Downregulation of prostaglandin E receptor subtype EP\textsubscript{3} during colon cancer development

Y Shoji, M Takahashi, T Kitamura, K Watanabe, T Kawamori, T Maruyama, Y Sugimoto, M Negishi, S Narumiya, T Sugimura, K Wakabayashi

Background and aims: Involvement of prostaglandin E\textsubscript{2} (PGE\textsubscript{2}) receptors EP\textsubscript{1}, EP\textsubscript{2}, and EP\textsubscript{3} in the formation of aberrant crypt foci (ACF) and/or intestinal polyps has been suggested. In contrast, EP\textsubscript{3} appears to have no influence on the early stages of colon carcinogenesis. In the present study, we examined expression of PGE\textsubscript{2} receptor subtypes EP\textsubscript{1}, EP\textsubscript{2}, EP\textsubscript{3}, and EP\textsubscript{4} in normal colon mucosa and colon cancers, and assessed the contribution of EP\textsubscript{3} to colon cancer development.

Methods: mRNA expression of PGE\textsubscript{2} receptor subtypes EP\textsubscript{1}, EP\textsubscript{2}, EP\textsubscript{3}, and EP\textsubscript{4} in normal colon mucosa and colon cancers in azoxymethane (AOM) treated mice and rats, and in humans, were examined by reverse transcription-polymerase chain reaction (RT-PCR), quantitative real time RT-PCR, and immunohistochemical analyses. Evaluation of the role of EP\textsubscript{3} was performed by intraperitoneal injection of AOM, using EP\textsubscript{3} receptor knockout mice. Effects of EP\textsubscript{3} receptor activation on cell growth of human colon cancer cell lines were examined using ONO-AE-248, an EP\textsubscript{3} selective agonist. Moreover, EP\textsubscript{3} expression in colon cancer cell lines was analysed with or without 5-aza-2′-deoxycytidine (5-aza-dC) treatment.

Results: Expression levels of EP\textsubscript{1} and EP\textsubscript{2} mRNA were increased in cancer tissues. EP\textsubscript{3} mRNA was constantly expressed in normal mucosa and cancers. In contrast, expression of EP\textsubscript{3} mRNA was markedly decreased in colon cancer tissues, being 5% in mice, 9% in rats, and 28% in humans compared with normal colon mucosa, analysed by quantitative real time RT-PCR. Immunohistochemical staining demonstrated the rat EP\textsubscript{3} receptor protein to be expressed in epithelial cells of normal mucosa and some parts of small carcinomas but hardly detectable in large carcinomas of the colon. Colon cancer development induced by AOM in EP\textsubscript{3} receptor knockout mice was enhanced compared with wild-type mice, with a higher incidence of colon tumours (78% v 57%) and mean number of tumours per mouse (2.17 (0.51) v 0.75 (0.15); p<0.05). Expression of EP\textsubscript{3} mRNA was detected in only one of 11 human colon cancer cell lines tested. Treatment with 5 μM of an EP\textsubscript{3} selective agonist, ONO-AE-248, resulted in a 30% decrease in viable cell numbers in the HCA-7 human colon cancer cell line in which EP\textsubscript{3} was expressed. Treatment with 5-aza-dC restored EP\textsubscript{3} expression in CACO-2, CW-2, and DLD-1 cells but not in WiDr cells, suggesting involvement of hypermethylation in the downregulation of EP\textsubscript{3} to some extent.

Conclusion: The PGE\textsubscript{2} receptor subtype EP\textsubscript{3} plays an important role in suppression of cell growth and its downregulation enhances colon carcinogenesis at a later stage. Hypermethylation of the EP\textsubscript{3} receptor gene could occur and may contribute towards downregulating EP\textsubscript{3} expression to some extent in colon cancers.

Clear benefits have been reported in epidemiological studies with non-steroidal anti-inflammatory drugs (NSAIDs) as chemopreventive agents against colon cancers, one of the most common malignancies in humans. Chemically induced colon carcinogenesis in rodents is also suppressed by administration of NSAIDs. Moreover, intestinal polyp formation in familial adenomatous polyposis coli patients is markedly reduced after application of agents such as sulindac or indomethacin. The common mechanism of action of NSAIDs is inhibition of cyclooxygenase (COX) activity, two distinct isosofrms of which have been reported: a constitutive enzyme, COX-1, and an inducible enzyme, COX-2. COX-1 and COX-2 are rate limiting enzymes in the synthesis of prostanoids which affect cell proliferation, tumour growth, apoptosis, and immune responsiveness, and both COX isoforms have been reported to be involved in colon carcinogenesis.

