cancer cells.

Increased tumorigenic potential of mPGES-1-overexpressing LLC cells in vitro — We next established LLC cells that stably overexpressed mPGES-1 and examined whether these cells (as opposed to mPGES-1 KD cells) would show increased proliferation and invasion. mPGES-1-transfected LLC cells expressed more mPGES-1 protein than did mock cells, whereas the expression of COX-2 protein was unaltered (Fig. 3A). PGE_2 release into the medium during culture was increased by ~2-fold in mPGES-1-overexpressing cells relative to mock cells (Fig. 3B). As shown in Fig. 3C, the growth rate of mPGES-1-overexpressing cells was significantly faster than that of mock cells. Moreover, the Matrigel™ invasion chamber assay revealed that the invasive activity of mPGES-1-overexpressing cells was notably higher than that of mock cells (Fig. 3D). Additionally, treatment of both mock and mPGES-1-overexpressing LLC cells with NS-398 reduced the invasion by ~40% (Fig. 3D). These data confirm that the increased production of PGE_2 through the COX-2/mPGES-1 pathway in cancer cells facilitates their proliferation and invasion.

Silencing of mPGES-1 in cancer cells reduces tumorigenesis in vivo — To assess the contribution of mPGES-1 in cancer cells to tumor development *in vivo*, we grafted mPGES-1 KD and mock LLC cells subcutaneously into BALB/c mice, and the development of the solid tumor around the injection sites was evaluated on day 14 after implantation. Remarkably, both the volume and weight of xenografts derived from mPGES-1 KD cells were significantly smaller than those derived from mock cells (Fig. 4A and B). The level of PGE_2 was consistently reduced in the homogenates of xenografts derived from mPGES-1 KD cells compared with those from mock cells (Fig. 4C). Interestingly, the level of 6-keto-$PGF_{1\alpha}$, but not $PGF_{2\alpha}$, was substantially increased in xenografts from mPGES-1 KD cells relative to those from mock cells (Fig. 4C), indicating a metabolic shunting effect from PGE_2 to PGI_2 *in vivo*. The expression of VEGF, as assessed by RT-PCR, was reduced in mPGES-1 KD xenografts as compared with control xenografts (Fig. 4D). Considering that VEGF expression was unaltered by silencing of mPGES-1 in LLC cells *in vitro* (Fig. 2B), the reduction of VEGF expression in mPGES-1 KD xenografts *in vivo* may reflect the action of mPGES-1-derived PGE_2 from cancer cells on proximal stromal cells in tumor microenvironments.

Reduced tumor growth and angiogenesis in mPGES-1 KO mice — The observations described above indicate that cancer cell-associated COX-2 and mPGES-1 cooperatively produce PGE_2, which accelerate multiple steps of malignant progression, including cell proliferation, invasion and ECM adhesion. In terms of the pathological circumstances, it has been reported that COX-2 and mPGES-1 are expressed not only in tumor cells but also in the stromal cells (mainly in infiltrating macrophages) surrounding them [14, 21]. To investigate the contribution of host-associated mPGES-1 to tumor growth, we next grafted parental LLC cells subcutaneously into either mPGES-1 KO or WT mice, and evaluated the development of solid tumor around the injection sites. Although the absence of mPGES-1 in the host mice influenced neither the engraftment rate nor the growth of tumors during the first 5 days after implantation, tumors grafted into the mPGES-1 KO mice were significantly smaller in size than those grafted in the WT mice after day 6 and beyond (Fig. 5A). On day 14 after implantation, the tumor weight in mPGES-1 KO mice was reduced to half of that in WT mice (Fig. 5B, *left*). The levels of PGE_2 in homogenates of the dissected tumor tissues were nearly 50% less in mPGES-1 KO mice than in WT mice, whereas those of $PGF_{2\alpha}$ were unaffected by the host mPGES-1 deficiency (Fig. 5B, *right*).

Histological examination of the xenografts revealed that the vascularization of tumor tissues was markedly reduced by the lack of host mPGES-1 expression (Fig. 5C, *arrowhead*). The vascular density was approximately 60% lower in tumors grafted in mPGES-1 KO mice than those grafted in WT mice (Fig.

5D, *left*). The hemoglobin contents in the tumor tissues, which appeared to be well correlated with tumor neovascularization upon histological examination (30, 31), were also reduced in mPGES-1 KO mice in comparison with that in WT mice (Fig. 5D, *right*). The reduction of tumor angiogenesis in mPGES-1 KO mice caused a large area of central necrosis in the xenografts of mPGES-1 KO, but not WT, mice (Fig. 5C, *asterisk*). We further found that the expression levels of the VEGF protein (Fig. 5E, *upper panel*) and mRNA (Fig. 5E, *lower panel*) in tumor tissues were also markedly lower in mPGES-1 KO mice than in WT mice. These data suggest that host mPGES-1-derived PGE_2 plays a pivotal role in tumor-associated VEGF production and accompanying angiogenesis.

