Published in final edited form as:

Dig Dis Sci. 2014 April ; 59(4): 753–759. doi:10.1007/s10620-013-2902-8.

The Importance of the Retinoid X Receptor Alpha in Modulating Inflammatory Signaling in Acute Murine Colitis

Rebecca Knackstedt, Sun Shaoli, Vondina Moseley, and Michael Wargovich

Medical University of South Carolina, Charleston, SC, USA

Rebecca Knackstedt: rew27@musc.edu

Abstract

Background—In order for vitamin D to signal and regulate inflammatory pathways, it must bind to its receptor (VDR) which must heterodimerize with the retinoid X receptor alpha (RXR α).

Although the role that vitamin D signaling plays in the development and progression of colitis, a disease characterized by excessive inflammation, has been suggested, little research has been done on determining the role that RXR α plays in acute colitis development.

Aims—This study sought to determine the effects that reduced availability of RXR α would have on the development of acute murine colitis. Expression of inflammatory markers, VDR and RXR α were investigated to determine if the reduction in expression of RXR α in RXR α ^{+/-} mice would result in increased inflammatory signaling and receptor downregulation as compared to their wild-type littermates.

Methods—An acute murine model of colitis, the axoymethane (AOM) and dextran sulfate sodium (DSS) model was utilized in wild-type and RXR α ^{+/-} mice. Gross manifestations of colitis measured included weight loss and colitis score. Immunoblots and real-time PCR were performed for inflammatory markers and receptor expression.

Results—RXR α ^{+/-} mice induced with AOM/DSS colitis demonstrated increased gene expression of Snail and Snail2, transcription factors downstream of inflammatory mediators, as compared to their wild-type littermates.

Conclusions—This demonstrates the importance of RXR α in regulating inflammation in acute colitis and also identifies RXR α expression as a new consideration when developing successful interventions for acute colitis due to the requirement of numerous receptors for RXR α .

Keywords

RXR α ; Colitis; Inflammation; Vitamin D receptor

Introduction

Retinoid X receptor alpha (RXR α), the receptor for 9-cis retinoic acid [1], is a member of the steroid nuclear receptor super family [2, 3]. RXR α is required by numerous receptors, including the vitamin D receptor (VDR) and the peroxisome proliferator-activated receptor gamma (PPAR γ) as a heterodimerization partner. Without RXR α to heterodimerize with, these receptor would not be able to allow their ligands to influence cellular signaling.

Vitamin D signals through VDR to control many pathways, including inflammation. After VDR and RXR α bind to their respective ligands and heterodimerize, they move to interact with vitamin D response elements (VDRE) in DNA. When binding VDRE, VDR binds the 3' half-site and RXR α binds the 5' half-site [4]. If this binding is reversed, gene repression results [5, 6]. Thus, RXR α availability and functionality is required for vitamin D to regulate gene expression [7]. Vitamin D has been linked to cancer development and progression through its regulation of proto-oncogene and tumor-suppressor genes [8–12], yet its role in disease states that can ultimately progress to cancer, such as colitis, remains less understood.

Ulcerative colitis (UC) is an inflammatory disease of the colon that can ultimately progress to colitis-associated cancer (CAC). The importance of vitamin D in colitis development was first proposed when it was observed that as distance from the equator increases, the prevalence of colitis also increases [13]. Up to 50 % of UC patients suffer from vitamin D deficiency with around 10 % having a severe deficiency that can impact quality of life [14]. It has yet to be determined if the observed vitamin D deficiency is a cause, effect, or both, of UC development and progression.

The roles of RXR α and PPAR γ in colon homeostasis and colitis development have been illustrated through in vivo experiments. PPAR γ ligands, thiazolidinediones, were able to ameliorate the dextran sulfate sodium (DSS) and 2,4,6-trinitrobenzene sulfonic acid (TNBS) models of colitis in mice [15–18]. RXR α agonists were also effective at reducing TNBS induced colitis with a synergistic effect observed when combined with PPAR γ agonists. PPAR γ ^{+/-} and RXR α ^{+/-} mice demonstrated an increased susceptibility to TNBS induced colitis [18]. However, the majority of this work was done in the TNBS model which more closely resembles Crohn's Disease with little research using the DSS model which models UC.

