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Differential response of flat and polypoid colitis-associated colorectal neoplasias to chemopreventive agents and heterocyclic amines

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Abstract

Individuals with ulcerative colitis face an increased risk of developing colorectal cancer and would benefit from early chemopreventive intervention. Results from preclinical studies in the mouse model of dextran sulfate sodium-induced colitis demonstrate that flat and polypoid colitis-associated dysplasias arise via distinct genetic pathways, impacted by the allelic status of p53. Furthermore, flat and polypoid dysplasias vary in their response to induction by the heterocyclic amine 2-amino-3-methylimidazo[4,5-*f*]quinoline (IQ) and inhibition by 5-aminosalicylic acid, a common therapy for the maintenance of colitis patients. These data suggest that use of combination therapy is essential for the optimal inhibition of colitis-associated colorectal cancer.

Keywords

Colorectal cancer; ulcerative colitis; chemoprevention; heterocyclic amines; 5-aminosalicylic acid; mouse

1. Introduction

Ulcerative colitis is a chronic form of inflammatory bowel disease of unknown etiology that affects approximately 700,000 individuals per year in the US [1]. Hallmarks of the disease include repeated flares of inflammation and inflammatory cell infiltration in the colorectum that severely disrupt the architecture of the colonic mucosa and promote cellular transformation [2]. Patients with ulcerative colitis face a risk of developing colorectal cancer

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that is significantly higher than that of the general population and increases with the extent of colonic involvement, disease severity, duration of colitis and a family history of colorectal cancer [3]. A meta-analysis of colorectal cancer risk in ulcerative colitis patients suggests a cumulative incidence of malignancy of 2% by 10 years, 8% by 20 years and 18% by 30 years of disease [4]. However, risk estimates from individual studies are as high as 27.5% after 45 years of disease [5] and 33% after 20 years for inflammatory bowel disease patients with primary sclerosing cholangitis [6]. While significant advancements have been made in managing the symptoms experienced by colitis patients, much less attention has been given to ameliorating the significant cancer risk associated with this disease.

Development of colitis-associated colorectal cancer in humans is known to progress in a stepwise manner, with the inflamed mucosa giving rise to flat and polypoid dysplasias and ultimately invasive cancers [7; 8]. While dysplasia, defined as neoplastic epithelium confined to the basement membrane, remains the most reliable marker of risk for colonic malignancy in colitis patients [9–11], current surveillance protocols fail to reliably identify the early stages of colitis-associated neoplasia [12]. In particular, detection of flat lesions that lack an elevated growth component is extremely challenging in patients with pancolitis, where as many as 50% can be missed during surveillance colonoscopy [13]. Furthermore, high-grade dysplasia or cancer was found in 27% of patients who had a colectomy within 6 months of a diagnosis of flat low-grade dysplasia [8]. Because the dysplasia-to-carcinoma sequence is not absolute [14], much controversy surrounds the management of flat low-grade dysplasias once they are identified [10]. These data, when combined with the current recommendation not to initiate endoscopic screening until at least seven years following diagnosis, speak clearly to the critical need to detect colitis-associated neoplasms more reliably and earlier when chemopreventive intervention may be efficacious.

Although significant overlap exists in the specific genetic aberrations that contribute to sporadic and colitis-associated colorectal cancer, the timing of oncogenic events in the two diseases differs dramatically. While mutation of APC represents the putative gate-keeping event in sporadic colorectal carcinogenesis, it occurs later and much less frequently in patients with ulcerative colitis (6% for colitis-associated neoplasms and 74% for sporadic tumors) [15]. In contrast to sporadic disease, alterations in p53 are not only found in the earliest colitis-associated dysplasias but may occur well in advance of the formation of dysplasia and confer cancer susceptibility [16; 17]. Disruption of the MAPK pathway through mutation of either RAS or BRAF is found in approximately 30% of all colitis-associated cancers [18].

