Tropisetron suppresses colitis-associated cancer in a mouse model in the remission stage

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ABSTRACT

Patients with inflammatory bowel disease (IBD) have a high risk for development of colitis-associated cancer (CAC). Serotonin is a neurotransmitter produced by enterochromaffin cells of the intestine. Serotonin and its receptors, mainly 5-HT3 receptor, are overexpressed in IBD and promote development of CAC through production of inflammatory cytokines. In the present study, we demonstrated the in vivo activity of tropisetron, a 5-HT3 receptor antagonist, against experimental CAC. CAC was induced by azoxymethane (AOM)/dextran sodium sulfate (DSS) in BALB/c mice. The histopathology of colon tissue was performed. Beta-catenin and Cox-2 expression was evaluated by immunohistochemistry as well as quantitative reverse transcription-PCR (qRT-PCR). Alterations in the expression of 5-HT3 receptor and inflammatory-associated genes such as IL-1β, Tnf-α, Tlr4 and Myd88 were determined by qRT-PCR. Our results showed that tumor development in tropisetron-treated CAC group was significantly lower than the controls. The qRT-PCR analysis demonstrated that the expression of 5-HT3 receptor was significantly increased following CAC induction. In addition, tropisetron reduced expression of β-catenin and Cox-2 in the CAC experimental group. The levels of IL-1β, Tnf-α, Tlr4 and Myd88 were significantly decreased upon tropisetron treatment in the AOM/DSS group. Taken together, our data show that tropisetron inhibits development of CAC probably by attenuation of inflammatory reactions in the colitis.

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1. Introduction

Colon cancer is the third most common type of cancer, accounting for approximately 70% of bowel neoplasms [1,2]. It has been shown that patients with inflammatory bowel disease (IBD) are at high risk of development of colitis-associated cancer (CAC) [3,4]. Due to improvement of treatment strategies against IBD, the patients have longer survival which leads to augmentation in the CAC incidence [5]. This indicates that there is a correlation between duration of IBD and increase in the CAC prevalence [6]. In this setting, identifying novel and efficacious preventive strategies against CAC-prone IBD patients might have substantial therapeutic benefit.

It is well-recognized that chronic inflammation promotes several stages of tumorigenesis, including transformation, proliferation, survival, angiogenesis, invasion and metastasis [7]. Previous studies have implied that inflammatory cytokines play a pivotal role in the pathogenesis of CAC [8,9]. Cytokines promote tumor growth and survival by stimulation of angiogenesis and suppression of the immune response [10]. For instance, it has been demonstrated that interleukin-1 beta (IL-1β) and tumor necrosis factor-alpha (TNF-α) induce secretion of numerous angiogenic factors as well as cyclooxygenase-2 (Cox-2) in CAC [11–14]. Therefore, decreasing the levels of TNF-α and IL-1β in patients with IBD should reduce the risk of CAC development.

Toll like receptors (TLRs) are pattern recognition receptors that recognize pathogen molecules and initiate the immune response [15]. TLR4/MYD88/NF-κB cascade is overexpressed in CAC [16]. Induction of Cox-2, activation of mitogen-associated protein (MAP) kinase signaling pathway and epidermal growth factor receptor (EGFR) as well as induction of production of pro-inflammatory cytokines such as TNF-α and IL-1β by immune cells are the mechanisms through which TLR4 promotes
In this regard, it has been reported that inhibition of TLR4 using a blocking antibody attenuates dysplasia and prevents CAC development [16]. Altogether, these studies suggest that TLR4/MYD88 loop is an attractive therapeutic target in CAC and drugs that modify its expression and functions are potential agents against CAC initiation and progression.

Cox-2 is an inducible enzyme which is overexpressed in CAC and plays a cardinal role in CAC tumorigenesis via inhibition of apoptosis, induction of proliferation and activation of β-catenin-dependent signaling [19,20]. It has been shown that Cox-2 inhibitors reduce risk of CAC development. Furthermore, recent studies indicate that treatment with Cox-2 inhibitors is an effective strategy for the treatment of CAC [21,22].

β-catenin is a transcriptional coactivator which is aberrantly overexpressed in CAC [23]. β-catenin is an effector in the WNT signaling pathway which has central role in cell growth and survival [24]. Overexpression and mutation in β-catenin/WNT pathway contribute to promotion of various types of cancers including colon cancer [25]. Several lines of evidence indicate that down-regulation of β-catenin attenuates CAC tumorigenesis [26,27].