We further examined the role of host-associated mPGES-1 in tumor metastasis by intravenous injection of parental LLC cells into mPGES-1 KO and WT mice. After 14 days, the macroscopic metastases, as assessed by Bouin's staining, were found in the lungs of both genotypes, but the number and size of metastatic foci (Fig. 6A) and the weight (Fig. 6B, *left*) of the lungs were significantly reduced in mPGES-1 KO mice compared with WT mice. Levels of PGE_2 were reduced by nearly 50% in homogenates of the lungs from mPGES-1 KO mice relative to WT mice (Fig. 6B, *right*). Moreover, as assessed by RT–PCR, VEGF expression in the metastasized lungs was significantly lower in mPGES-1 KO mice than in WT mice (Fig. 6C). Gelatin zymography revealed that the activity of MMP-2 in the metastasized lung tissues was significantly mitigated in mPGES-1 KO mice in comparison with that in WT mice, whereas that of MMP-9 was unchanged between the genotypes (Fig. 6D). Collectively, these results indicate that host mPGES-1-driven PGE_2 plays a role in promoting tumor development and metastasis, which was associated with increased VEGF expression and MMP-2 activation.

DISCUSSION

Although the concept that the PGE_2 produced through the COX-2-dependent pathway participates in the pathogenesis of several types of cancer has been well established in the past decade based on a series of genetic studies employing mice ablated for the biosynthetic enzymes (COX-2) and receptors (EPs) or pharmacological studies employing inhibitors or agonists/antagonists fairly specific for them, the contribution of a step between COX-2 and EP receptors, namely, PGES enzymes that convert COX-2-produced PGH_2 to PGE_2, to cancer development has remained incompletely understood. In this context, we have previously shown that co-transfection of mPGES-1 in combination with COX-2 into HEK293 cells leads to a malignant phenotype [14]. In our continuing efforts to gain further insights into the role of mPGES-1 in tumorigenesis, we used two approaches in the present study. First, we performed siRNA-mediated silencing and overexpression of mPGES-1, which enabled us to address the complementary effects of endogenous *versus* overexpressed mPGES-1 in cancer cells on tumorigenic potentials (growth, invasion and ECM binding *in vitro* and tumor xenograft propagation *in vivo*). Second, implantation of carcinoma cells into mPGES-1 KO mice and WT mice allowed us to evaluate the contribution of mPGES-1 expressed in tissue microenvironments to the development and metastasis of tumors in proximal and distant tissues. Our results clearly indicate that mPGES-1 expressed in both cancer cells and hosts is capable of promoting proliferation and invasion of cancer cells *in vitro* and *in vivo*.

Role of mPGES-1 in cancer cells. In this study, we took advantage of mouse lung carcinoma LLC cells, a cell line that has been shown to display PGE_2-dependent cell growth [27]. *In vitro* experiments showed that PGE_2 generation and cell proliferation of LLC cells stably expressing mPGES-1 siRNA are reduced to a level similar to that of mock cells treated with NS-398 (Fig. 1A, B and D). Conversely,

overexpression of mPGES-1 in LLC cells enhanced cell proliferation in association with an increase in PGE_2 generation (Fig. 3A- C). These results indicate the role of mPGES-1, acting downstream of COX-2, in providing the mitogenic PGE_2. The synthesis of PGI_2 (6-keto $PGF_{1\alpha}$) and $PGF_{2\alpha}$, other products of the COX pathway, in mPGES-1 KD cells was nearly the same as that in mock cells (Fig. 1B and C), arguing against the metabolic shunting of PGH_2 into an alternative prostanoid pathway due to mPGES-1 silencing, as has been demonstrated in several previous studies [12, 14].