The dextran sulfate sodium (DSS) mouse model of induced colitis represents a clinically relevant system in which to study the molecular basis of colitis-associated lesions. Similar to humans, colorectal lesions develop as both flat (nonpolypoid) and polypoid neoplasias (Fig. 1). Several observations in mice with DSS-induced colitis provide compelling evidence that these morphological subtypes of lesions arise via distinct genetic mechanisms (Table 1). First, flat dysplasias/cancers exhibit inflammation scores (a product of the intensity and extent of inflammatory infiltration) that are approximately 10-fold higher than those of polypoid lesions. Second, the majority of cancers arise from flat colonic dysplasias. Based on observations in the azoxymethane (AOM) mouse model of colon carcinogenesis, flat

dysplasias are expected to progress to invasive cancers without transitioning through a polypoid intermediate [19]. Large differences in the incidence of flat colorectal cancers among various mouse strains exposed to AOM provide evidence that the development of specific morphological subtypes of lesions is genetically predetermined. Third, β-catenin is localized to the nucleus of polypoid dysplasias induced by DSS, while flat lesions exhibit membranous staining. Of note, this differential is lost when DSS is administered in combination with AOM due to the ability of AOM to induce β-catenin mutations.

Results from previous studies by this group demonstrate that loss of p53 is a critical event in the commitment of colitis-associated neoplasias to develop as flat or polypoid morphological subtypes [20]. The role of p53 in colitis-associated tumorigenesis was examined by administering DSS to C57BL/6J mice that were either wild-type for p53 or p53 deficient (heterozygous or homozygous null) for 3–4 cycles followed by 120 days of untreated water. Complete loss of p53 led to an increase in the overall incidence (2.8-fold) and multiplicity (2.7 to 4.8-fold) of colitis-associated dysplasias and cancers as compared to animals wild-type or heterozygous for p53. Interestingly, a direct correlation was observed between loss of wild-type p53 and increasing multiplicity of flat dysplasias and cancers (Fig. 2). In mice bearing two copies of wild-type p53, 100% of the dysplasias were polypoid and no cancers were observed. The profile changed in mice heterozygous for p53, where the majority of the colitis-associated cancers were flat and the dysplasias polypoid. In p53 null mice, flat lesions were predominant, with only 15% of the lesions being polypoid. Furthermore, β-catenin mutations were not found in colitis-associated colorectal lesions from p53 null mice, while β-catenin mutations were detected in 75% of the lesions from p53 wild-type mice. These data provide strong evidence that flat and polypoid colitis-associated lesions arise via independent genetic pathways.

Consistent with differences in the genetic make-up of flat and polypoid lesions, data from this group indicate that morphological subtypes of colitis-associated lesions vary in their response to both carcinogens and chemopreventive agents. The following section describes the ability of the heterocyclic amine IQ to preferentially induce flat dysplasias while 5-aminosalicylic acid (5-ASA), a common maintenance therapy for patients with ulcerative colitis, is most effective in inhibiting flat lesions [21].

2. Contribution of meat and heterocyclic amines to colitis-associated neoplasia

Data from recent epidemiological studies [22–25] continue to confirm earlier reports of a strong association between the consumption of red meat and increased risk of colorectal cancer. This putative association is of great concern in ulcerative colitis patients who consume significantly more animal protein, dietary fat, cholesterol, cereals and simple carbohydrates/sugars in an attempt to alleviate the symptoms of their disease when in clinical remission [26]. Adoption of these dietary habits leads to a greater degree of colonic aneuploidy, an independent risk factor for the development of cancer among colitis patients [27]. Jowett and colleagues [28] reported that colitis patients who ate red and processed meats were six times more likely to relapse as compared to those who consumed much less meat. Consistent with this clinical observation, circulating levels of gamma-

glutamyltransferase, a marker of oxidative stress, were found to be elevated in subjects who consumed meat; an association that remained significant after adjusting for body mass index and other life style factors [29; 30]. Similar studies examining the effect of red meat consumption on plasma levels of C reactive protein in humans have yielded both positive and negative results [29; 31; 32]. It should be noted that there are numerous substances in meat that could potentially exacerbate colorectal inflammation and oxidative stress, including saturated fats, nitrates, nitrites, polycyclic aromatic hydrocarbons and heterocyclic amines.

