candidates for clinical application of chemoprevention against CRC development in patients with ulcerative colitis.

Figure 5. Incidences and multiplicities of mucosal ulcer and dysplastic crypts in the bezafibrate study.

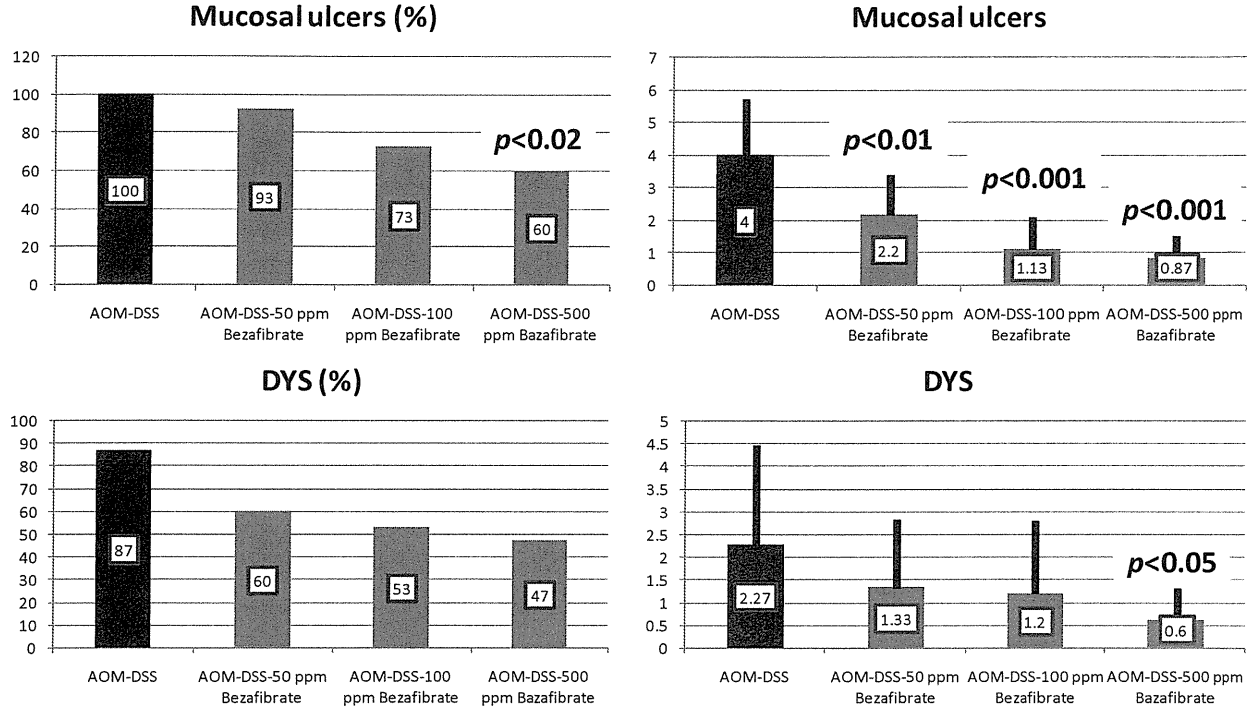
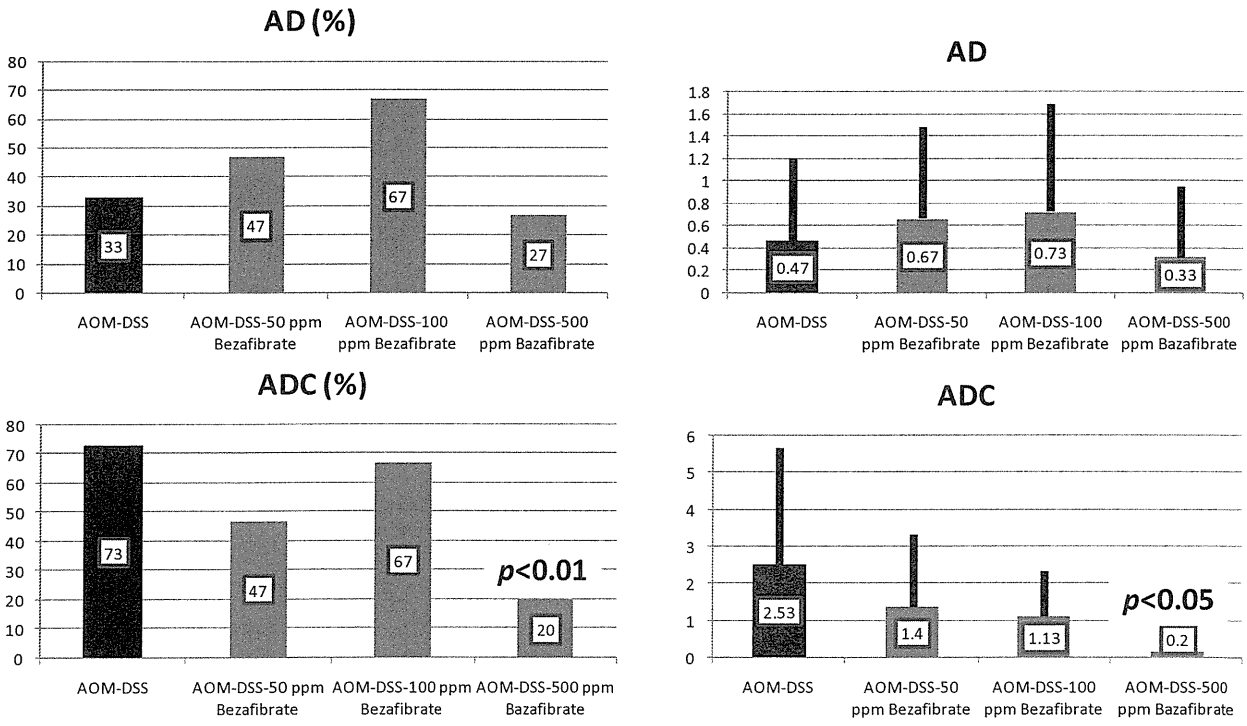


Figure 6. Incidences and multiplicities of adenoma (AD) and adenocarcinoma (ADC) in the bezafibrate study.



Accumulating animal experimental, human laboratory and epidemiologic data [50,63,79,82,83] support the hypothesis linking triglyceride levels and insulin resistance to the development of colon cancer. These facts emphasize the potential for this cancer to become a preventable disease not only via screening and removal of polyps but through relevant lifestyle changes and pharmacological interventions which can provide even more avenues for prevention [83–85].

