Chemoprevention of intestinal adenomas in the Apc<sup>Min</sup> mouse by piroxicam: kinetics, strain effects and resistance to chemosuppression

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Previous cancer chemoprevention studies have demonstrated that NSAIDs can be effective in suppressing the development of intestinal tumors. To further explore this issue, we performed cross-over chemoprevention studies using the drug piroxicam in the Apc<sup>Min</sup> mouse to evaluate the kinetics of NSAID-mediated tumor regression, the effects of genetic background and the incidence of resistance to chemoprevention. Starting at the time of weaning, C57Bl/6J-Apc<sup>Min</sup> mice were fed either the control diet (AIN-93G) or AIN-93G plus 200 p.p.m. piroxicam. Tumor multiplicity was significantly reduced in Apc<sup>Min</sup> mice that were fed 200 p.p.m. piroxicam until 100 or 200 days of age (94.4 and 95.7% reduction in tumor number, respectively; \( P < 0.001 \) versus AIN-93G controls). When the administration of piroxicam was delayed until 100 days of age and the mice were killed at 200 days of age, tumor multiplicity was reduced by 96.2% (\( P < 0.001 \) versus controls). Alternatively, when the administration of piroxicam was suspended at 100 days of age and the mice were killed at 200 days of age, tumor multiplicity was reduced by 68.0% (\( P < 0.001 \) versus controls). Short-term drug treatment periods for Apc<sup>Min</sup> animals with established tumors revealed that the kinetics of piroxicam-induced tumor regression were rapid: >90% reduction in tumor multiplicity was observed after 1 week of treatment with 200 p.p.m. piroxicam. The distribution of residual tumors in piroxicam-treated mice suggests that tumors of the duodenum and colon were relatively resistant to chemosuppression. Treatment of interspecific hybrid Apc<sup>Min</sup> mice with 200 p.p.m. piroxicam revealed that there was a strain-related effect on chemosuppression, suggesting the existence of genetic elements which modulate NSAID chemosensitivity. Finally, whole-genome allelic loss studies showed that there were few unique chromosomal deletions in the NSAID-resistant tumors from F1 mice, implying that loss-of-function mutations secondary to Apc inactivation are not likely to account for the observed difference in chemoresistance.

Introduction
Recent advances in molecular genetics and tumor biology have provided valuable information about the genetic etiology and fundamental biochemical pathways of carcinogenesis. These molecular insights provide new therapeutic targets and new opportunities to develop more effective methods of cancer management, including pre-symptomatic detection and prevention. One promising strategy for cancer prevention, chemoprevention, refers to the inhibition of carcinogenesis through the use of naturally occurring or synthetic chemical agents (1). A wide variety of chemical agents have been shown to possess chemopreventive properties against a broad spectrum of tumor types. These agents are thought to function through mechanisms which include carcinogen detoxification and suppression of genetic mutation, inhibition of cellular signal transduction and proliferation, and induction of apoptosis (reviewed in ref. 2). Some of the best characterized candidate chemopreventive agents to date are the non-steroidal anti-inflammatory drugs (NSAIDs), which have been shown to be effective against the development of several types of solid tumors, in particular bladder and intestinal cancers (reviewed in refs 3 and 4).

NSAIDs are a chemically diverse family of agents which share the ability to inhibit the activity of cyclooxygenase, a key enzyme in the metabolic conversion of arachidonic acid to a variety of bio-active lipids including prostaglandins, thromboxanes and leukotrienes (5). Although the precise role of eicosanoids in carcinogenesis remains poorly understood, it has been recognized that eicosanoid levels are frequently elevated in many different types of human tumors (reviewed in ref. 6) and that induction of cyclooxygenase is an early event in the development of intestinal tumors (7). It has recently been shown that there are two structurally distinct isoforms of cyclooxygenase (COX-1 and COX-2), and that these isoforms are encoded by separate genes and are likely to have different cellular functions (reviewed in ref. 8). In the case of intestinal tissues, the COX-1 isoform has been shown to be a constitutively expressed enzyme which is thought to function in tissue homeostasis by facilitating prostaglandin-mediated signaling functions, while the COX-2 isoform has been shown to be inducible upon stimulation through oncogenic pathways or through tissue damage, and is thought to function in wound repair and inflammation (reviewed in ref. 9). During tumor development, cyclooxygenase itself may function in carcinogen activation, while elevated levels of eicosanoids may act in a wide variety of carcinogenic pathways including immune system modulation, cellular signal transduction and cell proliferation (reviewed in refs 10 and 11). It is significant that COX-2 (but not COX-1) expression levels are frequently elevated in intestinal polyps and cancers (12,13), and that pharmacological inhibition or genetic ablation of COX-2 suppresses intestinal tumor formation (14–16).

