The Aryl Hydrocarbon Receptor is a Repressor of Inflammation-associated Colorectal Tumorigenesis in Mouse

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Objective: To determine the role of the aryl hydrocarbon receptor (AHR) in colitis-associated colorectal tumorigenesis.

Background: Colorectal cancer (CRC) is the third most commonly diagnosed cancer in the United States, Chronic intestinal inflammation increases the risk for the development of CRC. We investigated the involvement of AHR, a ligand-activated transcriptional regulator, in colitis-associated colorectal tumorigenesis.

Methods: We used a mouse model of colitis-associated colorectal tumorigenesis that employs treatment with azoxymethane and dextran sodium sulfate. We examined the role of AHR using both an Ahr-deletion mouse model (Ahr−/−) and treatment with the AHR pro-agonist indole-3-carbinol (I3C). Incidence, multiplicity, and location of tumors were visually counted. Tumors were defined as neoplasms. Intestinal inflammation was assessed by quantitative PCR for proinflammatory markers and colon length. Data were evaluated and compared using GraphPad Prism software (version 6, La Jolla, CA).

Results: Tumor incidence was increased 32% in Ahr null mice and tumor multiplicity was approximately increased 3-fold compared with wild-type mice (2.4 vs 7.0; P < 0.05). Furthermore, tumor multiplicity was reduced 92% by treatment of I3C in wild-type mice, whereas the suppressor effect of I3C was not observed in Ahr null mice (P < 0.05).

Conclusions: We found that AHR plays a protective role in colitis-associated colorectal tumorigenesis. This conclusion is based on the observations that Ahr null mice showed increased number of colorectal tumors, and mice treated with I3C exhibited fewer tumors. This study supports the use of AHR agonists such as I3C as a chemopreventive therapy for IBD-associated CRC in human patients.

Keywords: aryl hydrocarbon receptor, azoxymethane, chemoprevention, colorectal cancer, dextran sodium sulfate, indole-3-carbinol, inflammatory bowel disease


Inflammatory bowel disease (IBD) is characterized by persistent inflammation of the colon and small intestine, with its 2 major clinical manifestations being ulcerative colitis (UC) and Crohn disease (CD). The UC is described as a state of chronic inflammation of the colon and rectum, whereas CD involves inflammation in the mucosal lining of any part of the gastrointestinal tract. Combined, UC and CD afflict nearly 1.4 million people in the United States. Epidemiological studies have reported that patients with IBD have 6 times higher risk for colorectal cancer (CRC) than the general population, and further, that 10% to 15% of all deaths in IBD patients are associated with CRC.

Although the pathogenesis of IBD-associated CRC is not clearly understood, disturbances of the gut mucosa and dysregulation of intestinal immunity are associated with both the initiation and propagation of the disease. Recent investigations focusing on the aryl hydrocarbon receptor (AHR) have demonstrated its essential role in maintaining intestinal immunity by regulating the homeostasis of innate immune cells. The AHR is a member of the basic helix-loop-helix/Per-Arnt-Sim (PAS) superfamily of transcription factors and has been identified as a mediator of the toxic effects of environmental pollutants such as 2,3,7,8-tetrachlorodibenzo-p-dioxin and benzo(a)-pyrene. Upon ligand binding, AHR translocates into the nucleus where it forms a heterodimeric complex with the AHR nuclear translocator (ARNT) protein. This nuclear heterodimer recognizes and binds dioxin-response elements in the DNA and is followed by subsequent up-regulation of downstream target genes including phase I and phase II drug-metabolizing enzymes [eg, cytochrome P450 (Cyp)1a1, Cyp1a2, Cyp1b1, and the glutathione S-transferase Iα].

In addition to its role in xenobiotic metabolism, AHR is thought to be intimately involved in intestinal inflammation and colitis-associated colorectal tumorigenesis. Importantly, investigators have independently reported that Ahr-deficient mice display severe colitis in a chemically induced experimental model. In these mouse models, dextran sodium sulfate (DSS) induces colitis by generating local inflammation in the colon. This phenotype was ameliorated by treatment with AHR pro-ligands such as indole-3-carbinol (I3C) and 3,3′-diindolylmethane (DIM). Further, another group reported that treatment with both I3C and DIM suppressed tumor development in the cecum and small intestine in Apcmin mice, which model the human condition familial adenomatous polyposis (FAP).

