**Dysplasia and cancer in the dextran sulfate sodium mouse colitis model. Relevance to colitis-associated neoplasia in the human: a study of histopathology, B-catenin and p53 expression and the role of inflammation**

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Animal models of colitis, which develop dysplasia and cancer similar to human ulcerative colitis are needed to further investigate the dysplasia cancer sequence. This study describes the expression of B-catenin and p53 along with the histopathology and inflammation scores as they relate to dysplasia and cancer in the dextran sulfate sodium (DSS) colitis model. Swiss Webster mice were fed with 5% DSS as follows: group A, four cycles of DSS, 84 days total (1 cycle = 7 days DSS + 14 days H2O); group B, four cycles DSS followed by 120 days H2O, 204 days total; group C, 7 days DSS followed by 180 days H2O, 187 days total; group D, 7 days DSS followed by 90 days H2O, 97 days total. The incidences of dysplasia and/or cancer were 15.8, 37.5, 18.1 and 0% in groups A–D, respectively. Dysplasia and/or cancer occurred as flat lesions or as dysplasia-associated lesion or mass (DALM) as observed in the human. Thirty-three percent of cancers had associated dysplasia. Within group A, inflammation scores were significantly higher in animals with dysplasia and/or cancer compared with those without dysplasia and/or cancer (<0.05–<0.0001). Inflammation scores were significantly higher in animals with cancers versus those with dysplasia (<0.015) and in flat dysplasia and/or cancer versus DALM (<0.0042). B-catenin showed translocation from the cell membrane to the cytoplasm and/or nucleus in 100% of DALM and 5.8% of flat dysplasia and/or cancer. A total of 94.2% of flat dysplasia and/or cancer had exclusive cell membrane expression compared with 0% DALM (<0.0001). Only 7.4% of dysplasia and/or cancer showed nuclear expression of p53. In colitis-associated dysplasia and/or cancer in the DSS model: (i) histology resembles that in the human; (ii) inflammation plays a significant role in the dysplasia cancer sequence and whether dysplasia and/or cancer grows as a flat lesion or a DALM; (iii) the early molecular pathways are different for flat dysplasia and/or cancer versus DALM, with nuclear/cytoplasmic translocation of B-catenin as an early event in DALM but not flat dysplasia and/or cancer; and (iv) p53 has little or no role in dysplasia and/or cancer. This well characterized model provides an excellent vehicle for studying the roles of inflammation, the molecular events

and the role of chemopreventive agents in colitis-associated neoplasia.

Introduction

Ulcerative colitis (UC) is a form of chronic idiopathic inflammatory bowel disease (IBD) that usually has a course of intermittent exacerbation and remission, and less commonly an unremitting course or a course of only a single attack. (1) One of the complications of UC is an increased risk for the development of cancer of the colon and rectum. The cumulative risk for developing cancer in patients with longstanding pancolitis is ~10–13% at 25 years of disease (2–5). In most instances cancer evolves through a neoplastic process called dysplasia (2–9).

There are many animal models for IBD, but only a few of these models are applicable to study the dysplasia–cancer sequence. These animal models can be characterized as spontaneous, genetically engineered, and those induced by exogenous agents (10–22). Cotton top tamarins (Saguinus oedipus) are New World monkeys that develop colitis and cancer when in confinement. However, unlike in the human, these cancers tend to arise unassociated with dysplasia and it can take up to 4 years to develop cancers. The expensive nature of procuring and housing these endangered species precludes using this model in all laboratories (10–12). Recently, interleukin-10 knockout and Gα1 knockout (deficient) mice have been shown to develop colitis-associated adenocarcinoma (13,14). However, these models have not been fully characterized for cancer studies as they relate to the human. In recent years, a few investigators have described the appearance of dysplasia and/or cancers in mice, rats and hamsters when they are fed dextran sulfate sodium (DSS) (15–22). However, none of these studies has examined the incidence, distribution and multifocality of dysplasia and the association of colitis with dysplasia and cancer. Furthermore, none of these studies has correlated the clinical severity of disease, the role of single versus multiple attacks of colitis, the role of inflammation and the effect of disease inactivity in the development of dysplasia and cancer.

