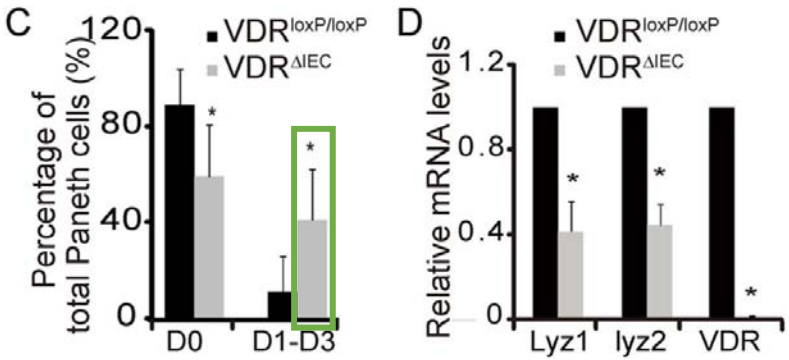
lysozyme staining for paneth cell counting



D1: disordered
D2: depleted
D3: diffuse



fewer than normal cells



decreased lysozyme in VDR$^{\Delta IEC}$

increased abnormal paneth cells

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

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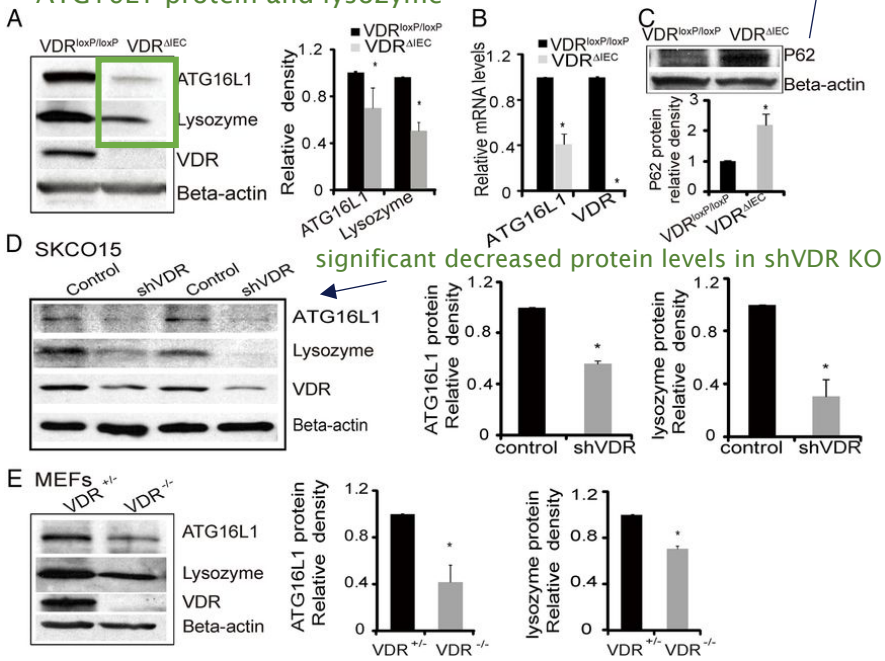
signaling adaptor that accumulates in autophagy deficient mice

