Roles of an inducible prostaglandin E synthase, mPGES-1 in host in enhancement of tumor-associated angiogenesis

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Background: Microsomal prostaglandin (PG) E synthase 1 (mPGES)-1 is a major PGE synthase and has recently been reported to be expressed at high levels in several cancer types. We previously reported that the PGE receptor EP3 is expressed in bone marrow (BM)-derived cells, enriched in stromal tissue, and enhances the potential for tumor angiogenesis.

Objective: In the present study, we examined the role of mPGES-1 in the host tissues in enhancement of tumor-associated angiogenesis.

Materials and Methods: We used 8-week-old male mPGES-1 KO mice backcrossed with their wild-type counterparts (WT, C57BL6). Lewis lung carcinoma (LLC) cells were injected into the subcutaneous dorsal tissue of mice. Tumor growth was evaluated over time. Serial sections were stained with hematoxylin and eosin (H&E). Microvessel density (MVD) and microvessel area (MVA) were quantified as parameters of angiogenesis. High-dose irradiated WT mice were divided into two groups. mPGES-1 BM cells collected from each of the femurs, tibias, and pelvis were injected via the tail vein into irradiated WT mice. Another group were injected with BM cells from WT Mice. Angiogenesis was also evaluated.

Results: Growth and tumor-associated angiogenesis were suppressed in mPGES-1 knockout mice (mPGES-1−/−) after the subcutaneous implantation of Lewis lung carcinoma cells, in comparison with those in wild-type (WT) mice. After lethal radiation, WT BMs were replaced with BM cells isolated from mPGES-1−/−. The levels of neoangiogenesis in the sponge implants measured in mPGES-1−/− BM chimeric mice were significantly reduced compared to those observed in WT BM chimeric mice. Tumor-associated angiogenesis as measured by histological analysis was localized to tumor stroma, and was significantly lower in mPGES-1−/− BM chimeric mice compared to that in WT BM chimeric mice. Tumor sections probed by immunohistochemistry revealed that vascular endothelial growth factor (VEGF) that was present in the stromal tissue was markedly reduced in mPGES-1−/− BM chimeric mice compared to wild-type BM chimeras.

Conclusions: These results suggest that host mPGES-1 enhanced tumor-associated angiogenesis, and that regulation of mPGES-1-expressing BM cell recruitment to the site of primary tumors may be a novel strategy for the treatment of solid tumors.

Key words: mPGES-1, anginogenesis, cancer, bone marrow-derived cells

Introduction

Recent evidence demonstrates that angiogenesis is an essential part of cancer development and is localized predominantly to the tumor microenvironment.1,4 A major component of the tumor microenvironment includes macrophages and fibroblasts,5 which play a definitive role in facilitating angiogenesis. Recently, bone marrow (BM)-derived hematopoietic cells were shown to be major components of tumor-associated stroma, and these cells can regulate the tumor microenvironment.6 However, the particular factors that
constitutively expressed and mPGES-1 is primarily expressed in various normal tissues, whereas COX-2 expression is induced by mitogens, cytokines, and stimuli with a concomitant increase in COX-2 expression. Disruption of COX-2 gene expression in mice was sufficient to reduce the size of mutated adenomatous polyposis (APC) derived intestinal polyps, suggesting that COX-2 is important for the development of colon cancers. COX-2 is expressed in various types of cancer tissues, and accumulating evidence suggests that COX-2 plays a significant role in the development of many cancer types, including colon cancer. COX-2 specific inhibitors have been predicted to act as better versions of aspirin with none of the adverse effects attributable to classical NSAIDs. However, more recent reports suggest that COX-2 inhibitors increase the incidence of adverse coronary events, which has hampered the identification and development of novel COX-2 inhibitors. Another therapeutic strategy aimed at circumventing these treatment considerations, however, involves targeting and inhibiting PG activity for the treatment of solid malignancies.

Previous studies, using a PG receptor (EP3) knockout (KO) mouse model, have demonstrated that stromal PGEs-EP3 receptor-mediated signaling is essential for angiogenesis and tumor growth. Growth of tumors and formation of tumor neovasculature are markedly suppressed in EP3 receptor KO mice (EP3−/−) when compared to wild-type (WT) counterparts. Furthermore, the angiogenesis-dependent wound healing process is significantly delayed in EP3−/− mice. Together, these results suggest that stromal PGEs plays an important role in tumor growth and angiogenesis. Thus, PGe biosynthesis is a promising therapeutic target for controlling tumor-associated angiogenesis.