Our laboratory has demonstrated that VDR is downregulated in murine colitis and that RXR α is downregulated in colon cancer cell lines, colorectal cancer [19], murine colitis and CAC [20]. The downregulation of VDR has been linked to Snail and Snail2, zinc-finger transcription factors [21–23] that are upregulated or stabilized by inflammatory mediators [24–28]. Snail and Snail2 are expressed in diseased tissue of UC and colorectal cancer (CRC) and their expression corresponds to a localized downregulation of VDR [22, 29–31]. No definitive mechanism for the downregulation of RXR α has emerged.

To investigate the role that RXR α plays in regulating inflammatory signaling and colitis development, RXR α ^{+/-} mice and their wild-type littermates were challenged with the well characterized azoxymethane (AOM) and dextran sulfate sodium (DSS) model of UC. An

acute model was chosen to elucidate changes that occur early in colitis development. Gross manifestations of colitis were measured to determine if heterozygotes were less capable of withstanding the AOM/DSS challenge. The expression of VDR and RXR α mRNA and protein, along with the mRNA of inflammatory markers and Snail and Snail2 were analyzed. These experiments demonstrate, for the first time, the importance of RXR α in regulating inflammatory markers activated in the AOM/DSS colitis model.

Materials and Methods

Mice

RXR $\alpha^{+/-}$ mice and their wild-type littermates were a gift from Dr. Steven Kubalal (Medical University of South Carolina, Department of Regenerative Medicine and Cell Biology). Geneotyping was performed in Dr. Kubalal's lab prior to transfer. These mice were induced to have acute colitis with 11 heterozygote or wild-type littermates per treatment modality (colitis or control). Mice were cared for within Institutional Animal Care Committee (IACUC) guidelines and all procedures were approved by the Medical University of South Carolina (MUSC) IACUC. Mice were housed in groups of five at 22–24 °C using a 12 h light–12 h dark cycle with lights on at 06:00. Animals were fed normal chow for the duration of the experiment (Harlan Teklad Diet 2918).

Induction of Colitis

To induce colitis, DSS (MW 36,000–50,000 D, MP Biomedical, Santa Ana, CA) and AOM (Sigma-Aldrich, St. Louis, MO) were utilized. Twenty-two heterozygote and 22 wild-type mice were divided into control and treatment groups based on sample size calculations. For the acute AOM/DSS model, mice were allowed to acclimate for 1 week and were then injected intraperitoneally with 10 mg/kg AOM or saline (control) on day eight. The mice recovered for 1 week with water, and on day fifteen, the mice injected with AOM were given 2 % DSS in water for 7 days and the mice injected with saline remained on normal water. The mice were sacrificed on the seventh day of the DSS/water treatment.

Study Design

Eleven heterozygote or wild-type mice were used per treatment group (colitis or control). The colons from six mice were used for RNA and protein extraction and the remaining five mice had their colons Swiss-rolled as described [32] for colitis scoring. All experiments were done in biological and technical duplicates.

Sacrifice and Tissue Harvesting

Mice were sacrificed via CO₂ inhalation followed by cervical dislocation. Blood was removed via cardiac puncture, allowed to clot at room temperature for 1 h, centrifuged for 15 min at 1,000×g and plasma was removed. The colon of each mouse was removed, measured, flushed with ice-cold PBS, flayed and the mucosa was scraped and separated into two fractions. One fraction was flash frozen in liquid nitrogen and the other fraction was placed in RNeasy lysis buffer (Qiagen, Grand Island, NY) and then flash frozen in liquid nitrogen.

Vitamin D Quantification

Plasma was transported to the laboratory of Dr. Bruce Hollis (Medical University of South Carolina, Department of Pediatrics) for quantification of systemic 25(OH)-vitamin D via a 25(OH)-vitamin D radioimmunoassay as described [33].

Colitis Scoring

The colons from mice reserved for colitis scoring were removed, flushed with ice-cold PBS, flayed and Swiss-rolled. Colons were fixed overnight in 70 % ethanol and paraffin embedded. Five-micrometer sections were cut and stained with H&E. H&E stained slides were scored blindly by Dr. Shaoli Sun (Medical University of South Carolina, Department of Pathology) on a scale from 0 to 4 as described [34]. Briefly, grade 0 was normal colon tissue, grade 1 was mild focal ulceration, grade 2 was moderate multifocal ulceration, grade 3 was moderate to severe multifocal ulceration and grade 4 was widespread ulceration.

