Short Communication

Simultaneous expression of COX-2 and mPGES-1 in mouse gastrointestinal hamartomas

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Cyclooxygenase (COX)-2 is induced in various types of cancer tissues. Here, we demonstrate stromal expression of both COX-2 and microsomal prostaglandin E₂ synthase (mPGES)-1 in gastrointestinal hamartomas developed in Lkb1⁺/⁻, Smad4⁺/⁻ and Cdx2⁺/⁻ mice. These results suggest that PGE₂ produced by COX-2 and mPGES-1 plays an important role in hamartoma development regardless of the mutated genes causing hamartomas.

British Journal of Cancer (2004) 90, 701 – 704. doi:10.1038/sj.bjc.6601584 www.bjcancer.com
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Keywords: hamartoma; LKB1; SMAD4; CDX2; COX-2; mPGES-1

Using Apc¹⁷¹⁶ mouse mutant, a model for familial adenomatous polyposis (FAP), we demonstrated earlier that disruption of the gene encoding cyclooxygenase (COX)-2 or prostaglandin E₂ (PGE₂) receptor EP2 suppresses intestinal polyposis (Oshima et al, 1996; Sonoshita et al, 2001). These results indicate that PGE₂ produced through the COX-2 pathway plays an important role in intestinal tumorigenesis. One of the PGE₂ synthases, microsomal prostaglandin E₂ synthase (mPGES)-1 appears to be responsible for PGE₂ production in tumour tissues, because this enzyme is induced and functionally coupled with COX-2 in a human embryonic kidney cell line (Murakami et al, 2000). Likewise, COX-2 and mPGES-1 are induced simultaneously in human colorectal cancer tissues (Yoshimatsu et al, 2001), intestinal-type gastric adenocarcinomas (Van Rees et al, 2003) and Apc¹⁷¹⁶ mouse intestinal adenomas (Takeda et al, 2003).

Peutz–Jeghers syndrome (PJS) and juvenile polyposis syndrome (JPS) are autosomal dominant diseases characterised by hamartomatous polyps of the gastrointestinal tract with an increased risk of cancer development. Germ line mutations in the LKB1 (Hemminki et al, 1998; Jenne et al, 1998) and SMAD4 (Howe et al, 1998) are responsible for subpopulations of PJS and JPS, respectively. Gene-targeted mice heterozygous for Lkb1 and Smad4 develop gastrointestinal hamartomas that have histological characteristics similar to those of PJS and JPS, respectively (Takaku et al, 1999; Miyoshi et al, 2002). Recently, it has been reported that COX-2 expression is induced in hamartomatous polyps of PJS patients and Lkb1⁻/⁻ mice (Rossi et al, 2002; de Leng et al, 2003; McGarrity et al, 2003), suggesting that production of PGE₂ is responsible for gastrointestinal hamartoma development as in intestinal adenomatous polyposis.

Here we show that both COX-2 and mPGES-1 are induced in gastric hamartoma tissues of Lkb1⁺/⁻ and Smad4⁺/⁻ mice. In addition, we demonstrate induction of these enzymes also in Cdx2⁺/⁻ mouse colonic hamartomas. These results strongly suggest that production of PGE₂ is responsible for gastrointestinal hamartoma development in intestinal adenomatous polyposis.

MATERIALS AND METHODS

All in vivo experiments were carried out with ethical committee approval and met the standards required by the UKCCCR guidelines (Workman et al, 1998). Constructions of Lkb1⁺/⁻, Smad4⁺/⁻ and Cdx2⁺/⁻ mutant mice have been described previously (Takaku et al, 1999; Tamai et al, 1999; Miyoshi et al, 2002). We used these mouse models to examine the expression patterns of COX-1, COX-2 and mPGES-1 in hamartomas that were caused by mutations in the putative genes, Lkb1, Smad4 and Cdx2, respectively. As the expression of COX-2 and mPGES-1 can be affected by various conditions such as infections, inflammations and host immune responses, it is important to use congenic mice bred in a specific pathogen-free (SPF) facility and compare them with the age-matched littermate controls. The results from these mouse experiments should provide important pieces of evidence applicable to human clinical research. Ages of mutant mice used in these experiments were 60 – 66, 76 – 90 and 20 – 35 weeks for Lkb1, Smad4 and Cdx2, respectively. Hamartomas were sampled from seven independent mice and used for further analysis.

For immunoblotting, tissue samples were homogenised and sonicated in lysis buffer (50 mM phosphate buffer pH 7.0, 100 mM NaCl, 2 mM EDTA) containing a protease inhibitor cocktail (Roche Diagnostics, Nonnenwald, Penzberg, Germany). After centrifugation at 10,000 × g at 4°C for 10 min, 40 μg of the supernatant protein was mixed with 6 × SDS sample buffer (350 mM Tris-HCl, pH 6.8, 36% glycerol, 10% SDS, 600 mM DTT), separated in SDS–polyacrylamide gels and transferred onto PVDF membranes. After blocking with 5% skimmed milk/Tris-buffered saline/Tween 20,
membranes were incubated with an antibody for COX-1 (Santa Cruz Biotechnology, Santa Cruz, CA, USA), COX-2, cPGES or mPGES-1 (Cayman Chemical, Ann Arbor, MI, USA) at 1000-fold dilution, or for β-actin (Sigma) at 5000-fold dilution. The ECL detection system (Amersham Pharmacia, Uppsala, Sweden) was used to detect the signals.

