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Host protein C inhibitor inhibits tumor growth, but promotes tumor metastasis, which is closely correlated with hypercoagulability

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ABSTRACT

Introduction: Protein C inhibitor (PCI), a member of the serine protease inhibitor family, is expressed in various human tissues, including liver and kidneys. In the plasma, PCI physiologically inhibits an anticoagulant serine protease, activated protein C (APC). PCI expressed by cancer cells suppresses tumor invasion by inhibiting urokinase-type plasminogen activator, and inhibits tumor growth and metastasis, which are independent of its protease-inhibitory activity. In the present study, we clarified the effects of host PCI on growth and metastasis of B16 melanoma (B16) cells by comparing between wild-type mice and mice transgenic for human PCI gene (hPCI-TG), which have a tissue distribution of PCI similar to that observed in humans.
Materials and Methods: Growth of intracutaneously-injected B16 cells was evaluated by measuring the tumor volume, and metastatic behavior of intravenously-injected B16 cells by counting the number of metastatic lung nodules.
Results: Growth of intracutaneously injected B16 cells was significantly faster in wild-type mice than in hPCI-TG mice; however, hPCI-TG mice developed more metastatic nodules of B16 cells in the lungs. Immunohistochemical analysis using anti-mouse fibrinogen antibody revealed more fibrin deposition in the lung in hPCI-TG mice than in wild-type mice. Furthermore, the more invasive behavior observed in hPCI-TG mice was reduced by rabbit anti-human PCI IgG, APC, or soluble TM administration for 3 consecutive days including the day that B16 cells were injected.
Conclusions: Our results suggest that like PCI expressed in tumor cells, host PCI also inhibits tumor growth, but host PCI promotes tumor metastasis via its procoagulant properties.

Introduction

The protein C anticoagulant pathway is the most important pathway for physiological blood coagulation [1]. The protein C inhibitor (PCI, SERPINA5), a member of the serine protease inhibitor (SERPIN) family, functions as a physiological inhibitor for activated protein C (APC), the main protease of the protein C anticoagulant pathway [2,3]. APC inactivates two blood coagulation cofactors, factors Va and VIIIa [4,5], in the presence of protein S [6,7]. PCI also inhibits other proteases of the blood coagulation and fibrinolysis system, including thrombin [8], thrombin–thrombomodulin complex [9], factor VIIa in complex with tissue factor [10], factor Xa [8], factor XIa [11], plasma kallikrein [11], and urinary plasminogen activator (uPA) [12].

Human plasma PCI is mainly synthesized in the liver [13], and it is also produced in kidneys and reproductive organs, including testes, seminal vesicles, and ovaries [14,15]. In the kidneys, PCI is expressed in renal proximal tubular epithelial cells (RPTECs) [16], and it inhibits uPA in urine [17]. uPA plays a key role in tumor invasion by activating plasminogen to plasmin, which promotes tumor invasion by degrading the extracellular matrix [18]. We previously reported that PCI expression disappears in RPTEC-derived renal carcinoma cells [16]; that PCI-expressing renal carcinoma cell and breast cancer cells have decreased invasive activity *in vitro* [16,19]; and that PCI-expressing breast cancer cells have decreased growth and metastatic activities *in vivo* [19], although the latter effect is not dependent on the protease-inhibitory activity of PCI. These results indicate that PCI expressed in tumor cells

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regulates not only tumor invasion, but also tumor growth and metastasis. However, the role of host PCI, especially in plasma, on tumor growth and metastasis is still unclear.

We have used mouse models to evaluate the growth and metastatic potential of tumor-expressed PCI [19]. In contrast to humans [16], in mice PCI is not expressed by the liver, but is instead solely expressed by reproductive organs such as testes and ovaries [20,21]; consequently, tumor behaviors are dependent on PCI expressed by the tumors themselves, because there is no PCI in mouse plasma. However, mouse is a suitable animal for investigating the role of PCI in fertilization, because mice do express PCI in the reproductive organs [20]. In fact, Uhrin et al. reported that PCI-knockout male mice are infertile due to abnormal spermatogenesis caused by destruction of the Sertoli-cell barrier [22]. In rats, as in mice, PCI is produced by males only in the testes and seminal vesicles [23], suggesting that rodents are inappropriate for studying the physiological role of PCI in plasma and other organs. To address this issue, we recently developed a human PCI gene transgenic (hPCI-TG) mouse in which the tissue distribution of PCI is similar to that in humans [21]. Using this animal model, we demonstrated that PCI is the physiological inhibitor of APC in plasma, and that plasma PCI promotes blood coagulation and inflammation in animals with endotoxemia [21]. Further, human PCI expression in kidney cells was quite similar to that in hPCI-TG mice [24]. These results demonstrate the advantage of using hPCI-TG mice to evaluate the *in vivo* roles of PCI on tumor growth and metastasis, rather than performing these investigations in humans.