In human UC-associated neoplasia, p53 mutation or overexpression is reported to occur commonly as an early event in the dysplasia cancer sequence (23–32). However, in sporadic colon cancer, it is a late event in the adenoma cancer sequence (23–25,27–29,32). There are only two reported studies that have looked at the role of p53 in animal models of colitis-associated neoplasia, one of these studies is reported in abstract form (33) and the other study reported only three cases (34). These studies have reported different results. The former study reported that p53-deficient mice with chronic DSS colitis had significantly higher numbers of dysplastic lesions than control mice with DSS colitis (33). The latter study reporting a 0% incidence of p53 mutations in colitis-induced rat colon cancers (34).
B-catenin is a 92 kDa protein that plays a role in both cell adhesion and intracellular signaling (35,36). Translocation of B-catenin from the cell membrane to the cytoplasm or nucleus is an early event in colorectal carcinogenesis in the human (37–40), mouse models possessing a germline mutation in the APC gene (Min) (41) and murine models treated with carcinogens (42,43). Data on the role of B-catenin in colitis-associated neoplasia are limited. There is only one reported study in the human (32) and none in animal models of colitis-associated neoplasia.

The sequence of events leading to dysplasia and cancer in animal models and its relationship to the human needs to be thoroughly investigated. The aims of this study are: (i) to establish the histopathological relevance of dysplasia/cancer in the DSS model to that of the human; (ii) to study the role of inflammation and the severity of attacks of colitis in colitis-associated neoplasia; and (iii) to study the expression of B-catenin and p53 in colitis-associated neoplasia.

Materials and methods

Animals

Female, 8-week-old Swiss Webster mice (FBR-Taconic) weighing 25–30 g were used in this study. The animals were housed in a vivarium with controlled temperature at 23 °C and light and dark cycles. Mice were fed standard mice chow pellets and had access to drinking tap water supplied in bottles. Animals with pain and distress were treated when necessary with buprenorphine or killed. This animal protocol was approved by the Institutional Animal Care and Use Committee, MCP Hahnemann University.

Study design (induction of colitis)

The design for inducing colitis-associated dysplasia/cancer is shown in Table I. Colitis was induced by feeding ad libitum 5% DSS, molecular weight 30–40,000 (ICN, Costa Mesa, CA) in the drinking water. The DSS solution was not sterilized; however, the solutions were changed daily. The groups were divided into groups A–F. Group A animals were exposed to four cycles of DSS (the basic cycle is 7 days of DSS followed by 14 days of H2O). Group A was subdivided into groups A1, A2 and A3. The intent was to modify the clinical severity of disease to see if clinical severity of disease correlated with dysplasia and/or cancer. The disease activity index (DAI; see below) is a measurement of clinical disease severity (activity) with scores ranging from 0 to 3 with 3 being the most severe. The DAIs for groups A1, A2 and A3 were maintained at 3.0, 2.0 and 1.5, respectively. To maintain a DAI of 1.5–2.0, the basic cycle was modified to 4–5 days of DSS feeding. Mice in group B were fed DSS for four cycles and killed 120 days after the end of the fourth cycle (204 days total). The intent here was to see the effect of inactive colitis on progression of dysplasia to cancer and the effect of longevity of disease in developing dysplasia and/or cancer. Group C was fed DSS for 7 days followed by 180 days of H2O (187 days total) and group D was fed DSS for 7 days followed by 90 days of H2O (97 days total). The intent of groups C and D was to see if a single attack of colitis was sufficient to induce dysplasia and/or cancer and its relationship to longevity of disease.

Controls (groups E and F) were mice of similar sex and age (100- and 200-day-old mice, respectively) that drank regular H2O. Animals were killed by an overdose of i.p. sodium pentobarbital.

Evaluation of severity of clinical colitis

A DAI was determined in all animals, by scoring body weight, hemocult reactivity or presence of gross blood and stool consistency, as detailed in our previous studies (19,22). The DAI is scored on a scale of 0–3, with 3 correlating with the most severe clinical disease and 0 the least severe clinical disease. This was measured during day 3 or 4 of DSS feeding, on the day DSS feeding was stopped and once a week thereafter. The individuals who examined the mice for DAI scores were blinded as to the experimental group in which the animal belonged.

Histopathological evaluation (inflammation, ulcer scores and dysplasia/cancer)

