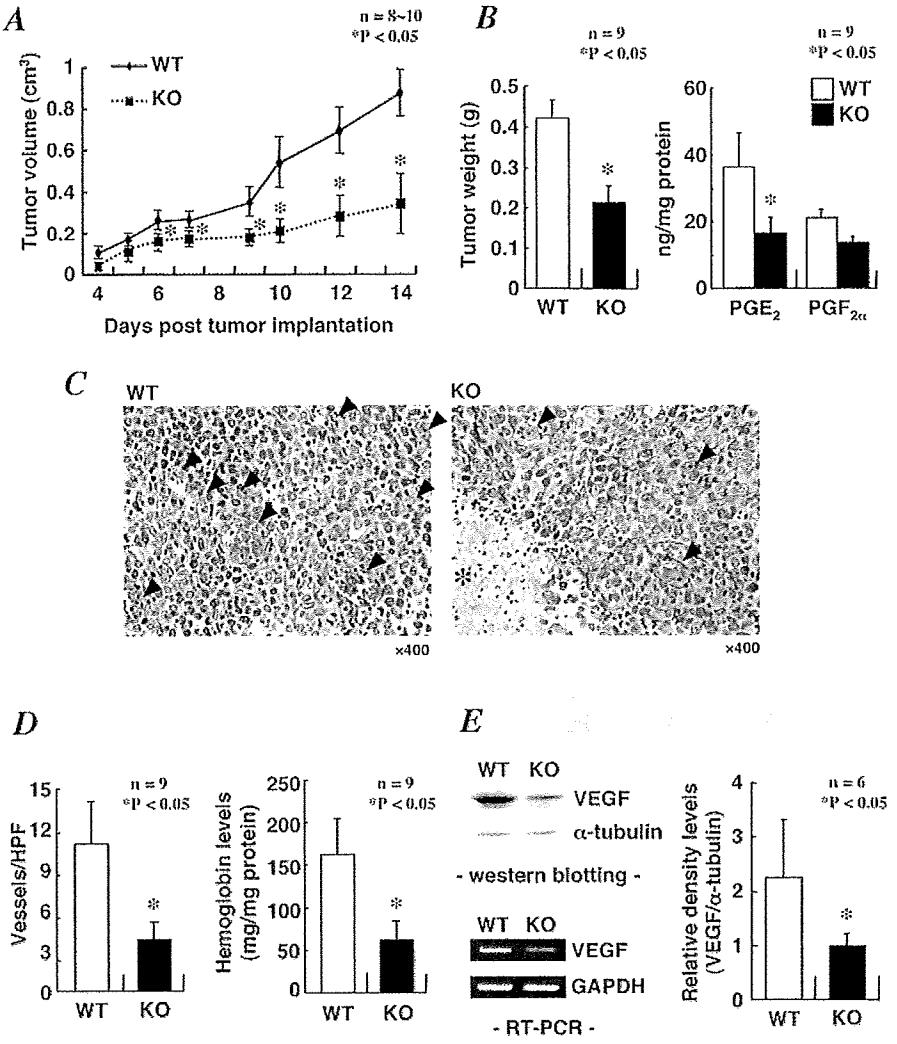

Figure 5 Growth of LLC cells subcutaneously implanted into mPGES-1-KO and WT mice

A total of 10^5 cells were injected into the subcutaneous tissue of female mPGES-1-KO and WT mice. (**A**) Tumour volumes were scored on the indicated days as described in the Experimental section. (**B**) On day 14 after implantation, the tumour tissues were dissected and weighed (left-hand panel). Amounts of PGE_2 and $PGF_{2\alpha}$ in homogenates of the tumour tissues were quantified by enzyme immunoassay (right-hand panel). Results are presented as the means ± S.E.M. ($n = 9$). (**C**) Tumour tissues were cut out, fixed in formalin, and stained with silver, haematoxylin and eosin for histochemical analysis. Microvessels and necrotic regions are shown by arrowheads and an asterisk respectively. (**D**) Vascular density (left-hand panel) and haemoglobin content (right-hand panel) were determined as described in the Experimental section. Results are presented as the means ± S.E.M. ($n = 9$). HPF, high-power field. (**E**) Expression levels of VEGF protein (upper panel) and mRNA (lower panel) in the tumour tissues were determined by Western blot and RT–PCR analysis respectively. Representative results of six independent experiments are shown. On Western blot analysis, the expression level of VEGF protein was quantified by a densitometer, with the expression level of α-tubulin used for normalization (means ± S.D., $n = 6$) (right-hand panel).

hosts is capable of promoting proliferation and invasion of cancer cells *in vitro* and *in vivo*.

Role of mPGES-1 in cancer cells

We found that PGE_2 generation and cell proliferation were reduced by mPGES-1 knockdown (Figures 1A, 1B and 1D) and conversely enhanced by mPGES-1 overexpression (Figures 3A–3C) in LLC cells (a PGE_2-dependent mouse lung carcinoma cell line; [27]), indicating the role of mPGES-1, acting downstream of COX-2, in providing the mitogenic PGE_2. In accordance with the impact on cell proliferation, the gene silencing of mPGES-1 decreased, whereas overexpression of mPGES-1 increased, the invasive activity of LLC cells (Figures 2A and 3D). The process by which tumour cells break out from their site of origin and metastasize to distant sites requires an ability to invade through the ECM and underlying mesenchymal cells. PGE_2 stimulates the invasion of cancer cells through up-regulating MMP-2 and MMP-9 (type IV collagenases), as shown by the observations that MMP-2 is increased in COX-2-overexpressing or PGE_2-treated tumour cells [25,26,28,29], and that MMP-9 is induced by COX-2/EP4 signalling in macrophages [30]. We found that the expression of MMP-2 and -9 in mPGES-1-KD cells were lower than that in mock cells (Figure 2B), suggesting the contribution of these matrix-degrading proteases to the mPGES-1-dependent invasiveness of LLC cells. In addition to adhesion to and degradation of the ECM, detachment from the ECM is also an important step in the metastasis of tumour cells [25,26]. As shown in Figure 2(C), both collagen- and fibronectin-adherent activities were significantly increased in mPGES-1-KD cells, and treatment with $dmPGE_2$ reversed these activities consistently. Accordingly, the expression of integrin $\alpha 5\beta 1$ (a major adhesion molecule of fibronectin) in mPGES-1-KD cells was higher than that in

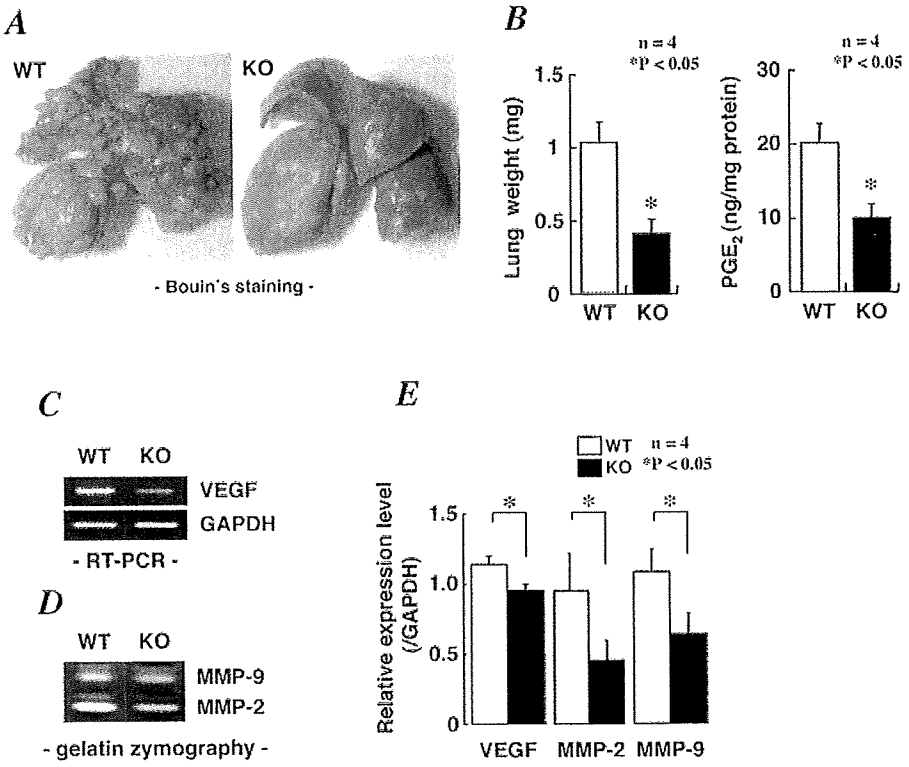

Figure 6 Reduced lung metastasis in mPGES-1-KO mice

A total of 10^5 cells were injected into the lateral tail veins of female mPGES-1-KO and WT mice on the BALB/c background. On day 14 after implantation, the mice were killed, and the removed lungs were weighed (**B**, left-hand panel) and then stained with Bouin's solution (**A**). Amounts of PGE_2 in homogenates of the metastasized lung tissues were measured by enzyme immunoassay (**B**, right-hand panel). (**C**) Expression of VEGF and GAPDH mRNAs were assessed by RT–PCR. (**D**) Activities of MMP-2 and -9 were evaluated by gelatin zymography. Results are presented as the means ± S.E.M. ($n = 4$) in (**B**) and representative results of four independent experiments are shown in (**A**, **C** and **D**). (**E**) The expression levels of mRNAs for VEGF, MMP-2 and MMP-9 were evaluated by quantitative RT–PCR, with GAPDH mRNA used for normalization (means ± S.D., $n = 4$).

mock cells (Figure 2D), an observation that is reminiscent of our previous report that co-overexpression of COX-2 and mPGES-1 in HEK-293 cells results in a marked reduction in a subset of integrins [14]. Thus mPGES-1-driven PGE_2 may regulate the ECM-adherent activity of cancer cells by altering the expression of integrins.

Importantly, *in vivo* tumour growth after subcutaneous engraftment of mPGES-1-KD cells was less obvious than that after engraftment of replicate mock cells (Figure 4), implying that mPGES-1 contributes to the supply of the major pool of the PGE_2 mediating tumour proliferation both *in vitro* and *in vivo*. Gene disruption of EP2 resulted in a reduced number and size of intestinal polyps in *Apc*-mutant mice, a model for human familial adenomatous polyposis [31], and the *Apc* mutation is accompanied by aberrant activation of β-catenin signalling, which is accelerated by PGE_2 through the EP2–Gsα axis [32,33]. In another model, disruption of the gene for EP1, EP2 or EP4 suppressed the development of colorectal cancer induced by carcinogens [34–36]. Transactivation of EGF (epidermal growth factor) receptors by the PGE_2/EP1, 2, or 4 signalling via protein kinase A and c-Src led to increased cell growth and invasion of carcinoma cells [37–39]. In a positive-feedback loop, COX-2-derived PGE_2 acts on EP2, leading to the elevation of intracellular cAMP, which in turn up-regulates the expressions of both COX-2 and EP2 [40]. Although the identification of EPs participating in the propagation of LLC xenografts is beyond the scope of the present study, the co-ordinated reduction of mPGES-1 and EP2 in mPGES-1-KD cells (Figure 1A) suggests that COX-2/mPGES-1-derived PGE_2 may stimulate EP2 signalling in an autocrine/paracrine fashion to facilitate LLC cell proliferation and, accordingly, defects in mPGES-1 may lead to down-regulation of EP2 and eventually reduce the cellular sensitivity to PGE_2.

While this paper was in preparation, two groups reported opposite effects of mPGES-1 deficiency in intestinal tumorigenesis. Nakanishi et al. [41] showed that the genetic deletion of mPGES-1 ameliorates the development of intestinal tumours in both $Apc^{\Delta 14}$-dependent and carcinogen-induced models, whereas Elander et al. [42] demonstrated that intestinal polyposis is exacerbated in mPGES-1-null Apc^{min} mutant mice, probably because of the metabolic shift from PGE_2 towards other pro-tumorigenic lipid mediators. Although the reason for the discrepancy between these two studies is unclear, our present results appear to be in line with the former study and thus support the feasibility of targeting mPGES-1 for cancer chemoprevention. Critically, although the experimental design of the previous studies did not allow a precise distinction between the contribution of cancer cell- and host-associated mPGES-1 to tumour development, our present approach clearly underscores the importance of mPGES-1 pools both in cancer cells (as discussed above) and in microenvironments (see below).

Role of mPGES-1 in host microenvironments

As is COX-2, mPGES-1 is expressed in stromal cells as well as in cancerous cells in several types of tumour [14,15]. Given that stromal COX-2 and PGE_2 of the host can influence the

development of grafted tumours [20,43], we used mPGES-1-KO mice to evaluate the potential roles of the host stromal mPGES-1 in tumour growth, and found that tumour growth at a proximal site and metastasis into a distant tissue were significantly reduced in mPGES-1-KO mice relative to WT mice (Figures 5 and 6). This result provides an additional line of genetic evidence that the COX-2/mPGES-1-derived PGE_2 from host stromal cells, in addition to that from cancer cells (see above), is important for tumorigenesis *in vivo*. The induction of mPGES-1 in tumour stromal tissues may be ascribed to the migration and expansion of host inflammatory cells and neovascular endothelial cells, since previous immunohistochemical analyses have demonstrated a preferential localization of mPGES-1 in macrophage-like cells infiltrating into the stromal tissues in proximity to cancer cells [14], and since massive macrophage infiltration and microvessel formation have been observed in the stroma of gastric hyperplasia in COX-2/mPGES-1-double transgenic mice [16,17].

