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Implication of intestinal VDR deficiency in inflammatory bowel disease

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ABSTRACT

Background: To investigate the function of the intestinal *Vdr* gene in inflammatory bowel disease (IBD), in conjunction with the discovery of possible metabolic markers for IBD using intestine-specific *Vdr* knockout mice.

Methods: $Vdr^{\Delta IEpC}$ mice were generated, phenotyped and treated with a time-course of 3% dextran sulfate sodium (DSS) to induce colitis. Colitis was diagnosed by evaluating clinical symptoms and intestinal histopathology. Gene expression analysis was carried out. In addition, metabolic markers of IBD were explored by metabolomics.

Results: $Vdr^{\Delta IEpC}$ mice showed abnormal body size, colon structures and feces color. Calcium, collagen, and intestinal proliferation-related gene expression were all decreased, and serum alkaline phosphatase was highly increased. In the acute model which was treated with 3% DSS for six days, $Vdr^{\Delta IEpC}$ mice showed a high score of IBD symptoms; enlarged mucosal layer and damaged muscularis layer. In the recovery experiment model, where mice were treated with 3% DSS for four days and water for three days, $Vdr^{\Delta IEpC}$ mice showed a high score of IBD symptoms; severe damage of mucosal layer and increased expression of genes encoding proinflammatory cytokines. Feces metabolomics revealed decreased concentrations of taurine, taurocholic acid, taurodeoxycholic acid and cholic acid in $Vdr^{\Delta IEpC}$ mice.

Conclusions: Disruption of the intestinal *Vdr* gene showed phenotypical changes that may exacerbate IBD. These results suggest that VDR may play an important role in IBD.

General significance

VDR function has been implicated in IBD. This is of value for understanding the etiology of IBD and for development of diagnostic biomarkers for IBD.

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1. Introduction

Inflammatory bowel disease (IBD) is a chronic disorder of the gastrointestinal tract and includes ulcerative colitis and Crohn's disease. Over 1.4 million people in the United States suffer from IBD [1]. Although the etiology of IBD is not fully understood, it is thought to involve a multi-factor interplay of inheritable components, environmental stress, microbial insults and autoimmune events [2–5]. The vitamin D receptor (Vdr), a nuclear hormone receptor, plays an essential role in normal calcium and bone homeostasis. It forms a heterodimer with the retinoid X receptor (Rxr) and binds to the vitamin D response element (VDRE). This is usually found upstream of target genes in the presence of 1,25-dihydroxyvitamin D3 [6]. Recently, genome wide association studies (GWAS) revealed that Vdr could play an important role in over 47 common diseases and traits including Crohn's disease, colorectal cancer and immune-related diseases [7]. Previously, reports have shown important links between Vdr and IBD using the mouse dextran sulfate sodium (DSS)-induced colitis model with whole body *Vdr* knockout mice [8–10]. These reports suggested that Vdr could play a critical role in innate immune responses to IBD. Furthermore, epidemiology studies revealed that calcium and vitamin D supplements could reduce the risk of IBD [11,12]. Thus, Vdr may play an important role in IBD. Although Vdr functions have been implicated in IBD using whole body VDR knockout mice, tissue-specific Vdr functions in IBD have not been investigated. Since Vdr expression may result in different pathophysiologies or metabolisms in IBD, an intestine-specific *Vdr*-null mouse was generated here to study IBD. In order to evaluate the susceptibility of these mice to colon disease, a DSS-induced colitis model was thus applied [13].