Prostanoids such as prostaglandin (PG)E\textsubscript{2}, PGD\textsubscript{2}, PGE\textsubscript{2}\textsubscript{α}, PGI\textsubscript{2}, and TXA\textsubscript{2} exert their biological actions through binding to nine specific receptors with seven transmembrane domains: the four subtypes EP\textsubscript{1}–EP\textsubscript{4} for PGE\textsubscript{2}, DP and CRTH2 for PGD\textsubscript{2}, FP for PGE\textsubscript{2}\textsubscript{α}, IP for PGI\textsubscript{2}, and TP for TXA\textsubscript{2}. Several reports have demonstrated increased levels of PGE\textsubscript{2} in human colon cancer tissues compared with surrounding normal mucosa. Signal transduction pathways of PGE\textsubscript{2} receptors have been studied by examining agonist induced changes in the levels of second messengers such as cAMP and free Ca\textsuperscript{2+} by identifying G protein coupling by various methods. The EP\textsubscript{1} receptor is known to mediate PGE\textsubscript{2} induced elevation of free Ca\textsuperscript{2+} concentration although the species of G protein to which EP\textsubscript{1} receptor is coupled remains unidentified. EP\textsubscript{2} and EP\textsubscript{4} receptors are coupled to Gs and stimulate CAMP production by adenylyl cyclase. In contrast, the major signalling pathway for the EP\textsubscript{3} receptor is inhibition of adenylyl cyclase via Gi. In addition, another function has been suggested for this receptor type in which cell phenotype is regulated through activation of Rho via G proteins other than Gi.

Abbreviations: PGE\textsubscript{2}, prostaglandin E\textsubscript{2}; ACF, aberrant crypt foci; AOM, azoxymethane; COX, cyclooxygenase; NSAIDs, non-steroidal anti-inflammatory drugs; RT-PCR, reverse transcription-polymerase chain reaction; 5-aza-dC, 5-aza-2′-deoxycytidine; FBS, fetal bovine serum.
Establishment of mice lacking the genes encoding prostanoid receptors has promoted understanding of the involvement of prostanoids and their receptors in the development of colon cancer. In previous studies, we demonstrated that deficiency of either EP1 or EP2 receptor decreases formation of azoxymethane (AOM) induced aberrant crypt foci (ACF), putative preneoplastic lesions in the colon. Moreover, antagonists of EP1 and EP2 receptors suppress formation of AOM induced ACF in the colon of mice and intestinal polyp formation in Apc deficient Min mice. Recently, it was also reported that homogenous deletion of the gene encoding the EP3 receptor resulted in a decrease in intestinal polyp formation in Apc knockout mice. As already mentioned, EP3 and EP4 stimulate adenylyl cyclase whereas EP1 exerts an inhibitory influence, suggesting a possible suppressive role against colon carcinogenesis. However, deficiency of EP3 did not affect AOM induced ACF formation in our previous study.

In the present study, we hypothesised that EP3 might act at a later stage in colon carcinogenesis. Examination of mRNA expression for EP1, EP2, EP3, and EP4 in colon carcinomas of mice, rats, and humans demonstrated that levels of EP3 were markedly decreased compared with normal mucosa. An increase in colon carcinoma formation induced by AOM was also demonstrated in EP3 receptor knockout mice. Furthermore, activation of the EP3 receptor showed a suppressive effect on cell growth in a colon cancer cell line in which EP3 was expressed. In mouse colon cancer cell lines tested, EP3 expression was not detected but treatment with 5-aza-2′-deoxycytidine (5-aza-dC) restored EP3 expression in some cell lines. On the basis of the results obtained, the role of the EP3 receptor in colon carcinogenesis is discussed.

MATERIALS AND METHODS

Animals

The mouse gene encoding the PGE2 receptor EP3 was disrupted by a gene knockout method using homologous recombination, as reported previously. The generated chimeric mice were backcrossed with C57BL/6Cr mice, and the resulting homozygous mutant mice of these F2 progeny were backcrossed into the C57BL/6Cr background for 10 generations. EP3 receptor deficient male mice were used at six weeks of age. Genotypes of the knockout mice were confirmed by polymerase chain reaction (PCR) according to the method described previously. Animals were housed in plastic cages at 24 ± 2°C and 55% relative humidity with a 12 h/12 h light/dark cycle. Water and basal diet (AIN-76A, Bio-Serv, Frenchtown, New Jersey, USA) were given ad libitum. Body weights and food intake were measured weekly.

