Vitamin D/Vitamin D Receptor Regulation of Gut Microbiome in Health and Diseases

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Innate Immune Responses and Bacterial-Epithelial Cell Interactions
Outlines

- Non-classical functions of vitamin D and VDR;
- Our recent findings on VDR regulation of microbiome and innate immunity in the context of health and inflammation; Mechanistic concepts that underlie inflammation;
- Potential therapeutic strategies to manipulate microbiota in IBD.
1. The non-classical roles of vitamin D and vitamin D receptor
Vitamin D and Health

Multiple sclerosis
(850 publications)

Colon cancer
(644 publications)

Immunity
(1170 publications)

Bone
(20344 publications)

Muscle
(2430 publications)

Blood pressure
(1518 publications)

Tuberculosis
(834 publications)

Diabetes
(3370 publications)

Searched on May 7, 2015

Most of the activity of 1,25(OH)$_2$D$_3$ is mediated by the vitamin D receptor (VDR), which presents in a wide variety of cells.
VDR action: molecular mechanism

VDR: Vitamin D receptor
RXR: Retinoid X receptor
VDRE: Vitamin D-response element

1,25 Vitamin D3

RNA Polymerase
DNA

RNA Polymerase
DNA
### Target genes of VDR signaling pathway

<table>
<thead>
<tr>
<th>Gene</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cyp27B1</td>
<td>Enzyme that catalyzes the conversion of inactive pro-vitamin D3 hormone into the active form</td>
</tr>
<tr>
<td>Cyp24</td>
<td>VDR-specific hydroxylase</td>
</tr>
<tr>
<td>Cathelicidin</td>
<td>Antimicrobial peptide</td>
</tr>
<tr>
<td>Beta defensin-4 (DEFB4)</td>
<td>Antimicrobial peptide</td>
</tr>
</tbody>
</table>
VDR and Its affected Genes

Active VDR affects transcription of at least 913 genes and impacts processes ranging from calcium metabolism to the expression of key antimicrobial peptides.

IBD pathogenesis

IBD includes Crohn’s disease (CD) and Ulcerative Colitis (UC)

Interaction of various factors contributing to chronic intestinal inflammation in a genetically susceptible host

Vitamin D and Inflammatory Bowel Diseases (IBD)

- Low vitamin D status has been reported in patients with IBD \(^1\).
- Vitamin D is an environmental factor that influences IBD \(^2\).

1. Sentongo et al., 2000
2. reviewed by Lim et al., 2005
VDR and IBD

- Polymorphisms in the VDR gene (chromosome 12) are associated with susceptibility to Crohn’s Disease\(^1\) and UC\(^2\).

- VDR IL-10 double knock out mice develop severe IBD that involved the whole intestinal tract\(^3\).

1. Simmons *et al.*, 2000
2. Dresner-Pollak *et al.*, 2004
# New target genes of VDR signaling pathway

<table>
<thead>
<tr>
<th>New target gene</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>Claudin 2</td>
<td>Tissue Barriers</td>
</tr>
<tr>
<td>ATG16L1</td>
<td>Epithelial tight junctions</td>
</tr>
<tr>
<td></td>
<td>Autophagy (self-eating), IBD risk</td>
</tr>
</tbody>
</table>

Papers from Sun lab
Wu, et al., *Gut*, 2014
The Functions of vitamin D/VDR

1,25-Dihydroxyvitamin D (1,25(OH)$_2$D$_3$), the hormonal form of vitamin D, is a multi-functional hormone

- **Calcium homeostasis and bone development**
- Immune response
- Anti-proliferation
- Anti-inflammation
- Anti-bacterial infection
- **Vitamin D and gut microbiome??**
The Good...