Protein Extraction and Immunoblotting

The colonic mucosa fraction not placed in RNA later was homogenized in 500 μ L of T-Per tissue protein extraction (Thermo Scientific, Rockport, IL) and 0.05 % protease inhibitor cocktail via sonification. The homogenate was centrifuged at 10,000 $\times$ g for 5 min and the supernatant was collected. Protein purity and concentration was quantified with a GE Nanovue. Protein samples (standardized to 50 μ g of nuclear protein per lane) were mixed in loading buffer containing 2 % sodium dodecyl sulfate and 10 % β -mercaptoethanol. Protein was denatured at 95 $^{\circ}$ C for 5 min and then run in a 10 % polyacrylamide gel with a Precision Plus Protein Standard (BioRad, Hercules, CA). Proteins were transferred to a nitrocellulose membrane at 65 mA for 4 h. The blot was saturated in PBS and 0.1 % Tween 20 (PBS-T buffer) containing 10 % nonfat dry milk at 4 $^{\circ}$ C for a minimum of 1 h and then incubated overnight at 4 $^{\circ}$ C with the appropriate primary antibody. Antibodies used were anti-VDR, -RXR α (Millipore, Billerica, MA) and -GAPDH (Santa Cruz Biotechnology, Santa Cruz, CA) as a loading control diluted 1:1,000–1:5,000 in 5 % nonfat dry milk. Blots were washed three times in PBS-T for 10 min at room temperature before incubating with the appropriate horseradish peroxidase secondary antibody (Santa Cruz Biotechnology, Santa Cruz, CA) diluted 1:10,000–1:20,000 in 5 % nonfat dry milk for 2 h at room temperature. Blots were then washed three times in PBS-T for 10 min each at room temperature and detection of protein was performed using West Pico and Femto blot detection reagents (Thermo Scientific, Pittsburgh, PA). Films were scanned and bands were quantified using Image J software. Proteins of interest were normalized to GAPDH for total cellular protein.

RNA Extraction and Real-Time PCR

RNA was isolated from the mucosa scraping placed in RNAlater with a miRNAeasy kit (Qiagen, Valencia, CA) according to manufacturer's instructions and each sample was resuspended in 40 μ L RNase-free water. The RNA purity and concentration was quantified with a GE Nanovue. Reactions were set up in duplicate for each sample. PCR was performed in a 25 μ L reaction containing 12.5 μ L of the 2X SYBR PCR reaction mix, 300 nM of each primer, 0.5 μ L of iScript reverse transcriptase and 25–150 ng of RNA. Reaction protocol was as follows: initial incubations at 50 $^{\circ}$ C for 10 min to allow for CDNA

synthesis, reverse transcriptase inactivation at 95 °C for 5 min and then 40 cycles of PCR cycling and detection with 95 °C for 10 s, 55 °C for 30 s and 95 °C for 1 min. The melt curve was done at 55 °C for 1 min and 80 cycles of 0.5 °C increments from 55 °C to 95 °C. Primer sequences were: RXR α 5'-CCATGAACCCTGTGAGCAG-3' (sense) and 5'-CCTCTTGAAGAAGCCCTTGC-3' (anti-sense), VDR 5'-ATGGCGGCCAGCACTTCCCTGCCTGAC-3' (sense) and 5'-CTCCTCCTTCCGCTTCAG GATCATCTC-3' (anti-sense), GAPDH 5'-CCCAGCAAGGACACTGAGCAAG-3' (sense) and 5'-AGGCCCCTCCTGTTATTATGGGG-3' (anti-sense), COX-2 5'-CCAGGGCCCTTCCCTCCCGTAG-3' (sense) and 5'-TGAGCCTTGGGGGTCAGGGA-3' (anti-sense), Snail 5'-TGTACCCGCCAGAGCCTCC-3' (sense) and 5'-CCCCTGAGCGGGGTTCAAGC-3' (anti-sense), Snail2 5'-GCCGGGTGACTTCAGAGGCG-3' (sense) and 5'-GATAACGGTCCAGGCGGCGG-3' (anti-sense) and TNF α 5'-TGTCCCTTTCACTCACTGGC-3' (sense) and 5'-CATCTTTTGGGGGAGTGCCT-3' (anti-sense).

Statistical Analysis

For the statistical analysis of immunoblotting and real time PCR data, expression levels from the animals were averaged in the control or treated group and the averages of treated and control mice were compared via a Student's *t* test. To perform comparisons between the wild-type and heterozygote mice, a two-way ANOVA with interactions was used to evaluate differences due to genotype.