Colon tumour samples and cell lines

Mouse colon tumours and normal colon mucosa tissues were obtained from C57BL/6J male mice treated with AOM, as previously reported. Rat colon tumours and normal colon mucosa tissues were obtained from eight F344 male rats treated with AOM, as previously reported. Frozen samples of mouse and rat tissues were used for reverse transcription (RT)-PCR analyses, and formalin fixed, paraffin embedded rat tissue samples were employed for immunohistochemical staining.

Surgical specimens of human colon cancer and adjacent normal colon mucosa tissues were taken from eight Japanese patients who had undergone surgical operations for colorectal cancers at the National Cancer Center Hospital, Tokyo, and samples were immediately frozen in liquid nitrogen.

Eleven human colon cancer cell lines were subjected to RT-PCR analysis. HCA-7 colony 29, a human colon adenocarcinoma cell line, was kindly provided by Dr Susan Kirkland, Imperial College of Science, Technology, and Medicine (London, UK). HCA-7 cells were maintained in Dulbecco’s minimum essential medium supplemented with 5% heat inactivated fetal bovine serum (FBS) (Hyclone Laboratories, Inc., Logan, Utah, USA) and antibiotics (100 μg/ml of streptomycin and 100 units/ml of penicillin) at 37°C in 5% CO2. Colo 201, DLD-1, HCT-116, SW48, SW480, SW620, WiDr (Dainippon Pharmaceutical Co., Ltd, Osaka, Japan), CACO-2, Colo 320, and CW-2 (Riken Cell Bank, Tsukuba, Japan) were purchased and cultured according to the manufacturer’s instructions.

Analysis of EP receptor expression in colon cancers by RT-PCR

Total RNA was extracted from tissues and cultured cells by direct homogenisation in IsoGene (Nippon Gene Co., Tokyo, Japan), and spectrophotometry was used for quantification. Aliquots (3 μg) of total RNA were subjected to the RT reaction with oligo-dT primer using an Omniscript Reverse Transcriptase kit (Qiagen, Hilden, Germany). After reverse transcription, PCR was carried out with Hotstartaq (Qiagen), according to the manufacturer’s instructions. To test cDNA integrity, the β-actin gene was amplified for each sample. Primers were designed using the computer program Oligo 4.0-5 (National Biosciences, Maryland, USA) and were based on published sequences in Genbank. Primers were designed to cross an exon-exon boundary or insertion of intron to ensure that genomic DNA was not being amplified. BLAST searches confirmed that the primers were specific for the target gene. Primers for the β-actin and EP receptor genes are listed in table 1. PCR amplifications were performed in a thermocycler (Gene Amp PCR System 9600; Perkin-Elmer Applied Biosystems, Foster City, California, USA), with 18–40 cycles of 94°C for 20 seconds, 60°C for 30 seconds, and 72°C for one min using the specific primer sets. PCR products were then analysed by electrophoresis on 2% agarose gel.

Quantitative real time RT-PCR analysis

Quantitative real time RT-PCR analysis was performed using the Smart Cycler system with the Ex Taq R-PCR version 2 kit and SYBR Green (Takara Shuzo Co., Shiga, Japan) according to the manufacturer’s instructions. Primers for the β-actin and EP receptor genes, and cycle conditions for PCR, are listed in table 2. To assess the specificity of each primer set, amplicons generated from the PCR reaction were analysed by their melting point curves and additionally run on 2% agarose gels to confirm the correct sizes of the PCR products. Each PCR product was subcloned into the TA cloning plasmid vector pGEN-T easy vector (Promega Co., Madison, Wisconsin, USA) and used as a positive control for real time PCR analyses. The number of molecules of specific gene products in each sample was determined using a standard curve generated by amplification of 107–1010 copies of the control plasmid. Each sample was analysed in triplicate.