Nevertheless, in accordance with the impacts on cell proliferation described above, the gene silencing of mPGES-1 decreased the invasive activity of LLC cells, whereas overexpression of mPGES-1 increased this activity (Figs. 2A and 3D). The process by which tumor 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, and PGE_2 has been shown to stimulate the invasion of cancer cells [25-27]. Degradation of ECM and penetration across the basement membrane are required for invasion and metastasis of the tumor. The MMP family is a group of proteolytic enzymes that have been associated with tumor invasion and metastasis, and the major component of the basement membrane, type IV collagen, serves as a substrate for MMP-2 and MMP-9 (type IV collagenases). Several reports have shown that the invasive activity of various cancer cells is correlated with expression levels of these two MMPs and that the level of MMP-2 is increased in COX-2-overexpressing tumor cells [25, 26]. Moreover, exogenous PGE_2 increases the expression level of MMP-2 in non-small cell lung cancer cells [28, 29], and the expression of MMP-9 is induced by COX-2/EP4-signaling in macrophages [30]. We herein found that the activity of MMP-2, but not that of MMP-9, in conditioned medium from mPGES-1 KD cells was lower than that from mock cells (Fig. 2B), suggesting the contribution of the former matrix-degrading protease 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 tumor cells. As such, malignant tumor cells show decreased ECM-adherent activity *in vitro* [25, 26]. As shown in Fig. 2C, both collagen- and fibronectin-adherent activities were significantly increased in mPGES-1 KD cells. Treatment with $dmPGE_2$ reversed these activities, even though it failed to fully restore the reduced growth rate of mPGES-1 KD cells. Consistently, the expression of integrin $\alpha5\beta1$ (a major adhesion molecule of fibronectin) in mPGES-1 KD cells was higher than that in mock cells (Fig. 2D), an observation that is reminiscent of our previous report that co-overexpression of COX-2 and mPGES-1 in HEK293 cells results in a marked reduction of 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* tumor growth after subcutaneous engraftment of mPGES-1 KD cells was less obvious than that after engraftment of replicate mock cells (Fig. 4), implying that mPGES-1 contributes to the supply of the major pool of the PGE_2 mediating tumor proliferation both *in vitro* and *in vivo*. There are four EP receptor subtypes (EP1, EP2, EP3, and EP4) whose roles in tumorigenesis have been investigated by gene targeting studies [31]. 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 [32]. The *Apc* mutation is accompanied by aberrant activation of β-catenin signaling, which is indirectly accelerated by PGE_2 through the EP2-Gsα axis [33, 34]. In another model, disruption of the gene for EP1, EP2, or EP4 suppressed the development of colorectal cancer induced by carcinogens [35-37]. It has also been reported that transactivation of EGF receptors by the PGE_2-EP1, -2, or -4 signaling via protein kinase A and c-Src led to

increased cell growth and invasion of carcinoma cells [38-40] Although the identification of EP receptors participating in the propagation of LLC xenografts is beyond the scope of this study, the coordinated reduction of mPGES-1 and EP2 in mPGES-1 KD cells (Fig. 1A) suggests that this receptor subtype plays a role in the xenograft propagation in our experimental setting. Indeed, in a previously reported positive feedback loop between COX-2 and EP2, 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 [41]. Thus, in LLC cells, COX-2/mPGES-1-derived PGE_2 may stimulate the EP2 signaling in an autocrine/paracrine fashion to facilitate cell proliferation, and accordingly, defect in mPGES-1 expression in LLC cells may lead to down-regulation of the expression of EP2 and eventually reduce the cellular sensitivity to PGE_2.