Metabolic perturbations to the intestine-specific *Vdr* knockout mice were analyzed using mass spectrometry-based metabolomics. Metabolomics is rapid, high-throughput, systems biology-based technique that can identify all the low molecular weight metabolites in

Abbreviations: VDR, vitamin D receptor; IBD, inflammatory bowel disease; ALP, alkaline phosphatase; ALT, alanine aminotransferase; qPCR, quantitative polymerase chain reaction; UPLC–ESI–QTOFMS, ultra-performance liquid chromatography–electrospray ionization–quadrupole time-of-flight mass spectrometry; DSS, dextran sulfate sodium; CHL, cholesterol; TBIL, total bilirubin; PCNA, proliferating cell nuclear antigen; LPS, lipopolysaccharides; FXR, farnesoid X receptor; H&E, hematoxylin and eosin

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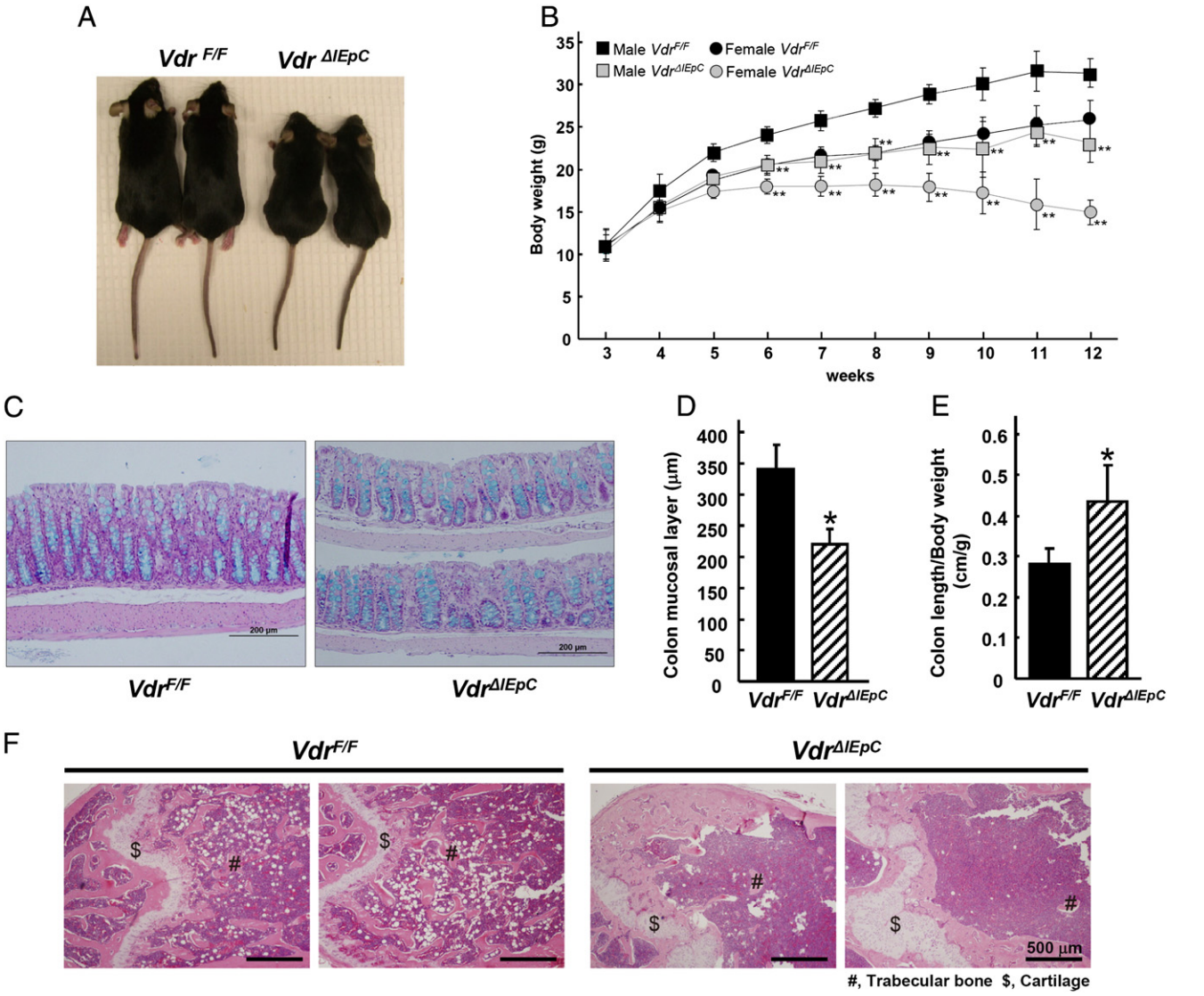