PGEs is the most abundant PG in the human body, and is formed from arachidonic acid by COX-catalyzed formation of PGHs and subsequent transformation by PGE synthases. The isomerization of the endoperoxide PGHs to PGEs is catalyzed by three different PGE synthases: the cytosolic PGE synthase (cPGES) and two membrane-bound PGE synthases, mPGES-1 and mPGES-2. Of these, cPGES and mPGES-2 are constitutively expressed and mPGES-1 is primarily induced. cPGES uses PGHs produced by COX-1, and mPGES-1 requires endoperoxide derived from COX-2. Interestingly, mPGES-2 can use both sources of PGHs. The enzyme mPGES-1 is a member of the membrane associated protein superfamily involved in eicosanoid and glutathione metabolism (MAPEG). mPGES-1 is upregulated in response to various proinflammatory stimuli with a concomitant increase in COX-2 expression. Thus, mPGES-1 inhibition may be a promising target in the treatment of COX-2-dependent pathological conditions with fewer coronary side effects.

We recently developed mPGES-1 KO mice (mPGES−1−/−) that revealed key roles of mPGES-1-generated PGEs in female reproduction and pathological conditions, such as inflammation, pain, stroke, and tumorigenesis. These findings suggested that mPGES-1 holds merit as a potential target for the development of therapeutic agents against several diseases. Here, we demonstrate that host stromal mPGES-1 enhances tumor-associated angiogenesis and that mPGES-1-expressing bone marrow cells participate in the development of tumors and the tumor-associated angiogenesis. The present study highlights the significance of mPGES-1 as a target of cancer angiogenesis, and suggests that the blockade of mPGES-1 activity and the recruitment of mPGES-1-expressing bone marrow cells may be a novel and effective strategy to treat solid cancers.

Materials and Methods

mPGES-1 KO mice
We used 8-week-old male mPGES-1 KO mice developed by us and backcrossed with their WT counterparts (C57BL6). All mice were housed with controlled levels of humidity (60 ± 5%) and temperature (25 ± 1°C), with a 12 h light/dark cycle. All animal experiments were performed in accordance with the guidelines for animal experiments of Kitasato University School of Medicine.

Determination of tumor-associated angiogenesis
Murine Lewis lung carcinoma (LLC) cells were cultured in Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 10% fetal bovine serum (Gibco BRL Life Technologies, Rockville, MD, USA). Cells were washed in phosphate-buffered saline and suspended in the same solution at a density of 2 × 10⁶ cells/ml, and 50 μl of the resulting suspension was injected into the subcutaneous dorsal tissue of mice. Each resulting tumor was allowed to grow, was excised, and subsequently fixed in formaldehyde. Serial sections were
stained with hematoxylin and eosin (H&E). Microvessel density (MVD) and microvessel area (MVA) were quantified as parameters of angiogenesis as described previously. All experiments were performed in accordance with the guidelines for animal experimentation of the Kitasato University School of Medicine.

**Sponge implantation model**

Sponge disks (thickness, 5 mm; diameter, 1.3 cm) were subcutaneously implanted into the dorsal tissue of mice anesthetized with Nembutal. After several days’ incubation the granulation tissues that had formed around the sponge were excised, and each granulation specimen was stained with H&E. MVD and MVA were assessed as previously described.

**Chamber implantation model**

LLC cells were washed as described above and suspended in PBS at a density of 10^6 cells/mL. Thereafter, 10^7 LLC cells in 0.1 mL of solution were placed in a round chamber (Plexiglas Rings), which consisted of a ring enclosed on both faces by cellulose ester filters (pore size, 0.45 μm; Millipore, Bedford, MA, USA). Chambers were subcutaneously implanted into the dorsum of mice. After several days’ incubation, the granulation tissues formed around the chamber were immediately excised. After the chamber was removed, half of each resulting granulation specimen was assayed using polymerase chain reaction (PCR), and the other half fixed, sectioned, and stained by H&E. MVD and MVA were assessed as previously described.

**Preparation of BM chimera mice**

BM cells were obtained by flushing with phosphate-buffered saline (PBS) the cavities of dissected femurs, tibias, and pelvic regions of donor mPGES-1 KO mice (mPGES-1^−/−). Sex was not a factor in harvesting BM cells. Donor BM cells of WT counterparts (8 week-old male C57BL/6 mice) were also harvested by the same method. The flushed BM cells from each donor were dispersed by pipetting and resuspended in PBS at a density of 10^7 cells/mL. WT mice (8 week-old male C57BL/6 mice) were lethally irradiated with 10.0 Gy using an MBR-1505R X-ray irradiator (Hitachi Medico Co., Tokyo), and a filter (copper, 0.5 mm; aluminum, 2 mm) monitored the cumulative radiation dose. BM mononuclear cells (2 × 10^6 cells) suspended in 200 μl of PBS were injected via the tail vein into irradiated WT mice.