As shown in Figure 5, vascular density and VEGF expression in tumour xenografts were decreased in mPGES-1-KO mice. It has been reported that angiogenesis induced by either endogenous COX-2 or exogenous PGE_2 was accompanied by increased VEGF expression and was abolished by a VEGF-directed antisense oligonucleotide [19]. Moreover, the growth, VEGF expression and angiogenesis of LLC-implanted tumours were markedly suppressed in EP3-KO mice [43], a phenotype that is reminiscent of mPGES-1-KO (the present study) and COX-2-KO mice [20]. These studies indicate that, in the tumour milieu, both cancer cells and adjacent stromal cells synthesize (via COX-2/mPGES-1) PGE_2, which in turn acts on the particular population of EP3-expressing stromal cells to induce the production of VEGF and consequent angiogenesis [43]. Consistently, Nakanishi et al. [41] have reported very recently that genetic deletion of mPGES-1 in *Apc*-mutant mice caused marked and persistent suppression of intestinal cancer growth in association with a disorganized vascular pattern. In addition to tumour growth and associated angiogenesis, lung metastasis of LLC cells across the blood circulation was also decreased in mPGES-1-KO mice as compared with replicate WT mice (Figure 6). The metastatic phenotype observed in mPGES-1-KO mice was similar to that observed in MMP-2-KO mice, in which focal xenograft propagation and lung metastasis of LLC cells were reduced [44]. In agreement, MMP-2, as well as MMP-9, expression in the metastasized lung tissues was markedly lower in mPGES-1-KO mice than in WT mice (Figures 6D and 6E). Thus, in the metastatic foci, the PGE_2 derived from the host mPGES-1 may lead to the increase in the activities of MMP-2 and -9, which, in co-operation with VEGF, may promote the invasion of cancerous cells into the adjacent and distant tissues, thereby allowing subsequent expansion and metastasis of the tumour.

In conclusion, the results of the present study suggest that both cancer cell-associated and host-derived mPGES-1 is critical for tumour growth and metastasis. The mPGES-1-driven PGE_2 signalling on stromal cells may be functionally linked to the induction of potent pro-angiogenic and matrix-degrading factors, which in turn would facilitate tumour development. Previous studies have shown that, unlike the specific inhibition of COX-2, which predisposes to cardiovascular risk, gene ablation of mPGES-1 in mice shows minimal unfavourable effects on the circulation system [12]. Therefore an mPGES-1 inhibitor would exhibit a chemopreventive action on various tumours by attenuating both cancer cell- and stromal cell-derived PGE_2, thereby serving as a novel therapeutic tool for cancer. Future clinical studies will address the important question of the efficacy and safety of mPGES-1 inhibition in human diseases.

AUTHOR CONTRIBUTION

Daisuke Kamei designed and performed the experiments, analysed the data and contributed to writing the manuscript; Yuka Sasaki also performed the experiments; Yoshihito Nakatani, Masataka Majima, Yukio Ishikawa and Toshiharu Ishii helped in manuscript and scientific discussions; Satoshi Uematsu and Shizuo Akira provided the resources for the work; Ichiro Kudo designed the experiments; and Makoto Murakami and Shuntaro Hara designed the experiments and also wrote the manuscript.

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REFERENCES

1 Levy, G. N. (1997) Prostaglandin H synthases, nonsteroidal anti-inflammatory drugs, and colon cancer. FASEB J. **11**, 234–247
2 Sheng, H., Shao, J., Washington, M. and DuBois, R. N. (2001) Prostaglandin E_2 increases growth and motility of colorectal carcinoma cells. J. Biol. Chem. **276**, 18075–18081
3 Murakami, M. and Kudo, I. (2004) Recent advances in molecular biology and physiology of the prostaglandin E_2-biosynthetic pathway. Prog. Lipid Res. **43**, 3–35
4 Tsujii, M. and DuBois, R. N. (1995) Alterations in cellular adhesion and apoptosis in epithelial cells overexpressing prostaglandin endoperoxide synthase 2. Cell **83**, 493–501
5 Tsujii, M., Kawano, S., Tsuji, S., Sawaoka, H., Hori, M. and DuBois, R. N. (1998) Cyclooxygenase regulates angiogenesis induced by colon cancer cells. Cell **93**, 705–716
6 Jakobsson, P. J., Thoren, S., Morgenstern, R. and Samuelsson, B. (1999) Identification of human prostaglandin E synthase: a microsomal, glutathione-dependent, inducible enzyme, constituting a potential novel drug target. Proc. Natl. Acad. Sci. U.S.A. **96**, 7220–7225
7 Murakami, M., Naraba, H., Tanioka, T., Semmyo, N., Nakatani, Y., Kojima, F., Ikeda, T., Fueki, M., Ueno, A., Oh-ishi, S. and Kudo, I. (2000) Regulation of prostaglandin E_2 biosynthesis by inducible membrane-associated prostaglandin E2 Synthase that acts in concert with cyclooxygenase-2. J. Biol. Chem. **275**, 32783–32792
8 Tanioka, T., Nakatani, Y., Semmyo, N., Murakami, M. and Kudo, I. (2000) Molecular identification of cytosolic prostaglandin E_2 synthase that is functionally coupled with cyclooxygenase-1 in immediate prostaglandin E_2 biosynthesis. J. Biol. Chem. **275**, 32775–32782
9 Tanikawa, N., Ohmiya, Y., Ohkubo, H., Hashimoto, K., Kangawa, K., Kojima, M., Ito, S. and Watanabe, K. (2002) Identification and characterization of a novel type of membrane-associated prostaglandin E synthase. Biochem. Biophys. Res. Commun. **291**, 884–889
10 Mancini, J. A., Blood, K., Guay, J., Gordon, R., Claveau, D., Chan, C. C. and Riendeau, D. (2001) Cloning, expression, and up-regulation of inducible rat prostaglandin E synthase during lipopolysaccharide-induced pyresis and adjuvant-induced arthritis. J. Biol. Chem. **276**, 4469–4475
11 Stichtenoth, D. O., Thoren, S., Bian, H., Peters-Golden, M., Jakobsson, P. J. and Crofford, L. J. (2001) Microsomal prostaglandin E synthase is regulated by proinflammatory cytokines and glucocorticoids in primary rheumatoid synovial cells. J. Immunol. **167**, 469–474
12 Samuelsson, B., Morgenstern, R. and Jakobsson, P. J. (2007) Membrane prostaglandin E synthase-1: a novel therapeutic target. Pharmacol. Rev. **59**, 207–224
13 Kamei, D., Yamakawa, K., Takegoshi, Y., Mikami-Nakanishi, M., Nakatani, Y., Oh-Ishi, S., Yasui, H., Azuma, Y., Hirasawa, N., Ohuchi, K. et al. (2004) Reduced pain hypersensitivity and inflammation in mice lacking microsomal prostaglandin E synthase-1. J. Biol. Chem. **279**, 33684–33695
14 Kamei, D., Murakami, M., Nakatani, Y., Ishikawa, Y., Ishii, T. and Kudo, I. (2003) Potential role of microsomal prostaglandin E synthase-1 in tumorigenesis. J. Biol. Chem. **278**, 19396–19405
15 Golijanin, D., Tan, J. Y., Kazior, A., Cohen, E. G., Russo, P., Dalbagni, G., Auborn, K. J., Subbaramaiah, K. and Dannenberg, A. J. (2004) Cyclooxygenase-2 and microsomal prostaglandin E synthase-1 are overexpressed in squamous cell carcinoma of the penis. Clin. Cancer Res. **10**, 1024–1031

16 Oshima, H., Oshima, M., Inaba, K. and Taketo, M. M. (2004) Hyperplastic gastric tumors induced by activated macrophages in COX-2/mPGES-1 transgenic mice. EMBO J. **23**, 1669–1678
17 Oshima, M., Oshima, H., Matsunaga, A. and Taketo, M. M. (2005) Hyperplastic gastric tumors with spasmolytic polypeptide-expressing metaplasia caused by tumor necrosis factor-α-dependent inflammation in cyclooxygenase-2/microsomal prostaglandin E synthase-1 transgenic mice. Cancer Res. **65**, 9147–9151
18 Hanahan, D. and Folkman, J. (1996) Patterns and emerging mechanisms of the angiogenic switch during tumorigenesis. Cell **86**, 353–364
19 Majima, M., Hayashi, I., Muramatsu, M., Katada, J., Yamashina, S. and Katori, M. (2000) Cyclo-oxygenase-2 enhances basic fibroblast growth factor-induced angiogenesis through induction of vascular endothelial growth factor in rat sponge implants. Br. J. Pharmacol. **130**, 641–649
20 Williams, C. S., Tsujii, M., Reese, J., Dey, S. K. and DuBois, R. N. (2000) Host cyclooxygenase-2 modulates carcinoma growth. J. Clin. Invest. **105**, 1589–1594
21 Murakami, M., Nakashima, K., Kamei, D., Masuda, S., Ishikawa, Y., Ishii, T., Ohmiya, Y., Watanabe, K. and Kudo, I. (2003) Cellular prostaglandin E_2 production by membrane-bound prostaglandin E synthase-2 via both cyclooxygenases-1 and -2. J. Biol. Chem. **278**, 37937–37947
22 Bombardier, C., Laine, L., Reicin, A., Shapiro, D., Burgos-Vargas, R., Davis, B., Day, R., Ferraz, M. B., Hawkey, C. J., Hochberg, M. C. et al. (2000) Comparison of upper gastrointestinal toxicity of rofecoxib and naproxen in patients with rheumatoid arthritis. N. Engl. J. Med. **343**, 1520–1528
23 Bresalier, R. S., Sandler, R. S., Quan, H., Bolognese, J. A., Oxenius, B., Horgan, K., Lines, C., Riddell, R., Morton, D., Lanas, A. et al. (2005) Cardiovascular events associated with rofecoxib in a colorectal adenoma chemoprevention trial. N. Engl. J. Med. **352**, 1092–1102
24 Narumiya, S., Sugimoto, Y. and Ushikubi, F. (1999) Prostanoid receptors: structures, properties, and functions. Physiol. Rev. **79**, 1193–1226
25 Steeg, P. S. (2006) Tumor metastasis: mechanistic insights and clinical challenges. Nat. Med. Rev. **12**, 895–904
26 Friedl, P. and Wolf, K. (2003) Tumour-cell invasion and migration: diversity and escape mechanisms. Nat. Rev. Cancer **3**, 362–374
27 Young, M. R. and Newby, M. (1986) Enhancement of Lewis lung carcinoma cell migration by prostaglandin E_2 produced by macrophages. Cancer Res. **46**, 160–164
28 Dohadwala, M., Batra, R. K., Luo, J., Lin, Y., Krysan, K., Pold, M., Sharma, S. and Dubinett, S. M. (2002) Autocrine/paracrine prostaglandin E_2 production by non-small cell lung cancer cells regulates matrix metalloproteinase-2 and CD44 in cyclo-oxygenase-2-dependent invasion. J. Biol. Chem. **277**, 50828–50833
29 Ito, H., Duxbury, M., Benoit, E., Clancy, T. E., Zinner, M. J., Ashley, S. W. and Whang, E. E. (2004) Prostaglandin E_2 enhances pancreatic cancer invasiveness through an Ets-1-dependent induction of matrix metalloproteinase-2. Cancer Res. **64**, 7439–7446
30 Pavlovic, S., Du, B., Sakamoto, K., Khan, K. M., Natarajan, C., Breyer, R. M., Dannenberg, A. J. and Falcone, D. J. (2006) Targeting prostaglandin E_2 receptors as an alternative strategy to block cyclooxygenase-2-dependent extracellular matrix-induced matrix metalloproteinase-9 expression by macrophages. J. Biol. Chem. **281**, 3321–3328
31 Sonoshita, M., Takaku, K., Sasaki, N., Sugimoto, Y., Ushikubi, F., Narumiya, S., Oshima, M. and Taketo, M. M. (2001) Acceleration of intestinal polyposis through prostaglandin receptor EP2 in *Apc* (*Delta 716*) knockout mice. Nat. Med. **7**, 1048–1051
32 Castellone, M. D., Teramoto, H., Williams, B. O., Druey, K. M. and Gutkind, J. S. (2005) Prostaglandin E_2 promotes colon cancer cell growth through a Gs-axin-β-catenin signaling axis. Science **310**, 1504–1510
33 Castellone, M. D., Teramoto, H. and Gutkind, J. S. (2006) Cyclooxygenase-2 and colorectal cancer chemoprevention: the β-catenin connection. Cancer Res. **66**, 11085–11088
34 Watanabe, K., Kawamori, T., Nakatsugi, S., Ohta, T., Ohuchida, S., Yamamoto, H., Maruyama, T., Kondo, K., Ushikubi, F., Narumiya, S. et al. (1999) Role of the prostaglandin E receptor subtype EP1 in colon carcinogenesis. Cancer Res. **59**, 5093–5096
35 Sung, Y. M., He, G. and Fischer, S. M. (2005) Lack of expression of the EP2 but not EP3 receptor for prostaglandin E_2 results in suppression of skin tumor development. Cancer Res. **65**, 9304–9311
36 Kawamori, T., Kitamura, T., Watanabe, K., Uchiya, N., Maruyama, T., Narumiya, S., Sugimura, T. and Wakabayashi, K. (2005) Prostaglandin E receptor subtype EP1 deficiency inhibits colon cancer development. Carcinogenesis **26**, 353–357
37 Pai, R., Soreghan, B., Szabo, I. L., Pavelka, M., Baatar, D. and Tarnawski, A. S. (2002) Prostaglandin E_2 transactivates EGF receptor: a novel mechanism for promoting colon cancer growth and gastrointestinal hypertrophy. Nat. Med. **8**, 289–293
38 Han, C., Demetris, A. J., Stolz, D. B., Xu, L., Lim, K. and Wu, T. (2006) Modulation of Stat3 activation by the cytosolic phospholipase $A_2\alpha$ and cyclooxygenase-2-controlled prostaglandin E_2 signaling pathway. J. Biol. Chem. **281**, 24831–24846
39 Sales, K. J., Maudsley, S. and Jabbour, H. N. (2004) Elevated prostaglandin EP2 receptor in endometrial adenocarcinoma cells promotes vascular endothelial growth factor expression via cyclic 3′,5′-adenosine monophosphate-mediated transactivation of the epidermal growth factor receptor and extracellular signal-regulated kinase 1/2 signaling pathways. Mol. Endocrinol. **18**, 1533–1545
40 Ansari, K. M., Sung, Y. M., He, G. and Fischer, S. M. (2007) Prostaglandin receptor EP2 is responsible for cyclooxygenase-2 induction by prostaglandin E_2 in mouse skin. Carcinogenesis **28**, 2063–2068
41 Nakanishi, M., Montrose, D. C., Clark, P., Nambiar, P. R., Belinsky, G. S., Claffey, K. P., Xu, D. and Rosenberg, D. W. (2008) Genetic deletion of mPGES-1 suppresses intestinal tumorigenesis. Cancer Res. **68**, 3251–3259
42 Elander, N., Ungerbäck, J., Olsson, H., Uematsu, S., Akira, S. and Söderkvist, P. (2008) Genetic deletion of mPGES-1 accelerates intestinal tumorigenesis in $APC^{Min/+}$ mice. Biochem. Biophys. Res. Commun. **372**, 249–253
43 Amano, H., Hayashi, I., Endo, H., Kitasato, H., Yamashina, S., Maruyama, T., Kobayashi, M., Satoh, K., Narita, M., Sugimoto, Y. et al. (2003) Host prostaglandin E_2-EP3 signaling regulates tumor-associated angiogenesis and tumor growth. J. Exp. Med. **197**, 221–232
44 Itoh, T., Tanioka, M., Yoshida, H., Yoshioka, T., Nishimoto, H. and Itohara, S. (1998) Reduced angiogenesis and tumor progression in gelatinase A-deficient mice. Cancer Res. **58**, 1048–1051