Results

Acute Challenge with AOM/DSS Results in Colitis in Heterozygote and Wild-Type Littermates

To study murine colitis, the widely accepted AOM/DSS murine model of UC was utilized. Mice were injected at 6 weeks of age intraperitoneally with 10 mg/kg of AOM and, after 7 days of recovery, were challenged with 2 % DSS for 7 days. This resulted in a decrease in body weight for heterozygote and wild-type mice associated with the DSS challenge with the difference observed between the heterozygotes and wild-types not statistically significant (Fig. 1). Gross blood loss, an indicator of disease severity, became apparent for both heterozygote and wild-type mice on the sixth day of the DSS challenge, 2 days prior to sacrifice. Both heterozygote and wild-type mice had a statistically significant decrease in systemic vitamin D. The average systemic vitamin D level for treated wild-type mice was 19.45 ± 7.05 ng/mL with the average for control wild-type mice being 25.65 ± 1.68 ng/mL ($p = 0.03$). The average for treated heterozygotes was 16.5 ± 2.26 ng/mL with the average for control heterozygotes being 25.45 ± 6.56 ng/mL ($p = 0.03$). The decreases measured in heterozygote and wild-type mice were not statistically different from one another. There was no statistical difference in the colitis scoring between the heterozygote and wild-type mice (data not shown).

Acute Colitis Results in Increased Inflammatory Marker Expression in Heterozygotes as Compared to Their Wild-Type Littermates

To analyze expression of receptors and inflammatory markers, real time PCR results or immunoblots were quantified and the average expression levels were calculated for the heterozygote and wild-type mice. Control heterozygote mice demonstrated a significant downregulation of RXR α mRNA ($p = 0.0004$) and protein trended toward downregulation, compared to wild-type mice (Fig. 2a, b). RXR α mRNA was not significantly downregulated in heterozygote or wild-type treated mice. VDR mRNA was significantly downregulated in both heterozygote and wild-type treated mice and TNF α and COX-2 expression were significantly upregulated in both heterozygote and wild-type treated mice. There was no statistical difference in the upregulation or downregulation in these genes when heterozygotes were compared to wild-type mice. Snail and Snail2 mRNA were both statistically upregulated in the heterozygote treated mice (Fig. 3a, b). RXR α protein was not significantly downregulated in either the treated heterozygote or wild-type mice whereas VDR protein was significantly downregulated in treated heterozygote and wild-type mice with no statistical difference between the heterozygote and wild-type mice (Fig. 4).

Discussion

The anti-inflammatory role of vitamin D has been well established [35–38] and its role in colitis development and progression is being explored. This is highlighted by our recent finding that a vitamin D deficient diet results in a more severe acute murine colitis with increased mortality, inflammatory signaling and receptor downregulation [20]. Although VDR's requirement for RXR α has long been recognized [7], the effects that a reduction in the availability of RXR α would have on vitamin D's regulation of inflammatory signaling in the context of acute AOM/DSS murine colitis have yet to be determined.

As RXR α knockout mice are embryonic lethal likely due to heart malformations [39], RXR α ^{+/-} mice are an ideal system in which to study the inflammatory effects that would result due to a reduction in the availability of RXR α . We found that heterozygote mice had decreased RXR α mRNA but not protein compared to wild-type mice. The lack of RXR α protein downregulation in our study is likely due to the small sample size. The AOM/DSS model is an accepted model for colitis-associated-cancer. DSS or AOM/DSS can be used acutely to produce an acute colitis; however, we and others have found that adding AOM to the regime can enhance lesion progression [40].

When challenged with acute AOM/DSS colitis, heterozygote mice and their wild-type littermates did not react grossly different to the AOM/DSS challenge as the weight loss and colitis scores observed in heterozygote and wild-type mice were not statistically significant from one another. RXR α mRNA and protein were not found to be significantly downregulated in either the heterozygote or wild-type treated mice. However, as RXR α ^{+/-} mice express less basal RXR α mRNA and likely protein, this degree of downregulation in treated mice may be more significant than in wild-type mice. Although the only statistically significant difference between heterozygote and wild-type mice was the upregulation of Snail and Snail2 mRNA in heterozygote mice, this could have important ramifications.

