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Genetic deletion of mPGES-1 accelerates intestinal tumorigenesis in APC$^{Min/+}$ mice

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ABSTRACT

The induced synthesis of bioactive prostanoids downstream of cyclooxygenase-2 (COX-2) and prostaglandin H_2 (PGH_2) exerts a critical event in colorectal carcinogenesis. Here we demonstrate that APC$^{Min/+}$ mice with genetic deletion of microsomal prostaglandin E synthase-1 (mPGES-1), which catalyses the terminal conversion of PGH_2 into PGE_2, surprisingly develop more and generally larger intestinal tumors than do mPGES-1 wild type littermates (mean number of tumors/intestine 80 vs. 38, $p < 0.0005$, mean tumor diameter 1.64 vs. 1.12 mm, $p < 0.0005$). No deviation regarding the expression of other PGE_2 related enzymes (COX-1, COX-2, mPGES-2, cPGES, and 15-PGDH) or receptors (EP1–4) was obvious among the mPGES-1 deficient mice. PGE_2 levels were suppressed in tumors of mPGES-1 deficient animals, but the concentrations of other PGH_2 derived prostanoids were generally enhanced, being most prominent for TxA_2 and PGD_2. Thus, we hypothesise that a redirected synthesis towards other lipid mediators might (over)compensate for loss of mPGES-1/PGE_2 during intestinal tumorigenesis. Nevertheless, our results question the suitability for mPGES-1 targeting therapy in the treatment or prevention of colorectal cancer.

Colorectal cancer is one of the leading malignancies in Western world, affecting about 1 000 000 new patients each year. It has for long been known that regular usage of non-steroidal anti-inflammatory drugs (NSAIDs) dramatically reduces the risk of developing colorectal tumors. This effect has mainly been attributed to the inhibition of cyclooxygenase-2 (COX-2), that is upregulated in inflammatory and neoplastic states and catalyses the synthesis of prostaglandin H_2 (PGH_2). Among other signalling molecules derived from PGH_2, prostaglandin E_2 (PGE_2) is present at high levels in colorectal tumors, and is thought to be essentially involved in intestinal carcinogenesis (reviewed in [1]) although recent reports suggest other bioactive PGH_2 metabolites to exert protumorigenic effects as well [2–10].

Though, treatment with NSAIDs or COX-2 selective inhibitors is associated with severe systemic side effects, most probably due to the altered balance of these non-PGE_2 metabolites downstream of COX generated PGH_2 [1]. In this context, the terminal prostaglandin E synthases provide attractive targets for specific modulation of PGE_2 production. In particular, microsomal prostaglandin E synthase-1 (mPGES-1) has attracted interest, since this isoform is markedly upregulated in neoplastic tissue, and thought to be critical for tumor PGE_2 generation [11,12], although this has not yet been fully assessed *in vivo*.

In the present study, the effect of homozygous mPGES-1 gene (PTGES) deletion was investigated with regard to intestinal tumor formation in APC$^{Min/+}$ mice. Notably, we found that the number and size of intestinal tumors were markedly increased in APC$^{Min/+}$ mPGES-1$^{-/-}$ mice, although PGE_2 levels were suppressed as expected. Enhanced concentrations of other COX-2 derived eicosanoids were present in tumors from mPGES-1 deleted animals, potentially (over)compensating for the loss of PGE_2. Nevertheless, our findings clearly challenge the suitability for mPGES-1 targeting therapy in the treatment or prevention of colorectal cancer.

Materials and methods

Animals. C57BL/6 mice with the APC$^{Min/+}$ genotype (The Jackson Laboratory, Bar Harbor, ME, USA), or C57BL/6 mice with targeted deletion of the mPGES-1 encoding gene (mPGES-1$^{-/-}$), previously described in [13], were used in the present study. APC$^{Min/+}$ and mPGES-1$^{-/-}$ mice were crossed, generating APC$^{Min/+}$mPGES-1$^{+/-}$ and APC$^{+/+}$mPGES-1$^{+/-}$ animals (F1 generation), which were subsequently intercrossed (F2 generation), yielding APC$^{Min/+}$mPGES-1$^{+/+}$, APC$^{Min/+}$mPGES-1$^{+/-}$ and APC$^{Min/+}$mPGES-1$^{-/-}$ littermates, which were included in the present analyses. Animals were housed in ventilated cages at 23 ± 1 °C with a 12-h light/dark cycle. Standard diet (CRME rodent, Special Diet Services Ltd., Witham, Essex, UK) and water were available *ad libitum*. DNA for genotyping was