**Immunochemistry**

Excised tumors and granulation tissues collected 7-21 days postimplantation were fixed with 4% paraformaldehyde in 0.1 mol/l sodium phosphate buffer (pH 7.4), dehydrated with a graded series of ethanol solutions, and embedded in paraffin. Sections (4 μm in thickness) were cut from the paraffin-embedded tissue and were mounted on glass slides. After removal of paraffin with xylene, the slides were placed in cold (4°C) acetone. Sections were subjected to hematoxylin and eosin (H&E) staining or immunostaining. For immunohistochemical analysis, sections were incubated with normal horse serum and subsequently incubated with rabbit anti-mouse mPGES-1 (Santa Cruz Biotechnology) or rabbit anti-mouse CD31 (Santa Cruz Biotechnology). Immune complexes were detected with a LSAB+System-HRP kit (DakoCytomation, Carpinteria, CA, USA).

**Reverse transcriptase (RT)-PCR**

Total RNA was extracted from mice tissues using TRIzol® Reagent (Invitrogen, San Diego, CA, USA) according to the manufacturer’s instructions, and cDNA was reverse transcribed from 1 μg of total RNA with ReverTra Ace-α™ (Toyobo Co., Ltd.). Quantitative PCR amplification was performed using SYBR® Premix Ex Taq™ (Takara Bio Inc., Shiga). PCR was performed in 20 μl reactions according to the method of the manufacturer. The reaction mixture was then subjected to 35 cycles of amplification in a DNA thermal cycler. Each cycle consisted of a heat-denaturation step at 95°C for 1 minute, an annealing of primers step at 55°C for 1 minute, and an extension step at 72°C for 1 minute. Primers used were as follows: 5’-AACCATGAACTTTCTGCTCTC-3’ (sense) and 5’-GTGATTTTCTGGCTTTGTTC-3’ (anti-sense) for VEGF, and 5’-CCCTTCATTGACCTCAACTACAATGGT-3’ (sense) and 5’-GAGGGGCCATCCACAGTCTTCTG-3’ (anti-sense) for GAPDH.

**Statistics**

Data are expressed as the mean ± SEM. Comparisons among multiple groups were performed by factorial analysis of variance (ANOVA) followed by Scheffé’s test. Comparisons between the two groups were performed with Student’s t-test. A P value of <0.05 was considered statistically significant.
Figure 1. Tumor growth and angiogenesis are blunted in tumors in mPGES-1 KO mice versus tumors in WT mice.
A. Typical representation of granulation sponge tissues excised from BM chimeric mice. Surgical sponges were subcutaneously implanted into WT (WT → WT) and mPGES-1−/− chimeric (KO → WT) mice. Granulation tissues became apparent 14 days postimplantation. The scale bar depicts 5 mm.

B. H&E staining of granulation tissues formed after sponge implantation in BM chimeric mice. The granulation tissues formed around the sponge implants in WT (WT → WT) mice and mPGES-1−/− (KO → WT) chimeric mice postimplantation were excised and stained with H&E. Arrows indicate neovascularization. The scale bar represents 100 μm.

C, D. Sponge implants were implanted as described, then excised after 14 days incubation. Measures of stromal angiogenesis were determined by calculating the Microvessel Density (C) and Microvessel Area (D). Data are expressed as the mean ± SEM (5 mice per group). *P < 0.05 compared to the WT → WT group (unpaired Student's t-test).

**Figure 2.** Sponge-induced angiogenesis, as quantified by MVD and MVA was reduced in cancer stroma from BM chimeric mice compared to tumor stroma from WT mice.
A. Gross excised granulation tissues that accumulated on tumor cell-containing chambers in mice. Chambers were implanted into the subcutaneous tissues in BM chimeric mice. Seven days postimplantation, chambers were excised and granulation tissues were photographed. Dotted circles indicate the site of chamber insertion. The scale bar denotes 5 mm.

B. H&E staining of granulation tissues formed around tumor cell chambers isolated from WT (WT → WT) and mPGES-1−/− (KO → WT) chimeric mice. Arrows denote loci of neovascularization. Bar represents 100 μm.