Heterocyclic amines (HCAs) are potent carcinogens and mutagens that are generated from the reaction of creatine, creatinine, amino acids and sugar when protein-rich foods, including meats, are cooked at a high temperature [33; 34]. The most common HCAs include 2-amino-3-methylimidazo[4,5-*f*]quinoline (IQ) and 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP), the latter of which induces gastrointestinal cancers when administered to animals [35–37]. Results from the Tennessee Colorectal Polyp Study, a large colonoscopy-based case-control study, indicate an association between exposure to HCAs (PhIP, MelQx and DiMelQx) in cooked meats and increased risk of colorectal adenomas (OR – 1.3–1.4; $P \leqslant 0.034$) [24]. The association of HCAs with colon cancer risk was stronger for patients with multiple adenomas, as compared to single adenomas, and for serrated vs. nonserrated polyps. Similar associations have been reported between PhIP and rectal adenoma (OR = 1.75, P = 0.02) [38] and MeIQx and DiMeIQx and colon cancer (HR = 1.19 and 1.17, respectively ($P_{trend} < 0.001$)) [23].

HCAs are procarcinogens that require metabolic activation to become mutagenic and carcinogenic [39]. Cytochrome P450 enzymes, primarily CYP1A2, metabolize HCAs to N-hydroxylamines that are further converted to esters by acetyltransferase and sulfotransferase. The resulting N-acetoxy metabolites of HCAs are spontaneously converted to arylnitrenium ions (R-NH+) and react with DNA to form adducts at the 8-position carbon of guanine (N-(deoxyguanosin-8-yl)-IQ). IQ and MeIQx can also form adducts by binding to the N2 position of guanine (i.e., 5-(deoxyguanosin-N2-yl)-IQ) [40–44]. Genetic alterations linked to HCA exposure include chromosomal aberrations, sister chromatid exchange [45] and microsatellite instability [46; 47]. Mutations in cancer-related genes, including *H-ras*, *p53*, *Apc* and β-catenin, have also been identified in tumors induced by HCAs [46–50].

3. Ability of the heterocyclic amine IQ to induce flat (but not polypoid) colitis-associated neoplasms

Previous studies from this group demonstrated that induction of colitis (by DSS) in mice bearing an Apc mutation ($Apc^{+/Min\text{-}FCCC}$) drives the progression of colorectal dysplasias to cancers, allowing an analysis of both the early and late phases of carcinogenesis [51]. The effect of IQ exposure on inflammation- associated tumorigenesis was examined in female $Apc^{+/Min\text{-}FCCC}$ mice (9 wks of age, N=131) that were obtained from an established colony at Fox Chase Cancer Center (FCCC) and maintained on Global 2018 diet (Harlan Teklad, Madison, WI) for the duration of the study. Mice were randomized to one of four groups (untreated (N=27); IQ alone (N=38); DSS alone (N=36); and DSS and IQ (N=30)) and treated as outlined in Fig. 3. Ulcerative colitis was induced by administering two cycles of

DSS (MW 30,000–40,000) (MP Biomedicals, Solon, OH). Cycle one consisted of 3 days of 4% DSS in the drinking water followed by 18 days of untreated water. Cycle two consisted of 2 days of 4% DSS in the drinking water plus 19 days of untreated water. Animals were gavaged with either IQ (40 mg/kg body weight) (Toronto Research Chemical, Inc., Ontario, Canada) or vehicle (DMSO/saline, volume ratio 1:1) on 2, 4 and 6 days post DSS treatment during each cycle of DSS. Body weights were recorded at the beginning and end of each cycle of DSS, and prior to gavage. At the time of sacrifice (16 weeks of age), the entire colon and rectum were examined grossly, fixed in 10% formalin and processed in their entirety for histopathologic evaluation.

IQ was well tolerated when administered in the absence of DSS; with animals gaining weight at a rate comparable to that of untreated control mice. Body weights dropped immediately following DSS exposure but recovered over time; consistent with previous observations [21]. The rate of survival among $Apc^{+/Min\text{-}FCCC}$ mice treated with IQ, DSS, or DSS and IQ did not differ significantly (60.5%, 61.1% and 70%, respectively ($P = 0.08$)).