The incidence of insulin resistance has been increasing in the Western world where colon cancer is the second leading cause of cancer death. This suggests the interrelationship of these conditions. The biological role of PPARs in various diseases, including inflammation and cancer, has been highlighted recently [50,63,78,85–87]. PPARs are members of the nuclear hormone receptor family of ligand-activated transcription factors that play a prominent role in the regulation of many metabolic processes. The PPAR isoformes α and γ are important regulators in lipid and glucose metabolism, cell differentiation and inflammatory response [87,88]. These data propose that PPAR may be associated with many aspects of colon cancer development including insulin- and inflammation-related mechanisms. The fibric acid derivative bezafibrate is the pan PPAR (α, β/δ, and γ) activator with predominantly PPARα (as all fibrates) and β/δ effects but also with perceptible PPARγ properties [88–90]. The use of bezafibrate is associated with triglyceride-lowering and high density-cholesterol raising effects resulting in decreased systemic availability of fatty acid, diminished of fatty acid uptake by muscle and improvement of insulin sensitization [91,92]. These direct and indirect effects may have contributed to the suppression of the development of colonic tumors in rodents by bezafibrate [50,63,79]. Recently, Tenenbaum *et al.* [93] have reported possible preventive effects of bezafibrate on the development of CRC from patients with coronary artery disease.

4.3. Valproic Acid (VPA) Study

Epigenetic modification plays an important role in tumorigenesis. Affecting epigenetic and tumorigenic alterations is a promising strategy for anticancer targeted therapy [94–96]. Among the key chromatin modifying enzymes which influence gene expression, histone acetyltransferases (HATs) and histone deacetylases (HDACs) have attracted interest because of their impact on tumor development and progression. Histone deacetylase inhibitors (HDACIs) represent a new and promising class of antitumor drugs that influence gene expression by enhancing acetylation of histones in specific chromatin domains. HDACIs also exert potent anticancer activities inducing cell cycle arrest and apoptosis. Moreover, HDACIs down-regulate genes involved in tumor progression, invasion and angiogenesis. Based on the ability of HDACIs to regulate many signaling pathways, co-treatment of these compounds that are currently under clinical investigation with molecular targeted drugs is a promising strategy against many types of tumors.

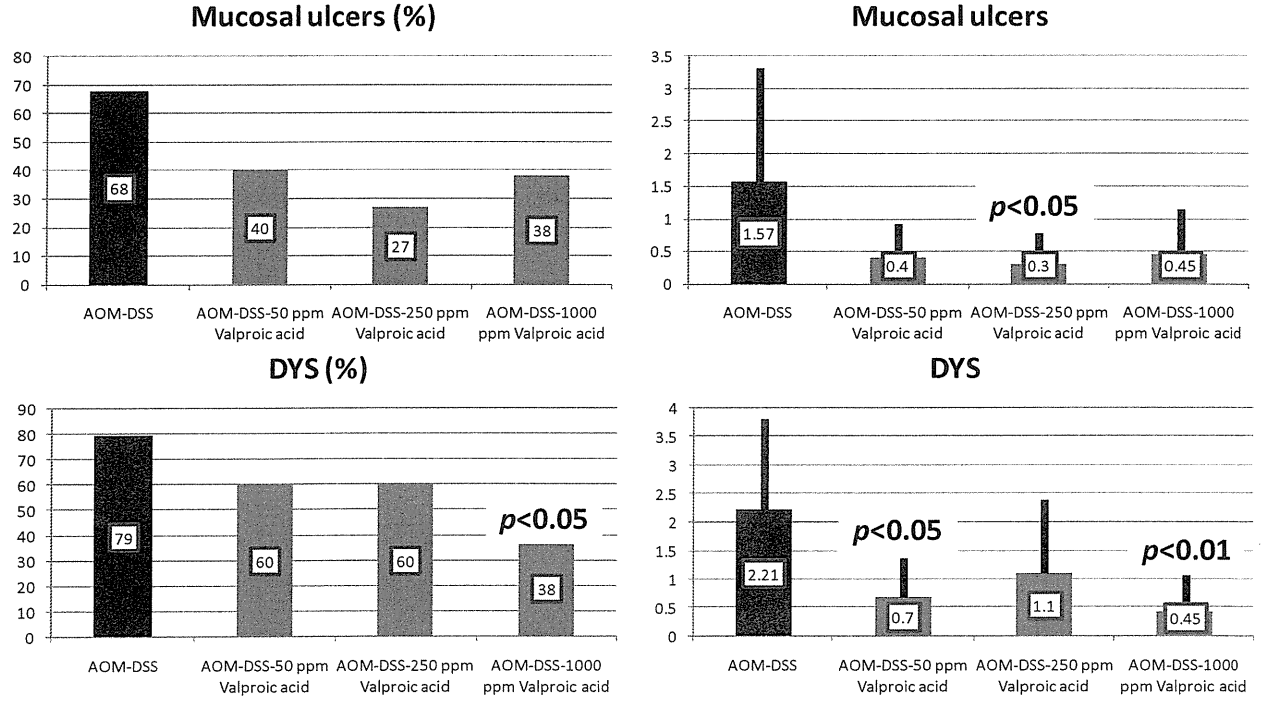
VPA (2-propylpentanoic acid) [97] is a well-established drug for the therapy of epilepsy. It is teratogenic when administered during early pregnancy and can induce birth defects such as neural tube closure defects and other malformations. This well-tolerated antiepileptic drug was found to be a powerful HDAC-1 inhibitor [97]. VPA induces differentiation of carcinoma cells, transformed hematopoietic progenitor cells and leukemic blasts from acute myeloid leukemia patients [98]. Our microarray analysis during the AOM-DSS carcinogenesis revealed alteration of Wif-1 expression [34]. VPA has been reported to modify the Wif-1 expression [99]. These findings may suggest possible

modifying effects of VPA on AOM-DSS colorectal carcinogenesis. In this study, we determined whether VPA is able to inhibit colitis-associated colon carcinogenesis in mice.

Materials and methods: A total of 85 mice aged five weeks was used and they were divided into 8 groups: AOM/2% DSS (n = 19), AOM/2% DSS/50 ppm VPA (n = 15), AOM/2% DSS/250 ppm VPA (n = 15), AOM/2% DSS/1,000 ppm VPA (n = 16), AOM alone (n = 5), 2% DSS alone (n = 5), 1,000 ppm VPA alone (n = 5), and untreated (n = 5) groups. Mice were initiated with a single s.c. injection of AOM (10 mg/kg bw) were promoted by 2% DSS in their drinking water for seven days. They were then given a basal diet containing 50, 250 or 1,000 ppm of VPA for 17 weeks. At the end (week 20) of the study histopathological examination of large bowel was performed on H&E-stained histological sections (3 μm in thickness). Immunofluorescence technique using anti-Mcm2 antibody (BD Biosciences PharMingen) for evaluating proliferating activity and fluorescein in situ tunnel method, TACS TdT kit (R&D Systems, Inc.) for detecting apoptosis cells were applied on histological sections of colonic ADCs. Polyamine levels [72] and mRNA expression of NF-κB, TNF-α, IL-1β, Stat3, and HIF-1α [73] in colonic mucosa were assayed in some mice of each group.At the end of the study (week 20), Measurements were statistically analyzed using either the Tukey multiple comparison post test or Fisher's extract probability test. Differences were considered to be statistically significant at $p < 0.05$.