C, D. Levels of cancer-induced neovascularization are reduced in mPGES-1−/− tumors compared to tumors in WT mice. Chambers containing LLC cancer cells were subcutaneously implanted as previously described. To measure angiogenesis in the granulation tissues surrounding the cancer cell chambers, MVD and MVA were calculated by examination of tumors excised 7 days postimplantation. Data are expressed as the mean ± SEM (5 mice per group). *P < 0.05 versus WT → WT chimeric mice as calculated using the unpaired Student's t-test.

E. VEGF-A expression levels are reduced in granulation tissues surrounding tumor cell chambers in BM chimeric mice compared to tumor chambers in WT mice. mRNA was isolated from granulation tissue harvested from tumor cell chambers excised from BM chimeric mice or WT mice, and cDNA was used to examine VEGF-A expression levels by real-time PCR. Data are expressed as the mean ± SEM (4 and 5 mice per group, respectively). *P < 0.05 compared to WT → WT chimeric mice, as measured by an unpaired Student's t-test.

Figure 3. BM chimeric mice implanted with tumor cell-containing chambers display marked neovascularization surrounding the site of the cancer.
A. Gross tumor appearance after excision from BM chimeric mice. LLC cells were subcutaneously implanted and allowed to grow for 21 days. Resulting tumors from WT (WT → WT) and mPGES-1−/− (KO → WT) chimeric mice were excised and photographed. The scale bar indicates 5 mm.

B. Changes in tumor volume in WT (WT → WT) and mPGES-1−/− (KO → WT) chimeric mice. Data are represented as the mean ± SEM (10 mice per group). *P < 0.05 compared to WT mice as measured by ANOVA.

C, D. Tumor-associated angiogenesis was determined by calculating MVD and MVA in LLC tumors excised after 7 days of tumor growth. Data are expressed as the mean ± SEM (7 mice per group). *P < 0.05 compared to WT mice as calculated using the unpaired Student’s t-test).

E, F. Tumor-associated angiogenesis determined by MVD and MVA 14 days after injection of LLC cells. Data are expressed as the mean ± SEM (7-8 mice per group). *P < 0.05; **P < 0.01 compared to WT mice as measured by the unpaired Student’s t-test).

G. VEGF-A expression was quantified by real-time PCR. mRNA was isolated from LLC tumors and surrounding stromal tissues excised from BM chimeric mice or WT mice. *P < 0.05 compared to wild-type mice, as measured by the unpaired Student’s t-test).

Figure 4. Tumor growth and angiogenesis are reduced in BM chimeras compared to tumors implanted in WT mice.
Results

Role of mPGES-1 in tumor-associated angiogenesis and tumor growth

Lewis lung carcinoma (LLC) cells were subcutaneously implanted on the dorsal skin of WT mice and mPGES-1 KO mice, and tumor growth and angiogenesis were examined. The neovascularization of tumors in mPGES-1−/− mice was reduced compared to that in WT mice (Figure 1A). In addition, LLC tumor growth in mPGES-1−/− mice was significantly reduced compared to that in WT mice (Figure 1B). Twenty-one days after LLC cell implantation, the primary tumor and surrounding stromal tissues were excised. H&E staining revealed that the vessel-like structures were predominantly localized to the stroma. mPGES-1−/− mice displayed less cancer neovasculature compared to that seen in WT animals (Figure 1C). Quantitation of angiogenesis by measurement of MVD (Figure 1D) and MVA (Figure 1E) revealed that these events are reduced in mPGES-1−/− mice compared to WT mice.

Reduced angiogenesis in granulation tissues formed around the sponge implants and chambers filled with LLC cells in mPGES-1 KO BM chimera mice

WT mice irradiated with were injected intravenously with BMCs isolated from mPGES-1 KO mice or from WT mice. After reconstitution of BM cells, we examined neoangiogenesis in granulation tissues on implanted circular sponge disks in mPGES-1−/− BM chimeric mice or in WT BM chimeric mice. Fourteen days after sponge implantation, newly formed granulation tissues were isolated and stained with H&E. We observed that the degree of redness of excised granulation tissues varied between mouse models, suggesting that angiogenesis in mPGES-1−/− BM chimeric mice was reduced compared

Figure 5. mPGES-1 expression is reduced in mPGES-1−/− BM chimeric mice compared with WT mice.
to that in WT BM chimeric mice (Figure 2A).