Serotonin, a key regulator of gastrointestinal tract motility, is mainly secreted from enterochromaffin cells of the gut [28]. Serotonin acts on the immune-inflammatory axis and modulates the immune response [29,30]. Serotonin induces inflammation through overexpression of prostaglandin E2 (PGE2) and up-regulation of IL-1β and TNF-α [31]. One of the known serotonin receptors is 5-HT3 receptor [32], and serotonin/5-HT3 receptor loop modulates the function of immune cells [33]. Evidence indicates that 5-HT3 receptor antagonists, especially tropisetron, have anti-inflammatory properties [34,35]. It has also been reported that tropisetron decreases production of pro-inflammatory cytokines such as TNF-α and IL-1β in the experimental model of colitis [36]. While previous studies have clarified the anti-inflammatory effects of tropisetron, there is no information about the chemopreventive effects of tropisetron in CAC. This study aimed to explore the in vivo activity of tropisetron against CAC in an experimental mouse model.
Fig. 3. The histopathological features provided from H & E-stained colon sections; H & E (×400). A; AOM/DSS, B; control normal, C; AOM/DSS + 5-ASA (100 mg/kg), D; AOM/DSS + tropisetron (10 mg/kg), E; AOM/DSS + tropisetron (5 mg/kg) and F; AOM/DSS + tropisetron (2 mg/kg).
2.8 Statistical analysis

Sections from each sample were examined for grading. Colon sample (n = 8) prepared for histopathological assessment. Five malady (none: 0, mild: 1, severe: 2). Of each experimental group, 8 depletion (null to mild: 0, moderate: 1, severe: 2); E: structural abnormality (none: 0, mild: 1, severe: 2); C: nuclear dispolarity (none: 0, mild: 1, severe: 2); D: goblet depletion (null to mild: 0, moderate: 1, severe: 2). Of each experimental group, 8 colon sample (n = 8) prepared for histopathological assessment. Five sections from each sample were examined for grading.

### Table 3

<table>
<thead>
<tr>
<th>No.</th>
<th>Treatment</th>
<th>Score median (min-max)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>AOM/DSS</td>
<td>3 (2–3)**</td>
</tr>
<tr>
<td>2</td>
<td>Control normal</td>
<td>0.5 (0–1)</td>
</tr>
<tr>
<td>3</td>
<td>AOM/DSS + 5-ASA (100 mg/kg)</td>
<td>1 (0–1)*</td>
</tr>
<tr>
<td>4</td>
<td>AOM/DSS + tropisetron (10 mg/kg)</td>
<td>1 (0–1)*</td>
</tr>
<tr>
<td>5</td>
<td>AOM/DSS + tropisetron (5 mg/kg)</td>
<td>1 (1–2)</td>
</tr>
<tr>
<td>6</td>
<td>AOM/DSS + tropisetron (2 mg/kg)</td>
<td>1.5 (1–3)</td>
</tr>
</tbody>
</table>

** P < 0.01 compared to control normal group, *

described by Alizadeh et al. [39]: A: nuclear/cytoplasmic ratio (<25%: 0, 25–50%: 1, >50%: 2); B: epithelial stratification (none: 0; mild: 1; severe: 2); C: nuclear dispolarity (none: 0, mild: 1, severe: 2); D: goblet depletion (null to mild: 0, moderate: 1, severe: 2); E: structural abnormality (none: 0, mild: 1, severe: 2). Of each experimental group, 8 colon sample (n = 8) prepared for histopathological assessment. Five sections from each sample were examined for grading.

### 3. Results

#### 3.1 Tumor development

Tumor induction was observed with high percentage in the AOM/DSS group (75%) compared to the controls. Tumor development was detected among different groups as follows: group 2 (62.5%), group 3 (50%) and group 5 (37.5%). The groups have been defined in the materials and methods. As shown in Fig. 1, development of tumor was inhibited following tropisetron (10 mg/kg) treatment.

#### 3.2 Body weight change

Mice were weighed weekly and reduction in the body weight was recorded as follows: 1, 1%–5%; 2, 5%–10%; 3, 10%–15%; and 4, >15%. We observed that AOM/DSS administration caused a statistically significant decrease in the body weight (Fig. 2). Body weights in the treatment groups were significantly increased on the 7th–10th weeks but did not reach the levels of the control normal group on the 10th week. However, there were no significant differences among the treatment groups for weeks 7–10.