While this manuscript was in preparation, two groups reported opposite effects of mPGES-1 deficiency in intestinal tunorigenesis. Nakanishi *et al.* showed that the genetic deletion of mPGES-1 ameliorates the development of intestinal tumors in both $Apc^{\Delta 14}$-dependent and carcinogen-induced models [42], whereas Elander *et al.* demonstrated that intestinal polyposis is exacerbated in mPGES-1-null Apc^{min} mutant mice, probably because of the metabolic shift from PGE_2 toward other pro-tumorigenic lipid mediators [43]. 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 tumor 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. We and several other investigators have found that mPGES-1 is expressed in stromal cells as well as in cancerous cells in several types of tumor [14, 15]. Given that stromal COX-2 and PGE_2 of the host can influence the development of grafted tumors [20, 44], we used mPGES-1 KO mice to evaluate the potential roles of the host stromal mPGES-1 in tumor growth, and found that tumor growth at a proximal site and metastasis into a distant tissue were significantly reduced in mPGES-1 KO mice in comparison with those in WT mice (Figs. 5 and 6). Like mPGES-1, COX-2 is highly expressed in tumor stromal cells [14-17], and a previous study employing COX-2-null mice has demonstrated that the host stromal COX-2 contributes to carcinoma growth [20]. Furthering these observations, our present results provide an additional line of genetic evidence that the COX-2/mPGES-1-derived PGE_2 from host stromal cells, as well as that from cancer cells *per se*, is important for tumorigenesis *in vivo*. As shown in Fig. 5, the levels of PGE_2 were reduced by nearly half in LLC-grafted tumor tissues, accompanied by concomitant reductions in tumor volume and weight, in mPGES-1 KO mice relative to replicate WT mice. These observations suggest that the ablation of host PGE_2 retards the proliferation of tumor cells, eventually leading to diminution of tumor cell-produced PGE_2. Previous immunohistochemical analyses have shown that mPGES-1 is preferentially distributed in macrophage-like cells infiltrating into the stromal tissues in proximity to cancer cells [16, 17]. The induction of mPGES-1 in stromal tissues in tumors may be mediated by the migration and expansion of host inflammatory cells and neovascular endothelial cells, 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 Fig. 5C and D, the vascular density in tumor xenografts was decreased in mPGES-1 KO mice. Nakanishi *et al.* have also 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 [42]. In addition to the decrease in vascular density, reduced expression of VEGF was also observed in tumors in mPGES-1 KO mice (Fig. 5E). It has been reported that angiogenesis induced by either endogenous COX-2 or exogenous PGE_2 was accompanied by increased expression of VEGF and that angiogenesis was abolished by administration of an antisense oligonucleotide specific for VEGF mRNA [19]. These results suggest that the host stromal COX-2/mPGES-1-derived PGE_2 regulates the expression of VEGF via EPs in tumor tissues, leading to increased tumor-associated angiogenesis. Amano *et al.* have found that the growth of LLC-implanted tumors was markedly suppressed in EP3 KO mice, in which both VEGF expression, possibly under the control of the transcription factor AP-1, and tumor-associated angiogenesis were concomitantly reduced [44]. This phenotype observed in EP3 KO mice is similar to those observed in mPGES-1 KO (this study) and COX-2 KO mice [20]. These studies indicate that, in the tumor milieu, both cancer cells and adjacent stromal cells synthesize (via COX-2/mPGES-1) and release PGE_2, which in turn acts on the particular population of stromal cells that express EP3 to induce the production of VEGF and consequent angiogenesis [44]. Besides such microenvironmental effect, the direct action of PGE_2 on the proliferation of adjacent tumor cells should also be taken into account.

In addition to tumor 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 (Fig. 6). VEGF, which is up-regulated in response to PGE_2 produced by the host stromal COX-2/mPGES-1, may participate in lung metastasis through its potent ability to enhance vascular permeability. The metastatic phenotype observed in mPGES-1 KO mice was similar to that observed in MMP-2 KO mice: in both models, focal xenograft propagation and lung metastasis of LLC cells were reduced [45]. Consistent with these findings, MMP-2 activity in the metastasized lung tissues was markedly lower in mPGES-1 KO mice than in WT mice (Fig. 6D). Thus, in the metastatic foci, the PGE_2 derived from the host mPGES-1 may lead to the increase in the activity of MMP-2, which, in cooperation with VEGF, may promote the invasion of cancerous cells into the adjacent and distant tissues, thereby allowing subsequent expansion and metastasis of the tumor.

In conclusion, our results suggest that both cancer cell-associated and host-derived mPGES-1 is critical for tumor growth and metastasis. The PGE_2 produced by cancer cell-associated mPGES-1 may increase the proliferation and malignant potential of these cells in an autocrine/paracrine manner and activate stromal cells in a paracrine manner. Then, the mPGES-1-driven PGE_2 signaling on stromal cells may be functionally linked to the induction of potent proangiogenic and matrix-degrading factors, which in turn would facilitate tumor development. Recent 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 unfavorable effects on the circulation system [12]. Therefore, an mPGES-1 inhibitor would exhibit a chemopreventive action on various tumors 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.

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*Prof. 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 Prof. Kudo who showed him kindness during his lifetime.

FIGURE CAPTION

Figure 1 PGE_2 production and cell proliferation in mPGES-1-silenced LLC cells *in vitro*.

A, expression of PGE_2-biosynthetic enzymes and PGE receptors in mPGES-1 KD (*right lane*) and control (mock, *left lane*) cells was assessed by immunoblotting. Equal amounts of cell lysates (20 μg protein equivalents) were separated by SDS-PAGE and Western blotted at the same time with the corresponding antibodies to allow for a direct comparison. Representative results of at least three experiments are shown. *B-D*, effects of mPGES-1 silencing and 10 nM NS-398, a COX-2 selective inhibitor, on production of PGE_2 (*B*), 6-keto $PGF_{1\alpha}$ and $PGF_{2\alpha}$ (*C*) and on cell proliferation (*D*). mPGES-1 KD and mock cells were seeded at 6 x 10^4 cells/well in 6-well culture dishes in the presence or absence of 10 nM NS-398. After culture for 3 days, the cells were collected and counted in a Bright-line hemocytometer in the presence of trypan blue, and the supernatants were taken for enzyme immunoassay of several PGs. *E*, effect of $dmPGE_2$ on cell proliferation. mPGES-1 KD and mock cells were seeded in T-25 culture flasks at 1.5 x 10^5 cells/flask in culture medium in the presence or absence of 1 μM $dmPGE_2$. After culture of 3 days, the cells were collected and counted. In *B-E*, values are the means ± S.E. of five independent experiments. Similar results were obtained in three independent cell line of mPGES-1 KD and control cells.