Immunohistochemical staining

Immunohistochemical analyses of colon tumours and normal mucosa samples from F344 male rats treated with AOM were performed with the avidin-biotin complex immunoperoxidase technique, as previously reported. As the primary antibody, a polyclonal rabbit anti-EP3 antibody raised against rat EP3 receptors was used at a 50× dilution. As the secondary antibody, biotinylated antirabbit IgG (H+L) raised in a goat, affinity purified, and absorbed with rat serum (Vector Laboratories, Inc., Burlingame, California, USA) was used at a 200× dilution. Staining was performed using avidin-biotin reagents (Vectastain ABC reagents; Vector Laboratories, 3,3′-diaminobenzidine, and hydrogen.
peroxide. Sections were counterstained with haematoxylin. As a negative control, the primary antibody was preincubated with a 16-fold (molar ratio) excess amount of the fusion protein used as the immunogen for one hour at room temperature prior to incubation of the sections.22

### AOM induced colon tumour development in EP3 receptor knockout mice

Male EP3 receptor deficient homozygous mice (EP3 −/−) and wild-type mice received AOM at a dose of 10 mg/kg body weight intraperitoneally once a week for six weeks. At 56 weeks of age, mice were sacrificed under ether anaesthesia and complete autopsy was performed. After laparotomy, the entire intestines were resected and opened longitudinally, and the contents were flushed with normal saline. Using a dissection microscope, colon tumours were noted grossly for their location, number, and diameter, measured with callipers. All tumours from AOM treated mice were subjected to histological examination after routine processing and haematoxylin and eosin staining. The experimental protocol was according to the guidelines for Animal Experiments in the National Cancer Center.

### Effects of ONO-AE-248 on growth of colon cancer cells

The EP3 receptor selective agonist 16-(3-methoxymethyl)-phenyl-o-tetranor-3,7-dithiapGE1 (ONO-AE-248) was chemically synthesised at Ono Pharmaceutical Co. Ltd. 23 DLD-1 and HCA-7 cells were seeded in plastic 96 well plates at a density of 5 × 10^4 cells/10 cm dish on day 0 and treated with 1 and 2 μM Aza-2′-deoxycytidine treatment.

COCO-2, CW-2, DLD-1, HCA-7, and WiDr cells were seeded at a density of 5 × 10^4 cells/10 cm dish on day 0 and treated with 1 and 2 μM Aza-2′-deoxycytidine treatment. Expression of PGE2 receptors EP1, EP2, EP3, and EP4 in normal colon mucosa and colon tumours. Expression of PGE2 receptors EP1, EP2, EP3, and EP4 in normal colon mucosa and colon tumours of AOM treated mice and rats, and in human tissues, were examined by RT-PCR (figs 1, 2). In the three mouse colon adenocarcinomas tested, expression of EP1 and EP2 receptor mRNAs was increased compared with levels in normal mucosa. EP4 mRNA was equally expressed in carcinomas and normal mucosa. In contrast, expression of EP3 mRNA was markedly decreased in all carcinoma samples compared with normal colon mucosa (fig 1A). Expression patterns of EP1, EP2, EP3,
and EP4 receptors were designed to target a sequence common to all EP3 receptor variants expressed.

Expression of EP3 receptor mRNA was significantly down-regulated in tumours, being 5% in mice (fig 1C), 9% in rats (fig 1D), and 28% in humans (fig 2C) of the average value of that in the respective normal colon mucosa.

**Localisation of EP3 receptor protein in rat colon tumours**

Immunohistochemical analysis of paraffin embedded specimens of eight colon tumours and normal colon mucosa in rats treated with AOM was performed. Slight background staining was widely detected in both negative controls, those stained without antirat EP3 receptor antibody (fig 3A, B) and those stained with anti-EP3 receptor antibody preabsorbed with fusion EP3 receptor protein (fig 3C, D). Moreover, slight non-specific staining was detected in red blood cells. In normal colon mucosa tissues, EP3 receptor expression was generated in epithelial cells (fig 3E), and the muscular coat was also positively stained. Similarly, positive staining of EP3 receptors was observed in hyperplastic ACF of the colon (data not shown). In contrast, staining was very faint, minimal, or absent in epithelial cells of colon adenocarcinomas (fig 3F), being totally lacking in seven cases, sized 3–9 mm in diameter. Only one carcinoma sample was weakly stained, and its size was 2 mm.