Several independent lines of experimental evidence now suggest that NSAIDs are effective inhibitors of intestinal tumor formation, and that they exert their chemoprotective effects through their inhibition of cyclooxygenase function and through the induction of apoptosis (reviewed in ref. 17). Many retrospective epidemiology studies have established an inverse

**Abbreviations:** 129 or 1, 129Sv/J; AAALAC, American Association for the Accreditation of Laboratory Animal Care; B6 or B, C57Bl/6J; CRC, colorectal cancer; F, FVB; LOH, loss of heterozygosity; NSAIDs, non-steroidal anti-inflammatory drugs; PCR, polymerase chain reaction; SPF, specific pathogen-free; TSG, tumor suppressor gene.
correlation between long-term NSAID use (in particular aspirin) and the risk for developing colorectal cancer (CRC). In addition, many prospective chemoprevention studies using animal colon tumor models have demonstrated the efficacy of several classical NSAIDs such as indomethacin, sulindac, and piroxicam (reviewed in ref. 5). More recently, several COX-2 selective inhibitors have also been shown to be effective in preventing the development of intestinal tumors (18,19). Human clinical trials using sulindac in the treatment of benign polyps in FAP patients have also demonstrated a chemopreventive benefit (reviewed in ref. 9), though the utility of NSAIDs against established CRC in humans remains controversial (20,21). One major limitation with the potential usefulness of classical NSAIDs for the purpose of cancer chemoprevention is the relatively high rate of adverse side effects associated with their long-term use, including gastrointestinal ulceration and renal toxicity (reviewed in ref. 22).

The ApcMin mouse model of intestinal neoplasia has provided a useful system for screening and characterizing candidate chemopreventive agents, and is described in detail elsewhere (23). This mouse was originally developed through chemical carcinogenesis in which a germline mutation was chemically induced at codon 850 of the Apc gene, resulting in the production of a truncated protein product (24). ApcMin mice develop multiple intestinal and colonic adenomas by 100 days of age, and thus provide a practical and genetically relevant model system for short-term chemoprevention studies. In addition, because genetic and environmental variables can be largely controlled in ApcMin mice, this model system provides the opportunity to gain information not readily available through human clinical trials alone. In order to make use of these important advantages, we have extended the studies of Jacoby et al. (25) by performing cross-over chemoprevention experiments using the NSAID piroxicam in the ApcMin mouse model to investigate the issues of kinetics of established polyp regression, resistance to NSAID suppression and genetic modulation of NSAID sensitivity.

Materials and methods

ApcMin mice

Male B6-ApcMin mice were purchased from the Jackson Laboratory (Bar Harbor, ME) and were mated to B6 wild-type females to maintain an inbred breeding colony from which to generate experimental animals. The interspecific crosses [(B6×FVB)F1-ApcMin and (B6×129SvJ)F1-ApcMin mice] were generated by mating male B6-ApcMin mice with FVB and 129SvJ wild-type females, respectively (for a complete description of these inbred mouse strains, see ref. 26). Because the ApcMin mutation is maintained in the heterozygous state, all progeny had to be screened for the presence of the ApcMin genotype at that time. Individual ApcMin mice were systematically assigned to drug treatment and control groups in order to normalize the distribution of males and females and to avoid the clustering of individual mice from single litters. At the end of the study, an intestinal tissue sample was taken from each animal and subjected to Min+/− screening PCR in order to verify the Min+/− genotype.