#### 3.3 Histopathology assessment

Histopathologic evaluations for CAC samples were performed on H & E-stained colon sections. The median number of nuclear/cytoplasmic ratio, epithelial stratification, nuclear dispolarity, goblet depletion and structural abnormality were determined in the CAC samples (Table 2, Fig. 3). All histopathologic parameters were increased significantly in the AOM/DSS group in comparison with the control normal group. Furthermore, 5-ASA and tropisetron (10 mg/kg) significantly decreased histopathologic parameters compared to the AOM/DSS group.

#### 3.4 Immunohistochemistry for β-catenin and COX-2

As summarized in Table 3 and shown in Figs. 4 and 5, AOM/DSS significantly increased the expression of β-catenin and Cox-2 in colon tissue compared to the control normal group. Treatment with 5-ASA and tropisetron (10 mg/kg) significantly reduced the expression of β-catenin and Cox-2 in comparison with the AOM/DSS group.

#### 3.5 qRT-PCR for expression of β-catenin and COX-2

As shown in Fig. 6, expression of β-catenin (A) and Cox-2 (B) was significantly increased in the AOM/DSS group in comparison with the control normal group. Furthermore, treatment with 5-ASA as well as tropisetron (10 mg/kg) significantly decreased expression of β-catenin and Cox-2 in colon tissue of the CAC experimental group. Moreover, tropisetron (5 mg/kg) significantly decreased expression of β-catenin as compared to the AOM/DSS group.

![Fig. 4](image-url). The immunohistochemical features of β-catenin expression in colon tissue. A; AOM/DSS (∗100), B; control normal (×400), C; AOM/DSS + 5-ASA (100 mg/kg) (×400), D; AOM/DSS + tropisetron (10 mg/kg) (×400), E; AOM/DSS + tropisetron (5 mg/kg) (×400) and F; AOM/DSS + tropisetron (2 mg/kg) (×400).
3.6. qRT-PCR for expression of 5-HT3 receptor

In order to determine expression of 5-HT3 receptor in colon tissue, qRT-PCR was performed. As Fig. 7 shows, expression of 5-HT3 receptor was significantly increased following induction of CAC.

3.7. qRT-PCR for expression of Il-1β, Tnf-α, Tlr4 and Myd88

As shown in Figs. 8, 5-ASA significantly reduced the levels of Il-1β, Tnf-α, Tlr4 and Myd88 as compared to the AOM/DSS group. Furthermore, treatment with tropisetron (2, 5 and 10 mg/kg) significantly decreased the expression of Il-1β, Tlr4 and Myd88 in comparison with the AOM/DSS group. Additionally, tropisetron (10 mg/kg) significantly attenuated the expression of Tnf-α in comparison with the AOM/DSS group. These results show that expression of Il-1β, Tnf-α, Tlr4 and Myd88 were significantly increased in the AOM/DSS group as compared to the control normal group.

4. Discussion

IBD is a debilitating disease with high morbidity rate and economic burden that its incidence is increasing during recent years [41,42]. Evidence indicates that IBD patients are at high risk for CAC development [43], suggesting that there is a direct association between IBD and CAC prevalence [6]. There is currently no effective treatment to prevent or reduce CAC development. It is therefore a pressing need to devise more efficacious preventive and therapeutic strategies against this fatal disease. It has been reported that 5-HT3 receptor antagonists have anti-inflammatory properties in certain inflammatory conditions such as colitis [36,44]. Regarding the direct association between inflammation and cancer [7], in the present study we aimed to explore the in vivo activity of tropisetron, a 5-HT3 receptor antagonist, against CAC in a mouse model of colitis.

IBD is a chronic relapsing condition which is pathologically considered by intestinal inflammation and epithelial injury. Cytokines are thought to play a central role in the pathogenesis of IBD and thereby,
modulation of these molecules might be applied for the treatment of IBD [45]. Among these cytokines, TNF-α and IL-1β are the most important mediators that link inflammation and cancer through activation of NF-κB and signal transducer and activator of transcription (STAT) pathways, leading to increased cell proliferation [46,47]. Chronically release of TNF-α and IL-1β furthers tumorigenesis via induction of angiogenic factors and increase in infiltration of leukocytes to inflammation sites. In consistent with this, previous studies have demonstrated that inhibition of TNF-α and IL-1β inhibits tumor growth and tumorigenicity [48–50].