**Colon tumour development in EP3 receptor knockout mice**

To assess the role of EP3 receptors in colon tumour development, EP3 receptor knockout mice were used in an in vivo model. Data for the incidence (percentage of mice with tumours) and multiplicity (number of tumours per
mouse) of colon tumours induced by AOM are summarised in table 3. Tumour incidence was increased to 78% in EP3 receptor knockout mice compared with 57% in wild-type mice. Regarding tumour multiplicity, values were 2.17 (0.51) for EP3 receptor knockout mice and 0.75 (0.15) for wild-type mice (p < 0.05). Histopathological examination revealed 20 colon tumours to be adenocarcinomas in wild-type, and 50 colon tumours to be three adenomas and 47 adenocarcinomas in EP3 receptor knockout mice. Figure 4 shows the size distribution, demonstrating a significant increase in tumours measuring > 2.0 mm in diameter in EP3 receptor knockout mice (2.00 (0.48) v 0.50 (0.11); p < 0.01) but not in those measuring < 2.0 mm in diameter (0.17 (0.08) v 0.25 (0.11)).

<table>
<thead>
<tr>
<th>Table 3</th>
<th>Colon tumour development in EP3 receptor knockout mice</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mice</td>
<td>Incidence†</td>
</tr>
<tr>
<td>---------</td>
<td>-------------</td>
</tr>
<tr>
<td>Wild-type</td>
<td>16/28 (57%)</td>
</tr>
<tr>
<td>EP3-/-</td>
<td>18/23 (78%)</td>
</tr>
</tbody>
</table>

†Number of mice bearing tumours per total number of mice. ‡Number of tumours per mouse. Data are mean (SEM). *Significantly different from the corresponding wild-type value (p < 0.05).

Expressions of PGE2 receptors in colon cancer cell lines, and effects of the EP3 selective agonist on growth of colon cancer cells

Expression of PGE2 receptors in 11 human colon cell lines was examined by RT-PCR. EP1, EP2, and EP4 were widely detected in the human colon cancer cell lines (in 10 of 11 for EP1, nine of 11 for EP2, and nine of 11 for EP4) but EP3 was only detected in HCA-7 (fig 5A).

To evaluate the physiological functions of the EP3 receptor, the effect of an EP3 receptor selective agonist ONO-AE-248 on viable cell numbers of DLD-1 and HCA-7 in monolayer cultures was examined. In the HCA-7 human colon adenocarcinoma cell line, expression of the EP3 receptor and other PGE2 receptors (EP1, EP2, and EP4) were detected by RT-PCR analysis (fig 5A). As shown in fig 5B, HCA-7 cell numbers were significantly decreased dose dependently by addition of ONO-AE-248, with 8%, 17%, and 30% decreases (p < 0.05, p < 0.01, and p < 0.01) in the presence of 1, 3, and 5 μM ONO-AE-248 on day 5, respectively. On the other hand, treatment with ONO-AE-248 did not affect growth of DLD-1 cells which were not expressing EP3 mRNA. The experiments were repeated three times and similar results were obtained.

Effect of 5-aza-dC on EP3 expression

To determine whether silencing by DNA methylation could be involved in reduced expression of EP3 receptor in colon tumours, we tested the effects of 5-aza-dC, a demethylating
agent, on EP1 receptor expression in colon cancer cell lines. Human colon cancer cell lines CACO-2, CW-2, DLD-1, HCA-7, and WiDr were treated with 5-aza-dC, and with 5-aza-dC, and expression levels of EP1 receptor were analysed by RT-PCR. Without 5-aza-dC treatment, expression of EP1 receptor was detected in HCA-7, but not in CACO-2, CW-2, DLD-1, or WiDr (fig 5A). After 5-aza-dC treatment, expression was restored in CACO-2, CW-2, and DLD-1, but not in WiDr (fig 6).

DISCUSSION

In the present study, examination of mRNA expression levels for EP1, EP2, EP3, and EP4 receptors in colon tissues in mice, rats, and humans by RT-PCR and quantitative RT-PCR provided evidence of a marked reduction in EP3 receptors in colon cancers, in clear contrast with the increase observed for EP1 and EP2. Additionally, results of mRNA expression of EP receptors in 11 human colon cancer cell lines support the above findings and further indicate the events may occur in colon cancer cells. Recently, we reported enhancement of tumour incidence and multiplicity of the EP3 receptor is widely distributed throughout the body, and its downregulation could be important to cancer development at a later stage. Moreover, the size of the tumours was significantly increased. Thus, based on our present and previous results, we suggest that the EP1 receptor does not influence the early stages of colon carcinogenesis, including ACF formation, but its downregulation could be important to cancer development at a later stage.