Figure 2 siRNA silencing of mPGES-1 in LLC cells reduces their malignant potential *in vitro*.

A, mPGES-1 KD and mock cells (1.2 x 10^6 cells in DMEM containing 3% (v/v) FCS) were seeded onto the upper wells of BD BioCoat™ Matrigel™ Invasion Chambers. DMEM containing 10% FCS was added as a source of chemoattractants into the bottom wells of the plates. After 16 h of incubation, cells that had invaded onto the lower surface of the chambers were fixed, stained with crystal violet, and counted (*left panel*). Values are the means ± S.E. of five independent experiments. Representative photographs of mPGES-1 KD and mock cells that invaded across the Matrigel™ -coated inserts are shown (*right panel*). *B*, the expression of VEGF protein in mPGES-1 KD and mock cells was assessed by immunoblotting (*upper panel*). The cell lysates (20 μg protein equivalents) were separated on SDS-PAGE and then subjected to Western blot analysis using anti-VEGF and anti-α-tubulin antibodies. The activities of MMP-2 and -9 in mPGES-1 KD and mock cells were assessed by gelatin zymography (*lower panel*). Lysates of cells (27 μg protein equivalents) were subjected to SDS-PAGE containing 1 mg/ml gelatin. Following electrophoresis, the gels were incubated in a bath containing 1 μM $ZnCl_2$ at 37°C for 18 h. The gels were then stained with 0.1% Coomassie blue. *C*, adhesion of mPGES-1 KD and mock cells to ECM proteins. mPGES-1 KD or mock cells (2 x 10^5 cells) were seeded onto 35 mm dishes coated with collagen, fibronectin or laminin in culture medium in the presence or absence of 1 μM $dmPGE_2$. After allowing cells to attach for 60 min at 37°C, non-adherent cells were removed by washing. Adherent cells were fixed and stained with Giemsa solution and counted in three fields at 40 x magnification using a microscope and J image software. Values are the means ± S.E. of five independent experiments. *D*, Expression of α5 and β1 integrins in mPGES-1 KD and mock cells. Total RNA was isolated from mPGES-1 KD and mock cells and subjected to quantitative RT-PCR using specific primers of α5 (*left*) and β1 (*right*) integrins and those of GAPDH as a reference. The results of quantitative RT-PCR were normalized with their expressions in mock cells being regarded as 1 (mean ± S.D., n = 3). Several representative results of two independent cell line of mPGES-1 KD and control cells.

Figure 3 Increased PGE_2 generation, proliferation and invasion of mPGES-1-overexpressing LLC

cells.

A, expression of COX-2 and mPGES-1 in mPGES-1-overexpressing and mock cells was assessed by Western blotting. Representative results of at least three experiments are shown. *B and C*, effects of mPGES-1 overexpression on PGE_2 generation (*B*) and cell growth (*C*). mPGES-1-overexpressing and mock cells were seeded at 6×10^4 cells/well in 6-well culture dishes. After culture for 2 days, the cells were collected and counted, and the supernatants were taken for PGE_2 enzyme immunoassay. Values are the means $\pm$ S.E. of five independent experiments. *D*, mPGES-1-overexpressing and mock cells (1.2×10^6 cells in the presence or absence of 10 nM NS-398) that had invaded through BD BioCoat™ Matrigel™ Invasion Chamber inserts over 16 h were counted. Values are the means $\pm$ S.E. of five independent experiments. The results are representative of two independent cell line of mPGES-1-overexpressing and mock cells.

Figure 4 siRNA silencing of mPGES-1 in cancer cells reduces tumorigenesis *in vivo*.

A-C, mPGES-1 KD or mock cells (10^6 cells) were injected into the subcutaneous tissue of female BALB/c mice. On day 14 after implantation, tumor volume was scored according to the formula (volume = (length x width^2) x 0.5) (*A*, *left panel*). The tumor tissues were photographed at x 100 magnification (*B*), dissected and weighed (*A*, *right panel*). *C*, amounts of PGE_2, $PGF_{2\alpha}$ and 6-keto $PGF_{1\alpha}$ in homogenates of the tumor tissues were quantified by enzyme immunoassay. *D*, expression of VEGF in tumor xenografts of mPGES-1 KD and mock cells was assessed by RT-PCR. Results are presented as the means $\pm$ SEM (n = 9) in *A and C*, and representative results are shown in *B and D*.