Results: VPA feeding inhibited the development of mucosal ulcer (Figure 7) and dysplastic crypts (the incidence at 1,000 ppm VPA, $p < 0.05$; and the multiplicity at 50 ppm, $p < 0.05$ and at 1,000 ppm, $p < 0.01$, Figure 7).

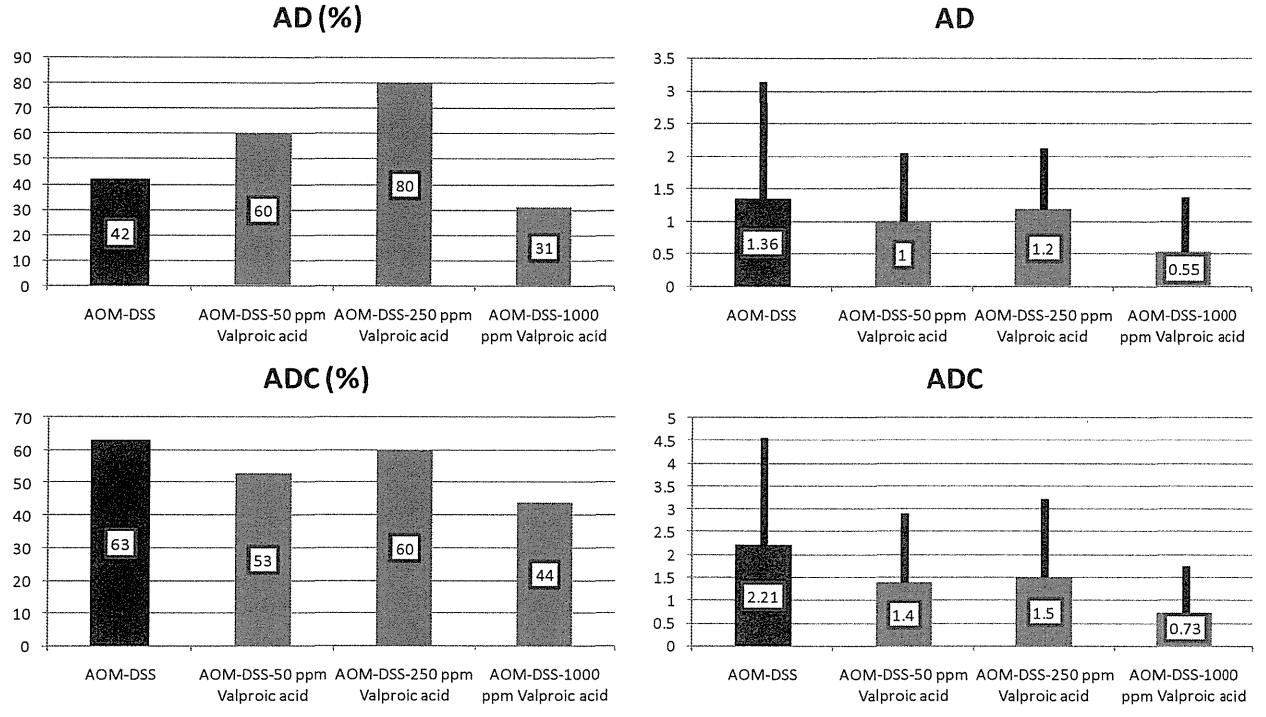
Figure 7. Incidences and multiplicities of mucosal ulcer and dysplastic crypts in the Valproic Acid (VPA) Study.



The development of colonic AD and ADC was lowered by feeding with VPA, but the reduction rates were statistically insignificant (Figure 8). When fed with VPA-containing diet, Mcm2 positive rates (%)

of ADCs were lower than that of the AOM and DSS group (n = 9, 80.1 ± 6.8): 125 ppm VPA (n = 7, 73.7 ± 8.0), 250 ppm VPA (n = 6, 64.2 ± 2.5, $p < 0.01$), and 1,000 ppm VPA (n = 5, 61.6 ± 9.6, $p < 0.001$). As to apoptotic index (%) of ADCs, the values of mice fed with 125 ppm VPA (n = 7, 10.67 ± 3.44), 250 ppm VPA (n = 6, 12.33 ± 1.75, $p < 0.05$), and 1,000 ppm VPA (n = 5, 12.80 ± 2.39, $p < 0.05$) were higher thab the AOM and DSS group (n = 9, 8.44 ± 1.33).

Figure 8. Incidences and multiplicities of adenoma (AD) and adenocarcinoma (ADC) in the Valproic Acid (VPA) Study.



Our findings suggest slight chemopreventive effects of VPA on colitis-related colon carcinogenesis in mice, suggesting that single use of a HDAC inhibitor VPA is not practical for inhibiting CRC development in inflamed colon. VPA combined with other known chemopreventive or chemotherapeutic agent(s) may exert to inhibit CRC that develop in colitic mucosa.

VPA was studied in combination with all-*trans* retinoid acid in patients with acute myeloid leukemia who were not candidates for intensive chemotherapy [100]. Using human hepatocellular carcinoma (HCC) cells, HepG2, combination treatment with acyclic retinoid and VPA is demonstrated to be an effective regimen for the chemoprevention and chemotherapy of HCC [101]. Acyclic retinoid and VPA cooperatively increase the expression of retinoid X receptor (RXR)-β and p21 (CIP1), while inhibiting the phosphorylation of RXRα, and these effects were associated with induction of apoptosis and the inhibition of cell growth in HepG2 cells. Several HDACIs seem to exert an antitumor effect in a synergistic manner with different anticancer compounds and to overcome the resistance induced by conventional chemotherapeutic drugs [102–105]. A phase I trial of single agent VPA was reported in patients with newly diagnosed cervical cancer [106]. Twelve patients were included. VPA doses ranged from 20 mg/kg to 40 mg/kg daily for five days. Tumor HDAC activity decreased in 8 patients. Many lines of evidence suggest that tumor cells are characterized by histone hypoacetylation and that

over-expression of HDACs is involved in tumorigenesis of various human malignancies [107,108]. Recently, a population-based case-control study with long-term users of VPA has not supported HDAC inhibition by VPA as a pharmacologic principle for general chemoprevention [109]. However, VPA doses (0.35–0.70 mmol/L) used in clinical practice could be too low to achieve cancer preventive effects in contrast the doses (0.50–3.0 mmol/L) that inhibit HDAC. Since VPA is reported to suppress progression of urological malignancies [110,111], it is worthy to evaluate the modifying effects of VPA on different stage of carcinogenesis.