Fig. 1. Phenotypes of intestine-specific *Vdr*-null mice. A) Photography shows 10-week-old female mice having $Vdr^{F/F}$ and $Vdr^{\Delta IEpC}$ genotypes. B) Body weight changes of $Vdr^{F/F}$ (male, n = 5; female, n = 6) and $Vdr^{\Delta IEpC}$ (male, n = 5; female, n = 6) mice are shown in different ages. C) Colons (middle) from 10-week-old male $Vdr^{F/F}$ (n = 8) and $Vdr^{\Delta IEpC}$ (n = 4) mice were stained with H&E plus Alcian blue (for goblet cells). D) Thickness of colon mucosal layer (middle) was measured from $Vdr^{F/F}$ (n = 3) and $Vdr^{\Delta IEpC}$ (n = 3) mice from the H&E stained slides using the DP Controller Program (ver. 3.1.1.267, Olympus corporation). E) Colon length from $Vdr^{F/F}$ (n = 14) and $Vdr^{\Delta IEpC}$ (n = 10) was measured. F) Distal femur were stained with H&E from 12-week-old male $Vdr^{F/F}$ and $Vdr^{\Delta IEpC}$ mice. Duo, Duodenum; Jej, ejunum; Ile, ileum; Co, colon; Ce, cecum; Sto, stomach; Bra, brain; Ki, kidney; He, heart; Lu, lung. Data are shown as mean ± SD.*, $p<0.00001$; **; $p<0.0001$ compared with $Vdr^{F/F}$ mice.

biofluids, cells, tissues and organisms. Metabolic profiles provide a meaningful snapshot for understanding the metabolic changes that occur following genetic variation, pathophysiological changes and xenobiotic challenges [14,15], and can show subtle changes between experimental groups. Previously, metabolomics has been applied for the diagnosis of disease [16,17] and the characterization of disease-related animal models [18–20].

2. Methods

2.1. Materials

DSS (35–44 kDa) was purchased from MP Biomedicals (Aurora, Ohio, USA). Taurine, taurocholic acid, taurodeoxycholic acid, cholic acid, deoxycholic acid and other chemicals were obtained from Sigma-Aldrich (St. Louis, MO, USA). Anti-VDR, anti-COX-2 and anti-actin were purchased from Santa Cruz Biotechnology (Santa Cruz, California, USA). Anti-p-ERK1/2, anti-ERK1/2, anti-p-p65, anti-p65, anti-p-Akt, anti-Akt and secondary antibody were obtained from Cell Signaling Technology (Beverly, MA, USA).

2.2. Generation of intestine-specific Vdr-null mice

Intestinal epithelial cell-specific *Vdr*-null mice, designated $Vdr^{\Delta IEpC}$, were generated by crossing *Vdr*-floxed mice, designated $Vdr^{F/F}$, with mice carrying the villin-Cre transgene [21]. Villin-Cre transgenic mice were provided by Deborah L. Gumucio (University of Michigan). Cre-mediated recombination resulted in the deletion of exon 2 of the *Vdr* gene. The male mice ($Vdr^{flox/WT}$; villin-Cre+) F1 mice were interbred with female $Vdr^{flox/flox}$ littermates lacking villin-Cre. All mice were genotyped by polymerase chain reaction (PCR). $Vdr^{flox/flox}$; villin-Cre (designated $Vdr^{\Delta IEpC}$) and $Vdr^{flox/flox}$; villin-Cre^{-} ($Vdr^{F/F}$) mice were used for the following experiments. PCR genotyping for the *Vdr* floxed and recombined alleles was carried out using specific designed primers,