After death the entire colonrectum from the colocolic junction to the anal verge was examined. The specimen was opened longitudinally and examined for gross lesions without the use of any magnification aid. The colon was divided into three equal portions (proximal, middle and distal) and each segment was fixed in neutral buffered formalin. Each segment was totally submitted as multiple transverse sections for histological processing. This averaged five to six pieces/segment and 15–18 pieces/total colon/rectum. All slides were stained with hematoxylin and eosin. Inflammation scores were determined as follows: the intensity of inflammation was graded 0–3 and its extent was estimated as 0–100%. The inflammation score was the product of these two parameters (range 0 to 300). This was performed for every piece of tissue on each slide and the total score was the sum of all pieces divided by the number of pieces of tissue in each segment. Ulcer scores were determined as the extent (0–100%) of ulcer for every piece of tissue on each slide and the total score was the sum of all the pieces divided by the number of pieces in each segment. Dysplasia was scored, negative, indefinite and positive for dysplasia, using the criteria that are used in the daily practice of clinical surgical pathology (44) with the following modifications: since we were examining the entire colon and rectum we were able with a high degree of accuracy to differentiate dysplasia from reactive changes. However, in order to relate our changes to that seen in the human, we retained the category of indefinite for dysplasia. This indefinite category consisted of cytologically atypical epithelium which for the most part was recognizable as reparative and/or regenerative in nature. Any dysplasia or cancer that had an elevated or polypoid growth pattern if seen grossly or microscopically was considered to be a dysplasia-associated lesion or mass (DALM). Flat lesions had no elevated component. Cancer was divided into early invasive and advanced cancer. Early invasive was defined as cancer cells invading into the muscularis mucosae and/or into the submucosa. Advanced cancer was defined as cancer cells invading into the muscularis propria or beyond. All cases were reviewed blindly by a single experienced gastrointestinal pathologist as to experimental group.

The evaluation of B-catenin and p53

Both B-catenin and p53 were evaluated by immunohistochemistry. The antibody for B-catenin was purchased from Sigma (St Louis, MO). This is a rabbit polyclonal antibody raised against B-catenin peptide amino acids 768–781. The p53 antibody, product cm-1 (Signet Laboratories, Dedham, MA), is a rabbit polyclonal that reacts with both wild-type and mutant p53. The B-catenin antibody was used at a dilution of 1:4000 and the p53 antibody was used at a dilution of 1:800. Heat epitope retrieval in a citrate buffer in a vegetable steamer was used. An automated Techmate 1000 was used for immunohistochemistry as per standard protocol with the exception that biotinylated sheep anti-rabbit (Vector labs) was substituted for the Techmate standard secondary antibody. A mouse benzo[a]pyrene-induced skin squamous cell carcinoma, which is known to express mutant p53 (45,46) and human colorectal adenocarcinoma were used as positive controls for the p53 antibody. Staining for p53 was considered positive if: (i) the intensity of nuclear staining was equal or greater to that of the mouse skin cancers and (ii) if >10% of cells were positive. Previous studies have shown that >10% positive nuclear staining correlates with the presence of mutated p53 (26,47). Controls for B-catenin expression were normal colon and colonic adenocarcinoma (human) and normal and neoplastic colonic lesions from mice possessing a germline mutation in the APC gene (Min). Non-immune rabbit IgG of similar protein concentrations were substituted for B-catenin and p53 as negative controls.

Statistical methods

All values are expressed as the mean ± SE. Comparisons were done using one-way ANOVA followed by Dunnet’s test. Univariate linear regression analysis of multiple squares was done to draw conclusions where appropriate. Odds ratios were done using 2×2 contingency tables followed by determining 95% confidence intervals (95%CI).
Results

Incidence and distribution of dysplasia and cancer

The data regarding incidence of indefinite for dysplasia, dysplasia and cancer, are presented in Table II. The incidence of dysplasia and/or cancer in animals exposed to only four cycles of DSS (84 days total) was 18.8% in group A1 (DAI 3.0), 15.7% in group A2 (DAI 2.0) and 10% in group A3 (DAI 1.5). The overall incidence of dysplasia and/or cancer in groups A1, A2 and A3 was 15.8%. The incidence of dysplasia and/or cancer in group B (four cycles followed by 120 days of H2O) was 37.5% compared to the control animals (groups E and F) was 0%. The incidence of dysplasia was 9.7, 31.2, 18.1 and 0% in groups A–D, respectively. The incidence of dysplasia and/or cancer in control animals (groups E and F) was 0%. Setting the DAI 0.01, in groups A1, A2 and A3, there was no statistically significant difference between these groups regarding the incidence of dysplasia and/or cancer. Similarly, there was no statistically significant difference between groups A, B and C.

Among all the animals with dysplasia and/or cancer, the incidence of dysplasia and/or cancer was 20, 44 and 36% in the proximal, middle and distal colon segments, respectively. In general, inflammation was most severe in the middle and distal segments, but this was not statistically significant for all groups. Eighteen of 21 (87.5%) animals with dysplasia and/or cancer had lesions limited to only one colon segment, one animal (4.7%) had lesions in two different segments and two animals (9.5%) had lesions in all three segments. Of the 12 animals with cancer, only two (16.6%) had two or more synchronous cancers.

Dysplasia/cancer relationships

Ten of 15 (66.6%) cancers arose in colons without associated dysplasia while only 33.3% (five of 15) cancers arose in colons with associated dysplasia. Of the five cancers with associated dysplasia, three (60%) actually arose out of dysplasia (two from DALM and one from flat dysplasia), one (20%) had dysplasia in the same segment of the colon and one (20%) had dysplasia in a different segment of the colon to the actual cancer.