5. Effects of Morin, Bezafibrate and VPA on Expression of Pro-Inflammatory Cytokines and HIF-1α and Content of Tissue Polyamines in the Inflamed Colon

IBD represents a dysregulated mucosal immune response to antigens derived from the commensal microbiota in a genetically susceptible host that initially derives from innate immune abnormalities leading to an excessive pro-inflammatory cytokines (T-helper 1, T-helper 2, and T-helper 17 cytokines) derived from $CD4^+$ T cells [9,112]. A key point in understanding IBD pathophysiology is to understand the immunoregulatory pathways associated with the intestinal immune system as they apply to IBD. Therefore, in addition to immunotherapy, pro-inflammatory cytokines secreted by innate and adaptive immune cells are targets for IBD treatment [73]. Similarly, cytokines, including NF-κB [73,113], TNF-α [9,35,64], and interleukin (IL)-1β [64,114,115] are potentially molecular targets for inflammation-associated CRC [14]. Potential cancer chemopreventive agents also modulate expression of signal transducer and activator of transcription (Stat3) [55,114], HIF-1α [116,117], and survivin [73] in the target tissues.

Polyamines are organic cations that control gene expression at the transcriptional, posttranscriptional, and translational levels. Multiple cellular carcinogenesis pathways are involved in regulation of transcription and translation of polyamine-metabolizing enzymes [118]. We have reported he importance of research utilizing pharmaceutical inhibitors and cancer chemoprevention against CRC targeting the polyamine pathway [72,119,120].

The findings in *in vivo* studies using the TANAKA models suggest that the order of chemopreventive potential of test compounds was bezafibrate > morin > VPA by estimating inhibition rate of CRC development. To investigate the effects of these chemicals on the molecules that are involved in carcinogenesis, we determined mRNA expression of the NF-κB (Figure 9), TNF-α, (Figure 10), IL-1β (Figure 11). Stat 3 (Figure 12), HIF-1α (Figure 13) in colorectal mucosa of mice or rats from the experiments with morin, bezafibrate, and VPA. At sacrifice, each colon was cut open longitudinally, and was flushed clean with PBS. Five animals of each group from three experiments were used for real-time quantitative RT-PCR analysis. Their distal colon was taken for total RNA isolation. For total RNA isolation, epithelial cells were scraped from the underlying muscle layer with a glass microscope slide, were homogenized on ice in lysis buffer (Qiagen, Tokyo, Japan), and were frozen at −80 °C until RNA was isolated. Total RNA was extracted from colonic mucosa using the RNeasy Mini Kit (Qiagen) according to the manufacturer's protocol. The cDNA was then synthesized from total RNA using the High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems Japan Ltd., Tokyo, Japan). Quantitative real time PCR analysis of individual cDNA was performed with ABI Prism 7500 (Applied Biosystems Japan Ltd., Tokyo, Japan) using TaqMan Gene Expression Assays (Applied Biosystems

Japan Ltd., Tokyo, Japan) and primers, which were chosen on the basis of rat or mouse nucleotide sequences in the GenBank database (Table 1). PCR cycling conditions were 50 °C for 2 min, 95 °C for 10 min, followed by 40 cycles of 95 °C for 15 s and 60 °C for 1 min. The relative mRNA expression was normalized by b-actin mRNA. All the test chemicals lowered mRNA expression of these proteins. Therefore, effects of these three test compounds on the development of mucosal ulcer, dysplastic crypts, and colonic neoplasms may be related to the expression that was modified by feeding with test chemicals.

Figure 9. mRNA expression of NF-κB in the colorectum of mice or rats from the morin, bezafibrate, and VPA studies.

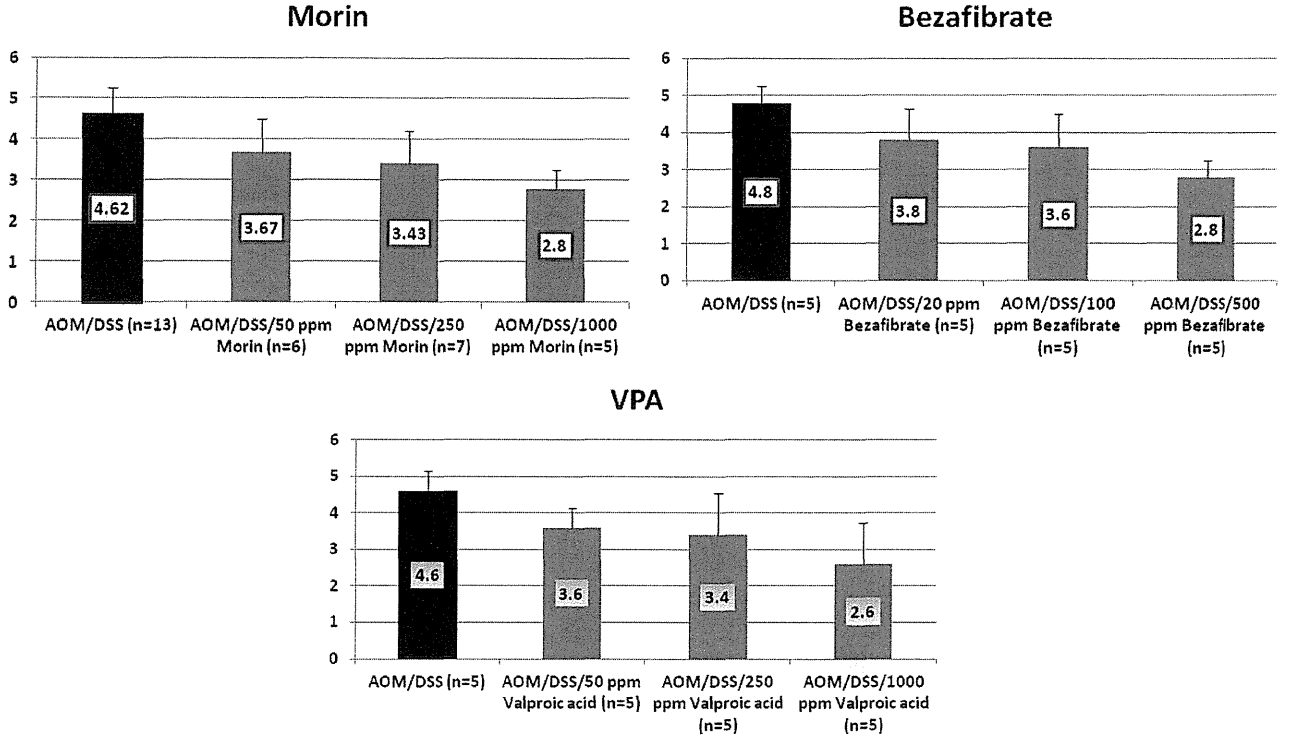


Figure 10. mRNA expression of TNF-α in the colorectum of mice or rats from the morin, bezafibrate, and VPA studies.