Given these observations, we investigated the idea that AHR plays a repressor role in colitis-associated colorectal tumorigenesis. To investigate this hypothesis, we compared the incidence and multiplicity of colorectal tumors between wild-type (WT) and Ahr null mice using a chemical-induced, colitis-associated colorectal tumorigenesis model. Further, we examined if activation of AHR by the pro-agonist I3C suppresses colorectal tumor incidence and multiplicity in the same model. Collectively, our data show that AHR plays a protective role in colitis-associated colorectal tumorigenesis.

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METHODS

Animals
C57BL/6J (WT) and Ahr null (Ahr\(^{\Delta2/\Delta2}\)) mice were housed in a selective pathogen-free facility on corncob bedding with chow diet and water ad libitum according to a protocol approved by University of Wisconsin Medical School’s Animal Care and Use Committee. The Ahr\(^{\Delta2/\Delta2}\) mouse has been previously described.\(^{16,17}\) The null mice in this study have been backcrossed to the C57BL/6J strain for more than 20 generations and are thus >99.9% C57BL/6J background.

Tumor Studies
The 2-stage colorectal tumor initiation-promotion assay (Fig. 1A) consisted of a single dose of 10 mg/kg body weight of azoxymethane (AOM) by intraperitoneal (IP) injection at 8 weeks of age, followed by 1% w/v DSS by drinking water on days 7 to 12 postinjection.\(^{15}\) Sixteen weeks later, the mice were sacrificed by CO\(_2\) euthanasia and the large intestine was harvested. The intestine was longitudinally incised along the main axis. The number, location, and size of tumors on the luminal surface of the colon and rectum were recorded. For analysis of spontaneous colon and cecum tumors, 52 to 56-week-old mice were evaluated. In these studies, tumor has been defined as a neoplasm.

Diets
To compare the incidence and multiplicity of colorectal tumors between WT and Ahr null mice, animals were fed with grain-based diet (5001, Test Diet, Richmond, IN). For the dietary treatment with I3C, and to eliminate the possibility of additional AHR ligands in the diet, mice were fed with a purified diet. Specifically, we used either I3C diet (0.1% w/w) in AIN-76A diet; Test Diet, Richmond, IN) or control diet (AIN-76A; Test Diet, Richmond, IN). For both experiments, mice were fed from birth continuously until completion of the colorectal tumorigenesis protocol (Fig. 1A). This means that dams were fed with the diet 1 week before mating and the progeny was then used for the experiments.

Assessment of DSS-induced Colitis
Eight-week old mice were weighed and treated with 1% w/v DSS in drinking water for 5 days followed by single oral gavage administration of 1% w/v DSS solution (3 mL) to normalize the time of last exposure. Twenty-four hours after the final DSS administration, mice were weighed, sacrificed, and the colon harvested. Colon length was measured and mucosal tissues were scraped with a surgical blade and removed from the distal colon segment. The colon mucosal tissues were subsequently used for gene expression analysis.

Gene Expression Analysis
Total RNA was isolated from mucosa using the Qiagen RNeasy kit (Qiagen, Valencia, CA). The isolated RNAs were reverse-transcribed using the High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster city, CA). The mRNA levels were measured with TaqMan Universal PCR Master Mix (Applied Biosystems) and custom-designed probes listed in Supplemental Digital Contest Methods (http://links.lww.com/SLA/B48, SDC Methods).

![Figure 1](image-url)

**FIGURE 1.** Susceptibility to AOM/DSS-induced colorectal tumorigenesis was increased in Ahr\(^{\Delta2/\Delta2}\) mice. A, Schematic protocol for AOM/DSS-induced colorectal tumorigenesis. At 8 weeks of age, Ahr\(^{\Delta2/\Delta2}\) (n = 20) and WT mice (n = 37) were treated with a single dose of 10 mg/kg body weight of AOM by IP injection. Seven days after the AOM injection, the mice were given 1% DSS by drinking water for 5 days. Sixteen weeks later, the mice were sacrificed for evaluation of colorectal tumorigenesis. B, Incidence of colorectal tumors in AOM/DSS-treated Ahr\(^{\Delta2/\Delta2}\) and WT mice. C–E, AOM/DSS-induced colorectal tumor multiplicity in Ahr\(^{\Delta2/\Delta2}\) and WT mice. C, Total colorectal tumor multiplicity; D, sex-based classification; E, segmentation-based classification. F, Size of colorectal tumors in AOM/DSS treated Ahr\(^{\Delta2/\Delta2}\) and WT mice. Bars represent mean values. Italic alphabets in the graphs indicate the following: a, significantly different relative to WT mice (P < 0.05); b, significantly different relative to the proximal colon of WT mice (P < 0.05); c, significantly different relative to the proximal colon of Ahr\(^{\Delta2/\Delta2}\) (P < 0.05).
Measurement of DNA Adducts