Inflammation, scores, ulcer scores and DAI

The inflammation scores for all groups are shown in Table III. There was no significant relationship between ulcer scores and dysplasia and/or cancer in any of the groups studied. Comparing the four cycle groups with different DAI the inflammation scores in groups A1 (DAI 3.0), A2 (DAI 2.0) and A3 (DAI 1.5) were 71.7 ± 11.4, 24.4 ± 12.5 and 26.8 ± 7.3, respectively. The inflammation scores in group A1 were statistically significantly higher than groups A2 and A3 (P < 0.012). The inflammation scores correlated with the DAI (r = 0.95, P < 0.01). In groups A1, A2 and A3, those animals with dysplasia and/or cancer had significantly higher inflammation scores than those without dysplasia and/or cancer (Figure 1). In contrast, inflammation scores in groups B, C and D did not correlate with the appearance of dysplasia and/or cancer. Combining all the groups together, the mean inflammation score for animals with cancer was 129 ± 38 compared with 21.0 ± 5.5 for those animals with dysplasia; this difference was significant (P < 0.015) (Figure 2).

Extended studies

The incidence of dysplasia and/or cancer in group B (four cycles DSS followed by 120 days of H2O) was 37.5% compared to...
with 15.8% in the combined four cycle group (A1, A2 and A3). These differences show a major trend towards an increased incidence of dysplasia and/or cancer with longevity of disease between groups with identical initial insult, even in the setting of colitis in clinical remission. The incidence of dysplasia and/or cancer in group C (7 days DSS followed by 180 days H2O) was 18.1% compared with 0% in group D (7 days DSS followed by 90 days H2O). Groups C and D show that a single attack of colitis is sufficient to induce dysplasia; however, dysplasia will develop only after longstanding disease (group C versus D). Groups B and C allow us to compare the incidence of dysplasia and/or cancer as it relates to multiple attacks of colitis versus a single attack among animals with longstanding colitis of approximately similar duration. The incidence of dysplasia and/or cancer was 37.5% in group B (multiple attacks of colitis) compared with 18.1% in group C (single attack of colitis).

Pathology
Gross lesions were noted in only four animals (three in group B and one in group C). These lesions were dome shaped and polypoid and ranged in size from 3 to 6 mm.

The dysplastic and cancer lesions could be divided into two major types, flat and elevated (polypoid); the latter were similar to the DALM seen in the human. Ten of 15 (67%) dysplastic lesions were DALMs and five of 15 (33%) dysplastic lesions were flat. Two of 15 (16.5%) cancers arose within a DALM and 13 of 15 (83.4%) cancers were flat. The odds ratio of cancer arising within a DALM is low at 0.08 (95% CI 0.012–0.48) while the odds ratio of cancer arising within a flat lesion is high at 13 (95% CI 2.07–81.52). The inflammation scores for the group of dysplasia and/or cancer lesions of the DALM type was significantly lower than for those of the flat lesions (18.2 ± 3.4 versus 183.7 ± 47.6; P < 0.0042) (Figure 3).

Histologically, DALMs showed proliferation of glands resulting in the formation of a polypoid mass. Architecturally, there was tubule formation, some with irregularity and cribriforming of glands. Cytologically, mucin production was often reduced and the nuclei were vesicular with nucleoli or elongated, hyperchromatic, and stratified to the surface of the cell (Figure 4). Foci of intraglandular necrosis could occasionally be identified. The flat dysplasias consisted of as little as three to four glands to diffuse areas. These flat lesions showed the same architectural and cytological changes as seen in the DALMs (Figure 5). Five dysplasias were categorized as low grade dysplasia and 10 dysplasias were categorized as high grade dysplasia.

The cancers that arose within DALMs consisted of glands that invaded into the muscularis mucosae and/or into the submucosa. These cancers consisted of well-differentiated glands (Figure 6). DALMs with invasive cancer were seen only in the group receiving four cycles of DSS followed by 120 days H2O (group B). The flat cancers consisted of malignant glands dropping off from the bottom of the crypts and invading into muscularis mucosae or into the submucosa and beyond, often eliciting a desmoplastic reaction. These invasive cancers were often associated with adjacent or overlying ulceration. Cytologically, these invasive glands showed loss of mucin production with vesicular nuclei, prominent nucleoli and central lumenal necrosis; however, others were extremely well differentiated (Figure 7). This pattern of cancers dropping off from the bottom of the crypts is quite reminiscent of cancers arising in human IBD (48). The majority of cancers were of the early invasive type. Mucinous-type carcinoma similar to that seen in human UC-related cancer was also present (48).