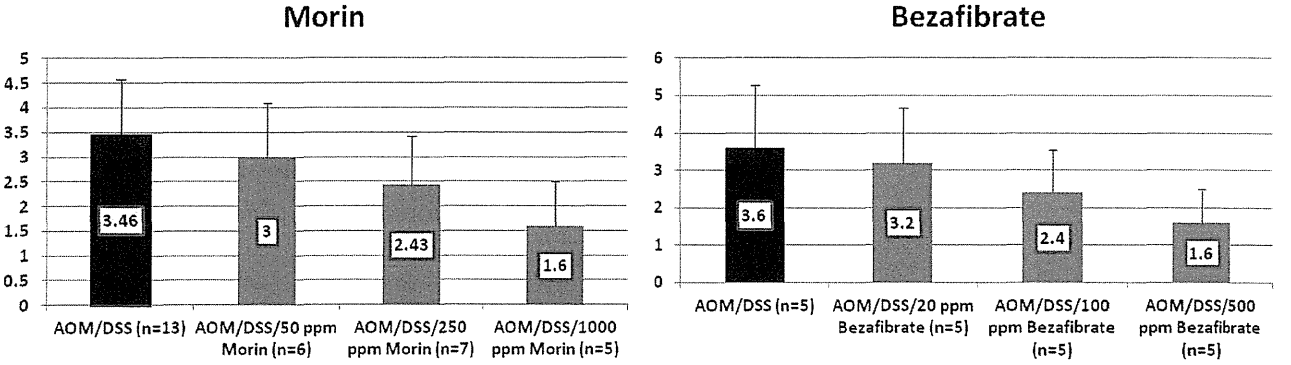


Figure 10. *Cont.*

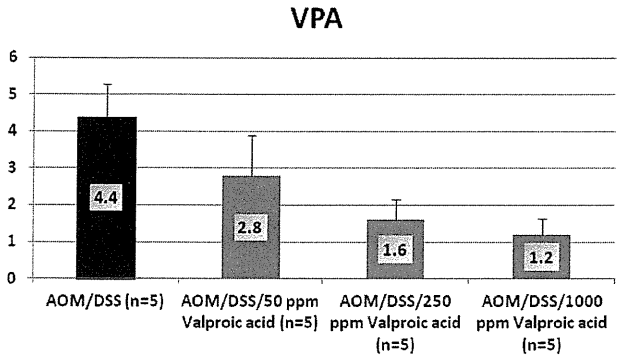


Figure 11. mRNA expression of IL-1β in the colorectum of mice or rats from the morin, bezafibrate, and VPA studies.

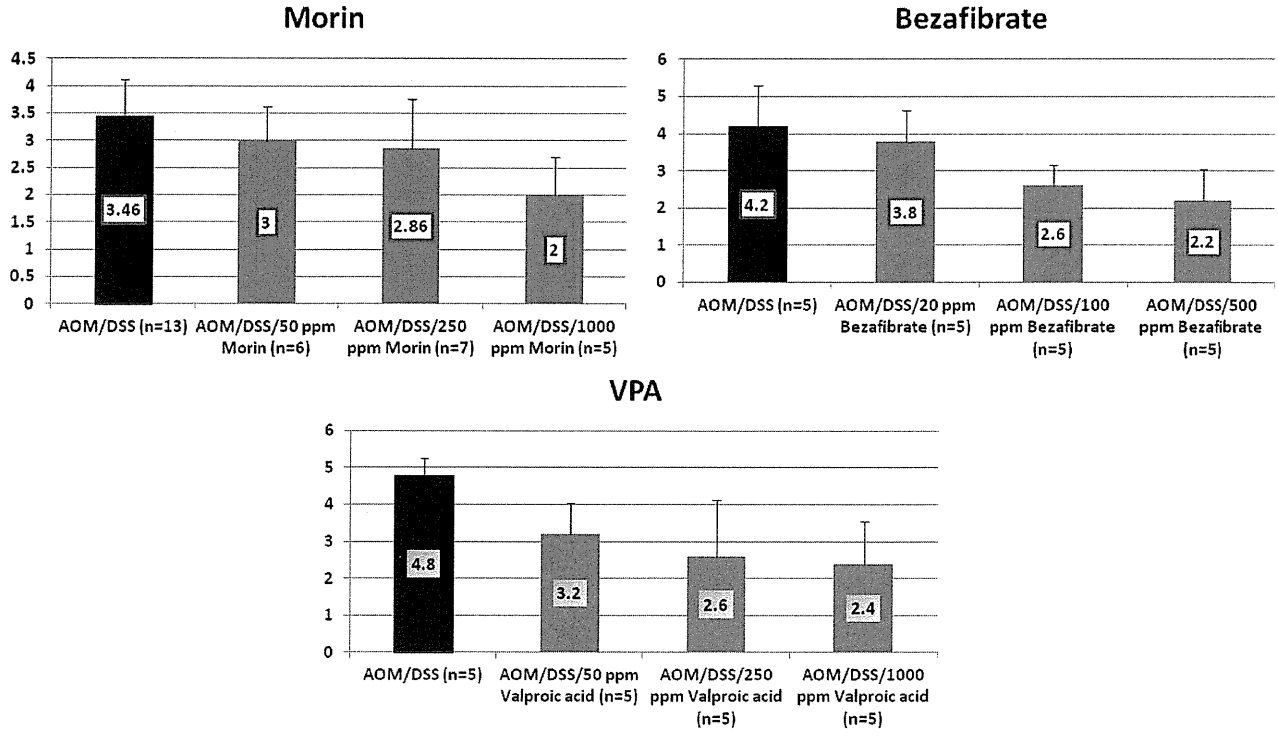


Figure 12. mRNA expression of Stat3 in the colorectum of mice or rats from the morin, bezafibrate, and VPA studies.