DNA pooled from colon and rectum mucosal cells were isolated from mice treated with AOM. Adducts quantitation (O\(^{\text{\textgreek{m}}}\)-methyl-guanine) was performed by capillary liquid chromatography–electrospray ionization tandem mass spectrometry (LC-ESI-MS/MS) in selected reaction monitoring (SRM) mode as described in SDC Methods (http://links.lww.com/SLA/B48). The amount of DNA in each sample was determined by measuring the amount of guanine by high-performance liquid chromatography-ultraviolet (HPLC-UV) spectrometric analysis. The amount of O\(^{\text{\textgreek{m}}}\)-methyl-guanine was normalized to the total amount of guanine in each sample and expressed as pmole O\(^{\text{\textgreek{m}}}\)-methyl-guanine per \(\mu\)mole guanine.

Statistical Analysis

All statistical analyses were performed using GraphPad Prism version 6.00 for Windows, GraphPad Software (La Jolla CA, www.graphpad.com). Intergroup comparisons were performed by Fisher exact test or the nonparametric Wilcoxon rank-sum test. Differences among groups were considered statistically significant when the \(P\) value was <0.05.

RESULTS

Ahr Null Mice have an Increased Susceptibility to Colitis-associated Colorectal Tumorigenesis

Using a colitis-associated colorectal tumorigenesis model\(^\text{19}\) (Fig. 1A), we demonstrated that AHR is protective against colorectal tumor development, as Ahr null mice have higher tumor incidence than WT mice (\(P < 0.005\)) (Fig. 1B). The average colon tumor multiplicity in Ahr null mice was nearly 3-fold higher than WT mice (\(P < 0.0001\)) (Fig. 1C). The results of colorectal tumor multiplicity were further classified by sex and colorectal segment (Fig. 2D–E). The increase of colorectal tumor multiplicity in Ahr null mice was independent of sex, as the 3-fold increase in tumor multiplicity was observed in both male and female mice (male, \(P < 0.01\); female, \(P < 0.01\)) (Fig. 1D). In both WT and Ahr null mice, most tumors were located in the distal colon and rectum segments (Fig. 1E). The tumor multiplicities of distal colon and rectum were significantly higher in Ahr null mice compared with WT mice (distal colon, \(P < 0.01\); rectum, \(P < 0.01\)), whereas the proximal colon tumor multiplicity was not significantly different between WT and Ahr null mice (\(P = 0.22\)). We also measured the size of colorectal tumors. Interestingly, the average size of colorectal tumors in Ahr null mice was approximately 23% smaller than that in WT mice (\(P = 0.03\)) (Fig. 1F). All tumors in both Ahr null and WT mice were adenomas (data not shown), indicating that AHR is likely involved in the early stage of colorectal tumorigenesis. In our model, spontaneous tumor development in WT and Ahr null mice were extremely low. We did not detect statistically significant differences in tumor multiplicity between 52-week-old Ahr null mice versus age-matched WT mice (Suppl. 1, http://links.lww.com/SLA/B48). In support of this observation, levels of \(\beta\)-catenin protein and \(c\)-myc mRNA, both involved in the molecular mechanism of CRC,\(^\text{14}\) were not significantly increased.

Ahr Null Mice Have Increased AOM-induced DNA Damage and DSS-induced Inflammation

The genotoxic agent AOM employed in this study is a potent colorectal tumor initiator.\(^\text{15}\) To compare the AOM-induced colorectal tumor initiation activity between Ahr null and WT mice, we measured levels of an established biomarker of DNA adduct formation, O\(^{\text{\textgreek{m}}}\)-methyl-guanine (Fig. 2).\(^\text{16}\) The amount of O\(^{\text{\textgreek{m}}}\)-methyl-guanine was undetectable in untreated animals, but was increased in AOM-treated Ahr null and WT mice (Ahr \(\Delta^{2/\Delta^2}\), \(P < 0.01\); WT: \(P < 0.01\)). The level of O\(^{\text{\textgreek{m}}}\)-methyl-guanine was almost 2-fold higher in AOM-treated Ahr null mice than treatment-matched WT mice (\(P < 0.02\)).