To investigate the effect of host PCI, especially in plasma, on tumor-cell behavior, we compared the growth and metastatic potentials of B16 melanoma cells in hPCI-TG mice to those in wild-type mice. Furthermore, we showed that the higher metastatic potential observed in hPCI-TG mice decreased upon treatment with anticoagulants such as human APC and recombinant soluble thrombomodulin (rsTM). The results of our investigation demonstrate that host PCI inhibits tumor growth, just as tumor cell–expressed PCI does, but stimulates tumor metastasis by promoting blood coagulation.

Materials and methods

Materials

Bovine serum albumin (BSA) and fetal bovine serum (FBS) were purchased from Sigma (St. Louis, MO, USA). Minimum essential medium (MEM) was purchased from GIBCO (Grand Island, NY, USA). Penicillin-streptomycin solution (×100) and Geneticin were obtained from Wako Pure Chemical Industries (Osaka, Japan) and Nacalai Tesque (Kyoto, Japan), respectively. Effectene transfection reagent was purchased from QIAGEN (Tokyo, Japan). Gelatin was purchased from Bio-Rad (Foster City, CA, USA). Protein A-Sepharose was acquired from GE Healthcare (Uppsala, Sweden). Biotinyl N-hydroxy succinimide ester and streptavidin–horseradish peroxidase conjugate were purchased from E Y Laboratories Inc. (San Mateo, CA, USA) and GE Healthcare (Buckinghamshire, UK), respectively. MaxiSorp 96-well microplates were purchased from Nunc (Roskilde, Denmark). SuperScript First Strand cDNA Synthesis System kit was obtained from Invitrogen (Carlsbad, CA, USA). Rabbit anti–mouse fibrinogen IgG and Alexa Fluor 594–labeled goat anti–rabbit IgG were purchased from Innovative Research, Inc. (Novi, MI, USA), and Molecular Probes (Eugene, OR, USA), respectively. Human APC and rsTM were kindly provided by The Chemo-Sero-Therapeutic Research Institute (Kumamoto, Japan) and Asahi Kasei Pharma Corp. (Tokyo, Japan), respectively. All other chemicals and reagents were of the highest quality commercially available.

Cell culture

B16 melanoma (B16) cells were obtained from the Japanese Cancer Research Resources Bank. B16 cells were cultured in Minimum Essential Medium (MEM) supplemented with 10% FBS, 100 IU/ml penicillin, and 100 μg/ml streptomycin, in a humidified atmosphere containing 5% CO_2 at 37°C.

Preparation of intact PCI-expressing B16 cells

Expression plasmid for human intact PCI was constructed as described previously [16]. Intact human PCI cDNA was inserted into the *ClaI/Xba* I site of the mammalian expression vector pRC/CMV (Invitrogen), and then transfected into B16 cells using the Effectene transfection reagent. After transfection, intact PCI-expressing B16 cell lines were selected using MEM containing 800 μg/ml geneticin, followed by measurement of PCI antigen in the culture medium of each cell line by enzyme immunoassay and/or by evaluating PCI mRNA expression by reverse transcriptase PCR (RT-PCR) as described below. Twenty to thirty cell lines expressing high levels of PCI (B16-PCI) were selected from among 240 transfected cell lines for use in the experiments. B16 cell lines expressing higher amounts of PCI were designated as B16-PCI 1 and B16-PCI 2. Following the same procedure, B16 cells transfected with pRC/CMV containing no DNA insert were prepared for use as negative controls; Two of these cell lines were designated as B16-Mock 1 and B16-Mock 2.

Enzyme immunoassay

PCI antigen levels in culture medium were determined by enzyme immunoassays using polyclonal anti–human PCI IgG, as described previously [16].