In our present study, PCR primers of mouse, rat, and human EP3 receptors targeted a common sequence in each species. PCR products would be expected to be derived from all species. Moreover, the size of the tumours was significantly increased. Thus, based on our present and previous results, we suggest that the EP1 receptor does not influence the early stages of colon carcinogenesis, including ACF formation, but its downregulation could be important to cancer development at a later stage.

In our present study, PCR primers of mouse, rat, and human EP3 receptors targeted a common sequence in each species. PCR products would be expected to be derived from all species. Moreover, the size of the tumours was significantly increased. Thus, based on our present and previous results, we suggest that the EP1 receptor does not influence the early stages of colon carcinogenesis, including ACF formation, but its downregulation could be important to cancer development at a later stage.

In our present study, PCR primers of mouse, rat, and human EP3 receptors targeted a common sequence in each species. PCR products would be expected to be derived from all species. Moreover, the size of the tumours was significantly increased. Thus, based on our present and previous results, we suggest that the EP1 receptor does not influence the early stages of colon carcinogenesis, including ACF formation, but its downregulation could be important to cancer development at a later stage.

In our present study, PCR primers of mouse, rat, and human EP3 receptors targeted a common sequence in each species. PCR products would be expected to be derived from all species. Moreover, the size of the tumours was significantly increased. Thus, based on our present and previous results, we suggest that the EP1 receptor does not influence the early stages of colon carcinogenesis, including ACF formation, but its downregulation could be important to cancer development at a later stage.

In our present study, PCR primers of mouse, rat, and human EP3 receptors targeted a common sequence in each species. PCR products would be expected to be derived from all species. Moreover, the size of the tumours was significantly increased. Thus, based on our present and previous results, we suggest that the EP1 receptor does not influence the early stages of colon carcinogenesis, including ACF formation, but its downregulation could be important to cancer development at a later stage.

In our present study, PCR primers of mouse, rat, and human EP3 receptors targeted a common sequence in each species. PCR products would be expected to be derived from all species. Moreover, the size of the tumours was significantly increased. Thus, based on our present and previous results, we suggest that the EP1 receptor does not influence the early stages of colon carcinogenesis, including ACF formation, but its downregulation could be important to cancer development at a later stage.

In our present study, PCR primers of mouse, rat, and human EP3 receptors targeted a common sequence in each species. PCR products would be expected to be derived from all species. Moreover, the size of the tumours was significantly increased. Thus, based on our present and previous results, we suggest that the EP1 receptor does not influence the early stages of colon carcinogenesis, including ACF formation, but its downregulation could be important to cancer development at a later stage.

In our present study, PCR primers of mouse, rat, and human EP3 receptors targeted a common sequence in each species. PCR products would be expected to be derived from all species. Moreover, the size of the tumours was significantly increased. Thus, based on our present and previous results, we suggest that the EP1 receptor does not influence the early stages of colon carcinogenesis, including ACF formation, but its downregulation could be important to cancer development at a later stage.

In our present study, PCR primers of mouse, rat, and human EP3 receptors targeted a common sequence in each species. PCR products would be expected to be derived from all species. Moreover, the size of the tumours was significantly increased. Thus, based on our present and previous results, we suggest that the EP1 receptor does not influence the early stages of colon carcinogenesis, including ACF formation, but its downregulation could be important to cancer development at a later stage.

In our present study, PCR primers of mouse, rat, and human EP3 receptors targeted a common sequence in each species. PCR products would be expected to be derived from all species. Moreover, the size of the tumours was significantly increased. Thus, based on our present and previous results, we suggest that the EP1 receptor does not influence the early stages of colon carcinogenesis, including ACF formation, but its downregulation could be important to cancer development at a later stage.

In our present study, PCR primers of mouse, rat, and human EP3 receptors targeted a common sequence in each species. PCR products would be expected to be derived from all species. Moreover, the size of the tumours was significantly increased. Thus, based on our present and previous results, we suggest that the EP1 receptor does not influence the early stages of colon carcinogenesis, including ACF formation, but its downregulation could be important to cancer development at a later stage.

In our present study, PCR primers of mouse, rat, and human EP3 receptors targeted a common sequence in each species. PCR products would be expected to be derived from all species. Moreover, the size of the tumours was significantly increased. Thus, based on our present and previous results, we suggest that the EP1 receptor does not influence the early stages of colon carcinogenesis, including ACF formation, but its downregulation could be important to cancer development at a later stage.