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online data

SUPPLEMENTARY ONLINE DATA

Microsomal prostaglandin E synthase-1 in both cancer cells and hosts contributes to tumour growth, invasion and metastasis

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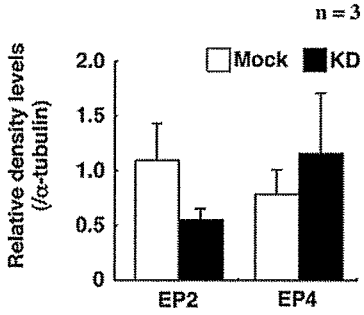

Figure S1 Reduced expression level of EP2 protein in mPGES-1-silenced LLC cells *in vitro*

Expression of EP2 and EP4 in mPGES-1-KD and control (mock) cells was assessed by immunoblotting. Representative results of at least three experiments are shown. On Western blot analysis the expression levels of EP2 and EP4 protein were quantified by a densitometer, with the expression level of α-tubulin used for normalization (means $\pm$ S.D., $n=3$).

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[2] Professor Kudo died on April 27, 2008. We greatly miss him as a scientist and a friend. We offer sincere thanks to all the friends, colleagues and former collaborators of Professor Kudo who showed him kindness during his lifetime.

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Review

Prostaglandin E synthases: Understanding their pathophysiological roles through mouse genetic models☆

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ABSTRACT

Prostaglandin E synthase (PGES), which converts cyclooxygenase (COX)-derived prostaglandin H_2 (PGH_2) to PGE_2, is known to comprise a group of at least three structurally and biologically distinct enzymes. Two of them are membrane-bound and have been designated as mPGES-1 and mPGES-2. mPGES-1 is a perinuclear protein that is markedly induced by proinflammatory stimuli and downregulated by anti-inflammatory glucocorticoids as in the case of COX-2. It is functionally coupled with COX-2 in marked preference to COX-1. mPGES-2 is synthesized as a Golgi membrane-associated protein, and the proteolytic removal of the N-terminal hydrophobic domain leads to the formation of a mature cytosolic enzyme. This enzyme is rather constitutively expressed in various cells and tissues and is functionally coupled with both COX-1 and COX-2. Cytosolic PGES (cPGES) is constitutively expressed in a wide variety of cells and is functionally linked to COX-1 to promote immediate PGE_2 production. Recently, mice have been engineered with specific deletions in each of these three PGES enzymes. In this review, we summarize the current understanding of the *in vivo* roles of PGES enzymes by knockout mouse studies and provide an overview of their biochemical properties.

1. Introduction

Prostaglandin E_2 (PGE_2) is the most common prostanoid produced by a variety of cells and tissues and has a broad range of biological activity. Three kinds of enzymes — phospholipase A_2 (PLA_2), cyclooxygenase (COX), and terminal PGE synthase (PGES) — are involved in the biosynthesis of PGE_2, and each of the three enzymatic steps involves multiple enzymes that can act in different phases of cell activation [1–5]. Arachidonic acid (AA) released from membrane glycerophospholipids by PLA_2 enzymes is then supplied to either of the two COX isozymes, COX-1 or COX-2. The constitutive COX-1 contributes mainly to immediate PG generation, whereas the inducible COX-2 mediates delayed PG generation (Fig. 1). The COX metabolite PGH_2 is then isomerized to PGE_2 by terminal PGES enzymes.

To date, three PGES enzymes, microsomal PGES-1 (mPGES-1) and -2 (mPGES-2) and cytosolic PGES (cPGES), have been identified [6–9]. Recently, mice with specific deletions in each of these three PGES enzymes have been engineered; these knockout mice have provided models to elucidate the physiological and pathophysiological roles of these enzymes [10–15]. This review summarizes the current understanding of the *in vivo* roles of PGES enzymes as revealed by experiments using knockout mice including a discussion of their biological structures and transcriptional regulation (Table 1).

2. mPGES-1

2.1. Biochemical properties of mPGES-1

In 1999, mPGES-1 was the first PGES identified. Jakobsson et al. reported that the recombinant human microsomal glutathione (GSH) *S*-transferase (GST) 1-like 1, a member of the MAPEG (membrane-associated proteins involved in eicosanoid and glutathione metabolism) superfamily that had been listed in nucleic acid data bases, can catalyze the conversion of PGH_2 to PGE_2 with strict substrate specificity [6]. We also cloned rat and mouse orthologs of this protein and showed that this enzyme is identical to

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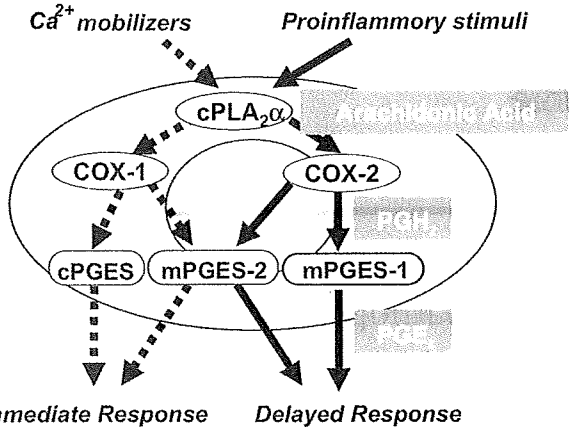

Fig. 1. Functional coupling of COX and PGES. Arachidonic acid released from membrane glycerophospholipids by cPLA$_2\alpha$ is then supplied to either of the two COX isozymes, COX-1 or COX-2. COX-1, a constitutive COX isozyme, contributes mainly to immediate PG generation in response to the Ca^{2+} mobilizer, whereas COX-2, an inducible enzyme, mediates delayed PG generation in response to proinflammatory stimuli. The COX metabolite PGH_2 is then isomerized to PGE_2 by three terminal PGES, mPGES-1, mPGES-2 and cPGES. mPGES-1 is functionally coupled with COX-2 in marked preference to COX-1, whereas cPGES is coupled preferentially with COX-1. mPGES-2 functions equally well with both COX-1 and COX-2.

a membrane-associated PGES, which we had originally detected in lipopolysaccharide (LPS)-stimulated macrophages [8].

mPGES-1 consists of 152–153 amino acids and shows significant homology with other MAPEG superfamily proteins, including MGST-1, MGST-2, MGST-3, 5-lipoxygenase-activating protein (FLAP), and leukotriene C_4 synthase (LTCS), with the highest homology being found with MGST-1 (38%). Furthermore, a projected structure of mPGES-1 at 10 Å revealed similar structural properties as had been determined for MGST-1, suggesting that the enzyme is a trimer of four helix bundles in which the hydrophobic helices traverse the membrane [16]. It was also shown that mutation of Arg^{110} in mPGES-1, which is the residue strictly conserved in all MAPEG protein, abrogates its catalytic function, implying this residue has an essential role [8].

mPGES-1 requires GSH as an essential cofactor for activity [6,8]. Purified recombinant mPGES-1 catalyzes rapid GSH-dependent conversion of PGH_2 to PGE_2 with a V_{max} of 170 μmol/min/mg and a high k_{cat}/K_m of 310 min^{-1} s^{-1} [16]. The enzyme also catalyzes GSH-dependent conversion of PGG_2 to 15-hydroperoxy-PGE_2. It displays weak GSH-dependent peroxidase activity toward cumene hydroperoxide, 5-hydroperoxyeicosatetraenoic acid, and 15-hydroperoxy-PGE_2 and catalyzes slow but significant conjugation of 1-chloro-2,4-dinitrobenzene to GSH.

2.2. Expression, transcriptional regulation, and cellular function of mPGES-1

Among PGES enzymes, only mPGES-1 is markedly induced by proinflammatory stimuli. Although the steady-state expression of mPGES-1 in normal rat tissues is very low, administration of LPS leads to a dramatic increase in mPGES-1 expression in various tissues [8,17,18]. Stimulation of various cultured cells with proinflammatory stimuli leads to a marked elevation of mPGES-1 expression, often with concomitant induction of COX-2. Concordant upregulation of COX-2 and mPGES-1 is accompanied by a marked increase in PGE_2 production [8,17,19–21]. It has also been shown that the coordinate upregulation of COX-2 and mPGES-1 and the attendant PGE_2 generation are downregulated by anti-inflammatory glucocorticoid [8,17,19,21]. However, there are some differences in the kinetics of induction between COX-2 and mPGES-1 [21], suggesting that the regulatory mechanisms for their expression are distinct. The promoter of the human mPGES-1 gene is GC-rich but lacks a TATA box, unlike the COX-2 gene promoter [22]. Furthermore, the 3′ region of the mPGES-1 gene lacks the AUUUA mRNA instability sequences that are found in the COX-2 gene. Our gene promoter analysis revealed that stimulus-inducible mPGES-1 expression required tandem GC boxes adjacent to the initiation site and that the inducible transcription factor Egr-1 is activated and bound to this GC box, leading to inducible mPGES-1 transcription.

It is also noteworthy that mPGES-1 is functionally coupled with COX-2, in marked preference to COX-1. When COX-2 and mPGES-1 are co-transfected into HEK293 cells, considerable amounts of PGE_2 are produced from both endogenous and exogenous AA relative to cells transfected with either enzyme alone [8]. In comparison, coupling between COX-1 and mPGES-1 occurs only when a large amount of AA is supplied exogenously, or if burst activation of cytosolic PLA$_2\alpha$ takes place, endogenously. Our co-transfection experiments revealed that mPGES-1 colocalized with both COX-1 and COX-2 in the perinuclear membrane [8]. The mechanism whereby mPGES-1 favors COX-2 over COX-1 cannot be explained simply by their subcellular localizations, but the presence of microdomains, in which mPGES-1 is located in closer proximity to COX-2 than to COX-1, within the perinuclear compartment cannot be ruled out. Colocalization of COX-2 and mPGES-1 in such a microdomain may allow efficient transfer of the unstable substrate PGH_2 between them. Further support for the COX-2/mPGES-1 coupling has been provided by studies using an antisense oligonucleotide or a small interfering RNA, in which the mPGES-1 knockdown markedly attenuates COX-2-mediated PGE_2 production in cultured cells [23,24].

2.3. Possible in vivo functions of mPGES-1

The first report of the critical role of mPGES-1 for induced PGE_2 production *in vivo* was published in 2002 [10]. Those authors generated mPGES-1-deficient mice and demonstrated that the biosynthesis of PGE_2 in wild-type peritoneal macrophages induced by LPS was abolished in mPGES-1-deficient cells. It was also shown that the increase in serum PGE_2 concentration by intraperitoneal administration of LPS was also suppressed in mPGES-1-deficient mice, although mPGES-1-deficient mice showed normal responses to the LPS-induced shock. Following this report, several investigators

Table 1
Characteristics of three PGES enzymes.

	mPGES-1	cPGES	mPGES-2
Gene map locus (human)	9q34.4	12q13.13	9q33–q34
Transcriptional regulation	Inducible	Constitutive	Constitutive
Protein molecular weight	16 kDa	23 kDa	33 kDa
Amino acid composition	152–153 aa	160 aa	377–384 aa
Subcellular localization	Perinuclear membrane	Cytoplasm	Golgi, cytoplasm
Preference for COX isozymes	COX-2 > COX-1	COX-1 > COX-2	No preference
Phenotypic changes in KO mice	Reduced inflammatory reactions, Impaired pain and febrile response, Suppression of tumorigenesis, etc.	Perinatal-lethal with poor lung development, delayed skin maturation, and growth retardation	No specific phenotype

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established additional mPGES-1-deficient mouse lines in order to unveil possible *in vivo* functions of mPGES-1.