Snail and Snail2 are transcription factors that are stabilized by inflammatory signaling [24–28] and are known to downregulate VDR expression [21–23]. They are expressed in the ulcerated tissue of UC patients corresponding with a localized downregulation of VDR expression [22, 29, 30]. The early upregulation of Snail and Snail2 observed in this study in heterozygote mice could lead to a vicious inflammatory cycle. If Snail and Snail2 are overexpressed early, as found in this acute colitis model which only lasted 2 weeks, this could lead to a further transcriptional downregulation of VDR that would incapacitate the anti-inflammatory effects of vitamin D, lead to increased inflammatory signaling as well as subsequent increased Snail and Snail2 expression. VDR was downregulated in both heterozygote and wild-type mice, whereas Snail and Snail2 were only upregulated in heterozygote mice, indicating another mechanism for early VDR downregulation was likely also at play; however, the upregulation of Snail and Snail2 could lead to further VDR downregulation in heterozygote mice if the model was allowed to progress. As RXR α does have numerous signaling partners, it is unclear if upregulation of Snail and Snail2 observed in our experiments are solely due to the reduced ability of vitamin D to quell the inflammatory cascade; however, it remains a valid explanation. Perhaps, if a chronic model of colitis or a CAC model was investigated, heterozygote mice would begin to demonstrate increased gross colitis symptomology, or, due to the early upregulation of Snail and Snail2, an increased downregulation of VDR.

One limitation of this study is the lack of an in vitro model for colitis. As colitis is a disease state that involves epithelial cells, stroma and inflammatory cells, it cannot simply be modeled in vitro. The availability of an in vitro model would allow for a further investigation into the mechanism of VDR downregulation observed in our in vivo model. With this limitation, we can only conclude from our experiments, the importance of RXR α in modulating acute colitis development.

Our lab has shown that RXR α is downregulated in colon cancer cell lines, human CRC [19] and murine colitis and CAC [20], yet a definite mechanism of downregulation has yet to emerge. Vitamin A deficiency and administration acute phase reactants have shown to downregulate RXR α in mice, but these mechanisms have yet to be fully explained [41–45]. If a mechanism through which RXR α is silenced is identified, targeted approaches to increasing RXR α could be explored, thus potentially restoring the signaling of vitamin D and PPAR λ ligands, allowing vitamin D to control inflammation and decrease the upregulation of Snail and Snail2. This may have clinical applicability in colon cancers that are unresponsive to vitamin D interventions [46] or if PPAR λ ligands are ever translated to the clinic. These experiments demonstrate, for the first time, the importance that VDR's signaling partner, RXR α , has in regulating inflammation in the setting of acute murine colitis.

Acknowledgments

The present work benefited from the input of Elizabeth Garrett-Mayer, PhD, a statistician (Medical University of South Carolina, Department of Public Health Sciences), from Bruce Hollis, PhD and Renee Washington (Medical University of South Carolina, Department of Pediatrics) who provided systemic vitamin D quantification and from Steven Kubalak, PhD and Jayne Bernake (Medical University of South Carolina, Department of Regenerative Medicine and Cell Biology) who provided the mice for experimentation as well as genotyping. This work was supported by NIH 2RO1CA96694 from the National Cancer Institute.