Diet and drug treatment

Study mice were housed in an American Association for the Accreditation of Laboratory Animal Care (AAALAC) approved specific pathogen-free (SPF) facility using forced air Thoren microisolator cages at a density of three mice per cage. Mice were provided with food and water ad libitum. Food and water were freely available at all times. Study mice were fed powdered AIN-93G diet (Dyets, Bethlehem, PA) or AIN-93G plus 200 p.p.m. piroxicam or 160 p.p.m. sulindac (Sigma, St Louis, MO) using food dispensers supplied by Dyets. All drug/food mixtures were prepared immediately before use, and all unused AIN-93G diet was stored at 4°C for a period not exceeding 3 months from the time of purchase. Fresh diet was provided every 2–3 days and piroxicam has been shown to be stable in this dietary mixture for at least a week (25).

Study groups and piroxicam treatment intervals

There were a total of 16 experimental groups included in this study (three AIN-93G control groups, one sulindac and 12 piroxicam treatment groups). For mice assigned to the 100 and 200 day drug treatment arms, piroxicam was provided continuously from the time of weaning to the time of death. For mice assigned to the 200-day cross-over study arms, piroxicam was provided either from weaning until 100 days of age (AIN-93G alone thereafter) or from 100–200 days of age (AIN-93G alone for the first 100 days). For mice included in the tumor regression kinetics study, piroxicam treatment began at 70–90 days of age and was continued until the time of death (2, 4, 6, 9 or 14 days later). For F1 mice included in the genetic modulation study, drug treatment began at 479–531 days of age and continued until the time of death (14 or 30 days later).

Tumor enumeration

Mice were killed by CO2 inhalation in accordance with current NIH guidelines. Necropsy was performed and the entire gastrointestinal tract was removed for dissection. The stomach and the cecum were omitted from the analysis due to their low tumor incidence. The small intestine was divided into three segments of approximately equal length (i.e. proximal, middle and distal small intestine) and the colon was left intact. All four intestinal segments were completely dissected using hemilobular paper as a support. Each segment was opened longitudinally using iris scissors and then washed extensively with PBS to remove intestinal contents. Tissues were fixed for 5 min in methacarn (60% methanol, 30% chloroform, 10% acetic acid) then rinsed in 70% ethanol. Tumor enumeration was performed using a Leica MZ3 stereo dissecting microscope, with darkfield trans-illumination (which substantially improved tumor contrast). All intestinal tissues were analyzed while mounted on a calibrated stage micrometer and were photographed at ×6.3 magnification in their entirety for a permanent record. The smallest tumors scored by this method were 0.2 mm in diameter and the site of each tumor was recorded to facilitate distribution analysis.

Allelic loss analysis

Microsatellite-based loss of heterozygosity analysis was performed using previously described methods (27). Briefly, polymorphic microsatellite loci were amplified by PCR and the resulting radiolabeled DNA fragments were resolved by denaturing polyacrylamide electrophoresis and visualized by autoradiography. Spleen was used as the source of normal tissue, while tumor DNAs were derived from intestinal polyps in which the surrounding normal mucosa and underlying muscularis mucosa were excluded by microdissection. Loss of heterozygosity was detected by comparing allelic intensities in matched normal versus tumor DNA samples, and a reduction of >50% in the relative intensity of one of the alleles was interpreted as evidence of allelic loss. The specific SSLP markers used in this study were: D1Mit3, D2Mit224, D3Mit127, D4Mit328, D5Mit164, D6Mit59, D7Mit297, D8Mit14, D9Mit154, D10Mit28, D11Mit67, D12Mit8, D13Mit147, D14Mit17, D15Mit158, D16Mit110, D17Mit39, D18Mit113, D19Mit47, D18Mit57, D18Mit119, D18Mit111, D19Mit30 and DXMit55.

Statistical analysis

Statistical comparisons of tumor multiplicity between chemoprevention study groups were performed using Wilcoxon rank-sum or two-sample t-tests. For the loss of heterozygosity study, rates of allelic loss on individual chromosomes were compared using a two-tailed Fisher’s exact test. All P-values <0.05 were considered significant.