The histological changes considered indefinite for dysplasia (see Materials and methods) were for the most part reactive and/or regenerative atypia associated with overlying or adjacent erosion or ulceration or less commonly two to three isolated crypts with decreased mucin production but with maturation towards the surface.

B-catenin expression (Table IV)
Thirteen cancers, 14 dysplasias, 15 indefinite for dysplasia, 15 negative for dysplasia and 10 control animals were studied for B-catenin expression. Of the 13 cancers, 12 were flat and one arose in a DALM, and of the 14 dysplasias, five were flat and nine were DALMs. In controls, B-catenin was expressed exclusively on the cell membrane (Figure 8). All 10 DALMs (100%) showed translocation of B-catenin to the cytoplasm and/or nucleus (Figure 9). Within each DALM the percentage of neoplastic cells showing cytoplasmic and/or nuclear expression of B-catenin ranged from 5 to 75% (mean 35%). Within the same group of neoplastic cells one could see cells with retained membrane expression adjacent to, or admixed with cells showing cytoplasmic and/or nuclear expression of B-catenin (Figure 10). The mean percentage of cells with cytoplasmic and/or nuclear expression was 42.6% for high-grade dysplasia and 21% for low-grade dysplasia. While showing a trend, this was not statistically significant (P = 0.08). The mean percentage of cells with cytoplasmic and/or nuclear expression was 30.25% in 84 day animals versus 48.25% in 204 day animals. Sixteen of 17 (94.2%) flat dysplasia/cancer lesions showed exclusively cell membrane expression of B-catenin (Figure 11) and one of 17 (5.8%) flat lesions showed cytoplasmic and/or nuclear expression of B-catenin. (This latter lesion was difficult to differentiate...
Dysplasia and cancer in mouse colitis

Fig. 4. (A) Whole-mount view of DALM. (B) High power view showing stratified dysplastic nuclei extending to cell surface.

Fig. 5. (A) Low power view showing dysplasia in flat mucosa. At this power one can also appreciate underlying transmural inflammation. (B) High power view showing gland in gland architecture. Nuclei are dysplastic and mitotic figures can be seen.

between a flat lesion or an early DALM.) The odds ratio for cytoplasmic and/or nuclear translocation of B-catenin in DALMs versus exclusive cell membrane expression in flat lesions was 1250 (95% CI 0.001–0.0068) and the Fisher’s exact two-tailed was \( P < 0.0001 \). All cases indefinite for dysplasia and negative for dysplasia showed an exclusive cell membrane pattern; however, all indefinite and negative lesions were from flat mucosa.

p53 Expression
Control animals (\( n = 10 \)), animals negative for dysplasia (\( n = 15 \)), and animals indefinite for dysplasia (\( n = 15 \)) all failed to show nuclear expression for p53. Nuclear expression of p53 was present in one DALM (dysplasia) and one flat dysplasia lesion. Overall nuclear expression of p53 was present in only two of 27 (7.4%) dysplastic and/or cancer lesions (Figure 12). P53 expression was not seen in any of the cancers.

Discussion
The focus of this study was to characterize the molecular events, histopathology and role of inflammation in the development of dysplasia and/or cancer in the DSS model of mouse colitis and to draw analogy to UC-associated dysplasia and/or cancer in the human with the intent to use this model for further molecular and chemopreventive research. Our findings show that our model and the human are similar regarding histopathology, the incidence of dysplasia and/or cancer and the increasing incidence of dysplasia and/or cancer with longevity of disease. Our model and the human are dissimilar regarding the role of...
Fig. 6. Whole-mount view of invasive cancer arising out of a DALM. One can appreciate malignant glands invading into the submucosa (arrow).

Fig. 7. Low power view showing adenocarcinoma arising in flat mucosa and invading deeply into the muscularis propria.

Table IV. Expression of B-catenin in DALM versus flat dysplasia/cancer

<table>
<thead>
<tr>
<th>B-catenin expression</th>
<th>DALM</th>
<th>Flat dysplasia/cancer</th>
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</thead>
<tbody>
<tr>
<td>Cell membrane*</td>
<td>0 (0%)</td>
<td>16 (94.2%)</td>
</tr>
<tr>
<td>Cytoplasmic and/or nucleus</td>
<td>10 (100%)</td>
<td>1 (5.8%)</td>
</tr>
<tr>
<td>Total</td>
<td>10 (100%)</td>
<td>17 (100%)</td>
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*Exclusive cell membrane expression: odds ratio 1250 (95%CI 0.001–0.0068) and Fisher’s exact two-tailed test $P < 0.0001$ for cytoplasmic and/or nuclear expression of B-catenin in DALM versus exclusive cell membrane expression in flat dysplasia/cancer.

p53, multifocality of disease and a different incidence of dysplasia-associated cancer. We were unable to draw a statistical conclusion regarding disease activity and dysplasia and/or cancer. Finally, we describe new and novel findings regarding the role of B-catenin in the early molecular pathway of DALM and the role of inflammation in flat versus DALM and in the development of dysplasia and/or cancer.