Challenge

the bad...

and the ugly.
Interaction among microbiome, vitamin D, and VDR signaling is a nearly unexplored area.
2. Our research on Vitamin D receptor and microbiota
VDR distribution in the normal mouse colon

DC: Distal colon
PC: Proximal colon

*P<0.05

Fermentation in the Colon

Proximal colon
- High concentration of substrates
- Saccharolysis
- Acid pH (5–6)
- Rapid bacterial growth

Distal colon
- Low substrate availability
- Proteolysis
- Neutral pH
- Slow bacterial growth

Bengmark S, et al., 1998
VDR relocation in the mouse colon after bacterial infection colonization

Germ-free

Conventionalized by bacteria

Human commensal *E. coli* F18 regulate expression of VDR in mono-associated mice

Relocation of VDR after *Salmonella* Infection

VDR+/+ Control  WT *Salmonella*  VDR-/- control

*Wu, et al. American J. of Pathology, 2010*
Salmonella directly increases VDR protein expression in intestinal cells in the absence of 1,25-vitamin D3

Salmonella colonization increased the VDR transcription activity in human epithelial cells

\[ \text{VDR Transcriptional Activity (Firefly/Renilla luciferase)} \]

- **Negative**
- **No treatment**
- **12h+SL**
- **16h+Salmonella(SL)**

**CaCO2 BBE**

**HCT116**

*Wu, et al. American J. of Pathology, 2010*
Our Central Hypothesis on Intestinal VDR

We hypothesize that VDR expression determines how host responds to bacterial triggers by inhibiting the proinflammatory signaling pathways.
VDR null mutant mice have worse outcomes with *Salmonella*-induced infection

*Wu, et al. American J. of Pathology, 2010*
VDR expression protects against *Salmonella* colonization and mucosal invasion
VDR expression decreased in UC patients
VDR, bacteria invasion, and intestinal inflammation

Wu, et al., 2010
LARGE INTESTINE

VDR signaling is altered by exposure to enteric bacteria

The vitamin D receptor (VDR) signaling pathway has important immunoregulatory and anti-inflammatory roles in gastrointestinal diseases, such as IBD and colorectal cancer. A mouse study published in the American Journal of Pathology now shows that VDR signaling protects against excessive immune responses to organisms in the intestinal lumen. “We found that VDR expression determines how intestinal epithelial cells respond to pathogenic bacterial triggers,” explains Jun Sun, the study’s corresponding author. Importantly, the effects of bacteria on VDR signaling seem to be independent of vitamin D₃, which is the VDR ligand.

Interestingly, VDR expression correlated with bacterial load, being highest in the proximal colon, where enteric bacteria grow strongly, and reduced in the distal colon, an area in which bacterial growth is limited. Furthermore, VDR-null mice exhibited a proinflammatory phenotype—indicated by increased activity of nuclear factor κB (NFκB) and high serum levels of interleukin 6—even in the absence of infection. VDR-null mice also showed a heightened response to infection with *Salmonella* compared with wild-type mice (mice lacking VDRs had greater cecal shortening, worse intestinal inflammation and increased mortality).

In wild-type mice, VDR is normally expressed by fully differentiated intestinal cells at the top of crypts. However, both VDR expression and transcriptional activity increased as a direct result of *Salmonella* infection, in conjunction with relocation of VDR expression to cells further down the crypts. “Intestinal VDR signaling responds to both commensal and pathogenic bacterial stimulation,” says Sun.

The VDR forms a complex with NFκB subunit p65 in osteoblasts, and Sun and colleagues demonstrated that this interaction also occurs in the mouse intestine *in vivo*. NFκB is an essential regulator of the innate and adaptive immune responses, but the functional relevance of this interaction has yet to be elucidated. The researchers showed that deletion of VDR completely abolished the formation of the complex with NFκB and allowed nuclear translocation of the p65 subunit, which might account for the proinflammatory features of VDR-null mice.

“VDR is an important contributor to intestinal homeostasis and host protection from bacterial invasion and infection,” conclude the researchers. “Future research could establish VDR signaling as a new target for treatment of infection and inflammatory bowel disease,” suggests Sun.