H&E staining revealed that the vessel-like structure was predominantly located in granulation tissues surrounding the implanted sponge. mPGES-1−/− BM chimeric mice displayed tumors that were less vascularized than tumors in WT BM chimeras (Figure 2B). Evaluation of angiogenesis, as measured by the quantitation of MVD and MVA, revealed that the MVD (Figure 2C) was significantly reduced in mPGES-1−/− BM chimeric mice compared to WT BM chimeric mice. Together, there was a reduced level of angiogenesis using the tumor cell-infiltrated chamber model when these chambers were implanted in BM chimeric mice that were recipients for BMCs isolated from mPGES-1 KO mice.

In an effort to identify the roles of mPGES-1 expressing BM-derived cells in tumor-associated angiogenesis, we compared angiogenesis in the granulation tissues formed around the chambers containing LLC cells in BM chimeric mice. Seven days after the chamber implantation, surrounding granulation tissues were isolated. The reddish appearance of granulation tissue in mPGES-1−/− BM chimeric mice was suppressed, compared with WT BM chimeric mice (Figure 3A), suggesting that tumor-initiated angiogenesis was reduced when mPGES-1 was present. H&E staining revealed that the vessel-like structure was predominantly located in the granulation tissues that faced to the membranes of the chambers. mPGES-1−/− BM chimeric mice displayed less vascularization compared with WT chimeric animals (Figure 3B). Evaluation of tumor-associated angiogenesis showed that the MVD (Figure 3C) and the MVA (Figure 3D) were reduced significantly in mPGES-1−/− BM chimeric mice when compared with WT BM chimeric mice. The expression of VEGF in the granulation tissues were reduced significantly in mPES-1−/− BM chimeric mice when compared with WT BM chimeric animals (Figure 3E).

Reduced tumor-associated angiogenesis and tumor growth in mPGES-1 KO BM chimera mice

We next compared the levels of tumor-associated angiogenesis in tumor models using BM chimeric mice. Seven to 21 days after LLC implantation, the primary cancer and surrounding stromal tissues were excised. The reddish appearance of tumors in mPGES-1−/− BM chimeric mice was reduced compared to that observed in WT BM chimeric mice (Figure 4A). Transplantation of BMCs from mPGES-1−/− animals significantly reduced tumor growth, in contrast with tumors harvested from mice possessing WT BM cell implantation (Figure 4B). Quantitative evaluation of characteristics of angiogenesis revealed that both the MVD (Figure 4C, 4E) and the MVA (Figure 4D, 4F) were reduced in mPGES-1−/− BM chimeric mice compared to WT BM chimeras. Real-time PCR analysis revealed that VEGF-A mRNA levels were drastically reduced in mPGES-1−/− BM chimeric mice compared to WT BM chimeric mice (Figure 4G). H&E staining of tumor and stromal sections revealed that the neovasculature was predominantly localized to the stroma surrounding the site of the primary tumor. mPGES-1−/− BM chimeric mice also displayed less vascularization than WT BM chimeric mice (Figure 5A). In addition, immunohistochemical detection of mPGES-1 demonstrated that expression of mPGES-1 14 days postimplantation was markedly suppressed in mPGES-1−/− BM chimeric mice compared to WT BM chimeric mice (Figure 5B), suggesting that mPGES-1-expressing cells accumulate in the stroma and may be recruited from BMs. Additionally, the reduction in stromal mPGES-1 expression in mPGES-1−/− BM chimeric mice was accompanied by impaired formation of CD31-positive vessels in mPGES-1−/− BM chimeras (Figure 5C).

Discussion

Non-steroidal anti-inflammatory drugs (NSAIDs) and specific inhibitors of cyclooxygenase (COX)-2 are therapeutic strategies used for the treatment of pain, inflammation and fever.10 Accumulating experimental and clinical evidence suggest that NSAIDs and COX-2 inhibitors also have anti-cancer activities.8 Epidemiological studies have shown that regular use of aspirin and other NSAIDs reduces the risk of developing cancers, in particular colon cancer. Molecular studies have revealed that COX-2 is expressed by cancer cells as well as by tumor stromal tissue, and may also be induced in response to chemotherapy or radiotherapy. Experimental studies have demonstrated that COX-2 overexpression contributes to tumorigenesis. In addition, studies have shown that NSAIDs and COX-2 inhibitors suppress tumorigenesis and tumor progression. More recently, results from clinical trials suggest that NSAIDs and COX-2 inhibitors suppress colon polyp formation and malignant progression in patients with familial adenomatous polyposis (FAP).11 Recent advances in the understanding of the cellular and molecular mechanisms of the anticancer effects of NSAIDs and COX-2 inhibitors have demonstrated that these drugs target the tumor vasculature suggesting their importance as novel anticancer therapeutics.