In various studies of human patients with universal long-standing UC, the incidence of dysplasia has ranged from 7.5 to 38% with a mean of ~15% (3–5,49–50) and the cumulative risk for cancer is reported at 7–12% at 20 years of disease (2–5,49,51). Although we realize that results will vary with the dosage and longevity of DSS treatment, our results are similar to those of dysplasia and cancer reported in human studies. The cumulative risk for dysplasia and cancer increases with length of disease with the former reported at 10.0% at 17 years, 14.2% at 25 years and 32.8% at 30 years (5) and the latter up to 12% for 20 years of disease. Our results are similar, as the incidence of dysplasia was approximately three times higher in those animals that were killed 120 days after four cycles (group B, 31%) versus those that were killed after the completion of four cycles (group A, 9.7%). Similarly, the incidence of dysplasia in animals that were exposed to a single attack of colitis and euthanized 180 days (group C) and 90 days later (group D) was 18.1 and 0%, respectively. Our incidence of cancer also increased with longevity; 9.7% in group A (84 days) and 25% in group B (204 days).

The histopathology of dysplasia and cancer in this model is very similar to that seen in the human (48). In our model, animals developed dysplasia in both flat mucosa and DALM identical to that seen in humans. The types of cancers seen in our animals are histologically identical to that seen in humans with chronic UC, namely cancers arising in flat mucosa with a dropping off or invasion from the bottom of the crypts, mucinous cancers and cancers arising out of DALMs (48).

In human UC, dysplasia is found in two or more segments in 42–75% of the cases and the incidence of multiple synchronous
Inflammation can lead to carcinogenesis through pathways of free radicals which can lead to damage to DNA, lipids and protein. This in a background of increased proliferation can lead to fixation of mutations (56–65). In chronic human UC there are no known studies that have looked at histopathological levels of inflammation as it relates to dysplasia and/or cancer. In fact, many dysplasias and cancers arise on a background of inactive colitis. In this study we showed that within each group receiving four cycles of DSS and of identical DAI (group A1, A2 and A3), those animals with dysplasia and/or cancer had significantly higher inflammation scores than those without dysplasia and/or cancer. Recent studies of cotton top tamarins (11,12) have shown that those animals which developed colitis-associated cancer had higher inflammation scores than those without cancer. However, among animals (group B) receiving four cycles of DSS followed by 120 days of H2O (204 days total) there was no significant difference in inflammation scores between those animals with dysplasia and/or cancer than those without dysplasia and/or cancer. This might be expected since colitis was resolving as the last insult with DSS occurred 120 days prior to killing. However, in this group there was progression of DALMs to invasive cancer and a higher incidence of synchronous cancers. It is generally believed that the development of dysplasia and/or cancer is not related to the clinical disease activity (54,55). In order to validate this finding, we designed studies that involved regulating the disease activity between groups A1 (DAI 3.0), A2 (DAI 2.0) and A3 (DAI 1.5). Whereas the data showed an increased incidence of dysplasia or dysplasia and/or cancer in group A1 (DAI 3.0), we believe that because of the sample size and a possible type II error, it is difficult to draw any conclusion on the association between disease activity and the incidence of dysplasia or dysplasia and/or cancer in this model. Interestingly, in humans (54,55), the incidence of dysplasia and cancer is not related to the severity of clinical activity.