2.3.1. Role of mPGES-1 in inflammatory reactions

The induction both *in vitro* and *in vivo* of mPGES-1 following proinflammatory stimuli, such as interleukin-1 (IL-1), tumor necrosis factor (TNF), and LPS, strongly suggests that mPGES-1 is an essential component of PGE_2 production during inflammatory reactions [6,8,17–21]. It has also been shown that mPGES-1 is abundantly expressed in synovial cells of patients with rheumatoid arthritis [23] and in the hind paws of rats with adjuvant arthritis [17]. We and others have shown that mPGES-1 plays an important role in synovial inflammation using mPGES-1-deficient mice. Trebino et al. induced arthritis by subcutaneous administration of chicken collagen type II and assessed symptoms of arthritis by clinical evaluation and histopathology [11]. They showed that in this collagen-induced arthritis (CIA) model, mPGES-1-deficient mice developed no or little arthritis. Similar CIA phenotypes have been observed in mice lacking $cPLA_2\alpha$ [24], COX-2 [25] or the PGE receptor EP4 [26], thus revealing the metabolic flow of the $cPLA_2\alpha$/COX-2/mPGES-1/EP4 pathway, leading to the development of inflammatory arthritis. It is possible that the results found in the CIA model are influenced in part by inadequate proximal lymphocyte-mediated responses in addition to the synovial symptoms, since the anti-collagen antibody formation in COX-2-deficient mice revealed a remarkable decrease in this humoral response. This view was further supported by the fact that COX-2-deficient mice exhibited altered helper T-cell development, a process that PGE_2 reversed [27]. Subsequently, it was indeed found that defective generation of a humoral immune response was associated with a reduced incidence and severity of the CIA model in mPGES-1-null mice [28]. Using the collagen antibody-induced arthritis (CAIA) model, in which the influence of lymphocyte-mediated humoral responses could be minimal, we also showed that the severity of synovial inflammation, including bone destruction and juxta-articular bone loss, were mild in mPGES-1-deficient mice as compared with replicate wild-type mice, although the incidence of inflammation was unaffected (Fig. 2A) [29]. mPGES-1-mediated PGE_2 production by osteoblasts plays a critical role in LPS-induced bone loss associated with inflammation [30,31]. Moreover, mPGES-1 deficiency was associated with impaired fracture healing, but not with bone loss or osteoarthritis, in mouse models of skeletal disorders [32].

Antigen-induced paw edema was markedly reduced in mPGES-1-deficient mice as compared with replicate wild-type mice [11]. This deficit in edema was accompanied by a marked reduction in the infiltration of white blood cells into the inflamed site. Likewise, the migration of macrophages following peritoneal injection of thioglycollate was strikingly reduced in mPGES-1-null mice relative to replicate wild-type mice [29]. We recently found that exudate accumulation and leukocyte migration into the pleural cavity after intrapleural injection of carrageenan was also attenuated in mPGES-1-deficient mice (Fig. 2B). These observations indicate that mPGES-1-derived PGE_2 increases vascular permeability in acute inflammatory reactions.

The formation of inflammatory granulation tissue and attendant angiogenesis in the dorsum induced by subcutaneous implantation of a cotton thread was significantly reduced in mPGES-1 knockout mice as compared with wild-type mice [29]. In this model, mPGES-1 deficiency was also associated with reduced induction of vascular endothelial cell growth factor (VEGF) in the granulation tissue. These results indicated that mPGES-1-derived PGE_2, in cooperation with VEGF, may play a critical role in the development of inflammatory granulation and angiogenesis, thus eventually contributing to tissue remodeling.

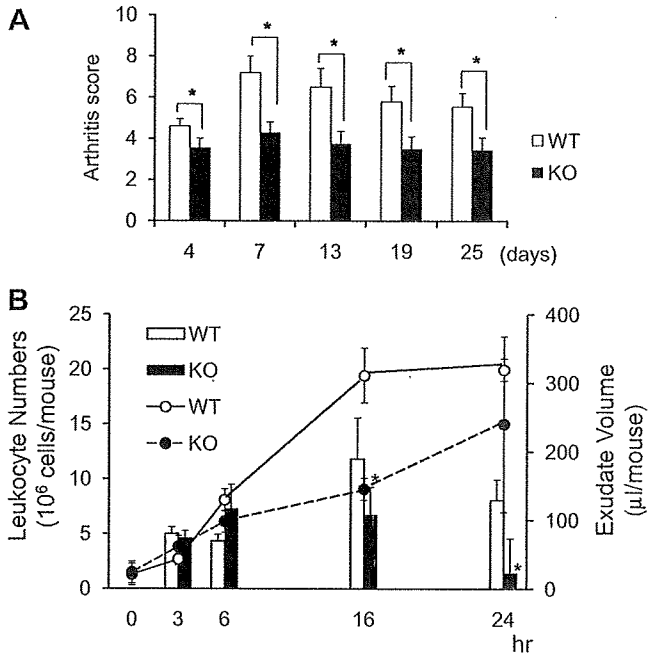

Fig. 2. Impaired inflammatory reactions in mPGES-1 knockout mice. A. mPGES-1 knockout and wild-type mice were injected intraperitoneally with 10 mg of anti-collagen monoclonal antibodies on day 0 and boosted with 50 μg of LPS on days 2 and 7, followed by intermittent LPS injections every 3 days. The clinical arthritis score was graded on a 0–3 scale as follows: 0, normal; 1, swelling of ankle or wrist, or limited to digits; 2, swelling of the entire paw; 3, maximal swelling. Each limb was graded, allowing a maximum arthritis score of 12 for each animal. Data were analyzed by Student's *t*-test. The results are expressed as means ± standard error ($n = 3$; *, $p < 0.05$ versus wild-type mice). For details, see Ref. [29]. B. mPGES-1 knockout and wild-type mice had been injected intrapleurally with 0.1 ml of 1% carrageenan saline solution. Immediately after the animals were killed by exsanguination at specified time points after the carrageenan injection, pleural exudates and saline wash (0.5 ml) were collected, and the exudate volume (bars) and leukocyte number in the exudates (lines) were measured. Data were analyzed by Student's *t*-test. The results are expressed as means ± standard error ($n = 3$–9; *, $p < 0.05$ versus wild-type mice).

2.3.2. Role of mPGES-1 in pain

PGE_2 is known to mediate inflammatory pain. The inflammatory pain response has been assessed using an acetic acid writhing model, in which diluted acetic acid was injected into the peritoneal cavity of mice. The pain response as recorded by counting the number of writhing motions was significantly reduced in mPGES-1-deficient mice compared with wild-type mice [11,29]. This phenotype was particularly evident when these mice were primed with LPS, where the stretching behavior and the peritoneal PGE_2 level were significantly reduced in knockout mice relative to wild-type mice [29]; this is being consistent with elevated expression of COX-2 and mPGES-1 in response to LPS. The basal (i.e., LPS-non-primed) writhing response was also partially reduced in mPGES-1-null mice [11,29], where COX-1-mPGES-1 coupling takes place.

The effect of mPGES-1 deficiency on neuropathic pain was also investigated in a neuropathic pain model prepared by spinal nerve transection [33]. The left L5 spinal nerve was transected, and after recovery, the mice were subjected thermal and mechanical sensitivity testing. The results showed that, unlike wild-type mice, mPGES-1-null mice did not exhibit mechanical allodynia or thermal hyperalgesia.

2.3.3. Role of mPGES-1 in fever

Genetic inactivation of COX-2, but not of COX-1, reduced PGE_2 levels in the central nervous system (CNS), in association with impaired LPS-induced febrile response [34]. Likewise, mPGES-1

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knockout mice showed no fever and no central PGE_2 synthesis after peripheral injection of LPS [35]. It was also shown that injection of PGE_2 into the brain ventricles elicited a fever response in mPGES-1 knockout mice. In rats, LPS treatment led to an increase in mPGES-1 expression in blood vessels, especially in veins and venules, in the whole brain [18]. Thus, cerebral vascular endothelial cells express components enabling blood-borne cytokines to stimulate the synthesis of PGE_2, whose small size and lipophilic property allow it to pass across the blood–brain barrier and to diffuse into the CNS neurons, thereby evoking the febrile response. Furthermore, Saha et al. reported that both induction of febrile responses by subcutaneous injection of turpentine (a model for an aseptic cytokine-induced pyresis) and intraperitoneal injection of IL-1β were also strongly impaired in mPGES-1-deficient mice [36]. In contrast, wild-type and mPGES-1-deficient mice showed similar psychogenic stress-induced hyperthermia, diurnal temperature variations, and reduced motor activity following injection of turpentine. These results indicate that mPGES-1-derived PGE_2 plays a critical role in fever during infectious and inflammatory conditions but is functionally dissociated from circadian temperature regulation, stress-induced hyperthermia, and inflammation-induced activity depression.

2.3.4. Role of mPGES-1 in neurological disorders

The role of PGE_2 in brain diseases, including ischemic injury and several neurodegenerative diseases has been established in models using mice lacking individual PGE receptors [37]. Ikeda-Matsuo et al. demonstrated that mPGES-1 was induced in neurons, microglia, and endothelial cells in the cerebral cortex after transient focal ischemia and indicated that mPGES-1-derived PGE_2 may be a critical determinant of postischemic neurological dysfunctions [38]. In mPGES-1 knockout mice, in which postischemic PGE_2 production in the cortex was completely absent, infarction, edema, apoptotic cell death, and caspase-3 activation in the cortex after ischemia were all reduced compared with those in wild-type mice. Furthermore, the behavioral neurological dysfunctions observed after ischemia in wild-type mice were significantly ameliorated in mPGES-1 knockout mice.

Induction of mPGES-1 was observed in various areas and various kinds of cells in the brain. Treatment of rat astrocytes with β-amyloid also induced mPGES-1 expression [39]. In clinical views, treatment with COX inhibitors slowed progression of Alzheimer's disease, and both COX-2 expression and PGE_2 levels were elevated in brains from Alzheimer's disease patients [40,41]. A recent immunohistochemical analysis revealed that mPGES-1 expression was also elevated in the brains of patients with Alzheimer's disease [42]. In this context, mPGES-1 might participate in the progression of Alzheimer's disease.

2.3.5. Role of mPGES-1 in tumorigenesis

COX-2 plays a critical role in the development of colorectal cancer and likely other types of cancer [43]. Pharmacological or genetic inactivation of COX-2 led to the suppression of cell growth and survival as well as to reductions in tumor size, invasion, and metastasis [44]. Disruption of $cPLA_2\alpha$ [45] or the PGE receptor EP2 [46] also resulted in a reduced incidence of gastrointestinal polyps, providing strong evidence for a link between PGE_2 signaling and oncogenesis. The tumorigenic potential of mPGES-1 has been suggested by several observations: that transfection of mPGES-1 in combination with COX-2, but not with COX-1, into HEK293 cells led to cellular transformation with a concomitant increase in PGE_2 [8]; that the COX-2/mPGES-1-co-transfected cells formed a number of large colonies in soft agar culture and were tumorigenic when implanted into nude mice [47]; and that transgenic mice overexpressing both COX-2 and mPGES-1 developed metaplasia, hyperplasia, and tumorous growth in the glandular stomach with heavy macrophage infiltration [48]. We recently showed that PGE_2 synthesis, cell proliferation and invasive activity *in vitro* and xenograft formation *in vivo* were reduced by mPGES-1 knockdown and conversely enhanced by mPGES-1 overexpression in Lewis lung carcinoma (LLC) cells [49].

Furthermore, we recently found that, like cancer cell-associated mPGES-1, host-associated mPGES-1 contributes to tumor growth, invasion, and metastasis using mPGES-1-deficient mice [49]. LLC tumors grafted subcutaneously into mPGES-1 knockout mice grew more slowly than did those grafted into wild-type mice, with concomitant decreases in the density of microvascular networks, the expression of proangiogenic VEGF, and the activity of matrix metalloproteinase-2. Lung metastasis of intravenously injected LLC cells was also significantly less obvious in mPGES-1 knockout mice than in wild-type mice. mPGES-1-driven PGE_2 signaling on host stromal cells may be functionally linked to the induction of potent proangiogenic and matrix-degrading factors, which in turn would facilitate tumor development.

The effect of mPGES-1 deficiency on intestinal tumorigenesis was also reported. Nakanishi et al. showed that the genetic deletion of mPGES-1 ameliorated the development of intestinal tumors in both $Apc^{\Delta 14}$-dependent and carcinogen-induced models [50]. In contrast, Elander et al. demonstrated that intestinal polyposis was exacerbated in mPGES-1-null Apc^{Min} mutant mice, probably because of the metabolic shift from PGE_2 toward other pro-tumorigenic lipid mediators [51]. Although the reason for the discrepancy between these two studies is unclear, our recent results appear to be in line with those of the former study.

2.3.6. Role of mPGES-1 in cardiovascular diseases

The selective inhibition, knockout, or mutation of COX-2, or the deletion of the receptor for COX-2-derived PGI_2, accelerates thrombogenesis and elevates blood pressure in mice, whereas these responses are attenuated by COX-1 knockdown, which mimics the beneficial effects of low-dose aspirin. The deletion of mPGES-1 in mice reduced PGE_2 and increased PGI_2 in circulation, had no effect on thromboxane biosynthesis, and affected neither thrombogenesis nor blood pressure [52]. Furthermore, the deletion of mPGES-1 led to eccentric cardiac myocyte hypertrophy, left ventricular dilation, and impaired left ventricular contractile function after acute myocardial infarction [53]. In mice with a low-density lipoprotein receptor knockout background, the deletion of mPGES-1 also augmented PGI_2 and retarded atherosclerosis development without an attendant impact on blood pressure [54], and protected against abdominal aortic aneurysm formation induced by angiotensin II in hyperlipidemic mice, coincident with a reduction in oxidative stress [55]. These results suggest that inhibitors of mPGES-1 may retain their anti-inflammatory efficacy by depressing PGE_2, while avoiding the adverse cardiovascular consequences associated with COX-2-mediated PGI_2 suppression.