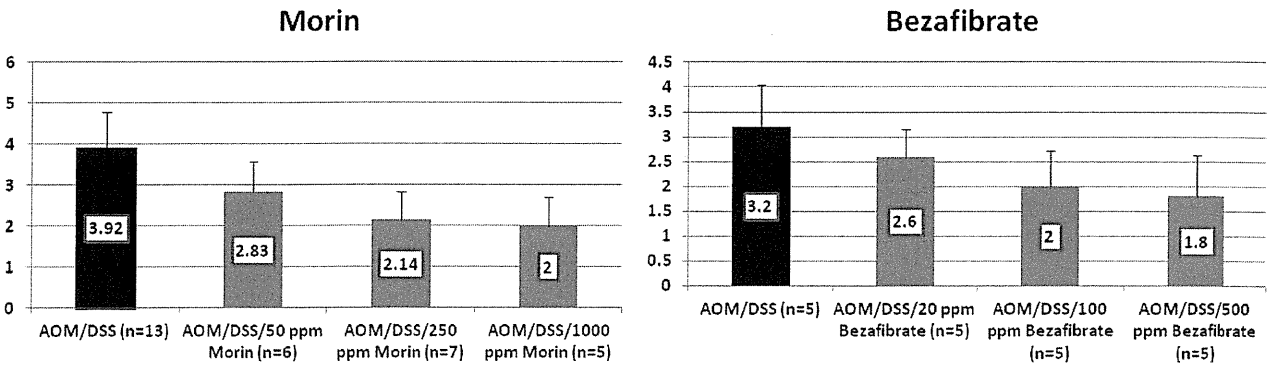


Figure 12. *Cont.*

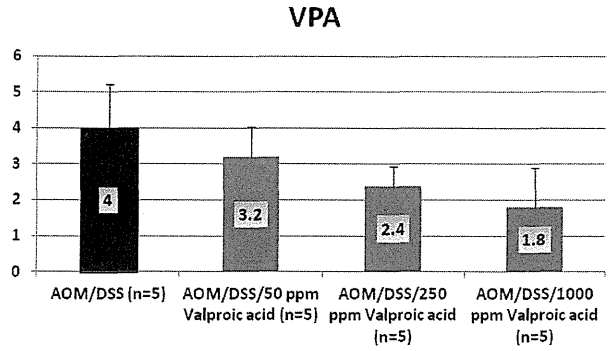


Figure 13. mRNA expression of HIF-1α in the colorectum of mice or rats from the morin, bezafibrate, and VPA studies.

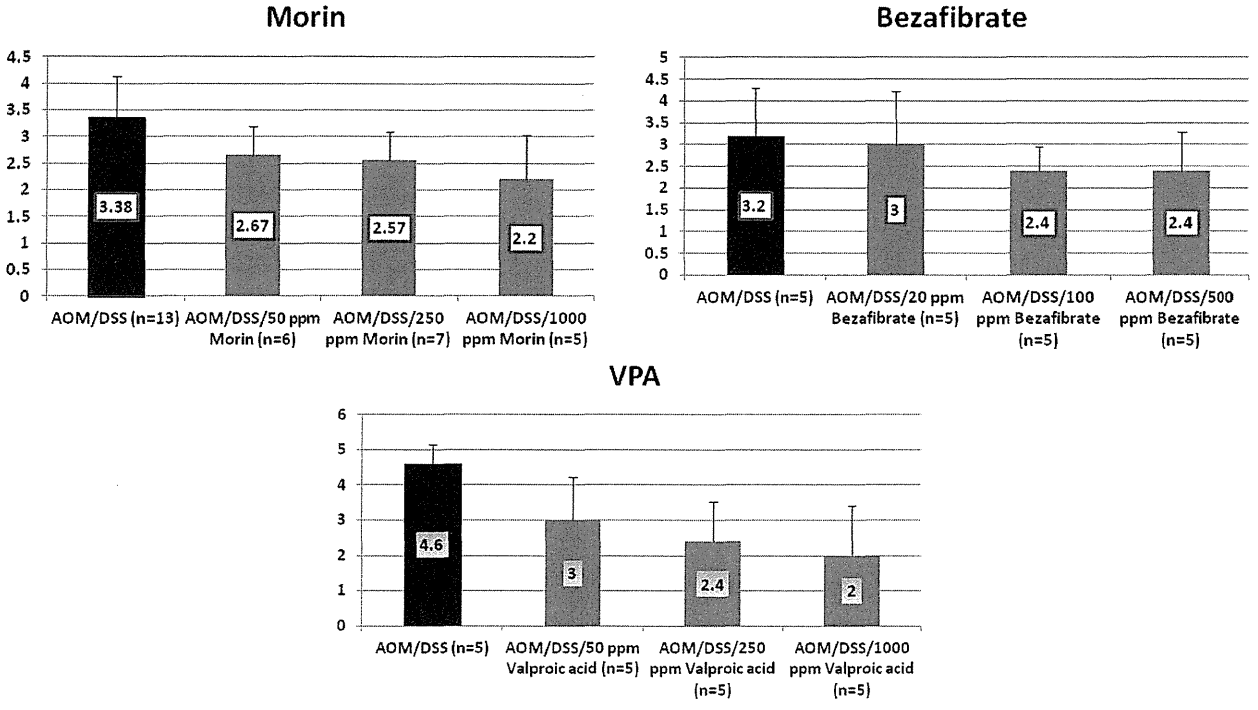


Table 1. Primer sequences used for real-time PCR assays.

Gene symbol	Primers	
	Rat	**Mouse**
NF-κB	Forward: 5'-ctggcagctcttctcaaagc-3' Reverse: 5'-ccaggtcatagagaggctcaa-3'	Mm00476361_m1 *
Tnf-α	Forward: 5'-cgagatgtggaactggcaga-3' Reverse: 5'-ctacgggcttgtcactcga-3'	Mm00443258_m1 *
IL-1β	Rn00 580432_m1 *	Mm00434228_m1 *
Stat3	Forward: 5'-ttgtgatgcctccttgattgtc-3' Reverse: 5'-atcggaggcttagtgaagaagttc-3'	Mm00456961_m1 *
Hif-1α	Rn00577560_ml *	Forward: 5'-cctggaaacgagtgaaagga-3' Reverse: 5'-tggtcagctgtggtaatcca-3'
βactin	Forward: 5'-tcaggtcatcactatcggcaat-3' Reverse: 5'-aaagaaagggtgtaaaacgca-3'	Mm00607939_s1*

* TaqMan (Assay ID#).

Treatments with morin, bezafibrate, and VPA also lowered immunohistochemical positivity of survivin (Figure 14) in ADCs and polyamine contents (Figure 15) of colorectal mucosa. These effects may be also responsible for modulatory effects of these three compounds on colonic tumor development. However, further studies including dose selection and toxicity of the compounds should be conducted before going to clinical trials.

Figure 14. Immunohistochemical positivity (%) against survivin in colonic ADC cells that developed in mice or rats in the morin, bezafibrate, and VPA studies.