RNA extraction

Total RNA was extracted from cultured cells by a modified guanidinium thiocyanate-phenol chloroform technique using the RNAzol B reagent (TEL-TEST, Friendswood, TX, USA) as described previously [25]. Total RNA was quantitated spectrophotometrically and stored at -80°C until use.

Reverse transcriptase–polymerase chain reaction (RT-PCR)

To evaluate mRNA expression of PCI in B16 cells transfected with human PCI expression vector, RT-PCR was carried out as described previously [16] using total RNA prepared from B16 cells as a template.

Animals

hPCI-TG mice were produced as described previously [21]. Founder mice were then back-crossed at least 30 times by mating with C57BL/6J mice. The transgene was analyzed by detecting the human PCI antigen in plasma using an enzyme immunoassay specific for human PCI. Mice were housed under a 12-h light/12-h dark constant cycle and allowed free access to standard food and water. The experiments were approved by the Mie University Review Board for animal investigation and were conducted according to the National Institutes of Health guidelines for animal experiments. In every study, we used littermates of hPCI-TG mice that did not express the human PCI antigen in plasma as wild-type mice.

Evaluation of tumor growth in vivo

Sub-confluent cells were harvested with EDTA solution and resuspended at an appropriate density (2.5×10^6 cells/ml) using serum-free MEM; then 5×10^5 cells in 0.2 ml of sterilized MEM were intracutaneously injected into the abdominal wall of 6-week-old male hPCI-TG and wild-type mice. Each cell line was injected into at least four mice. Tumor growth was monitored by weekly measurement of tumor size

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using calipers. Tumor volume was determined using the following equation: volume = (smallest diameter)^2 × (largest diameter) × 0.52.

Model of experimental lung metastasis

Sub-confluent cells were harvested with EDTA solution and resuspended at an appropriate density (7.5 × 10^5 cells/ml) in serum-free MEM. The cells (1.5 × 10^5 cells in 0.2 ml of sterilized MEM) were then injected into the tail veins of male wild-type and hPCI-TG mice. Rabbit anti–human PCI IgG (200 μg/mouse), human APC (70 μg/mouse), or human rsTM (200 μg/mouse) were administered for 3 consecutive days, starting on the day the B16 cells were introduced. Fourteen days after tumor injection, the mice were anesthetized with pentobarbital and sacrificed. The lungs were excised, fixed in formaldehyde neutral buffer solution, and nodules visible as black spots in the lungs were counted using a magnifying glass. Each group consisted of at least four mice.

Immunohistochemical staining

Immunoreactivity of fibrin in tumor-including tissues was assessed by single-immunofluorescence labeling. Briefly, deparaffinized and dehydrated sections (5-μm thickness) were incubated with 5% skim milk for 60 min, and then incubated with a rabbit anti–mouse fibrinogen IgG (1:400) at 4°C for 24 h. Subsequently, the sections were incubated at room temperature for 3 h with Alexa Fluor 594–labeled goat antibody against rabbit IgG (1:400). The sections were washed with PBS between each step. The stained sections were examined by fluorescence microscopy (BX53, Olympus, Tokyo, Japan). As a control for the specificity of the immunoreaction, normal rabbit IgG was used instead of primary antibody.

Statistical analysis

All values were expressed as means ± standard deviation of the mean. All experiments were repeated at least three times. Student's t-test was used to evaluate significant differences. Values of $p < 0.05$ were considered to be statistically significant.

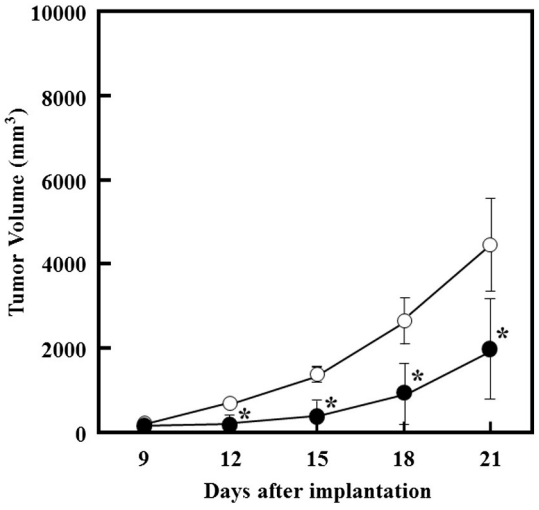

Fig. 1. Growth of B16 cells intracutaneously injected into wild-type and hPCI-TG mice. B16 cells (5 × 10^5 cells in 200 μl of sterilized MEM) were injected intracutaneously into the abdominal wall of 6-week-old male wild-type (open circles) and hPCI-TG mice (closed circles), and tumor volume was measured using calipers. The data represent tumor volumes (means ± S.D.) from at least four mice. *, $p < 0.05$ relative to wild-type mice.