Results

The experimental groups and drug treatment intervals used in this study are summarized in Figure 1. For the B6-ApcMin study groups depicted in Figure 1A, 200 p.p.m. piroxicam was supplied through either continuous or discontinuous drug treatment schedules (detailed in Materials and methods). The purpose of the B6-ApcMin study was to evaluate the kinetics, timing and duration of piroxicam-mediated intestinal tumor suppression in the inbred background. For the [B6F1-ApcMin and [B1]F1-ApcMin study groups depicted in Figure 1B, 200 p.p.m. piroxicam was supplied through short interval treatment periods (14 or 30 days in duration) initiated at 16–17 months of age. The purpose of the F1-ApcMin study was to evaluate strain-dependent resistance to piroxicam-mediated tumor regression.
B6-ApcMin mice treated with 200 p.p.m. piroxicam showed statistically significant reductions in tumor multiplicity versus controls for all four of the long-term piroxicam treatment groups (Figure 2). There was no significant difference in tumor multiplicity between the 100 and 200 day age end-point groups, implying that continuous piroxicam treatment maintains intestinal tumor suppression well beyond the normal lifespan of an untreated B6-ApcMin mouse (~130–150 days). There was also no significant difference in tumor multiplicity between the AIN→piroxicam cross-over and the continuous drug treatment groups, suggesting that piroxicam was equally effective at inducing regression of established adenomatous polyps as at preventing the development of nascent polyps. The piroxicam →AIN cross-over group showed an intermediate level of tumor suppression, indicating that piroxicam may cause irreversible regression of some neoplastic foci, and may thus confer a long-term anti-tumor benefit from discontinuous drug treatment. Average weight loss (defined as weight at death versus weekly maximum weight) was ~4% in each of the control and drug treatment groups at the 100 day age end-point, reflecting the facts that tumor-related cachexia has a relatively late onset under these experimental conditions and that 200 p.p.m.
piroxicam does not frequently cause acute short-term toxicity in these animals.

Although piroxicam was effective against tumors in all regions of the intestine, it was most effective against tumors of the jejunum and ileum and was significantly less effective against tumors of the duodenum and colon ($P < 0.05$ by $\chi^2$ analysis; data not shown). Chemoresistant tumors (those present after 14 days or more of drug treatment) were morphologically and histopathologically similar to untreated tumors (i.e. there was no consistent change in the histologic features of chemoresistant versus untreated tumors). Figure 3 shows low power photomicrographs and histology of representative piroxicam-resistant intestinal tumors. In addition to its anti-tumor activity, piroxicam frequently caused mucosal ulceration in the small intestine of treated mice (Figure 4), which was detectable as early as 6 days after the initiation of drug treatment. These small bowel ulcers were histologically characterized as superficial mucosal erosions showing signs of acute inflammation and fibrosis. Corresponding colonic lesions in piroxicam-treated mice had the gross appearance of polypoid growths (Figure 4), but were histologically characterized as benign mucosal hyperplasias with no signs of dysplasia. It is important to note that piroxicam-associated ulcers and colonic hyperplasias occurred in both B6 (wild-type) and B6-Apc$^{Min}$ mice, suggesting that ulceration was not an artifact of tumor regression. Finally, intestinal ulcers were clearly distinguishable from tumors at both macroscopic and histologic levels.

The kinetics of piroxicam-induced regression of established intestinal adenomas in B6-Apc$^{Min}$ mice were very rapid, as shown in Figure 5. Gross morphologic changes in the appearance and size of the tumors were apparent after only 2 days of piroxicam treatment, and maximum tumor regression was achieved by treatment day 6.

In order to investigate the effects of genetic background on tumor chemosensitivity, we performed cross-over chemoprevention studies using F1-Apc$^{Min}$ mice (as illustrated in Figure 1). While intestinal adenomas from B6-Apc$^{Min}$ and [B1]F1-Apc$^{Min}$ mice shared a similar degree of sensitivity to piroxicam-induced tumor regression (96.2 and 93.7% reduction in tumor number versus controls after 14 days of drug treatment, respectively), intestinal tumors from [BF]F1-Apc$^{Min}$ mice were significantly more resistant to NSAID suppression (36.3–54.0% reduction in tumor number versus controls after 14 or 30 days of drug treatment, respectively; $P < 0.01$ versus B6 or [B1]F1 treatment groups). This was true for both 200 p.p.m. piroxicam and 160 p.p.m. sulindac, and for both 14 and 30 day treatment periods (Figure 6).
Piroxicam in the Apc<sup>Min</sup> mouse

Fig. 4. Intestinal ulceration and colonic hyperplasia in piroxicam-treated mice. (A–C) Low power photomicrographs of transilluminated intestinal tissues from piroxicam-treated wild-type B6 mice (100 day drug treatment group). (A) Duodenal ulcer; (B) ileal ulcer; (C) colonic hyperplasia. Arrows indicate the approximate location of the corresponding histological sections. (D–F) Matched hematoxylin and eosin-stained sections for these intestinal lesions.