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isolated from tail biopsies with the Extract-N-Amp Tissue PCR Kit™ (Sigma, St. Louis, MO, USA), according to supplier's recommendations. The APC and mPGES-1 encoding genes were genotyped as previously described [13,14]. All experimental procedures were approved by the animal Care and Use Committee at the Linköping University.

Tumor quantitation. At age 125 ± 5 days, mice were sacrificed with CO_2 and whole intestines were collected. Intestines were held on ice and immediately cut longitudinally and carefully rinsed with ice-cold 0.9% saline. Small intestines were subdivided in proximal, middle, and distal third, respectively. Tumor frequency and size were determined under a 20× dissection microscope (Leica Microsystems GmbH, Wetzlar, Germany) by a single examiner (NE) who was blinded to the genotype. Tumor size was approximated by measuring the maximum tumor diameter with a calibrated eyepiece reticule (Leica).

Histopathological classification. A subset of tumors were put in 4% formaldehyde solution and embedded in paraffin. Standard H&E staining was performed, and tumors were classified by an experienced pathologist (HO) who was blinded to the genotype.

RNA isolation. Tumors and non-tumor intestinal mucosa specimens were collected and immediately placed in RNAlater (Ambion Inc., Austin, TX, USA). Specimens were homogenised through shaking with 5 mm stainless steal beads (Qiagen, Hilden, Germany) in a TissueLyser (Qiagen) at 20 Hz for 2 × 2 min. RNA was then isolated with RNeasy Mini Kit (Qiagen) according to the supplier's recommendations. Total RNA concentrations were determined spectrophotometrically.

cDNA synthesis and real-time PCR. Five hundred nanograms of total RNA from each sample was reversely transcribed into cDNA with Superscript III (Invitrogen, Carlsbad, CA, USA) according to manufacturer's protocol. mRNA expression of mPGES-1, mPGES-2, cPGES, COX-1, COX-2, 15-PGDH, and PGE_2 receptors (EP) 1–4 was subsequently determined with the 7500 Fast Real-Time PCR System (Applied Biosystems, Foster City, CA, USA), using predesigned primer/probe assays purchased from Applied Biosystems (sequences available at request). C_t values were related to the endogenous control gene GAPDH (ΔC_t), and relative expression ($2^{-\Delta Ct}$) was normalised to the average expression in non-tumor intestinal mucosa of $APC^{Min/+}$mPGES-$1^{+/+}$ mice ($2^{-\Delta\Delta Ct}$). To validate GAPDH as a reliable endogenous control gene, a randomly chosen subset of tumors and non-tumor mucosal specimens were co-analysed with regard to three control genes, namely GAPDH, β-actin, and β-glucuronidase (Applied Biosysems), revealing similar relationships between Ct values of the different genes among all specimens (data not shown).

Measurement of PGE_2, TxB_2, PGD_2-MOX, 6-keto-$PGF_{1\alpha}$, and $PGF_{2\alpha}$. Whole tumor specimens and adjacent mucosa samples were homogenised in 0.1 M phosphate buffer (pH 7.4, containing 1 mM EDTA and 10 μL indomethacin) through shaking in a TissueLyser (Qiagen, for details see above). Fractions of the lysate were diluted in EIA buffer and PGE_2, thromboxane B_2 (TxB_2), 6-keto-prostaglandin $F_{1\alpha}$ ($PGF_{1\alpha}$), and prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) concentrations were determined with appropriate Monoclonal EIA Kits (Cayman Chemical, Ann Arbor, MI, USA) according to the manufacturer's recommendations. With regard to PGD_2, conversion to a more stable methoxylamine (MOX)-derivative (PGD_2-MOX) was performed according to supplier's recommendations. All samples were analysed in duplicate and at least at two different dilutions to ensure reliable results within the spectrum of the assays (B/B_0 between 20% and 80%).