References

- Heyman RA, Mangelsdorf DJ, Dyck JA, Stein RB, et al. 9-cis retinoic acid is a high affinity ligand for the retinoid X receptor. *Cell*. 1992; 68:397–406. [PubMed: 1310260]
- Mangelsdorf DJ, Thummel C, Beato M, Herrlich P, et al. The nuclear receptor superfamily: the second decade. *Cell*. 1995; 83:835–839. [PubMed: 8521507]
- Carlberg C, Polly P. Gene regulation by vitamin D3. *Crit Rev Eukaryot Gene Expr*. 1998; 8:19–42. [PubMed: 9673449]
- Kurokawa R, Yu VC, Naar A, Kyakumoto S, et al. Differential orientations of the DNA-binding domain and carboxy-terminal dimerization interface regulate binding site selection by nuclear receptor heterodimers. *Genes Dev*. 1993; 7:1423–1435. [PubMed: 8392479]
- Mackey SL, Heymont JL, Kronenberg HM, Demay MB. Vitamin D receptor binding to the negative human parathyroid hormone vitamin D response element does not require the retinoid x receptor. *Mol Endocrinol*. 1996; 10:298–305. [PubMed: 8833658]
- Demay MB, Kiernan MS, DeLuca HF, Kronenberg HM. Sequences in the human parathyroid hormone gene that bind the 1,25-dihydroxyvitamin D3 receptor and mediate transcriptional repression in response to 1,25-dihydroxyvitamin D3. *Proc Natl Acad Sci USA*. 1992; 89:8097–8101. [PubMed: 1325645]
- Haussler MR, Haussler CA, Jurutka PW, Thompson PD, et al. The vitamin D hormone and its nuclear receptor: molecular actions and disease states. *J Endocrinol*. 1997; 154:S57–S73. [PubMed: 9379138]
- Muto A, Kizaki M, Yamato K, Kawai Y, et al. 1,25-Dihydroxyvitamin D3 induces differentiation of a retinoic acid-resistant acute promyelocytic leukemia cell line (UF-) associated with expression of p21(WAF1/CIP1) and p27(KIP1). *Blood*. 1999; 93:2225–2233. [PubMed: 10090931]
- Liu M, Lee MH, Cohen M, Bommakanti M, et al. Transcriptional activation of the Cdk inhibitor p21 by vitamin D3 leads to the induced differentiation of the myelomonocytic cell line U937. *Genes Dev*. 1996; 10:142–153. [PubMed: 8566748]
- Sinkkonen L, Malinen M, Saavalainen K, Vaisanen S, et al. Regulation of the human cyclin C gene via multiple vitamin D3-responsive regions in its promoter. *Nucleic Acids Res*. 2005; 33:2440–2451. [PubMed: 15863722]
- Tu-Yu AH, Morris RC, Ives HE. Differential modulation of fos and jun gene expression by 1,25-dihydroxyvitamin D3. *Biochem Biophys Res Commun*. 1993; 193:161–166. [PubMed: 8503903]
- Yanagisawa J, Yanagi Y, Masuhiro Y, Suzawa M, et al. Convergence of transforming growth factor-beta and vitamin D signaling pathways on SMAD transcriptional coactivators. *Science*. 1999; 283:1317–1321. [PubMed: 10037600]
- Levin AD, Wadhwa V, Leach ST, Woodhead HJ, et al. Vitamin D deficiency in children with inflammatory bowel disease. *Dig Dis Sci*. 2011; 56:830–836. [PubMed: 21222159]
- Ulitsky A, Ananthakrishnan AN, Naik A, Skaros S, et al. Vitamin D deficiency in patients with inflammatory bowel disease: association with disease activity and quality of life. *JPEN J Parenter Enteral Nutr*. 2011; 35:308–316. [PubMed: 21527593]
- Su CG, Wen X, Bailey ST, Jiang W, et al. A novel therapy for colitis utilizing PPAR-gamma ligands to inhibit the epithelial inflammatory response. *J Clin Invest*. 1999; 104:383–389. [PubMed: 10449430]
- Saubermann LJ, Nakajima A, Wada K, Zhao S, et al. Peroxisome proliferator-activated receptor gamma agonist ligands stimulate a Th2 cytokine response and prevent acute colitis. *Inflamm Bowel Dis*. 2002; 8:330–339. [PubMed: 12479648]
- Schaefer KL, Denevich S, Ma C, Cooley SR, et al. Intestinal antiinflammatory effects of thiazolidinedione peroxisome proliferator-activated receptor-gamma ligands on T helper type 1 chemokine regulation include nontranscriptional control mechanisms. *Inflamm Bowel Dis*. 2005; 11:244–252. [PubMed: 15735430]
- Desreumaux P, Dubuquoy L, Nutten S, Peuchmaur M, et al. Attenuation of colon inflammation through activators of the retinoid X receptor (RXR)/peroxisome proliferator-activated receptor gamma (PPARgamma) heterodimer. A basis for new therapeutic strategies. *J Exp Med*. 2001; 193:827–838. [PubMed: 11283155]