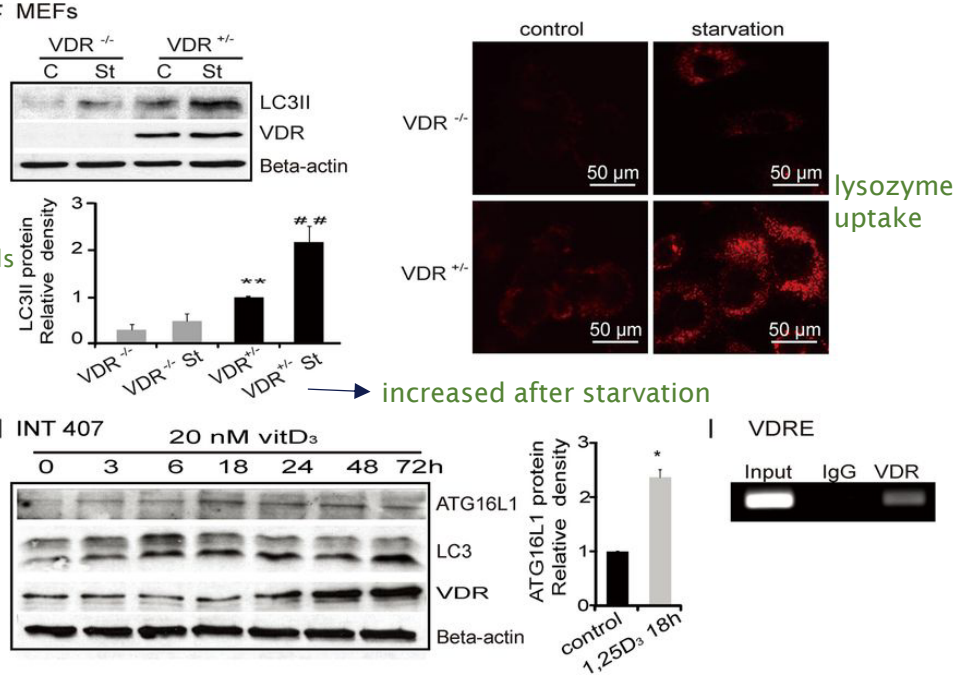
in vivo: significant reduction of ATG16L1 protein and lysozyme



significant decreased protein levels in shVDR KO cells

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

starvation-induced autophagy model



increased after starvation

VitD$_3$ 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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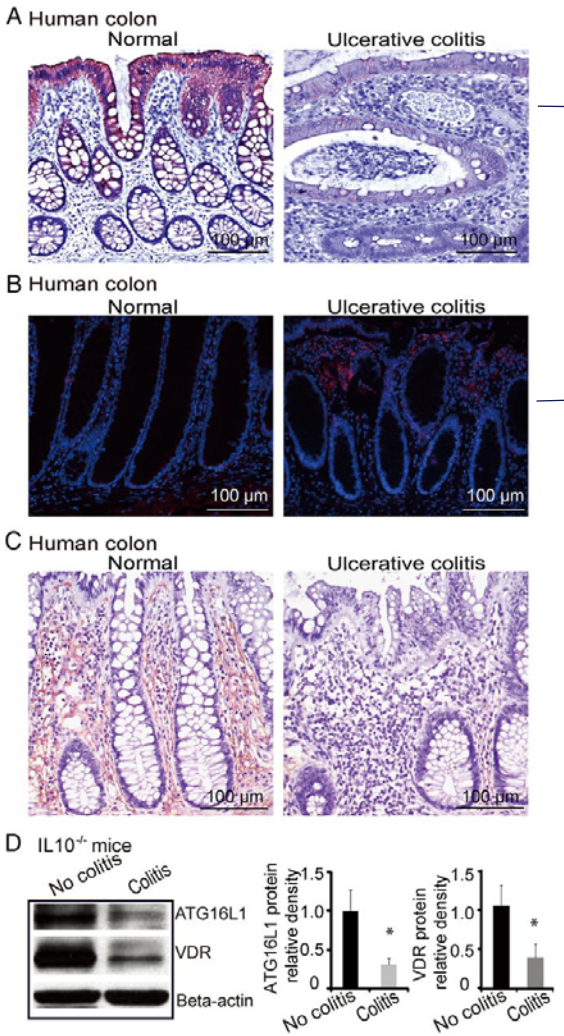

VDR expression is decreased in patients with ulcreative colitis

inflamed intestine with low VDR → increased *bacteroides* were found by FISH

staining for ATG16L1, very low signal

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Bacterial product butyrate activates vitamin D receptor (VDR) signalling pathway in human intestinal epithelial cells.