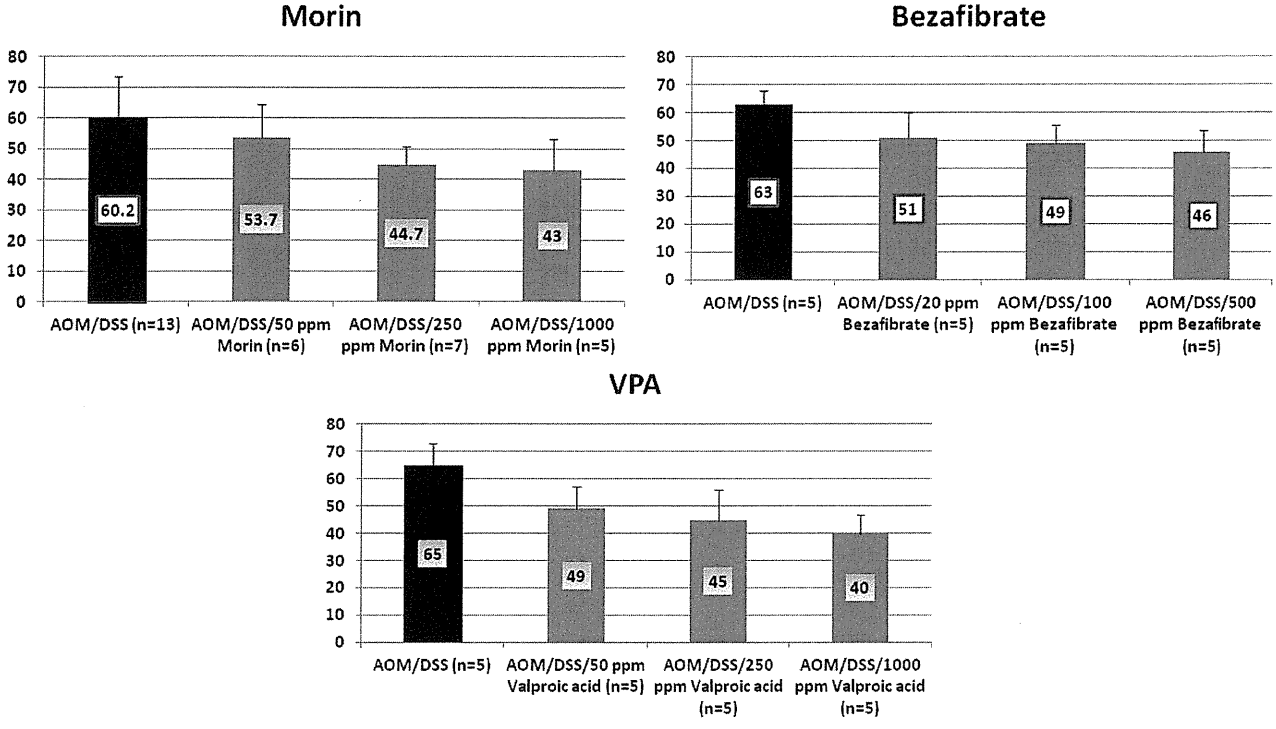


Figure 15. Polyamine content (nmol/mg) in the colonic mucosa of mice or rats in the morin, bezafibrate, and VPA studies.

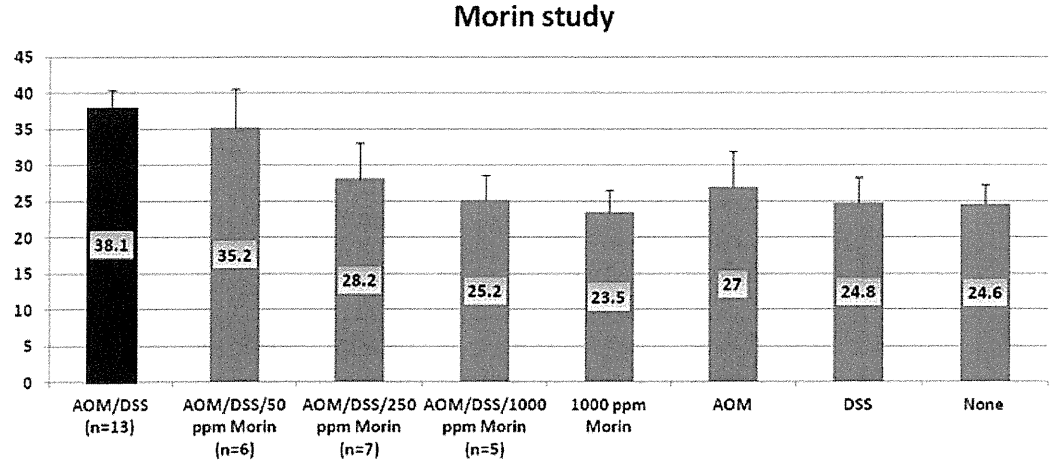
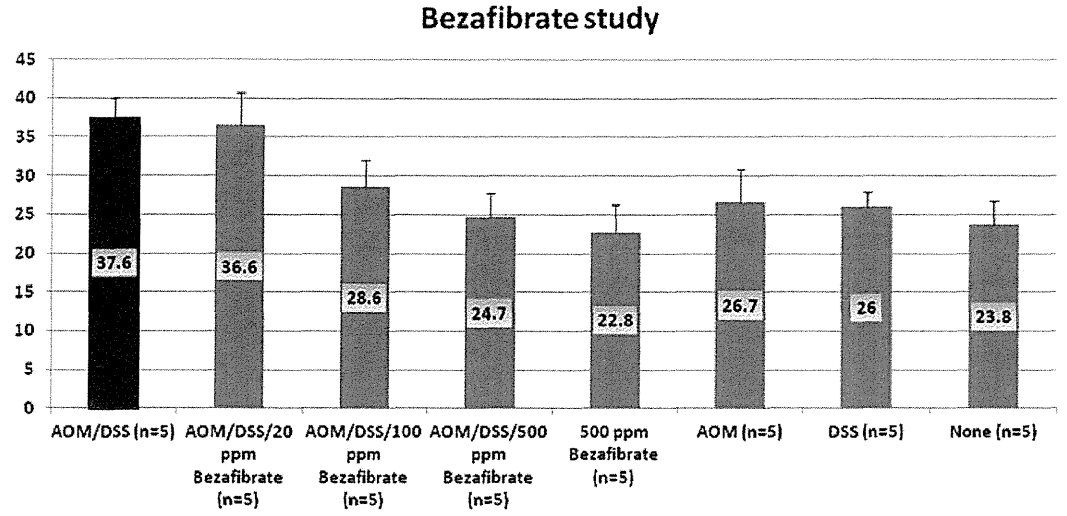
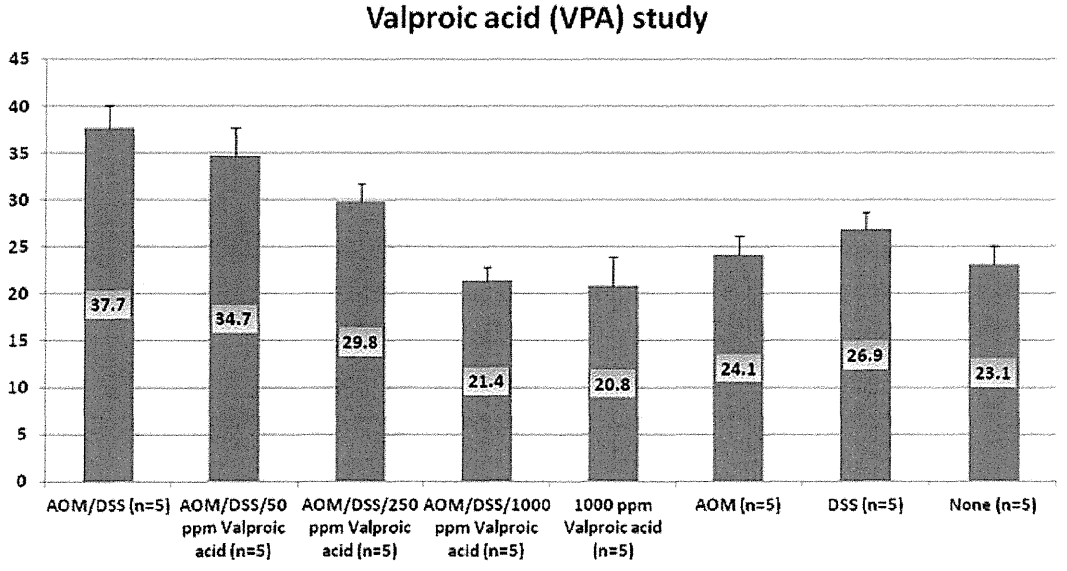