3.1. Incidence, multiplicity and types of dysplasia

The incidence of colorectal dysplasias in $Apc^{+/Min\text{-}FCCC}$ mice treated with IQ alone (65%) was comparable to that of untreated control mice (61%). Consistent with previous results [51], the incidence of dysplasias in mice administered DSS alone or in combination with IQ was 100%. No cancers were observed in either untreated mice or mice treated with IQ alone. However, exposure to IQ and DSS in combination resulted in a trend towards accelerated disease progression, increasing the incidence of cancers in DSS-treated mice from 22.7% to 37% ($P = 0.3219$).

The multiplicity of colonic dysplasias in $Apc^{+/Min\text{-}FCCC}$ mice administered IQ alone was comparable to that of untreated controls (Mean ± SEM - 0.96 ± 0.24 and 1.13 ± 0.25, respectively) ($P > 0.05$), with number of lesions per female as expected for this strain (Fig. 4A). Treatment with DSS led to a 30-fold increase in the number of dysplastic lesions per mouse (Mean SEM - 29.64 ± 3.00), as compared to controls (untreated or IQ alone) ($P < 0.0001$). Administration of IQ to $Apc^{+/Min\text{-}FCCC}$ mice with DSS-induced colitis further increased tumor multiplicity to 36.63 ± 2.98.

Lesions were classified as flat or polypoid based on standardized morphology and nomenclature for the human pathology of colitis-associated colorectal neoplasia [52], as described previously [53]. Any dysplasia or cancer exhibiting an elevated growth pattern, either grossly or microscopically, was considered polypoid. Flat (nonpolypoid) lesions did not have an elevated growth component, with a height less than 2-fold that of the adjacent nonneoplastic mucosa. While polypoid lesions were the predominant subtype in both untreated (82%) and IQ-treated (no DSS) (65%) animals, the number of flat colonic lesions was 2-fold higher in mice treated with IQ alone as compared to untreated $Apc^{+/Min\text{-}FCCC}$ mice. In contrast, flat lesions were the major morphological subtype present in DSS-treated animals (Fig. 4B). Exposure to IQ led to a 1.3-fold increase in the percentage of flat colorectal lesions in mice with DSS-induced colitis (61%) as compared to animals administered only DSS (48%). Linear regression analyses yielded significant trends of decreased polypoid lesions ($P = 0.03$) and increased flat lesions ($P = 0.002$) across the

treatment groups (untreated, IQ alone, DSS alone and DSS/IQ) as illustrated in Fig. 4B). Interestingly, all flat lesions in untreated mice and mice treated with IQ alone were microadenomas (1–2 glands). Based on the established prevalence of wild-type p53 in polypoid vs. flat lesions and the known ability of IQ to induce p53 mutations [50; 54], one can speculate that IQ alters the status of p53, thus promoting the growth of flat lesions preferentially.

3.2 Effect of IQ on the proliferation and apoptosis of colitis-associated lesions

In order to investigate the mechanism by which IQ promotes flat lesions on a background of colitis, the effect of IQ on cell proliferation (Ki-67) and apoptosis (active caspase-3) was investigated. Immunohistochemical staining was performed on specimens of the distal colon (nonneoplastic mucosa, polypoid lesions and flat lesions) from mice treated with DSS or DSS and IQ. Tissue sections were subjected to antigen retrieval (Reagent cc-1 for 30 minutes) and incubated with either primary antibody against caspase 3 (active) (AF835, R & D Systems, Minneapolis, MN 1:800 dilution) or Ki-67 (VP-K451, Vector Laboratories, Inc. Burlington, CA. 1:1500) for 1 hr at 37°C. Non-immune rabbit Ig at the same concentration as the primary antibody served as a negative control. As anticipated, the proliferative index of flat and polypoid lesions was higher than that of nonneoplastic colonic mucosa (Fig. 5). IQ increased the proliferative index of both the nonneoplastic colonic mucosa and polypoid lesions significantly ($P = 0.05$ and 0.017, respectively) but had no effect on cell proliferation in flat lesions ($P = 0.60$).