In our 2-stage colorectal tumor protocol, DSS-induced intestinal inflammation plays an important role in colorectal tumor promotion.\(^\text{15,16}\) To investigate the effect of Ahr deletion on the susceptibility of DSS-induced inflammation, we assessed reduced colon length as a measure of the severity of DSS-induced acute colitis. After oral administration of 1% DSS for 5 days, Ahr null mice developed a reduction in colon length compared with untreated Ahr null mice (\(P < 0.05\)). In contrast, colon length was not significantly changed by DSS treatment in WT mice (Fig. 3A). Additionally, we measured mRNA levels of proinflammatory markers including the cytokines interleukin (IL)-\(1\)\(\beta\), tumor necrosis factor (TNF\(\alpha\)), and IL-6,\(^\text{20}\) in colonic mucosa of Ahr null and WT mice, to further characterize the extent of acute DSS-induced colitis (Fig. 3B–D). In the colon of Ahr null mice, IL-1\(\beta\), TNF\(\alpha\), and IL-6 mRNA levels were markedly increased by DSS treatment (\(P < 0.05\)). In contrast, expression of IL-1\(\beta\) and IL-6 was not significantly increased by DSS treatment in WT mice, although an increase in TNF\(\alpha\) mRNA was observed.

Previous studies have reported that DSS treatment may induce gene expression of Ahr and the AHR-driven gene Cyp1a1, the highly sensitive and specific surrogate for Ahr activation, in murine colon mucosa.\(^\text{21}\) To investigate whether AOM or DSS treatment stimulated Ahr gene expression or AHR activation, we measured the Ahr and Cyp1a1 mRNA levels in the colon of both AOM or DSS-treated WT mice. Under these conditions, we did not see AOM and DSS-mediated induction of Ahr-driven gene expression or enhancement of AHR expression (Suppl. 2, http://links.lww.com/SLA/B48).

AHR-dependent Protection From Colorectal Tumor Development by I3C

To investigate the role of AHR in I3C protection from colitis-associated colorectal tumors, we compared tumor incidence and multiplicity in mice fed with an I3C-supplemented diet to those

\[\text{AOM} \rightarrow + \quad \text{WT} \quad \text{Ahr}^{\Delta^{2/\Delta^2}}\]

FIGURE 2. Ahr \(\Delta^{2/\Delta^2}\) mice showed higher levels of AOM-induced DNA adduct than WT mice. Levels of O\(^{\text{\textgreek{m}}}\)-methyl-guanine in colonic DNA of Ahr \(\Delta^{2/\Delta^2}\) and WT mice. At 8 weeks of age, Ahr \(\Delta^{2/\Delta^2}\) and WT mice were administered a single IP injection of PBS or 10 mg/kg body weight of AOM. Twelve hours after the injection, mice were sacrificed. Italic alphabets in the graph indicate the following: \(a\), significantly different relative to the no AOM treated mice (\(P < 0.05\)); \(b\), significantly different compared with the AOM-treated WT mice (\(P < 0.05\)). Bars represent mean values.
on control diet (Fig. 4). The colorectal tumor incidence was decreased in 92% in I3C-fed WT mice compared with control diet-fed WT mice (P < 0.0001) (Fig. 4A). However, the colorectal tumor incidence was not significantly influenced by I3C treatment in Ahr null mice. The colorectal tumor multiplicity in I3C-diet fed WT mice was 33-fold lower than control diet-fed mice (P < 0.0001) (Fig. 4B). In contrast, there were no decrease of colorectal tumors in Ahr null mice when supplemented with dietary I3C (P = 0.35). We confirmed that I3C activates AHR in this model by demonstrating a significant increase of Cyp1a1 mRNA in the colon of mice fed with I3C supplementation versus mice fed with the control diet (P < 0.0001) (Suppl. 3, http://links.lww.com/SLA/B48).