2.3.7. Role of mPGES-1 in renal homeostasis

PGE_2 influences a wide range of physiologic functions in the kidney, and mPGES-1 colocalizes with both COX-1 and COX-2 in distal convoluted tubules, medullary interstitial cells, and cortical and medullary collecting ducts [56]. Although there was a 50% decrease in basal urinary excretion of PGE_2 in mPGES-1-null mice [57], these mice were similar to wild-type mice in their urinary osmolalities at baseline and after water deprivation, as well as in their blood pressure on control, high-salt, or low-salt diets [58]. In contrast, another study showed that mPGES-1-deficient mice developed severe and progressive hypertension associated with an inappropriate increase in sodium balance when fed a high-salt diet, with a marked reduction of urinary PGE_2 [59]. These mice exhibited a significantly impaired ability to excrete an acute enteral load of NaCl as well as a remarkable inhibition of high salt-induced increase in the gene expression of all three NO synthase isoforms. Chronic salt

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loading remarkably induced mPGES-1 protein expression exclusively in the distal nephron. In addition, mPGES-1 deficiency sensitized the hypertensive effect of angiotensin II. Overall, this study characterized the natriuretic and antihypertensive role of the mPGES-1/PGE_2/NO axis that likely contributes to blood pressure homeostasis.

It was also shown that mPGES-1 deletion impairs diuretic response to acute water loading [60]. Compared with wild-type mice, mPGES-1-null mice exhibited an impaired ability to excrete an acute water load, but not a chronic water load. In response to acute water loading, the renal aquaporin-2 protein level in wild-type mice was significantly reduced, but this reduction was blunted in mPGES-1-null mice. That study of mPGES-1-null mice also revealed a delayed increase in urinary PGE_2 excretion by acute water loading, coinciding with the stimulation of renal medullary expression of cPGES but not of mPGES-2. These results suggested that cPGES may act in concert with mPGES-1 to sustain renal PGE_2 production, particularly in the face of water excess.

2.3.8. Role of mPGES-1 in gastrointestinal homeostasis

PGE_2 is crucial for maintaining gastrointestinal mucosal homeostasis. Inflammatory bowel disease, including Crohn's disease and ulcerative colitis, is characterized by chronic, relapsing inflammation of the gastrointestinal tract. Increased amounts of PGE_2 were detected at sites of intestinal inflammation and correlated with disease activity [61], but COX inhibitors triggered or worsened the disease [62]. Experimental models for inflammatory bowel disease indicate that PGE_2 appears to have a dual effect. High levels of PGE_2 exacerbate the inflammatory process [63]. On the other hand, PGE_2 signaling is required for suppressing colitis symptoms and protecting mucosal damage. Mice engineered to be deficient in COX-2 or the PGE_2 receptor EP4 were sensitized to the development of experimental colitis, whereas treatment of EP4-selective agonist ameliorated colitis in wild-type mice [64,65]. mPGES-1 expression was also increased in inflamed intestinal mucosa from patients with inflammatory bowel disease [66]. To reveal whether or not mPGES-1 exhibits a beneficial role in mucosal protection, we examined the roles and functions of mPGES-1 in ulcerative colitis using a dextran sodium sulfate (DSS)-induced colitis model in mice (Fig. 3). Colitis was induced in wild-type or mPGES-1-deficient mice by continuous oral administration of 5% DSS in drinking water. DSS-induced expression of mPGES-1 was found throughout the large intestine in wild-type mice. The hemoccult scores of DSS-treated mPGES-1-deficient mice were significantly higher than those of wild-type mice, and mPGES-1-deficient mice exhibited significantly more severe anemia and splenomegaly than wild-type mice. The decrease in PGE_2 levels in descending colon and increased expression levels of proinflammatory cytokines were also found in mPGES-1-deficient mice. Furthermore, mPGES-1 deficiency induced severe epithelial loss and crypt abscess in the colon. These results indicate that mPGES-1-derived PGE_2 contributes to mucosal defense in colitis and negatively modulates acute colonic injury.

It was also shown that basal COX-1-dependent PGE_2 production in the stomachs of mPGES-1-deficient mice was decreased by 80–90% relative to that in wild-type mice [57], suggesting that COX-1/mPGES-1-derived PGE_2 might also contribute to the homeostatic protection of gastric mucosa.

2.3.9. Role of mPGES-1 in reproduction: a remaining mystery

Ovulation and fertilization are key steps in female reproduction, which, in turn, is regulated by several hormones, including gonadotropins. PGE_2, a dominant prostanoid in the ovary, has been implicated as a mediator of the ovulatory actions of gonadotropins. Gonadotropins induced COX-2 expression in granulosa cells of ovarian follicles prior to ovulation *in vivo* [67,68]. COX-2-deficient mice have a reduced ability to ovulate and severe problems in fertilization as well as defects in the uterus's ability to receive implants and to undergo decidualization [69]. Furthermore, studies using PGE receptor EP2-deficient mice revealed that PGE_2 cooperates with gonadotropins to complete cumulus expansion for successful fertilization [70,71]. Like COX-2, mPGES-1 was markedly upregulated in the granulose cell layer of bovine follicles after treatment with gonadotropins in the hours just prior to ovulation, a period when EP2 may be critically involved [72]. It was also reported that mPGES-1 is highly expressed in the female reproductive organs of various animal species, including humans [73–75]. However, mPGES-1-null mice showed no apparent reproduction abnormality [10,11], even under the inflammatory preterm delivery model in which mPGES-1 expression was markedly increased in the myometrium and fetal membrane [76].

Also, although both COX-2 and mPGES-1 were increased at the ductus arteriosus in perinatal mice [77] and patent ductus arteriosus and associated neonatal death occurred in EP4-deficient mice [78], this phenotype did not occur in mPGES-1-null mice probably due to compensation by mPGES-2 [79]. Thus, even though the contributions of mPGES-1 to these reproductive events are currently unclear, an mPGES-1 inhibitor may stand out as a better prospective tool than the currently used COX inhibitors for the management of pregnant women as well as premature infants with persistent ductus.

3. cPGES

3.1. Biochemical properties of cPGES

In 2000, we purified cPGES as a cytosolic form of PGES from LPS-treated rat brain and sequence analysis of the 23 kDa purified protein revealed that it is identical to the heat shock protein 90 (Hsp90)-associated protein p23 [7], which had been originally implicated as a cofactor for the molecular chaperone function of Hsp90 [80,81].

Like mPGES-1, cPGES requires GSH as an essential cofactor for its activity [7]. As with the classical cytosolic GSTs, cPGES activity is inhibited by 1-chloro-2,4-dinitrobenzene, even though 1-chloro-2,4-dinitrobenzene itself is a poor substrate for cPGES. Although the homology between cPGES and other known cytosolic GSTs is low, near its N-terminus cPGES has a tyrosine residue (Tyr^9) that is conserved in several other cytosolic GSTs. Tyr^9 mutation abrogates cPGES activity, suggesting that this tyrosine serves as a GSH acceptor, as it does in other cytosolic GSTs [82].

cPGES is directly associated with and phosphorylated by protein kinase CK2, resulting in a marked reduction of K_m for the substrate PGH_2 ($K_m = 66.6$ μM for cPGES alone compared to $K_m = 35.7$ μM for cPGES plus CK2) [83]. In activated cells, CK2-directed phosphorylation of cPGES occurs in parallel with increased cPGES enzymatic activity and PGE_2 production, and these processes are facilitated by interaction with Hsp90. cPGES, CK2, and Hsp90 form a stoichiometric complex of 1:1:1 immediately after cell activation. In this context, Hsp90 may act as an essential scaffold protein that brings cPGES and CK2 in proximity, thereby spatially allowing their efficient functional interaction under physiological conditions. *In vitro* coincubation of Hsp90, CK2 and cPGES results in maximal cPGES activity ($K_m = 14.9$ μM). On the other hand, pharmacologic inhibition of CK2 or Hsp90 or mutation of two CK2-directed phosphorylation sites (Ser^{113} and Ser^{118}) on cPGES results in poor activation of cPGES [83,84]. These results indicate that the tertiary complex formation and attendant phosphorylation are essential for cPGES to act in cells.

3.2. Expression and cellular function of cPGES

cPGES is expressed ubiquitously and in abundance in the cytosol of various tissues and cells [7]. Although the expression of cPGES is constitutive and is unaffected by proinflammatory stimuli in most

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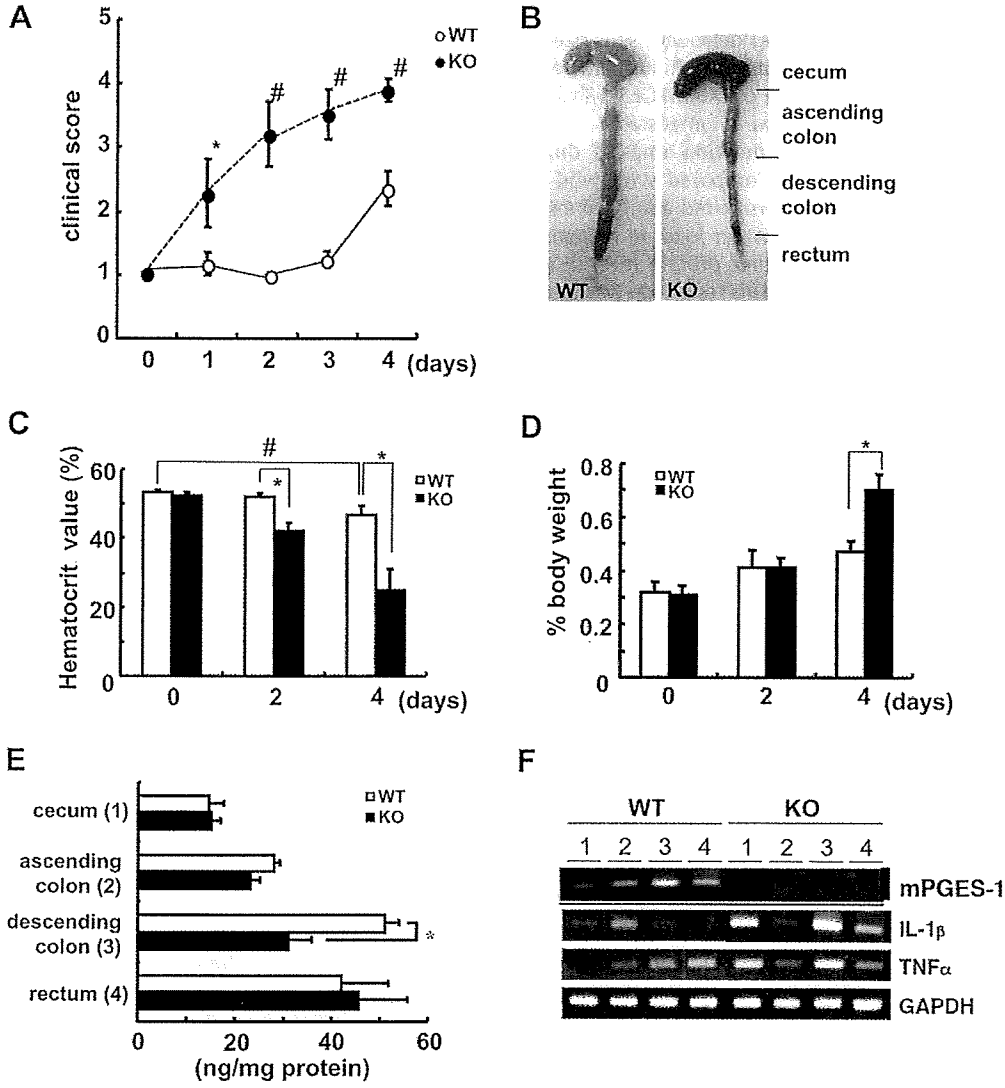

Fig. 3. Exacerbation of DSS-induced colitis in mPGES-1 knockout mice. DSS of the average molecular weight of 5000, at 5% concentration in the drinking water, was administered orally into mPGES-1 wild-type or knockout mice for 4 days. A. The fecal blood score was monitored daily. Fecal blood was scored as follows: 1, normal; 2, trace positive; 3, positive; 4, strong positive; 5, gross bleeding. B. The macroscopic examination of the large intestines on day 4 is shown. Hemorrhagic redness throughout the cecum to the rectum in the large intestine of mPGES-1 knockout mice. C and D. On days 0, 2, and 4, hematocrit values and spleen weights were measured. The weights of spleen are shown as % body weight. E and F. On day 4, the large intestines were divided into four regions. PGE_2 levels and mPGES-1, IL-1β, TNFα mRNA levels in each region (cecum (lane 1), ascending colon (lane 2), descending colon (lane 3), and rectum (lane 4)) were analyzed by EIA and RT-PCR, respectively. Data were analyzed by Student's *t*-test. The results are expressed as means ± standard error ($n = 8–12$; *, $p < 0.05$ versus wild-type mice).

cases, some exceptions have been reported. Treatment of rats with LPS resulted in elevated expression of cPGES in the brain [7]. Administration of IL-1 into the mouse cortex via intraparenchymal microinjection led to an increase in PGE_2, which was accompanied by elevated expression of cPGES as well as of COX-2 and mPGES-1 with different kinetics [85]. In pregnant female mice, cPGES was strongly detected in the stroma underlying the luminal epithelium surrounding the implanting blastocyst at the implantation site and in decidualized cells under artificial decidualization, whereas only a basal level of cPGES was observed in the control horn [86]. The mouse cPGES gene promoter is GC-rich and contains many Sp1 sites, but lacks an obvious TATA box motif [87]. At present, the transcriptional regulation of cPGES is still unknown.