The number of caspase-3 (active) positive cells in the nonneoplastic inflamed colonic mucosa of animals administered DSS was very low; less than one per field (60×) (Fig. 6). In mice exposed to DSS alone or in combination with IQ, the number of apoptotic cells was at least 15-fold higher in both polypoid and flat lesions than in the nonneoplastic colonic mucosa. Treatment with IQ had no effect on the level of apoptosis in the nonneoplastic colonic mucosa. IQ significantly increased the number of caspase-3 positive cells in polypoid lesions ($P = 0.002$) but not in flat lesions ($P = 0.13$). In the case of polypoid lesions, the effect of IQ on cell proliferation is counterbalanced by an effect of this heterocyclic amine on apoptosis, resulting in a comparable multiplicity of polypoid dysplasias in animals receiving DSS alone or in combination with IQ. In contrast, flat lesions from mice treated with DSS/IQ had less apoptotic activity but a similar rate of cell proliferation as compared to mice treated with only DSS. Inhibition of apoptosis by IQ resulted in a significant increase (56%) in the number of flat lesions in mice treated with DSS and IQ (22.3%) as compared to those treated with DSS alone (14.3%; $P = 0.004$). These data when combined with the inability of standard white light endoscopy to reliably detect flat dysplasias and cancers speak clearly to the need to both improve current methods of clinical surveillance and establish dietary recommendations for ulcerative colitis patients who are at increased risk for colorectal cancer.

3.3 Impact of inhibition of inducible nitric oxide synthase on the formation of colitis-associated tumors

Inducible nitric oxide synthase (iNOS) is overexpressed within the colon of ulcerative colitis patients and may contribute to the development of colitis-associated neoplasia [55; 56].

Chronic activation of iNOS leads to the sustained production of nitric oxide at cytotoxic levels. The potential ability of IQ to be converted to a more potent carcinogen in the presence of free radical oxygen was investigated by administering S,S'-1,4-phenylene-bis(1,2-ethanediyl)bis-isothiourea (PBIT), an inhibitor of inducible nitric oxide synthase (iNOS), to IQ-treated mice with DSS-induced colitis. Administration of PBIT failed to decrease either the multiplicity of total lesions (DSS/IQ – 36.6 ± 3.0; DSS/IQ/PBIT – 38.4 ± 3.5) or the percentage of flat lesions (DSS/IQ – 61%); DSS/IQ/PBIT – 58%). These data suggest that IQ specifically induces flat lesions in a manner that is independent of iNOS activity.

4. 5-aminosalicylic acid is most effective in inhibiting flat colitis-associated neoplasias

5-aminosalicylic acid (5-ASA), a common therapy for the treatment of flare-ups and maintenance of disease remission in patients with ulcerative colitis, is structurally similar to aspirin. Results from several clinical studies suggest that long-term treatment with 5-ASA decreases the risk of developing colorectal cancer in patients with ulcerative colitis [57–59]. However, results from a recent comprehensive population-based analysis of long-term 5-ASA use ($\geqslant$ 5 years) by patients with inflammatory bowel disease revealed no chemoprophylactic activity against colorectal cancer [60].

5-ASA and other nonsteroidal anti-inflammatory drugs (NSAIDs) have common molecular targets including inflammation, proliferation, angiogenesis, and/or apoptosis. Specific pathways targeted by 5-ASA include TNF-α, TGF-β, NF-κB and Wnt/β-catenin signaling as well as cell cycle control, scavenging of reactive oxygen and nitrogen species and antimicrobiol activity [61]. While the growth inhibitory effects of 5-ASA are associated with downregulation of COX-2 mRNA and protein, the ability of 5-ASA to inhibit the growth of colorectal carcinoma cells that do not express COX-2 has been demonstrated [62]. Additional potential mechanisms of action of 5-ASA include enhanced apoptosis via inhibition of NF-kappaB and p38/MAP kinases, decreased Wnt/beta-catenin activity and elevation of SH-PTP-2, a phosphatase that targets and inactivates the EGF receptor [63]. Results from other studies attribute the antineoplastic activity of 5-ASA to its ability to improve the fidelity of DNA replication by reducing frameshift mutations at microsatellites [64] and inhibit the formation of reactive oxygen species by polymorphonuclear leukocytes [65; 66].