19. Volate SR, Muga SJ, Issa AY, Nitcheva D, et al. Epigenetic modulation of the retinoid X receptor alpha by green tea in the azoxymethane-Apc Min/+ mouse model of intestinal cancer. *Mol Carcinog.* 2009; 48:920–933. [PubMed: 19378291]
20. Knackstedt RW, Moseley VR, Sun S, Wargovich MJ. Vitamin D receptor and retinoid X receptor alpha status and vitamin D insufficiency in models of murine colitis. *Cancer Prev Res (Phila).* 2013; 6:585–593. [PubMed: 23585425]
21. Larriba MJ, Bonilla F, Munoz A. The transcription factors Snail1 and Snail2 repress vitamin D receptor during colon cancer progression. *J Steroid Biochem Mol Biol.* 2010; 121:106–109. [PubMed: 20138990]
22. Palmer HG, Larriba MJ, Garcia JM, Ordonez-Moran P, et al. The transcription factor SNAIL represses vitamin D receptor expression and responsiveness in human colon cancer. *Nat Med.* 2004; 10:917–919. [PubMed: 15322538]
23. Pena C, Garcia JM, Silva J, Garcia V, et al. E-cadherin and vitamin D receptor regulation by SNAIL and ZEB1 in colon cancer: clinicopathological correlations. *Hum Mol Genet.* 2005; 14:3361–3370. [PubMed: 16203744]
24. Wu Y, Deng J, Rychahou PG, Qiu S, et al. Stabilization of snail by NF-kappaB is required for inflammation-induced cell migration and invasion. *Cancer Cell.* 2009; 15:416–428. [PubMed: 19411070]
25. Barbera MJ, Puig I, Dominguez D, Julien-Grille S, et al. Regulation of Snail transcription during epithelial to mesenchymal transition of tumor cells. *Oncogene.* 2004; 23:7345–7354. [PubMed: 15286702]
26. Hotz B, Visekruna A, Buhr HJ, Hotz HG. Beyond epithelial to mesenchymal transition: a novel role for the transcription factor Snail in inflammation and wound healing. *J Gastrointest Surg.* 2010; 14:388–397. [PubMed: 19856033]
27. Thuault S, Tan EJ, Peinado H, Cano A, et al. HMGA2 and Smads co-regulate SNAIL1 expression during induction of epithelial-to-mesenchymal transition. *J Biol Chem.* 2008; 283:33437–33446. [PubMed: 18832382]
28. Thuault S, Valcourt U, Petersen M, Manfioletti G, et al. Transforming growth factor-beta employs HMGA2 to elicit epithelial-mesenchymal transition. *J Cell Biol.* 2006; 174:175–183. [PubMed: 16831886]
29. Roy HK, Smyrk TC, Koetsier J, Victor TA, et al. The transcriptional repressor SNAIL is overexpressed in human colon cancer. *Dig Dis Sci.* 2005; 50:42–46. [PubMed: 15712635]
30. Pena C, Garcia JM, Larriba MJ, Barderas R, et al. SNAIL expression in colon cancer related with CDH1 and VDR downregulation in normal adjacent tissue. *Oncogene.* 2009; 28:4375–4385. [PubMed: 19802011]
31. Larriba MJ, Martin-Villar E, Garcia JM, Pereira F, et al. Snail2 cooperates with Snail1 in the repression of vitamin D receptor in colon cancer. *Carcinogenesis.* 2009; 30:1459–1468. [PubMed: 19502595]
32. Moolenbeek C, Ruitenberg EJ. The “Swiss roll”: a simple technique for histological studies of the rodent intestine. *Lab Anim.* 1981; 15:57–59. [PubMed: 7022018]
33. Hollis BW, Kamerud JQ, Selvaag SR, Lorenz JD, et al. Determination of vitamin D status by radioimmunoassay with an 125I-labeled tracer. *Clin Chem.* 1993; 39:529–533. [PubMed: 8448871]
34. Osburn WO, Karim B, Dolan PM, Liu G, et al. Increased colonic inflammatory injury and formation of aberrant crypt foci in Nrf2-deficient mice upon dextran sulfate treatment. *Int J Cancer.* 2007; 121:1883–1891. [PubMed: 17631644]
35. D'Ambrosio D, Cippitelli M, Cocciolo MG, Mazzeo D, et al. Inhibition of IL-12 production by 1,25-dihydroxyvitamin D3. Involvement of NF-kappaB downregulation in transcriptional repression of the p40 gene. *J Clin Invest.* 1998; 101:252–262. [PubMed: 9421488]
36. Harant H, Wolff B, Lindley IJ. 1 Alpha,25-dihydroxyvitamin D3 decreases DNA binding of nuclear factor-kappaB in human fibroblasts. *FEBS Lett.* 1998; 436:329–334. [PubMed: 9801142]
37. Cohen-Lahav M, Shany S, Tobvin D, Chaimovitz C, et al. Vitamin D decreases NFkappaB activity by increasing IkappaBalpha levels. *Nephrol Dial Transpl.* 2006; 21:889–897.