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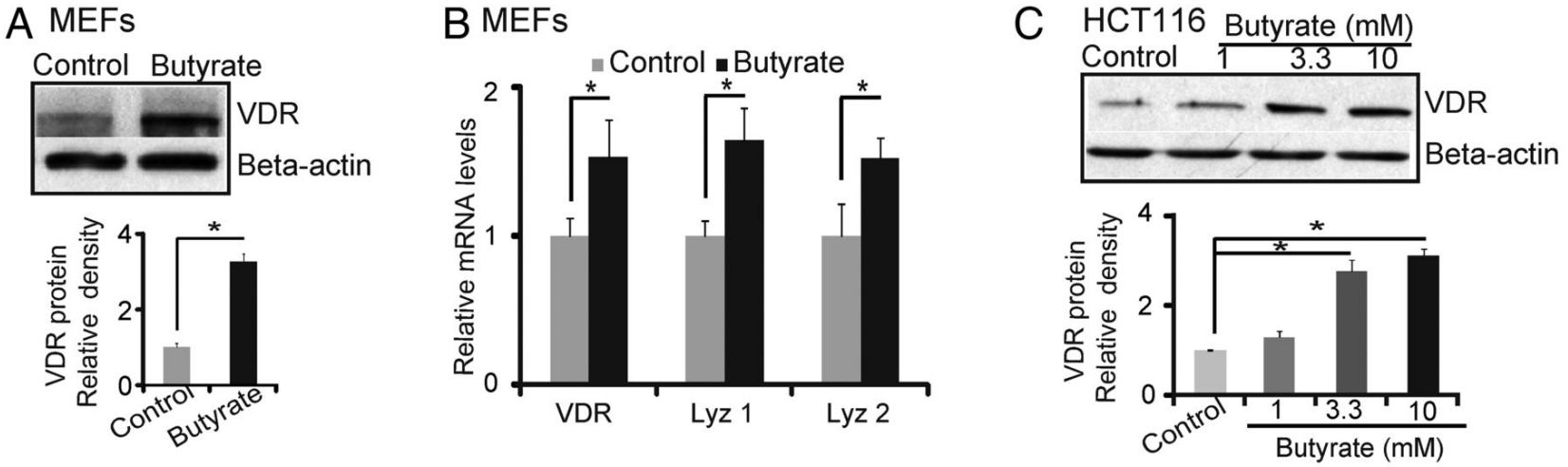
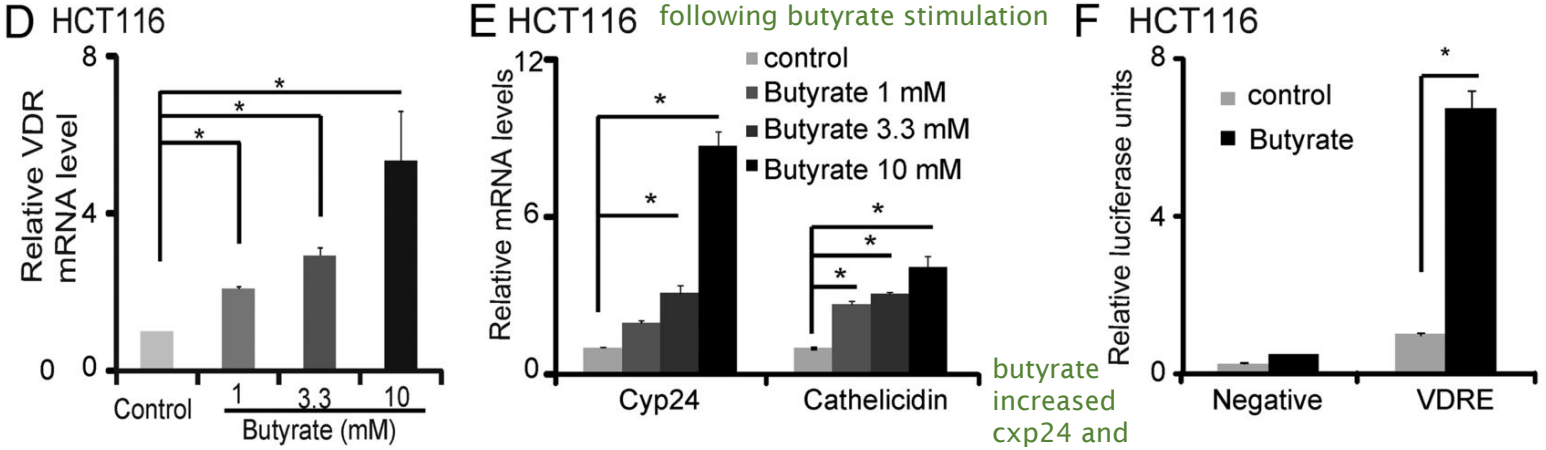
butyrate pretreated human intestinal epithelial cells → increased expression of VDR