The efficacy of 5-ASA in inhibiting colitis-associated neoplasia has been evaluated in the mouse model of AOM/DSS-induced colitis [21]. Swiss Webster mice were administered 5-ASA in the drinking water at various doses starting one week prior to three cycles of DSS treatment. A 55% reduction in the size of polypoid colonic dysplasias was observed in mice receiving chronic 5-ASA at a dose (75 mg) comparable to that used for the treatment of patients with colitis. Interestingly, 5-ASA had no effect on the multiplicity of polypoid lesions. In contrast, the multiplicity of flat lesions was significantly decreased (44%) in mice administered 5-ASA at the same dose, while no reduction in lesion size was observed. Comprehensive analyses of the genome-wide RNA and microRNA expression profiles of flat and polypoid lesions are anticipated to enhance our understanding of the molecular

mechanisms that underlie their differential response to 5-ASA exposure. These data strongly support the need to rationally design combination therapies for the prevention of colitis-associated cancer.

In summary, emerging data from in-depth molecular and pathological analyses continue to suggest that flat and polypoid colitis-associated colorectal neoplasms arise via distinct genetic mechanisms, with the mutational status of p53 being a major determinant of their ultimate morphology. Both the nuclear localization of β-catenin in DSS-induced polypoid lesions and the enhanced prevalence of infiltrating inflammatory cells in flat lesions provide further evidence of the inherent heterogeneity of colitis-associated neoplasms. The functional significance of the biological differences noted between flat and polypoid lesions is confirmed by their differential response to induction by the carcinogen IQ and chemoprevention by 5-ASA. These data provide unique insight into the essential need for combination therapy to target both flat and polypoid neoplasms and effectively inhibit the risk of colorectal cancer among patients with ulcerative colitis.

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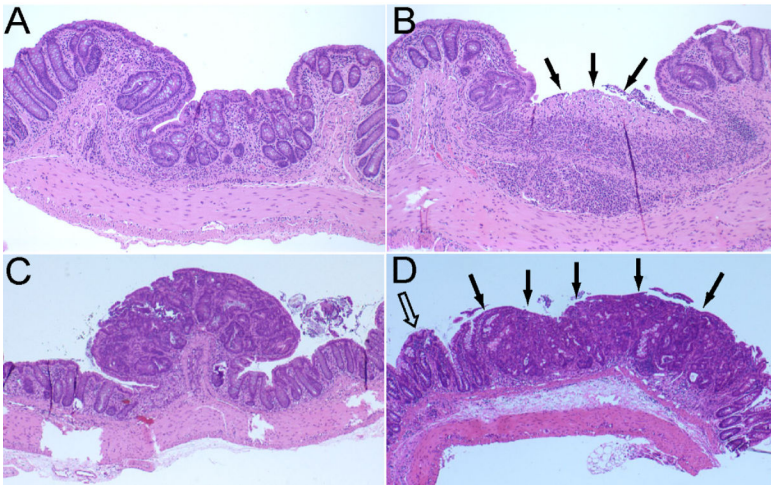

Fig. 1.

Histopathology of representative colorectal lesions in Swiss Webster mice with AOM/DSS-induced colitis. A) Chronic colitis characterized by thickening of the colonic mucosa, crypt distortion, and chronic inflammation (10× magnification). B) Ulcer in a background of chronic colitis (10× magnification). C) Polypoid colitis-associated dysplasia (4× magnification). Note: The lesion projects above the mucosa (compare to panel D). D) Flat (nonpolypoid) dysplasia. Note: The flat dysplasia (solid arrows) has a contour and thickness similar to that of the adjacent nonneoplastic mucosa (open arrow) (compare to panel C).