Fig. 5. Kinetics of intestinal tumor suppression by piroxicam. B6-Apc<sup>Min</sup> mice with pre-existing intestinal tumors (70–90 days of age) were treated with 200 p.p.m. dietary piroxicam for short intervals (2, 4, 6, 9 or 14 day periods) prior to death. The histogram compares tumor multiplicity from these treatment groups to the control (no drug) group. Error bars are standard deviations. n, number of mice per treatment group; *, statistical significance (P < 0.01 using the Wilcoxon rank-sum test).

Finally, in order to determine whether loss-of-function mutations secondary to inactivation of the wild-type Apc allele might account for the observed resistance to piroxicam-induced tumor regression in the F1-Apc<sup>Min</sup> mice, we performed allelic loss studies on microdissected intestinal tumor tissues. A total of 37 intestinal tumors (including seven piroxicam-resistant tumors) from F1-Apc<sup>Min</sup> mice were genotyped using 24 microsatellite markers representing every autosome in the mouse genome (including five markers on chromosome 18). Although loss of heterozygosity (LOH) was frequently observed for markers on chromosome 18 (which harbors the Apc gene), very few losses and no microsatellite instability was observed for any of the other autosomes. There were no significant differences in the allelic loss profiles of piroxicam-resistant versus unselected tumors. Examples of chromosome 18 LOH in Apc<sup>Min</sup> tumors are shown in Figure 7A, and the results of the entire allelotype analysis are summarized in Figure 7B.
Fig. 6. Strain-related sensitivity to chemosuppression by piroxicam. Interspecific hybrid (F1)-Apc<sub>Min</sub> mice were evaluated for their sensitivity to piroxicam-mediated tumor regression as compared with B6-inbred Apc<sub>Min</sub> mice. B6×(P14), B6×Apc<sub>Min</sub> mice fed 200 p.p.m. piroxicam for 14 days; B×1×(P14), [B6×129]F1-Apc<sub>Min</sub> mice fed 200 p.p.m. piroxicam for 14 days; B×F(P14), [B6×FV]B1-Apc<sub>Min</sub> mice fed 200 p.p.m. piroxicam for 14 days; B×F(S30), [B6×FV]B1-Apc<sub>Min</sub> mice fed 200 p.p.m. piroxicam for 30 days; B×F(S30), [B6×FV]B1-Apc<sub>Min</sub> mice fed 160 p.p.m. sulindac for 30 days. *, statistical significance (P < 0.01 versus the strain-matched control group); NS, not significant (P > 0.05 versus controls).

Fig. 7. (A) LOH on chromosome 18 in F1-Apc<sub>Min</sub> mice. Example chromatograms show allelic imbalance for polymorphic loci on mouse chromosome 18. In all cases of chromosome 18 LOH in these F1-Apc<sub>Min</sub> tumors, it was the FVB or 129 allele which was deleted, most likely reflecting somatic inactivation of the wild-type copy of the Apc gene. (B) Histogram of allelic loss in Apc<sub>Min</sub> mice. Whole-genome loss of heterozygosity analysis using intestinal tumors derived from [B6×129]F1-Apc<sub>Min</sub> and [B6×FV]B1-Apc<sub>Min</sub> mice showed a very low background rate of allelic loss with the notable exception of chromosome 18, which was frequently deleted (77.8%, 12.1% SD; *P < 0.01 versus background rate of loss). No other single chromosome had a rate of loss significantly greater than background. Y error bars are standard deviations.

Discussion

Continued development of effective chemopreventive agents offers a promising new strategy for improving current cancer treatment methods. The most useful chemopreventive agents will have minimal long-term toxicity while significantly reducing tumor incidence, delaying tumor onset or preventing tumor progression. A large body of experimental evidence suggests that some NSAIDs can be effective in the chemoprevention of intestinal tumors. The specific purpose of this study was to further characterize the duration, kinetics and timing of chemopreventive activity by piroxicam using the Apc<sub>Min</sub> mouse model of intestinal neoplasia.