Results

Effect of host PCI on the growth of B16 cells

To evaluate the effect of host PCI on tumor growth, we monitored the growth of B16 cells implanted into the abdominal wall of male hPCI-TG or wild-type mice. As shown in Fig. 1, B16 cells grew significantly more slowly in hPCI-TG mice than in wild-type mice. In hPCI-TG mice, whose PCI concentration in plasma was 19.0 ± 3.6 μg/ml, the growth of B16-PCI 1 (which secreted 5.25 ng PCI/10^4 cells/24 h) was slower than that of B16-Mock 1 (Fig. 2A), and the growth of B16-PCI 2 (which secreted 0.69 ng PCI/10^4 cells/24 h) was also slower than that of B16-Mock 2 (Fig. 2B). These results show that PCI expressed by the cells was more effective than PCI in the host.

Effect of host PCI on the metastasis of B16 cells

Next, we examined the effect of host PCI on the metastatic potential of B16 cells by counting the number of metastatic lung nodules after intravenous injection of B16 cells into hPCI-TG and wild-type mice. As shown in Fig. 3, the number of lung nodules in hPCI-TG mice was

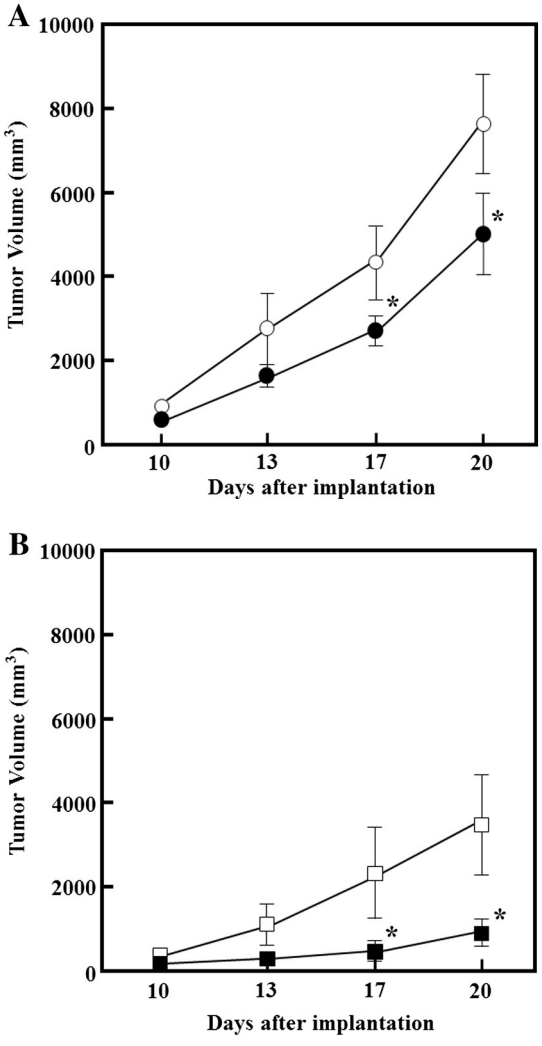

Fig. 2. Growth of intracutaneously injected B16-Mock or B16-PCI cells in the hPCI-TG mice. (A) B16-Mock 1 (open circles) or B16-PCI 1 (closed circles) (5 × 10^5 cells in 200 μl of sterilized MEM), and (B) B16-Mock 2 (open squares) or B16-PCI 2 (closed squares) (5 × 10^5 cells in 200 μl of sterilized MEM) were injected intracutaneously into the abdominal wall of 6-week-old male hPCI-TG mice, and tumor volume was measured using calipers. The data represent tumor volumes (means ± S.D.) from at least five mice. *, $p < 0.05$ relative to respective B16-Mock.