Shreya Nanda

Established intestinal epithelial cell VDR KO mice

Wu, et al., Gut 2014
Alterations of the gut microbiome in the intestinal epithelial VDR deficient (VDR^{ΔIEC}) mice

Wu, et al., Gut 2014
Deletion of intestinal VDR leads to abnormal Paneth cells

Wu, et al., Gut, 2014
Deletion of intestinal VDR leads to abnormal Paneth cells and decreased lyz.
VDR deletion leads to less autophagy protein LC3-RFP activation *in vitro*

Wu, et al., Gut, 2014
ATG16L1 is a VDR target gene
Increased VDR protein by vitamin D enhances ATG16L1 in the VDR$^{+/+}$ organoids.
VDR and ATG16L1 in human colon

Human colon Normal

Ulcerative colitis

VDR IHC

Human colon Normal

Ulcerative colitis

ATG16L1

100 µm
Working model of intestinal VDR in inflammation and dysbiosis

Wu et al., *Gut*, 2014
Mutual interactions between VDR and gut bacteria

• VDR expression, distribution, transcriptional activity, and target genes were regulated by bacterial stimulation.

• Absence of VDR leads to dysbiosis and dysfunction of innate immunity (through Paneth cells).
Limitation of identify associations between disease and microbiome

- Enormous complexity of the colonic microbiota
- Inadequate understanding of what constitutes exposure risks for commensals
- Inadequate understanding of functions of microbiome
3. The potential therapeutic role of microbiome and intestinal VDR
Probiotics LGG-CM enhances VDR protein expression *in vitro*

**Graph:**
- X-axis: Time (hours) with values 0, 0.5, 1, 2
- Y-axis: Relative Density of VDR
- C, 0.5, 1, 2 (hours) shown with corresponding bars
- VDR and β-actin bands on the gel

**Legend:**
- C: Control
- 0.5, 1, 2 (hours)
- * indicates significant difference
VDR protein expression is increased in LGG mono-associated pig model.
Vitamin D receptor pathway is required for probiotic protection in colitis

Bacterial natural product butyrate restore VDR and Paneth cells \textit{in vivo}

\textbf{Figure 3.} Butyrate treatment increases VDR mRNA and protein levels in intestinal epithelial cells in IL10\textsuperscript{/-} mice. (A) Protein and (B) mRNA of VDR in IL10\textsuperscript{/-} mice treated with butyrate. (C) Increased mRNA of ATG16L1 in butyrate treated intestine. (D) Abnormal Paneth cells were reduced in butyrate treated IL10\textsuperscript{/-} mice. n=5. *P < 0.05.
Co-housing and fecal microbiome transplantation (FMT) in colitis
Future Clinical Application

- Intestinal VDR as a clinical biomarker for identifying patients who might benefit from currently available interventions.

- Novel strategies for the prevention and treatment of human IBD and colon cancer by restoring the healthy host-microbiome interactions:

  Prebiotics, probiotics, fecal microbiome transplantation (FMT).
Target Microbiome in Prevention and Treatment of Human Diseases

Jun Sun and Eugene Chang. *Genes and Diseases* 2014
Take-home messages

- Vitamin D/VDR is an important contributor to host protection and intestinal homeostasis.

- There are interactions between microbiome and the VDR signaling pathway in health and inflammation.

- Studies on vitamin D/VDR and microbiome will provide insights for the novel therapeutic strategies in controlling chronic inflammation.
Our Team, Collaborators, and Grants

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Elaine Petrof, M.D.

**Katholieke Universiteit Leuven**
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**University of Rochester**
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**Current and Previous Sun Lab Members**
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Swim Across America Research Award
ALS Association, and Piccolo Cancer Award (J.S.)
Many Experimental Faces in Sun Lab

- Function of vitamin D receptor (VDR) in inflammatory bowel diseases and cancer.
- Novel role of bacterial protein AvrA in inhibiting inflammation and promoting cancer.
- HIV infection and cancer overgrowth
- Microbiome and intestinal stem cells.
- Alcohol injury and intestinal stem cells.
- Novel role of microbiome in ALS.
Our recent publications related to VDR

- Shaoping Wu et al., Gut, 2014
- Sonia Yoon and Jun Sun. Gastroenterology Research and Practice, 2011
- R Lu, S Wu, Y Xia, J Sun. Current Opinion in Gastro., 2010
- Shaoping Wu et al., AJ of Pathology, 2010 (Research Highlight, Nature Reviews Gastroenterology and Hepatology 7, 532 (October 2010))
- Shaoping Wu et al., International Journal of Biochemistry & Cell Biology, 2009
Summary for Vitamin D

• Prevailing theories of vitamin D are imprecise and suggest contradictory understandings of vitamin D metabolism.