**Influence of I3C on AOM-induced DNA Damage and on DSS-induced Inflammation**

To understand the effect of I3C in the colitis-associated colorectal tumorigenesis model, we measured the levels of DNA adduct formation in AOM-treated WT mice fed with I3C or control diet. In WT mice supplemented with I3C, AOM-induced DNA adducts were decreased by nearly 38% compared with WT mice on the control diet (P = 0.0006) (Fig. 5). In untreated WT mice, DNA adducts formation did not change based on I3C supplementation. Additionally, we evaluated the effect of I3C in DSS-induced inflammation (Fig. 6). We observed that the DSS-treated WT mice on I3C diet showed longer colons than WT mice on control diet (P < 0.002) (Fig. 6A). The mRNA levels of TNFα and IL-1β were not affected by I3C treatment, though mRNA levels of IL-6 were significantly reduced in WT mice on I3C-supplemented diet compared with WT mice on control diet (P = 0.03) (Fig. 6B–D).

**DISCUSSION**

Inflammatory bowel disease is a well-established risk factor for the development of CRC. 21,22 Although the etiology of IBD is not fully understood, prior investigations demonstrate that dysregulation of intestinal immunity is a significant contributor to its pathophysiology. 23,24 In the past several years, experiments using Ahr deletion mouse models have revealed that AHR is a fundamental player in mediating intestinal inflammation through regulation of both intraepithelial lymphocytes (IELs) and innate lymphoid cells (ILCs). 7,8 The relationship between AHR and intestinal immunity, and the importance of inflammation in IBD led us to hypothesize that the presence and activation of AHR may impact the development of IBD-associated CRC. We therefore postulated that the absence of AHR would yield an increase in colorectal tumors, whereas the activation of the receptor by agonists like I3C will protect against tumor development. Importantly, we observed an increased
susceptibility to AOM/DSS-induced colorectal tumor development in our Ahr null mice (Fig. 1), whereas activation of AHR by dietary I3C protected against colorectal tumor development in an AHR-dependent manner (Fig. 4).

The role of AHR in AOM/DSS-induced colorectal tumors has not been previously investigated, and its mechanism may be multifactorial, including an immunological/inflammatory component and a xenobiotic metabolism component. With regard to xenobiotic metabolism, we have previously reported that Ahr null mice display a patent ductus venosus and therefore have a decreased rate of first-pass metabolism for many foreign compounds, potentially including AOM (Fig. 4). The role of AHR in AOM/DSS-induced colorectal tumors has not been previously investigated, and its mechanism may be multifactorial, including an immunological/inflammatory component and a xenobiotic metabolism component. With regard to xenobiotic metabolism, we have previously reported that Ahr null mice display a patent ductus venosus and therefore have a decreased rate of first-pass metabolism for many foreign compounds, potentially including AOM (Fig. 4). The role of AHR in AOM/DSS-induced colorectal tumors has not been previously investigated, and its mechanism may be multifactorial, including an immunological/inflammatory component and a xenobiotic metabolism component. With regard to xenobiotic metabolism, we have previously reported that Ahr null mice display a patent ductus venosus and therefore have a decreased rate of first-pass metabolism for many foreign compounds, potentially including AOM. This is a reflection of its ability to influence AOM bioactivation through first-pass metabolism of AOM, which, as previously mentioned, may be attributed to the hepatic shunt. Additionally, it is reported that the hepatic metabolizing enzymes, such as Cyp2e1, and the β-glucuronidases from intestinal flora are required for the generation of the electrophilic AOM metabolite responsible for alkylating DNA. Thus, it is possible that altered intestinal immunity in AHR nulls, possibly through decrease in the number of resident IELs, is influencing the bacterial loads of intestinal flora. In turn, this could lead to alterations of microbial populations expressing β-glucuronidase (eg, Clostridium, Peptostreptococcus, Bacteroides fragilis, Staphylococcus) in the colons of Ahr null mice, making them more susceptible to the alkylating and therefore toxic effects of AOM.

Collectively, these observations suggest that the high susceptibility of Ahr null mice to AOM/DSS may be due to altered xenobiotic metabolism, altered intestinal microbiota, and aberrant response to chemically-induced inflammation.