Statistics. Data were expressed as means ± standard error of the mean (SEM). For comparative analyses, Student's *t*-test was used. All calculations were performed with the SPSS Software 15.0 (SPSS Inc., Chicago, IL, USA). $p < 0.05$ was considered statistically significant.

Results

Animal characteristics

At age 125 ± 5 days, mice were sacrificed and intestines were collected. With few exceptions, mice of all investigated genotypes survived until this time point, although the body weight was significantly lower among both male and female $APC^{Min/+}$mPGES-$1^{-/-}$ animals (Table 1).

Tumor multiplicity, size, and histopathology

$APC^{Min/+}$mPGES-$1^{+/+}$ mice displayed a total of 38.19 ± 2.22 (mean ± SEM, n = 20) intestinal tumors per mouse. Surprisingly, the intestinal tumor frequency was twofold elevated among $APC^{Min/+}$mPGES-$1^{-/-}$ animals, reaching 79.56 ± 9.21 ($n = 12$, $p < 0.0005$).

The increased tumor frequency among mPGES-1 deleted animals was observed in all parts of small intestine (proximal, middle, and distal third), with most of the tumors located in the distal third (Fig. 1A), and results were consistent among both males and females (data not shown). Colonic tumor frequency barely differed between genotypes (2.56 ± 0.72 mm [mean ± SEM] vs. 2.42 mm ± 0.33, $p = 0.836$, Fig. 1A).

Further, tumors were found to be significantly larger in small intestines of $APC^{Min/+}$mPGES-$1^{-/-}$ mice (1.64 ± 0.043 mm [mean tumor diameter ± SEM], n = 276) in comparison with $APC^{min/+}$mPGES-$1^{+/+}$ littermates (1.12 ± 0.034 mm, $n = 351$, $p < 0.0005$, Fig. 1B). The same tendency was observed with regard to colonic tumor size, although this difference did not reach statistical significance (2.29 ± 0.29 mm, $n = 54$ vs. 1.78 ± 0.33 mm, $n = 30$, $p = 0.331$, Fig. 1B). In addition, a limited number of $APC^{Min/+}$mPGES-$1^{+/-}$ mice were analysed with regard to tumor multiplicity and size, revealing a phenotype in between $APC^{Min/+}$mPGES-$1^{+/+}$ and $APC^{Min/+}$mPGES-$1^{-/-}$ animals (Fig. 1A–B).

A subset of tumors ($n = 30$) from $APC^{Min/+}$mPGES-$1^{+/+}$ and $APC^{Min/+}$mPGES-$1^{-/-}$ mice were classified histopathologically. In total, 80% of all tumors were classified as high grade tubular adenomas and 20% as invasive adenocarcinomas. These findings did not differ between $APC^{Min/+}$mPGES-$1^{+/+}$ and $APC^{Min/+}$mPGES-$1^{-/-}$ animals (data not shown).

PGE_2 measurements

In accordance with previous reports on human and murine intestinal tumors [15–17], the PGE_2 levels were nearly threefold elevated in $APC^{Min/+}$mPGES-$1^{+/+}$ tumors compared to non-tumor mucosa, but, as expected, similarly low in normal mucosa and tumor tissue of mPGES-1 deleted mice (Fig. 2).

mRNA expression assays

To determine whether the $APC^{Min/+}$mPGES-$1^{-/-}$ individuals exhibited any deviations in the expression of other genes critically involved in PGE_2 signalling, a panel of mRNA expression assays was performed on tumors and corresponding non-tumor mucosa spec-

Table 1
Mean body weight ± SEM (g)

Genotype	$APC^{Min/+}$mPGES-$1^{+/+}$	$APC^{Min/+}$mPGES-$1^{-/-}$	*p* Value[a]
Sex			
Male	34.00 (±0.63) ($n = 12$)	30.14 (±0.67) ($n = 8$)	0.002
Female	26.75 (±0.59) ($n = 8$)	23.25 (±0.63) ($n = 4$)	0.004

[a] Mean body weights were compared with Student's *t*-test.