Figure 15. *Cont.*





6. Conclusions

Chemoprevention is an important approach to decreasing cancer morbidity and mortality by the use of non-toxic natural or synthetic substances to reverse the processes of initiation, promotion, and subsequent progression of cancer. Evidence is rapidly accumulating that chronic inflammation contributes to carcinogenesis through increase of cell proliferation, angiogenesis, and metastasis in a number of neoplasms, including colorectal carcinoma. To investigate pathobiology of CRC developed in inflamed colorectum and search effective cancer chemopreventive agents against such colitis-related CRC, we developed mouse and rat models (TANAKA models) of inflammation-associated colorectal carcinogenesis. In this article, powerful tumor-promotion effect of inflammation induced by DSS in rodents that are initiated with a low dose of a colonic carcinogen is described. Also, our recent data on the modifying effects of morin, bezafibrate, and VPA on AOM/DSS-induced colorectal carcinogenesis is presented. I would stress that inflammatory stress and several cytokines produced by inflammatory

cells are important in pathobiology of CRC development in colitic mucosa. These could be molecular targets for chemoprevention and/or therapy in CRC in IBD patients.

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Conflict of interest

None declared.

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Loss of HITS (FAM107B) expression in cancers of multiple organs: tissue microarray analysis

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Abstract. Family with sequence similarity 107 (FAM107) proteins consist of two subtypes, FAM107A and FAM107B in mammals, possessing a conserved N-terminal domain of unknown function. Recently we found that FAM107B, an 18 kDa nuclear protein, is expressed in a broad range of tissues and is downregulated in gastrointestinal cancer. Because FAM107B expression is amplified by heat-shock stimulation, we designated it heat shock-inducible tumor small protein (HITS). Although data related to FAM107A as a candidate tumor suppressor have been accumulated, little biological information is available for HITS. In the present study, we examined HITS expression using immunohistochemistry with tissue microarrays and performed detailed statistical analyses. By screening a high-density multiple organ tumor and normal tissue microarray, HITS expression was decreased in tumor tissues of the breast, thyroid, testis and uterine cervix as well as the stomach and colon. Further analysis of tissue microarrays of individual organs showed that loss of HITS expression in cancer tissues was statistically significant and commonly observed in distinct organs in a histological type-specific manner. The HITS expression intensity was inversely correlated with the primary tumor size in breast and thyroid cancers. In addition, effects of tetracycline-inducible HITS expression on tumor growth were investigated *in vivo*. Forced expression of HITS inhibited tumor xenograft proliferation, compared with the mock-treated tumor xenograft model. These results show that loss of HITS expression is a common phenomenon observed in cancers of distinct organs and involved in tumor development and proliferation.

Introduction

Family with sequence similarity 107 (FAM107) proteins are conserved beyond species: they are found in mammals, *Xenopus*, fish and *Drosophila*. They are characterized by a common N-terminal domain of unknown function (DUF1151) with no homology match to other functional conserved domains. Mammals have two genes, FAM107A and FAM107B, respectively encoding the proteins of 144 amino acids (aa) and 131 aa (http://www.uniprot.org/uniprot). C-terminal variable regions of FAM107 carry a coiled-coil domain that has been identified in many nuclear proteins including transcription factors, suggesting a role of FAM107 in regulating gene transcription. Analysis of protein-protein interactions revealed that FAM107A and FAM107B interact, respectively, with transcriptional adaptor 2α and 3α (Tada2α and Tada3α) (1-3). In fact, Tada2α and Tada3α are reciprocal binding partners and core proteins of the histone acetyltransferase (HAT) complex, suggesting that FAM107 family proteins can modulate the structure and function of HAT complexes that are involved in chromatin structure modification for gene transcription and acetylation of many proteins (4,5).

Because of epigenetic silencing, FAM107A is downregulated or deleted from cancer of many types such as non-small cell lung, renal cell, and prostate cancers and astrocytoma (6-10).

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Abbreviations: FAM107, family with sequence similarity 107; HITS, heat-shock-inducible tumor small protein; HSP, heat-shock protein; TSG, tumor suppressor gene; DUF, domain of unknown function; HAT, histone acetyltransferase; ER, estrogen receptor; PR, progesterone receptor; HER2, human epidermal growth factor receptor 2; IDC, invasive ductal carcinoma; DCIS, ductal carcinoma *in situ*; CIN, cervical intraepithelial neoplasia; HPV, human papilloma virus; PCNA, proliferating cell nuclear antigen; FNAC, fine needle aspiration cytology; FA, focal adhesion

Key words: family with sequence similarity 107B, heat-shock protein, tumor suppressor gene, tissue array