Inflammation can lead to carcinogenesis through pathways of free radicals which can lead to damage to DNA, lipids and protein. This in a background of increased proliferation can lead to fixation of mutations (56–65). In chronic human UC there are no known studies that have looked at histopathological levels of inflammation as it relates to dysplasia and/or cancer. In fact, many dysplasias and cancers arise on a background of inactive colitis. In this study we showed that within each group receiving four cycles of DSS and of identical DAI (group A1, A2 and A3), those animals with dysplasia and/or cancer had significantly higher inflammation scores than those without dysplasia and/or cancer. Recent studies of cotton top tamarins (11,12) have shown that those animals which developed colitis-associated cancer had higher inflammation scores than those without cancer. However, among animals (group B) receiving four cycles of DSS followed by 120 days of H2O (204 days total) there was no significant difference in inflammation scores between those animals with dysplasia and/or cancer than those without dysplasia and/or cancer. This might be expected since colitis was resolving as the last insult with DSS occurred 120 days prior to killing. However, in this group there was progression of DALMs to invasive cancer and a higher incidence of synchronous cancers. It is generally believed that the development of dysplasia and/or cancer is not related to the clinical disease activity (54,55). In order to validate this finding, we designed studies that involved regulating the disease activity between groups A1 (DAI 3.0), A2 (DAI 2.0) and A3 (DAI 1.5). Whereas the data showed an increased incidence of dysplasia or dysplasia and/or cancer in group A1 (DAI 3.0), we believe that because of the sample size and a possible type II error, it is difficult to draw any conclusion on the association between disease activity and the incidence of dysplasia or dysplasia and/or cancer in this model. Interestingly, in humans (54,55), the incidence of dysplasia and cancer is not related to the severity of clinical activity.
incidence of dysplasia and/or cancer (37%) compared with animals (15.8%) receiving only four cycles of DSS (84 days). Wilcox et al. (66) fed rats poligenan, a non-genotoxic, low molecular weight sulfated polysaccharide. They showed that animals fed poligenan had significantly increased cellular proliferation as measured by proliferating cell nuclear antigen and thymidine kinase activity. After stopping the poligenan diet, the increased levels of cellular proliferation continued with some labeling still at the surface. These findings might indicate that high levels of inflammation could be an important factor in the earlier stages of initiation of dysplasia while in longstanding colitis, continued inflammation may not be an important factor in the development of dysplasia and/or cancer. Analogous to our model this might explain why, in human UC, one frequently finds dysplasia and/or cancer on a background of inactive colitis.

In this study we found statistically significant higher inflammation scores in flat versus DALM lesions and in animals with cancers versus those with dysplasia. However, this latter finding might be interrelated to the flat versus DALM type of lesions as the odds ratio of cancer arising within a DALM is at 0.08 but the odds ratio of cancer arising within a flat lesion is 13.

The number of attacks of colitis may also play a role in the length of time needed to develop dysplasia and/or cancer. We were able to induce a 15.8% incidence of dysplasia and/or cancer after 84 days in those animals given four cycles of DSS (group A); however, we could not induce dysplasia and/or cancer after 97 days in the group of animals (group D) treated with DSS for only 7 days followed by 90 days of H2O. Interestingly, in this model a single episode of colitis also resulted in the development of dysplasia albeit after a prolonged period. Sequential killing of mice after a single attack of colitis showed that at 90 days none of the mice had developed dysplasia or cancer. However, after 180 days, 18% of mice had developed dysplasia, but not cancer, suggesting that a single attack of colitis was sufficient for some mice to develop dysplasia. Whether this phenomenon is true in humans is not clear.

B-catenin is a 92 kDa protein that has a high sequence similarity to the polarity gene Armadillo in Drosophila (35). In both the human and Drosophila, B-catenin plays a role in cell–cell adhesion and is involved in intracellular signaling (36). Wild-type APC is responsible for homeostatic control of degradation of B-catenin. With loss of APC function, B-catenin accumulates in the cytoplasm and nucleus and activates Tcf4 and c-myc. In the human, sporadic (non-colitic) colorectal adenomas and carcinomas show translocation of B-catenin from the cell membrane to the cytoplasm/nucleus, which, along with loss of APC function, is believed to be an early event in the development of colorectal neoplasia (37–40). This translocation has been shown to be associated with loss of function of APC; however, it has been shown that 48% of human colorectal adenoma/cancers lacking mutation of APC were found to have mutations of the B-catenin gene (67). In rats, cytoplasmic/nuclear translocation of B-catenin has been reported in azoxymethane-induced adenomas and carcinomas; however, in this model loss of APC function is rare (42). Rat colon tumors induced by methylazoxymethanol acetate and 1-hydroxyanthraquine have frequent mutations in the B-catenin gene in the absence of APC mutation (43). Karayiannakis et al. (68) studied the expression of B-catenin in humans with UC and Crohn’s disease. They found that in inactive or active colitis, B-catenin was always localized to the cell membrane; however, they did not study dysplastic or cancerous lesions. To date the only study of B-catenin in UC-associated neoplasia reported loss of heterozygosity of B-catenin in 7% of human UC-related cancers compared with 31% sporadic cancers. However, they did not study B-catenin immunohistochemically (32). In our study, we found translocation of B-catenin to the cytoplasm/nucleus in 100% of DALMs; however, 94.2% of flat dysplasia and/or cancers exclusively expressed B-catenin on the cell membrane ($P < 0.0001$). Our findings suggest that translocation of B-catenin is involved in polypoid dysplasia/cancer rather than flat lesions.
and polypoid lesions might be explained by different molecular pathways. Kobayashi et al. (69) report that in humans, K-ras mutations (codon 12) were significantly less common \( (P < 0.005) \) in sporadic non-polypoid tumors versus polypoid tumors. Sparks et al. (70) and Iwao et al. (71) have presented data indicating the existence of pathways of colorectal carcinogenesis independent of both APC and B-catenin. In our study we do not know whether the translocation of B-catenin was due to loss of APC function or directly through a mutation of B-catenin itself. In our study, the mean percentage of cells with cytoplasmic and/or nuclear expression of B-catenin was 42.6% for high-grade dysplasia versus 21% for low-grade dysplasia and 30.3% in 84 day animals versus 48.3% in 204 day animals. Hao et al. (40) showed that both carcinomas and adenomas with high grade dysplasia had a significantly greater percentage of lesions showing cytoplasmic/nuclear expression of B-catenin than did adenoma with low-grade dysplasia. This might suggest that this change may be associated with progression to cancer. In our study we noted that in the DALMs not all cells showed cytoplasmic/nuclear translocation (5–75%) of B-catenin. This phenomena has been noted in the human also (39,40).