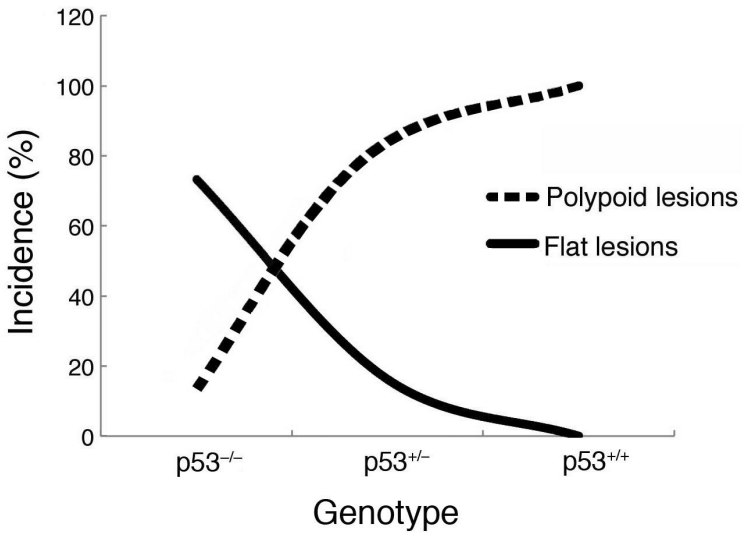

Fig. 2.

Comparison of the morphological subtypes of colorectal dysplasias and cancers in C57B1/6J mice with DSS-induced colitis that are either wild-type ($p53^{+/+}$) or deficient ($p53^{+/-}$ or $p53^{-/-}$). A direct correlation exists between the number of alleles of wild-type p53 present and the percentage of polypoid colorectal lesions, while an inverse association is observed with flat lesions.

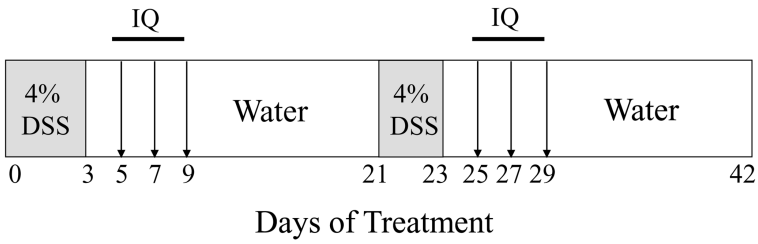

Fig. 3.

Regimen for the administration of IQ to animals with DSS-induced colitis. All animals were fed a Teklad Global 2018 diet for 5 weeks prior to the initiation of 2 cycles of DSS and for the duration of the study. DSS was added to the drinking water, and IQ (40 mg/kg body weight) was administered by gavage.

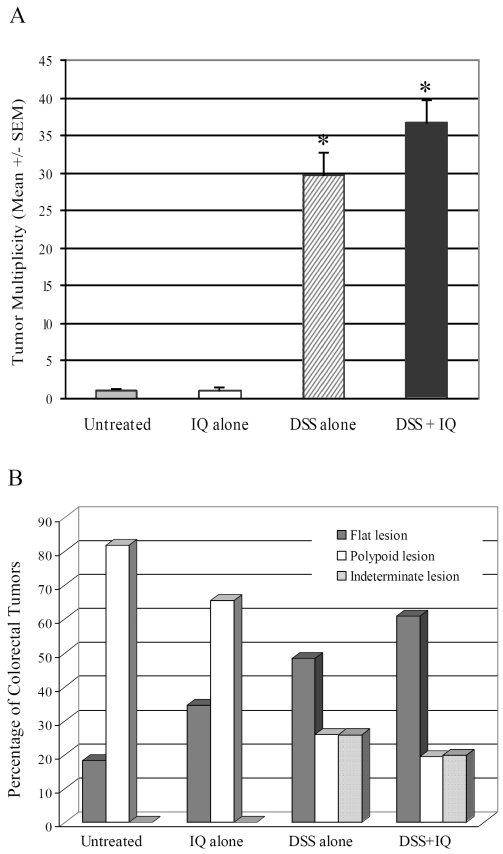

Fig. 4.

Effect of IQ on colonic tumorigenesis in $APC^{+/Min\text{-}FCCC}$ mice with DSS-induced colitis. A) Total tumor multiplicity is based on histopathological reviews. Asterisk: Significantly different from untreated control mice and mice treated with IQ alone ($P < 0.05$) as determined by the Wilcoxon 2-sample test. B) Linear regression analyses revealed trends of decreased polypoid lesions ($P = 0.03$) and increased flat lesions ($P = 0.002$) across the treatment groups.