Co-transfection and antisense experiments indicated that cPGES is capable of converting COX-1-, but not COX-2-derived PGH_2 to PGE_2 in cells, particularly during the immediate PGE_2-biosynthetic response elicited by Ca^{2+}-evoked stimuli [7]. Localization of cPGES in the cytosol may allow coupling with proximal COX-1 in the ER in preference to distal COX-2 in the perinuclear envelope, although other regulatory mechanisms could be involved.

3.3. Possible in vivo functions of cPGES

Functional coupling of cPGES with COX-1 suggests that the functions of cPGES *in vivo* overlap significantly, if not entirely, with COX-1. COX-1-derived PGE_2 may be involved in gastrointestinal protection, reproduction, and some neuronal functions, leading to acute pain in the periphery and affecting the central nervous system, which in turn affects daily activities. To elucidate the *in vivo* function of cPGES, cPGES-deficient mice were developed [12–14], but cPGES deletion studies have not been particularly informative in addressing the roles of cPGES-derived PGE_2, because cPGES-deficient mice were perinatal-lethal with poor lung development, delayed skin maturation, and growth retardation (Fig. 4). The lung phenotype was consistent with a defective glucocorticoid response and with p23 acting as a co-chaperone for the glucocorticoid

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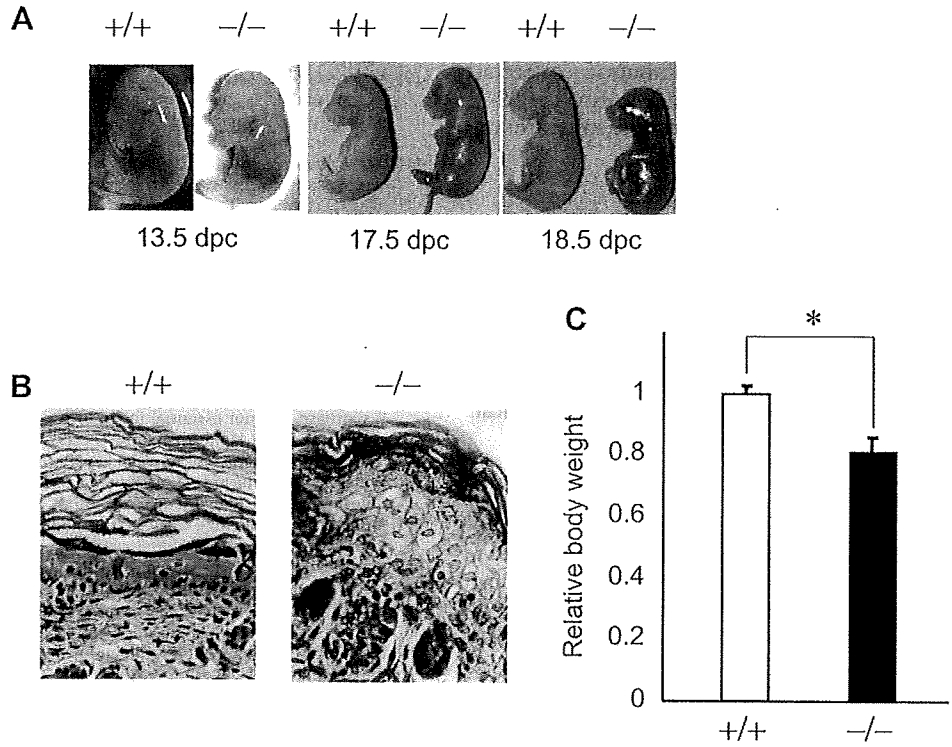

Fig. 4. Phenotypes observed in cPGES/p23 knockout mice. A. Side view of embryos from the same littermates. After 17.5 dpc, cPGES/p23 knockout embryos began to exhibit glossy and sticky skin. B. HE staining of skin sections of wild-type (+/+) and cPGES/p23 knockout (−/−) neonates. Original magnification: 400×. Compared with wild-type mice, the epidermis of knockout mice displayed remarkable abnormalities, including spinous layer hyperplasia and cornified envelope deformation. Such epidermal malformation causes the loss of the epidermal water barrier, leading to neonatal death. C. Growth retardation of cPGES/p23 knockout embryos. Relative whole-body weight of wild-type (+/+) and cPGES/p23 knockout (−/−) embryos between 17.5 and 18.5 dpc. Data were analyzed by Student's *t*-test. The results are expressed as means ± standard error, with $p = 0.05$ as the limit of significance. For details, see Ref. [13].

receptor/Hsp90 complex. In fibroblasts and tissues from cPGES-deficient mice, the expression of glucocorticoid-responsive genes, as well as the glucocorticoid transcriptional activation of reporter plasmids, was reduced [12,14]. Defective nuclear translocation of the glucocorticoid receptor in cPGES-deficient fibroblasts was also observed. Although PGE_2 levels were reduced in lung and other tissues in cPGES-null mice, primary fibroblasts from these mice showed increased, rather than decreased, PGE_2 production [13,14].

Interestingly, the reduction of cPGES in rat spinal cord with intrathecal application of cPGES antisense oligonucleotides reduced nociceptive behavior in zymosan-evoked thermal hyperalgesia and in the formalin assay [88]. These results indicate that cPGES plays an important role in mediating early responses during spinal nociceptive processing. Further studies using tissue-specific cPGES-knockout mice will be needed to clarify the *in vivo* roles of cPGES-derived PGE_2.

4. mPGES-2

4.1. Biochemical properties of mPGES-2

The third PGES, mPGES-2, was initially purified from the microsomal fraction of bovine heart [89], and cDNAs encoding human and monkey homologs were subsequently identified [9]. mPGES-2 is a 41 kDa protein consisting of 378–385 amino acids, which is structurally distinct from mPGES-1; moreover, unlike mPGES-1, mPGES-2 does not exclusively depend on GSH for its catalytic activity [9]. mPGES-2 has an N-terminal hydrophobic domain, followed by a glutaredoxin/thioredoxin homology region, in which the consensus thioredoxin homology sequence of Cys^{110}-X-X-Cys^{113} is present. A mutagenesis study indicated that Cys^{110}, but not Cys^{113}, is essential for the enzymatic activity [90]. The V_{max} and K_m values for PGH_2 of the purified recombinant mPGES-2 are about 3.3 μmol/min/mg and 28 μM, respectively [9]. The recombinant enzyme is activated by various SH-reducing reagents, such as dithiothreitol, GSH, and β-mercaptoethanol in the order of decreasing effectiveness. In addition, the reduced form of lipoic acid (dihydrolipoic acid) serves as one of the natural activators of mPGES-2 in the cells [90].

Crystallization of mPGES-2 reveals that it forms a dimer and attaches to the lipid membrane by anchoring the N-terminal section [91]. Two hydrophobic pockets connected to form a V shape are located at the bottom of a large cavity. The geometry suggests that the SH of Cys^{110} in the glutaredoxin/thioredoxin-like domain is most likely the catalytic site of mPGES-2. PGH_2 fits well into the V-shaped pockets and its endoperoxide moiety interacts with the SH of Cys^{110}. The fold of mPGES-2 is quite similar to that of GSH-dependent hematopoietic PGD synthase, except for the two large loop sections.

4.2. Expression and cellular function of mPGES-2

mPGES-2 is synthesized as a Golgi membrane-associated protein, and the proteolytic removal of the N-terminal hydrophobic domain leads to the formation of a mature cytosolic enzyme [92]. When transfected in several cell lines, mPGES-2 is coupled with both COX-1 and COX-2, leading to PGE_2 production [92]. Since the mature mPGES-2 exists as an N-terminally truncated cytosolic form, mPGES-2 can function as a cytosolic enzyme rather than a membrane-bound enzyme.

The transcript for mPGES-2 is more abundantly distributed in the brain, heart, skeletal muscle, kidney, and liver than in other tissues [9]; this differs from the expression profile of mPGES-1. mPGES-2 expression is rather constitutive in various cells and tissues and is not elevated appreciably during inflammation or tissue damage.

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This suggests that these two enzymes are not always redundant but rather exhibit tissue-specific functions. However, a considerable increase in mPGES-2 expression is observed in human colorectal cancer, in which mPGES-1 is also overexpressed [92]. Also, mPGES-2 expression is dramatically increased in mouse bone marrow stromal cells in accordance with *in vitro* culture [93].

4.3. Possible in vivo functions of mPGES-2

Very recently, mPGES-2-deficient mice showed no specific phenotype and no alteration in PGE_2 levels in several tissues (including liver, kidney, heart, and brain) or in LPS-stimulated macrophages [15]. These results suggest that mPGES-2 is not involved in PGE_2 synthesis under the physiological and pathological conditions tested thus far. However, the possibility of tissue-specific or particular pathological roles of mPGES-2 has not yet been ruled out.

5. Concluding remarks

It has become apparent that there are three PGES enzymes in mammalian cells and that they display distinct functional coupling with upstream COX enzymes. Distinct PGES enzymes may control the spatial and temporal production of PGE_2 in different pathophysiological aspects in particular tissues and cells. Therefore, it is very important to understand the functions specific to each PGES. Although COX-2 inhibitors have reduced gastrointestinal toxicity as compared with traditional NSAIDs, there are also some adverse effects associated with this new group of drugs. Specific inhibition of COX-2 alters the balance between platelet-derived thromboxane A_2 and endothelium-derived PGI_2, leading to increases in the risk of thrombosis due to altered vascular tone [94]. Thus, more selective modulation of the prostanoid pathway appears to be desirable. Theoretically, a pharmacologic blockade of PGES could decrease pathological PGE_2 production while sparing other prostanoids including PGI_2 and thromboxane A_2. Further investigation into the biochemical properties, transcriptional regulation, and *in vitro* and *in vivo* functions of each PGES enzymes may illuminate the potential utility of clinically targeting PGES.