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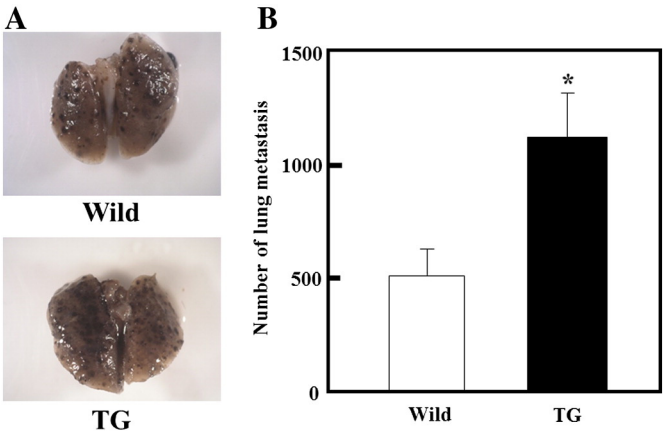

Fig. 3. Lung metastasis of B16 cells intravenously injected into hPCI-TG mice. B16 cells (1.5×10^5 cells in 200 μl of sterilized MEM) were intravenously injected into 6-week-old male wild-type and hPCI-TG mice. Lungs were isolated after 14 days and fixed, following which pulmonary metastasis formation was quantitated by counting the number of tumor foci. (A) Representative results and (B) data representing the number of foci (means ± S.D.) from at least five mice. *, $p < 0.05$ relative to wild-type mice.

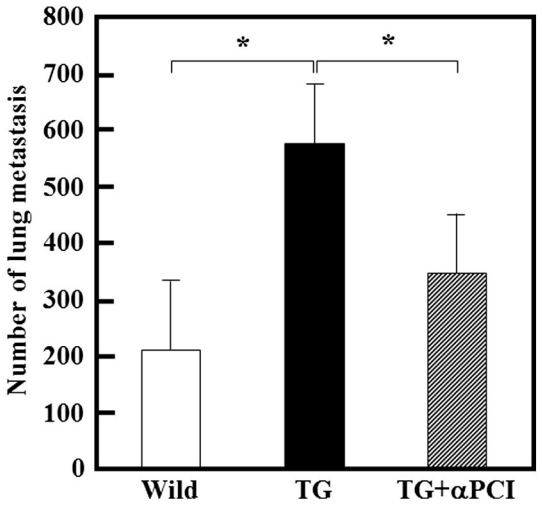

Fig. 5. Effect of anti-PCI IgG on lung metastasis of B16 cells intravenously injected into hPCI-TG mice. B16 cells (1.5×10^5 cells in 200 μl of sterilized MEM) were injected into the tail vein of male wild-type and hPCI-TG mice. Rabbit anti–human PCI IgG (200 μg/mouse) was administered for 3 consecutive days, starting on the day the B16 cells were introduced. Fourteen days after tumor injection, the mice were anesthetized with pentobarbital and sacrificed. After lungs were isolated and fixed, pulmonary metastasis formation was quantitated by counting the number of tumor foci. The data represent the number of foci (means ± S.D.) from at least four mice. *, $p < 0.05$.

significantly higher than in wild-type mice. Furthermore, immunostaining revealed significantly more fibrin deposition in the lungs of hPCI-TG mice (Fig. 4A and 4B). Furthermore, this increase in the number of lung nodules was mitigated in hPCI-TG mice treated with rabbit anti–human PCI IgG (Fig. 5), which had shown no effect on the metastasis of B16 cells observed in wild-type mice (data not shown). In hPCI-TG mice, metastasis of B16-PCI 1 was significantly lower than that of B16-Mock 1 (Fig. 6). These results show that PCI expressed by tumor cells is a more important determinant of metastatic potential than PCI expressed in the host.

Effect of human APC and rsTM on metastasis of B16 cells observed in hPCI-TG mice

The hPCI-TG mice exhibited a hypercoagulative phenotype relative to wild-type mice [21], and there was more fibrin deposition in the lungs of hPCI-TG mice than in wild-type mice. Therefore, we examined the effect of the coagulative tendency of the hPCI-TG mice on metastasis of B16 cells by pre-injecting several anticoagulants, such as human APC and rsTM. As shown in Fig. 7A and Fig. 7B, the number of lung nodules in hPCI-TG mice was greater than in wild-type mice, but this number decreased in hPCI-TG mice treated with APC or rsTM. Immunohistochemical analysis revealed that APC or rsTM treatment also reduced fibrin deposition in the lungs of hPCI-TG mice (data not shown). In addition, both human APC and rsTM also inhibited the metastasis of B16 cells observed in wild-type mice (data not shown). These results confirmed the relationship between blood coagulation and tumor cell metastasis.