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*, tumor volumes were scored on the indicated days as described in the Materials and Methods. *B*, on day 14 after implantation, the tumor tissues were dissected and weighed (*left panel*). Amounts of PGE_2 and $PGF_{2\alpha}$ in homogenates of the tumor tissues were quantified by enzyme immunoassay (*right panel*). *C*, tumor tissues were cut out, fixed in formalin, and stained with silver, hematoxylin and eosin for histochemical analysis. Microvessels and necrotic regions are shown by *arrowheads* and an *asterisk*, respectively. *D*, vascular density (*left panel*) and hemoglobin content (*right panel*) were determined as described in the Materials and Methods. *E*, expression of VEGF protein (*upper panel*) and mRNA (*lower panel*) in the tumor tissues was determined by Western blotting and RT-PCR analysis, respectively. Results are presented as the means $\pm$ SEM (n = 9) in *A, B, and D* and representative results are shown in *C and E*.

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 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 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 $\pm$ SEM (n = 4) in B, and representative results are shown in *A, C and D*.

FIGURES

Figure. 1

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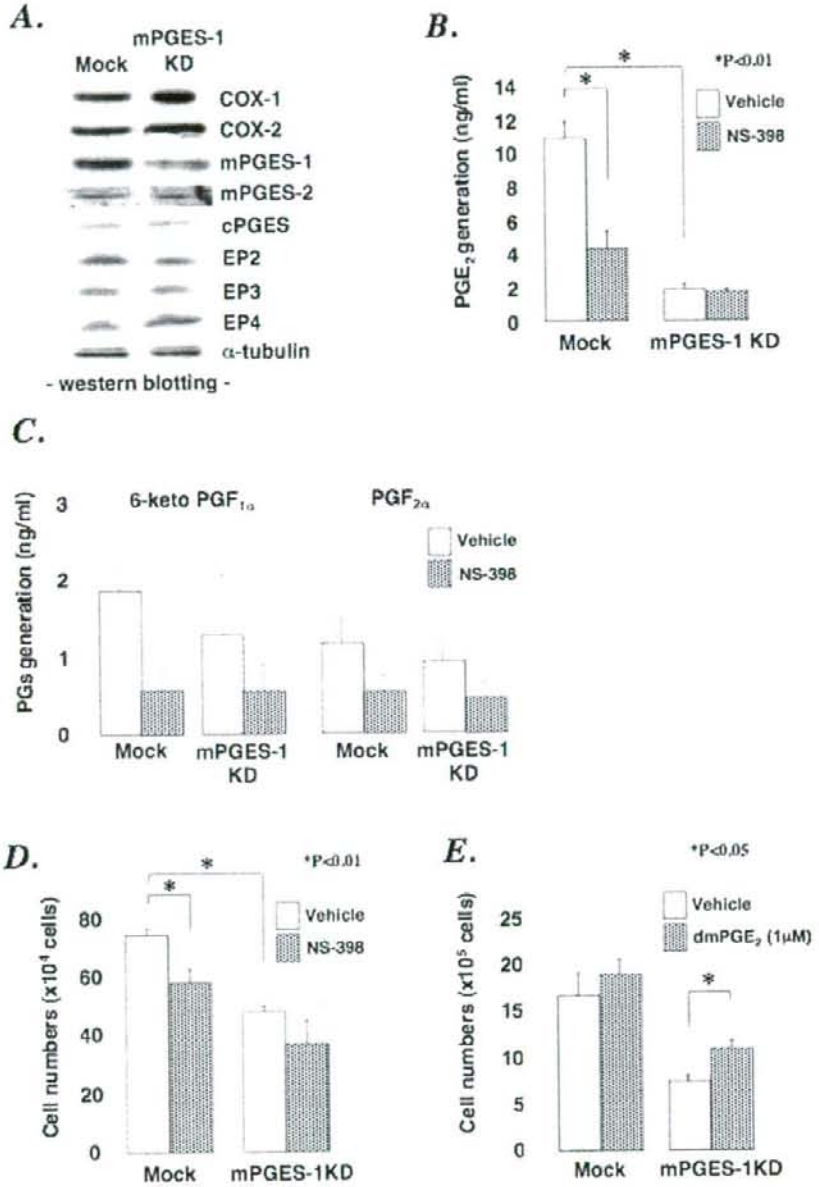