In our present study, PCR primers of mouse, rat, and human EP3 receptors targeted a common sequence in each species. PCR products would be expected to be derived from all species. Moreover, the size of the tumours was significantly increased. Thus, based on our present and previous results, we suggest that the EP1 receptor does not influence the early stages of colon carcinogenesis, including ACF formation, but its downregulation could be important to cancer development at a later stage.

In our present study, PCR primers of mouse, rat, and human EP3 receptors targeted a common sequence in each species. PCR products would be expected to be derived from all species. Moreover, the size of the tumours was significantly increased. Thus, based on our present and previous results, we suggest that the EP1 receptor does not influence the early stages of colon carcinogenesis, including ACF formation, but its downregulation could be important to cancer development at a later stage.

In our present study, PCR primers of mouse, rat, and human EP3 receptors targeted a common sequence in each species. PCR products would be expected to be derived from all species. Moreover, the size of the tumours was significantly increased. Thus, based on our present and previous results, we suggest that the EP1 receptor does not influence the early stages of colon carcinogenesis, including ACF formation, but its downregulation could be important to cancer development at a later stage.

In our present study, PCR primers of mouse, rat, and human EP3 receptors targeted a common sequence in each species. PCR products would be expected to be derived from all species. Moreover, the size of the tumours was significantly increased. Thus, based on our present and previous results, we suggest that the EP1 receptor does not influence the early stages of colon carcinogenesis, including ACF formation, but its downregulation could be important to cancer development at a later stage.

In our present study, PCR primers of mouse, rat, and human EP3 receptors targeted a common sequence in each species. PCR products would be expected to be derived from all species. Moreover, the size of the tumours was significantly increased. Thus, based on our present and previous results, we suggest that the EP1 receptor does not influence the early stages of colon carcinogenesis, including ACF formation, but its downregulation could be important to cancer development at a later stage.

In our present study, PCR primers of mouse, rat, and human EP3 receptors targeted a common sequence in each species. PCR products would be expected to be derived from all species. Moreover, the size of the tumours was significantly increased. Thus, based on our present and previous results, we suggest that the EP1 receptor does not influence the early stages of colon carcinogenesis, including ACF formation, but its downregulation could be important to cancer development at a later stage.

In our present study, PCR primers of mouse, rat, and human EP3 receptors targeted a common sequence in each species. PCR products would be expected to be derived from all species. Moreover, the size of the tumours was significantly increased. Thus, based on our present and previous results, we suggest that the EP1 receptor does not influence the early stages of colon carcinogenesis, including ACF formation, but its downregulation could be important to cancer development at a later stage.
are different in the carboxy terminal tail, and the amino acid sequence has an important role in G protein coupling specificity.10 11 Two of the three variants of the mouse EP receptors are EP3α and EP3β, which are coupled to Gi and cause inhibition of adenylate cyclase.10 The mouse EP3γ receptor, in contrast, is coupled to Go in addition to Gi and evokes pertussis toxin insensitive cAMP production.10 Preliminary, we examined expression of three splice variants of mouse EP3 receptors by RT-PCR using specific primers for each variant, and found EP3γ to be the major form in mouse normal mucosa (data not shown). These observations support the conclusion that the major splice variants of EP3 receptors are coupled to Gi and act to inhibit adenylate cyclase in normal colon mucosa in mice. On the other hand, EP1 and EP2 receptors are coupled to Go and stimulate cAMP production by this enzyme. Increased cAMP results in activation of cAMP dependent protein kinase (PKA) and transcriptional factors that bind to cAMP responsive elements to transactivate the transcription of specific primary response genes that initiate cell proliferation.12 In our previous study,13 the EP4 receptor selective agonist ONO-AE-329 was shown to enhance colony formation by the HCA-7 human colon adenocarcinoma cell line. The EP1 receptor selective agonist ONO-AE-248 was demonstrated to suppress cell growth in HCA-7 in the present study. It has been reported that ONO-AE-248 attenuates the rise in intracellular cAMP induced by forskolin, an activator of adenylate cyclase, in CHO cells transfected with EP3 receptor.25 Therefore, the EP3 receptor pathway may play an important role in counteracting the effects of EP1 and EP4 receptors, and its downregulation in later stages of colon carcinogenesis may enhance cancer development. Additional studies are needed to investigate interactions between the EP3 receptor signalling pathway and others linked to EP receptors.