For immunohistochemistry, tissue samples were fixed in 4% paraformaldehyde, embedded in paraffin wax and sectioned at 4 μm. After pretreatment in 3% H2O2 in methanol, sections were boiled in 10 mM citrate buffer (pH 6.0) in a microwave oven for 5 min. Sections were blocked with 3% BSA-10% normal serum for 1 h and incubated with the primary antibody for COX-1, COX-2 or mPGES-1 at 400-fold dilution. Immunostaining signals were visualised using Vectastain Elite Kit (Vector Laboratories, Burlingame, CA, USA).

RESULTS AND DISCUSSION

Hamartomatous polyp tissues were excised from the stomach of Lkb1+/−/− and Smad4+/−/− mice and from the colon of Cdx2+/−/− mice. We first examined the expression levels of COX enzymes and PGE2 synthases in these tumour tissues by immunoblotting (Figure 1). The expression of COX-2 and mPGES-1 was detected in all hamartomatous polyps, whereas these enzymes were rarely expressed in the normal tissues. On the other hand, COX-1 and cPGES were detected in both normal and hamartoma tissues. These results are consistent with our recent report that COX-1 and cPGES are expressed constitutively in the normal mouse intestines, whereas COX-2 and mPGES-1 are induced in the intestinal polyps of ApcMin mice (Takeda et al., 2003).

We next determined by immunohistochemistry the localisation of COX-1, COX-2 and mPGES-1 in the hamartoma tissues of the Lkb1+/−/−, Smad4+/−/− and Cdx2+/−/− mice, respectively (Figure 2).
Expression of COX-2 and mPGES-1 in gastrointestinal hamartomas

H Takeda et al

all samples, COX-1 was expressed in the stromal cells of hamartomas (Figure 2A, D, G) as well as in those of the normal mucosa (data not shown). Expression of COX-2 and mPGES-1 was detected in the stroma of hamartomas near the intestinal lumen, overlapping partly with the COX-1-expressing cells (Figure 2B–I). Moreover, cells expressing COX-2 and mPGES-1 appeared to be the same stromal cells showing a fibroblast-like morphology. There was no apparent difference in the expression patterns of COX-1, COX-2 and mPGES-1 among the hamartomas developing in different mutants. We did not find any difference in the expression levels and cell types among the individual mice of each model. These results indicate that COX-2 and mPGES-1 are induced simultaneously in the hamartomas, stromal PGE₂ production appears to play a key role in the hamartoma expansion.

The PGE₂ signalling stimulates tumour angiogenesis through the EP2 receptor (Seno et al, 2002), increases cell survival and motility (Sheng et al, 2001), inhibits host immune responses (Huang et al, 1998) and activates epidermal growth factor receptor (EGFR) (Pai et al, 2002). Accordingly, it is conceivable that stromal PGE₂ in the hamartomas contributes to tumour expansion through these effects.

Inhibition of COX-2 by NSAIDs or COX-2-selective inhibitors suppresses intestinal polypyosis in Apc<sup>–/–</sup> mice and FAP patients (Oshima et al, 1996; Steinbach et al, 2000). In addition, administration of COX-2 inhibitor to trefoil factor 1 (TFF1)-deficient mice suppresses gastric adenomas that are caused without Wnt signalling activation (Saukkonen et al, 2003). The results suggest that COX-2 induction in the tumour stroma is independent of the molecular mechanism that initiates tumorigenesis in the epithelial cells. These results, taken together, strongly suggest that COX-2 inhibitors, and possibly EP antagonists, are therapeutic agents effective for not only adenomatous polyps but also hamartomas of the gastrointestinal tract. As hamartomaticus polyps can progress into neoplastic tumours (Wang et al, 1999), COX-2 inhibitors may also turn out to be cancer chemopreventive agents suitable for hereditary hamartoma syndromes.

ACKNOWLEDGEMENTS

This work was supported by grants from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

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Jenne DE, Reimann H, Nezu J, Friedel W, Loff S, Jeschke R, Muller O, Back McGarrity TJ, Peiffer LP, Amos CI, Frazier ML, Ward MG, Howett MK (2000) CDX 2 mutations have not been detected in any hereditary hamartoma syndromes, it is conceivable that a subset of sporadic hamartomas contains CDX2 mutations. Regardless of the mutated genes that caused hamartomas, stromal PGE₂ production appears to play a key role in the hamartoma expansion.

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