Discussion

Previously, we reported that PCI expressed by the breast cancer cell line MDA-231 inhibits invasion, growth, and metastasis; furthermore, inhibition of invasion by PCI, but not inhibition of growth and

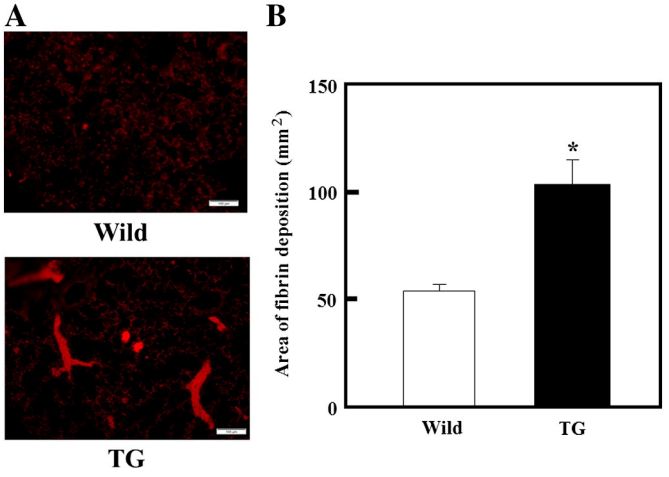

Fig. 4. Fibrin deposition in lung of the wild type and the hPCI-TG mice. Fixed tumor tissues embedded in paraffin were cut into 5-μm sections using microtome. The sections were incubated with rabbit anti–mouse fibrinogen IgG (1:400), followed by incubation with Alexa Fluor 594–labeled goat IgG specific for rabbit IgG (1:400). Finally, labeled sections were examined under an inverted laser-scanning confocal microscope. (A) Representative results and (B) data representing the area of fibrin deposition (means ± S.D.) from five different areas. Scale bar shows 100 μm. *, $p < 0.05$ relative to wild-type mice.

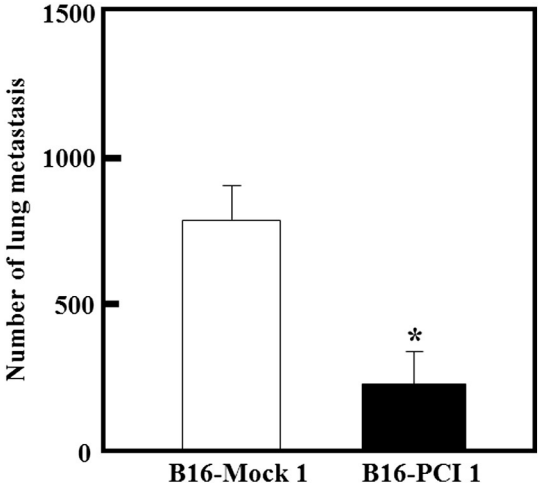

Fig. 6. Lung metastasis of B16-Mock or B16-PCI cells intravenously injected into hPCI-TG mice. B16-Mock 1 (open bar) or B16-PCI 1 (closed bar) (1.5×10^5 cells in 200 μl of sterilized MEM) were intravenously injected into 6-week-old male hPCI-TG mice. Lungs were isolated after 14 days and fixed, following which pulmonary metastasis formation was quantitated by counting the number of tumor foci. Data representing the number of foci (means ± S.D.) from at least five mice. *, $p < 0.05$ relative to wild-type mice.