Hypermethylation of CpG islands in promoter regions is known to cause silencing of genes in various human cancers,14 15 and silencing of COX-2 and APOC genes by hypermethylation has been reported in human colon cancer.16 17 Although hypermethylation of the prostaglandin receptor gene has not been reported,18 19 DNA sequences in the promoter region and exons 1 of the human EP3A gene are GC rich (Genbank AL051429). Therefore, in the present study, we examined the effects of demethylation of DNA with 5-aza-dC on EP3 expression in human colon cancer cell lines. Demethylation of five cell lines by 5-aza-dC treatment resulted in restoration of EP3 receptor expression in three cell lines. These findings suggest that the DNA sequence of the EP3 receptor may be methylated but further studies are needed to clarify whether hypermethylation of the EP3 receptor gene occurs and regulates EP3 expression in colon cancers.

In conclusion, data obtained in our present and previous studies suggest that the PGE2 receptor subtype EP3 plays an important role in suppression of cell growth and that its downregulation enhances colon carcinogenesis at a later stage. The underlying mechanisms clearly warrant further investigation.

ACKNOWLEDGEMENTS

This work was supported in part by Grants-in-Aid for Cancer Research, for the Second-Term Comprehensive 10-Year Strategy for Cancer Control, and for the Research on Advanced Medical Technology from the Ministry of Health, Labor and Welfare of Japan.

Authors’ affiliations

Y Shoji, M Takahashi, T Kitamura, K Watanabe, T Kawamori, T Sugimura, K Wakabayashi, Cancer Prevention Basic Research Project, National Cancer Center Research Institute, Tokyo, Japan

T Maruyama, Minase Research Institute, Ono Pharmaceutical Co. Ltd, Osaka, Japan

Y Sugimoto, Department of Physiological Chemistry, Faculty of Pharmaceutical Sciences, Kyoto University, Kyoto, Japan

M Negishi, Laboratory of Molecular Neurobiology, Graduate School of Biomedical Sciences, Kyoto University, Kyoto, Japan

S Narumiya, Department of Pharmacology, School of Medicine, Kyoto University, Kyoto, Japan

REFERENCES


www.gutjnl.com

Downloaded from http://gut.bmj.com/ on June 26, 2017 - Published by group.bmj.com
EDITOR’S QUIZ: GI SNAPSHOT

Answer

From question on page 1150

An emergency operation was performed which revealed foreign material which had penetrated into the ileum. A wedge resection of the perforated bowel region was undertaken, and intraperitoneal drainage was performed. The patient was discharged from our hospital nine days postoperatively in good condition.

The object that had been imaged on the computed tomography scan was found to be the foot of a soft shelled turtle (fig 2), commonly referred to as “Supon” in Japanese (scientific name *Trionyx sinensis*). This turtle is only served on special occasions and is an expensive item for cuisine. Discussions with the patient indicated that he had eaten soft shelled turtle two months before the operation during a new year festival in January. As an aid in identifying this type of situation, it is important to also make use of preoperative computed tomography scans, review the patient’s history in light of any prior operations and, where possible, evaluate the patient’s menu or discuss with the family to recollect any sources of hard body parts that could be an immediate source of the problem.

Figure 2 A picture of the foot of a soft shelled turtle.

doi: 10.1136/gut.2003.023929
Downregulation of prostaglandin E receptor subtype EP3 during colon cancer development

Y Shoji, M Takahashi, T Kitamura, K Watanabe, T Kawamori, T Maruyama, Y Sugimoto, M Negishi, S Narumiya, T Sugimura and K Wakabayashi

Gut 2004 53: 1151-1158
doi: 10.1136/gut.2003.028787

Updated information and services can be found at:
http://gut.bmj.com/content/53/8/1151

These include:

References
This article cites 38 articles, 18 of which you can access for free at:
http://gut.bmj.com/content/53/8/1151#BIBL

Email alerting service
Receive free email alerts when new articles cite this article. Sign up in the box at the top right corner of the online article.

Topic Collections
Articles on similar topics can be found in the following collections

- Colon cancer (1547)
- Cancer: small intestine (189)

Notes

To request permissions go to:
http://group.bmj.com/group/rights-licensing/permissions

To order reprints go to:
http://journals.bmj.com/cgi/reprintform

To subscribe to BMJ go to:
http://group.bmj.com/subscribe/