One thing that is clear from these studies is that most, if not all, of the anticarcinogenic activity of I3C observed in these studies is mediated through the AHR. I3C is a pro-agonist found in the Brassica family of vegetables. After ingestion, I3C undergoes condensation reactions within the acidic gastric environment, resulting in several different metabolites including 3,3'-diindolylmethane (DIM) and indolo[3,2-b]carbazole (ICZ), both of which have high affinity for AHR. In an effort to determine if the AHR mediated the protective effects of I3C in our model of IBD-associated CRC, we compared the intestinal tumor burden in WT and Ahr null mice after AOM/DSS treatment. Our observation that Ahr null mice were not influenced by the treatment of I3C is consistent with the idea that the anticarcinogenic effect of I3C is dependent upon the AHR (Fig. 4). Furthermore, we investigated the effect of I3C in AOM-induced DNA adduct formation and measured intestinal inflammation. Our data demonstrate that I3C confers protection against electrophilic damage by AOM (Fig. 5). The I3C-mediated inflammation is unique in that inhibition of IL-6 alone was observed (Fig. 6). Together, these observations suggest that a major component of the anticarcinogenic effect of I3C in colitis-associated colorectal tumorigenesis model is a reflection of its ability to influence AOM bioactivation through first-pass metabolism of AOM, which, as previously mentioned, may be attributed to the hepatic shunt. Additionally, it is reported that the hepatic metabolizing enzymes, such as Cyp2e1, and the β-glucuronidases from intestinal flora are required for the generation of the electrophilic AOM metabolite responsible for alkylating DNA. Thus, it is possible that altered intestinal immunity in AHR nulls, possibly through decrease in the number of resident IELs, is influencing the bacterial loads of intestinal flora. In turn, this could lead to alterations of microbial populations expressing β-glucuronidase (eg, Clostridium, Peptostreptococcus, Bacteroides fragilis, Staphylococcus) in the colons of Ahr null mice, making them more susceptible to the alkylating and therefore toxic effects of AOM. Collectively, these observations suggest that the high susceptibility of Ahr null mice to AOM/DSS may be due to altered xenobiotic metabolism, altered intestinal microbiota, and aberrant response to chemically-induced inflammation.

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FIGURE 4. I3C-suppressed tumor development induced by AOM/DSS in WT mice. Mice were fed with I3C or control diet from birth. At 8 weeks of age, WT (control) (n = 24), WT (I3C) (n = 29), Ahr<sup>Δ/Δ</sup> (control) (n = 14), Ahr<sup>Δ/Δ</sup> (I3C) (n = 11) were treated with a single dose of 10 mg/kg body weight of AOM by IP injection. Seven days after the AOM injection, the mice were given 1% DSS by drinking water for 5 days. Sixteen weeks later, the mice were sacrificed for evaluation of colorectal tumorigenesis. A, Incidence of colorectal tumors in AOM/DSS-treated Ahr<sup>Δ/Δ</sup> and WT mice. B, AOM/DSS-induced colorectal tumor multiplicity in Ahr<sup>Δ/Δ</sup> and WT mice. Italic alphabets in the graph indicate the following: a, significantly different relative to WT mice treated with control diet (P < 0.0001); b, significantly different relative to Ahr<sup>Δ/Δ</sup> mice.

FIGURE 5. I3C treatment decreased AOM-induced DNA adducts. Levels of O<sup>6</sup>-methyl-guanine in colonic DNA of WT mice treated with I3C and control diet (n = 5–10). At 8 weeks of age, both groups of WT mice were administered a single IP injection of PBS or 10 mg/kg body weight of AOM. Twelve hours after the injection, mice were sacrificed. Bars represent mean values. Italic alphabets in the graph indicate the following: a, significantly different relative to no AOM treatment group (P < 0.0001); b, significantly different relative to WT mice treated with control diet (P < 0.0006).
response to chemically-induced colitis in Ahr null mice. For example, 1 group reported that an Ahr deletion mouse model displayed significant spontaneous cecal and colon tumor development.4 The study demonstrated that 100% of 11-week-old Ahr null mice (Ahr<sup>Δ2/Δ2</sup>, corresponding to the excision of exon 1 in the Ahr gene) bore spontaneous colon or cecal tumors. In our Ahr null mouse model (Ahr<sup>Δ2/Δ2</sup>, corresponding to excision of exon 2 in the Ahr gene), spontaneous cecal and colon tumors were rare even at 52 weeks of age (Suppl. 1, http://links.lww.com/SLA/B48). Although tumor incidence in Ahr<sup>Δ2/Δ2</sup> was approximately 3 times higher than in our WT mice, these differences were not significant (Suppl. 1A, http://links.lww.com/SLA/B48). Another example of a difference between our study and current literature is that several reports have demonstrated that treatment with I3C and DIM rescue DSS-induced colitis.7,12,13 Interestingly, we found that the treatment of I3C in our model minimally influenced the DSS-induced inflammation (Fig. 6). These discrepancies may be due to differences in Ahr null allele, mouse genetic background, intestinal microbiota, housing condition, and protocols used for DSS exposure.