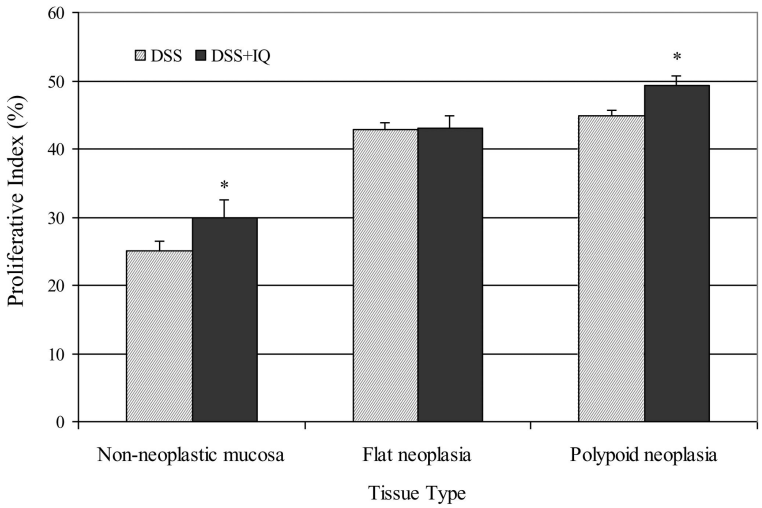

Fig. 5.

Impact of IQ exposure on cell proliferation (immunostaining for Ki-67). Values represent the number of Ki-67 positive nuclei per 300 tumor cells for polypoid or flat lesions and 6–7 normal colonic crypts for nonneoplastic mucosa (mean ± SEM. Asterisk: Significantly different from the DSS control group ($P < 0.05$) as determined by the Wilcoxon 2-sample test.

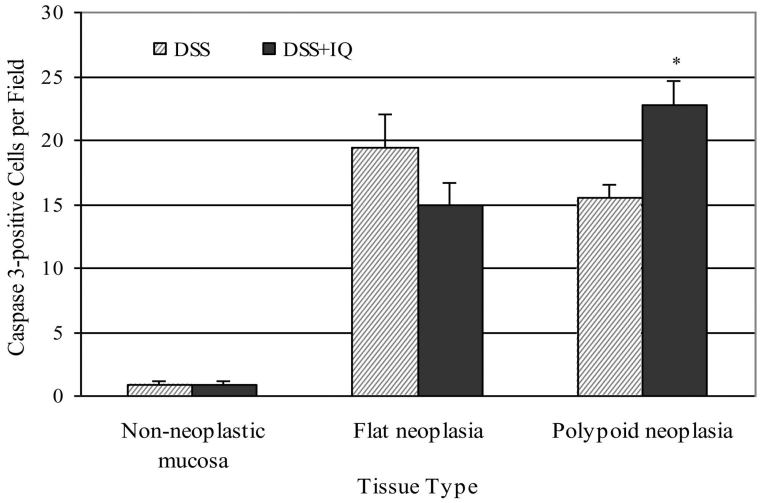

Fig. 6.

Effect of IQ on apoptosis as determined by immunohistochemistry using antibodies against caspase 3 (active form). Values represent the number of cells positive for caspase 3 per field (600×). Asterisk: Significantly different from the DSS control group (P = 0.0017) as determined by the Wilcoxon 2-sample test.

Table 1

Comparison of the characteristics of flat and polypoid colitis-associated colorectal lesions in the DSS model*

	Strain	Treatment	Flat	Polypoid
Inflammation	SW	DSS	10×	×
Nuclear β-cat	SW/C57BL/6J	DSS	−	+
Cancers	SW	DSS	+++	+
p53 mutation	C57BL/6J ($p53^{-/-}$)	DSS	+	−
	($p53^{+/+}$ or $p53^{+/-}$)	DSS	−	+
β-catenin mutation	C57BL/6J ($p53^{-/-}$)	DSS	+	−
	($P53^{+/+}$, $p53^{+/-}$)	DSS	−	+
IQ	$Apc^{+/Min\text{-}FCCC}$	DSS	Increase	No change
5-ASA	SW	AOM/DSS	Decrease	Trend

* DSS – dextran sulfate sodium was added to the drinking water and administered in cycles.