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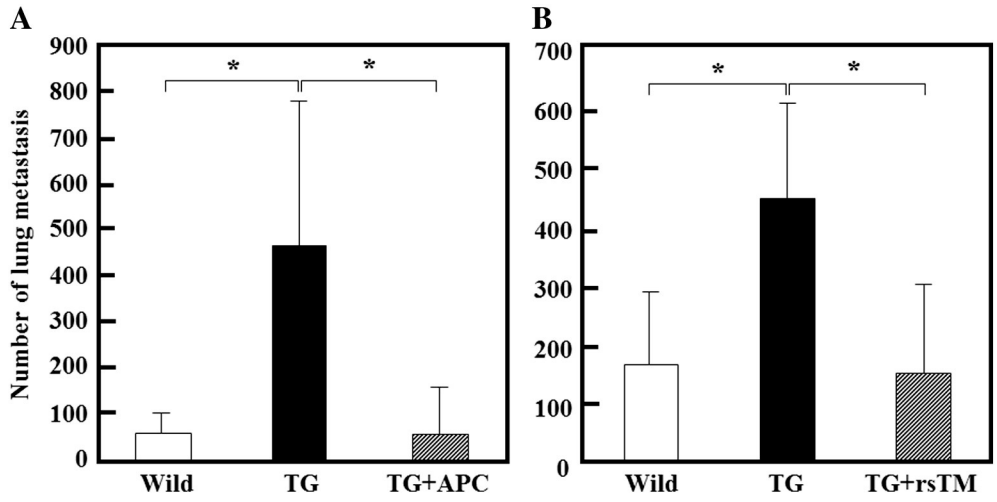

Fig. 7. Effect of APC and rsTM on lung metastasis of B16 cells intravenously injected into hPCI-TG mice. B16 cells (1.5×10^5 cells in 200 μl of sterilized MEM) were injected into the tail vein of male wild-type and hPCI-TG mice. APC (70 μg/mouse)(A) or rsTM (200 μg/mouse) (B) was administered for 3 consecutive days, starting on the day the B16 cells were introduced. Fourteen days after tumor injection, the mice were anesthetized with pentobarbital and sacrificed. After lungs were isolated and fixed, pulmonary metastasis formation was quantitated by counting the number of tumor foci. The data represent the number of foci (means ± S.D.) from at least four mice. *, $p < 0.05$.

metastasis, is dependent on its protease-inhibitory activity [19]. PCI also inhibits angiogenesis; consequently, PCI-expressing tumor cells exhibit decreased growth potential relative to Mock-expressing tumor cells [19]. Various proteins of the SERPIN family, including cleaved antithrombin (AT) [26], α1-antitrypsin [27], plasminogen activator inhibitor-1 [28], and pigment epithelium derived factor [29], also inhibit tumor growth by inhibiting angiogenesis. Furthermore, the heparin-binding site of cleaved AT is crucial for its anti-angiogenic activity [30], and kallistatin, another heparin-binding SERPIN, loses its anti-angiogenic activity if its heparin-binding site is mutated [31]. PCI also contains a heparin-binding site, located on its N-terminal H and D helices, that contains positively charged residues [32,33]; as in the cases of cleaved AT and kallistatin, the heparin-binding site in PCI is likely to be very important for its anti-angiogenic activity. The results of these studies suggest that host PCI, especially plasma PCI, also inhibits tumor growth, and that this inhibition might be caused by its anti-angiogenic activity.

Rodents such as mouse and rat do not express PCI in the liver [21,23] and have no PCI in plasma; therefore, it is impossible to investigate the effects of host PCI, especially PCI in plasma, on tumor growth and metastasis by observing the phenotype of PCI-knockout rodents. Humans have PCI in plasma, and we are interested in the effects of plasma PCI on tumor growth and metastasis. Previously, we generated hPCI-TG mice, which have a PCI tissue distribution similar to that observed in human; the transgenic animals have human PCI in the plasma [21], making them appropriate for analysis of the effects of plasma PCI on tumor growth and metastasis. In this study, we used the hPCI-TG mice to investigate the effects of plasma PCI on tumor behaviors such as growth and metastasis. Our results indicated that B16 cells grew more slowly in hPCI-TG mice than in wild-type mice, and that growth of B16-PCI 1 (which secreted approximately 5.25 ng PCI/10^4 cells/24 h) was also lower than that of B16-Mock 1 in the hPCI-TG mice. PCI concentration in the plasma of male hPCI-TG mice was approximately 20 μg/ml, which is much higher than the amount of PCI expressed by B16-PCI cells. These results also suggest that PCI in plasma might have a different effect on tumor growth than tumor cell–expressed PCI, which inhibits tumor growth by reducing the angiogenesis. Recently, we showed that PCI effectively inhibits hepatocyte growth factor activator (HGFA) [34], which converts hepatocyte growth factor (HGF) precursor to active HGF [35]; the resultant HGF promotes growth not only in normal hepatocytes [36], but also in various tumor cells [37], such as hepatocellular carcinoma [38] and non–small cell lung cancer [39], *via* c-Met. The physiological importance of HGF/c-Met was confirmed by the use of the c-Met inhibitor, ARQ 197 (tivantinib), for medical treatment of cancer [40]. ARQ 197 inhibits the tyrosine kinase activity of c-Met, resulting in inhibition of cancer growth *in vivo* [41]. It is well-known that HGFA is produced by activation of proHGFA by thrombin [42] which is the main protease of blood coagulation cascade, and that tumor cells activate blood coagulation [43]. Our results suggest that plasma PCI inhibits tumor growth by inhibiting HGFA, leading to a reduction of HGF-mediated c-Met phosphorylation. The detailed mechanism of inhibition of tumor growth by plasma PCI including the contribution of HGFA inhibition by PCI to inhibition of tumor growth is now under investigation.