TLR4/MYD88/NF-κB loop is overexpressed in CAC and promotes tumorigenesis via production of the inflammatory cytokines such as TNF-α and IL-1β. This signaling network is a potential therapeutic target to be exploited in translation research to establish novel and effective treatment regimens against CAC [16]. Our results show that tropisetron decreased mRNA levels of Il-1β, Tnf-α, Tlr4 and Myd88 in the experimental model of CAC.

Canonical WNT/β-catenin signaling is essential for cell-cycle progression, proliferation, differentiation of stem cells and development of uterus [51]. Alteration in WNT/β-catenin cascade promotes tumor initiation and progression [52]. Mutation and overexpression of β-catenin cause abundant proliferation and inhibition of differentiation in various types of cancer cells such as colon cancer and is associated with tumor progression and metastasis [53–55]. Therefore, inhibition of WNT/β-catenin signaling is an attractive therapeutic and preventive approach for the treatment of neoplasms. Our results show that expression of β-catenin was reduced following treatment with tropisetron in the CAC model.

Expression of Cox-2 is increased in chronic inflammation as well as cancer. Cox-2 creates an immune suppressive and proinflammatory microenvironment which plays a key role in inflammation-driven cancers [56]. Accumulating evidence suggests that median survival is shorter in patients with Cox-2-expressing colon cancer [57]. Cox-2 is overexpressed in CAC and promotes tumorigenesis by decreasing pro-apoptotic proteins and enhancement of anti-apoptotic signals [58]. A large body of evidence indicates that the risk of colon carcinogenesis is significantly reduced by use of Cox-2 inhibitors such as aspirin [59,60]. Our results demonstrate that tropisetron treatment reduced the expression of Cox-2 in a mouse model of CAC.

Serotonin is found in the immune-inflammatory axis and influences the immune response [30,61]. 5-HT3 receptors are expressed in immune cells including T-cells [33,62] and modulate the effects of serotonin on T-cells [32]. These data suggest that tropisetron might affect T-cells function through 5-HT3 receptor. It is determined that serotonin
exerts mitogenic properties and promotes inflammation via induction of pro-inflammatory cytokines [63,64]. Rahimian et al. determined that protective properties of tropisetron are associated with its anti-inflammatory properties against stroke [65]. Stratz et al. have shown that blocking 5-HT3 receptors possess anti-inflammatory properties in chondrocytes via decreasing IL-1β, IL-6 and Cox-2 expression [34]. Previous studies have reported that tropisetron exerts anti-inflammatory effects in acute colitis and inhibits expression of TNF-α and IL-1β [36,66]. Atae et al. have shown that 5-HT3 receptor agonists have mitogenic effects and increase proliferation of colon cancer cells which was reversed by 5-HT3 receptor antagonists [67]. It is well-determined that serotonin acts as a regulator of inflammation, proliferation and regeneration [68,69]. In this regard, it has been shown that serotonin plays a key role in growth of colon cancer allografts through increase in angiogenesis by reducing the expression of matrix metalloproteinase 12 (MMP-12) in tumor-infiltrating macrophages [63]. Our findings show that tropisetron attenuates tumorigenesis in a mouse model of CAC through reduction of β-catenin, Cox-2 as well as the inflammation-associated cancer genes such as Tnf-α, Il-1β, Myd88 and Tilr4.

The common symptom in patients with colon cancer is weight loss [70]. Our findings show that tropisetron decreased weight loss in CAC. Histopathological evaluation show that tropisetron improved pathologic parameters in CAC. In current study we chose 5-ASA as reference histology. Tumor necrosis factor (TNF-α) and interleukin-1β (IL-1β) elicit pro-inflammatory responses that play critical roles in colitis-associated cancer (CAC) [71]. Tumor necrosis factor (TNF-α) and interleukin-1β (IL-1β) elicit pro-inflammatory responses that play critical roles in colitis-associated cancer (CAC) [71].

5. Conclusion

We found that tropisetron, a 5-HT3 receptor antagonist, given in the remission stage of colitis, possess chemopreventive properties and suppress CAC development. We conclude that tropisetron exerts these preventive effects by decreasing the levels of IL-1β, Tnf-α, Tilr4 and Myd88 along with reduction in the expression of β-catenin and Cox-2.

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References