38. Sun J, Kong J, Duan Y, Szeto FL, et al. Increased NF-kappaB activity in fibroblasts lacking the vitamin D receptor. *Am J Physiol Endocrinol Metab.* 2006; 291:E315–E322. [PubMed: 16507601]
39. Kastner P, Messaddeq N, Mark M, Wendling O, et al. Vitamin A deficiency and mutations of RXRalpha, RXRbeta and RARalpha lead to early differentiation of embryonic ventricular cardiomyocytes. *Development.* 1997; 124:4749–4758. [PubMed: 9428411]
40. Clapper ML, Cooper HS, Chang WC. Dextran sulfate sodium-induced colitis-associated neoplasia: a promising model for the development of chemopreventive interventions. *Acta Pharmacol Sin.* 2007; 28:1450–1459. [PubMed: 17723178]
41. Kuwata T, Wang IM, Tamura T, Ponnampertuma RM, et al. Vitamin A deficiency in mice causes a systemic expansion of myeloid cells. *Blood.* 2000; 95:3349–3356. [PubMed: 10828015]
42. Wang Y, Moser AH, Shigenaga JK, Grunfeld C, et al. Downregulation of liver X receptor-alpha in mouse kidney and HK-2 proximal tubular cells by LPS and cytokines. *J Lipid Res.* 2005; 46:2377–2387. [PubMed: 16106051]
43. Beigneux AP, Moser AH, Shigenaga JK, Grunfeld C, et al. The acute phase response is associated with retinoid X receptor repression in rodent liver. *J Biol Chem.* 2000; 275:16390–16399. [PubMed: 10747970]
44. Feingold K, Kim MS, Shigenaga J, Moser A, et al. Altered expression of nuclear hormone receptors and coactivators in mouse heart during the acute-phase response. *Am J Physiol Endocrinol Metab.* 2004; 286:E201–E207. [PubMed: 14701665]
45. Pospechova K, Rozehnal V, Stejskalova L, Vrzal R, et al. Expression and activity of vitamin D receptor in the human placenta and in choriocarcinoma BeWo and JEG-3 cell lines. *Mol Cell Endocrinol.* 2009; 299:178–187. [PubMed: 19133314]
46. Palmer HG, Gonzalez-Sancho JM, Espada J, Berciano MT, et al. Vitamin D(3) promotes the differentiation of colon carcinoma cells by the induction of E-cadherin and the inhibition of beta-catenin signaling. *J Cell Biol.* 2001; 154:369–387. [PubMed: 11470825]

Fig. 1.

Acute AOM/DSS colitis results in a weight loss for heterozygote and wild-type mice. To model an acute colitis, 10 mg/kg of AOM (treated) or saline (control) was injected into mice intraperitoneally at 6 weeks of age. Mice were allowed to recover for 1 week and then treated mice were challenged with 2 % DSS. Wild-type and heterozygote mice challenged with AOM/DSS experience non-significant difference in weight loss associated with the DSS challenge

Fig. 2.

Expression of RXRα in heterozygote and wild-type mice. Heterozygote mice demonstrate a significant decrease in RXRα mRNA (a) and protein trends on downregulation (b) compared to wild-type mice. Wild-type and heterozygotes, $n = 4$

Fig. 3.

Acute AOM/DSS colitis results in increased inflammatory marker mRNA expression in colonic mucosa of treated heterozygote mice and a downregulation of VDR mRNA in colonic mucosa of heterozygote and wild-type treated mice. Both wild-type (a) and heterozygote (b) treated mice demonstrate an increase in inflammatory marker mRNA expression and downregulation of VDR mRNA expression. Snail and Snail2 mRNA was only significantly upregulated in heterozygote mice indicating increased inflammatory signaling in heterozygote mice. Fold change reflective of difference in expression for gene of interest between treated and control wild-type or heterozygote mice. Wild-type, $n = 3$ treated and 4 controls. Heterozygotes, $n = 4$ treated and 4 controls

Fig. 4.

Acute AOM/DSS colitis results in a downregulation of VDR protein in colonic mucosa of treated heterozygote and wild-type mice. Both wild-type (a) and heterozygote mice (b) demonstrate a similar reduction in VDR protein expression while RXRα is not downregulated in wild-type or heterozygote mice. Fold change reflective of difference in expression for protein of interest between treated and control wild-type or heterozygote mice. Wild-type and heterozygotes, $n = 5$ treated and 4 controls