The long duration of chemosuppressive efficacy by continuous treatment with 200 p.p.m. dietary piroxicam was demonstrated by the observation that tumor multiplicity in the 100 and 200 day age groups was essentially identical (no statistically significant difference). The fact that no additional tumors had developed in the 200 day age group (by comparison to the 100 day age group) suggests that pre-existing neoplastic foci do not frequently develop acquired resistance to piroxicam suppression and that chemoresistance may thus be determined early in the tumorigenic process.

The short interval drug treatments using Apc<sub>Min</sub> mice with established tumors (70–90 days of age at drug initiation) demonstrated that the kinetics of piroxicam-induced tumor regression are very rapid. A statistically significant reduction in tumor multiplicity was observed after only 4 days of treatment with 200 p.p.m. piroxicam, and maximum suppression was reached after only 6 days of treatment. This observation is consistent with the recent report of Chiu et al. (28), who showed that 320 p.p.m. dietary sulindac induced significant tumor regression in Apc<sub>Min</sub> mice after only 4 days of treatment. The rapid induction of tumor regression may reflect the fact that piroxicam is thought to induce apoptosis (29) and that the rate of cellular turnover in the intestine is very high [i.e. complete renewal of the epithelial lining occurs every 2–3 days in the mouse (30)]. The rapid kinetics of NSAID-mediated chemosuppression might be particularly useful for neo-adjuvant therapy in human patients, where drug-based tumor debulking preceding surgical or endoscopic excision may be desirable.

The cross-over treatments included in this study suggest that piroxicam can be equally effective against both nascent and established adenomatous polyps. This hypothesis is supported by the observation that there was no significant difference in tumor reduction for animals treated with piroxicam beginning at the time of weaning versus animals in which the initiation of piroxicam treatment was delayed until 100 days of age. However, it is important to recognize that the intestinal tumors arising in Apc<sub>Min</sub> mice have generally not undergone extensive genetic or phenotypic progression (31), and thus the chemosensitivity of established adenomas in these animals might not be applicable to more advanced tumors.

The cross-over studies also revealed that there may be a lasting chemopreventive benefit to piroxicam treatment early in life, even long after the cessation of drug. This effect is demonstrated by the fact that animals treated with piroxicam until 100 days of age and then crossed onto AIN-93G diet alone until 200 days of age had a 68% reduction in intestinal tumor multiplicity versus control animals. Although most intestinal tumor initiation is thought to occur within the first month of life in Apc<sub>Min</sub> mice (32), it has been reported that new tumors continue to appear throughout the lifespan of B6-Apc<sub>Min</sub> mice (33). Therefore, it is not known whether the tumors present in the piroxicam→AIN-93G cross-over mice represent tumors arising as the result of de novo transformation events or whether they instead reflect the outgrowth of pre-existing (but
macroscopically undetectable) neoplastic elements held in stasis by piroxicam. However, in the case of human FAP patients treated with sulindac, it has been reported that polyposis regression is reversible upon the suspension of treatment (i.e. it is thought that the original colonic polyps recur once sulindac is removed; reviewed in ref. 34). If piroxicam-induced tumor regression were likewise reversible in the Apc\textsuperscript{Min} mouse, we might have expected to see a full complement of tumors in the 200-day old piroxicam→AIN-93G mice. The fact that there was a significant reduction in tumor burden as the result of discontinuous 200 p.p.m. piroxicam treatment suggests that high dose piroxicam can induce irreversible regression of intestinal adenomas in this model system.