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References

[1] I. Kudo, M. Murakami, Phospholipase A_2 enzymes. Prostaglandins Other Lipid Mediat. 68–69 (2002) 3–58.
[2] W.I. Smith, D.L. DeWitt, R.M. Garavito, Cyclooxygenases: structural, cellular, and molecular biology. Annu. Rev. Biochem. 69 (2000) 145–182.
[3] I. Kudo, M. Murakami, Prostaglandin E synthase, a terminal enzyme for prostaglandin E_2 biosynthesis. J. Biochem. Mol. Biol. 38 (2005) 633–638.
[4] M. Murakami, T. Kambe, S. Shimbara, I. Kudo, Functional coupling between various phospholipase A_2s and cyclooxygenases in immediate and delayed prostanoid biosynthetic pathways. J. Biol. Chem. 274 (1999) 3103–3115.
[5] Y. Nakatani, Y. Chin, S. Hara, I. Kudo, Immediate prostaglandin E_2 synthesis in rat 3Y1 fibroblasts following vasopressin V1a receptor stimulation. Biochem. Biophys. Res. Commun. 354 (2007) 676–680.
[6] P.J. Jakobsson, S. Thorén, R. Morgenstern, B. Samuelsson, Identification of human prostaglandin E synthase: a microsomal, glutathione-dependent, inducible enzyme, constituting a potential novel drug target. Proc. Natl. Acad. Sci. U. S. A. 96 (1999) 7220–7225.
[7] T. Tanioka, Y. Nakatani, N. Semmyo, M. Murakami, I. Kudo, Molecular identification of cytosolic prostaglandin E_2 synthase that is functionally coupled with cyclooxygenase-1 in immediate prostaglandin E_2 biosynthesis. J. Biol. Chem. 275 (2000) 32775–32782.
[8] M. Murakami, H. Naraba, T. Tanioka, et al., Regulation of prostaglandin E_2 biosynthesis by inducible membrane-associated prostaglandin E_2 synthase that acts in concert with cyclooxygenase-2. J. Biol. Chem. 275 (2000) 32783–32792.
[9] N. Tanikawa, Y. Ohmiya, H. Ohkubo, et al., Identification and characterization of a novel type of membrane-associated prostaglandin E synthase. Biochem. Biophys. Res. Commun. 291 (2002) 884–889.
[10] S. Uematsu, M. Matsumoto, K. Takeda, S. Akira, Lipopolysaccharide-dependent prostaglandin E_2 production is regulated by the glutathione-dependent prostaglandin E_2 synthase gene induced by the Toll-like receptor 4/MyD88/NF-IL6 pathway. J. Immunol. 168 (2002) 5811–5816.
[11] C.E. Trebino, J.L. Stock, C.P. Gibbons, et al., Impaired inflammatory and pain responses in mice lacking an inducible prostaglandin E synthase. Proc. Natl. Acad. Sci. U. S. A. 100 (2003) 9044–9049.
[12] I. Grad, T.A. McKee, S.M. Ludwig, et al., The Hsp90 cochaperone p23 is essential for perinatal survival. Mol. Cell. Biol. 26 (2006) 8976–8983.
[13] Y. Nakatani, Y. Hokonohara, S. Kakuta, K. Sudo, Y. Iwakura, I. Kudo, Knockout mice lacking cPGES/p23, a constitutively expressed PGE_2 synthetic enzyme, are perinatally lethal. Biochem. Biophys. Res. Commun. 362 (2007) 387–392.
[14] A.K. Lovgren, M. Kovarova, B.H. Koller, cPGES/p23 is required for glucocorticoid receptor function and embryonic growth but not prostaglandin E_2 synthesis. Mol. Cell. Biol. 27 (2007) 4416–4430.
[15] L.A. Jania, S. Chandrasekharan, M.G. Backlund, et al., Microsomal prostaglandin E synthase-2 is not essential for in vivo prostaglandin E_2 biosynthesis. Prostaglandins Other Lipid Mediat. 88 (2009) 73–81.
[16] S. Thorén, R. Weinander, S. Saha, et al., Human microsomal prostaglandin E synthase-1: purification, functional characterization, and projection structure determination. J. Biol. Chem. 278 (2003) 22199–22209.
[17] J.A. Mancini, K. Blood, J. Guay, et al., Cloning, expression, and up-regulation of inducible rat prostaglandin E synthase during lipopolysaccharide-induced pyresis and adjuvant-induced arthritis. J. Biol. Chem. 276 (2001) 4469–4475.
[18] K. Yamagata, K. Matsumura, W. Inoue, et al., Coexpression of microsomal-type prostaglandin E synthase with cyclooxygenase-2 in brain endothelial cells of rats during endotoxin-induced fever. J. Neurosci. 21 (2001) 2669–2677.
[19] S. Thorén, P.J. Jakobsson, Coordinate up- and down-regulation of glutathione-dependent prostaglandin E synthase and cyclooxygenase-2 in A549 cells. Inhibition by NS-398 and leukotriene C_4. Eur. J. Biochem. 267 (2000) 6428–6434.
[20] M. Soler, M. Camacho, J.R. Escudero, M.A. Iñiguez, L. Vila, Human vascular smooth muscle cells but not endothelial cells express prostaglandin E synthase. Circ. Res. 87 (2000) 504–507.
[21] D.O. Stichtenoth, S. Thorén, H. Bian, M. Peters-Golden, P.J. Jakobsson, L.J. Crofford, Microsomal prostaglandin E synthase is regulated by proinflammatory cytokines and glucocorticoids in primary rheumatoid synovial cells. J. Immunol. 167 (2001) 469–474.
[22] H. Naraba, C. Yokoyama, N. Tago, et al., Transcriptional regulation of the membrane-associated prostaglandin E_2 synthase gene. Essential role of the transcription factor Egr-1. J. Biol. Chem. 277 (2002) 28601–28608.
[23] F.J. Sweeney, T.S. Wachtmann, J.D. Eskra, et al., Inhibition of IL-1 β-dependent prostaglandin E_2 release by antisense microsomal prostaglandin E synthase 1 oligonucleotides in A549 cells. Mol. Cell. Endocrinol. 205 (2003) 151–157.
[24] M. Hegen, L. Sun, N. Uozumi, et al., Cytosolic phospholipase A_2-deficient mice are resistant to collagen-induced arthritis. J. Exp. Med. 197 (2003) 1297–1302.
[25] L.K. Myers, A.H. Kang, A.E. Postlethwaite, et al., The genetic ablation of cyclooxygenase 2 prevents the development of autoimmune arthritis. Arthritis Rheum. 43 (2000) 2687–2693.
[26] J.M. McCoy, J.R. Wicks, J.P. Audoly, The role of prostaglandin E_2 receptors in the pathogenesis of rheumatoid arthritis. J. Clin. Invest. 110 (2002) 651–658.
[27] B. Rocca, L.M. Spain, E. Pure, R. Langenbach, C. Patrono, G.A. FitzGerald, Distinct roles of prostaglandin H synthases 1 and 2 in T-cell development. J. Clin. Invest. 103 (1999) 1469–1477.
[28] F. Kojima, M. Kapoor, L. Yang, et al., Defective generation of a humoral immune response is associated with a reduced incidence and severity of collagen-induced arthritis in microsomal prostaglandin E synthase-1 null mice. J. Immunol. 180 (2008) 8361–8368.
[29] D. Kamei, K. Yamakawa, Y. Takegoshi, et al., Reduced pain hypersensitivity and inflammation in mice lacking microsomal prostaglandin E synthase-1. J. Biol. Chem. 279 (2004) 33684–33695.
[30] M. Saegusa, M. Murakami, Y. Nakatani, et al., Contribution of membrane-associated prostaglandin E_2 synthase to bone resorption. J. Cell. Physiol. 197 (2003) 348–356.
[31] M. Inada, C. Matsumoto, S. Uematsu, S. Akira, C. Miyaura, Membrane-bound prostaglandin E synthase-1-mediated prostaglandin E_2 production by osteoblast plays a critical role in lipopolysaccharide-induced bone loss associated with inflammation. J. Immunol. 177 (2006) 1879–1885.
[32] K. Yamakawa, S. Kamekura, N. Kawamura, et al., Association of microsomal prostaglandin E synthase 1 deficiency with impaired fracture healing, but not with bone loss or osteoarthritis, in mouse models of skeletal disorders. Arthritis Rheum. 58 (2008) 172–183.
[33] T. Mabuchi, H. Kojima, T. Abe, et al., Membrane-associated prostaglandin E synthase-1 is required for neuropathic pain. Neuroreport 15 (2004) 1395–1398.
[34] S. Li, Y. Wang, K. Matsumura, L.R. Ballou, S.G. Morham, C.M. Blatteis, The febrile response to lipopolysaccharide is blocked in cyclooxygenase-2 (−/−), but not in cyclooxygenase-1 (−/−) mice. Brain Res. 825 (1999) 86–94.
[35] D. Engblom, S. Saha, L. Engstrom, et al., Microsomal prostaglandin E synthase-1 is the central switch during immune-induced pyresis. Nat. Neurosci. 6 (2003) 1137–1138.
[36] S. Saha, L. Engström, L. Mackerlova, P.J. Jakobsson, A. Blomqvist, Impaired febrile responses to immune challenge in mice deficient in microsomal prostaglandin E synthase-1. Am. J. Physiol. 288 (2005) R1100–R1107.

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[37] P.J. Cimino, C.D. Keene, R.M. Breyer, K.S. Montine, T.J. Montine, Therapeutic targets in prostaglandin E_2 signaling for neurologic disease. Curr. Med. Chem. 15 (2008) 1863–1869.
[38] Y. Ikeda-Matsuo, A. Ota, T. Fukada, S. Uematsu, S. Akira, Y. Sasaki, Microsomal prostaglandin E synthase-1 is a critical factor of stroke-reperfusion injury. Proc. Natl. Acad. Sci. U. S. A. 103 (2006) 11790–11795.
[39] K. Satoh, Y. Nagano, C. Shimomura, N. Suzuki, Y. Saeki, H. Yokota, Expression of prostaglandin E synthase mRNA is induced in beta-amyloid treated rat astrocytes. Neurosci. Lett. 283 (2000) 221–223.
[40] G.M. Pasinetti, P.S. Aisen, Cyclooxygenase-2 expression is increased in frontal cortex of Alzheimer's disease. Neuroscience 87 (1998) 319–324.
[41] T.J. Montine, K.R. Sidell, B.C. Crews, et al., Elevated CSF prostaglandin E_2 levels in patients with probable AD. Neurology 53 (1999) 1495–1498.
[42] U.A. Chaudhry, H. Zhuang, B.J. Crain, S. Doré, Elevated microsomal prostaglandin-E synthase-1 in Alzheimer's disease. Alzheimers Dement. 4 (2008) 6–13.
[43] Y.I. Cha, R.N. DuBois, NSAIDs and cancer prevention: targets downstream of COX-2. Annu. Rev. Med. 58 (2007) 239–252.
[44] M. Oshima, J.E. Dinchuk, S.L. Kargman, et al., Suppression of intestinal polyposis in Apc^{716} knockout mice by inhibition of cyclooxygenase 2 (COX-2). Cell 87 (1996) 803–809.
[45] K. Takaku, M. Sonoshita, N. Sasaki, et al., Suppression of intestinal polyposis in Apc^{716} knockout mice by an additional mutation in the cytosolic phospholipase A_2 gene. J. Biol. Chem. 275 (2002) 34013–34016.
[46] M. Sonoshita, K. Takaku, N. Sasaki, et al., Acceleration of intestinal polyposis through prostaglandin receptor EP2 in Apc^{716} knockout mice. Nat. Med. 7 (2001) 1048–1051.
[47] D. Kamei, M. Murakami, Y. Nakatani, Y. Ishikawa, T. Ishii, I. Kudo, Potential role of microsomal prostaglandin E synthase-1 in tumorigenesis. J. Biol. Chem. 278 (2003) 19396–19405.
[48] H. Oshima, M. Oshima, K. Inaba, M.M. Taketo, Hyperplastic gastric tumors induced by activated macrophages in COX-2/mPGES-1 transgenic mice. EMBO J. 23 (2004) 1669–1678.
[49] D. Kamei, M. Murakami, Y. Sasaki, et al., Microsomal prostaglandin E synthase-1 in both cancer cells and hosts contributes to tumor growth, invasion and metastasis. Biochem. J. 425 (2010) 361–371.
[50] M. Nakanishi, D.C. Montrose, P. Clark, et al., Genetic deletion of mPGES-1 suppresses intestinal tumorigenesis. Cancer Res. 68 (2008) 3251–3259.
[51] N. Elander, J. Ungerbäck, H. Olsson, S. Uematsu, S. Akira, P. Söderkvist, Genetic deletion of mPGES-1 accelerates intestinal tumorigenesis in $APC^{Min/+}$ mice. Biochem. Biophys. Res. Commun. 372 (2008) 249–253.
[52] Y. Cheng, M. Wang, Y. Yu, J. Lawson, C.D. Funk, G.A. Fitzgerald, Cyclooxygenases, microsomal prostaglandin E synthase-1, and cardiovascular function. J. Clin. Invest. 116 (2006) 1391–1399.
[53] N. Degousee, S. Fazel, D. Angoulvant, et al., Microsomal prostaglandin E_2 synthase-1 deletion leads to adverse left ventricular remodeling after myocardial infarction. Circulation 117 (2008) 1701–1710.
[54] M. Wang, A.M. Zukas, Y. Hui, E. Ricciotti, E. Puré, G.A. FitzGerald, Deletion of microsomal prostaglandin E synthase-1 augments prostacyclin and retards atherogenesis. Proc. Natl. Acad. Sci. U. S. A. 103 (2006) 14507–14512.
[55] M. Wang, E. Lee, W. Song, et al., Microsomal prostaglandin E synthase-1 deletion suppresses oxidative stress and angiotensin II-induced abdominal aortic aneurysm formation. Circulation 117 (2008) 1302–1309.
[56] A. Schneider, Y. Zhang, M. Zhang, et al., Membrane-associated PGE synthase-1 (mPGES-1) is coexpressed with both COX-1 and COX-2 in the kidney. Kidney Int. 65 (2004) 1205–1213.
[57] L. Boulet, M. Ouellet, K.P. Bateman, et al., Deletion of microsomal prostaglandin E_2 (PGE_2) synthase-1 reduces inducible and basal PGE_2 production and alters the gastric prostanoid profile. J. Biol. Chem. 279 (2004) 23229–23237.
[58] H. Francois, C. Facemire, A. Kumar, L. Audoly, B. Koller, T. Coffman, Role of microsomal prostaglandin E synthase 1 in the kidney. J. Am. Soc. Nephrol. 18 (2007) 1466–1475.
[59] Z. Jia, A. Zhang, H. Zhang, Z. Dong, T. Yang, Deletion of microsomal prostaglandin E synthase-1 increases sensitivity to salt loading and angiotensin II infusion. Circ. Res. 99 (2006) 1243–1251.
[60] S. Soodvilai, Z. Jia, M.H. Wang, Z. Dang, T. Yang, mPGES-1 deletion impairs diuretic response to acute water loading. Am. J. Physiol. 296 (2009) F1129–F1135.
[61] P. Sharon, M. Ligumsky, D. Rachmilewitz, U. Zor, Role of prostaglandins in ulcerative colitis: enhanced production during active disease and inhibition by sulfasalazine. Gastroenterology 75 (1978) 638–640.
[62] I. Bjarnason, J. Hayllar, A.J. MacPherson, A.S. Russell, Side effects of non-steroidal anti-inflammatory drugs on the small and large intestine in humans. Gastroenterology 104 (1993) 1832–1847.
[63] A.F. Sheibanie, J.H. Yen, T. Khayrullina, et al., The proinflammatory effect of prostaglandin E_2 in experimental inflammatory bowel disease is mediated through the IL-23 –> IL-17 axis,. J. Immunol. 178 (2007) 8138–8147.
[64] O. Morteau, S.G. Morham, R. Sellon, et al., Impaired mucosal defense to acute colonic injury in mice lacking cyclooxygenase-1 or cyclooxygenase-2. J. Clin. Invest. 105 (2000) 469–478.
[65] K. Kabashima, T. Saji, T. Murata, et al., The prostaglandin receptor EP4 suppresses colitis, mucosal damage and CD4 cell activation in the gut. J. Clin. Invest. 109 (2002) 883–893.
[66] K. Subbaramaiah, K. Yoshimatsu, E. Scherl, et al., Microsomal prostaglandin E synthase-1 is overexpressed in inflammatory bowel disease: evidence for involvement of the transcription factor Egr-1. J. Biol. Chem. 279 (2004) 12647–12658.
[67] J. Sirois, Induction of prostaglandin endoperoxide synthase-2 by human chorionic gonadotropin in bovine preovulatory follicles *in vivo*. Endocrinology 135 (1994) 841–848.
[68] J. Sirois, M. Doré, The late induction of prostaglandin G/H synthase-2 in equine preovulatory follicles supports its role as a determinant of the ovulatory process. Endocrinology 138 (1997) 4427–4434.
[69] H. Lim, B.C. Paria, S.K. Das, et al., Multiple female reproductive failures in cyclooxygenase 2-deficient mice. Cell 91 (1997) 197–208.
[70] C.R. Kennedy, Y. Zhang, S. Brandon, et al., Salt-sensitive hypertension and reduced fertility in mice lacking the prostaglandin EP2 receptor. Nat. Med. 5 (1999) 217–220.
[71] H. Hizaki, E. Segi, Y. Sugimoto, et al., Abortive expansion of the cumulus and impaired fertility in mice lacking the prostaglandin E receptor subtype EP_2. Proc. Natl. Acad. Sci. U. S. A. 96 (1999) 10501–10506.
[72] F. Filion, N. Bouchard, A.K. Goff, J.G. Lussier, J. Sirois, Molecular cloning and induction of bovine prostaglandin E synthase by gonadotropins in ovarian follicles prior to ovulation *in vivo*. J. Biol. Chem. 276 (2001) 34323–34330.
[73] J.W. Meadows, B. Pitzer, D.E. Brockman, L. Myatt, Differential localization of prostaglandin E synthase isoforms in human placental cell types. Placenta 25 (2004) 259–265.
[74] J.W. Meadows, A.L. Eis, D.E. Brockman, L. Myatt, Expression and localization of prostaglandin E synthase isoforms in human fetal membranes in term and preterm labor. J. Clin. Endocrinol. Metab. 88 (2003) 433–439.
[75] N. Alfaidy, M. Sun, J.R. Challis, W. Gibb, Expression of membrane prostaglandin E synthase in human placenta and fetal membranes and effect of labor. Endocrine 20 (2003) 219–225.
[76] K. Kubota, T. Kubota, D. Kamei, et al., Change in prostaglandin E synthases (PGESs) in microsomal PGES-1 knockout mice in a preterm delivery model. J. Endocrinol. 187 (2005) 339–345.
[77] A. Bouayad, J.C. Fouron, X. Hou, et al., Developmental regulation of prostaglandin E_2 synthase in porcine ductus arteriosus. Am. J. Physiol. 286 (2004) R903–R909.
[78] E. Segi, Y. Sugimoto, A. Yamasaki, et al., Patent ductus arteriosus and neonatal death in prostaglandin receptor EP4-deficient mice. Biochem. Biophys. Res. Commun. 246 (1998) 7–12.
[79] B. Baragatti, D. Sodini, S. Uematsu, F. Coceani, Role of microsomal prostaglandin E synthase-1 (mPGES1)-derived PGE_2 in patency of the ductus arteriosus in the mouse. Pediatr. Res. 64 (2008) 523–527.
[80] J.L. Johnson, T.G. Beito, C.J. Krco, D.O. Toft, Characterization of a novel 23-kilodalton protein of unactive progesterone receptor complexes. Mol. Cell. Biol. 14 (1994) 1956–1963.
[81] K.A. Hutchison, L.F. Stancato, J.K. Owens-Grillo, et al., The 23-kDa acidic protein in reticulocyte lysate is the weakly bound component of the hsp foldosome that is required for assembly of the glucocorticoid receptor into a functional heterocomplex with hsp90. J. Biol. Chem. 270 (1995) 18841–18847.
[82] J.A. Johnson, A. el Barbary, S.E. Kornguth, J.F. Brugge, F.L. Siegel, Glutathione *S*-transferase isoenzymes in rat brain neurons and glia. J. Neurosci. 13 (1993) 2013–2023.
[83] T. Kobayashi, Y. Nakatani, T. Tanioka, et al., Regulation of cytosolic prostaglandin E synthase by phosphorylation. Biochem. J. 381 (2004) 59–69.
[84] T. Tanioka, Y. Nakatani, T. Kobayashi, et al., Regulation of cytosolic prostaglandin E_2 synthase by 90-kDa heat shock protein. Biochem. Biophys. Res. Commun. 303 (2003) 1018–1023.
[85] A.H. Moore, J.A. Olschowka, M.K. O'Banion, Intraparenchymal administration of interleukin-1β induces cyclooxygenase-2-mediated expression of membrane- and cytosolic-associated prostaglandin E synthases in mouse brain. J. Neuroimmunol. 148 (2004) 32–40.
[86] H. Ni, T. Sun, X.H. Ma, Z.M. Yang, Expression and regulation of cytosolic prostaglandin E synthase in mouse uterus during the peri-implantation period. Biol. Reprod. 68 (2003) 744–750.
[87] Y. Zhang, A. Schneider, R. Rao, et al., Genomic structure and genitourinary expression of mouse cytosolic prostaglandin E_2 synthase gene. Biochim. Biophys. Acta 1634 (2003) 15–23.
[88] A. Hofacker, O. Coste, H.V. Nguyen, C. Marian, K. Scholich, G. Geisslinger, Downregulation of cytosolic prostaglandin E_2 synthase results in decreased nociceptive behavior in rats. J. Neurosci. 25 (2005) 9005–9009.
[89] K. Watanabe, K. Kurihara, T. Suzuki, Purification and characterization of membrane-bound prostaglandin E synthase from bovine heart. Biochim. Biophys. Acta 1439 (1999) 406–414.
[90] K. Watanabe, H. Ohkubo, H. Niwa, et al., Essential ^{110}Cys in active site of membrane-associated prostaglandin E synthase-2. Biochem. Biophys. Res. Commun. 306 (2003) 577–581.
[91] T. Yamada, J. Komoto, K. Watanabe, Y. Ohmiya, F. Takusagawa, Crystal structure and possible catalytic mechanism of microsomal prostaglandin E synthase type 2 (mPGES-2). J. Mol. Biol. 348 (2005) 1163–1176.
[92] M. Murakami, K. Nakashima, K. Kamei, et al., Cellular prostaglandin E_2 production by membrane-bound prostaglandin E synthase-2 via both cyclooxygenases-1 and -2. J. Biol. Chem. 278 (2003) 37937–37947.
[93] N. Ueno, Y. Takegoshi, D. Kamei, I. Kudo, M. Murakami, Coupling between cyclooxygenases and terminal prostanoid synthases. Biochem. Biophys. Res. Commun. 338 (2005) 70–76.
[94] S. Hara, I. Kudo, COX-2 inhibitors and the risk of cardiovascular events. Jpn. Med. Assoc. J. 49 (2006) 276–278.