Angiogenesis, or the formation of new blood vessels from pre-existing microvasculature, is an important
mechanism that increases the blood supply carrying nutrients and oxygen that are required for cancer growth and development. In addition, angiogenesis is initiated in early stages of cancer growth. Major components of the tumor microenvironment include macrophages and fibroblasts, which both contribute to angiogenesis. Recently, BM-derived hematopoietic cells were found to be major components of tumor-associated stroma, and are important regulators of the tumor microenvironment. However, the particular factors that enhance the functions of BM-derived precursor cells and the mechanism of recruitment of these cells during tumor angiogenesis are not well understood.

mPGES-1 is a member of the MAPEG (Membrane-Associated Proteins involved in Eicosanoid and Glutathione metabolism) superfamily. This family of proteins contains other proteins involved in arachidonic acid metabolism, such as 5-lipoxygenase-activating protein (FLAP) and leukotriene C4 synthase. mPGES-1 expression is predicted to be associated with various pathophysiological events, such as rheumatoid arthritis, febrile response, reproduction, bone metabolism, and Alzheimer’s disease, in which COX-2-derived PGE2 has been implicated. A recent gene-targeting study of mPGES-1 demonstrated that PGE2 production by lipopolysaccharide-stimulated peritoneal macrophages depends almost entirely on this enzyme. Induced expression of mPGES-1 is regulated by the NF-IL-6 pathway and by the mitogen-activated protein kinase pathway, the latter of which may switch on the inducible transcription factor Egr-1 that in turn binds to the proximal GC box in the mPGES-1 gene promoter region, leading to mPGES-1 transcription. mPGES-1 has been linked with tumorigenesis with the recent observation that mPGES-1 is constitutively expressed in several cancers, many of which also constitutively express COX-2.

Previous work with PG receptor KO mice has revealed that PGE2-stimulated EP receptors have proangiogenic effects. Thus, to examine the role of PGE2 as a functional regulator of tumor microenvironment, we studied the effect of this gene on tumor growth and angiogenesis in mPGES-1 KO mice with LLC tumors. Both tumor proliferation and tumor-induced angiogenesis were reduced in mice lacking mPGES-1, suggesting that host mPGES-1 plays a significant role in suppressing tumor growth and tumor associated angiogenesis. This was consistent with our previous findings. Further, BM-derived cells may enable or enhance tumor-induced angiogenesis. Therefore, we constructed a BM chimeric mouse model by replacing host BM cells with the BM cells from mPGES-1 KO mice. Based on the data from these models, PGs may be important regulators of tumor-host communication.

To clarify the functional relevance of mPGES-1 in BM-derived cells, selective knockdown of stromal mPGES-1 was achieved in BM-derived cells from mPGES-1 KO chimeric mice. Data demonstrated that the recruitment of mPGES-1-expressing BM cells to stromal tissues is crucial for tumor growth and tumor-associated angiogenesis, and concomitantly occurs with gene expression of stromal VEGF (Figures 4, 5). Recently, it was reported that mice deficient in cytosolic phospholipase A(2) are protected against the development of lung tumors. Mouse lung cancer cells (CMT167 and Lewis lung carcinoma cells) injected directly into lungs of syngeneic mice formed a primary tumor at the site of injection, and subsequently metastasized to other lobes of the lung as well as to mediastinal lymph nodes. WT mice transplanted with cPLA(2)-KO BM had a marked survival advantage after inoculation with tumor cells compared to mice that received WT BM. Together, these results suggest that stromal cPLA(2) from BM plays a critical role in tumor progression by altering tumor-macrophage interactions and cytokine production. This suggested that mPGES-1 that links stromal cPLA(2) is a downstream molecule involved in the PGE generation in tumor microenvironment.

In conclusion, we have shown that stromal mPGES-1 is a key regulator of tumor-associated angiogenesis and tumor growth. Present results also indicate that the recruitment of mPGES-1-expressing BM cells to stromal tissues is crucial for tumor growth and tumor-associated angiogenesis and also involves VEGF gene expression. Together, present study suggested that inhibition of host stromal mPGES-1 activity and of the recruitment of mPGES-1-expressing BM cells to the site of a primary tumor may be a novel strategy for the treatment of solid tumors.

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