Figure. 2 D. Kamei et al

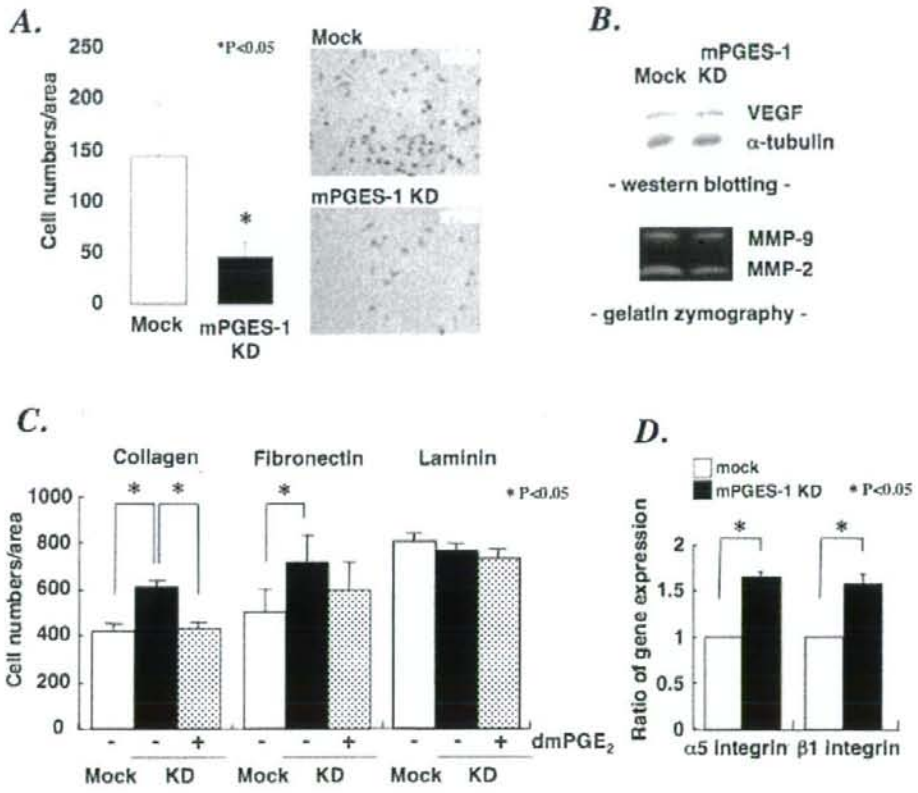

Figure. 3

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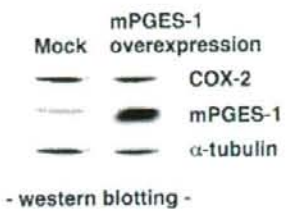
A.



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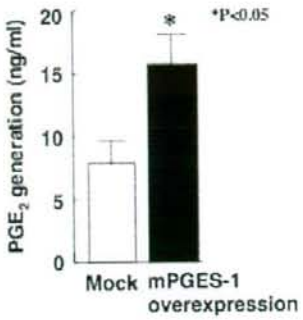

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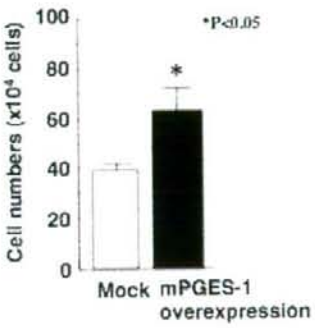

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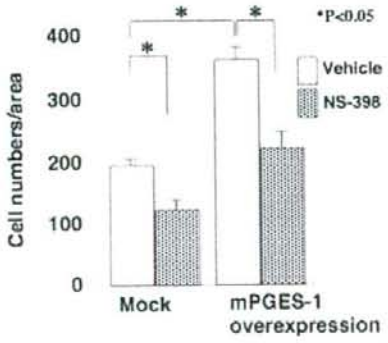