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Microsomal prostaglandin E synthase-1 deficiency attenuates neuronal cell death and mitigates Alzheimer's disease-like pathology in a mouse model

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Epidemiological and clinical studies have suggested that long-term use of non-steroidal anti-inflammatory drugs (NSAIDs) moderate the onset or progression of Alzheimer's disease (AD). As NSAIDs inhibit cyclooxygenase (COX) activity, it was suggested that prostaglandin E_2 (PGE_2), a major end-product of COX, may have a pathogenic role in AD. However, the involvement of PGE synthase (PGES), a terminal enzyme downstream of COX, has not been fully elucidated. We here found that among three PGES isozymes, only microsomal PGES-1 (mPGES-1) expression is induced and associated with β-amyloid (Aβ) plaques in cerebral cortex from human AD patients and Tg2576 mice, a transgenic AD mouse model. Treatment of primary mouse cerebri neuronal cells with Aβ induced mPGES-1 gene expression and PGE_2 production, following significant apoptotic cell death. To evaluate potential roles of mPGES-1 in the development of AD, we have used mPGES-1 deficient mice and found that deletion of mPGES-1 attenuates Aβ-induced neuronal cell death *in vitro* and mitigates AD-like pathology in Tg2576 mice. Accumulation of microglia around senile plaques was reduced and learning impairments were attenuated in mPGES-1 deficient Tg2576 mice. These results indicated that mPGES-1 is induced in AD brains and then plays a role in AD pathology. Blockage of mPGES-1 could form the basis of a novel therapeutic strategy for AD, which may represent a safer approach than therapies involving NSAIDs.

Introduction

Alzheimer's disease (AD) is the most common neurodegenerative disorder of the elderly and is characterized clinically by a progressive memory loss, as well as other cognitive impairments. The neuropathological hallmarks of AD include abundant deposits of β-amyloid peptide (Aβ) fibrils in senile plaques (SPs), accumulation of abnormal tau protein filaments in neurofibrillary tangles (NFTs), and extensive neuronal degradation and loss (1). AD brains also exhibit a number of pathological abnormalities, including a profound loss of synapses, profuse reactive gliosis, microglial activation, and inflammatory process.

Many epidemiological studies have shown that long-term use of nonsteroidal anti-inflammatory drugs (NSAIDs) reduces the risk of developing AD and delays its onset (2, 3). It has been demonstrated that neurodegeneration of AD is accompanied by inflammatory reactions, which is indicated by accumulation of microglia and astrocytes around senile plaques and elevated levels of inflammatory cytokines, chemokines, proteases, and reactive oxygen species (4, 5). The preventing effect of NSAIDs has also been modeled in transgenic mice expressing mutant forms of amyloid precursor protein (APP) and presenilin 1 (PS1), and in these models, NSAIDs significantly reduce amyloid deposition and microglial activation (6, 7). However, the mechanism by which NSAIDs may affect these or other pathophysiogical processes relevant to AD is unclear.

NSAIDs inhibit the activity of cyclooxygenase (COX), which catalyzes the conversion of arachidonic acid to prostaglandin H_2 (PGH_2), the precursor of the five PGs: PGE_2, PGD_2, PGI_2, $PGF_{2\alpha}$, and thromboxane A_2 (TXA_2). Of the two COX isoforms, COX-1 is expressed

constitutively in most tissues and is generally responsible for the production of PGs that control normal physiological functions, while COX-2 is inducible in response to mitogens, cytokines, and cellular transformation (8). Although it remains uncertain as to how NSAIDs prevent the development of AD, several studies have shown that elevated COX-2 expression is present in cerebral cortex of AD brain and correlate with amyloid plaque density and NFTs (9-13). Significantly, levels of PGE_2, which is the main product of COX, have been found to be elevated in cerebrospinal fluid of AD patients (14), suggesting that PGE_2 signaling may function in the development of AD.

PGE_2 is synthesized from COX-derived PGH_2 by the action of PGE synthase (PGES) (15). Thus far, three PGES enzymes, microsomal PGES (mPGES)-1, mPGES-2, and cytosolic PGES (cPGES), have been identified (16). Among these PGES isozymes, mPGES-1 is induced by proinflammatory stimuli and down-regulated by anti-inflammatory glucocorticoids as in the case of COX-2, and is functionally coupled with COX-2 in marked preference to COX-1 (16, 17). Induction of mPGES-1 expression has been observed in various systems in which COX-2-driven PGE_2 has been implicated, such as rheumatoid arthritis, febrile response, reproduction, bone metabolism, and cardiovascular function (18). Furthermore, Satoh et al. reported that Aβ induced mPGES-1 expression in rat astrocytes (19). However, little is known about the involvement of mPGES-1, downstream enzyme of COX-2, in AD.

In the present study, to examine the expression and distribution of mPGES-1 in AD brain, we performed immunohistochemical analysis using brain tissues from human AD patients and Tg2576 mice. Tg2576 mice harbor the Swedish mutation of APP and exhibit some of the

pathological features of the AD brain (20). We here found that among three PGES isozymes, only mPGES-1 expression is induced and associated with Aβ plaques in cerebral cortex from both human AD patients and Tg2576 mice. The induction of mPGES-1 was also observed in Aβ-treated primary mouse nerve cells. Furthermore, we have used mPGES-1 deficient mice (21, 22) to evaluate potential roles of mPGES-1 in the development of AD, and found that mPGES-1 deficiency attenuates Aβ-induced neuronal cell death *in vitro* and mitigates AD-like pathology in Tg2576 mice. These results indicated that mPGES-1 is induced in AD brains and then plays a role in AD pathology.

Results

High Expression of mPGES-1 in Brains of Human AD and Tg2576 Mice. First, immunohistochemical studies were performed in human brain tissues from 18 patients. Positive immunoreactivity for mPGES-1 and -2 was observed in all tested brain tissues, but definite immunostaining with cPGES antibody was not found.

As shown in Fig. 1A, even in case of normal control (NC) hippocampus, dense immunostaining with the mPGES-1 antibody was observed in the cytoplasm and neurites in the CA2-3 (CA: cornu ammonis) region, but less intense staining in the CA4 and sparse positive staining in the CA1 were found. No positive staining was detected in the dentate gyrus and subiculum. In AD hippocampus, a similar staining pattern could be seen with much more intense labeling. Very clear cytoplasmic staining with many areas of neurite labeling was seen in the CA2-3 (Fig. 1A), and to a lesser extent in the CA4, and sparse but definite staining was also seen

in the CA1 and subiculum regions. No staining was seen in the dentate gyrus. In non-AD hippocampus, the intensity of the labeling was equivalent to that of NC (data not shown). There were no differences in the staining of representative tissue samples of frontal and parietal lobes of NC, AD and non-AD cases, except mild to very weak cytoplasmic staining of the cortices, and no labeling of the white matter. Quantitative image analysis confirmed that the expression of mPGES-1 was more extensive in AD brains than in NC and non-AD brains (Fig. 1B). Furthermore, we performed double-immunostaining using antibodies that recognized mPGES-1 and phosphorylated tau or Aβ to investigate whether the mPGES-1 expression was implicated in the pathogenesis of AD. As the results, we found that mPGES-1 was expressed with dystrophic neurites around senile Aβ plaques in AD brains (Fig. 1C).

On the other hand, with the anti-mPGES-2 antibody, the hippocampus of all groups exhibited consistent staining pattern of similar intensity (data not shown). Through the CA to the subiculum, glial and neuronal cytoplasmic staining was uniformly observed with many areas of neurite labeling. Mild cytoplasmic staining was seen in the dentate gyrus. Some glial neurite labeling extended to vessels to form a perivascular pattern, suggesting that the positive staining was of astrocytes. In the frontal and parietal lobes, neuronal and glial staining was seen in the cortical region, and glial staining in the white matter was observed. Some perivascular staining was also seen. With the anti-cPGES antibody, there was no definite staining of any tissue samples of hippocampus, frontal and parietal lobes from NC, AD and non-AD groups (data not shown). The expression levels of mPGES-2 and cPGES in AD brains were similar to those in the other brains. Neither mPGES-2 nor cPGES expressions were associated with Aβ plaques.