We demonstrated that AHR plays a protective role in IBD-associated colorectal tumorigenesis; the implication of which is that AHR is a requisite for resistance to IBD-associated tumor formation. The clinical importance of this consideration stems from the evolutionary conserved nature of the AHR such that it is highly conserved among mammals including humans.9,10 Accordingly, genetic or epigenetic gene silencing or environmental inhibition of AHR could represent novel health modifiers for IBD and IBD-associated CRC in humans. In general, our data support the previous described hypothesis that I3C-mediates suppression of AOM/DSS-induced colorectal tumorigenesis. In addition, this is the first study to establish that the protective effect of I3C within the colon is dependent on AHR. This study supports the application of I3C as a chemopreventive agent for IBD-associated CRC in humans and also suggests that the activation of AHR may be used as a chemopreventive strategy against CRC.

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REFERENCES


DISCUSSANT

S. Gandalgi (Louisville, KY):

Dr Kennedy and his colleagues discuss the role of an aryl hydrocarbon receptor in colitis-associated colorectal tumorigenesis.

This topic is important for many of us, not only those of us interested in inflammatory bowel disease. Cancers arising in a field of chronic inflammation are responsible for roughly 20% of malignancies worldwide. With respect to this study, Dr Kennedy shows using a dextran sulfate murine model of colitis that the aryl hydrocarbon receptor plays a protective role and that indole-3-carbinol protects against azoxymethane/dextran sodium sulfate (DSS)-induced colorectal tumorigenesis.

I have 4 brief questions for the authors.

I note in the manuscript there is mention that the only neoplasms that were actually identified in animals were adenomas. Your model used 1% DSS given only for days 7 to 12 after azoxymethane was injected intraperitoneally. This is a short, single course of DSS, and this transient inflammation does not mimic the human situation where cancer develops in a field of ongoing chronic inflammation. How transposable or relevant are your findings for patients, particularly in light of the findings of the “glue grant” about the applicability of mouse models?

The second question, your aryl hydrocarbon receptor knockout mouse model was an exon 2 knockout as compared with the exon 1 knockout that had been previously used. Can you comment upon why this model was chosen?

Thirdly, why do you believe that the colorectal tumors were larger in wild-type mice than in AHR knockout mice?

Finally, the indole-3-carbinol was given from birth, so to speak, before administration of the carcinogen and before the inflammatory stimulus. Have you or will you plan to do this as a therapeutic intervention after these have occurred to more closely mimic the real-life scenario?

Response From G.D. Kennedy (Birmingham, AL):

Thank you for those questions, Dr Gandalgi. Very astute observations. The abnormal model, of course, it would be great if we had a clear carcinoma model, and they are fairly rare and hard to come by in mice.

The 1 cycle of DSS is chosen because it turns out the mice do not particularly like DSS, and we are just trying to optimize our numbers that survive the protocol.

In fact, many in the literature have used 3% DSS. And we are a slave to our environment, and the microbiome or whatever else is going on, and my mice just didn’t tolerate more than 1% for 1 reason or another. So the model is what we could develop, basically.

The knockout mouse that we have chosen is the Delta 2, as you note, and the Japanese group reported actually 3 different model knockouts of HR. Why did I choose Delta 2?

The simple answer, and perhaps simplistic, is that is what my mentor had in the lab, and it was free, so that is what we used.
The Delta 1 did show a different phenotype, as you probably read, and the Delta 1 actually developed spontaneous cecal tumors, all of which developed over the course of about the first 12 weeks of life. Our mice did not. We let our mice go for over a year. And although there was some increase in spontaneous tumors, it did not reach statistical significance. So again, is it the Delta 1 or is it something about the environment? I do not know the answer. I would like to get the model to test.

Why are the tumors larger? I would love to know the answer to that. There is likely some other genetic abnormalities in these tumors that we have not clearly investigated yet, but it probably has something to do with it for sure.

And finally, the therapeutic interventions and how we have been given the indole-3-carbinol, you are very astute in noticing that we basically put down the mothers on indole-3-carbinol so the mice were born to mothers on indole-3-carbinol. We can talk later. I am sure people are not terribly interested in my thoughts on exposure in utero, but that’s really what it comes down to. So yes, we are doing experiments where we are treating the mice at the time and following exposure as well to try to see the impacts.