Next, we investigated the effect of host plasma PCI on metastasis of B16 cells in hPCI-TG mice. The results indicated that more metastatic foci was observed in hPCI-TG mice than in wild-type mice; this increase was mitigated by treatment with anti–human PCI IgG, which had shown no effect on the metastasis observed in wild-type mice. These results suggest that plasma PCI promotes tumor metastasis. In the process of experimental tumor metastasis used in our study, the first step is the attachment of tumor cells to pulmonary endothelium, which occurs near sites of coagulation consisting of fibrin clots and platelet aggregates, after the intravenous injection of tumor cells. We previously showed that plasma PCI plays a role as a procoagulant and proinflammatory factor by inhibiting APC [21]. Furthermore, immunohistochemical staining revealed larger numbers of fibrin clots in hPCI-TG mice that in wild-type mice. These results suggest that the procoagulant character of the hPCI-TG mice, induced by APC inhibition, promotes metastasis of B16 cells. Subsequently, again using the hPCI-TG mice, we examined the effect of anticoagulants on promotion of tumor metastasis by PCI. Our results indicated that anticoagulant APC inhibits the elevation in the number of metastatic foci observed in the hPCI-TG mice. This result suggests that the protease-inhibitory activity of PCI is very important for the promotion of metastasis observed in the hPCI-TG mice, and that blood coagulation plays an important role in tumor metastasis. To further confirm the importance of coagulation on tumor metastasis, we showed that rsTM also inhibited the tumor metastasis observed in the hPCI-TG mice. In addition, immunohistochemical analysis using anti–mouse fibrinogen antibody revealed less fibrin deposition in mice treated with human APC or rsTM (data not shown).

Many studies have reported that anticoagulants, such as warfarin and thrombin inhibitor, inhibit experimental tumor metastasis; in clinical trials, however, the extent of improvement in cancer patients' survival rates remains controversial [43]. These findings suggest that coagulation is at least related to the metastasis of tumor cells. Coagulation

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contributes to metastasis through several mechanisms, including expression of tissue factor in tumor cells and P-selectin on platelets [43]. The aggregation of platelets around tumor cells is important in order to protect them from killing by natural killer cells [43]. These clots contained both platelets and fibrin, which promotes the attachment of tumor cells at an early step of metastasis. In addition to the clots surrounding tumor cells, disturbance of blood flow by clots induced by tumor cells is also a critical determinant of metastasis. It is not obvious whether PCI promotes coagulation around tumor cells, which might enhance tumor cell attachment to the endothelium, or coagulation in the small vessels, which might induce tumor cell congestion, thereby facilitating the invasion of the endothelium. Further investigation is needed to elucidate this point. Overall, our results suggest that the procoagulant character of the hPCI-TG mice promotes tumor metastasis, and that plasma PCI plays a critical role in the enhanced metastasis observed in these mice.

Conclusions

In brief, the results of the present study demonstrate that host PCI, especially PCI in plasma, inhibits tumor growth by a mechanism distinct from that of PCI expressed by cells, but promotes tumor metastasis via its procoagulant activity. These findings provide important insight into the overall effect of PCI on tumor growth and metastasis in human.

Disclosure of conflict of interests

The authors state that they have no conflict of interests.

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