• Vitamin D is not 25-Dihydroxyvitamin D or 1,25-Dihydroxyvitamin D.

• 25-hydroxyvitamin D is immunosuppressive.

• Molecular biology suggests that low levels of 25-D are a result rather than a cause of the autoimmune disease process.

• Elevated levels of 1,25-D exist at the site of disease and are an indication that the innate immune system is responding to an infection.

• The importance of sunlight.
The Truth About Vitamin D Toxicity

Is vitamin D toxic?
--Not if we take the same amount nature intended when we go out in the sun.

“Vitamin D is toxic, as it may cause hypercalcemia.”

http://www.vitamindcouncil.org/vitaminDToxicity.shtml
Butyrate can be produced

**Directly** by certain groups of bacteria:

Butyricicoccus pullicaecorum; Faecalibacterium prausnitzii, Rosebuia

**Indirectly.** By cross-feeding some butyrate producers with lactate.

Eubacterium ballii, Anaerostipes caccae, Escherichia coli.
Host-bacterial interactions that potentially mediate the gut microbiota in local intestine and distant organs in diseases

Environment/Host factors

Gut microbiota (Dysbiosis) → local → distant

Metabolites → Inflammatory cytokines → PAMPS

NEC, IBD, IBS, GI cancer, etc.

Heart diseases
Liver infection cancer
Brain Parkinson's disease Alzheimer's disease
Prostate Cancer
Kidney infection
Lung Allergy (Asthma) Infection (TB)
Islet Obesity Diabetes

Metabolites: secondary bile acids, SFAC, vitamin, etc
PAMPS: LPS, flagelin, peptidglycan, etc
Proinflammatory cytokines: IL-6, TNF-α, etc.
?: mutual interactions

Sun and Chang. Genes and Diseases, 2014
## Altered microbiota in IBD and colon cancer

<table>
<thead>
<tr>
<th>Microbiota</th>
<th>Diseases</th>
<th>Effects</th>
<th>ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>Firmicutes/Bacteroidetes ratio</td>
<td>IBD</td>
<td>↓</td>
<td>1,2</td>
</tr>
<tr>
<td>Proteobacteria</td>
<td>IBD</td>
<td>↑</td>
<td>1</td>
</tr>
<tr>
<td><em>Faecalibacterium praunitzii</em></td>
<td>CD</td>
<td>↓</td>
<td>1</td>
</tr>
<tr>
<td><em>Clostridium leptum</em></td>
<td>IBD</td>
<td>↓</td>
<td>1</td>
</tr>
<tr>
<td><em>Clostridium coccoides</em></td>
<td>IBD</td>
<td>↓</td>
<td>1</td>
</tr>
<tr>
<td><em>Enterobacteriaceae</em></td>
<td>CD</td>
<td>↑</td>
<td>1</td>
</tr>
<tr>
<td><em>Ruminococcus</em></td>
<td>CD</td>
<td>↑</td>
<td>1</td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td>UC</td>
<td>↑</td>
<td>1</td>
</tr>
<tr>
<td>sulfate-reducing bacteria</td>
<td>UC</td>
<td>↑</td>
<td>1</td>
</tr>
<tr>
<td>Coprococcus</td>
<td>UC</td>
<td>↓</td>
<td>2</td>
</tr>
<tr>
<td>Dorea</td>
<td>UC</td>
<td>↓</td>
<td>2</td>
</tr>
<tr>
<td><em>Fusobacterium nucleatum</em></td>
<td>colorectal cancer</td>
<td>↑</td>
<td>3</td>
</tr>
</tbody>
</table>

2. Inflamm Bowel Dis 2013;19:2906–2918
3. Curr Opin Gastroenterol 2013, 29:49–54
Crypt-derived intestinal organoid culture system for host–bacterial interactions
Intestinal epithelial cell VDR KO mice have higher tumor numbers

All mice treated with AOM+DSS

Azoxymethane (AOM) is a colon-specific carcinogen, dextran sulfate sodium. (DSS) can strongly promote colitis-associated colon cancer.
VDR protein expression is increased in probiotic LGG mono-associated pig model.