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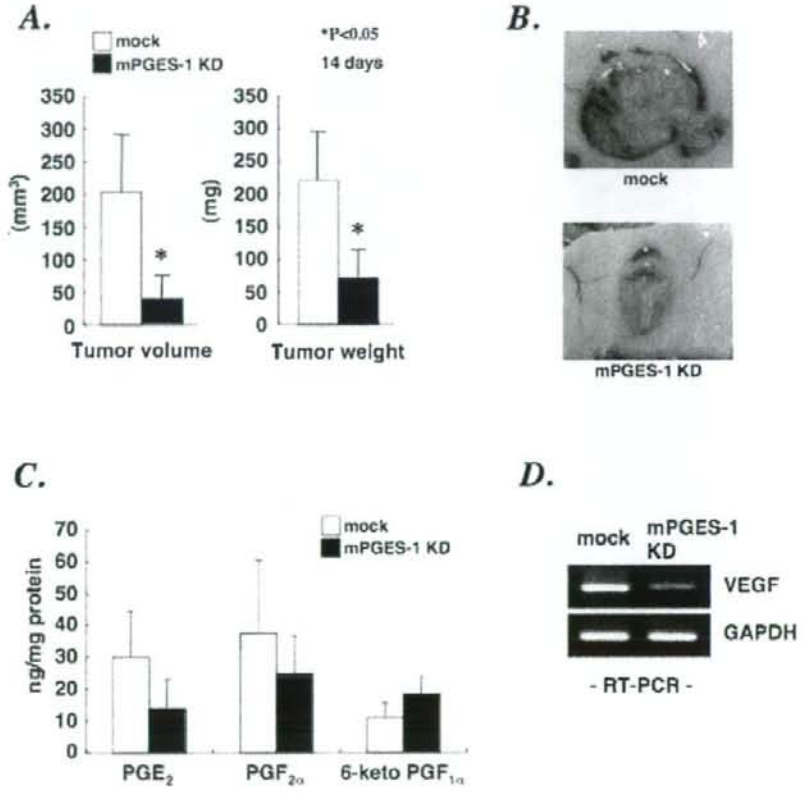

Figure. 5

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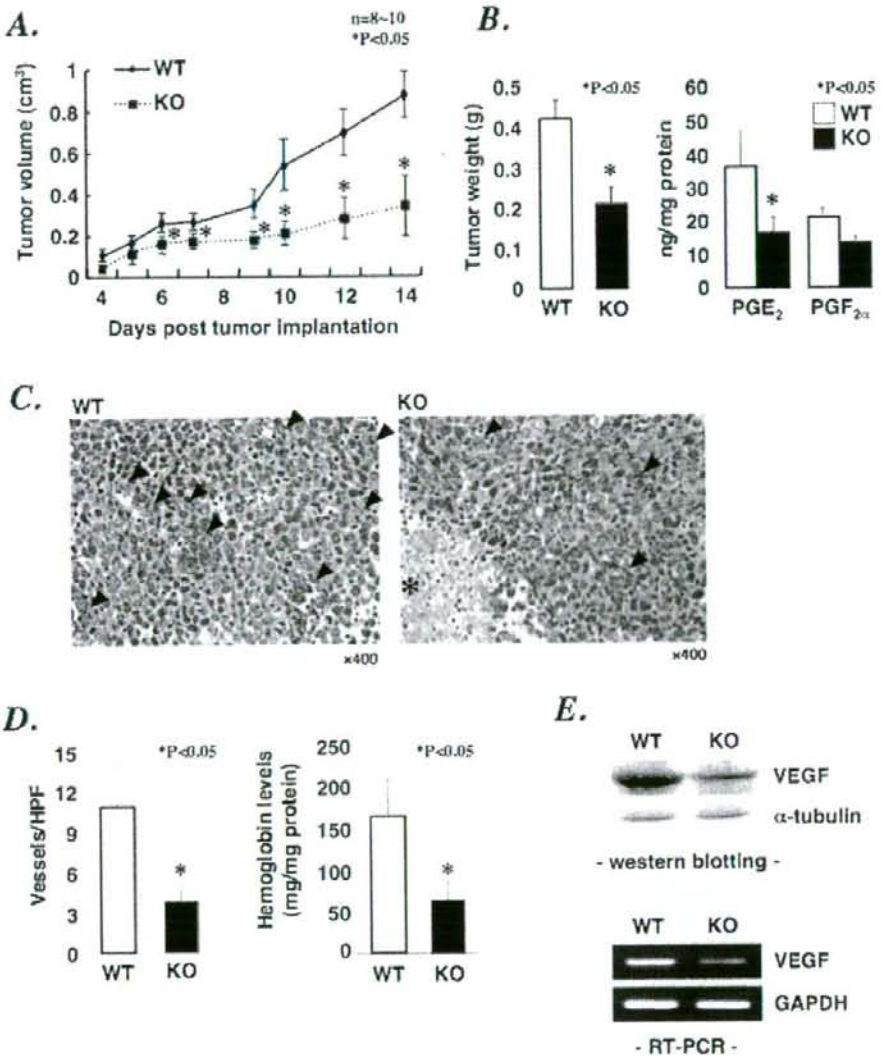

Figure. 6

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