The role of p53 in human colitis-associated neoplasia has been studied by both immunohistochemistry and molecular techniques. Immunohistochemical and molecular studies have reported that nuclear expression of p53 and both mutations and LOH of p53 occur as early events in human UC-related neoplasia in contrast to colorectal neoplasia in the non-colitic where they occur as a late event (23–24,26,27–31). By immunohistochemistry, nuclear expression of p53 has been reported in 33–60% and 7–45% of human UC cancers and dysplasia, respectively (23,26,30,31). In our study, nuclear expression of p53 was seen in 0% of cancers and in only 2/14 dysplasias (14.2%). The frequency of p53 alterations in murine primary epithelial tumors is generally lower than that seen in the human (72). Carcinogen-induced tumorigenesis of colorectal cancer in both mice and rats has resulted in conflicting reports regarding molecular alterations and nuclear expression of p53 with some authors reporting no role for p53 while others report a role for p53 (73–77). Studies of mice heterozygous for p53 show a 2–3% incidence of spontaneous colon cancers (78,79); however, colon cancers are not reported in mice homozygous deficient for p53 (80). Min mice crossed with p53-deficient mice (p53\(^{-/-}\)) do not show an increased number of colonic adenomas or an increased rate of colonic carcinoma when compared with Min mice with intact p53 (p53\(^{+/+}\)) (81). Reaves et al. (33) report in abstract form, that p53-deficient mice (p53\(^{-/-}\)) with chronic colitis induced by DSS had significantly higher numbers of dysplastic lesions than control mice with chronic DSS colitis. Suzui et al. (34) reported a 0% incidence of p53 mutations in colitis-associated rat colon tumors induced solely by 1-hydroxyanthraquinone; however, they studied only three tumors. In our model we found that p53 mutations (as detected by immunohistochemistry) are extremely uncommon in dysplasia and cancer in contrast to that seen in human UC-related neoplasia (23–24,27–29). Our

![Fig. 12.](image-url) **Fig. 12.** (A) Area of dysplasia showing nuclear expression of p53 in majority of cells. (B) Adjacent serial section stained with rabbit IgG as negative control. There is no nuclear staining.
findings may be due to: (i) a different molecular pathway regarding p53 involvement in colitis-associated neoplasia in the mouse as compared with the human; (ii) the low incidence of p53 alterations in murine colon cancer as noted in the literature; or (iii) a nonsense mutation of p53 that could account for the low incidence of immunoreactivity for nuclear p53.

In summary: (i) this model is similar to human UC-associated dysplasia and cancer regarding histopathology, the incidence of dysplasia and/or cancer and an increasing incidence of dysplasia and/or cancer with longevity of disease. We were unable to draw any conclusion regarding disease activity and the incidence of dysplasia and/or cancer. (ii) This model is dissimilar to human UC-associated neoplasia regarding the role of p53, multifocality of disease and a lower incidence of dysplasia associated cancer. (iii) Higher immunohistogen scores are significantly associated with dysplasia and/or cancer in animals fed DSS for four cycles, with flat dysplasia and/or cancer versus the DALM type, and with cancers versus dysplasia. (iv) The translocation of B-catenin to the nucleus/cytoplasm is an important role in the early molecular events of DALM but not flat dysplasia/cancer. (v) One attack of colitis is sufficient to induce dysplasia and/or cancer. (vi) Dysplasia of the DALM type can progress to cancer in the setting of ‘clinical remission’.

This study provides a well characterized model (with both similarities and dissimilarities to human UC-related neoplasia) to study the molecular mechanisms, the effects of inflammation in colitis-associated neoplasia and chemopreventive agents in preventing dysplasia and/or cancer. It also provides a model to study pathways of neoplasia which are either dependent or independent of B-catenin and independent of p53.

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References


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