VDR transcriptional activity following butyrate stimulation



butyrate increased cxp24 and cathelicidin mRNA

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Butyrate treatment restores vitamin D receptor (VDR) expression in colitis and inhibits inflammation.

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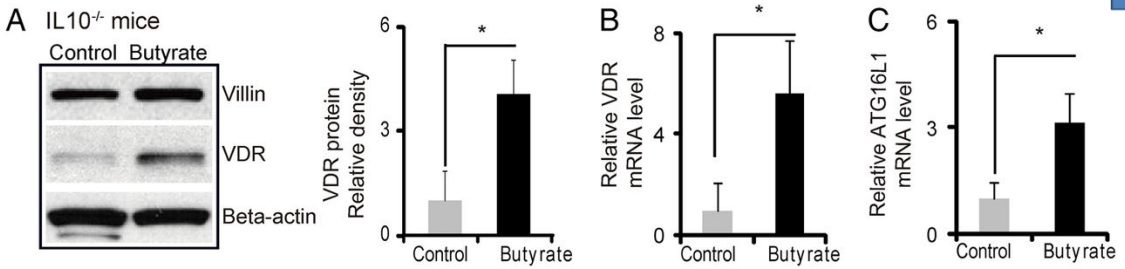
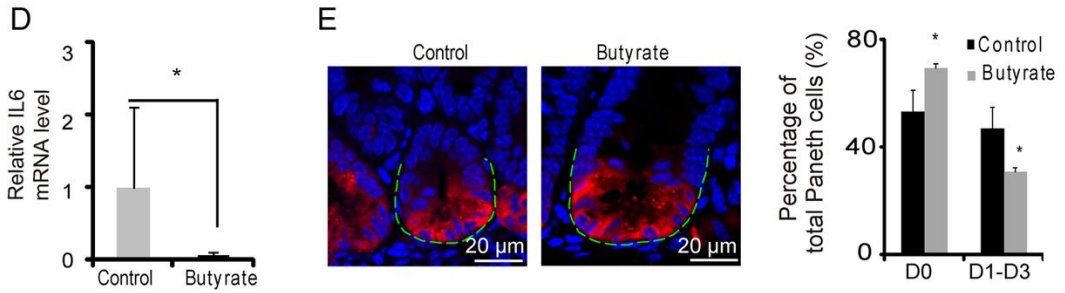
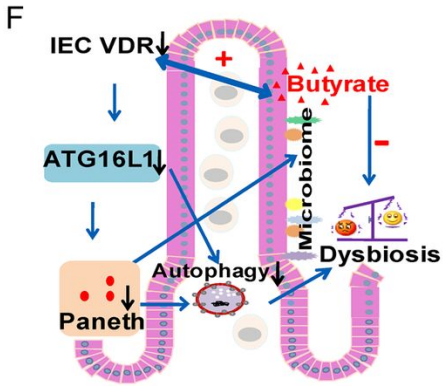
butyrate increased VDR and ATG16L1 protein in IL10$^{-/-}$ mice



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

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Discussion

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- 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 $VDR^{\Delta IEC}$ mice increase risk for colitis

1. Parkes M. *Evidence from genetics for a role of autophagy and innate immunity in IBD pathogenesis. Dig Dis 2012;30:330–3*
2. Randall-Demllo S, Chieppa M, Eri R. *Intestinal epithelium and autophagy: partners in Gut homeostasis. Front Immunol 2013;4:301.*

Discussion

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- 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

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- 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

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END. Thank you for the attention!