While the vast majority of intestinal tumors in Apc\textsuperscript{Min} mice are susceptible to suppression by 200 p.p.m. piroxicam, ~5% of the individual tumors in B6-Apc\textsuperscript{Min} mice are resistant to chemoprevention, even after extended treatment periods. This suggests that these resistant tumors have a pre-existing or acquired chemoresistance and a reduced reliance on cyclooxygenase activity. One possible explanation for this phenomenon would be the accumulation of genetic mutations (such as tumor suppressor gene inactivation) which might circumvent the need for continued cyclooxygenase activity. In order to address this possibility, we performed whole-genome loss of heterozygosity analysis in chemoresistant F1 tumors in order to detect chromosomal loss or deletion, which is often associated with loss of function mutations. Although frequent whole-chromosome loss was observed on chromosome 18 in these Apc\textsuperscript{Min} mice (most likely reflecting the obligate inactivation of the wild-type allele of the Apc gene), LOH elsewhere in the genome was rare and there was no consistent differential in the pattern of LOH observed between unselected versus chemoresistant tumors. Thus, our comparative allelotype suggests that tumor suppressor gene (TSG) inactivation through chromosomal deletion is not a likely mechanism of acquired NSAID resistance in this model system. However, the possibility that gain of function mutations (such as oncogene activation) may play a role in chemoresistance cannot be excluded by this analysis.

There appears to be a strain-dependent effect on the sensitivity of intestinal tumors to NSAID suppression in Apc\textsuperscript{Min} mice, suggesting the existence of genetic biological response modifiers. Tumors arising in [B6×FVB]F1-Apc\textsuperscript{Min} animals are significantly more resistant to piroxicam suppression than either B6-Apc\textsuperscript{Min} or [B6×129]F1-Apc\textsuperscript{Min} derived tumors. It is important to note that expression of the tumor phenotype in Apc\textsuperscript{Min} mice has previously been demonstrated to be dependent on several genetic modifier loci including Mom-1 (35), and that these genetic elements affect both tumor latency and multiplicity. The fact that B6-Apc\textsuperscript{Min} and [B6×129]F1-Apc\textsuperscript{Min} mice share a susceptible allele of Mom-1 and are both highly sensitive to NSAID suppression, while [B6×FVB]F1-Apc\textsuperscript{Min} mice have a resistant allele of Mom-1 and are relatively resistant to NSAID suppression may suggest that chemosensitivity is related to Mom-1 status. However, it is also possible that genetic modifier loci distinct from Mom-1 are involved in the strain-dependent modulation of chemosensitivity.

There also appears to be a regional bias in the efficacy of piroxicam-induced tumor suppression in Apc\textsuperscript{Min} mice. Although neoplasms in all regions of the intestine were at least partially responsive to piroxicam treatment, tumors of the duodenum and colon were relatively resistant by comparison to tumors of the jejunum and ileum. Because piroxicam is incompletely absorbed in the gut and may act through either topical or systemic mechanisms, it is possible that differential drug concentrations along the gastrointestinal tract account for some of the observed regional bias. However, it is also possible that biological mechanisms contribute to chemoresistance in tumors of the duodenum and colon, as these lesions are morphologically distinct from polypos of the distal small intestine. A similar region-specific sensitivity to chemoprevention has been observed for colonic tumors arising in azoxymethane-treated rats (36).

Finally, an important consideration in any cancer chemoprevention trial is the issue of toxicity. In human patients taking long-term therapeutic doses of piroxicam (20 mg/day), ~20% of individuals experience significant adverse gastrointestinal side effects (ranging in severity from dyspepsia to intestinal perforation; reviewed in ref. 37). Likewise, treatment with 200 p.p.m. piroxicam in Apc\textsuperscript{Min} mice (corresponding to ~33 mg/kg/day) resulted in grossly detectable intestinal ulceration in >90% of treated animals after 6 or more days of treatment. Ulcers ranging in size from 0.2 mm (the lower scorable limit of our macroscopic detection) to >3 mm were observed in all regions of the small intestine, with the most severe lesions occurring in the duodenum. In addition to intestinal ulceration, >50% of piroxicam-treated mice also had regions of benign polyoid hyperplasia in the proximal colon. Morphologically similar intestinal lesions also developed in wild-type B6 animals treated with 200 p.p.m. piroxicam, suggesting that the observed ulcers and hyperplasias were not an artifact of tumor regression in Apc\textsuperscript{Min} mice. Although unexpected mortality in piroxicam-treated animals was rare, necropsy revealed that on at least one occasion intestinal perforation was the likely cause of death. Because NSAID-associated gastrointestinal toxicity is a major concern in human patients (reviewed in ref. 22), the Apc\textsuperscript{Min} mouse model may be particularly useful for identifying NSAID formulations, dosage levels, and treatment intervals which provide the best therapeutic margin by minimizing intestinal toxicity while maximizing anti-tumor efficacy.

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